Expert Opinion

The role of biomimetism in developing nanostructured inorganic matrices for drug delivery

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Background: Biomimetism of synthetic biomaterials can be carried out at different levels, such as composition, structure, morphology, bulk and surface chemical–physical properties. Biomaterials can be turned into biomimetic imprinting of all these characteristics in order not only to optimise their interaction with biological tissues, but also to mimic biogenic materials in their functionalities. *Objective*: This review outlines the biomimetic chemical–physical properties of inorganic matrices in controlling drug release. *Methods*: This review is restricted to phosphates and silica among inorganic biomaterials proposed as drug delivery vehicles. *Conclusion*: By mimicking nature, we can design and synthesise inorganic smart materials that are reactive towards biological tissues and can release bioactive molecules by a kinetic that is controlled not only by the matrix tailored chemical–physical properties, but also by the response to stimuli induced by physiological or pathological processes.

Keywords: apatite, biomimetism, controlled release, nanostructures, silica

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1. Introduction

Ideal drug delivery systems should control the rate and period of release of drugs in specific body areas through the chemical–physical characteristics of the carrier material. Traditional therapies instead produce in plasma a drug concentration profile characterised by the typical burst effect and are indubitably necessary only when acute pathologies require an immediate high pharmacological dose.

In biomedical materials science, controlled drug delivery represents an ever-evolving field in which the study of drug delivery from implantable prosthetic systems is one of the most attractive and developed areas [1-7].

Mimicking nature and designing bioinspired materials represents a promising way to reach technological innovations in biomaterials, which can optimise their interface with biological tissues and contemporary release bioactive molecules *in situ* with a tailored kinetic [8-14]. In fact, biological materials exhibit a hierarchical structure from nano- to macro-scopic dimensions and nanotechnology has recently motivated studies to develop new nanostructured materials for biomedical applications [15-22]. Nanotechnologies that allow a 'bottom-up' approach can supply valid tools to synthesise innovative biomimetic materials, mimicking nature not only in composition but also in size, morphology, structure and surface bioactivity [23-39].

Inorganic biomaterials appear to be more suitable than polymeric ones in replacing hard tissues due to their mechanical behaviour, even if synthetic inorganic–polymeric hybrid materials can mimic the natural bone chemical–physical properties more

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closely [40,41]. Among synthetic inorganic biomaterials, apatite and silica are in particular leading to the development of new implantable materials that can also act as therapeutic agents, releasing biological active molecules *in situ* with a controlled kinetic [42-51].

In specific biomedical prosthetic systems, the drug release rate *in loco* is dependent on the disease evolution. For this purpose, inorganic biomimetic materials are ideal for long-term implantable devices which could require a modulated rate of drug release. In fact, they could exhibit a tailored drug delivery controlled by the response to physiological or pathological processes [2,40,41,52]. For example, phosphates and silica biomimetic nanostructured materials can be surface functionalised with different linking agents to anchor biologically active molecules. These can be released by breaking the linkage as a consequence of external stimuli or internal chemical factors, such as pH and ionic force variation due to physiological or pathological processes [2,5,11].

In the following sections, biomimetic approach, biogenic apatite and silica will be described, before the achieved potentialities of biomimetic nanostructured synthetic apatite and silica as advanced drug delivery agents are discussed.

2. Biomimetism

Chemists, biologists, physicists and engineers interested in material science are amazed by the high degree of sophistication, miniaturisation, hierarchical organisation, hybridisation, reliability, efficiency, resistance and adaptability that characterise natural materials. These properties, which biogenic materials have achieved through specific building principles selected by evolution, can only partially be possessed in man-made materials by present synthetic processes. For this reason nature is a school for material science. Biomimetism and bioinspiration represent important tools for the design and the synthesis of innovative materials and devices [8-11]. The highly elaborate performances of biologically occurring materials are the result of an evolved convergence on limited constituents, which occur at a precise moment and are available at that time. Nature produces soft and hard materials exhibiting remarkable functional properties by controlling the hierarchical assembly of simple molecular building blocks from the nano- to the macro-scale [12]. Biomineral morphogenesis is related to specific strategies for the long-range chemical construction of well-organised architectures from preformed nano- or micro-crystalline inorganic building blocks. In fact, many biologically complex structures are obtained by promoting specific links induced by the conformation variability at the nanometre scale of biological macromolecules. The concept of 'interfacial molecular recognition' observed in the biogenic materials has led many scientists to perform synthesis by functionalised organic matrices for the template directed control of inorganic compound nucleation and crystal growth. Biosystems reveal a high level of integration of three fundamental aspects: the nano–micro 'spatial confinement' of biochemical reactions, the inorganic and organic 'hybridisation' compounds and the 'hierarchy' from nano- to macro-scale, in order to produce a biomaterial able to exhibit the appropriate chemical–physical properties at any different scale level [13-16]. As reported in publications from the past decade, these aspects have been translated into synthetic methods and strategies for the laboratory construction of materials organised across a range of length scale. Synthetic vesicles, artificial ferritins, bacterial threads and polymer sponges have been prepared by biomimetic spatially confined synthetic methods of natural phospholipids vesicles, ferritin, cellular assemblies and protein frameworks respectively [17-31]. The template-directed synthesis has been utilised to assemble textured organic–inorganic composites, silica–lipid lamellar mesophase with helicoidal morphology [32], inorganic–organic nanotube composites from template mineralisation of tobacco mosaic virus [33], DNA-driven self-assembled multifunctional nanoparticle networks using metallic and bioinorganic building blocks [34,35], self-assembled nanoparticles structured in linear chains [36], self-assembled inorganic nano-filament networks [37] and self-assembling materials using peptide–amphiphile nanofibres [38]. Besides, self-directed immobilisation of molecular constructs have been obtained using both nanoparticles and flat inorganic substrates containing multimaterials patterned at the nano–micro scales [39].

Biogenic materials are nucleated in defined nano–micro-dimensioned sites inside the biological environments in which chemistry can be spatially controlled. The spatial delimitation is essential to biological mechanisms for controlling the size, shape and structural organisation of biomaterials. With the development of nanotechnology, this strategy employing natural material genesis has attracted a lot of attention in designing bioinspired materials such as polymeric micelles, nanoparticles, dendrimers and nanocrystals synthesised in nanoscale dimensions [52-57]. These biomaterials can represent the highlighted 'nano' drug carriers called 'nanovehicles' [1]. Inorganic biomimetic nanovehicles are prevalently constituted of phosphates or silica, which mimic inorganic components of bone tissues and silica sponges.

3. Biogenic apatite

Vertebrate bones and teeth are biological hybrid materials where a calcium phosphate, in the form of hydroxyapatite (HA), represents the inorganic component intimately inter-grown with the organic matter prevalently constituted of proteins and polysaccharides [58,59]. Biological HA is not stoichiometric according to the ideal formula $Ca_{10}(PO_4)_6(OH)_2$, but Ca^{2+} is replaced at a low extent by other ions such as Na^+, K^+, Mg^{2+}, Sr^{2+}, while PO_4^{3-} and OH^- can be partially substituted by other anions such as CO_3^{2-}, HPO_4^{2-}, $P_2O_7^{4-}$ and SiO_4^{2-}.

