Hierarchical biomineralization: from nature's design to synthetic materials for regenerative medicine and dentistry

Sherif Elsharkawy[1,2,3] & Alvaro Mata[1,2*]

[1]Institute of Bioengineering, Queen Mary University of London, London E1 4NS, United Kingdom. [2]School of Engineering and Materials Science, Queen Mary University of London, London E1 4NS, United Kingdom. [3]Institute of Dentistry, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London E1 4NS, United Kingdom. *e-mail: a.mata@qmul.ac.uk

Abstract

Biomineralization is a highly dynamic, yet controlled, process that many living creatures employ to develop functional tissues such as tooth enamel, bone, and others. A major goal in materials science is to create bioinspired functional structures based on the precise organization of building-blocks across multiple length-scales. Therefore, learning how nature has evolved to use biomineralization could inspire new ways to design and develop synthetic hierarchical materials with enhanced functionality. Towards this goal, we dissect the current understanding of structure-function relationships of dental enamel and bone from a materials science perspective and discuss a wide range of synthetic technologies that aim to recreate their hierarchical organization and functionality. We also provide insights into how these strategies could be applied for regenerative medicine and dentistry.

I. Introduction

Nature is rich with examples of sophisticated materials displaying outstanding properties that emerge from their specific hierarchical structure[1]. Millions of years of evolution have allowed biological structures to not only optimize performance, but also provide astonishing solutions to address structural and functional problems. Therefore, a deep understanding of such natural materials would provide invaluable insight to design new ways to generate advanced synthetic materials[2]. Tissues such as dental enamel, bone, dentin, and nacre possess distinct structural organization at different length-scales, which enhances their bulk material properties and functionality[3]. The capacity to create synthetic structures that emulate such ingenious architectures represents a major goal in materials science and an opportunity to profoundly improve functionality[4]. In particular, the field of biomaterials would greatly benefit from the functionalities that can emerge from well-defined hierarchical organizations[5]. Therefore, many research groups have attempted to develop hierarchical structures with a great variety of highly organized multiscale microstructures[6, 7], which may lead to potential advanced healthcare applications.

II. Biomineralization in nature

Biomineralization, the process by which minerals are formed by living organisms under strict biological control, is responsible for the well-defined structure and subsequent function of mineralized tissues[8]. This process is based on a highly dynamic environment regulated by an organic matrix that nucleates and directs the hierarchical growth and morphogenesis of mineralized tissue[8]. The charge[9], conformation[10], supramolecular assembly[11], and post-translational cross-linking[12] of specific macromolecules of the organic matrix play key multifunctional roles during the biomineralization process. For example, negatively-charged domains in non-collagenous[13] and non-amelogenin[10] proteins are known to stabilise crystal

nucleation while the degree of collagen cross-linking in bone is known to affect its mineral density, microarchitecture, and stiffness[14]. The resultant mineralized tissue is species-specific and performs appropriately according to their functional needs whether protection, structural, navigation, vision, or even reproduction[15]. The next section describes how nature produces some of the most complex and functional hierarchical materials and aims to identify key structural features and bioprocessing steps that may help in the design of new synthetic materials.

Dental enamel

1. Structure

Dental enamel is a highly inorganic non-vital structure that has no cellular regeneration. Unlike dentin and bone, enamel loses its forming cells (ameloblasts) just after its matrix formation and maturation. Dental enamel forms the hard cover of the crowns of teeth, varying in thickness from about 2.5 mm over the cusps to a tapering edge at the cemento-enamel junction. It is the most mineralized hard tissue in the human body consisting about 96-98% by weight of apatitic calcium phosphate, which is carbonated hydroxyapatite (HAp) and the remainder is about 3% non-amelogenin proteins and 1% water [16]. Enamel is a hierarchical structure with different length-scales ranging from atomic or molecular scale to nanometre, micrometre, and up to macroscopic scale (Figure 1). The chemical structure of HAp with different substitutions in the lattice varies according to location. At the microscale, enamel consists of enamel prisms (also known as enamel rods), each of them about 5 μm in diameter containing a few thousand nanocrystals. The predominant pattern of the human enamel prism is the keyhole shape, where the core of the prism is wider than its tail[16].

Since enamel is highly inorganic with almost no organic matrix, it can lose almost all its ionic content when it dissolves during acidic attacks leading to dental caries or erosion. It is well-known that salivary secretions, which protect the oral cavity, play a critical role in maintaining enamel integrity by providing an appropriate ionic content to remineralize. On the other hand, the absence of an organic scaffold, renders enamel regeneration very challenging to occur naturally. Therefore, the creation of enamel-like substitutes is considered a major goal in materials science, tissue engineering, and regenerative medicine.

Apatite crystals possess hexagonal crystallographic symmetry with a space group known as ($P6_3/m$), where the 6-fold *c*- axis is perpendicular to 3-fold *a*- axes at 120˚. Apatites are flexible structures with wide range of substitutions that can happen within their lattice at both cation and anion positions. They have a general formula of $A_{10}(BO_n)_6X_2$; where A is a divalent cation like Ca^{2+}, Sr^{2+}, Ba^{2+}, and Pb^{2+}, while the anionic complexes BO_n = $(PO_4)^{3-}$, $(AsO_4)^{3-}$, $(VO_4)^{3-}$ or $(CO_3)^{-2}$, and X= OH^-, F^- or Cl^-. Hence this chemical variability can lead to tailored properties for various applications[17]. For example, the enamel mineral (96% by weight) is formed of a stable apatitic calcium phosphate phase; hydroxyapatite (HAp), which is $(Ca,Z)_{10}(PO_4,Y)_6(OH,X)_2$; where Z could be Na^+, Mg^{2+}, Sr^{2+}, or K^+; Y could be $(CO_3)^{2-}$ or $(HPO_4)^-$, and also the hydroxyl group $(OH)^-$ could be substituted by F^- or Cl^-[18]. Robinson *et al.*[19, 20] were the first to determine the distribution of $(CO_3)^{-2}$, Ca^{2+}, $(PO_4)^{3-}$, Mg^{2+}, and protein within enamel quantitatively via micro-sampling and micro-analysis. They found that carbonate $(CO_3)^{2-}$ diminishes from the dentino-enamel junction (DEJ) to the enamel surface, due to the reduction of metabolic activity of ameloblasts when approaching the surface. This further leads to less CO_2 production, consequently generating less incorporation of carbonate near the surface. Similarly, Ca^{2+} and $(PO_4)^{3-}$ contents increase from the DEJ towards the surface of enamel, reflecting the rate of enamel formation. This increase could be explained further by

the slowing down of ameloblasts near the surface, as more ions can be released in this location. The fluoride content of enamel is also higher near the surface of enamel compared to its deeper portion due to the higher fluoride uptake at the surface as soon as the tooth erupts and contacts the oral environment. This provides the surface of enamel with superb anti-cariogenicity and low solubility properties[21]. On the other hand, enamel proteins exhibit an opposite trend to that of the ions mentioned above, where high concentrations of proteins are to be found beneath the fissures and towards the DEJ. Furthermore, organic material and magnesium ions have been found to be entrapped within the core of the apatite crystals, which can greatly affect the solubility of the crystals[20]. At this scale, enamel is composed of HAp crystals with dimensions of 70 nm in width and 25 nm in thickness, and with lengths that can extend across the full width of enamel[22].

At the micrometre scale, enamel is composed of prismatic and interprismatic microstructures extending from the deepest portions near the DEJ up to the enamel surface. These microstructures reflect the secretory territories of ameloblasts[16]. Each enamel prism is about 5 μm in diameter and contains a few thousand nanocrystals. The nanocrystals are more likely to be perpendicular to the surface they grow from, hence their parallel orientation at the core of the enamel prism. The orientation of the nanocrystals changes when moving towards interprismatic areas of about 40˚-60˚. Due to this difference in orientation, the nanocrystals form areas of discontinuity at the edge of the enamel prisms. This area is called the prism boundary and is known to contain a higher amount of organic material[23]. Previous attempts have been done in order to track the direction and arrangement of enamel prisms and to understand their packing from the DEJ towards the surface of enamel. The techniques used include sectioning[24], serial photographing of sections[25], x-ray diffraction[26], graphical computer modelling[27], combined computer modelling with scanning electron microscopy

(SEM)[28], and synchrotron x-ray microtomography[29]. For example, Raue *et al.*[30] used synchrotron x-ray diffraction to understand the orientation of nanocrystals through texture analysis, which in turn provides insights into the orientation of the enamel prisms. Moreover, Hunter-Shreger band (HSB) is a group of 10-13 enamel prisms, which causes an optical phenomenon due to the difference of the directionality of the enamel prisms. HSBs render dental enamel more resistant against wear and fracture, therefore dentition has evolved by increasing the packing of HSB[31]. In addition, the micrometre length-scale of enamel may provide insight into the life history and the directionality of ameloblasts; therefore enhancing our understanding of enamel biomineralization, evolution, and biomechanical properties (i.e. anti-abrasiveness).

2. Structure-function

Characterization of the structure of enamel has been critical to investigate its mechanical properties across multiple length-scales and to understand its functional need and behaviour. The degree of mineralization, crystallographic texture, and different substitutions within the nanocrystal lattice have enabled enamel to perform according to its needs at different specific locations[32]. In this regard, Cuy et *al.*[32] studied the hardness (H) and the elastic modulus (E) of dental enamel across the axial cross-sections in the maxillary 2nd and 3rd molars by using nanoindentation at high spatial resolution (~ 1 μm). They found that both E and H properties decrease from the surface going down towards the DEJ. Interestingly, the palatal cusp (functional cusp) of the upper molar tooth was found to possess the highest H and E in order to suit its functional need as a functional cusp in maxillary teeth (Figure 2a). Recently, the changes in the lattice parameter at the crystallographic scale has been studied by using synchrotron x-ray diffraction, which allows to investigate structural changes as function of

location[33]. Structural variation in *a*- lattice is more significant than *c*- lattice from the enamel surface towards the DEJ by an average value of -0.6% and +0.3%, respectively. This observation reflects the spatial control of biomineralization to achieve its distinctive mechanical properties in order to oppose masticatory forces. As mentioned above, dental enamel comprises of aligned elongated nanocrystals[22]. The nanocrystals' preferred orientation was measured by synchrotron x-ray diffraction (Figure 2b), where the nanocrystals' texture is perpendicular to the DEJ[33]. The magnitude of the crystal texture is also observed to be less intense (corresponding to a more random organization) at the external surface of the teeth. A similar trend is observed in deeper portions of the dental enamel near the dentinal horns. Moreover, it is found that the nanocrystals situated at the functional cusps (i.e. palatal cusps of upper molar teeth) possess high crystal texture, which are perpendicular to the surface of the tooth. This observation would further explain the importance of the preferred orientation at this lengthscale in order to allow the teeth to withstand the high masticatory forces applied during function[33, 34]. Therefore, it is believed that the spatial variations observed on the mechanical properties of enamel are correlated with its chemical composition and microstructure.

