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Nanostructured Platforms for the Sustained and Local Delivery of Antibiotics in the Treatment of Osteomyelitis

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Abstract

This article provides a critical view of the current state of the development of nanoparticulate and other solid-state carriers for the local delivery of antibiotics in the treatment of osteomyelitis. Mentioned are the downsides of traditional means for treating bone infection, which involve systemic administration of antibiotics and surgical debridement, along with the rather imperfect local delivery options currently available in the clinic. Envisaged are more sophisticated carriers for the local and sustained delivery of antimicrobials, including bioresorbable polymeric, collagenous, liquid crystalline, and bioglass- and nanotube-based carriers, as well as those composed of calcium phosphate, the mineral component of bone and teeth. A special emphasis is placed on composite multifunctional antibiotic carriers of a nanoparticulate nature and on their ability to induce osteogenesis of hard tissues demineralized due to disease. An ideal carrier of this type would prevent the long-term, repetitive, and systemic administration of antibiotics and either minimize or completely eliminate the need for surgical debridement of necrotic tissue. Potential problems faced by even hypothetically "perfect" antibiotic delivery vehicles are mentioned too, including (i) intracellular bacterial colonies involved in recurrent, chronic osteomyelitis; (ii) the need for mechanical and release properties to be adjusted to the area of surgical placement; (iii) different environments in which *in vitro* and *in vivo* testings are carried out; (iv) unpredictable synergies between drug delivery system components; and (v) experimental sensitivity issues entailing the increasing subtlety of the design of nanoplatforms for the controlled delivery of therapeutics.

Keywords

calcium phosphate; composites; controlled drug delivery; nanoparticle; osteomyelitis

I. INTRODUCTION

Osteomyelitis, the infectious inflammation of bone and one of the oldest documented diseases, the earliest descriptions of which date back to Hippocrates (fifth century BC),[1] is an illness particularly prevalent among the elderly, diabetics, children, and indigenes of

Third World countries (Fig. 1a). Before the advent of antibiotics, the mortality rate because of osteomyelitis was 25–45%. Although morbidity due to chronic bone infection has drastically decreased from the pre-penicillin era, down to ~3% in the past 20 years,[2] it is still high on the global scale, and treating the disease continues to be considerably challenging.[3] The incidence of osteomyelitis in the United States is 1–2%, but the disease is far more prevalent in developing countries, as well as among particular patient populations: approximately 1 in 5000 children, 1 in 1000 neonates, 1 in 250 patients with sickle cell disease, 1 in 7 diabetics, and 1 in 3 patients with punctured foot.[4–7] Its comparatively low prevalence can be explained by the fact that bone is an organ well protected from external pathogens and is not readily prone to infection. The difficulty faced by invasive pathogens in an attempt to colonize the bone is, however, directly proportional to the difficulty faced by clinicians in ensuring the delivery of antibiotics to the site of infection and curing it. The prevalence of chronic osteomyelitis among patients treated for at least one episode of acute osteomyelitis is consequently high, in the range of 5–25%.[8] Strategies for improving the therapeutic approach in the treatment of osteomyelitis have thus been explored for over a century,[9] with a steadily increasing annual number of publications related to it—from 1 to 10 until 1944 to 100–300 from 1944 to 1974 to 713 in 2012 (US National Library of Medicine), more than in any of the preceding years—going in step with the anticipated increase in the number of cases of bone disease as the corollary of the aging population worldwide (Fig. 1b). The number of hip and knee replacement procedures performed in the United States has, for example, doubled in the past decade, whereas the number of the reported cases of bone infection accompanying those procedures also has steadily increased in proportion to the number of surgeries performed (Fig. 2). In spite of using aseptic techniques and antibiotic prophylaxis, osteomyelitis is estimated to develop in 22–66% of patients following orthopedic operations, and the corresponding mortality rate could be as high as 2%.[10] This review describes (1) the pathologies that cause osteomyelitis; (2) the traditional therapeutic approach to curing it; and (3) advanced therapeutic methods based on the design of nanostructured platforms for the sustained and local delivery of antibiotics.

II. PATHOLOGIES AND THE DOWNSIDES OF THE TRADITIONAL CLINICAL APPROACH

Osteomyelitis is mainly caused by pyogenic bacteria found in healthy oral flora, although cases of infection caused by fungi are also common.[13–15] Bone infections caused by *Brucella suis*,[16] *Haemophilus influenzae*,[17] *Mycobacterium tuberculosis*,[18] *Mycobacterium ulcerans*,[19] and pox viruses,[20,21] as well as those whereby bone lesions are secondary to Bacille Calmette-Guérin[22] or smallpox[23] vaccination, also have been reported in the literature. Although many gram-negative and gram-positive bacteria were reported to have caused osteomyelitis; the great majority of bone infections, however, is staphylococcal in origin and mostly caused by a single bacterium: *Staphylococcus aureus*.[24,25] In addition to *S. aureus*, *S. epidermidis* is another common cause of osteomyelitis; it is present in up to 90% of bone infections following intraoperative implantation of a foreign material. Because most cases of osteomyelitis are caused by bacteria that reside on healthy skin and in healthy oral flora, osteomyelitis is an illness often caused by a bizarrely small scratch or a bite where

by body fluids become exposed to external pathogens, which then go on to induce septic arthritis and/or osteomyelitis.[26]

The onset of the infection induces an acute suppurative inflammation, and numerous factors synergistically contribute to the necrosis of the hard tissues, demineralization of the bone, and degradation of its collagen matrix: bacteria, pH change, local edema that accumulates under pressure, vascular obstruction, and leukocyte collagenase.[27] As the infection progresses locally, it extends to the adjacent osseous structures through the Haversian and Volkmann canals, leading to an increased obstruction of vascular channels and necrosis of more osteocytes in the lacunae. By the time the infection reaches the outer part of the cortex, it has already caused an inversion of the periosteal blood flow and gained access to the subperiosteal space, which results in a subperiosteal abscess and the formation of involucrum, a layer of new bone grown from periosteum stripped from the original bone under the pressure of pus. Figure 3 shows radiological images of cases of acute and chronic osteomyelitis (the former came from a clinic and the latter from an animal model),[28] along with involucrum formed around the area of necrotic infection and a periosteal reaction in the proximal area of the bone, respectively.

Osteomyelitis is particularly prevalent in the facial skeleton because of its accessibility to a variety of external pathogens and commensal microorganisms.[29] It also presents a major complication following orthopedic and maxillofacial surgeries,[30] including even the most routine dental extractions.[31] Although the resistance to infection of healthy bone is naturally high, implants reduce it by a factor of 10^3 (i.e., the number of pathogens sufficient to cause an infection is reduced from 10^8 to 10^5). As a result, intraoperative introduction of bacteria accounts for the largest number of osteomyelitis cases, with the hip being a particularly common orthopedic site of infection. Timely treatment of osteomyelitis is required to prevent its spread to new sites in the body and to avoid systemic osteonecrosis or unaesthetic facial disfigurement in the case of maxillofacial infection. The typical treatment regimen for bone infection consists of (1) intravenous administration of antibiotics lasting 2–6 weeks, frequently followed by a 6-month course of oral antibiotics in the case of chronic infection; and (2) surgical removal of bone that has undergone necrosis due to restriction of blood flow by the formed abscesses.[32,33] Correspondingly, the major downsides of the conventional therapeutic approach include (1) systemic administration of the therapeutic agent and its side effects; (2) low concentration of the therapeutic agent around the site of infection, potentially inducing resistance of the pathogen to the antibiotic therapy; and (3) irretrievable bone loss that often requires the insertion of implants or prostheses as lasting bone substitutes. Moreover, in the advanced stages of infection, when bone necrosis has become significant, the blood supply to the infected area is inadequate and the lesion is largely inaccessible to antimicrobial agents transported by the blood stream. All these downsides provide strong incentives in favor of the development of appropriate carriers for the local delivery of antibiotics in the treatment of osteomyelitis.

III. CLINICALLY AVAILABLE MATERIALS FOR LOCAL DELIVERY

Because of the apparent downsides of traditional therapy, including primarily the systemic and repetitive administration of antibiotics whereby the therapeutic concentrations in the

target area constantly fluctuate between toxic and ineffective, steps have been taken to develop particulate carriers for the local and sustained delivery of antibiotics following their implantation directly at the zone of infection. Ever since the pioneering research in this field carried out in Europe in the 1970s,[34,35] poly(methyl methacrylate) (PMMA) beads, first clinically applied in 1972, have been the gold standard for the local delivery of antibiotics to bone cavities. Currently, there is no clinical alternative to PMMA as a local delivery carrier for osteomyelitis since they are the only preloaded option approved by the US Food and Drug Administration (FDA).[36]

PMMA beads loaded with hydrophilic antibiotics, including gentamicin, ceftriaxone, tobramycin, and vancomycin, have been used with experimental and clinical success in the past.[37–41] Despite this, numerous limitations are associated with the use of PMMA beads. First, they are not biodegradable and require a secondary surgical procedure for removal after the antibiotic is released through their porous polymeric structure. Second, PMMA beads and spacers exhibit burst release[42] that depletes the drug from the carrier and is followed by a rather insubstantial release period that may be insufficient to maintain a therapeutic concentration for the desired 3–4 weeks and may even promote antibiotic resistance.[43] Although release kinetics could be extended by increasing the size of the beads and increasing the polymerization time, burst release has seemed unavoidable so far.[44] Because the release is conditioned by the diffusion of the drug through the porous polymeric network and microscopic cracks in the cement—and not by the degradation of the polymer the elution profiles show broad variations depending on the nature of the antibiotic, exhibiting intense burst release and a prompt decrease in concentration below the therapeutic level in some cases.[45,46]

Relatively low toxicity results from the absorption of methyl methacrylate monomers and the associated carboxylesterase-mediated conversion of methyl methacrylate to methacrylic acid,[47,48] whereas biofilm frequently forms on antibiotic-laden PMMA beads, hindering the antimicrobial action.[49,50] Although products preloaded with gentamicin are available on the market (Septopal), most clinically applied PMMA beads are loaded with the antibiotic just before surgical insertion[51] (Fig. 4), which can lead to inconsistent release profiles.[52] This nullifies the producer's liability for the product and makes it therapeutically applicable only with the patient's consent.[53] Finally, a comprehensive clinical study has yet to prove that PMMA beads are more effective than the systemic antibiotic delivery approach in treating orthopedic infections.[54] No significant difference in the treatment success rate was typically observed when debridement was followed by the implantation of antibiotic-containing PMMA beads for local release or the prescription of systemic antibiotics.[55] The lower cost of the therapy is often considered the only advantage of local delivery using PMMA beads.[56] Consequently, a large population of clinicians is skeptical about the benefits of local delivery in the management of osteomyelitis compared with the traditional approach and resorts to the latter in their practice.

With no nonbiodegradable bone substitutes for load-bearing applications in sight for either the biomedical device market or anywhere in clinical testing, for many decades now the greatest potential among the bone engineers has been logically ascribed to bioresorbable implants. However, the only currently clinically available bioresorbable alternative to

PMMA beads, predominantly used outside the United States, is calcium sulfate cements.[57] Unlike PMMA, whose monomeric absorption has previously caused intraoperative cardiopulmonary complications during arthroplasty,[58] calcium sulfates are nontoxic and inexpensive and have been successfully used as a drug carrier in the treatment of osteomyelitis.[59,60] In addition to being used since the late 19th century as a bone filler in their hemihydrate form, also known as plaster of Paris,[61] calcium sulfates have been applied in reparative dentistry for maxillary sinus floor augmentation[62] and for the repair of periodontal defects[63] and root perforations.[64] In addition to their exceptional softness and poor handling features, the main downside is that they are resorbed rapidly, in a matter of weeks—faster than the bone ingrowth rate—which can lead to mechanical implant failure.[65] An ideal bioresorbable implant provides a mechanical support that is gradually transferred to the newly formed bone, a requirement that calcium sulfates do not satisfy. They can also cause severe drainage at the wound site after the surgical implantation,[66] as well as the formation of a fibrous gap in the area where the slowly ingrowing bone replaces the rapidly resorbing cement,[67] the same effect that is expected to result from the use of aragonite[68] or calcium phosphate phases, such as tricalcium[69] or dicalcium[70,71] phosphates, as bone fillers; these are more soluble than hydroxyapatite, the calcium phosphate constituent of bone. Also, as a result of their relatively fast degradation in the body, the concentration of the antibiotic at the target site and its mean blood serum concentration over the first month following implantation are lower when compared with hydroxyapatite.[72] For this reason, and in view of the fact that calcium sulfates have not led to therapeutic outcomes any better than those of PMMA implants,[73] their use as an ideal bioresorbable delivery vehicle for antibiotics and a void filler in bony defects has been questioned.[74]

IV. ADVANCED DRUG DELIVERY PLATFORMS IN THE RESEARCH STAGE

As noted earlier, two main disadvantages of the traditional treatment of osteomyelitis include (1) systemic distribution of the therapeutic agent and (2) the need for surgical removal of necrotic bone. Options for sustained antibiotic release that can ensure high local concentrations and low serum concentrations of the drug[75] already exist, and progress in terms of promoting the osteogenic activity of the carrier is expected in the future. Whereas local and sustained release of the drug could overcome the need for prolonged oral and/or intravenous antibiotic therapies, the induction of osteogenesis by the carrier itself or the growth factors released from it could eliminate or at least minimize the surgical removal of affected bone, along with the frequent skeletal deformations and unaesthetic physical disfigurements it entails. Patients with diabetic neuropathy are prone to developing osteomyelitis of the forefoot, which often leads to minor amputation[76]; with the development of osteogenic carriers that could revitalize the diseased bone, however, such clinical cases could be coped with in a manner less traumatic for the patient. For example, in parallel with the drug release process, the particles may decompose, dissipating their osteogenic contents and thus fostering the bone healing process and natural restoration of the portion of bone damaged by the pathogen. The therapeutic approach to treating osteomyelitis would clearly yield a whole new dimension by using one such osteogenic drug delivery platform. After all, with an ideal therapeutic agent serving a dual purpose of (1) eliminating the source of illness and (2) revitalizing the organism, the conception of drug

delivery carriers that exhibit simultaneous bactericidal and osteogenic performance is natural.