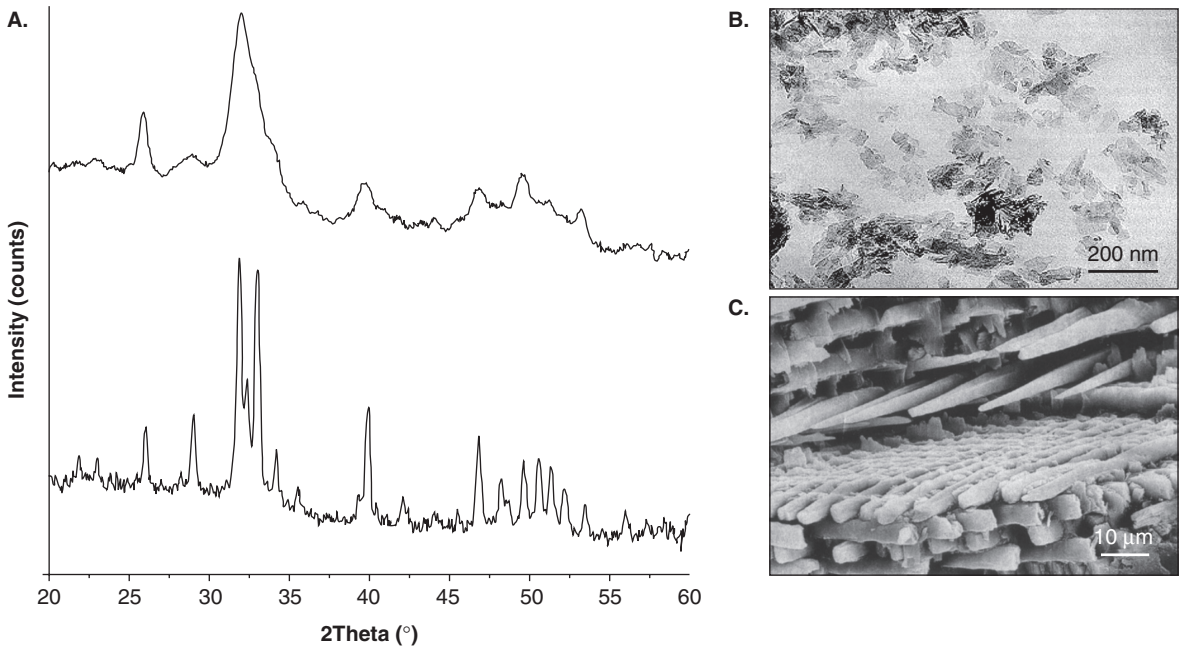

Figure 1. A. Crystal structure of natural carbonate hydroxyapatite. Powder X-ray diffraction patterns of bone (top) and enamel (bottom) apatite nanocrystals. **B.** Deproteinised bone hydroxyapatite nanocrystals observed by transmission electron microscopy (scale bar 100 nm). **C.** Scanning electron micrograph of enamel carbonated hydroxyapatite 'spaghetti-shaped nanocrystals' arranged in bundles oriented along three different directions (scale bar 10 µm).
B and **C** reproduced by kind permission of [59].

The bone mineral phase is more correctly called carbonate hydroxyapatite. Carbonate is the prevalent foreigner anion and represents about 4 – 8 wt% [60,61]. The substitution of CO_3^{2-} groups into the PO_4^{3-} sites (type B carbonate apatite) is prevalent in young humans, while the carbonate replacement to OH^- groups (type A carbonate apatite) increases with the age of the individual [62].

The bone carbonate hydroxyapatite nanocrystals, which can represent a typical example of an 'organic matrix-mediated' biogenic material, have a blade shape of approximately 25 nm width, 2 – 5 nm thickness and about 60 nm length. Biogenic hydroxyapatite crystals exhibit non-stoichiometric composition, structured carbonate ions in the crystal lattice, a low degree of crystallinity, plate acicular morphology and a nano-size that confers a large surface area of about 120 m^2/g (**Figure 1A, B**) [40,48,59].

Vertebrate bone can be considered a 'living biomaterial' since it contains a network of different cells under permanent activity, living within the mineralised structure, which are interconnected through pores and channels. Osteocytes respond to changes in mechanical pressure activating another type of cells (osteoblasts) to start the mineralisation process through the synthesis and release of a protein mixture, which is primarily composed of type I collagen. Subsequently this is mineralised by a controlled nucleation and growth of apatitic nanocrystals. Osteoblasts remain trapped inside the mineral phase until this is degraded through a heady mixture of acid and enzymes secreted by another type of cells (osteoclasts), which are proposed to catabolise the bone. This dynamic process of bone formation and destruction accounts for the growth of bone during the body's development and regeneration after fractures.

Dentine resides within the central region of the tooth and is similar to bone in composition and structure [63-72]. Enamel, the tooth external surface coating, has a much larger inorganic content than bone and dentine, close to 95% wt, which is mainly constituted of long thin ribbon-like prismatic crystals of hydroxyapatite that exhibit a higher degree of crystallinity and a lower carbonate content than bone and dentine apatite crystals (**Figure 1A, C**). Amelogenins, present in relatively large numbers in the early stages of enamel formation, are enzymically degraded and removed up to 5% wt as the hydroxyapatite crystals grow [73]. Adult dental enamel, considered to be the most resistant and tough material in the biological world, does not contain cells and therefore cannot be regenerated by itself. There is no biological process that can repair degraded or damaged enamel, evidencing the need for synthetic enamel biocompatible materials able to repair teeth decay [74-76].

In mineralised tissues, the organic–mineral phase ratio can vary depending on species, tissue location, age, diet and pathologies [64,65]. In cortical bone, the organic matrix represents about 20% wt, mineral phase about 70% wt and the rest is the water associated with the constituents. The organic

matrix is mainly composed from type I collagen that acts as a template for the nucleation of the inorganic crystals [66]. Collagen mineralisation may be considered a sequence of events requiring the interaction of many different promoting or inhibiting factors [67], through the formation of matrix vesicles [68-71], inside which calcium and phosphate raise to saturation favouring the deposition of amorphous calcium phosphate, octacalcium phosphate and/or brushite, with later transformation into carbonate hydroxyapatite [72]. Type I collagen is produced by osteoblasts in the surrounding extracellular space, where molecules and microfibrils self-assemble into mature collagen fibrils. Single collagen molecules, constituted of a triple-stranded helical chain characterised by the (Glycine-X-Y), where X and Y are commonly proline and hydroxyproline, exhibit an uniform size: 280 nm in length, 1.5 nm in width and a molecular mass of about 285 kD, including N and C non-helicoidal short-terminal domains [77]. In the collagen microfibril structure, single molecules are lined up head-to-tail in rows, which are staggered by 64 nm along their long axis. Stabilised by strong intermolecular crosslinks, this arrangement produces a regular array of small gaps 40 nm long and about 5 nm wide referred to as 'hole zones', which are considered to be the loci of the nucleation and growth of hydroxyapatite nanocrystals. The overlapping of adjacent hole zones form grooves oriented parallelly to the main collagen fibre axis. The three-dimensional fibril structure allows hydroxyapatite growing into plate-shaped nano-crystals with the *c* crystallographic axis preferentially oriented along the protein fibril axis. Utilising the orientation coincidence between HA crystallographic *c* axis and collagen fibrils main axis, using conventional and synchrotron radiation sources at both wide and low angles from single osteons and osteonic lamellae, recorded X-ray diffraction patterns have allowed the determination of the orientation of hydroxyapatite crystallites and consequently collagen fibrils in bone [78-82].