In order to compare E and H between prismatic and interprismatic areas, Habelitz *et al.*[35] combined AFM with nanoindentation with spatial resolution just beyond 500 nm. Both properties were found to be lower at the tail of the prism compared to the body of the prism. This could be further explained by the different packing and organization of the nanocrystals at these locations, as well as a higher protein content within the prism boundaries. Moreover, the strength of dental enamel can reach the theoretical strength of pure apatite thanks to the small thickness of enamel nanocrystals[36]. In addition, enamel is 3 times tougher than geologic apatite due to the biological protein component of enamel along with the high degree of structural anisotropy. This anisotropy protects enamel by redirecting functional stresses into

the resilient underlying dentin. The organic remnants of enamel play a significant role (i.e. plasticizing effect) in preventing catastrophic fractures by spreading the load laterally over larger areas instead of focusing and advancing the damage straight through the enamel[37].

Dental hard tissue destruction is primarily caused by the acid produced during dental caries and dental erosion either from acids produced during the metabolic activity of cariogenic bacteria or from dietary sources, respectively[38]. Lippert *et al.*[39], studied extensively the E and H of surface enamel during demineralization and remineralization using AFM-based nanoindentation. The group demonstrated a significant decrease in both E (From ~110 GPa down to ~70 GPa) and H (from ~4.5 GPa down to ~1 GPa) of dental enamel when exposed to acid attack for 2 minutes. On the other hand, they also found a slight re-stiffening/re-hardening of the surface of enamel after remineralization for 4 hours.

Moreover, enamel is an anisotropic tissue, which facilitates the dissipation of masticatory forces and optimises its compressive and tensile strengths in the necessary directions[40]. Studies have reported that cracks induced by microindentations parallel to the long axes of the enamel prisms are longer than those developed from microindentations taking place perpendicular to the long axes of the prisms[41, 42]. It is assumed that cracks can travel easier along the prism direction rather than across the prisms (semi-circles) (Figure 2c-d). In addition, HSBs are not only important for evolutionary needs (being species-specific), but are also found to increase the durability and lifespan of dental enamel in order to better withstand masticatory forces[31, 43].

Bone

1. Structure

Bone is a well-characterised hierarchical tissue[44] (Figure 3). The basic building block of bone is a hybrid organic-inorganic material based on hydrated mineralized collagen (type I) fibrils (around 80 – 100 nm in diameter). The chemical composition of bone consists of about 62% of the inorganic mineral, whereas the rest 38% is mainly organic material and water. The organic component is mainly collagen type I, which represents about 90% by weight of the total protein in bone while the other 10% by weight are non-collagenous proteins (NCPs)[45]. The bone's collagen fibres are organized in a triple helix conformation within which crystals made of carbonated apatite (Ca_5 (PO_4, CO_3)$_3$(OH)) are found[2, 46]. These crystals are plate-like in morphology with uniform length and width of ~35 nm and 1.5 nm, respectively[47]. In addition, the crystallographic unit cell dimensions of bone apatite are about 9.47 and 6.80 angstroms for *a*- and *c*- axes, respectively[48]. These crystals are plate-like in morphology with uniform length and width of ~35 nm and 1.5 nm, respectively[47]. On the other hand, non-biological synthetic apatite is hexagonal in shape and does not acquire the plate-like crystal morphology, which suggests that bone apatite forms from a plate-like crystalline precursor. This precursor may potentially be the plate-like octacalcium phosphate[49]. However, recent evidence suggests the phase transformation from amorphous calcium phosphate (ACP) can proceed directly into apatite[50]. Non collagenous proteins found in bone such as osteopontin are well-known to stabilize ACP during phase transformation[51]. The mineralized collagen fibrils are hierarchically organized further into multiple length scales forming the overall structure of bone. First, the crystallographic *c*- axes are well aligned parallel with the fibril long axis in a layered arrangement[52]. Furthermore, the mineralized collagen fibrils are grouped into bundles (or lamellae) of about 3–7 μm in diameter[45]. These bundles are organized further into patterns/arrays aligned along their long axes[53]. These arrays could be parallel[54], cylindrical

(osteons)[55], woven[56], or plywood-like[57] (Figure 3e). Osteons are about 200–300 μm in diameter and a few millimetres in length, which are aligned along the long axis of the bone[45]. The plywood-like structure is one the interesting geometrical features that is found not only in bone, but in many other natural structures[57]. For example, plywood-like structures can also be acquired by asymmetrical elongated molecules in the form of liquid crystals in concentrated solutions[58]. For instance, polypeptides[59], polysaccharides[60], and DNA[61] are able to form liquid crystalline structures within their phase diagrams[62]. This self-assembly process can be tuned based on different factors including concentration[58], temperature[63], pH[64], and ionic interactions[63]. In addition, this liquid crystalline phase is anisotropic in nature, where molecular crosslinks can further stabilise the structures[65]. Interestingly, essential cellular and tissue morphogenesis can be generated as a result of a similar self-assembly process[66]. For instance, osteonal bone comprises successive layers of parallel mineralized fibrils arranged in a regular angle of 30° (in average) from one layer to the next[67], which has great mechanical implications for the overall tissue[53] and represents an attractive strategy for materials design.

2. Structure-function

The hierarchical organization of bone crystals plays a major role in the structure-function relationship of bone (Figures 4-5). Every lengthscale is known to be involved in the tissues' ultimate mechanical performance. The apatite phase of bone is stiff and strong but brittle, although the collagen is soft but highly deformable[45]. The role of water in bone is not entirely understood; nevertheless, it is thought to act as a plasticizer that contributes to the overall toughness of the tissue[45]. Tertuliano and Greer[68] revealed the presence of strength transition in bone from plastic deformation into brittle failure. This could be further explained by the presence of both ordered crystalline (plastic) and disordered amorphous (brittle) phases in bone. In addition, Fantner *et al.*[69] investigated the nanoscale structural organization of bone

including its constituents and corresponding interactions. They discovered the presence of a non-fibrillar organic matrix based on non-collagenous proteins (NCP) mainly composed of osteopontin (OPN), which acts as a glue that holds the mineralized fibrils together. Recently, Cavalier *et al.*[70] discovered an important mechanism suggesting that OPN crosslinking enhances the interfacial organic-inorganic adhesion, hence increases the fracture toughness of bone. The effectiveness of this mechanism increases with the presence of Ca^{2+} ions. Therefore, these calcium-mediated bonds within the organic matrix also contributes significantly to the ultimate mechanical properties of the tissue as confirmed by NMR[71]. This new mechanism is based on energy-dissipation from the nanomechanical heterogeneity, which can further offer a means for ductility enhancement, damage evolution, and toughening. Furthermore, Tai *et al.*[72] quantified the spatial distribution of the heterogenous nanomechanical properties of bone and demonstrated the presence of distinct stiffness patterns within the tissue ranging between ~2 and 30 GPa. Interestingly, these patterns do not correlate with topographical features but are instead attributed to the underlying local structural and compositional variations within the tissue. At the macroscale, Liebi *et al.*[73] observed high degrees of orientation of mineralized fibril domains of several tens of micrometres in diameter at areas with higher curvature (i.e. articulating surfaces), where the collagen fibrils follow closely the trabecular bone microstructure. This finding further confirms how nanostructure can dictate structure-function relationships.

The concentric arrangement of osteonal bone renders the growth of cracks, not to be trivial and tends to follow a zig-zag path[74]. In order to provide insights on the mechanisms associated with bone fracture, it is necessary to investigate how crack propagation interacts with the bone microstructure[75]. Towards this goal, Nalla *et al.*[75] found that the plane of the crack and the crack front are parallel to the long axis of the osteons. The path taken by the growing crack is

not influenced neither by the central region of region of osteon known as harversian canal nor by their concentric lamellar rings. Crack deviation/bridging and microcracking are possible toughening mechanisms that can hinder crack propagation. For example, in bone, the formation of micro-cracks around the main crack consumes a high amount of energy and therefore increases the energy needed to advance the main crack forward. However, further accumulation of these micro-cracks can also contribute to fatigue fractures at later stages. Furthermore, when bone is exposed to tensile forces, shear deformation is dissipated as a result of the intimate co-alignment and interaction between the collagen and the mineral. On the other hand, Tai *et al.*[76] suggested that cohesion originates from within the organic matrix itself, rather than collagen-mineral interaction, and that bone strength is mainly related to nanogranular friction between the mineral itself.

III. Biomimetics and synthetic biomineralization platforms

Nature has developed materials/tissues with high performance and functional design[77]. Biomimetics, a word derived from the Greek word '*biomimesis*' (imitating '*mimesis*' life '*bio*'), is a field that focuses on learning from natural processes to develop synthetic materials. Otto Schmitt proposed this concept using a physical device that recreated the synapses and impulses present in different marine creatures[78]. Schmitt believed that biophysics is not a subject of matter, but a perspective towards finding solutions to biological problems and learning from biologic processes[79].

Tissues such as bone and nacre have motivated the development of synthetic mineralizing materials[80]. For example, several research groups have investigated ways to mineralize collagen intrafibrillarly in order to mimic the natural mineralization process of bone tissue [81]. Others have reported materials that resemble the hierarchical structure and chemical

composition of nacre using a β-chitin matrix[7] and layer-by-layer polyelectrolyte-clay dispersions[82]. A particularly inspiring challenge has been, and continues to be, the pursuit of approaches that can recreate the distinctive apatite composition, hierarchical architecture, and corresponding properties of enamel[35]. Towards this goal, Yamagishi *et al.*[83] and Chen *et al.*[84] have developed inorganic chemical methods to grow aligned enamel-like apatite nanocrystals on dental enamel. However, approaches based on organic matrices offer the possibility to guide mineralization through biomimetic routes based on tuneable organic-inorganic interactions[85]. Pioneering work by Moradian-Oldak *et al.* using amelogenin[86] and Kniep *et al.* using gelatin[87] has enabled the growth of aligned apatite nanocrystals directly on enamel surface. Nonetheless, the development of organized apatite nanocrystals with the distinctive hierarchical order of enamel expanding from the crystallographic-, nano-, micro-, and macro-scale, is still an exciting, yet unattained, goal[86].

Hierarchical apatite structures

Ordered structures have gained a great interest within materials science and bioengineering. For example, Hu *et al.*[88] grew ordered HAp crystals displaying high stiffness, excellent bioactivity, and outstanding biocompatibility. Inspired by this study, Liu *et al.*[89] synthesised highly ordered aligned fluorapatite crystals on metallic substrates and investigated their effect on the cell adhesion, growth and mineralization. They found that ordered FAp crystals promote higher cellular attachment and stronger bonding to the substrate than disordered crystals. Furthermore, the FAp ordered crystals seemed to trigger an increase in the expression of bone mineralization markers, as well as accelerated osseointegration compared to metallic surfacess[90]. However, the mechanism behind this enhanced bioactivity of ordered crystals over disordered ones remain vague. Biomineralized tissues such as teeth and bones comprise of hierarchical structures that can dictate crucial mechanical, biological, and chemical

functions[91]. In particular, dental enamel offers a unique structure/function relation, which has not yet been recreated[35]. Failures in restorative dentistry including fractures, rocking of restorations, and marginal damage can lead to secondary caries of tooth enamel/dentin and therefore further loss of dental tissues. These clinical problems are mainly due to the physical mismatch between artificial dental materials (isotropic in nature) and the dental hard tissues (anisotropic in nature)[92]. The design of biomimetic materials that can recreate the complexity and functionality of tissues such as enamel will require a multidisciplinary approach that integrates a fundamental understanding of the structure-function relationships observed in nature with new ways to engineer and grow materials[93].