A. Calcium Phosphates

Calcium phosphates occupy a special place among the biodegradable drug carriers of antibiotics in bone repair. They have been traditionally considered a convenient choice for the synthetic substitute of hard tissues because of their excellent biocompatibility, osteoconductivity, lack of cytotoxicity, nonimmunogenicity, and sufficient loading capacities, thanks to which hydroxyapatite, their least soluble phase, has been used as a chromatographic adsorbent of proteins,[77–79] nucleic acids,[80–82] and microorganisms.[83] Excellent adsorption properties of hydroxyapatite are the result of its positively charged surface Ca^{2+} ions engaging in an anion-exchange interaction with deprotonated carboxyl groups of proteins and the negatively charged PO_4^{3-} groups engaging in a cation-exchange interaction with protonated amino groups of proteins.[84] Moreover, hydroxyapatite possesses different net charges on the *a* and *c* planes of its hexagonal crystal lattice—positive and negative, respectively,[85] which renders it effective in the crystallographically selective binding of multiple molecular entities. Other variations of hydroxyapatite, such as carbonated apatite[86] and biphasic calcium phosphate,[87] possessed an even greater protein adsorption capacity, given an identical particle size and specific surface area, which was hypothesized to be due to their greater solubility, which increases the ionic strength in the medium and the surface exposition of the polar residues of proteins, thus increasing the binding efficacy.[88] Hydroxyapatite also has been used as an amphiphilic stabilizer in Pickering emulsions, suggesting its ability to interact with both hydrophilic and hydrophobic compounds.[89]

Unlike PMMA, calcium phosphates are fully bioresorbable, and the rate of their degradation could be tentatively tuned by controlling the phase composition of the compound.[90] Namely, as can be seen in Table 1, calcium phosphates can adopt a variety of stoichiometries, covering a range of solubility product values, from 0.07 for anhydrous and monohydrous monocalcium phosphates to 10^{-7} for monetite and brushite to 10^{-25} for α-tricalcium phosphate to 10^{-117} for hydroxyapatite.[91] Of course, because more ionic species exist in the stoichiometric formulas of the less soluble phases, the difference in solubility is of a lesser magnitude than that in the solubility product, amounting to approximately $6 \cdot 10^4$, $1.6 \cdot 10^2$, and 8.3 times higher solubility for monocalcium phosphate, monetite, and α-tricalcium phosphate, respectively, compared with hydroxyapatite (0.3 mg/dm^3) in water at 37°C and at a physiological pH.

Particle size presents an important consideration in the design of most optimal degradation and release profiles, and nanosized calcium phosphates have proved to be far more advantageous than the microsized ones,[92] a natural consequence of the fact that bone itself contains apatite particles with nanosized dimensions[93] ($20 \times 10 \times 2$ nm, on average[94]). Furthermore, porosity that is controllable via sintering at elevated temperatures could be used to vary the degradation rate *in vivo* within a wide window of values; again, nanosized and fully dispersed hydroxyapatite is highly bioresorbable and the denser formulations are resorbable to a significantly lesser degree,[95] leading to hypotheses that nonporous, sintered

hydroxyapatite blocks should be stable in biological milieus for centuries.[96] In this case the drug release rate tends to be directly proportional to the resorption rate, that is, significantly higher for the more porous calcium phosphate microstructures.[97] Stoichiometry of single-phase compositions, implant geometry, ionic substitutions, crystallinity, and macro- and microporosity are other factors known to greatly affect the degradation rate of calcium phosphates *in vivo*.[98–100] When self-setting calcium phosphates pastes are used, the powder-to-liquid ratio, initial viscosity, pH, and the presence of additives, such as crystallization seeds, inhibitors, or dispersants, are additional factors that influence the hardening properties, the degradation kinetics, and the rates of resorption and new bone ingrowth,[101] which usually range anywhere between 3 months and 3 years.[102]

Calcium phosphates are also a component of the mineral phase of hard tissues, which makes them a natural candidate for bone-filling drug carriers. With bone acting as a natural reservoir for calcium and phosphate ions,[109] any excessive amounts thereof could be regulated in favor of new bone growth. Calcium and phosphate ions released upon the degradation of these compounds can also stimulate osteoblastic differentiation[110,111] and proliferation[112] and be used as ionic ingredients for the formation of new bone. Another advantage of calcium phosphates is that they could be sterilized by a variety of techniques, including γ-irradiation, gas plasma, supercritical carbon dioxide, or even steam autoclaving (in the case of hydroxyapatite), without causing adverse effects to their structure and properties. By contrast, in general there is currently no established sterilization procedure for polymers that does not modify their structure to some degree, due to (1) physical deformations and chemical changes—scission and cross-linking that occur upon autoclaving,[113] alongside practically inevitable degradation of an encapsulated drug[114]; (2) surface chemistry modifications that occur upon the application of ethylene oxide, hydrogen peroxide, or ozone[115]; (3) bulk structural changes and a decrease in the molecular weight that occur during γ-irradiation,[116] while a difficult regulatory path is posed before novel or nontraditional sterilization methods.

Calcium phosphates are also relatively easy to prepare in a variety of morphological forms,[117] although not at a particle size below 20 nm, as is the case with metals. Different calcium phosphate particle morphologies possess different bioactivities,[118,119] which allows for the optimization of their biological response by means of controlling morphological and specific crystal face exposition. Calcium phosphates are also naturally precipitated in a nanosized form, and the use of nanoparticulate calcium phosphates could be considered as a win–win solution in the quest for simultaneous bactericidal and osteogenic properties. Namely, the drug adsorption efficiency is directly proportional to the specific surface area of the adsorbent and inversely proportional to the particle size.[120] The large surface area of nanosized calcium phosphates thus increases their drug-loading capacity and makes them a more effective bactericidal agent.[121] At the same time, nanosized calcium phosphates possess higher bioactivity than their microsized counterparts,[122,123] an insight that is natural in view of the nanosized dimensions ($30 \times 20 \times 2$ nm)[124] of mineral particles in bone. Last but not least, calcium phosphates are one of the safest nanomaterials evaluated for toxicity so far.[125] Figure 5a displays round hydroxyapatite nanoparticles obtained by precipitation from alkaline aqueous solutions and highlights their ability to capture large amounts of drug

molecules in the pores between the particles upon desiccation at low pressure. Mechanistically similar intraporous loading of hydroxyapatite with a drug was reported earlier for isepamacin sulfate, an aminoglycoside antibiotic.[126] The same effect of extended release could be achieved by compacting the antibiotic-loaded calcium phosphate powders under pressure.[127] PerOssal, a commercial mixture comprising 51.5% nanocrystalline hydroxyapatite and 48.5% calcium sulfate, for example, relies on such compaction of nanoparticles to ensure sustained release of antibiotics.[128]

1. Concerns Pertaining to the Use of Calcium Phosphates—The application of calcium phosphate particles as drug delivery carriers naturally has its downsides, and the main one comes from their difficult surface functionalization. This is, in part, the effect of their ionic nature, which dictates that the surface layers undergo rapid reorganization via dissolution/reprecipitation phenomena in ionic media. As evidence of this effect, ζ potential of hydroxyapatite particles changed with the immersion time, indicating an exchange of ions across the interface layer and its restructuring following local changes in the solvent medium.[129,130] Despite the presence of calcium, phosphate, and hydroxyl ionic groups on the particle surface, which, in theory, would allow the binding of an array of functional groups, the intense ionic exchange between the particle surface and its ionic milieu renders this approach inoperative for dispersed particles. Compared with calcium phosphate nanoparticles, silanol groups on the surface of silica nanoparticles offer greater stability and more facile functionalization with organic molecules, having the same role as monolayers of thiol groups chemisorbed on the surface of silver, copper, or gold[132] and carboxylic or phosphonic acid moieties on the surface of metal oxides or quantum dots. Their downside, however, is an uncertain fate in the body and an array of inflammatory and oxidative stresses possibly induced in it, ranging from mitochondrial dysfunction to genotoxicity to pulmonary congestion to hepatocyte necrosis.[133–135]

Unlike polymeric materials (e.g., hyaluronic acid), whose viscosity could be controlled to a greater degree by means of chemical or photochemical cross-linking, thixotropic calcium phosphate cements exhibit a far narrower window of setting rates, which significantly limits the flexibility of their surgical handling. Variations in the concentration of plasticizing additives, liquid-to-solid ratio, particle size and sphericity, and ionic strength of the liquid phase have all been studied in a search for the optimal conditions for the fabrication of injectable but cohesive calcium phosphate pastes and putties.[136] On the other side of the spectrum, occupied by solid and strictly implantable materials, nonsintered calcium phosphates in particular—which are strong but fragile and most interesting for drug delivery applications are hardly formable and also are difficult to surgically attach to bone with screws and intramedullary rods, for which reason they are often combined with a tougher, more ductile organic phase to mimic the mechanical properties of bone itself.[137] Also, as a consequence of uncontrolled ripening in the nucleation and crystal growth stages, monodisperse calcium phosphates are difficult to prepare in a broad array of sizes, which poses obstacles to systematic studies of the effect of calcium phosphate particle size on bioactivity.[138]

Last but not least, the most important disadvantage of calcium phosphates is that they have little or no ability to be loaded with organic molecules via intercalation, which limits the

loading mechanism to physisorption only and makes prolonged release difficult to achieve. Namely, burst release typically results when the drug is adsorbed on the surface of the carrier, and while, on one hand, this effect is favorable in terms of ensuring that the minimal inhibitory concentration for the given pathogen is exceeded, it can also deplete the carrier from the antibiotic and make its further release therapeutically ineffective. Still, a tremendous difference between microsized and nanosized calcium phosphate particles was found: Whereas the concentration of vancomycin released from the former was below the detection limit 10 days after the implantation, nanoparticles of the same composition were able to sustain the therapeutic level of release for up to 6 weeks.[139] Extended release from porous calcium phosphate cements and its therapeutic effects *in vivo* were confirmed on numerous other occasions.[140–143] Finally, because of relatively low ζ potentials (<15 mV on the absolute scale), calcium phosphates form sols of low stability; simple and rapid precipitation procedures for their formation in the low crystalline and nanoparticulate form, on the other hand, enable them to be prepared before their clinical application.

2. Calcium Phosphate as an Intrinsically Osteoinductive Material—Calcium phosphates have been generally considered as osteoconductive materials in the sense that they support bone growth on them, although their ability to upregulate the expression of osteogenic markers and boost osteoblastic differentiation, making them osteoinductive, too, has been reported on numerous occasions.[144–146] The addition of growth factors, such as bone morphogenetic proteins (BMPs), also has made calcium phosphates osteoinductive,[147,148] although the same osteoinductive effect achieved by BMP-2 on human mesenchymal stem cells was accomplished by nanosized hydroxyapatite particles.[149] In a corresponding study composites for the delivery of recombinant human BMP-2 (rH-BMP-2) to mice and rabbits, comprising poly(D, L-lactic acid), p-dioxanone, polyethylene glycol (PEG), and β-tricalcium phosphate needed less of the BMP than the same composites that excluded hydroxyapatite from their composition to induce the same osteopromoting effect and new bone formation.[150,151] Another study demonstrated that the expression of BMP-2 in human periodontal ligament cells increased upon stimulation with nanosized hydroxyapatite.[152] Optimization of substrate topography was able to yield the same differentiation–induction effect as the chemical differentiation agents in the transformation of mesenchymal stem cells to osteoblastic ones,[153] and a similar approach that could be applied to ensure induced osteogenic response of bone cells without the use of expensive growth factors would be great news, especially since bone infection is an illness known to be particularly prevalent among patients in the Third World countries, for whom affordability presents a vital feature of a marketed drug. This does not even consider that the use of rHBMP-2 in bone augmentation procedures has induced ectopic bone formation, osteolysis, pseudoarthrosis, inflammatory reactions in soft tissues, increased risk of malignancies, and other adverse effects,[154,155] raising significant concerns over its safety in the recent years.[156] The combination of rHBMP-2 with calcium phosphates has, however, mitigated these adversities associated with the direct infusion of the given growth factor or its delivery using organic carriers.[157] The naturally bactericidal citrate ion, accounting for 5.5 wt% of the organic content of bone, where it coats hydroxyapatite crystals at 0.5 molecules/nm^2 and stabilizes them in the collagen matrix,[158] increases in concentration in parallel with the differentiation of mesenchymal stem cells into osteoblasts[159] and has been

proposed as an alternative to BMPs in view of the ability of composites comprising hydroxyapatite, in combination with polymers based on citric acid, to facilely regenerate necrotic bone.[160]

To render calcium phosphates as a base for an authentically osteogenic material by including cell components capable of bone production, such as osteoprogenitor cells or differentiated osteoblasts, however, the formation of porous scaffolds based on calcium phosphates is needed, comprising a difficult but not impossible task.[161–164] For example, with the addition of only 3 vol% gelatin, electrospinning, the method traditionally used to obtain polymeric scaffolds, could be used to prepare calcium phosphate scaffolds as well.[165] Biomimetic methods based on the usage of porous biological hard tissues as casting molds for the synthesis of structurally similar inorganic scaffolds also have recently gained popularity.[166] In addition, a combination of self-setting pastes and porogens, such as mannitol crystals,[167,168] pectin,[169] hydrogen peroxide,[170] inorganic crystals,[171] surfactants,[172,173] poly-(D,L-lactide-co-glycolide) (PLGA),[174,175] oils,[176] or other hydrophobic compounds, was also used to create macroporous calcium phosphate formulations. Calcium phosphate nanoparticles were successfully incorporated in polymeric,[177] collagen,[178] or carbon nanotube[179] scaffolds with the purpose of promoting greater adsorption of adhesive serum proteins and inducing bone growth. Simple admixing of microsized polymeric spheres into calcium phosphate cements is another method used to produce macroporosity sufficient to provide a proliferation milieu for host cells after the degradation of the polymeric phase.[180]

