Biomimetics and Marine Materials in Drug Delivery and Tissue Engineering

18

Andy H. Choi, Sophie Cazalbou, and Besim Ben-Nissan

Contents

Abstract

During the last two decades, biomimetics has provided mankind new directions for the utilization of natural organic and inorganic skeletons for novel drug delivery systems and new medical treatment approaches with unique designs ranging from the macro- to the nanoscale. The use of ready-made organic and

A.H. Choi (✉) • B. Ben-Nissan
School of Chemistry and Forensic Science, Faculty of Science, University of Technology Sydney, Ultimo, NSW, Australia
e-mail: ahchoi@hotmail.com; besim.ben-nissan@uts.edu.au

S. Cazalbou
CIRIMAT Carnot Institute, CNRS-INPT-UPS, Faculty of Pharmacie, University of Toulouse, Toulouse, France

I.V. Antoniac (ed.), *Handbook of Bioceramics and Biocomposites*,
DOI 10.1007/978-3-319-12460-5_26

inorganic marine skeletons has potentially created an opportunity of presenting one of the simplest cures to fundamental issues hampering the future development of regenerative medicine, dentistry, and orthopedics such as providing a richness of framework designs and devices and abundant and available sources of osteopromotive analogues and biomineralization proteins. Organic matrix and inorganic marine skeletons possess a habitat ideal for the proliferation of added mesenchymal stem cell populations and promoting clinically acceptable bone formation. It has been proven that self-sustaining musculoskeletal tissues can be supported by coral and marine sponge skeletons, and bone mineralization can be promoted by the extracts of spongin collagen and nacre seashell organic matrices. This idea is reinforced by the fact that bone morphogenetic protein molecules are produced by endodermal cells into the developing skeleton. Furthermore, the regenerative signaling proteins in bone therapeutics such as TGF and Wnt are also present in early marine sponge development and instrumental to the activation of stem cells in cnidarians. This chapter aims to give a brief background into the nature, morphology, and application of some of these structures in bone grafts, drug delivery, tissue engineering, and specific extracts such as proteins for regenerative medicine.

Keywords
Hydroxyapatite (HAp) • Calcium phosphate • Marine sponge • Marine shell • Foraminifera • Coral • Tissue scaffold • Urchin

Introduction

Marine organisms are created and organized by materials containing a wide variety of characteristic and properties which might warrant their possible use within the biomedical arena. In addition, the pledge to exploit natural marine resources in a sustainable manner generates a highly interesting stage for the development of novel biomaterials together with both environmental and economic benefits. As a result, a growing number of compounds of different types are being isolated from aquatic organisms and converted into products for health applications including tissue engineering and controlled drug delivery devices.

The fabrication of highly capable scaffolds that can function at the macro-, micro-, and nanoscopic level will play a pivotal role in making regenerative medicine a clinical success in the future. These scaffolds will be able to arrange and organize cells into tissues and in a targeted manner releasing encapsulated chemical signals and conveying them into the body.

Abundant sources of materials and structures are currently available that can be utilized to perform different function than their original proposed one. A simple strategy is to use a predesigned and preformed structure such as unique marine structures and modify it in a specific manner to suit its new intended purpose [1]. Moreover, we can learn from nature and attempt to duplicate the essential

components and reinvent in a laboratory. Additionally, we strive to learn more from nature the principle of minimizing energy usage during the synthesis process, the importance of structural organization, and the execution of transformative self-assembly and nonequilibrium chemistry.

These materials as well as its designs have played an instrumental role in the introduction of one of the easiest resolutions to crucial problems in regenerative medicine and in providing frameworks and highly accessible avenues of osteopromotive analogues, nanofibers, micro- and macrospheres, and mineralizing proteins. This is demonstrated by the biological efficiency of marine structures such as shells, corals, and sponge skeletons to accommodate self-sustaining musculoskeletal tissues and to promote bone formation through the use of nacre seashells and sponging extracts.