Towards this goal, Yin *et al.*[94] developed a wet chemical method to synthesise the prism-like structure of enamel. The group succeeded in creating a similar morphology but the dimensions of the synthesised crystals were found to be larger than those of natural human enamel. In addition, the authors employed a hydrothermal method (high temperature and pressure) to produce their synthetic structures, which renders limited clinical use. Recently, Elsharkawy and Al-Jawad[95] synthesised ordered fluorapatite crystals at near physiological conditions, the crystals have comparable size and morphology to those found in dental enamel with a similar keyhole enamel prism pattern. Other attempts to regenerate dental enamel encompassed the use of glycerine gelatine[96], self-assembling monolayers[97], agarose hydrogels[93], peptide amphiphiles with the Arg-Gly-Asp (-RGD) motif[98], amelogenin in presence of fluoride[99], and amelogenin-chitosan hydrogels[86]. Nevertheless, none of these previous attempts have successfully recreated the highly-organized mineralized apatite structure across multiple lengthscales. This further evidences not only the need for robust and functional materials for dental applications but also new strategies to design and engineer materials with this kind of complexity and functionality.

Synthetic strategies to mimic hierarchical biomineralization

1. Inorganic strategies

While synthetic inorganic strategies have not yet reproduced the structure of enamel or bone, they provide insight into important physico-chemical rules that could be used to generate hierarchically-organized biomimetic structures.

a- Non-crystallographic architectures

Oscillating precipitation in nature, whether temporal or spatial, can produce complex structures known as non-crystallographic architectures [6]. These architectures are arranged in a highly ordered fashion at the nanoscale, microscale, and up to the macroscale, generating sophisticated morphologies. Interestingly, these hierarchical minerals are formed in the absence of any organic scaffold. A chemical feedback process is highly thought to act as the driving force for the self-assembly process of non-crystallographic architectures[100]. This feedback process is dynamic and pH-dependent and can selectively precipitate one crystal phase over another present within the system on a pre-designed sequential order. The nanocrystals achieved by this method are formed by alkali-earth metal-carbonate minerals and silica within an alkaline media[101]. The sensitivity of silicate (acidic) and carbonate (basic) species to pH oscillations, gradients at the mineralizing front, along with their different solubility products (K_{sp}), is of central importance for such system. This phenomenon is known as pH-mineralization feedback, where silica can precipitate early on, inhibiting the metal-carbonate nucleation. Afterwards, upon silica precipitation, the pH fluctuates and further promotes the nucleation of the metal-carbonate. The pursuit for finding alternative reagent pairs can also be applied for this phenomena beyond those related to pH effects and precipitation[102]. Furthermore, the

formation of these geometries is also thought to be a result of osmotic pressure and the interplay between localized crystal growth and inhibition at the fluid–solid interface. Nonetheless, the exact mechanism of the non-crystallographic architectures remains elusive[101]. Garcia- Ruiz *et al.*[100, 102, 103] have reported a series of remarkable studies on various extraordinary morphologies including helices, curved sheets, and twisted ribbons of alkali–earth carbonate crystals formed in silica gel. In a similar manner, Terada *et al*.[104] reported non crystallographic morphologies that comprise self-assembled fibrous crystals individually enveloped with a nanoscale silicate sheath. They discovered that the nanoscale fibrous subunits originated from a spherulite and are essential for the formation of the non-crystallographic architectures including curved sheets, petal-like, and twisted morphologies (Figure 6a-b).

It is believed that this morphological evolution is caused by diffusion gradients and the instability of the growing surface in a non-equilibrium condition. For example, calcium carbonate hierarchical structures were successfully grown in alkaline silica solutions. These remarkable hierarchically structured morphologies comprise of self-assembled curly sheet-like structures made up from ordered aragonite (calcium carbonate) nanocrystals. Interestingly, these hierarchical structures mimic natural coral forms[105] (Figure 6c). Similarly, Noorduin *et al.*[6] used the diffusion of carbon dioxide (CO_2) in a solution of barium salt and silicate to develop carbonate-silica microstructures with a variety of hierarchical geometries. The system depends on CO_2 concentration, pH, and temperature in order to switch between different systems and create a selection of hierarchically assembled multiscale microstructures (Figure 6d-h).

b- Ice-templated hierarchical materials

Through a bioinspired approach, Bouville *et al.*[106] fabricated a hierarchical layered ceramic material with an outstanding combination of properties including high stiffness (290 GPa), strength (470 MPa), and toughness (22 $MPa/m^{1/2}$). This material design is inspired in the hierarchical organization of nacre, where closely packed sub-micrometre ceramic tablets attach to each other through defined ceramic bonds, generating a brick-and-mortar arrangement (Figure 7a). Interestingly, this group employed an ice-templating approach where they exploited and tuned the growth of ice crystals to assemble their ceramic tablets. Furthermore, they introduced a low stiffness phase at the surface of the ceramic tablets in order to ensure crack deflection and redistribution, resulting in a material with high toughness.

c- Magnetic field-controlled materials

Biological materials are anisotropic in nature in order to accomplish crucial functional requirements. These structures are mainly formed by the layer-by-layer methodology employed by living cells to construct biomimetic composite materials. In contrast, synthetic material analogues have traditionally lacked this attractive heterogeneous property. In this context, Studart and co-workers[107] demonstrated the capabilities of an additive manufacturing approach to produce synthetic composites capable of recreating the typical twisted-plywood hierarchical architectures found in teeth, bone, and seashells[107]. The method consists of a liquid that comprises iron oxide nanoparticles or alumina platelets, which passes into a porous scaffold through capillary forces, packing the particles within the walls (250 μm thick) of the scaffold. Subsequently, by applying a time-dependent magnetic field, the particles aligned anisotropically and in a controlled manner, resulting in enhanced mechanical properties[108]. Since typical reinforcing particles are often diamagnetic and require extremely high magnetic fields for alignment (1 Tesla), Erb *et al.*[108] used more responsive superparamagnetic

nanoparticles. Though this method, they reduced the magnetic field down to 0.8 mT, a value that is only an order of magnitude above the Earth's natural magnetic field (0.05 mT). Furthermore, through ultralow magnetic fields (1 to 10 mT) the group produced synthetic composites with tuneable three-dimensional orientation and distribution, wear resistant, and shape memory properties. In addition, Le Ferrant *et al.*[107] have unprecedentedly fabricated reinforced composites with high volume fractions of inorganic phase (up to 100%) in a ceramic, metal, or polymer functional matrix. They have generated proof-of-concept experiments that include bulk composites with periodic patterns of tuneable orientation and tooth-like structures with complex shapes exhibiting site-specific composition and texture (Figure 7b). However, the clinical performance of these materials remains unknown.

d- Bone-like steel

Fatigue is a major contributor to the failure of most biological mineralized tissues and engineered biomimetic structures. Inspired by the excellent fracture toughness of bone, Koyama *et al.*[109] successfully grew multiphase steel microstructures (i.e. martensite and austenite) that are hierarchical and similar to those of bone, but with superior crack resistance (Figure 8). Their outstanding mechanical properties emerge from the capability of tuning the phase structure, stability, and distribution of the material, resulting in a resistance of crack propagation at the microscale. The group found that this resistance emerges from a transformation induced crack termination and roughness-dependent toughening mechanisms. These results represent a significant leap forward for steels by, inspired in functional biological materials, improving the functionality of structures that are exposed to enormous cyclic loads.

e- Lightweight 3D hierarchical materials

Despite being lightweight and porous, several siliceous skeleton species including diatoms, sea sponges, and radiolarians have remarkably high strength when compared to synthetic materials of the same composition. In this context, Jang et *al.*[110] reported on the development of a multi-step nanofabrication process and the fabrication of 3D octahedral hollow metamaterials that possess rigid crystallographic periodically-arranged structures. These structures mimic the hierarchy of natural siliceous diatoms at multiple lengthscales. Furthermore, these metamaterials can attain exceptionally high strength that may offer a new class of damage-tolerant lightweight engineering materials[106].

f- Bio-templated hierarchical materials

There is great interest to develop hierarchical materials based on bio-templates that direct their growth.[111] This strategy is considered to be a bio-exploitation rather than bio-inspiration approach. This method is fairly simple, inexpensive, and opens new routes to use renewable resources with an implausible variety of complexities. A variety of biological templates such as DNA, proteins, microorganizms, pollens, bioskeletons, plants, insects, or even a full animal embryo have been utilised for this purpose[112]. For example, Bao *et al.*[113] converted 3D nanostructured silica diatom micro-assemblies into nanocrystalline silicon or silicon/magnesia composites at low temperature (Figure 9a-b). In a similar manner, Goodwin *et al.*[114] reported on the synthesis of 3D nanocrystalline iron oxide replicas of pollen microparticles, where these replicas can show outstanding ferromagnetic properties (Figure 9c-d). Similarly, wood is a highly sophisticated and hierarchical material. Therefore, Deshpande *et al.*[115] employed wood as a bio-template to cast hierarchical ceramic materials. This group successfully managed to cast cerium zirconia ceramics into the original fibrillar structure of wood at multiple lengthscales by replacing the hemicellulose/lignin matrix of wood with ceria/zirconia mixed

oxide ceramic, enabling nano-casting with high precision (Figure 9e-f). On the other hand, Kamata *et al.*[116] attempted to bio-template the spiral vessels in plants' stems to fabricate metal microcoils with efficient electromagnetic properties thanks to the sophisticated hierarchical organization of the natural spiral vessels (Figure 9g-h). Furthermore, a magnetic leaf skeleton was produced with iron carbide while replicating the microstructure of the leaf veins[117].

2. Organic strategies

a- Non-biologic polymers

Polycarboxylate diamino hydroxypropane tetraacetate (HPDTA)

Polycarboxylate diamino-hydroxypropane tetraacetate (HPDTA) is a ligand that acts as a chelating agent where its six binding groups, are capable to attach to a metal ion. This behaviour resembles that of hydroxyethyl-ethylenediaminetriacetic acid (HEDTA), which is a strong chelator that possesses four carboxylates and two nitrogen donors. However, the alcohol provides a nucleating site for mineralization by bridging between two Ca^{2+} ions. Mukkamala and Powell[118] mineralized calcium carbonate in the presence of HPDTA, which resulted in the formation of self-assembled 'microtrumpets' composed of nanocrystalline calcite. This study provides insight into the role of chelating agents on calcium carbonate mineralization. In this case, the nanocrystals evolved from a rhombohedral to a hexagonal morphology, which consequently builds up the hierarchical trumpet (Figure 10a-b).

Poly(styrene-alt-maleic acid) (PS-MA)

Xu *et al.*[119] reported the growth of hierarchical 3D calcium carbonate superstructures. The structures further exhibited pyramidal units with sharp facets and edges. The authors utilised poly(styrene-alt-maleic acid) (PS-MA) as a crystal growth modifier. Their mechanism of formation was based on the mesoscopic transformation of nanoparticles in the presence of the

polymer. Furthermore, the selective adsorption of PS-MA molecules seemed to play an important role in this mesoscale transformation up to the formation of the complex higher-order structures (Figure 10c-f). These superstructures provide insight into the significance of mesoscopic processes in biomineralization.