3. Prospect of Ion-Substituted Calcium Phosphates—By affecting their lattice parameters, crystallinity, and the solubility product, ionic substitutions in calcium phosphates seem to have a large effect on a range of their physicochemical properties.[181–183] While geological apatite can accommodate half of all the elements of the periodic table in its crystal lattice,[184] biological apatite contains about a dozen different ions as impurities, which has provided a rationale for the expected improvement in the biological response to ion-substituted calcium phosphates.[185] Substitution of Ca^{2+} with K^+, Na^+, or other alkali ions can, for example, increase the solubility of hydroxyapatite beyond that of tricalcium phosphate.[186] Like Na^+, Mg^{2+} is an ion that inhibits the nucleation of apatite.[187,188] However, it is also the ion for which bone is the biggest reservoir in the body and whose deficiency logically reduces bone growth,[189] explaining numerous attempts to augment existing calcium phosphate formulations by doping them with Mg^{2+}.[190,191] Together with Mg^{2+}, Zn^{2+} has been found in subnormal concentrations in osteoporotic patients, suggesting the vital role of these two cations in proper bone remodeling.[192,193] Because of the essential role of Zn^{2+} in the production of more than one bone growth protein, including the zinc finger containing transcription factor Osterix,[194] the deficiency of this micronutrient was proven to have detrimental repercussions on bone development, as well,[195] which is another argument in favor of its incorporation into calcium phosphates designed for bone substitutes. Zinc-substituted hydroxyapatite containing 1.6 wt% of Zn^{2+} possessed a more pronounced antibacterial effect against *S. aureus* compared with pure hydroxyapatite.[196] Selenium is another element with strong antimicrobial properties that has been introduced to carbonated hydroxyapatite via $CO_3^{2-} \rightarrow SeO_3^{2-}$ substitution, with the

resulting material being able to inhibit the formation of *Pseudomonas aeruginosa* and *S. aureus* biofilm on its surface.[197]

Silicon (Si) and strontium (Sr) are present in newly formed bone in the amounts of 0.5[198] and 0.03 wt%, respectively, and a more viable biological response was detected upon the implantation of Si-doped and Sr-doped hydroxyapatite compared with pure hydroxyapatite.[199–201] The most probable reason for this lies in the osteopromotive properties of Si and Sr ions per se; Si has been demonstrated to increase bone mass density and angiogenesis during new bone growth,[202] whereas Sr upregulates the expression of the osteoblastic protein osteoprotegerin, which inhibits the production of RANKL and hinders the differentiation and activation of osteoclasts.[203] Incorporation of either of these two ions in the crystal lattice of hydroxyapatite increased the degradation of the compound *in vitro*.[204,205] Vanadium is another element critical for healthy bone development because of its ability to stimulate mineralization of collagen and proliferation of osteoblasts,[206] but the bioactivity of vanadium-doped calcium phosphates[207] has yet to be assessed.

Calcium phosphates are able to sequester heavy ions from the environment, such as Pb^{2+} and As^{5+}, which is why they have been used as adsorbents in water purification.[208] Calcium phosphate particles could thus be easily doped with Eu^{3+}, Tb^{3+}, Gd^{3+}, La^{3+}, or other lanthanides and be made luminescent and used for imaging applications.[209,210] Hydroxyapatite labeled with ^{99}Tm, ^{125}I, ^{90}Yt, ^{153}Sm, or ^{3}H radionuclides could also be considered for simultaneous bone substitution and imaging applications.[211–213] Doping hydroxyapatite with alkaline earth metals and magnetic elements, such as cobalt[214] or iron,[215–217] yielded other impure forms of calcium phosphate that have been intensively researched for their unique bioactive properties.[218–220] Superparamagnetic hydroxyapatite obtained by doping with approximately 10 wt% Fe^{2+}/Fe^{3+} was hailed as a far less toxic alternative to magnetite when used as a heating material for hyperthermia-based bone cancer therapies.[221] Finally, carbonated hydroxyapatite, structurally similar to its biomineralized form, has been frequently demonstrated to be superior in terms of its bioactivity compared with its stoichiometric, noncarbonated counterpart.[222,223]

Explored alternatives to calcium phosphates and the two aforementioned materials in actual clinical use, PMMA and calcium sulfate, include mainly various polymeric materials, bioactive glasses, liquid crystals, collagen, and titanium nanotubes; these are discussed in the sections that follow.

B. Synthetic Biodegradable Polymers

Synthetic biodegradable polymers proposed as potential antibiotic carriers in the site-specific treatment of osteomyelitis are predominantly poly(α-hydroxy esters).[224–226] Among them, poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), PLGA,[227] and poly(ε-caprolactone)[228] (PCL) have been studied most. All of these compositions have a proven history of encapsulating arrays of both hydrophilic and hydrophobic compounds, including antibiotics,[229] and enabling their sustained, first-order release over prolonged periods of time.[230] While being formable in situ and capable of fitting practically any shape of a bone defect to be filled, they also allow for fine-tuning of their mechanical and degradation properties via control over their chemical structure, including parameters such as molecular

weight, crystallinity, cross-linking ratio, and end-group identity. For example, the decomposition kinetics of PLGA could be easily controlled by varying the lactide-to-glycolide ratio and made to match the rate of new bone formation; namely, while PLLA has a relatively lengthy degradation time scale, ranging from 3 months to over a year, depending on the molecular weight, crystallinity, and other physicochemical factors, a gradual increase in PGA content shortens this degradation to a matter of weeks for PLGA 50:50 as a result of the decreased crystallinity and higher hydrolysis rate of PGA, after which the crystallinity and resistance to degradation increase again at higher PGA contents, producing the characteristic *U*-shaped curve (Fig. 6a).[231,232] An example of how sensitive the kinetics of degradation and drug release could be to cross-linking ratio is shown in Fig. 6b; whereas 0.5 % of cross-linking in an acrylic hydrogel completes release in less than 5 hours, 1% of cross-linking promotes sustained release over a period of 8 days.[233] Other biodegradable synthetic polymers developed and tested as potential carriers of antibiotics in the treatment of osteomyelitis include poly(trimethylene carbonate)[234,235]; polyamide fibers[236]; polyhydroxyalkanoates, e.g., poly(3-hydroxybutyrate-co-3-hydroxyvalerate)[237]; and polyanhydrides, e.g., poly(sebacic anhydride)[238]; poly(sebacic-co-ricinoleic-ester-anhydride)[239]; or Septacin,[240] a copolymer of dimeric erucic acid and sebacic acid. Polymeric composites are also the subjects of intense research. For example, a layer-by-layer technique was used to grow multilayered polyelectrolyte films incorporating gentamicin and comprising a cationic poly(β-amino ester) and anionic poly(acrylic acid) on top of nondegradable poly(ethyleneimine) and poly(sodium 4-styrenesulfonate). Despite the fact that more than two-thirds of the drug content were released in the first 3 days, the implants were successful in treating *S. aureus* infection in a rabbit bone model.[241]

1. Concerns Pertaining to the Use of Aliphatic Polyesters—Although poly(α-hydroxy esters) have been successfully used in bone tissue engineering since the early 1990s,[242–244] there exists a concern that their acidic degradation products may favor bacterial growth and promote hard-tissue resorption and bone mass loss,[245,246] effects experimentally evidenced in the past. In spite of the supposed safe inclusion of the byproducts of the degradation of PLLA-based polymers in the metabolic cycles of the host organism (e.g., lactic acid is secreted by osteoclasts to resorb bone and is also one of the compounds in the Krebs cycle), chronic inflammation has often resulted as a response to their implantation in bone tissue engineering.[247–249] Another concern is that this acidification effect may render rather ineffective antibiotics whose antimicrobial effectiveness exists within only a narrow window of pH values. A decrease in pH from 7.4 to 5.5, for example, has led to a 16-fold increase in the minimum inhibitory concentration of clindamycin with respect to *S. aureus*.[250] Poly(α-hydroxy esters) also lack the mechanical properties required for load-bearing applications; PLGA, combining the adsorptive stability of PLA with the mechanical strength of PGA, is the most favored and thus the most researched option with respect to this intrinsic drawback.

C. Gels and Bioderived Polymers

Aqueous monoolein gels are an example of a liquid crystal system that was used to deliver gentamicin sulfate for 3 weeks without the burst effect.[252] A mannosylated poly-phosphoester gel with the capability of targeting macrophages and releasing the antibiotic

payload in a site-activated manner, that is, only after being degraded by the bacterial enzymes, was developed.[253] Use of the osteointegrating effects of calcium phosphates was attempted by incorporating them into cubic liquid crystals of gentamicin–mono-olein–water formulations.[254] Various combinations of calcium phosphates with gelatins, that is, mixtures of peptides and proteins resulting from partial degradation of collagen, also were investigated.[255,256] Among other bioderived polymers, some have been used to encapsulate antibiotics, such as albumin[257,258] or dextran,[259] but have not been reported in bone-related experimental trials, except in combination with more mechanically stable, inorganic phases. Albumin coatings around allografts, for example, improved cell adhesion and proliferation[260] and enhanced bone healing,[261] whereas the use of dextran as a porogen in PMMA beads boosted the release of vancomycin, daptomycin, and amika-cin.[262] Conversely, silk fibroin coating around PCL microspheres managed to reduce the initial burst release of vancomycin and extend the timeframe of its release.[263] Silk–alginate copolymers are particularly interesting because of their tunable stiffness as the function of the silk-to-alginate ratio and the concentration of the crosslinker,[264] but they have not been used yet for the controlled delivery of antibiotics. Other natural polysaccharides, such as chitosan,[265–267] pectin,[268] amylose,[269] alginate,[270] and hyaluronic acid,[271] have been both used for the controlled release of antibiotics *in vitro* and tested as a component of antimicrobial bone grafts *in vivo*. Pectin microspheres, alone and in combination with chitosan, were used to encapsulate ciprofloxacin and were more effective in treating osteomyelitis than intramuscularly administered antibiotic in a rat model.[272] A cross-linked amylose starch matrix loaded with ciprofloxacin prevented and eradicated infection more effectively than oral ciprofloxacin treatments in dogs with an infected femur.[273] Vancomycin encapsulated within alginate beads and distributed in a fibrin gel scaffold was used to treat infected tibiae in rabbits.[274] Still, the most researched among bioderived polymers as a potential carrier of antibiotics in the treatment and prevention of orthopedic infection is collagen.

D. Collagen Sponges

Outside the United States in the 1980s, collagen sponges, also known as fleeces, began to be used as the major alternative to PMMA beads for the local delivery of antibiotics. Their application has been justified by a moderate number of clinical and *in vivo* studies.[275] For example, compared with PMMA beads, sponge-like collagen carriers of gentamicin were 7 times more effective in reducing the bacterial colony count in the treatment of osteomyelitis caused by *S. aureus* in the tibiae of rats.[276] Also, the placement of gentamicin-eluting collagen fleece around the fixation plate during the surgical treatment of open bone fracture prevented surgical site infection from occurring and promoted bone union in a large population of patients.[277] In fact, rather than as a bone filler, collagen has been mostly used as a material for postsurgical prophylaxis in the treatment of infectious disease.

1. Concerns Pertaining to the Use of Collagen—In spite of (1) the viable tensile strength of collagen, (2) its ability to foster cellular attachment, and (3) the fact that collagen sponges have been successfully used in the past,[278] the choice of antibiotics in their clinically applicable versions has been limited to gentamicin only, alongside other disadvantages that collagen intrinsically possesses. The main problem associated with the

application of collagen and its derivatives as bone fillers comes from the intrinsic immunogenicity of the collagen molecule,[279] namely, most of it is xenogenic in origin because it is difficult to obtain directly from a patient, and recombinant technologies and the methods to extract the immunogenic, telopeptide portion of collagen molecules are not only of limited availability but also lead to reduced bioactivity of the protein.[280] Although collagen has been successfully applied topically, for example, in biodegradable sutures and as a prophylactic wound dressing carrier of antibiotics,[281–283] its mere subcutaneous epithelialization may lead to undesired immunogenic or antigenic responses.[284,285] While it can lead to antigenic and inflammatory responses, collagen is also typified by comparatively uncontrolled degradation and drug release rates in the body.[286,287] For this reason, a combination of collagen sponges with other polymers has been used to render more sustained drug release profiles. One such composite material enriched with chitosan microspheres and delivering recombinant human BMP-2 considerably outperformed a pure collagen sponge loaded with the same growth factor in terms of new bone growth enhancement, bone/implant integration, and the duration of drug release.[288]