4. Biogenic silica

Among biogenic minerals, silica is singular for its amorphous state of metal oxide, which is formed by complicated inorganic polymerisation processes, unlike biogenic carbonates and phosphates, which are commonly crystalline solids [83]. Chemists have only recently given particular attention to the chemical process involved in the natural formation of biogenic silica structures, characterised by a charming morphology and showing a highly ordered network of nanopores, as in the diatom algae and in the spicules of sponges (**Figure 2**) [84]. Using new biomimetic synthesises, chemists have discovered the possibility of designing mesoporous silica materials with a highly ordered pattern of orifices resembling the siliceous structure found in unicellular diatom algae [85-88]. Only in the last few years have mesoporous materials been studied and suggested for biomedical applications. Biomimetic synthetic silica MCM-41 was first proposed as a drug delivery device in 2001 [49], but SBA-15 and MCM-48 have also been successfully considered as drug carriers and controlled release systems [2].

Bacteria, algae, protozoa and plants use silica for different functions and the biochemistry of the silica formation is different. The envelopes of bacteria, which live in different environments, present a coating of amorphous silica about 100 nm thick [89]. This coating has been observed in bacteria that live in extreme environments, suggesting it may represent a protective shell [90].

Different possible chemical processes have been suggested for this silica formation, for example for the direct interaction of soluble anionic silicates with the positively charged groups of peptidoglycane of the bacteria envelopes. Alternatively, the formation of hydrogen bonding between silanol groups and the polysaccharide hydroxyl groups of the peptidoglycane has been proposed. Finally, for Gram-positive bacteria (*Bacillus subtilis*), where the superficial bacterial layers are predominantly negatively charged, it has been supposed that foreign metallic ions may provide nucleation sites for mineralisation where they interact on the superficial bacterial envelope [91].

Microscopic and macroscopic specific amorphous silica structures are generated from marine sponges and represent a characteristic of the single specie.

They form spicules of glassy rods from a few microns to several millimetres in diameter and lengths reaching up to some metres, useful for sponges anchoring to the sea floor. Spicules are constituted of concentric layers of hydrated silica. The intersilica layers contain a thin organic component called *silicatein*, meaning a silica-based protein [92]. If the spicule mechanical response is compared to that of a synthetic monolithic silica rod, we can observe that the spicule breaking stress is four times higher than the monolithic silica one. Monolithic silica breaks in a single catastrophic event, while spicule breaks gradually with progressive load drops, showing the effect of an 'onion' layer structure in arresting the fracture through the energy absorption at the interfaces [93-96]. They exhibit low elastic modulus, which leads to flexibilities of material for which there is no equivalent man-made synthetic silica-based material. Spicules are multifunctional materials and carry light-showing optical properties very close to those of modern optics fibres [94,95]. A growing spicule is embedded in a membrane called silicalemma and silica polycondensation takes place on poly protein fibres which catalyse the hydrolysis, orienting the tetraethoxysilane polymerisation parallelly to the proteases lineament [97-99]. Diatom 'glass box' deposits represent the main contributors to the global silica biogenic cycle. They consist of two halves overlapping like a Petri dish and are structured with nano-particles ranging from 40 to 200 nm [100,101]. The diatom frustules surface exhibits a homogeneous patterned network of nano–micro metric orifices quite uniform in shape [102,103]. Even if the utilisation of amorphous silica rather than a crystalline

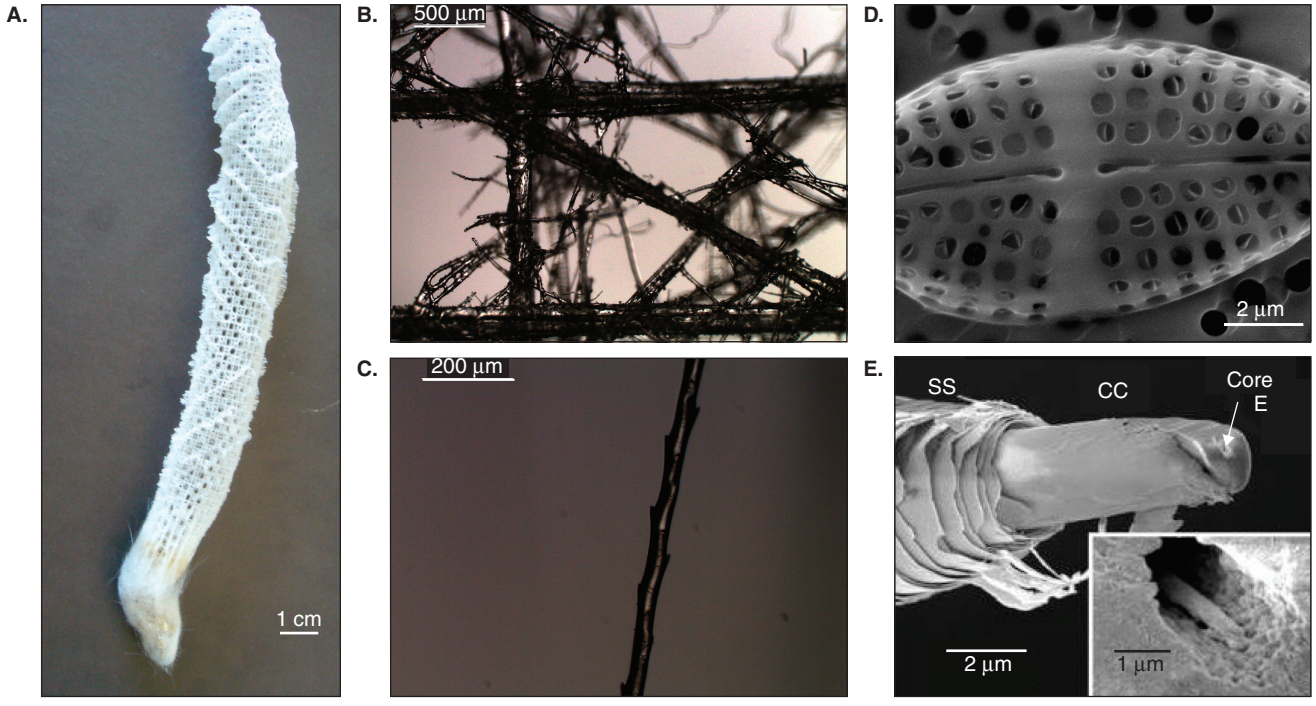

Figure 2. A. Photograph of a typical specimen of *E. aspergillum*, showing the silica rigid basket cage, subject to mechanical stresses, ocean current and the flexible silica spicules, which anchor to sea floor. Optical micrographs of a portion of lattice-like skeleton of fused siliceous spicule (**B**) and of a barb-like spine (**C**). **D.** Scanning electron micrograph (SEM) image of the surface of silica diatom showing self-organised pattern of orifices. SEM images produced after fluoridric acid treatment on a spicule. **E.** The exposed CC (central cylinder) region with the hollow core (indicated by an arrow) and surrounded by a receding series of SS (striated shell) layers can be seen. The inset shows a high-magnification SEM of the etched core region (2 µm in diameter), exposing the organic filament.
E from [94]. Copyright (2004) National Academy of Sciences, USA.

inorganic mineral, such as carbonate and phosphate, in producing the wide variety of biogenic silica complex architectures is unknown, probably an amorphous material can be easier moulded in specific microscopic textures by the Si-O-Si higher bound angle variability in amorphous than in crystalline structures. This finding can be usefully utilised in designing biomimetic silica-based drug carriers.