Poly(hydroxyethyl methacrylate) (pHEMA)/pHEMA methacrylamide (pHEMAm)

Bone development occurs through templated mineralization of apatite crystals within a protein scaffold, a process that can be mimicked using synthetic biomimetic hydrogel polymers. For instance, Bertozzi and co-workers[120, 121] conducted a series of studies based on pHEMA and pHEMAm, where they formed stable and robust 3D hydrogel copolymers crosslinked with either ester (EGDMA) or amide (EGDMAm). The anionic monomers present in the hydrogels can tune the overall polarity and number of negatively charged carboxylate groups, and hence control the distance between potential nucleation sites for binding calcium phosphates. The generated materials exhibited a bone-like morphology but both the mechanism and the levels of hierarchical organization are not yet well understood (Figure 12p-r).

Polyacrylic acid (PAA)

Mao *et al.*[7] developed a synthetic nacre that possesses striking resemblance to natural nacre. Their synthetic system is hierarchically organized, where each mineral layer is made up of aragonite platelets (2-4 μm thick) similar to the microstructure of natural nacre. The overall thickness of the bulk synthetic nacre is about 1-2 mm. Their materials demonstrated outstanding ultimate strength and fracture toughness, yet still well below those of natural nacre. The group's strategy was to employ a β-chitin scaffold to be mineralized in a peristaltic pump-driven circulatory system in the presence of polyacrylic acid (PAA). Initially, carboxyl groups

act as nucleation sites for $CaCO_3$ selectively, then the crystals grow laterally to form a boundary. Subsequently, as the matrix gradually mineralizes, the organic material is pushed between the aragonite layers (Figure 10g-n).

Polymethylmethacrylate (PMMA)

Recently, Bai *et al.*[122] developed a bidirectional freezing technique capable of assembling HAp into a centimetre-scale with long range ordered structures that resemble that of natural nacre in morphology. However these structures did not resemble this tissue in terms of its chemical composition nor its hierarchical structure. This group fabricated their scaffolds by sandwiching polydimethylsiloxane (PDMS) layers, creating gaps where the HAp could grow into the pre-designed mould. Subsequently, they introduced PMMA and densified further their composites in order to not only increase the weight fraction of the mineral but also to increase the E of their material to about 20 GPa. In addition, the composite HAp/PMMA demonstrated a toughening mechanism that could inhibit catastrophic fracture as a result of its predesigned architecture.

Poly-hydroxyethylacrylate (l) triethylene glycol dimethacrylate (PHEA-l-TEG)

Lately, Rauner *et al.*[123] reported novel organic-inorganic composites, where amorphous calcium phosphate (ACP) nanostructures grew within a polymer hydrogel in a homogenous manner. They exploited the use of a biocompatible polymer based on (PHEA-l-TEG), where the mineralization process can take place via an enzyme-induced mechanism (alkaline phosphatase). Their mineralized materials showed exceptional fracture toughness above all other water swollen synthetic materials reported in the literature. Therefore, these materials may find applications to generate biomedical implants with tuneable mechanical properties.

b- Biological polymers

Chirality and liquid crystal templating

Chirality can be found in various natural structures including DNA[124], phages[125], polysaccharides[126], and proteins[58]. Chirality is responsible for many of the unique properties of various hierarchically-ordered structures. In nature, helical molecules such as collagen, chitin, and cellulose have the capabilities to self-template and to produce non-equilibrium structures. For example, collagen type I, can form either transparent tissues from orthogonally aligned fibres (i.e. cornea) or colourful tissues from cholesteric phase fibre bundles (i.e. skin)[125]. In addition, the outstanding colourful exoskeletons of beetles arise from the chiral organization of chitin [126]. Therefore, hierarchical functional structures can be templated using the self-assembly of chiral molecules that generate lyotropic liquid crystals (LC)[127, 128].

DNA

DNA is one of the most striking chiral biomolecules, it can self-assemble into multiple liquid-crystal phases, including blue phases, chiral cholesteric phases, and 2D columnar phases[124]. Liu *et al.*[111, 129] were inspired by the chiral capabilities of DNA, where they discovered a novel method to self-assemble 2D silica-DNA platelets hierarchically. Furthermore, they employed a top-down lithographic technique in addition to the bottom-up assembly of silica-DNA platelets, in order to selectively control the placement and arrangement of the mineral (Figure 11a-b). Interestingly, these materials could find various applications attempting to generate hard templates for the fabrication of various hierarchical oriented inorganic structures.

Phages

Bacteriophages are well-known for their chirality, monodispercity, helical nanofibrous shape, and their capacity to exhibit several functional domains. They also act as model for liquid crystal systems[130]. Hence, Chung *et al.*[125] self-templated a chiral phage system into functional materials, where long range order, helical twist, and several levels of hierarchical organization can be achieved. For example, the phage films were mineralized in a supersaturated solution in respect to apatite, resulting in organic–inorganic hybrids that mimic to some extent tooth enamel.

Polysaccharides

By taking advantage of a chiral cholesteric liquid crystal of cellulose as a template, Shopsowitz *et al.*[128] developed a hierarchical silica-based material. These hierarchical films exhibited outstanding tuneable photonic properties depending on the porosity at the mesoscale along with the long-range chiral ordering. These materials will open opportunities in developing new classes of biosensors, lasers, and advanced displays. Similarly, Sugawara *et al.*[131] fabricated thin films of chitosan that serve as substrates to guide the nucleation and crystal growth of calcium carbonate ($CaCO_3$) in an organized fashion. The mineral grew in a radial pattern where the crystals were about 20 nm in size. In addition, they investigated the synergistic effect of polyaspartic acid and chitosan where the addition of polyaspartic acid triggered the formation of another rhythmic orientation parallel to that of the radial pattern of calcium carbonate disks. A similar diffusion approach was employed by Manjubala *et al.*[132] using chitosan scaffolds in order to generate biomimetic apatite structures. The authors observed entangled apatite crystals that were not only formed on the surface of the scaffold but also in the bulk of the porous scaffolds.

Proteins, polypeptides, and amino-acids

Collagen matrix in bone self-assemble to generate lyotropic liquid crystals (LC) that are highly crucial for bone formation[58] (Figure 12). Inspired by collagen, He *et al.*[133] utilised an amphiphilic triblock copolymer that can self-assemble and exhibit LC behaviour. Subsequently, the authors UV crosslinked the LCs in order to utilized the physical properties of their scaffolds. Then, they mineralized the scaffolds via ACP that later transforms into apatite crystals. The generated apatite crystals are stable nanocomposites and mimic the nanostructure of bone. Similarly, Bellomo and Deming[134] used a water soluble poly-lysine that self-assembles and in turn templates amorphous silica into hierarchical silica-polypeptide structures (Figure 11c-d). Likewise, peptides and proteins containing phosphoserines (Ser(P)) are well-known to play a major role in controlling the nucleation and morphology of biominerals[135]. In that context, Sugawara *et al.*[127] studied the effects of Ser(P) containing polypeptides on the crystallisation of $CaCO_3$. During mineralization, they used a copolymer based on phosphoserines and aspartic acid copoly[Ser(P)-Asp], where unique spiral mineralized structures were developed in the presence of the co-polymer. Furthermore, they elegantly studied the effect of the chirality of the copolymers on the mineralization. Interestingly, when an L-copolymer was used, a clockwise twisted spiral morphology was formed, while the D-copolymer induced the formation of a counter-clockwise twisted spiral morphology. However, the detailed mechanisms of the formation of the different spiral orientation using the chiral copolymers of Ser(P) and Asp remains vague. Furthermore, Ling *et al*.[136] reported the fabrication of sophisticated and hierarchically ordered HAp based on silk multilayer membranes with nanoporous features by combining protein self-assembly and in situ biomineralization. Recently, Jiang *et al.*[137] discovered and studied novel chiral, hierarchically organized toroidal calcium carbonate (vaterite) structures, which can be tuned

based on chiral acidic amino acids such as Aspartic and Glutamic amino acids. The structures can have either counter-clockwise or clockwise spiralling morphology induced by L-enantiomers or D-enantiomers, respectively (Fig. 11e).

Polymer-induced liquid precursor (PILP)

Laurie Gower and colleagues have introduced a novel process within the field of synthetic biomineralization termed polymer-induced liquid precursor (PILP), which has been used to study the effect of different polymers on the mineralization process. For example, they discovered in a ground-breaking study[138] that poly-aspartic acid can lead to unique helical morphologies of calcium carbonate, mimicking those of biominerals. The calcium carbonate structures demonstrated a spherulitic twisted crystal growth, in which the polymer seemed to stabilise the metastable calcium carbonate phases. In a later study led by same group[139], they investigated the mechanism behind these formations and concluded that poly-aspartic acid triggers a liquid-liquid phase separation along with the mineral amorphous phase precursor, leading to non-equilibrium morphologies and the emergence of texture of the crystalline phase. In a similar way, Li *et al.*[81] mineralized densified highly-crosslinked collagen films using the PILP process in an attempt to mineralize collagen intrafibrillarly in a homogenous manner, similarly to the nanostructure of bone. They concluded that collagen cross-links played a major role not only in optimising the packing of the crystals within the collagen during the mineralization process, but also in generating stiff mineralized scaffolds of about 9.1 ± 1.4 GPa.

c- Intrinsically disordered proteins (IDPs)

IDPs are a class of natural proteins that do not adopt a characteristic conformation along their secondary structure. In addition, IDPs are highly variable and can contain both unstructured

(disordered) and structured (ordered) regions. It has been demonstrated that IDPs play a key role in many biological processes in human physiology and pathology. Therefore, there is an increasing interest in investigating extensively the mechanisms by which IDPs operate[140, 141]. For example, there is growing evidence that IDPs play a fundamental role in mineralization[141]. These proteins contribute in intermolecular interactions at the protein–mineral interface[142]. A major group of IDPs are the small integrin binding N-glycosylated proteins known as the SIBLING proteins[141]. The SIBLING family, which comprises osteopontin[143], dentin matrix protein 1[144], and bone sialoprotein[13] is well-known to bind hydroxyapatite through strong electrostatic and hydrophobic interactions. SIBLINGs share the common repeat sequence: aspartate–serine–serine (DSS) or glutamate–serine–serine (ESS), where the serine can be phosphorylated, which renders the protein to become highly acidic[141].

Furthermore, Beniash *et al.*[145] reported that amelogenin, a highly conserved IDP[142], undergoes a conformational change from disordered random coils to ordered β-sheet structures upon interaction with the developing enamel crystals. This conformational change is known to guide crystal growth in enamel formation[146]. Another example has been reported by Habelitz *et al.*[147], who demonstrated that the distinctive hierarchical structure of mature enamel may require further conformational organization of amelogenin into amyloid-like nanoribbons. Synthetic mineralization platforms that can emulate features of these dynamic supramolecular organic matrices, including these disorder-order transitions, may lead to more complex materials capable of recreating the structure and properties of biomineralized tissues[80, 141].

Amelogenin

Amelogenin plays a major biological role in enamel biomineralization (Figure 13). It is about 7-25 kDa with an isoelectric point of about 6.7[148]. Its primary structure is composed of 3 main

regions; a hydrophobic N-terminal tyrosine rich (TRAP) domain, a hydrophobic core, and an acidic hydrophilic C-terminal leucine rich (C-telopeptide) domain. This bipolarity in nature enables the hydrophobic core to be localised interiorly while the hydrophilic segments are positioned exteriorly. However, the secondary and tertiary structures are still poorly defined[11, 146, 149], which limits understanding of its function and mechanism of action. Towards this goal, Fincham *et al.*[146] prepared TEM sections of enamel matrix, where they observed that amelogenin self-assembles into nanospheres. These nanospheres (quaternary structure) prevent mineral growth in width and thickness, inhibit crystal fusion and fractures, and promote the elongation of crystals in *c*- axis when exposed to inorganic ions from the secretory ameloblasts. Additionally, Fang *et al.*[11] and Brookes *et al.*[150] confirmed the critical role of C-telopeptide in self-assembling the amelogenin protein into an intermediate stage of oligomers. These oligomers in turn control the pre-nucleation clusters, their assembly into nanospheres, and the overall organization of enamel crystals.