It has been discovered that molecules essential for regulating and guiding bone morphogenesis and in particular the actions accompanying mineral metabolism and deposition also exist in the earliest marine organisms. This is based on the fact that they symbolize the first molecular components recognized for calcification, morphogenesis, and wound healing. It has emerged that bone morphogenetic protein (BMP), the main cluster of bone growth factors for human bone morphogenesis, are secreted by endodermal cells into the developing skeleton. Furthermore, off-the-shelf organic and inorganic marine skeletons possess an ideal environment for the proliferation of added mesenchymal stem cell populations and promoting bone formation that is clinically acceptable.

The marine environment is distinctively rich in highly functional architectural structures with interconnected open pores. The chemical compositions and high mechanical strength of these structures make them ideal to be used for human implantation either in its original form or convert to materials more suitable for biomedical applications.

Areas such as new pharmaceutical drug delivery systems with enhanced properties and a more efficient design, hard and soft tissue engineering, and the discovery of a new generation of organic molecules have been the major emphases in the field of marine-based structures during the last two decades. More and more investigations on proteins and biopolymers produced by marine organisms have been carried out to examine its applications in the biomedical arena. At the moment, a growing number of compounds and materials are being identified from marine organisms such as calcium carbonates and proteins and applied to medical applications [2].

In tissue engineering applications, converted coralline apatites and coral skeletons are perfect examples [3]. They have demonstrated substantial clinical success as templates for tissue reconstruction. This has encouraged researchers to explore other skeletons with improved mechanical and/or biological properties. These unique three-dimensional marine structures are able to support the growth as well as an enhancement in differentiation of stem cell progenitors into bone cells. This is different to standard carbonate frameworks which do not induce stem cell differentiation.

Biomimetics

In nature, biomaterials possess desirable properties such as complexity and sophistication, and we are gradually discovering ways to imitate nature to create similar levels of sophistication even though it is to a limited extent. Current 3-D printing methods are good examples; however, we are only able to recreate microscopic structures with some level of biomimetic detail. For the replication of bioinorganic structures, this has been particularly true. The utilization of biological microstructures as templates for the recreation of inorganic structures with identical features has emerged as a versatile approach. Through this technique, ordered silica microstructures have been produced using bacterial filaments and nanotubes produced from tobacco mosaic virus [4].

The main purpose in biomimetics is to synthetically duplicate the structures of selected inorganic biomatrices [4], and they play a clear part in the production of replacements for calcified tissues. This can be accomplished using techniques in biomineral-inspired materials chemistry. The idea is to make skeletons from molecules into macroscopic structures by utilizing the consecutive developmental pathway of systems that nature employs. The space of construction is defined by the foundations which are laid. Constant delivery of all the necessary building materials is provided and maintained throughout construction. In nature, the process begins with supramolecular pre-organization, interfacial recognition, and vectorial regulation lending to multi-level processing [1]. The continual multiplication of these assemblies accumulates into the emergence of morphology and macroscale biomimetic forms. In the fabrication of the simplest skeletons, researchers have realized the great benefits of using emulsion droplets to create porous hollow shells, foams, and bead templates in conjunction with using bio-continuous microemulsions to produce microskeletal networks [5].

Investigators have also examined another approach that uses the controlled mineralization of adapted organic matrices from natural skeletons [4] and has generated clinically relevant end results. These bio-imitation approaches and strategies are being examined with cellular and extracellular matrix inputs such as mineralizations of reverse microemulsions [6] and bi-liquid foams and bio-continuous microemulsions [7, 8] and template-mediated biomineralization of organic biomatrices [9]. Biomimetic microspheres synthesized within self-organizing microemulsions were routinely employed as highly functional constructs for the localized delivery of growth factors and genes to primary human cells. These unique particles were also capable of producing osteoid and neocartilage [10].

Artificial Devices

A hundred years ago, artificial devices were produced from all sorts of materials such as gold and wood, and the development of these devices has reached a point where they could be used to replace different components of the human body. These

materials are capable of being in contact with bodily fluids and tissues for prolonged periods of time while demonstrating little or if any adverse reactions.