5. Biomimetic nanostructured synthetic apatite

Synthetic hydroxyapatite exhibits good properties as a biomaterial, such as biocompatibility, bioactivity, osteo-conductivity, direct bonding to bone, etc., exciting the applications of HA in the fields of bone tissue engineering and orthopaedic therapies [40]. There are many synthetic strategies to produce HA and substituted HA, including wet producing, hydrothermal, electrochemical and ultrasonic mobilisation methods, sol-gel and solid-state synthesis. Apatites with different stoichiometry and morphology have been prepared and the effects of varying powder synthesis conditions on stoichiometry, crystallinity and morphology, have been analysed. The effects of varying the concentration of the reagents, the reaction temperature and time, initial pH, ageing time and the atmosphere within the reaction vessel have also been studied [46,47,104-106]. In order to optimise its specific biomedical applications, especially drug delivery function, the physical–chemical features that should be tailored in synthetic biomimetic HA are dimensions, porosity, morphology and surface properties [3,41].

For the last 30 years calcium phosphate ceramics have been, and still are today, very popular implant materials for diverse clinical applications. Porous HA simulating spongy bone morphology (porosity varying from a microporosity > 1 µm to a macroporosity ranging from 300 to 2000 µm) has been prepared using various technologies to control pore dimension, shape, distribution and interconnections. HA ceramics processed by high-temperature treatment [107] present a significant reduction of bioreactivity and growth kinetics of new bone due to the lack of resorbability. New formation methods at lower temperatures have been developed, allowing one to obtain porous bioceramics with a low degree of crystallinity. Colloidal processing [108], starch consolidation [50], gel casting and foam out [109] have yielded excellent results, producing bioceramics with a bimodal distribution of the pore size that can be modified as a function of the sintering conditions.

Different types of CaP-ceramics are available, although they can be classified as either hydroxyapatite (HA), beta-tricalcium

Table 1. Z potential measurements for hydroxyapatite (HA) synthesised in the absence of amino acids and amino acid functionalised HA nanocrystals.

	pH of suspension	ζ-potential (mV)
HA	7.5	-21.2 ± 1.4
HA/alanine	7.8	-5.8 ± 1.5
HA/aspartic acid	7.6	-6.9 ± 1.2
HA/arginine	7.6	-4.1 ± 1.0

phosphate (β-TCP), biphasic calcium phosphate (BCP), amorphous calcium phosphate (ACP), carbonated apatite (CA) or calcium deficient HA (CDHA) [4]. The use of these materials for tissue engineering purposes is still being explored. Most researchers are aware that the low resorbability of sintered CaP-ceramics, and in particular the incomplete resorbability of ceramic HA, appear useful when a biomaterial has to be implanted with a defined 3D form. The use of highly porous implants induces bone formation inside the implant and increases degradation, but the complete resorption in most cases is very difficult, due to the crystalline architecture.

Porous coralline HA can be synthesised by a hydrothermal method for HA formation directly from natural sea corals [110] and HA replaces aragonite whilst preserving its porous structure. The biaxial strength of coralline apatite could be improved with a unique double treatment that includes a nano-coating layer to cover meso- and nano-pores. In this two-stage process, the coral is fully converted into hydroxyapatite and then coated with a sol-gel-derived apatite. This new material can be applied to bone graft applications where high strength requirements and longevity are pertinent [49,111].

The interconnected network of pores promotes bone in-growth, but also allows bioceramics to be utilised as drug delivery agents by inserting different bioactive molecules. Many studies have demonstrated that hydroxyapatite ceramics can be used to deliver steroids, antibiotics, proteins, hormones and anticancer drugs. Porous ceramics closely mimicking spongy bone morphology have been synthesised by the impregnation of cellulosic sponges with poorly crystalline HA water suspension [39]. These porous ceramics have been tested as controlled drug delivery bone grafts to evaluate the fundamental parameters that control release kinetics. A theoretical approach, based on the use of the Finite Element Method, was adopted to describe the ibuprofen–lysine and hydrocortisone Na-succinate release kinetics, comparing numerical results with experimental results [5]. An alternative approach to tissue engineering, which uses cells seeded onto macropores of these HA scaffolds to promote bone growth, is represented by filling the macro- and micro-pores with gelatine, which can act as a cell nutrient and/or delivery agent of bioactive molecules [6]. When powder bioceramics are used for bone filling applications, they are usually mixed with a polymeric carrier matrix to avoid migration out of the implant region. Both non-absorbable (poly(methyl methacrylate) [112], polyethylene [113] and polysulfone) and biodegradable (poly(lactic acid) [114], polyglycolic acid, collagen, cellulose and starch [43,44]) polymeric matrices can be used, even if the non-biodegradability drastically reduces the HA crystal bioactivity.

The chemical and biological properties of the latter are strictly linked to their dimensions, the regulation of which requires a high level of biological and chemical control at the nano-scale. Thus, the recent trend in biomaterials research is focused on overcoming the limitations of calcium phosphates, or more precisely hydroxyapatite ceramics, and in improving their biological properties via exploring the unique advantages of nanotechnology [45]. The trend is shifting towards nanotechnology to improve the biological responses of HA, because nano-HA is a constituent of bone, improving the biomaterial–bone interface. The use of biomimetic HA and CA in orthopaedics is therefore considered to be very promising, owing to its composition, structure, dimensional and morphology similarity with the bone crystals (**Table 1**). It has been established that biomimetism offers a unique approach to overcome many traditional materials shortcomings. Nanostructured biomimetic materials offer much higher performances than their larger particle-sized counterparts, due to their large surface to volume ratio and unusual chemical/electronic synergistic effects (**Figure 3**).

In addition, the surface adsorption properties of these materials has led to applications in affinity chromatography [115], waste-water remediation [116] and drug delivery systems [7].

The surface functionalisation of HA nano-crystals with bioactive molecules makes them able to transfer information to and to act selectively on the biological environment, and this represents a main challenge for innovative bone substitute materials. In this way HA nanocrystals will not only guarantee, for instance, either osteo-integration or osteo-induction enhanced properties, but they will also perform at the molecular level by stimulating specific cellular responses.

Only in recent years have scientists begun to use biomolecules for the synergistic coupling of crystals synthesis and functionalisation. In fact, previous studies have limited the use of biomolecules as simple growth inhibitors of HA crystallisation, rather than considering their use as a strategy to fine-tune the bioactivity of the nanoparticles [117,118]. Studies of the effect of biological molecules onto hydroxy-apatite crystal growth have been related directly to physiological or pathological calcification processes.