Elastin-like recombinamers (ELRs)

Elastin-like recombinamers (ELRs), also known as elastin-like polypeptides (ELPs), are recombinant macromolecules based on the natural elastin recurrent motif Val-Pro-Gly-X-Gly (VPGXG), where X can be any amino acid apart from proline[151, 152]. These molecules exhibit comparable biological[153] and mechanical[154] properties as natural elastin and have generated great interest due to their biocompatibility, biodegradability, and capacity to be synthesised with a high level of molecular control (Figure 14)[151]. These molecules exhibit an inverse transition temperature (ITT) across which the ELR transitions from a soluble to an insoluble phase[152]. Below the ITT, the polymer chains are extended into hydrated randomly-ordered coils[155] while above it, they acquire dynamic, non-random β-spiral structures. The

recombinant nature of ELRs have enabled the incorporation of bioactive epitopes to provide specific functionalities[156] such as RGDS to promote cell adhesion[157] or the statherin-derived peptide DDDEEKFLRRIGRFG to promote mineralization[158-160]. Taking advantage of these characteristics and the PILP process, Li *et al.*[161] demonstrated the possibility to assemble ELRs into fibres that are able to undergo intrafibrillar mineralization, recreating the biomineralization process of collagen (Figure 14). The group concluded that the spatial confinement formed by the acquired β-spiral structures in the fibrous form plays a key role in the observed mineralization.

For the last five years, our group has focused on exploiting ELRs to explore different ways to control and guide mineralization both *in vitro* through chemical and physical features[158, 162] as well as *in vivo* demonstrating its potential in bone regeneration[159] (Figure 14). Recently, inspired by the possibility to use ELRs as models of IDPs[163] and to generate materials with dynamic properties[164, 165], we have discovered that the intrinsically disordered nature of ELRs enables modulation over their molecular conformation in a tuneable manner, which can be used to guide organic-inorganic interactions (Figure 15a-c)[166]. By systematically modulating levels of molecular order and disorder, we were able to assemble supramolecular ELR matrices that can trigger calcium phosphate nucleation and template the hierarchical growth of HAp (Figure 15d) into materials with tuneable properties (Figure 15g). In this way, the intrinsically-disordered ELRs have the capability to stabilise a precursor single crystal phase (brushite)[165], which can then template the growth of a polycrystalline phase (apatite), a behaviour that has been previously suggested by other IDPs in biomineralization[167]. The mineralizing platform is capable of growing materials comprising elongated apatite nanocrystals that are aligned and organised into microscopic prisms, which grow together into spherulite-like structures hundreds of microns in diameter that come together to fill macroscopic areas (Figure 15e). The

structures can be grown over large uneven surfaces and native tissues (Figure 15f) as acid resistant membranes or coatings with tuneable hierarchy and stiffness that can recreate those of native tissues (Figure 15g)[166].

IV. Conclusion

The level of hierarchical organization of a material or tissue can dictate its corresponding functionality. Examples of this phenomenon can readily be seen in nature. With this in mind, understanding how biomineralization processes work and generate such sophisticated structures can provide promising tools for the creation of the new generation of robust functional materials. Therefore, extensive amount of research has been undertaken to characterize the structural hierarchy, mechanisms of formation, and properties of various natural biomineralized tissues. Simultaneously, other research groups have focused on mimicking these hierarchical materials to generate an extraordinary realm of superstructures.

In this review, we have attempted to use a materials science perspective to compare the design rules that nature has so effectively evolved with synthetic state-of-the-art strategies that are aiming to recreate structures and properties found in nature. Furthermore, hierarchical organization plays a fundamental role in human tissues such as enamel and bone to provide unique structure-function relationships that have not yet been recreated. Given, the strong clinical need and the potential impact of being able to recreate or replace these kinds of tissues, we believe that it is critical to develop new material platforms that will enable control and guidance of mineralization across multiple scales. These approaches will not only open opportunities in regenerative medicine and dentistry but also push the boundaries of advanced healthcare materials science.

Figure Legends:

Figure 1 Hierarchical structure of dental enamel at multiple lengthscales.

a) Bright-field transmission electron microscope (TEM) image of a focused-ion-beam (FIB) prepared thin section and selected-area diffraction pattern (SAED) of the edge of one enamel prism, showing that the alignment of nanocrystals is parallel to the crystallographic *c*- axis of the apatite lattice. Reproduced with permission [168]. Copyright © 2015, American Association for the Advancement of Science. b) SEM image showing the oriented enamel crystals. Reproduced with permission[168]. Copyright © 2015, American Association for the Advancement of Science. c) SEM image of human enamel prisms from the inner enamel, collected at the higher magnification of x5000. Reproduced with permission[169]. Copyright © 2013, Elsevier Ltd. d) Schematic showing the different layers of tooth structures including enamel, dentin, and dental pulp. Reproduced with permission[170]. Copyright © 2012, Elsevier Ltd.

Figure 2 Structure-function relationship of dental enamel.

a) Nanoindentation measurement maps of dental enamel including E (left) and H (right), noting the wide variation between the enamel surface and the DEJ. Higher values were observed for the palatal cusp of the upper molar as a functional cusp. Reproduced with permission[32]. Copyright © 2002, Elsevier Science Ltd. The mechanical properties were well-matched with the crystallographic texture of dental enamel (b) as conducted by synchrotron x-ray diffraction maps. Reproduced with permission[33]. Copyright © 2007, Elsevier Ltd. c-d) Crack path in a transverse enamel sample. In order to initiate a notch, cracks propagated at about 45° and branched, which is evidence for a toughening mechanism. Also, un-cracked bridging can also be observed in addition to the meandering cracks, which are crack branches that can re-join the main crack after some propagation. As these images show, crack propagation mainly occurred around the enamel prism (arrow) within the protein-rich prism boundary. Reproduced with permission[41]. Copyright © 2009, Elsevier Ltd.

Figure 3 Hierarchical structure of bone.

a-b) Electron microscopy images of human cortical osteonal bone showing that disordered regions populate the spaces between the ordered fibril arrays. Reproduced with permission[44]. Copyright © 2014, Elsevier Ltd. c) SEM image of a transverse section of human femur after osteoclastic resorption showing lamellae containing transversely oriented collagen (T), other thicker lamellae comprising of fibres that are arranged longitudinally (L), and the Haversian canal (HC). d) SEM image of a transverse section of human femur showing the opening of a Haversian canal. Reproduced with permission[171]. Copyright © 1986, Springer Nature. e) Drawing showing an example of a model of osteon as seen in cross-section in polarising light microscopy (PLM), where the fibrils in one lamella have a transversal course; in the next lamella they have a longitudinal course. Reproduced with permission[62]. Copyright © 1988, Springer Nature. f) PLM image of a ground section of human rib showing a characteristic Maltese-Cross, where the lamellation present indicating a change of collagen fibre orientation between adjacent lamellae. Reproduced with permission[171]. Copyright © 1986, Springer Nature. g) PLM images of ground sections of human femur showing multiple lamellae with the characteristic Maltese-Crosses. Reproduced with permission[171]. Copyright © 1986, Springer Nature. h) SEM image showing the

macroscale architecture of cancellous bone. Reproduced with permission[172]. Copyright © 2003, Springer Nature. (Courtesy of Prof. A. Boyde, QMUL).

Figure 4 Structure-function relationship of bone.

A schematic illustrating the fundamental toughening mechanisms of bone at multiple lengthscales. At the nanoscale level, toughening is achieved through molecular uncoiling and intermolecular sliding of tropocollagen molecules in addition to the microcracking and fibrillar sliding of the fibril arrays. At the microscale, breaking of sacrificial bonds at the interfaces of fibril arrays together with crack bridging by collagen fibrils contribute to increased energy dissipation. At the macroscale, the toughening is attained through the extensive crack deflection and crack bridging by uncracked ligaments. Reproduced with permission[173]. Copyright © 2011, Springer Nature.

Figure 5 Structure-function relationship of bone (2).

a) A schematic showing the orientation of collagen fibrils within trabecular bone and its impact on the functionality. Reproduced with permission[73]. Copyright © 2015, Springer Nature. b) Epi-fluorescence images showing the crack propagation present in osteonal bone under compression with the arc-shaped circumferential microcracks (bright green) arranged in the quasi-orthogonal pattern (a) that propagates across neighboring osteons (b). c, d) SEM images showing arc-shaped microcracks, and short microradial cracks in the thick lamellae and a circumferential microcrack[174].

Figure 6 Non-crystallographic branching of inorganic hierarchical architectures.

SEM images of a typical petal-like calcium carbonate crystals grown in silica gel (a). Helical forms (b) were observed at the top of the petals. Reproduced with permission[104]. Copyright © 2003 Elsevier Science B.V. c) Self-assembled silica-calcium carbonate coral-like structures showing the highly-ordered nanocrystals (insets). Reproduced with permission[105]. Copyright © 2008, American Chemical Society. d, e, and f) Different morphologies can be achieved by changing the orientation of the substrate in distinct growth steps to load different morphologies on top of each other. (g) A spiral grown by lowering the pH of the bulk solution. (h) A transmission electron microscopy image showing a grid that is decorated with the hierarchical structures. Reproduced with permission[175]. Copyright © 2013, American Association for the Advancement of Science

Figure 7 Ice-templated mineralization and bio-inspired composites for teeth restoration.

A) Ice-templated (freezing) strategy to generate nacre-like hierarchical materials. The growth of ordered-ice crystals elicits the local alignment of alumina platelets. Alumina nanoparticles and liquid-phase precursors are entrapped between the platelets (Top). Natural nacre (a-c); nacre-like alumina (d-f). A liquid-phase film is present even when the platelets are close, mimicking the protein layer in the nacre structure. Scale bars, 10 um (a,d); 500 nm (b,e); 250 nm (c,f). Reproduced with permission[106]. Copyright © 2014, Springer Nature. B) Bio-inspired composite that resembles the complex architecture of natural tooth, made up of two layers of both alumina and silica with distinct platelet orientation. Reproduced with permission[107]. Copyright © 2015, Springer Nature.

Figure 8 Hierarchical organization of bone-like steel compared to natural bone at multiple lengthscales.

Hierarchical organization of bone-like steel compared to natural bone at multiple lengthscales (a-b). SEM image showing a 760 MPa fatigue crack on the multiphase hierarchical steel and how it relates to natural bone (c-d). Graph showing the propagation rate of the fatigue crack, a behaviour that mimics that of natural bone (e). Reproduced with permission[109]. Copyright © 2017, American Association for the Advancement of Science.

Figure 9 Exploiting biological materials for bio-templating hierarchical materials.