E. Silicon-Based Materials

Porous bioactive glass scaffolds loaded with ceftriaxone demonstrated a higher local concentration of the antibiotic 6 weeks after the implantation compared with a parenteral treatment composed of two injections per day.[289] Silicate-to-borate replacement in bioactive glasses produced materials that also were used for the controlled delivery of vancomycin or teicoplanin and repair of infected bone in rabbits.[290,291] Partial substitution of PO_4^{3-} groups of hydroxyapatite with SiO_4^{4-} species resulted in a calcium phosphate–based glass ceramic able to release vancomycin in a sustained manner over 2 weeks after cross-linking with chitosan.[292] The addition of Ag^+ ions to phosphate-based glasses led to their sustained release and bactericidal effect against *S. aureus* biofilms. [293] Further research will, however, be necessary to show whether such antibiotic-free methods are capable of acting against severe bone infections. As far as silica-containing materials are concerned, xerogels obtainable from a solgel process were used to encapsulate and ensure the prolonged release of vancomycin, with the water-to-alkoxysilane molar ratio being discerned as a parameter for the control of release kinetics.[294] Zeolites, microporous aluminosilicates with pronounced (1) antibacterial,[295] (2) adsorptive,[296] and (3) bone-protective dietary[297] properties, inhibited osteoclast-mediated bone resorption *in vitro*,[298] but their application as a component of bone fillers in combination with calcium phosphates or other osteoconductive phases is still a largely unexplored area. Metal-organic frameworks, mesoporous materials structurally related to zeolites,[299] have been proposed as potentially efficient drug delivery carriers,[300] including in applications that pertain to bone regeneration,[301] which, however, they have yet to be tested for. Their main weakness is rapid degradability in aqueous media, and structural variants with increased stability in water are being intensively sought.[302]

F. Metals

The widespread rise in the resistance of common pathogens to organic antibiotics has led to a greater degree of consideration of the use of metals to prevent or treat infection,[303] with many of them, such as silver-impregnated fabrics used as prophylactic dressings during

wound healing,[304] regularly applied in the clinic. Because of its antimicrobial properties,[305] the first metal proposed for use in the treatment of osteomyelitis was silver; be it alone or in the form of nylon wire composites, clinical testing resulted in a 65% success rate and no evidence of postoperative argyria.[306] Titania nanotubes formed by electrochemical anodization on the surface of titanium nanowires used for bone fixation were capable of being loaded with gentamicin and releasing it over a period of 2 weeks.[307] In another study, however, the same combination of gentamicin and TiO_2 nanotubes with an 80-nm diameter and 400-nm length led to prompt release of the drug in only 1–2 hours, but it still reduced the adhesion of *Staphylococcus epidermis* on the surface compared with pure titanium.[308] Surface etching and anodization parameters could be used to modify the diameter of the nanotubes and thereby control the rate of diffusion of the drug stored in them into the biological environment.[309]

Surface texture of the material is also a property of interest; for example, electropolishing of a Ti-6Al-7Nb alloy decreased the amount of *S. aureus* adhering to it.[310] A tradeoff, however, is expected to arise because osteoblasts, which compete with the bacteria for the bioactive surface of the implant in a process that greatly determines the clinical outcome,[311] also prefer to attach to rougher surfaces, such as those that typify naturally topographically irregular calcium phosphates.[312] To that end, titanium implants are being subjected to sandblasting and etching procedures,[313,314] as well as coated with bioactive layers, predominantly hydroxyapatite,[315–317] to make up for their intrinsic bioinertness and have their bioactivity boosted before surgical insertion.

G. Composites

In the design of nanoparticles for biomedical applications great emphasis has been placed on particles capable of simultaneously aiding in prevention, early detection, and treatment of a medical condition. With antibiotic calcium sulfate cements already in use in prophylaxis against surgical wound infection, it can be expected that theranostic particles able to simultaneously prevent, monitor, or diagnose the onset of infection and release antimicrobial agents to prevent its early spread may be developed in the future. In that sense fantastic multifunctional composite nanoparticles can be considered to be the ideal toward which the nanoparticle fabrication field will advance (Fig. 7a). The difficulties in achieving stable, chemical functionalization of calcium phosphate particles with therapeutic ligands can be mitigated by coating them with a chemically bondable layer, such as PLGA[318,319] or PCL[320] or by forming around the calcium phosphate core multilayered composite particle structures[321] with an ability to carry various therapeutic agents either between the calcium phosphate layers or within the polymeric coatings (Fig. 5b–d). Through a simple series of chemical steps, polymeric coatings can also be conjugated with various targeting or therapeutic ligands.[322] Binding amino acids with appropriate physical properties via PEG linkers, for example, phenylalanine as a hydrophobic residue, lysine as a positively charged one, and glutamic acid as a negatively charged one, can be used to increase the drug-binding affinity of the polymeric surface.[323] Tailoring of the nanodiamond particle surface with carboxylic or amino groups to render it negatively or positively charged under physiological conditions, respectively, greatly affected the binding and release of various drugs; binding of the negatively charged drug by physisorption to an amine-functionalized surface is so

intensive that virtually no release *in vitro* occurred.[324] Such combinations of various loading locations on the particle could potentially yield multiple-stage release profiles that might not only favor the antimicrobial efficacy of the particles *in vivo* but also prove to be beneficial in increasing the regenerative capacity of the carriers, given that the tissue regeneration process following injury can be divided into multiple stages (Fig. 7b), each of which could be targeted and augmented by a specific particle additive released within a precisely tailored time window. To avoid adverse outcomes resulting from obviation or incompletion of any single one of the interconnected steps in the bone-healing cascade (Fig. 7c, d), the biomolecular machinery involved in every one of these stages could be targeted separately and triggered at the right time by using such a smart composite particle that sequentially releases its multiple payload in a highly controlled, spatiotemporal manner. An interesting approach to achieving such multimodal release profiles is through cooperative assembly of block copolymers as elemental building blocks of the particle, each of which carries a unique therapeutic payload and degrades at a different rate.[325]

The combination of calcium phosphates with a polymeric component can also be beneficial for the second essential function to be achieved by these nanoparticulate drug carriers, in addition to their antibacterial role: assistance in bone regeneration. Namely, since bone itself is a composite material comprising a soft, collagenous component and a hard, ceramic one, it is natural to expect that a soft/hard composite of a similar nature should prove an ideal material for bone replacement therapies. In view of this, a range of properties of calcium phosphates is improved upon their combination with a polymeric phase, starting, most essentially, with the mechanical ones. Namely, it is generally assumed that the microstructure and nanoarchitecture of calcium phosphates alone cannot be modified in such a manner as to make the material mechanically compatible with the grafted bone and prevent the frequent fracture of the filler upon its surgical placement to substitute natural bone.[326] Only a combination with a soft component is thought to be able to ameliorate these fundamental issues associated with the clinical application of calcium phosphates. The combination of viscoelastic properties of the polymers and osteoconductivity of calcium phosphates has yielded composites that surpassed the resistance to fracture, structural integrity, and stiffness of the individual components,[327] making up for the low compressive strength of the former and the brittleness and lack of malleability of the latter.[328] Reinforcement with polypropylene fumarate,[329] for example, improved the flexural strength of brushite from 1.8 to 16.1 MPa and increased the fracture surface energy from 2.7 to 249 J/m^2. Although calcium phosphates exhibit relatively high values of compressive strength (10–100 MPa), as opposed to tensile and shear strengths (1–10 MPa), even these values could be improved with the addition of a polymer, as exemplified by the doubling of the compressive strength of a biphasic calcium phosphate cement, from 35 to 60 MPa, upon the incorporation of only 0.5 vol% of a superplasticizer based on a vinyl-modified copolymer, [330] as well as upon the addition of gelatin[331] or ammonium polyacrylate.[332] Polymers could also increase the plastic flow and enhance the viscosity of the material, thus making possible its preparation in the form of an injectable self-setting paste,[333] although there is usually a fine line dividing an excessive increase in the setting time from improved mechanical properties.[334,335] Finally, the resorption time and the corresponding bone ingrowth rate significantly increased when hydroxyapatite was implanted as a bone

substitute *in vivo* in a composite form, in combination with PLGA,[336,337] a polymer that is able to accelerate the resorption of calcium phosphates by releasing its acidic degradation products.[338] Yet another tradeoff exists here: An increase in the porosity of the ceramic substructure of the composite leads to improved bioresorption characteristics but simultaneously entails an increased susceptibility of the material to crack propagation and the corresponding proneness to fail under load-bearing conditions.[339]

Such composite particles showed promise in earlier research. Gentamicin-containing granules composed of hydroxyapatite nanoparticles, chitosan, and ethyl cellulose, for example, were effective in the treatment of chronic osteomyelitis.[340] Another prospective hybrid organic–inorganic system was formed by dispersing silsesquioxane microspheres loaded with acetylsalicylic acid as an anti-inflammatory model drug in a calcium phosphate cement.[341] Similar composites reduced in size to the nano scale may be recognized as a trend toward which this field will be moving (Fig. 8). Polymeric coatings may also increase the loading capacity and prevent the burst release of the drug merely adsorbed on the particle surface. Coating chitosan/tricalcium phosphate composites with 2.5w/v% PCL has thus mitigated the burst release effect and promoted zero-order kinetics for the release of vancomycin during the first 6 weeks. [342] Impregnation of the poly(α-hydroxy esters) with bone morphogenetic proteins has been shown to (1) overcome the inflammatory response, (2) induce full bioresorption of the polymer, and (3) enhance bone growth,[343–345] while the addition of demineralized bone particles to PLGA reduced (1) inflammation, (2) fibrous tissue encapsulation, and (3) foreign body giant cell response.[346] The combinations of alkaline calcium phosphate phases, such as hydroxyapatite or octacalcium phosphate, with acidic poly(α-hydroxy esters) are thus particularly interesting because of their ability to mutually compensate for potentially harmful pH changes that follow their degradation. The wide range of pH conditions provided by the synergetic action of osteoblasts and osteoclasts in the degradation of calcium phosphates *in vivo* makes the use of pH-sensitive coatings potentially interesting, too. Poly(aspartic acid) presents one such pH-sensitive polymer; its swelling is more pronounced at the physiological pH than at pH ~3 and can be facilely controlled by the degree of cross-linking.[347] Another type of environmentally responsive polymers are thermosensitive polymers, which transform from sols to hydrogels at body temperature and enable in situ gelling at the target site promptly after injection.[348] Some of the biodegradable polymers of this type include *N*-isopropylacrylamide copolymers, poly(ethylene oxide)/poly(propylene oxide) block copolymers, and PEG/poly(D,L-lactide-co-glycolide) block copolymers, the latter of which have been successfully applied to encapsulate teicoplanin with 100% efficacy and treat osteomyelitis in rabbits.[349]

That even the activity of antibiotics can be improved with a proper coating is illustrated by the more effective prevention of the formation of *S. aureus* biofilms when vancomycin was delivered encapsulated within cationic liposomes and carried in a porous nano-hydroxyapatite/chitosan/konjac glucomannan scaffold.[350] The same antibiofilm effect was achieved by the delivery of liposomal gentamicin from scaffolds containing β-tricalcium phosphate with release kinetics able to be controlled by the liposome size.[351] Functionalization of particulate carriers with anionic amphiphiles that may disrupt the bacterial biofilm and neutralize the carbohydrates by the action of which bacteria penetrates

the cell membrane is another unexplored but potentially fruitful direction for research. With antioxidant therapy being one of the hotspots of medicine, endowing carriers with reducing agents, such as ceria domains[352] or ascorbic acid,[353] could be an avenue for abating the reactive oxygen species and minimizing the oxidative stress that entail infectious disease. Nanoparticle uptake by the cells can be controlled using ζ potential,[354] a ubiquitous physical property,[355] but precise correlations between the surface charge and the therapeutic efficacy of nanoparticles in the treatment of osteomyelitis have yet to be established, even though wound healing could be enhanced by endowing cells with relatively high ζ potentials.[356] The usage of dispersion agents or the application of other strategies from the repertoire of colloid chemistry[357] to promote greater dispersion and penetration of the antibiotic-carrying particle to the infected tissue—a general challenge for the developers of injectable drug delivery materials[358]—is another unexplored avenue.

Conjugation of the carrier particles to moieties that would have an affinity for various bone components[359] and act as either targeting agents or metabologens is yet another unexplored research directive in the design of antiosteomyelitis composite particles. Human recombinant BMPs, two of which—BMP-2 and BMP-7—were approved for specific clinical cases by the FDA, have been successfully delivered using various types of nanoparticles, ranging from poly(α-hydroxy esters) to PEG-based hydrogels to dextran to polymeric composites with hydroxyapatite to calcium phosphates alone,[360] and their covalent binding on the polymeric particle surface may prove to be a more effective approach for their delivery compared with internal encapsulation, especially in view of the extraordinary sensitivity of their osteoinductive effect to the release kinetics.[361] These conjugates could also include biomolecules that inhibit specific bacterial ingredients, such as (1) lipoteichoic acids, components of gram-positive cell walls that induce bone resorption; (2) polysaccharides in the bacterial capsules, which play a role in the adhesion of bacteria onto an osseous or implant surface and the formation of a biofilm, the basis for proliferation of pathogens in hard tissues; or (3) other osteolytic factors, including cytokines or other signaling molecules, which may interfere with the pathway of the osteoblast lineage.[364] Such efforts may have a chance to bring researchers from drug delivery and drug discovery fields closer because, after all, the synergy between the drug and the particle will prove to be of ever more vital importance in the design of ultrapotent therapeutic agents in general.

Inclusion of peptides with strong antibacterial properties, which tend to be more immune to promoting bacterial resistance if delivered in concentrations lower than minimal inhibitory ones, would present another interesting approach.[365,366] The polymeric surface of a composite particle could be functionalization with arginylglycylaspartic acid, a tripeptide involved in cellular recognition and capable of triggerinxg adhesion of fibroblasts.[367] Bisphosphonates, molecules with a strong affinity for the mineral component of bone[368,369] and most commonly prescribed in the prevention and treatment of osteoporosis and other conditions featuring bone loss and fragility,[370] could be used further to ensure particle localization and the delivery of therapeutics directly in the area of infected tissue. Although a possible concern comes from the clinically observed adverse consequences of oversuppressed bone resorption and disrupted bone metabolism by the prolonged use of bisphosphonates,[371] the risk for developing these side effects is still small compared to the benefits.[372]

ZnO has been added to implantable PMMA beads as a radiographic contrast medium for the past 30 years,[373] yet calcium phosphates and other carriers could be doped with heavy metal atoms such as ^{111}In, ^{99m}Tc, Gd, or Mn or bound to optically active molecules and used for the same imaging purpose with far greater sensitivity. Combinations of calcium phosphate, PLGA, and semiconductor quantum dots[374,375] at the nanoparticle scale have enabled monitoring of the particle route in the body, and the distribution of the locally implanted therapeutics could be monitored in a similar manner. Quantum dots are, however, known for their cytotoxic nature,[376] with only a few exceptions, including silica-based compositions, the only type currently approved for use in clinical trials by the FDA.[377] Proposed as bioimaging alternatives to inherently toxic quantum dots and nonbiodegradable aromatic polymers are aliphatic, biodegradable, and tunably photoluminescent oligomers,[378] but they have yet to be explored as components of bone tissue substitutes.