Particular interest has been dedicated to amino acids, which are compounds of major importance for living organisms, because their concentration is controlled by physiological mechanisms and they enter the cell environment by simple diffusion [119,120]. Considering that the presence of proteins, (and hence amino acids) in biological materials

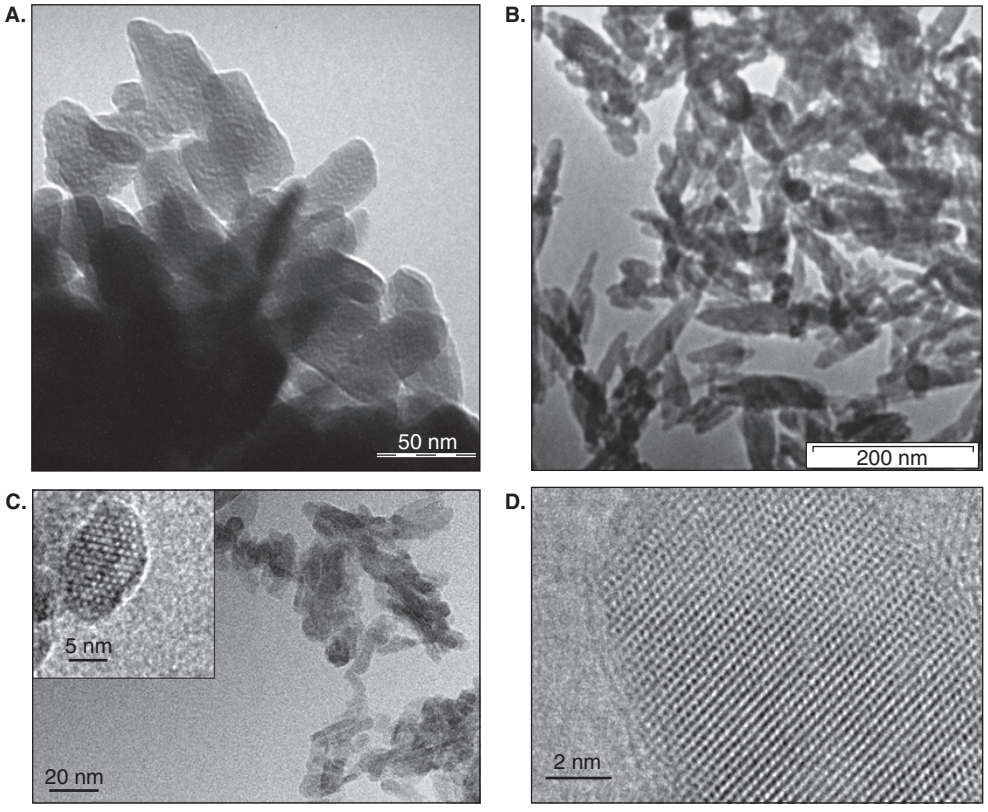

Figure 3. Transmission Electron Microscopy (TEM) images of biomimetic hydroxyapatite (HA) nano-particles with different morphological and dimensional features (A – C). Scale bars are 50 nm (**A**), 200 nm (**B**) and 20 nm (**C**). High resolution images (HRTEM) of bone and dentine-like HA nanocrystals with the *c* axis perpendicular or parallel to the image plane (inset **C, D**).
From [123].

is intrinsic to the bioactivity of HA, amino acids can be considered as agents that can increase the bioactivity of the synthetic HA [121,122]. Hydroxyapatite nanocrystals with amino acid surface functionalities and different morphology in dependence on the amino acid used as co-reagent during the synthesis have been obtained. A self-assembly mechanism could be supposed, considering that the crystals domain size appeared slightly decreased by the amino acid presence, while the nano-crystal grows one-directionally. The ζ-potential measurements showed that the amino acid functionalised HA surface charge is inverted, being shifted towards neutrality in respect to the HA (**Table 1**). This chemical surface modification should dramatically affect the hydroxyapatite biological properties and should offer the potential for the nanocrystals to be used as carriers of bioactive molecules linked to the amino acidic residue. Amino acid is the anchoring agent between apatite and bioactive molecules, dramatically affecting the adsorption and released kinetics [123].

Hydroxyapatite is also known for its binding capability to a wide variety of molecules [120]. It is an attractive goal to develop new biomimetic apatite nanocrystals for potential use in bone implantation, which in addition function as a local targeted delivery system for anticancer and antimetastatic drugs with controlled release properties. For example, the application of such a material in the chemotherapeutic treatments of osteosarcoma could result in tumour inhibition accompanied by low levels of systemic toxicity.

The adsorption and release of bioactive molecules are strongly affected not only by the chemical properties of the drug molecule, but also by the chemical and structural characteristics of the HA substrates (**Table 2** and **Figure 3**). Thus, the adsorption and release of cisplatin (CDDP), alendronate, and di(ethylendiamineplatinum)medronate (DPM) have been investigated using two bio-mimetic synthetic hydroxyapatite nanocrystal materials with either plate-shaped or needle-shaped morphologies and with different physico-chemical surface properties. These bioactive molecules were chosen in order to compare the behaviour of metal-based drugs (CDDP and DPM) to that of a classical organic drug (alendronate), evaluating the effect of the drug molecule overall charge in influencing the drug affinity for apatite nanocrystals with variable structural and chemical different properties. The HA surface area and surface charge (Ca/P ratio), as well as the charge on the adsorbed molecules and their mode of interaction with the HA surface, influence the

Table 2. Physicochemical characteristics of different kinds of synthetic hydroxyapatite (HA) nanocrystals: comparison with bone HA.

	D_{002} (nm)	D_{310} (nm)	Length dimensions TEM (nm)	Surface area (m^2/g)	Bulk Ca/P (ICP-OES)	Surface Ca/P (XPS)
HA plate shape	22 ± 5	5 ± 2	10 ± 5	120 ± 12	1.62 ± 0.05	1.45 ± 0.05
HA needle shape	35 ± 5	8 ± 3	100 ± 10	100 ± 10	1.65 ± 0.05	1.30 ± 0.05
Calcium deficient HA	23 ± 3	7 ± 2	15 ± 5	110 ± 11	1.50 ± 0.05	1.20 ± 0.05
High crystallinity HA	50 ± 7	23 ± 3	80 ± 10	60 ± 6	1.65 ± 0.05	1.30 ± 0.05
Bone HA [59,78]	18 – 25	3 – 5	20 – 150	85 – 170	1.58 – 1.71	1.30 – 1.60

adsorption and release kinetics of the three drugs investigated. The results demonstrated that HA nanocrystals and antitumour drugs can be selected in such a way that the bioactivity of the drug–HA conjugate could be tailored for specific therapeutic applications (**Figure 5D**) [3].

The exposure of biomaterials to plasma proteins, blood or biological fluids normally leads to the adsorption of blood proteins into the biomaterial surface. The adsorbed protein layer can further mediate additional biological responses, such as cell attachment and activation, and can create unpredicted perturbations to device operation [124]. Although protein adsorption on solid surfaces has been widely studied for decades, its mechanisms are still far from being fully understood. This is because adsorption of protein on a solid surface is a complicated process consisting of many events, such as conformational changes in protein molecules and coadsorption of ions. In particular, the protein conformational change, which results in entropic gain, is thought to be important for driving forces to the protein surface adsorption [125]. Here, the knowledge of the underlying principles of protein interaction with calcium phosphates is required not only in evaluating their potential application, but also their ability to act as a carrier for the biomolecules.

In evaluating the interaction of HA with serum and bone morphogenetic proteins [126-131] the interaction of HA with myoglobin (Mb) has been clearly elucidated. In fact, Mb can be considered as a model protein because of its well-known structure and properties, commercial availability and relatively small sizes [125].

A new trend of research is to compare the protein interaction not only with the 'clean' substrate, but also with the functionalised materials. In particular, the Mb affinity versus the alendronate surface functionalised biomimetic HA with respect to unfunctionalised hydroxyapatite has been tested. The Mb recognising and assembling on drug-loaded apatite crystals not only allows the better understanding of the protein adhesion mechanism, but it could also aid the development of surface coatings to improve the biocompatibility of bone implantable biomaterials and for hard-tissue engineering and regeneration technologies.