SEM images showing shape-preserving magnesiothermic reduction of silica diatom frustules. A natural diatom skeleton (a) has been exploited to achieve MgO/Si composite replica (b) after magnesiothermic reduction of the diatom with Magnesium at 650C for 2.5 h. Reproduced with permission[113]. Copyright © 2007, Springer Nature. c, d) Iron oxide hierarchical materials templated by a pollen grain. Reproduced with permission[114]. Copyright © 2013, American Chemical Society. e, f) SEM images of bio-templated ceramic material obtained from the bio-templating of normal wood[115]. g, h) Silver spiral bio-templated by the spiral vessel of Nelumbo nucifera plant's rhizome. The electric conductivity of this microcoil was examined and exhibits outstanding bio-electrical properties[116].

Figure 10 Hierarchical organization of superstructures including coccolith-like and nacre-like materials.

SEM images showing hierarchical coccolith-like (a) calcite formation in comparison to the microtrumpet of natural coccolithophore Discosphaera tubifera (b). Reproduced with permission[118]. Copyright © 2004, Royal Chemical Society. c-f) SEM images of the pyramidal building blocks made of calcite mesocrystals in the presence of PS-MA[119]. Hierarchical organization of synthetic nacre (h) that resembles that of Anodonta woodiana natural nacre (g) at multiple lengthscales. The synthetic nacre mimic the brick-mortar architecture (i-j), Voronoi pattern (k-l) of that of natural nacre. Scale bars are 1 cm, 1 cm, 3 μm, 3 μm, 5 μm, 100 μm, 100 nm, and 100 nm for (g) to (n), respectively. Reproduced with permission[7]. Copyright © 2016, American Association for the Advancement of Science.

Figure 11 Chiral materials and hierarchical organization.

SEM images showing hierarchical silica-DNA ordered platelet mesostructures (a) without and (b) with addition of Mg^{2+} ions. According to circular dichroism, right-handedness is evidenced in the DNA similar to chiral cholesteric organization where left- and right-handed structures are denoted by + and -, respectively[129]. c, d) SEM images showing the different morphologies of silica-poly-lysine composites with only D- arrangement showing the concentric arrangement (Scale bars c= 10 μm, d=2 μm). Reproduced with permission[134]. Copyright © 2006, American Chemical Society. e) SEM images showing the hierarchical vaterite toroid structures exhibiting chiral orientations. Scale bars, 6 μm (a,b,f) and 8 μm (c–e). Reproduced from CC-BY open access publication[137]. Copyright © 2017, Springer Nature.

Figure 12 Collagen hierarchical mineralization and bone-like materials.

a, b, c) CryoTEM images of collagen at different stages of mineralization in the presence of poly aspartic acid, at different timepoints; (a) 24 h, b) 48 h, c) 72h. Scale bar=100 nm. Reproduced with permission[176]. Copyright © 2010, Springer Nature. SEM and TEM images of the cross section of CaP/polymerized Liquid crystals composites before (d,g) and after

(e,h) the aging procedure. The bright dots represent CaP nanoparticles within the polymer matrix. (i) Selected area electron diffraction (SAED) pattern of aged composite. f) TEM image showing the arrangement of the rods into fibre-like morphology mimicking that of bone[133]. j-o) SEM and TEM images of mineralized collagen cross-linked fibrils using the PILP method. Reproduced with permission[81]. Copyright © 2012, American Chemical Society. p-r) SEM images showing circular mineralized bone-like structures grown on top of pHEMA-based hydrogel containing various anionic residues. Two-dimensional outward growth of circular calcium phosphate mineral structures from multiple nucleation sites (indicated by arrows) were observed. Reproduced with permission[121]. Copyright © 2005, American Chemical Society.

Figure 13 Amelogenin self-assembly and its mineralization

a) Hypothesized schematic of dental enamel mineral deposition processes near the DEJ. In the dentin, plate-like apatite crystals grow in the periodic gap spaces along the collagen fibrils and fibril bundles. The apatite crystal *c*- axis is mostly aligned with the long axis of the collagen fibril. In the enamel, it is proposed that that linear aggregates of self-assembled amelogenin nanospheres form a negatively charged template that induces apatite formation. Reproduced with permission[177]. Copyright © 2005, American Association for the Advancement of Science. b) Amelogenin self-assembly model and its role during the enamel biomineralization. 1- Secretion of amelogenin extracellularly. 2- Amelogenin monomer assembly to form the nanospheres, where the hydrophilic part (carboxy-terminal) is at the exterior. 3- Electrostatic interactions between the nanospheres and the apatite crystals preventing them to grow in thickness but in length. 4- Degradation of amelogenin nanospheres by the action of proteinases (enamelysin). 5- Enamel maturation, where the crystals can grow in width. Reproduced with permission[178]. Copyright © 1999 Academic Press.

Figure 14 Regenerative mineralizing capacity of elastin-like recombinamers (ELR).

a) SEM and TEM images of the mineralized elastin-like fibrils via the PILP process. The minerals were hydroxyapatite nanocrystals oriented parallel to the longitudinal axis of the fibril. Reproduced with permission[161]. Copyright © 2017, American Chemical Society. b) Preferential nucleation and crystal growth on top microfabricated channels made from ELR membranes. Reproduced with permission[162]. Copyright © 2016, Elsevier Ltd. c) An orthotopic critical-size rat calvarial defect model to the bone regeneration capacity of membranes made of ELR membranes. Statherin-rich ELR membranes exhibited higher bone mineral density within the defect. Reproduced with permission[159]. Copyright © 2014, Elsevier Ltd.

Figure 15 Hierarchical mineralization platform based on the interplay between molecular order and disorder.

a,b) Graph and schematics showing the different levels of ELR order and disorder as a function of cross-linking. The levels of ELR spherulites' ordered β-sheet structure and disordered random coil can be modulated and tuned, while maintaining β-turn and α-helix conformations nearly constant. c) SEM observations revealed the abundant presence of a dense pattern of spherulite-like structures with a granulated central region at the bulk of the membranes' cross-sections, which template the growth of fluorapatite spherulites. Near the

membrane surface, mineralised structures with nanocrystals grow vertically towards the surface of the membrane. TEM image from a FIB milling liftout of the mineralised structures illustrating the change in growth direction of the nanocrystals from parallel to the surface towards the bulk of the ELR membrane. d) SEM images of the top of an ELR membrane after mineralisation showing the hierarchical organisation of the mineralised structures including aligned fluorapatite nanocrystals that are grouped into prism-like microstructures that further grow into macroscopic circular structures. e) The hierarchical structures grow until they meet each other. f) Application of the in-situ cross-linked ELR membrane conformed over the rough and uneven surface of exposed human dentine, exhibiting the hierarchical mineralised structures as a coating on top of the native tissue, where the nanocrystals infiltrating, binding, and occluding the open dentinal tubule structures. g) Young's modulus and hardness relationship between the mineralised structures and different mineralised tissues[166].

References

[1] U. G. K. Wegst, H. Bai, E. Saiz, A. P. Tomsia, R. O. Ritchie, Nature Materials 2014, 23.
[2] S. Weiner, H. D. Wagner, Annual Review of Materials Science 1998, 28, 271.
[3] R. Lakes, Nature 1993, 361, 511.
[4] S. Weiner, L. Addadi, Journal of Materials Chemistry 1997, 7, 689.
[5] J. Aizenberg, P. Fratzl, Advanced Materials 2009, 21, 187.
[6] W. L. Noorduin, A. Grinthal, L. Mahadevan, J. Aizenberg, Science 2013, 340, 832.
[7] L. B. Mao, H. L. Gao, H. B. Yao, L. Liu, H. Cölfen, G. Liu, S. M. Chen, S. K. Li, Y. X. Yan, Y. Y. Liu, S. H. Yu, Science 2016, 1.
[8] L. Addadi, S. Weiner, Angewandte Chemie International Edition in English 1992, 31, 153.
[9] K. Naka, Y. Chujo, Chemistry of Materials 2001, 13, 3245.
[10] D. Fan, R. Lakshminarayanan, J. Moradian-Oldak, Journal of Structural Biology 2008, 163, 109.
[11] P. A. Fang, J. F. Conway, H. C. Margolis, J. P. Simmer, E. Beniash, Proceedings of the National Academy of Sciences of the United States of America 2011, 108, 14097.
[12] M. T. Kaartinen, W. Sun, N. Kaipatur, M. D. McKee, Journal of Dental Research 2005, 84, 607.
[13] H. A. Goldberg, K. J. Warner, M. J. Stillman, G. K. Hunter, Connective Tissue Research 1996, 35, 385.
[14] M. Saito, K. Marumo, Calcified Tissue International 2015, 97, 242.
[15] S. Mann, *Biomineralization : principles and concepts in bioinorganic materials chemistry*, Oxford University Press, Oxford 2001; H. A. Lowenstam, S. Weiner, *On biomineralization*, Oxford University Press, 1989; C. Tamerler, M. Sarikaya, Acta Biomaterialia 2007, 3, 289.
[16] A. Boyde, in *The Dentition and Dental Care*, Vol. 3 (Ed: R. J. Elderton), Heinemann Medical Books, 1990, 30.
[17] C. M. B. Henderson, A. M. T. Bell, J. M. Charnock, K. S. Knight, R. F. Wendlandt, D. A. Plant, W. J. Harrison, Mineralogical Magazine 2009, 73, 433.
[18] R. Z. LeGeros, Monographs in oral science 1991, 15, 1.
[19] C. Robinson, J. A. Weatherell, A. S. Hallsworth, Caries Research 1971, 5, 44.
[20] C. Robinson, J. A. Weatherell, A. S. Hallsworth, Caries Research 1981, 15, 70.
[21] C. Robinson, J. Kirkham, R. Shore, *Dental enamel : formation to destruction*, CRC Press, Boca Raton 1995.
[22] B. K. B. Berkovitz, G. R. Holland, B. J. Moxham, *Oral anatomy, histology and embryology*, Mosby/Elsevier, Edinburgh ; New York 2009.