During the last two decades, acknowledging the importance of tissue-implant interactions on the nanoscale has led to the extensive development and application of nanotechnology in science and engineering. This is also based on the consideration that functional nanostructured materials are capable of being modified and incorporated into a range of biomedical devices. In addition, most biological systems such as viruses, protein complex, and membrane exhibit natural nanostructures. The synthesis method and the processing routes will govern the microstructure and properties of these new-generation nanostructured materials. Therefore, it is vital that the most appropriate technique is selected for the fabrication of materials with preferred design and property combinations.

Techniques such as solid-state, liquid-state, and gaseous ionic-state processing methods are frequently used for the synthesis of inorganic materials such as advanced ceramics. Additionally, wet chemical processing techniques such as sol-gel and coprecipitation have also been employed to obtain nanocoatings, nanoparticles, and nanostructured solid shapes and blocks. In modern ceramic technology, pressing is achieved by placing the powder into a dye and compacting it through the exertion of pressure. For the production of bioceramics, the most commonly used methods are hot pressing and hot isostatic pressing. Compared to hot pressing, higher-density and smaller grain structure, essential for obtaining good mechanical properties, can be achieved through the use of hot isostatic pressure, whereby heat and pressure are applied simultaneously with the pressure being applied from all directions via a pressurized gas such as argon or helium. It is relatively easy to produce flat plates or blocks and nonuniform components using hot pressing or hot isostatic pressing.

Sol-gel processing is unique in that it can be utilized to synthesize various forms of the same composition such as coatings, fibers, powders, platelets, and monoliths simply by changing factors such as chemistry and viscosity of a given solution [11]. The sol-gel technique possesses a number of advantages, for instance, it is of the nanoscale and it results in a stoichiometric, homogeneous, and pure product as mixing takes place on the molecular scale. It also has the ability to produce uniform fine-grained structures and can be easily applied to complex shapes with a range of coating techniques.

Appropriate surface finish is required for most biomaterials intended to be utilized within the physiological environment to permit the attachment of soft or hard tissue without any adverse reaction. Furthermore, biomaterials with a similar chemical composition to bone are needed for hard tissue attachment. For the majority of the animals, the microstructure of bone is composed of interconnected pores micro- and nanoscopic in size. The hard tissues contain calcium and phosphate ions and their combined form as calcium phosphate compounds with a variety of minerals. With our currently available production techniques, it is of great difficulty or in some cases almost impossible to copy and produce synthetically these complicated designs as a consequence of the limitations in resolution. However, in the near future, this could be achieved through the use of the new-generation three-dimensional printing techniques.

Nature, on the other hand, has provided a solution to obtaining these intricately fine porous structures. As a product to their natural need, some marine structures contain excellent interconnected pores and architectures that can meet and answer the problems discussed previously. These marine structures have very fine interconnected pores from nanometer to a few hundred microns in range as well as excellent mechanical properties. More importantly, most of them are composed of or contain inorganic materials such as calcium phosphates and calcium carbonate with a range of minerals containing magnesium, strontium, and silicon which assist in improving the properties of hard tissues after implantation. Although small, the organic matter within the marine skeletons contains a variety of materials, for example, proteins with very promising possible medical applications [12, 13].

Mankind is facing the creation of new biomimetic material synthesis systems using living cells, and producing tailor-made biomaterials to our specifications and requirements accurately in a beaker or test tube can be considered to be one of the most fascinating bio-inspired approaches ever known [12]. This can be accomplished by careful adjustment in the growing environment. The convenient starting points are single and multicelled organisms such as Foraminifera, Diatoms, and coccolithophores as they are the most basic and elementary organisms to grow and support in artificial culture and provide enough utility for providing this approach as practically beneficial [2].

Of great interest for the advancement of new strategies in nanotechnology and molecular assembly are diatoms as they offer modes of construction at these scales that can potentially benefit the research and development of new-generation drug delivery devices as a result of their microscopic size and internal pore network structure [13]. Discovered throughout marine and freshwater environments, diatoms are photosynthetic secondary endosymbionts and are believed to be responsible for approximately one-fifth of the primary productivity on the planet. It was recently reported the genome sequence of the marine centric diatom *Thalassiosira pseudonana*, revealing a wealth of information about diatom biology.