The HA–myoglobin interaction mechanism has been elucidated and, in particular, using UV/Vis and surface-enhanced Raman spectroscopy, it has been found that the spin state of Mb haem moiety changes from the six-coordination high spin native state to six-coordination low spin state as a consequence of the interaction with biomimetic hydroxyapatite nanocrystals. The spin state of myoglobin is relevant in relation to the catalytic activity of the protein. The surface electrostatic potential map of protein allows us to hypothesise a preferential interactive mechanism through one defined region of protein surface, in spite of random adhesion mechanisms towards other inorganic supports. Haem moiety is attracted towards the apatitic surface as a consequence of the surface disorder of the nanocrystals, which is connected to a negative surface charge in respect to the crystalline core. These results have highlighted that the immobilisation of bisphosphonates onto the HA is an important strategy to set up a bone-specific drug delivery device. Considering that the interaction of protein with HA/biomolecules conjugates tailored for specific therapeutic applications plays a key role as biological probe, the Mb affinity towards the HA/alendronate bioconjugates has been tested [132]. The alendronate avoids both the adsorption and the conformational changes of Mb haem moiety. Myoglobin behaviour towards alendronate grafted HA crystals evidences that this functionalisation imprints surface selectivity to HA and drives the biological environment response towards it [132].

Considering the functionalisation effects, one goal could be to obtain a drug delivery characterised by a stimuli responsive kinetic. This aims to surface functionalise HA with different linking agents, such as bisphosphonates, to anchor biologically active molecules which can be released, breaking the linkage as a consequence of external stimuli or internal chemical factors, such as pH and ionic force variation due to physiological or pathological biological process.

6. Biomimetic nanostructured synthetic silica

The biomimetism of some silica-based materials can be considered intrinsic in view of the fact that various organisms utilise amorphous silica rather than a crystalline mineral as a structural material, producing arrangements based on a covalently linked polymer of randomly set tetrahedral

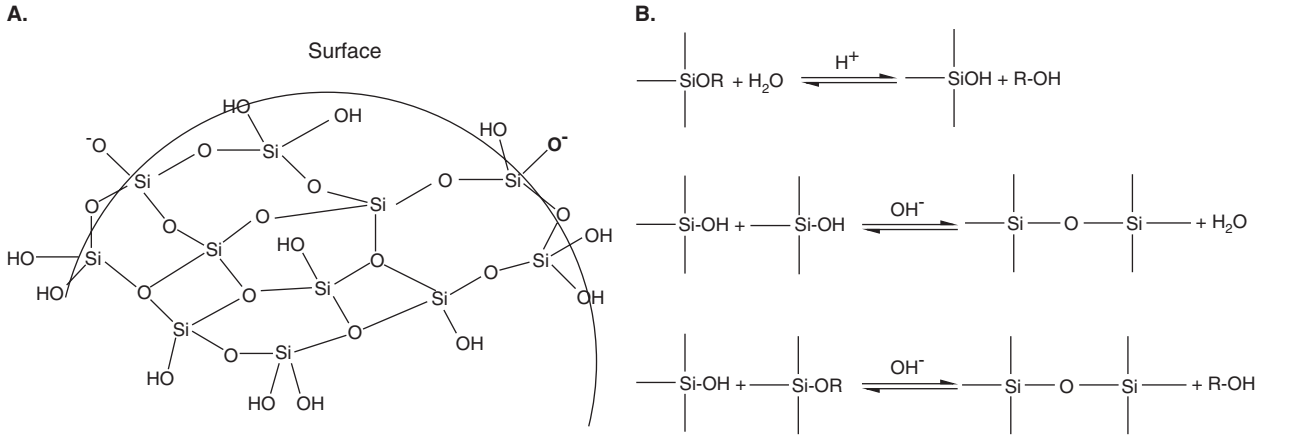

Figure 4. The chemical structure of the amorphous silica (A) and hydrolysis and condensation reactions involved in the sol-gel polymerization of silica (B).

coordinated siloxane centres with variable levels of hydroxylation (**Figure 4A**).

This is probably because the amorphous biomineral can be subsequently moulded into a wide variety of complex architectures without any loss of strength. This concept demonstrates how getting close to biogenic silica in generating materials can represent an enormous advantage in producing drug delivery systems. The advantage arises primarily from the possibility of tailoring the internal microstructure of the matrix. It is obvious that a flexible technique is necessary to serve such versatility: in this sense the sol-gel technology, an ambient-temperature inorganic polymerisation technique, results biomimetic in its adaptability to produce silica-based biomimetic structures [133]. The chemical reactions that take place during the synthesis of silica gels include hydrolysis and condensation reactions. The hydrolysis reaction can be either acid or base catalyzed, an unusual finding which reveals another feature of silica versatility. During the hydrolysis, the metal alkoxide reacts with H_2O and therefore new alcohol molecules appear and Si-OH groups are formed (**Figure 4B**). During the poly-condensation reactions, the Si-OH groups react, forming Si-O-Si bonds, which yield to SiO_2 nanoparticle formation. These solutions result from the balance between particle growth, yielding to sols, and particle aggregation, leading to gels. The gel network is highly pH dependant, dense networks being obtained in acidic conditions (pore size < 2 nm), whereas alkaline conditions favour particulate porous gels (pore size ranging from 2 to 50 nm) [134,135].

Sol-gel technology has allowed the difficulty in using the classical glass-based biomaterials as drug delivery systems to be overcome, the problems being due to the high process temperature employed in traditional methods of synthesis and the consequent difficulty of manipulating the internal microstructure of the matrix. Sol-gel processes allow the entrapment of bioactive molecules inside the silica matrix at room temperature during the formation of the oxide backbone, leading to the production of a composite gel with the active ingredient being homogeneously distributed throughout the resulting gel (or xerogel).

The physical characteristics (including density, pore size and nanostructure) of the oxides can be tailored by controlling the sol-gel reaction kinetics and in particular the relative rates of hydrolysis and condensation. The silanol-rich pore surface can furthermore be functionalised either by post-synthesis functionalisation methods [136,137] or, more commonly, by the co-condensation method [138], where alkyl residues are introduced in the synthesis by replacing part of the tetra-alkoxysilane precursor with organically-modified alkoxides, although these techniques are more commonly used for mesoporous materials. R_n'-$Si(OR)_{4-n}$, where R′ ranges from alkyl chain to amino- or thiol-bearing groups can be used. The Si-R′ covalent bond is not sensitive to hydrolysis and is therefore maintained in the final material, providing functional sites for further binding. In contrast, these additional functional sites can give rise either to interactions with silanol groups (i.e., protonated terminal amine groups can interact with silanol groups to form zwitterions (NH_3^+-$^-$OSi) [139]) or steric impedance. These cause profound effects both on the local structure of the silicate framework (pore size shrinkage or enlargement) and in terms of steric hindrance or chemical affinity towards the host molecules [140].