[23] A. Nanci, A. R. Ten Cate, *Ten Cate's oral histology : development, structure, and function*, Elsevier, St. Louis, Mo. 2013.
[24] J. W. Osborn, Journal of Dental Research 1968, 47, 395.
[25] J. W. Osborn, Journal of Dental Research 1968, 47, 223.
[26] F. Hirota, Archives of Oral Biology 1982, 27, 931.
[27] G. A. Macho, Y. Jiang, I. R. Spears, Journal of Human Evolution 2003, 45, 81.
[28] Y. Jiang, I. R. Spears, G. A. Macho, Archives of Oral Biology 2003, 48, 449.
[29] P. Tafforeau, J. P. Zermeno, T. M. Smith, in *J Hum Evol*, Vol. 62, England 2012, 424.
[30] L. Raue, N. Gersdorff, M. Rödiger, H. Klein, Archives of Oral Biology 2012, 57, 271.
[31] C. D. Lynch, V. R. O'Sullivan, P. Dockery, C. T. McGillycuddy, J. S. Rees, A. J. Sloan, Journal of Oral Rehabilitation 2011, 38, 359.
[32] J. L. Cuy, A. B. Mann, K. J. Livi, M. F. Teaford, T. P. Weihs, Archives of Oral Biology 2002, 47, 281.
[33] M. Al-Jawad, A. Steuwer, S. H. Kilcoyne, R. C. Shore, R. Cywinski, D. J. Wood, Biomaterials 2007, 28, 2908.
[34] L. M. Simmons, M. Al-Jawad, S. H. Kilcoyne, D. J. Wood, Eur J Oral Sci 2011, 119 Suppl 1, 19.
[35] S. Habelitz, S. J. Marshall, Marshall G.W, Jr., M. Balooch, Archives of Oral Biology 2001, 46, 173.
[36] H. Gao, B. Ji, I. L. Jäger, E. Arzt, P. Fratzl, Proceedings of the National Academy of Sciences of the United States of America 2003, 100, 5597.
[37] S. N. White, W. Luo, M. L. Paine, H. Fong, M. Sarikaya, M. L. Snead, Journal of Dental Research 2001, 80, 321.
[38] E. A. M. Kidd, *Essentials of dental caries*, Oxford University Press, Oxford ; New York 2005.
[39] F. Lippert, D. M. Parker, K. D. Jandt, Journal of Colloid and Interface Science 2004, 280, 442.
[40] I. R. Spears, R. van Noort, R. H. Crompton, G. E. Cardew, I. C. Howard, Journal of Dental Research 1993, 72, 1526.
[41] S. Bechtle, S. Habelitz, A. Klocke, T. Fett, G. A. Schneider, Biomaterials 2010, 31, 375.
[42] H. H. K. Xu, D. T. Smith, S. Jahanmir, E. Romberg, J. R. Kelly, V. P. Thompson, E. D. Rekow, Journal of Dental Research 1998, 77, 472.
[43] L. He, *Human Enamel - Elegantly designed natural biocomposite*, VDM Verlag Dr. Muller, 2009.
[44] N. Reznikov, R. Shahar, S. Weiner, Acta Biomaterialia 2014, 10, 3815.
[45] E. Hamed, Y. Lee, I. Jasiuk, Acta Mechanica 2010, 213, 131.
[46] N. Reznikov, J. A. M. Steele, P. Fratzl, M. M. Stevens, Nature Reviews Materials 2016, 1.
[47] D. Erts, L. J. Gathercole, E. D. T. Atkins, Journal of Materials Science: Materials in Medicine 1994, 5, 200.
[48] R. A. Robinson, The Journal of bone and joint surgery. American volume 1952, 34 A, 389.
[49] B. O. Fowler, E. C. Moreno, W. E. Brown, Archives of Oral Biology 1966, 11, 477.
[50] E. Beniash, R. A. Metzler, R. S. K. Lam, P. U. P. A. Gilbert, Journal of Structural Biology 2009, 166, 133.
[51] C. J. S. Ibsen, D. Gebauer, H. Birkedal, Chemistry of Materials 2016, 28, 8550.
[52] X. Su, K. Sun, F. Z. Cui, W. J. Landis, Bone 2003, 32, 150; R. A. Robinson, M. L. Watson, The Anatomical record 1952, 114, 383.
[53] V. Ziv, H. D. Wagner, S. Weiner, Bone 1996, 18, 417.
[54] S. Weiner, W. Traub, H. D. Wagner, Journal of Structural Biology 1999, 126, 241.
[55] D. B. Burr, Bone 2002, 31, 8.
[56] A. Boyde, in *Gray's Anatomy*, (Eds: P. Williams, H. Bannister), Churchill Livingstone, New York 1995.
[57] M. M. Giraud-Guille, Microscopy Research and Technique 1994, 27, 420.
[58] M. M. Giraud-Guille, Biology of the Cell 1989, 67, 97.
[59] C. Robinson, Tetrahedron 1961, 13, 219.

[60] Y. Dong, J. Wang, Q. Yuan, Progress in Chemistry 1999, 11, 416.
[61] Y. M. Yevdokimov, M. A. Zakharov, V. I. Salyanov, Crystallography Reports 2006, 51, 1016.
[62] M. M. Giraud-Guille, Calcified Tissue International 1988, 42, 167.
[63] L. Wang, X. Chen, Y. Chai, J. Hao, Z. Sui, W. Zhuang, Z. Sun, Chemical Communications 2004, 2840.
[64] Y. Xu, H. P. C. Van Kuringen, D. J. Mulder, A. P. H. J. Schenning, N. A. J. M. Sommerdijk, RSC Advances 2016, 6, 13953.
[65] M. Kondo, M. Sugimoto, M. Yamada, Y. Naka, J. I. Mamiya, M. Kinoshita, A. Shishido, Y. Yu, T. Ikeda, Journal of Materials Chemistry 2010, 20, 117.
[66] Y. Bouligand, Comptes Rendus Chimie 2008, 11, 281.
[67] I. Sabanay, T. Arad, S. Weiner, B. Geiger, Journal of Cell Science 1991, 100, 227.
[68] O. A. Tertuliano, J. R. Greer, Nature Materials 2016, 15, 1195.
[69] G. E. Fantner, T. Hassenkam, J. H. Kindt, J. C. Weaver, H. Birkedal, L. Pechenik, J. A. Cutroni, G. A. G. Cidade, G. D. Stucky, D. E. Morse, P. K. Hansma, Nature Materials 2005, 4, 612.
[70] S. Cavelier, A. K. Dastjerdi, M. D. McKee, F. Barthelat, Bone 2018, 110, 304.
[71] O. Nikel, D. Laurencin, S. A. McCallum, C. M. Gundberg, D. Vashishth, Langmuir 2013, 29, 13873.
[72] K. Tai, M. Dao, S. Suresh, A. Palazoglu, C. Ortiz, Nature Materials 2007, 6, 454.
[73] M. Liebi, M. Georgiadis, A. Menzel, P. Schneider, J. Kohlbrecher, O. Bunk, M. Guizar-Sicairos, Nature 2015, 527, 349.
[74] W. Wagermaier, K. Klaushofer, P. Fratzl, Calcified Tissue International 2015, 97, 201.
[75] R. K. Nalla, J. H. Kinney, R. O. Ritchie, Nature Materials 2003, 2, 164.
[76] K. Tai, F. J. Ulm, C. Ortiz, Nano Letters 2006, 6, 2520.
[77] B. Bhushan, Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences 2009, 367, 1445.
[78] O. H. Schmitt, Science 1948, 107, 432.
[79] J. M. Harkness, IEEE engineering in medicine and biology magazine : the quarterly magazine of the Engineering in Medicine & Biology Society 2004, 23, 20; J. F. V. Vincent, Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine 2009, 223, 919.
[80] P. Fratzl, R. Weinkamer, Progress in Materials Science 2007, 52, 1263.
[81] Y. Li, T. T. Thula, S. Jee, S. L. Perkins, C. Aparicio, E. P. Douglas, L. B. Gower, Biomacromolecules 2012, 13, 49.
[82] Z. Tang, N. A. Kotov, S. Magonov, B. Ozturk, Nature Materials 2003, 2, 413.
[83] K. Yamagishi, K. Onuma, T. Suzuki, F. Okada, J. Tagami, M. Otsuki, P. Senawangse, Nature 2005, 433, 819.
[84] Y. Yin, S. Yun, J. Fang, H. Chen, Chemical Communications 2009, 5892.
[85] L. C. Palmer, C. J. Newcomb, S. R. Kaltz, E. D. Spoerke, S. I. Stupp, Chemical Reviews 2008, 108, 4754.
[86] Q. Ruan, Y. Zhang, X. Yang, S. Nutt, J. Moradian-Oldak, Acta Biomaterialia 2013, 9, 7289.
[87] S. Busch, U. Schwarz, R. Kniep, Chemistry of Materials 2001, 13, 3260.
[88] R. Hu, C. J. Lin, H. Y. Shi, Journal of Biomedical Materials Research - Part A 2007, 80, 687.
[89] J. Liu, T. Jin, S. Chang, A. Czajka-Jakubowska, Z. Zhang, J. E. Nör, B. H. Clarkson, Tissue Engineering - Part A 2010, 16, 2977; J. Liu, T. C. Jin, S. Chang, A. Czajka-Jakubowska, B. H. Clarkson, J Biomed Mater Res A 2011, 96, 528.
[90] J. Liu, X. Wang, Q. Jin, T. Jin, S. Chang, Z. Zhang, A. Czajka-Jakubowska, W. V. Giannobile, J. E. Nor, B. H. Clarkson, Biomaterials 2012, 33, 5036.
[91] S. Mann, S. Weiner, in *J Struct Biol*, Vol. 126, United states 1999, 179.
[92] A. Kishen, A. Asundi, Journal of X-Ray Science and Technology 2002, 10, 153.
[93] Y. Cao, M. L. Mei, Q. L. Li, E. C. M. Lo, C. H. Chu, ACS Applied Materials and Interfaces 2014, 6, 410.

[94] H. Chen, Z. Tang, J. Liu, K. Sun, S. R. Chang, M. C. Peters, J. F. Mansfield, A. Czajka-Jakubowska, B. H. Clarkson, Advanced Materials 2006, 18, 1846.
[95] S. Elsharkawy, M. Al-Jawad, Journal of Dental Research, 94 Special Issue A, 2015.
[96] S. Busch, Angewandte Chemie - International Edition 2004, 43, 1428.
[97] H. Li, W. Huang, Y. Zhang, M. Zhong, Materials Science and Engineering C 2007, 27, 756.
[98] Z. Huang, T. D. Sargeant, J. F. Hulvat, A. Mata, P. Bringas Jr, C. Y. Koh, S. I. Stupp, M. L. Snead, Journal of Bone and Mineral Research 2008, 23, 1995.
[99] Y. Fan, Z. Sun, J. Moradian-Oldak, Biomaterials 2009, 30, 478.
[100] J. M. García-Ruiz, S. T. Hyde, A. M. Carnerup, A. G. Christy, M. J. Van Kranendonk, N. J. Welham, Science 2003, 302, 1194.
[101] W. Kunz, M. Kellermeier, Science 2009, 323, 344.
[102] J. M. García-Ruiz, E. Melero-García, S. T. Hyde, Science 2009, 323, 362.
[103] J. M. Garcia-Ruiz, Journal of Crystal Growth 1985, 73, 251.
[104] T. Terada, S. Yamabi, H. Imai, Journal of Crystal Growth 2003, 253, 435.
[105] A. E. Voinescu, M. Kellermeier, B. Bartel, A. M. Carnerup, A. K. Larsson, D. Touraud, W. Kunz, L. Kienle, A. Pfitzner, S. T. Hyde, Crystal Growth and Design 2008, 8, 1515.
[106] F. Bouville, E. Maire, S. Meille, B. Van de Moortele, A. J. Stevenson, S. Deville, Nat Mater 2014, 13, 508.
[107] H. Le Ferrand, F. Bouville, T. P. Niebel, A. R. Studart, Nature Materials 2015, 14, 1172.
[108] R. M. Erb, R. Libanori, N. Rothfuchs, A. R. Studart, Science 2012, 335, 199.
[109] M. Koyama, Z. Zhang, M. Wang, D. Ponge, D. Raabe, K. Tsuzaki, H. Noguchi, C. C. Tasan, Science 2017, 355, 1055.
[110] D. Jang, L. R. Meza, F. Greer, J. R. Greer, Nature Materials 2013, 12, 893.
[111] B. Liu, Y. Yao, S. Che, Angewandte Chemie - International Edition 2013, 52, 14186.
[112] L. Chen, K. Liang, J. Li, D. Wu, X. Zhou, Arch Oral Biol 2013, 58, 975.
[113] Z. Bao, M. R. Weatherspoon, S. Shian, Y. Cai, P. D. Graham, S. M. Allan, G. Ahmad, M. B. Dickerson, B. C. Church, Z. Kang, H. W. Abernathy Iii, C. J. Summers, M. Liu, K. H. Sandhage, Nature 2007, 446, 172.
[114] W. Brandon Goodwin, I. J. Gomez, Y. Fang, J. C. Meredith, K. H. Sandhage, Chemistry of Materials 2013, 25, 4529.
[115] A. S. Deshpande, I. Burgert, O. Paris, Small 2006, 2, 994.
[116] K. Kamata, S. Suzuki, M. Ohtsuka, M. Nakagawa, T. Iyoda, A. Yamada, Advanced Materials 2011, 23, 5509.
[117] Z. Schnepp, W. Yang, M. Antonietti, C. Giordano, Angewandte Chemie - International Edition 2010, 49, 6564.
[118] S. B. Mukkamala, A. K. Powell, Chemical Communications 2004, 10, 918.
[119] A. W. Xu, M. Antonietti, S. H. Yu, H. Cölfen, Advanced Materials 2008, 20, 1333.
[120] J. Song, E. Saiz, C. R. Bertozzi, Journal of the American Chemical Society 2003, 125, 1236; J. Song, E. Saiz, C. R. Bertozzi, Journal of the European Ceramic Society 2003, 23, 2905; A. P. Tomsia, E. Saiz, J. Song, C. R. Bertozzi, Advanced Engineering Materials 2005, 7, 999.
[121] J. Song, V. Malathong, C. R. Bertozzi, Journal of the American Chemical Society 2005, 127, 3366.
[122] H. Bai, F. Walsh, B. Gludovatz, B. Delattre, C. Huang, Y. Chen, A. P. Tomsia, R. O. Ritchie, Advanced Materials 2016, 28, 50.
[123] N. Rauner, M. Meuris, M. Zoric, J. C. Tiller, Nature 2017, 543, 407.
[124] Z. Reich, E. J. Wachtel, A. Minsky, Science 1994, 264, 1460.
[125] W. J. Chung, J. W. Oh, K. Kwak, B. Y. Lee, J. Meyer, E. Wang, A. Hexemer, S. W. Lee, Nature 2011, 478, 364.
[126] V. Sharma, M. Crne, J. O. Park, M. Srinivasarao, Science 2009, 325, 449.
[127] T. Sugawara, Y. Suwa, K. Ohkawa, H. Yamamoto, Macromolecular Rapid Communications 2003, 24, 847.