Porosity of composites in the compact, fully set form could be modified using other additives, such as glucose,[379] calcium sulfate,[380] calcite,[381] gelatin,[382] or others, and set to a specific pore size, pore size distribution, and pore interconnectivity that maximize the internal cell proliferation and the transfer of nutrients and metabolic products. For example, combinations of silica and calcium phosphate allowed for a control over porosity of the resulting gentamicin-loaded nanocomposites in the mesoporous (2–50 nm) and macroporous (>50 nm) ranges by means of controlling their silica content.[383] Porosity also could be limited to the surface only[384] to promote a bioactive response while preserving the compactness and stability of the core of the system against attack from the corrosive biological environment, or the other way around, porous on the inside and compact on the outside,[385] like bone itself. It could be also made gradient, extending throughout the bulk of the composite in different ways.[386] As a matter of fact, Janus-faced[387] and functionally gradient structuring[388] on the nano and molecular scales are other largely unexplored, yet incredibly potent features of the next generation of advanced materials. Finally, enriching antibiotic carriers with pluripotent cells, such as mesenchymal stem cells able to differentiate into osteoblasts,[389,390] would be another research step in the direction of advanced therapeutic platforms for simultaneous bactericidal and osteogenic performance. In that sense, understanding the role of the extracellular matrix and an array of microenvironmental cues in directing the pluripotent cell fate currently stands as a major challenge to be overcome to minimize cells' chances of acquiring neoplasticity and to maximize their chances of acquiring the best possible phenotype for the given therapy.[391]

V. ADDITIONAL CHALLENGES

A. Inconsistencies Arising from Different Analytical Contexts

An essential wisdom conveyed from the drug delivery field is that context is everything. When not delivered in a proper manner, even the most effective therapeutics will be deprived of their remedial effectiveness. A direct corollary of this insight is that drug discovery and drug delivery could be imagined as two sides of the same coin, complementing each other in a complete drug therapy. In other words, in a wrong setting even the most therapeutically potent agent will be ineffective, whereas even the most toxic chemicals applied in the right amount and setting could strengthen an organism, as data in

support of the effect of hormesis could indicate. Therefore, it should not be surprising that problems and challenges will continue to abound even if we were to succeed in the design of a perfect drug delivery carrier.

An example of the effect of the environmental context on a drug elution profile from a particulate carrier is shown in Fig. 9. Namely, two kinetically distinct release profiles from an identical drug-containing powder result, depending on the release measurement method applied: zero-order when a comparatively small volume of the solvent is replenished daily and first-order when a larger volume of the solvent is used without its daily replenishments. Whereas the drug is released with a burst effect when large amounts of solvent surround the powder, a limited volume of the solvent limits the maximal amount of the drug that could be released before saturation, leading to identical concentrations of the drug in the solution sampled at regular time spans (24 hours). In such a manner reprecipitation of sparsely soluble hydroxyapatite on the surface of more soluble di- and tricalcium phosphates in the form of a protective layer that hinders further dissolution, which might have occurred *in vivo* or at smaller solvent volumes, and higher corresponding degrees of supersaturation can be prevented by frequently replenishing the solvent and used to speed up the dissolution of these calcium phosphate phases under physiological conditions. Neither of these two methods, however, mimics the biological conditions under which fast clearance of the released drug is typically observed, nor do they account for the effects of the complex interface between the device and various macromolecules and cells of the host organism on the drug release. Although the size, shape, and elasticity of nanoparticles in biological milieus do influence their biodistribution profiles, the route of uptake and the mechanism of interaction with a cell are mainly determined by the protein corona adsorbed on the particles and the surface propensities that it endows them with.[394,395] How to devise *in vitro* drug release testing procedures that would be able to replicate *in vivo* conditions better, typically characterized by (1) a more dynamic flow of fluids, (2) specific local pH profiles that are often disease-dependent, and (3) much more complex and selective media, is another colossal challenge for the drug delivery field.

Incompatibility between *in vitro* and *in vivo* tests has been frequently observed[396]; what has been shown as toxic or inflammatory *in vitro* can have the same effect *in vivo* but can also provide the right level of inflammation that follows every successful reaction toward unity between the bone and the implant.[397] The trivial observation that pure water momentarily destroys cells in culture via osmotic rupture of the cell membrane, whereas we consume it every day without serious consequences, could be used to illustrate the inevitable discrepancy between testing materials in culture and in a more complex, organismic environment. Moreover, not only do different lines of the same cell type often respond differently to identical chemical stimuli[398]; the same insight applies to cells from the same line but at different stages of cell cycle progression.[399] On the other hand, techniques for replicating the exact microenvironment in which cocultured primary cells exist in the body, which is one of the key factors that determine their fate,[400] have yet to be developed.

As for animal models of osteomyelitis, different species and experimental protocols have been used in the past. The rabbit is the oldest animal model of osteomyelitis, dating back to 1941,[401] and it has traditionally involved injections of a suspension comprising *S. aureus*

and 5 wt% sodium morrhuate, a sclerosing agent causing aseptic necrosis of bone, to induce suppuration and subperiosteal abscesses in the bone. Fibrin glue and other sealants often are used to prevent bacterial leakage.[402] The most common, however, is the rat model, typically involving the insertion of screws or other fixation devices inoculated with *S. aureus*.[403] An alternative method involves the creation of a defect in the tibia or femur by drilling a hole in it and stabilizing it with screws, plates, and wires. The defect then is filled with collagen or gelatin sponges soaked in *S. aureus* and kept there for up to 24 hours, which is superseded by injection or implantation of an antibiotic-releasing material.[404] Mice and chickens are other common small-animal models, whereas dogs, goats, and sheep are the most common large-animal models, whose main advantage is the ability to accommodate real-size devices and tolerate multiple interventions, alongside more veritably mimicking the mechanical loads born by bones in the human body. A central challenge in all of these models is how to create bone lysis that is sufficient but not excessive and does not threaten the fixation stability. Avoiding the formation of virtually untreatable biofilm on fixation devices is cited as another challenge faced by these animal models,[405] even though it presents a more faithful model for cases in which infection is secondary to surgical foreign body implantation. Yet another detail common to these models is that chemically induced necrosis or mechanical trauma, such as fracture (as in most mouse models) or the insertion of intramedullary pins, plates, or other fixation devices, need to be coupled to dispersal of the pathogen to induce chronic infection.[406] Still, in spite of more than 70 years since the first reproducible animal model of chronic osteomyelitis was reported and continuous advancements since then, inconsistent correlations between *in vitro* and *in vivo* antibacterial efficacies of therapeutic agents still commonly occur.[407]

As for the drug elution rate, values obtained *in vitro* may drastically increase *in vivo* for various reasons, including the complex interface with chemical and biological species (e.g., enzymatic activity, the concentration of free radicals that induce oxidative scission of covalent bonds between monomers, the types of antibodies adsorbed, or the extent of fibrous capsule formation, if any) or different rheological properties, demanding new strategies to ensure the optimal 4–6 weeks of release time. PLGA scaffolds, for instance, degraded faster *in vivo* than *in vitro*,[408] and in addition to phagocytosis, enzymatic hydrolysis, the regions of low pH at the cell–material interface, and biomechanical stress, increased wetting in biological conditions can be another factor responsible for this effect.[409] This is especially relevant for hydrophobic polymers, the category to which unmodified PLGA belongs. This disparity between the carrier degradation and the drug release kinetics estimated using *in vitro* and *in vivo* measurement modes is expected to be even higher for calcium phosphates than for polymers because while the degradation of the latter is primarily driven by hydration and hydrolysis both *in vivo* and *in vitro*, the degradation of ceramic implants is mainly caused by the phagocytic and acidifying (pH 7.4 $\rightarrow$ 3–4)[410] action of multinucleated osteoclasts and macrophages.[411] This discrepancy is further added up to by knowing that calcium phosphate phases more soluble than octacalcium phosphate transform to a certain degree into the most stable phase, hydroxyapatite under physiological conditions.[412,413] Factors that dictate to what degree this transformation takes place *in vivo* are not clearly defined; on one hand its precursors are classified as biosoluble ceramics,[414] unlike bioresorbable hydroxyapatite, and indisputably degrade faster than the latter, whereas on the

other hand this transformation has been both theoretically predicted and experimentally verified on numerous occasions.[415–417] Comparatively soluble calcium phosphates, including monetite, brushite, and amorphous calcium phosphate, were also found *in vivo*,[418] suggesting that this transformation may frequently be limited to a few surface layers that undergo intense dissolution/reprecipitation, thus protecting the inherently unstable and soluble particle or implant core. The chemical propensity of more soluble calcium phosphates to be resorbed at a higher rate, however, is complicated by knowing that osteoclasts anchor more steadily on the less soluble calcium phosphate phases; the substantial release of Ca^{2+} ions from the surface of the more soluble ones disrupts the ordering of actin microfilaments in the osteoclast podosomes, leading to the periodic detachment of the cells from the material surface.[419] Similarly, whereas the degradation of aliphatic polyesters(e.g., PLGA)is slowed under fluid flow because of dissipation of acidic byproducts that would have sped up the degradation process, fluid flow prevents local supersaturation and accelerates the degradation of alkaline calcium phosphates such as hydroxyapatite. Hence, despite the excellent release profiles *in vitro*, the possibility that the minimum inhibitory concentration in sections of the target tissue may not be exceeded in a time-sustained manner, be it due to biofilm formation, intracellular colonization, premature biodegradation, or other factors, will always exist. In such a way exist threats that antibiotic resistance could be inadvertently promoted, an effect that directly contributes to the global loss of antibiotic efficacy in use.[420] In those cases even a direct injection of a bolus dose of the antibiotic may lead to more favorable outcomes than the implantation of the drug–carrier composite.[421]

B. Antibiotic Specificities

Antibiotics differ according to their mechanism of action; some, such as β-lactam antimicrobials, are time-dependent, requiring prolonged presence in the target zone for effective suppression or eradication of the pathogen, whereas others, such as quinolones and aminoglycosides, are concentration-dependent, requiring higher concentrations over shorter periods of time.[422] Therefore, depending on the nature of the drug, differently structured carriers may prove to be most optimal. As expected from the synergetic background of drug carrier interaction, the properties of the carrier influence the efficacy of the drug therapy, but the drug identity, amount, and binding mechanism in turn influence the properties of the carrier. The anionic group of gentamicin sulfate, for example, had an inhibitory effect on the crystallization of brushite during coprecipitation of the antibiotic and the carrier, resulting in smaller particles, lower porosity, and slower drug release compared with pure gentamicin.[423] The morphology, crystallinity, and dispersability of particles coprecipitated with the drug may thus all be affected by the drug properties. The loading efficiency and the release rate of a drug from a particle is consequently dependent on the molecular nature of the drug, as exemplified by the different elution profiles for different antibiotics [424] The release from PMMA beads, for example, varied drastically, reaching completion anywhere between 3 and 21 days, depending on the antibiotic admixed.[425] Also, whereas the release of vancomycin from a brushite cement reached completion between days 1 and 2, only one quarter of tetracycline loaded within the same carrier was released on day 5.[426] Synergetic effects are important here; for example, the elution rates for tobramycin and vancomycin released together from a PMMA cement were higher than those when the drugs were

released alone.[427] Then, not only does the identity of the drug have an effect on release profiles, but its amount can drastically modify them, too, as exemplified the case where 25 μg/cm^2 of paclitaxel deposited on the surface of a phosphonohexadecanoic acid coated cobalt chromium alloy released 90% of the drug in the first week, whereas quadrupling the paclitaxel concentration to 100 μg/cm^2 resulted in zero-order release throughout a 5-week period of time.[428]

The release zone of the antibiotic following surgical insertion is generally only a few centimeters, and the rheology of the fluid in which the drug carrier is immersed, including hematoma and seroma, might redirect the released drug away from the target site. Therefore, continuous-flow chambers have been designed to assess drug elution profiles under more dynamic conditions that resemble the *in vivo* context to a greater extent.[429] Still, different implantation sites greatly affect the biodegradation rate of the implant, including the release profile of the drug that it contains.[430] Subcutaneous implantations of a biomaterial composed of PEG and poly(butylene terephthalate) thus degraded faster than the intramuscular ones,[431] whereas polydioxanone orthopedic pins were resorbed faster when implanted in the medullar canal rather than intramuscularly or subcutaneously.[432] Mechanical loading, sheer, and friction are other factors that contribute to the different release from orthopedic drug delivery devices implanted at different sites in the body.[433] Also, hypochlorites generated smaller polymeric fragments with higher toxicity than peroxides,[434] suggesting that the dominant reactive oxygen species as inflammatory compounds in the implantation area, which are involved in the degradation of polymeric carriers, inevitably define the toxic propensities of the biomaterial in question. In that sense the art of surgical implantation has to complement the reliability of the drug release pattern of the implanted drug–carrier composite. That different target areas in the body require unique drug delivery platforms for optimal release has been confirmed many times, and it is logical to expect that the same will prove to be true in the treatment of different segments of bone. Mandibular infection, for example, typically requires a shorter duration of antibiotic therapy compared with long-bone infection. Intensely vascular cancellous bone, with a comparatively high rate of turnover, may thus be expected to require more intense release kinetics compared with less vascular and more slowly remodeled cortical bone.[435] In general, the principle *similia similibus curantur*, dictating the substitution of like with like, is expected to apply in every aspect of tissue engineering, including the province of bone.