The size and the organisation of the host pores are key parameters controlling loaded drug diffusion and this has attracted attention towards silica-based mesoporous materials [141] for delivery systems. Combining silicon alkoxides with surfactants, these molecules self-organise in micellar systems that can further assemble to form liquid crystal phases. When the hydrolysis/condensation of silicon alkoxides is triggered, silica formation occurs at the interface with these structures, resulting in a silica gel containing an ordered array of micellar systems. Upon surfactant removal, an open porosity remains, which replicates the initial organisation of the organic phase [142]. The biomimetism of this ordered

network can be observed in how this mimics, in the nanoscale, the hierarchically ordered pore structures with dimensions ranging from the nanometre to the micrometre domain, present in unicellular organisms, such as diatoms silica shells.

In the case of mesoporous materials, the synthetic procedure is not compatible with the direct encapsulation of the drug; thus, these materials are first formed and then impregnated with a solution of the bioactive molecule [49].

Release systems in the form of particles, as opposed to implants, are required to target specific sites in the body. The sol-gel technology combined with water-in-oil emulsion technique has allowed the development of microreactors constituted by the sol-gel solution droplet in which a normal sol-gel transition has been conducting. As a consequence, the size of the droplet dictates the size of the particles, while the internal nanostructure of the resulting micro- and nano-spheres is completely controlled by the sol-gel chemistry [143]. Porous hollow nanoparticles (with pores connected to one large reservoir) [144], or templated mesoporous silica nanoparticles (where the unique hexagonally ordered pore structures of the mesoporous materials are reproduced), have been recently developed [145].

The release of a drug dispersed in the gel matrix occurs according to a combined process of diffusion through solvent-filled capillarity channels and the dissolution process of the matrix. Therefore, both chemical–structural characteristics of the silica xerogel and chemical interactions between the gel and embedded molecules strongly affect the drug release behaviour.

Keeping this in mind, for example, a heparin release silica xerogels system, whose properties could be modified and the release adjusted according to therapeutic needs, was defined. Tailoring the gel microstructure has led to diffusive (fittable with Higuchi model) or zero order release kinetics by varying the combination of the two parameters: molecular weight of heparin embedded and surface area of the silica matrix (**Figure 5C**) [146].

A step forward in the design of drug release systems is the modification of the pore surface [2], aimed either to increase the drug–surface interaction or to impede the drug transport out of the matrix by functionalisation with hydrophobic species that disfavour the aqueous medium penetration inside the pores [147].

The possibility of tailoring the silica gel–drug affinity factor by using calcium ions has been recently developed [148]. A new platinum(II)-bisphosphonate complex was embedded into two different silica-based polymers to obtain biocompatible hybrid materials to be used for the local treatment of bone tumours. This investigation was aimed at controlling the release properties of the hybrid material by changing the inorganic network composition, either pure silica or Ca^{2+}-added silica. The presence of calcium in the matrix was found to reduce its loading capacity but to improve its stability upon storage, a property that is fundamental for practical applications. In addition, the presence of calcium affected the nature of the platinum complex released in the slow diffusion controlled process following the initial burst. When present, Ca^{2+} was able to retain the bisphosphonic ligand so that only the platinum-ethylen-diammine residue was released from the xerogel. In contrast, in the absence of calcium, the platinum complex was released in its original dinuclear form with bridging bisphosphonate. Hopefully, the platinum complex concentration will be sufficient to exert therapeutic activity only at the site of the implant, while it will be too low to exert undesired toxic effects on the neighbouring tissues and at a systemic level (**Figure 5A, B**) [148].

However, the idea to control the kinetic release of the drug through external stimuli is a recent, fascinating and ambitious possibility, which opens a wide field of possibilities for long-term therapies and represents an almost biomimetic strategy of administering bioactive molecules that are released at and when they are needed. Thermo-responsive mesoporous materials have been developed as hybrid systems that combine the silica inorganic phase with thermally active polymers, such as poly(*N*isopropylacrylamide) (PNIPAm), to produce sponge-like phases [149]. Magnetic field responsive mesoporous materials have been developed by capping their pores with magnetic Fe_3O_4 nanoparticles [150]. Mesopore entrances can also be modified by other types of organic functionalities to develop supramolecular mesoporous materials that respond to several chemical signals [151].

Furthermore, many delivery systems, such as those for highly toxic antitumour drugs, should require both a site-selective tool and a zero release before the targeted cells or tissues are reached. In other words, an important prerequisite for designing an efficient delivery system is the ability to transport the desired guest molecules to the targeted site and release the cargo in a controlled manner. In the case of mesoporous silica particles [152], the ideal delivery system should present a site-directing capability in order to be attracted to the specific site of interest, for example by a magnetic field, a perfect capping of all of the pore openings, in order to achieve a 'zero premature release' and contemporary a stimuli-responsive controlled release property [153].

7. Conclusion

Advanced implantable bio-mimetic biomaterials are expected to interface and integrate well with biological tissues, frequently to be bio-resorbable, in order to be completely replaced by new formed tissue, but always have to be bioactive, inducing a specific cellular response. This goal can be reached either through the biomaterial surface functionalisation or by the controlled release of biologically active molecules.

The biomimetic approach represents an important tool for the design of innovative, nonviable materials that temporarily or permanently provide a part of the body. Through their similarities with biological tissues, these