[128] K. E. Shopsowitz, H. Qi, W. Y. Hamad, M. J. MacLachlan, Nature 2010, 468, 422.
[129] B. Liu, L. Han, S. Che, Angewandte Chemie - International Edition 2012, 51, 923.
[130] Z. Dogic, S. Fraden, Physical Review Letters 1997, 78, 2417.
[131] A. Sugawara, A. Oichi, H. Suzuki, Y. Shigesato, T. Kogure, T. Kato, Journal of Polymer Science, Part A: Polymer Chemistry 2006, 44, 5153.
[132] I. Manjubala, S. Scheler, J. Bössert, K. D. Jandt, Acta Biomaterialia 2006, 2, 75; I. Manjubala, I. Ponomarev, I. Wilke, K. D. Jandt, Journal of Biomedical Materials Research - Part B Applied Biomaterials 2008, 84, 7.
[133] W. X. He, A. K. Rajasekharan, A. R. Tehrani-Bagha, M. Andersson, Advanced Materials 2015, 27, 2260.
[134] E. G. Bellomo, T. J. Deming, Journal of the American Chemical Society 2006, 128, 2276.
[135] E. D. Spoerke, S. G. Anthony, S. I. Stupp, Advanced Materials 2009, 21, 425; A. Mata, Y. Geng, K. J. Henrikson, C. Aparicio, S. R. Stock, R. L. Satcher, S. I. Stupp, Biomaterials 2010, 31, 6004.
[136] S. Ling, Z. Qin, W. Huang, S. Cao, D. L. Kaplan, M. J. Buehler, Science Advances 2017, 3, 1DUMMY.
[137] W. Jiang, M. S. Pacella, D. Athanasiadou, V. Nelea, H. Vali, R. M. Hazen, J. J. Gray, M. D. McKee, Nature Communications 2017, 8.
[138] L. A. Gower, D. A. Tirrell, Journal of Crystal Growth 1998, 191, 153.
[139] L. B. Gower, D. J. Odom, Journal of Crystal Growth 2000, 210, 719.
[140] H. J. Dyson, P. E. Wright, Nature Reviews Molecular Cell Biology 2005, 6, 197.
[141] A. L. Boskey, E. Villarreal-Ramirez, Matrix Biology 2016, 52-54, 43.
[142] K. Delak, C. Harcup, R. Lakshminarayanan, Z. Sun, Y. Fan, J. Moradian-Oldak, J. S. Evans, Biochemistry 2009, 48, 2272.
[143] M. D. McKee, A. Nanci, Microscopy Research and Technique 1995, 33, 141.
[144] G. He, S. Gajjeraman, D. Schultz, D. Cookson, C. Qin, W. T. Butler, J. Hao, A. George, Biochemistry 2005, 44, 16140.
[145] E. Beniash, J. P. Simmer, H. C. Margolis, Journal of Dental Research 2012, 91, 967.
[146] A. G. Fincham, J. Moradian-Oldak, T. G. H. Diekwisch, D. M. Lyaruu, J. T. Wright, P. Bringas Jr, H. C. Slavkin, Journal of Structural Biology 1995, 115, 50.
[147] K. M. M. Carneiro, H. Zhai, L. Zhu, J. A. Horst, M. Sitlin, M. Nguyen, M. Wagner, C. Simpliciano, M. Milder, C. L. Chen, P. Ashby, J. Bonde, W. Li, S. Habelitz, Scientific Reports 2016, 6.
[148] J. P. Simmer, A. G. Fincham, Critical Reviews in Oral Biology and Medicine 1995, 6, 84.
[149] A. G. Fincham, J. P. Simmer, CIBA Foundation Symposia 1997, 118; X. Yang, L. Wang, Y. Qin, Z. Sun, Z. J. Henneman, J. Moradian-Oldak, G. H. Nancollas, Journal of Physical Chemistry B 2010, 114, 2293.
[150] S. J. Brookes, C. Robinson, J. Kirkham, W. A. Bonass, Archives of Oral Biology 1995, 40, 1.
[151] A. Girotti, J. Reguera, J. C. Rodríguez-Cabello, F. J. Arias, M. Alonso, A. M. Testera, Journal of Materials Science: Materials in Medicine 2004, 15, 479.
[152] D. W. Urry, *What sustains life?: Consilient mechanisms for protein-based machines and materials*, 2006.
[153] J. F. Almine, D. V. Bax, S. M. Mithieux, L. Nivison-Smith, J. Rnjak, A. Waterhouse, S. G. Wise, A. S. Weiss, Chemical Society Reviews 2010, 39, 3371.
[154] J. Lee, C. W. Macosko, D. W. Urry, Macromolecules 2001, 34, 5968.
[155] D. W. Urry, Angewandte Chemie (International Edition in English) 1993, 32, 819.
[156] A. Panitch, T. Yamaoka, M. J. Fournier, T. L. Mason, D. A. Tirrell, Macromolecules 1999, 32, 1701.
[157] A. Nicol, C. Gowda, D. W. Urry, Journal of Vascular Surgery 1991, 13, 746.
[158] E. Tejeda-Montes, K. H. Smith, M. Poch, M. J. López-Bosque, L. Martín, M. Alonso, E. Engel, A. Mata, Acta Biomaterialia 2012, 8, 998; E. Tejeda-Montes, K. H. Smith, E. Rebollo, R. Gómez, M. Alonso, J. C. Rodriguez-Cabello, E. Engel, A. Mata, Acta Biomaterialia 2014, 10, 134.

[159] E. Tejeda-Montes, A. Klymov, M. R. Nejadnik, M. Alonso, J. Rodriguez-Cabello, X. F. Walboomers, A. Mata, Biomaterials 2014, 35, 8339.
[160] Y. Li, X. Chen, A. J. Ribeiro, E. D. Jensen, K. V. Holmberg, J. C. Rodriguez-Cabello, C. Aparicio, Advanced Healthcare Materials 2014, 3, 1638.
[161] Y. Li, J. C. Rodriguez-Cabello, C. Aparicio, ACS Applied Materials and Interfaces 2017, 9, 5838.
[162] S. Elsharkawy, E. Tejeda-Montes, M. Al-Jawad, A. Mata, Materials Today 2016, 19, 478.
[163] S. Roberts, M. Dzuricky, A. Chilkoti, FEBS Letters 2015, 589, 2477.
[164] J. R. Simon, N. J. Carroll, M. Rubinstein, A. Chilkoti, G. P. López, Nature Chemistry 2017, 9, 509; K. E. Inostroza-Brito, E. Collin, O. Siton-Mendelson, K. H. Smith, A. Monge-Marcet, D. S. Ferreira, R. P. Rodríguez, M. Alonso, J. C. Rodríguez-Cabello, R. L. Reis, F. Sagués, L. Botto, R. Bitton, H. S. Azevedo, A. Mata, Nature Chemistry 2015, 7, 897.
[165] K. Shuturminska, N. V. Tarakina, H. S. Azevedo, A. J. Bushby, A. Mata, P. Anderson, M. Al-Jawad, Frontiers in Physiology 2017, 8.
[166] E. S., A.-J. M., P. M.F., T.-M. E., M. K., J. H., A. S., S. K., R. A., T. N.V., W. R.M., B. A.J., A. M., R.-C. J.C., B. E., d. R. H. A., S. M.M., P. N.M., A. P., M. A., Nature Communications 2018, accepted.
[167] E. P. Chang, J. A. Russ, A. Verch, R. Kröger, L. A. Estroff, J. S. Evans, Biochemistry 2014, 53, 4317.
[168] L. M. Gordon, M. J. Cohen, K. W. MacRenaris, J. D. Pasteris, T. Seda, D. Joester, Science 2015, 347, 746.
[169] L. H. He, Z. H. Yin, L. Jansen Van Vuuren, E. A. Carter, X. W. Liang, Acta Biomaterialia 2013, 9, 6330.
[170] H. Eimar, E. Ghadimi, B. Marelli, H. Vali, S. N. Nazhat, W. M. Amin, J. Torres, O. Ciobanu, R. F. Albuquerque Junior, F. Tamimi, Acta Biomaterialia 2012, 8, 3400.
[171] S. A. Reid, Anatomy and Embryology 1986, 174, 329.
[172] A. M. Stoneham, J. H. Harding, Nature Materials 2003, 2, 77.
[173] R. O. Ritchie, Nat Mater 2011, 10, 817.
[174] V. Ebacher, R. Wang, Advanced Functional Materials 2009, 19, 57.
[175] W. L. Noorduin, Science (New York, N.Y.) 2013, 340, 832.
[176] F. Nudelman, K. Pieterse, A. George, P. H. H. Bomans, H. Friedrich, L. J. Brylka, P. A. J. Hilbers, G. De With, N. A. J. M. Sommerdijk, Nature Materials 2010, 9, 1004.
[177] A. Veis, Science 2005, 307, 1419.
[178] A. G. Fincham, J. Moradian-Oldak, J. P. Simmer, Journal of Structural Biology 1999, 126, 270.