C. Intracellular Colonization by *S. aureus*

Another potential difficulty arises from the fact that *S. aureus*, the main causative agent of osteomyelitis, found in healthy oral and nasal flora has the ability to penetrate endothelial, epithelial, and osteoblastic cells and thrive in the intracellular environment, where it is less susceptible to the antibiotic therapy.[436–438] Different but in all cases finite uptake efficiency and kinetics were observed for different strains of *S. aureus*, and common to all of them was the role of fibronectin-binding proteins in intracellular colonization, the blocking of which completely prevented the latter from occurring.[439] *S. aureus* internalized by the cells and shielded from the host immune system is thought to provide a reservoir of bacteria in recurring osteomyelitis and inhibit the immunological role of osteoblasts in releasing cytokines and attracting leukocytes to the infection site. Targeting the antibiotic therapy to

these intracellular colonies may thus prove to be more relevant for treating chronic bone infection than eliminating only pathogens that colonize the bone matrix.[440] Nanoparticles, including calcium phosphate ones, have shown great promise in the intracellular delivery of plasmids,[441–443] and they may similarly prove to be effective carriers of antibiotics inside the cell where bacterial microcolonies are localized. Correspondingly, a recent study showed that clindamycin-loaded hydroxyapatite and amorphous calcium phosphate particles are more effective in reducing the intracellular bacterial population and slowing the growth of *S. aureus* cocultured with osteoblastic MC3T3-E1 cells than the pure antibiotic.[444] Figure 10a shows a schematic description of their uptake by the cell and the delivery of a genetic material to it, whereas 10b shows the intracellular localization of calcium phosphate nanoparticles in an immunofluorescent analysis of osteoblastic MC3T3-E1–calcium phosphate interface. A reduction in the number of intracellular bacteria has already been demonstrated for nanosized PLGA particles loaded with nafcillin.[445] The rapid degradation of calcium phosphate carriers in the acidic milieu of a lysosome–endosome complex following uptake may also increase the osmotic pressure and enable the plasmid or protein cargo to escape swiftly into the cytoplasm before it is enzymatically hydrolyzed, which could be considered yet another advantage of calcium phosphates as intracellular delivery agents.

D. Synergetic and Sensitivity Effects

Immunofluorescent labeling and confocal microscopy, along with other *in vitro* assays, including real-time polymerase chain reaction, can provide good insight into the cell–material interface on which the bone regeneration aspect of therapy ultimately depends[446] (Fig. 11a). Nanoparticles have, however, been notorious in terms of resisting any clear-cut descriptions of their biological effects, as exemplified by the recently derived inverse, dose-dependent toxicity relationship for 100-nm silica nanoparticles in the human epithelial intestinal HT-29 cell line.[447] In a similarly counterintuitive fashion, the uptake efficiency and the expression of plasmids could occasionally be out of proportion: Efficient uptake may lead to low gene transfection and vice versa.[449] Synergetic effects resulting from the minor amounts of impurities left over from synthesis procedures or supposedly inert product components may prove to be equally important in determining therapeutic outcomes. For example, the osteogenic effect of calcium phosphate as a drug carrier was able not only to mitigate but also to fully reverse the unviable effect that the pure antibiotic exerted on osteoblastic cells, while retaining its antimicrobial potency through a more sustained release of the antibiotic (Fig. 11b).The addition of calcium hydroxide to tobramycin-containing PMMA beads similarly had a protective effect on bone against the high concentrations of the antibiotic.[450]

A related problem occurring in parallel with the sophistication of the carrier particle is the difficulty transferring the synthesis methods from the laboratory to the clinic or any large-scale fabrication setting. Namely, the more intricate the particle, the greater the range of experimental variables to which its preparation is sensitive.[451] The case of Abbott Labs losing hundreds of millions of dollars trying to restore the polymorph of their proprietary AIDS drug, Ritonavir, after a new polymorph had begun to appear in their synthesis batches and before being forced to withdraw the drug from the market and lose another half a billion

dollars,[452] can thus be instructive, if not ominous, in its foreshadowing the trend of a pervasive irreproducibility toward which the science of synthesis of fine particles streams. Startups in organic photovoltaics have, for instance, failed to make profit mainly because of the unsurpassable variability in processing outcomes. A systematic analysis of two donor materials synthesized by the same manufacturer resulted in confirmation of the same crystallinity, side-chain variation, interface composition, and domain morphology, yet their performance was radically different because of a difference in the nano scale that was too fine to be probed by state-of-the-art instrumentation.[454] Note also that the purity of solvents and compounds used as precursors for the synthesis of nanostructured powders has had a drastic effect on their morphology,[455] whereas cell detachment or excellent spreading result depending on whether cultured cells are grown on ordinary or tissue culture–grade polystyrene.[456] Alongside the chemical effects exemplified by different reaction outcomes when reactants produced by different manufacturers are used, there exist examples of the following physical effects on synthesis reactions: stirring rate[457,458]; the Earth's magnetic field[459,460]; gravity,[461,462] whose intensity appears to be directly proportional to the osteogenic potency of mesenchymal stem cells and boney tissues[463–465]; the seasonal variations in (1) humidity,[466] (2) barometric pressure, and (3) temperature; micrometric differences in the positioning of samples in furnaces during annealing[467]; experimental animal behavior; and circadian rhythm, on which the expression of almost 50% of genes in certain cell types has been shown to depend[468]; and the reaction vessel composition,[469] texture,[470] and dimensions,[471,472] one of the most critical parameters in the transfer of synthesis methods from a small-scale setup in a laboratory to a large-scale fabrication setting in an industrial milieu. For example, overly tall reaction volumes increased the probability of the formation of aragonite or vaterite, two of the less thermodynamically stable calcium carbonate phases during the precipitation of this compound from a solution in the presence of an organic matrix, whereas flattened volumes were more prone to yield calcite, the least soluble calcium carbonate phase.[473] Agitation, a traditional means for dispersing particles, has occasionally had the opposite effect, inducing the aggregation of both polymeric and inorganic nanoparticles,[474] which may explain cases in which the efficiency of drug loading via adsorption is inversely proportional to the stirring rate.[475] Similarly antagonistic effects of specific physicochemical synthesis parameters present more of a rule than an exception in the field of nanoscience.[476] Assessments of biological responses are particularly prone to exhibit such antagonistic intricacies, as exemplified by the case in which doubling the dose of calcium phosphate nanoparticles transformed the response of human bronchial epithelial cells from unviable to viable.[477] Biological systems in general are, in fact, notorious for their sensitivity to the slightest change in their homeostatic equilibria, as demonstrated by the long-familiar finding that bone reaches its maximum toughness in compositions that leave behind 66.5 wt% of ash, twice higher than the value at 65 and 68 wt% of ash content.[478,479] On the characterization side, this sensitivity results in ever more difficult separation of the effects of the measurement system from the properties of the measured objects, and this will increasingly apply to all 3 essential characterization aspects toward which materials science progresses: single-particle spectroscopic microscopy, high-throughput analysis, and in situ analyses. Of course, each of these problems conceals a gateway to an exciting opportunity, as exemplified by the fact that ultrafine microscopic methods do regularly create or remove single vacancies[480] or shift adatoms[481,482] on the

surfaces of analyzed materials, but measuring the energy required for these local transformations to take place has allowed for the construction of atomic-scale images of local phase properties,[483,484] which is state-of-the-art in the field of materials imaging.

In view of this one thing is certain: the design of a new generation of biomaterials will need to cope with the very same issues pertaining to the extraordinary sensitivity of function to the finest structural and compositional variations if its products are to match perfectly the properties of the natural tissues they are meant to replace. Yet, as common wisdom has it, a path without risks, perplexities, and challenges is not worth taking, and difficulties arising from attempts to design nanoscale platforms for the ultrasensitive delivery of pharmaceutics can certainly be seen as signs that those involved in these endeavors are heading in the right direction.

VI. SUMMARY

We approach a time of a prolific confluence of materials science and medicine. It is anticipated that materials science will provide the foundations for the design and development of advanced diagnostic and therapeutic methods. Within the frame of its objective, this review has provided a critical view of the current state of affairs in the development of nanoparticulate and other solid-state carriers for the local delivery of antibiotics in the treatment of osteomyelitis. In a broader picture extending outside of this narrow frame, however, is a view of ongoing progress in the way a relatively modest field of medicine, in terms of the complexity of therapeutic materials used, is being revolutionized by recent advancements in materials science and engineering. The surgical implantation of PMMA beads or, occasionally, plaster of Paris still presents the most popular method for the local and sustained delivery of antibiotics in the treatment of osteomyelitis, but this is about to change as more sophisticated materials, nanostructured in essence, are being developed on laboratory benches and sent down the translational path toward the direction of the bedside.

The two principal downsides of the traditional means of treating bone infection are systemic and long-term administration of antibiotics and the necessity for surgical debridement. The new generation of carriers for the delivery of antimicrobials envisaged during this discourse is expected to tackle these issues by first promoting the sustained release of antibiotics limited to the target site. Another vital feature of advanced carriers for the controlled release of antibiotics is their ability to contribute to the osteogenesis of the adjacent tissue in parallel with their degradation and replacement with regenerated bone. Thus antibiotic delivery and tissue regeneration are two central aspects of therapies for osteomyelitis in which milestone improvements are to be expected using the new generation of nanostructured carriers. Next steps in their development would be tuning their structure to an environmentally responsive and spatiotemporally targeted performance, as well as integrating them with alternatives to traditional antibiotics, whose ineffectiveness against increasingly resistant opportunistic pathogens is approaching critical scales. If we look at this trend in the development of drug delivery carriers for treating a particular disease from a broader angle, outside of the frame once again, we might conclude that structurally complex, multifunctional, theranostic composite nanoparticles present an object of interest toward which scientific efforts

colliding at this multidisciplinary junction of medical significance will be converging in the years and perhaps decades to come.

For a long time the degradation of polymeric biomaterials had been considered an unfavorable process, a cause of the deterioration of their properties and performance in the body. It took a fresh, new look at them to turn these demerits into advantages and present their degradation as a colossal value and untapped potential for the biomedical community. Using their degradation as a pathway to controlled drug release and tuning it to the rate of new tissue in growth nowadays presents the basic approach to tissue engineering and regenerative medicine. It goes without saying, of course, that the time for the reversal of this paradigm and inauguration of equally legitimate strivings to create bionic tissue-engineered materials—whose purpose would be not only to restore the lost functionality in a segment of the body or the body as a whole but also to augment and raise it far beyond the levels of ordinariness via lasting assimilation of the new interfaces—has yet to come. Be that as it may, potential problems faced by even the hypothetically perfect antibiotic delivery vehicles mentioned toward the end of this discourse include (1) the propensity of *S. aureus*, the main causative agent of osteomyelitis, to form intracellular colonies involved in recurrent, chronic osteomyelitis; (2) the need for the mechanical and release properties of a carrier to be adjusted to the target area of surgical implantation or injection; (3) the disparity between environments in which *in vitro* and *in vivo* drug–carrier composite testing is carried out; (4) unpredictable synergetic effects of the delivery system components or foreign agents; and (5) experimental sensitivity issues posed in parallel with the increasing subtleness of nanoplatforms designed for the controlled delivery of therapeutics. Inspired by the parable of biodegradable polymers, we could conclude that all of these problems, were they considered from a fresh, new angle, might either stimulate the development of bioengineered systems that will solve them and many other problems at the same time or, even more amazingly, be glimpsed as solutions per se to problems existing in a different domain. For if a new, multidisciplinary model for a prolific lifetime in science teaches us something, it is that two gaps in knowledge, when combined, can create a bridge to much greater knowledge lying far beyond the horizon.

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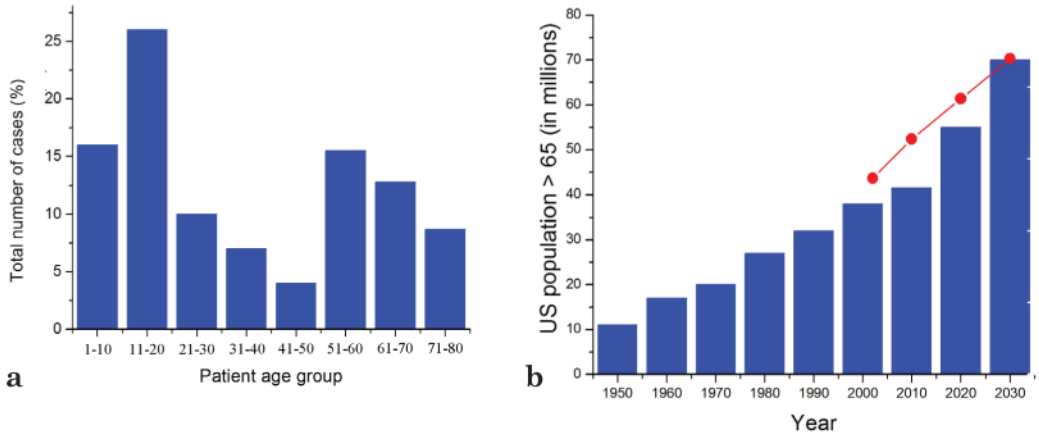

FIG. 1.

(a) Age of patients with osteomyelitis.[11] (b) US population ≥65 years old (bars), along with a projected increase in the number of patients with bone disease. Sources: US Bureau of the Census and Office of the Surgeon General.