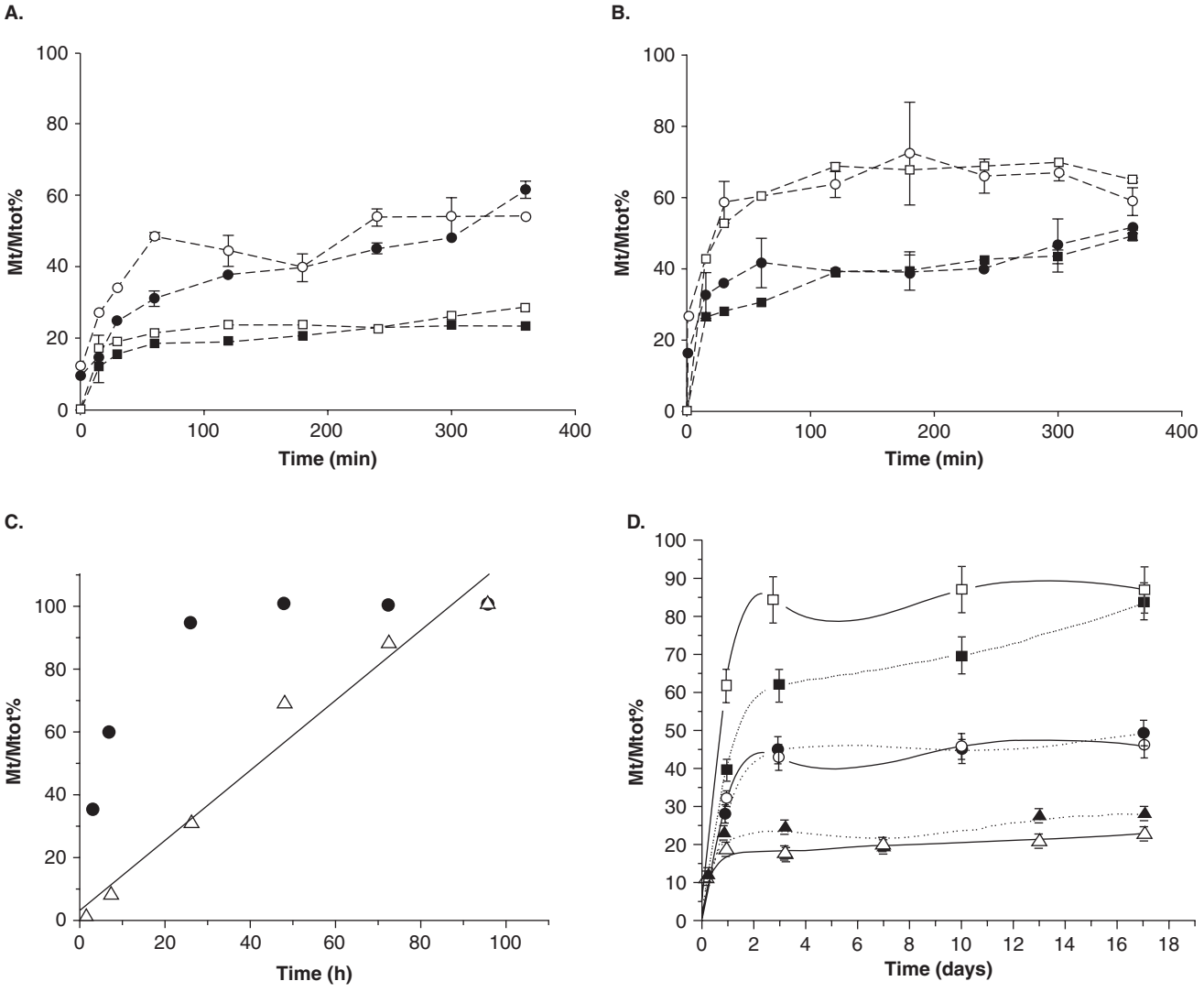

Figure 5. The role of biomimetism in controlling the release behaviour from different biomimetic nanostructured matrices. The possibility of establishing the delivery timescale, which ranges in these cases from minutes (**A, B**) to hours (**C**), to days (**D**) is shown. The possibility of modulating the release trend (which can be either diffusive, linear, burst release, or zero-premature release characterised) is represented. **A** and **B**. Kinetics of a platinum dinuclear complex containing a geminal bisphosphonate release from silica xerogels (**A**) and from calcium containing silica xerogel (**B**). The formulations contain Pt : Si ratio of ca. 1.5×10^{-4} (full symbols ●, ■) and 3.0×10^{-4} (open symbols □, ○) as measured one day after xerogel preparation (circles ●, ○) and 30 days after preparation (squares ■, □). (From [148]. Reproduced by kind permission of The Royal Society of Chemistry). **C.** Mass percentage of not fractioned heparin released from silica xerogels obtained by using higher (filled circles ●) or lower (empty triangles ▽) catalyst/tetraethoxysilane molar ratio. **D.** Released mass percentages normalised with respect to the hydroxyapatite (HA) surface areas of cisplatin (circles ●, ○), platinum(II) di(ethylendiamineplatinum)medronate (□, ■ squares), and alendronate (triangles ▽, ▼) as a function of time for plate-shaped (full symbols ●, ▼, ■) and needle-shaped (empty symbols ○, ▽, □) HA nanoparticles. (From [3]. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission).

biomaterials not only improve the interface with natural tissues, being more acceptable in the biological environment, but can also utilise these peculiarities, offering innovative opportunities as drug delivery systems.

In engineering implantable materials for hard tissues replacement, inorganic materials appear particularly suitable considering their mechanical behaviour. Among these, synthetic biomimetic nanostructured apatite and silica are leading to the development of prostheses and protective coatings which can contemporarily act as delivery agents with the double function of releasing biological active molecules *in situ* and controlling kinetics.

The modulation of the biomimetic characteristics of synthetic apatite such as crystal dimensions and morphology, and of silica such as network organisation and pore dimensions, is closely related to the drug delivery efficiency and can also

affect the therapeutic agents' release profile. The possibility of establishing the delivery timescale (which can range from minutes to days), and the possibility of modulating the release trend (which can be either diffusive, linear, burst release characterised, or zero-premature release characterised) can be achieved by modulating the above features.

A step forward is the functionalisation of nanostructured apatite and silica, linking agents able to anchor biologically active molecules that can be released by breaking the linkage, in order to prepare innovative systems delivering biologically active molecules with a release profile tailored for specific therapeutic applications and controlled by the response to a physiological or pathological process induced stimulus.

8. Expert opinion

The biomimetism of inorganic polymeric and composite biomaterials could be carried out at different levels: composition, structure, morphology and surface reactivity. The aim of a researcher is to realise a biomaterial that is biomimetic in all these characteristics in order; not only to optimise the interaction of synthetic materials with biological materials but also, and more ambitiously, to mimic the biogenic materials in its functionality. This concept should be utilised in designing and preparing synthetic inorganic and polymeric biomaterials in replacing hard and soft tissues respectively. Nanotechnology has great potential in the biomimetism field to do just that: increase in efficacy by orders of magnitude. In fact, the nano-size of biological tissues' building blocks is one of the bases of their self-organisational ability and one that needs to be replaced by synthetic materials in the synthesis of structured architectures with controlled organisation on a multiple-length scale.

In order to improve their functionality when used as implant devices, the above biomaterials can be functionalised in order to release drugs, growth factors, enzymes, nanoparticles and general bioactive agents into the surrounding environment, lowering systemic toxicity. In biomaterials with a drug delivery function field, nanostructured materials offer a much improved performance over their larger particle sized counterparts, due to their large surface to volume ratio and unusual chemical/electronic synergistic effects. The role of some of these features (porosity and surface area) has been clarified in the literature, while some parameters linked to other surface characteristics have still to be evaluated (surface electrification, hydrophobicity, hydrophilicity).

Biomimetic structural surface features of the nanostructured matrices are strongly connected with the loading and release of biologically active agents. In the case of nano-dimensioned matrices, some of the biomimetic characteristics of such biomaterials – that is surface area due to the nanometric scale and structural and compositional surface disorder – need to be moulded in order to tailor the release of bioactive molecules with the aim of obtaining a controlled kinetic. Moreover, in the case of nanostructured porous materials, the nanoporosity allows the absorption of a consistent amount of bioactive molecules, while macroporosity can be utilised for cell growth, transforming these materials into a real cellular scaffold for tissue engineering.

An ideal biomaterial with drug delivery function should be able to target these biological agents towards specific organism sites without compromising their efficacy. Moreover, it should be able to release the biologically active agents with kinetics that can be controlled by the matrices' structural characteristic modifications or by means of either a physiological or chemical trigger. In this way, researchers could obtain a biomimetic release where the kinetic release of bioactive molecules is controlled by physiological–pathological factors, such as variation of pH, ionic force, and enzyme and protein activity.

Therefore we could obtain a 'stimuli-responsive' nano-device system, in which the stimulus is represented by a change induced by a biological, pathological process (inflammation, infection, metastasis, etc.). Nowadays, many 'smart materials' result from the assembly of different components into a single system: one material acts as a sensor; another one as an actuator; the third one is the processor. However, it is possible to design a unique material integrating all of these functions.

In consideration of the above, nanotechnology coupled with a biomimetic approach offers a unique, innovative strategy to overcome the shortcomings of many conventional drug delivery materials. From nanomedicine to nanofabrics, this promising technology has encompassed almost all disciplines of human life. In the case of biomaterials science, it offers the double advantage of mimicking nature and improving material performance.

Declaration of interest

The authors state no conflict of interest and have received no payment in the preparation of this manuscript.

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•• **Excellent review on the recent research progress on the design of functional mesoporous silica materials for stimuli-responsive controlled release delivery of pharmaceutical drugs, genes and other chemicals.**

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