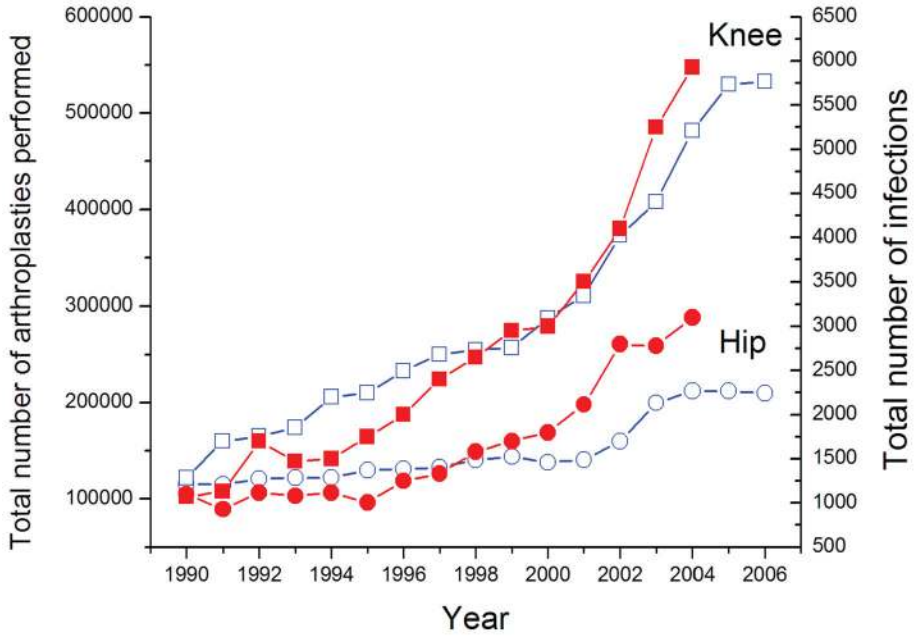

FIG. 2.

The number of prosthetic joint infections (solid circles and squares), increasing in direct proportion with the total number of knee (squares) and hip arthroplasties (circles) performed.[12]

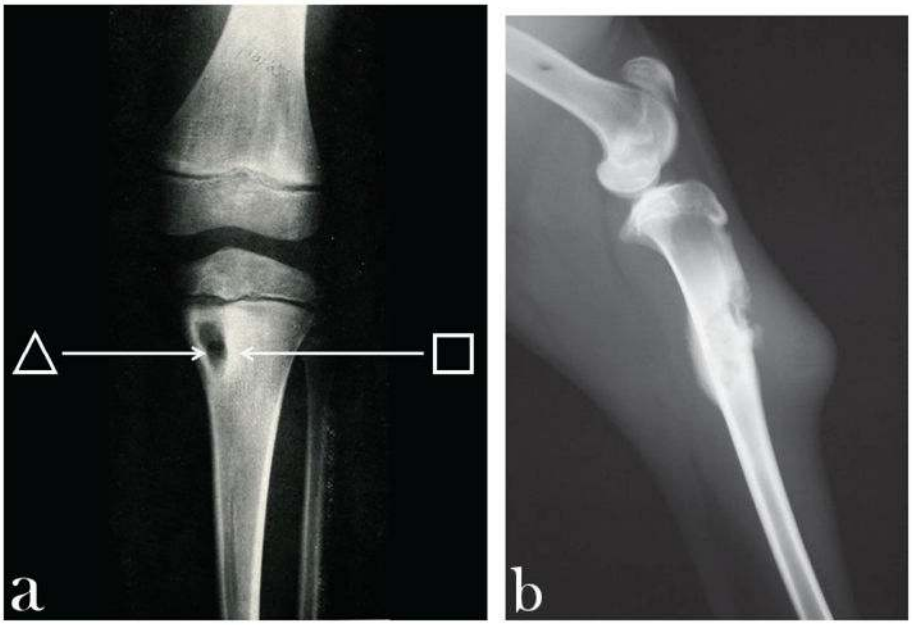

FIG. 3.

Radiographs of tibiae displaying a clinical case of acute pyogenic hematogenous osteomyelitis, also known as Brodie's abscess (△), along with an area of increased bone density around the lytic lesion due to periosteal reaction and osteosclerosis (□) (a), and evidence of chronic osteomyelitis from a goat model, manifesting as a periosteal reaction in the proximal area of the bone (b).

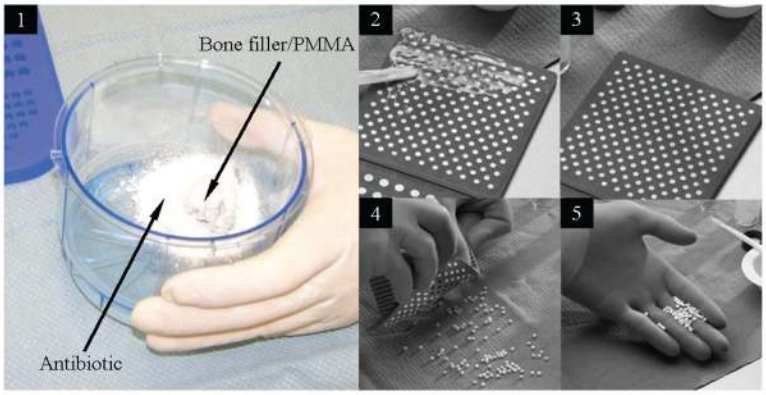

FIG. 4.

A typical preoperative preparation of poly(methyl methacrylate) (PMMA) beads encapsulating an antibiotic of choice, consisting of (1) manual admixing of the antibiotic with the bone filler; (2) filling a mold with the solid mixture; (3) hardening; (4) removing the beads; (5) collecting the beads.

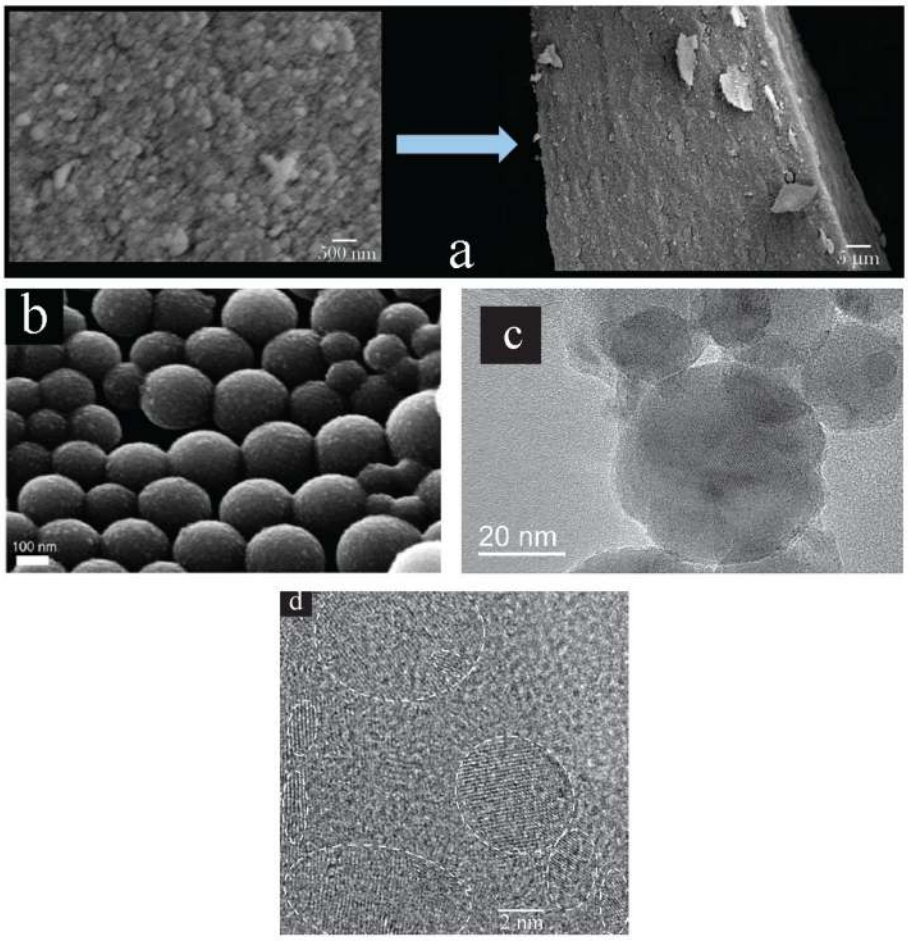

FIG. 5.

(a) Narrowly dispersed calcium phosphate nanoparticles prepared by precipitation form aggregates upon desiccation at low pressure, capturing the antibiotic clindamycin inside of the resulting pellet pores, thus ensuring sustained release over prolonged periods of time. (b) Increased loading efficiency and sustained release from well-dispersed particles could be obtained by coating calcium phosphate particles with clindamycin adsorbed on them with a layer of polymer, in this case poly-(D,L-lactide-co-glycolide) (PLGA). High-resolution transmission electron microscopic images of PLGA-coated hydroxyapatite (c) and hydroxyapatite nanoparticles (encircled by dashed lines) dispersed in a chitosan matrix (d).[131] Reprinted with permission from Elsevier (Vukomanović M, Škapin S, Jančar B, Maksin T, Ignjatović N, Uskoković V, Uskoković D. Poly(D,L-lactide-co-glycolide)/ hydroxyapatite core-shell nanospheres. Part 1: A multifunctional system for controlled drug delivery. Coll Surf B Biointerfaces. 2011;82(2): 404–13).

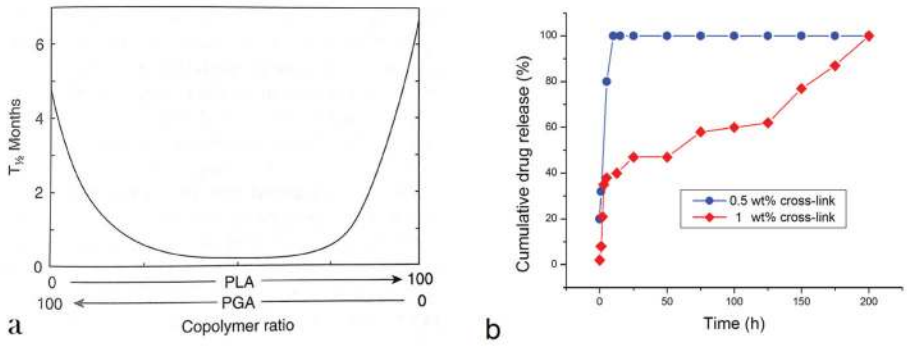

FIG. 6.

An approximate degradation half-life ($T_{1/2}$) for pure poly(lactic acid) (PLA), pure poly(glycolic acid) (PGA), and their copolymers at various weight ratios (a) and a drastic difference in the kinetics of drug release from polyethylene glycol diacrylate with 2 different percentages of cross-linking: 0.5 and 1 (b).[251] Reprinted with permission from Elsevier; Middleton JC, Tripton AJ. Synthetic biodegradable polymers as orthopaedic devices. Biomaterials. 2000;21:2334–46.

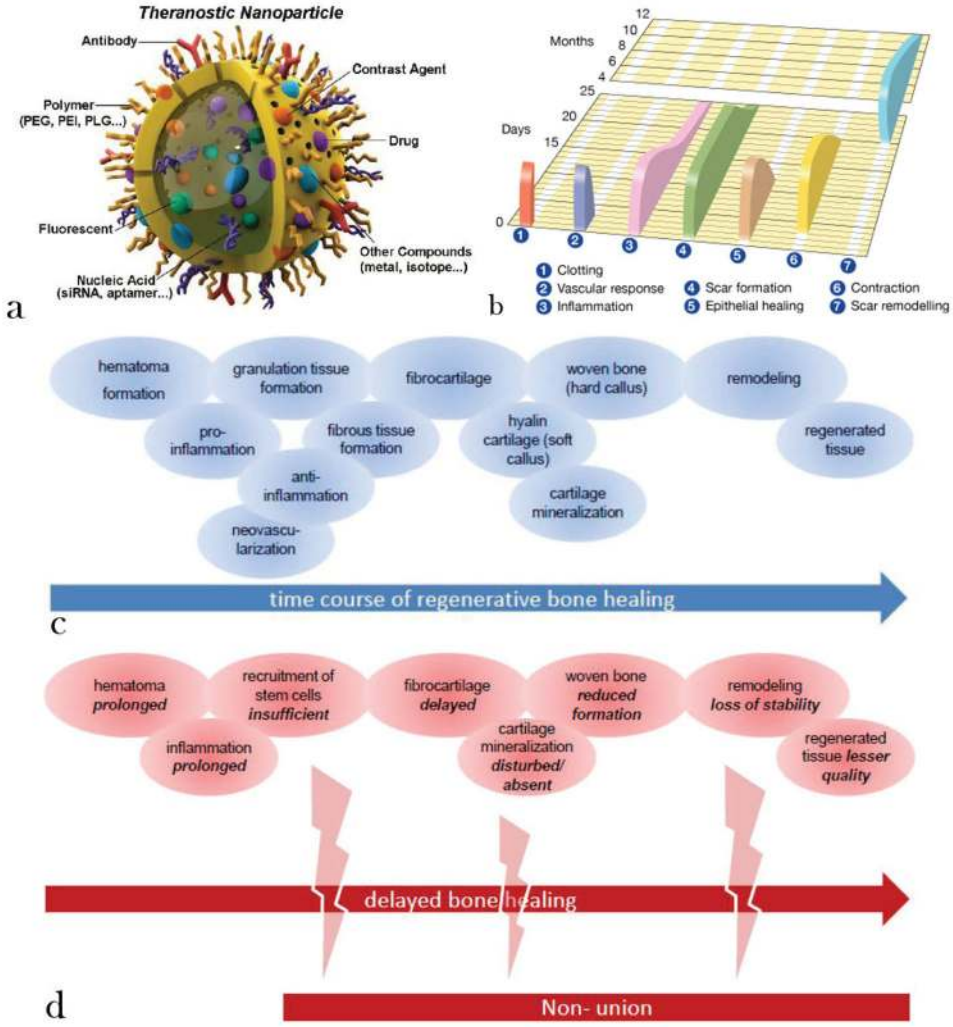

FIG. 7.

Schematic description of a hypothetical multifunctional composite nanoparticle (a) and the subdivision of the tissue regeneration process following injury at multiple stages (b). Stages specific to the bone regeneration process (c) and adverse outcomes of their obviation or incompletion (d) are shown.[362,363] Reprinted with permission from American Cancer Society; Ma X, Zhao Y, Liang XJ. Theranostic nanoparticles engineered for clinic and pharmaceutics. Acc Chem Res 2011;44(10):1114–22, and Elsevier; Mehta M, Schmidt-Bleek K, Duda GN, Mooney DJ. Biomaterial delivery of morphogens to mimic the natural healing cascade in bone. Adv Drug Deliv Rev. 2012;64(12):1257–76.

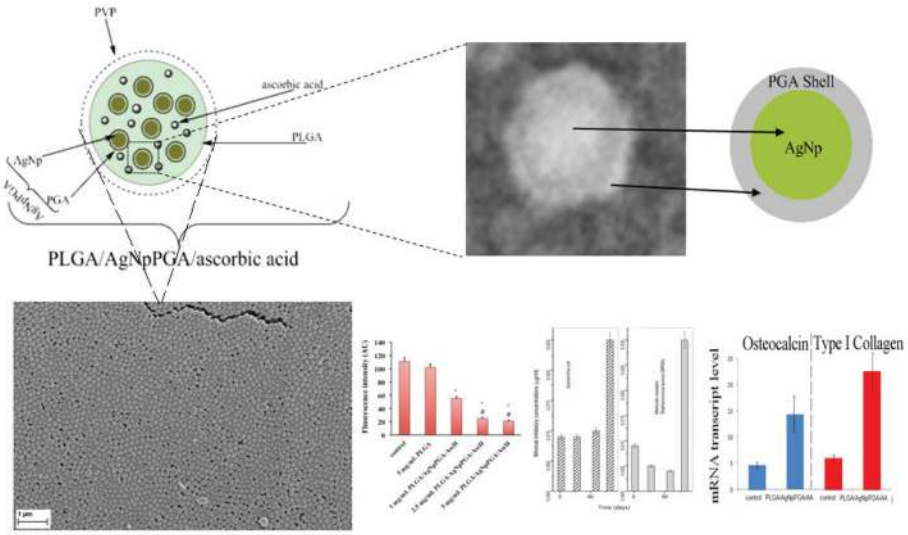

FIG. 8.

An example of composite monodisperse nanoparticles with simultaneous antioxidative, antibacterial, and osteoinductive properties. These nanoparticles are formed by coating poly-(D,L-lactide-co-glycolide) (PLGA) around silver (Ag) poly(glycolic acid) (PGA) core-shell nanoparticles (Nps) with ascorbic acid dispersed therein. The particles reduced the concentration of superoxide in human umbilical vein endothelial cells, suppressed the growth of Escherichia coli and methicillin-resistant Staphylococcus aureus, and upregulated the expression of 2 osteogenic markers: osteocalcin and protocollagen type I.[392,393] Adapted and reprinted with permission from Springer (Stevanović M, Savanović I, Uskoković V, Škapin SD, Bračko I, Jovanović U, Uskoković D. A new, simple, green and one-pot four-component synthesis of bare and poly(α, γ, L-glutamic acid) capped silver nanoparticles. Coll Polym Sci. 2012;290(3):221–31) and the American Chemical Society (Stevanović M, Uskoković V, Filipović M, Škapin SD, Uskoković DP. Composite PLGA/AgNpPGA/AscH nanospheres with combined osteoinductive, antioxidative and antimicrobial activities. ACS Appl Mat Interfaces. 2013;5(18):9034–42).

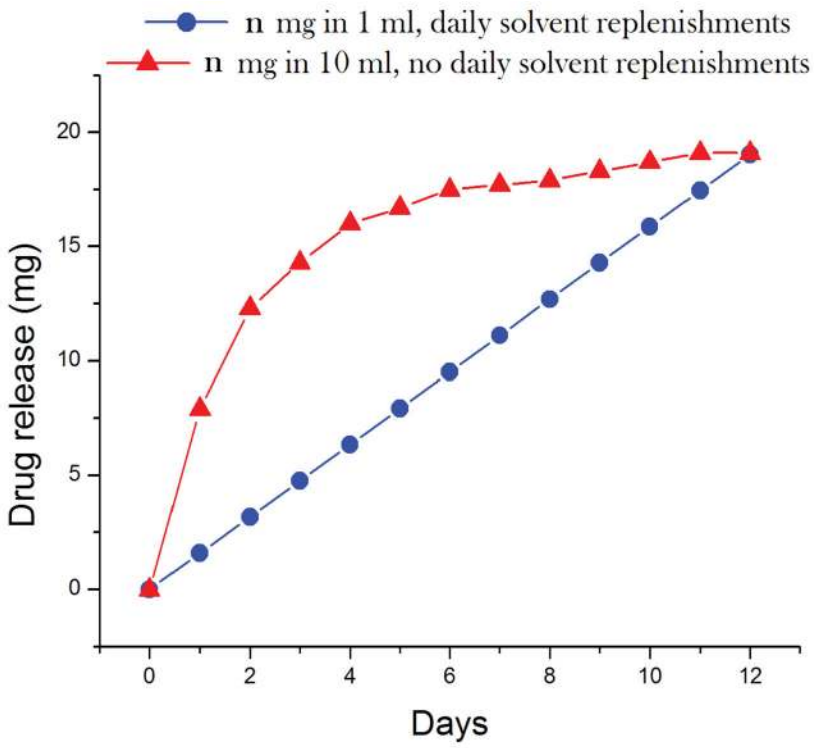

FIG. 9.

Hypothetic release curves for a drug delivery device under two different measurement regimens: daily replacements of a comparatively small volume of the solvent (circles) and usage of a considerably larger volume of solvent with no daily replenishments (triangles).

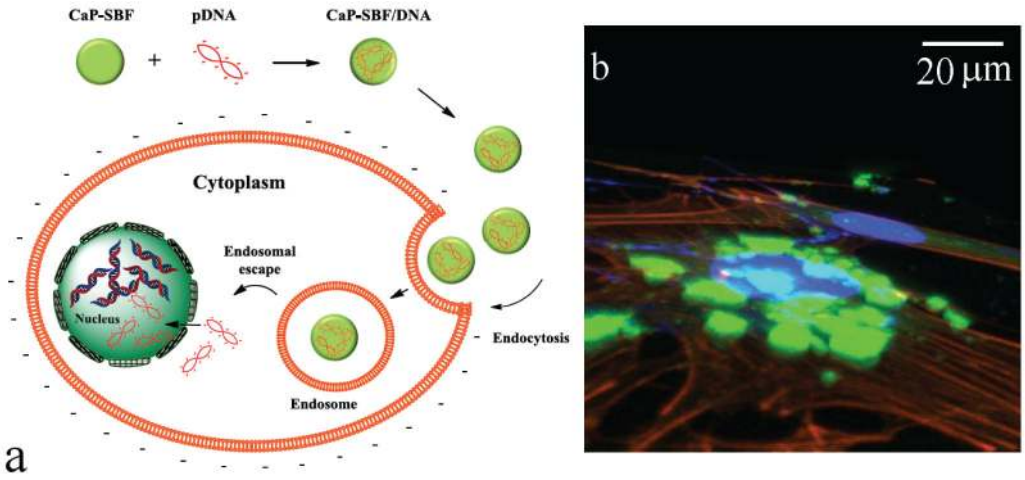

FIG. 10.

(a) A schematic description of the uptake of a calcium phosphate nanoparticle (CaPs) by the cell and its gene transfection with a DNA plasmid attached to the nanoparticle.[448] (b) A single-plane confocal optical image of fluorescently stained osteoblastic cell nuclei, cytoskeletal f-actin, and CaP aggregates containing clindamycin following 48 hours of incubation. Part A is reprinted with permission from Elsevier (Nouri A, Castro R, Santos JL, Fernandes C, Rodrigues J, Tomás H. Calcium phosphate-mediated gene delivery using simulated body fluid (SBF). Int J Pharm. 2012;434(1–2):199–208).

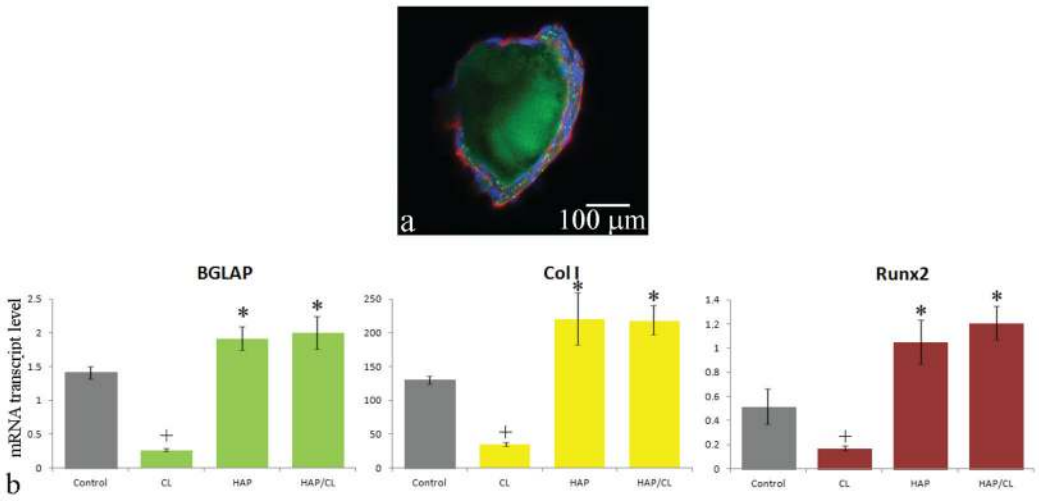

FIG. 11.

(a) A single-plane confocal optical micrograph of a fluorescently stained calcium phosphate nanoparticle conglomerate loaded with clindamycin and osteoblastic MC3T3-E1 cells (f-actin; nucleus) following 21 days of incubation in differentiation medium. This image shows intimate contact between the cells and the material, a direct indication of the osteoconductivity of the latter. (b) Results of a gene expression study performed using quantitative reverse transcriptase polymerase chain reaction and showing diminished expression of three different osteogenic markers BGLAP (left), Col I (middle), and Runx2 (right) in osteoblastic MC3T3-E1 cells incubated with an antibiotic, clindamycin phosphate (CL). The effect was compensated for when incubation was carried out in the presence of either hydroxyapatite nanoparticles (HAP) per se or HAP loaded with CL (HAP/CL). Messenger RNA expression was detected relative to the housekeeping gene ACTB. *Genes are significantly upregulated ($P < 0.05$) with respect to the control group. +Genes are significantly down-regulated ($P < 0.05$) with respect to the control group.[453] Reprinted with permission from John Wiley and Sons (Uskoković V, Desai TA. Phase composition control of calcium phosphate nanoparticles for tunable drug delivery kinetics and treatment of osteomyelitis. Part 2: antibacterial and osteoblastic response. J Biomed Mat Res Part A. 2013;101:1427–36).

Table 1

Main Calcium Phosphate Phases[103–108]

Phase	Chemical Formula	Space Group	pK_{sp} at 37°C	Solubility (mg/dm^3)
MCPA	$Ca(H_2PO_4)_2$	Triclinic P^{1-}	1.14	$17 \cdot 10^3$
MCPM	$Ca(H_2PO_4)_2 \cdot H_2O$	Triclinic P^{1-}	1.14	$18 \cdot 10^3$
DCPD	$CaHPO_4 \cdot 2H_2O$	Monoclinic I_a	6.6	88
DCPA	$CaHPO_4$	Triclinic P^{1-}	7.0	48
β-CPP	$Ca_2P_2O_7$	Tetragonal $P4_1$	18.5	7.6
ACP	$Ca_3(PO_4)_2 \cdot nH_2O$	/	25	0.8
α-TCP	$Ca_3(PO_4)_2$	Monoclinic $P2_1/a$	25.5	2.5
β-TCP	$Ca_3(PO_4)_2$	Rhombohedral R3cH	29.5	0.5
TTCP	$Ca_4(PO_4)_2O$	Monoclinic $P2_1$	37.5	0.7
OA	$Ca_{10}(PO_4)_6O$	Pseudo-hexagonal $P6_3/m$	69	87
CDHA	$Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$ $(0 < x < 1)$	Pseudo-hexagonal $P6_3/m$	85	9.4
OCP	$Ca_8H_2(PO_4)_6 \cdot 5H_2O$	Triclinic P^{1-}	97.4	8.1
HA	$Ca_{10}(PO_4)_6(OH)_2$	Pseudo-hexagonal $P6_3/m$	117.3	0.3
FA	$Ca_{10}(PO_4)_6F_2$	Pseudo-hexagonal $P6_3/m$	120	0.2

ACP, amorphous calcium phosphate (data pertain to the phase obtainable at pH 9–11); CDHA, calcium-deficient hydroxyapatite; CPP, calcium pyrophosphate; DCPA, dicalcium phosphate anhydrous, also known as monetite; DCPD, dicalcium phosphate dihydrate, also known as brushite; FA, fluoroapatite; HA, hydroxyapatite; MCPA, monocalcium phosphate anhydrous; MCPM, monocalcium phosphate monohydrate; OA, oxyapatite; OCP, octacalcium phosphate; TCP, tricalcium phosphate; TTCP, tetracalcium phosphate.