Diatoms have also been labeled as “natural-born” lithographers [14] and inspired the fabrication of nanostructured templates for nano-imprint processes where large structural areas with nanometer precision are required. Sussman et al. [14] exploited the mechanisms of patterning by diatoms for applications in patterning microchips, while Belegratis et al. [15] investigated the technical capabilities of the precise 3-D laser lithography based on two-photon polymerization of organic materials. This approach enabled the fabrication of arbitrary artificial diatom-inspired micro- and nanostructures and the design of an inverse structure. Therefore, only one replication step is required to obtain a template for nano-imprint processes.

There is also a vision to grow materials with living cells integrated during synthesis and construction in the field of biomimetic photonic materials. This represents an attractive proposition, and through this approach, the directed evolution may be conceivable with specific organisms that reproduce rapidly so that many thousands of generations are produced in short experimental time frames. At the moment, protocols are well established for the mass production of new proteins using site random mutagenesis combined with high-throughput screening [16].

Marine Materials and Synthetic Tissue Biology

One of the most fundamental and effective way of resolving scientific and technological problems is the conversion of products from nature into technology. Natural history collections provide a unique and abundant source of practical ideas and answers aimed at the early stages of tissue reassembly in artificial culture. Gaining an understanding into the chemistry and evolution of tissues and organs as well as their design and function will create a new path in providing elements that can be utilized in the reconstruction of tissues in the most simple and practical manner feasible. Fossilized marine organisms can also be utilized as excellent templates for providing new materials. Despite the fact that nature cannot create the perfect designs, it can, however, produce the most optimized, ideal, and functional adaptive ones [1].

A relatively newly emerging discipline, synthetic tissue biology aims to engineer tissues and transform them into complex biological assemblies [17]. An approach being investigated is the reverse engineering of biological materials, tissues, organs, or systems and to interpret how they are constructed and how they function at the highest level of detail. It can be suggested that it can revolutionize the concept and approach for reengineering biological systems.

The formation of multicellular tissues via synthetic biology utilizes the most advanced techniques available to construct extracellular environments to direct morphogenesis of tissues and cells. From a different perspective, cells are designed and produced with innovative functions and combined into multicellular organizations. In the vast diversity of nature, there are numerous recognized blueprints into the production and arrangement of cells and tissues into organs. Through natural selection, the evolution of tissues has provided mankind with an insight into how different development strategies have been exploited by organisms according to function. For that reason, we should be able to develop simplified assembly strategies to recreate functional approximations of every human tissue.

Hard Tissue Scaffold Development

Until now, naturally occurring biomatrices with wide-ranging chemical homologies and structural analogies to human extracellular matrices and whole tissues have been identified as a candidate in our quest of scaffolding materials. Examples of such biomatrices include marine shell and sponge skeletons, coral skeletons, and echinoderm skeletal elements. Certain species of these marine animals has been applied to the regeneration of human bone and cartilage as a result of their usefulness. On the other hand, the full effectiveness in these tissues and other marine animal and structure tissues has yet to be exploited and harnessed.

There are limitations when it comes to the regeneration potential of human bone especially in the case of repairing large bone defects. In general, autogenous and allogeneic bones are used as bone grafts. In spite of this, only a limited amount of autogenous bones can be used within the body, and there is a possibility of donor site morbidity. There is also the likelihood of transmission of infection from allografts if

they are based on demineralized bone. Consequently, synthetic bone-graft materials such as calcium phosphate ceramics, composites, and polymers have been widely developed [18].

The main inorganic mineral constituent close to human bone is hydroxyapatite (HAp), which is also an outstanding synthetic bone substitute because of its osteoconductive properties. HAp ceramics can be produced synthetically from its constituents using a variety of production techniques. In addition, demineralizing bovine or human hard tissues and natural marine structures such as corals and seashells and sea urchin to name just a few have also been used in the manufacture of HAp [1]. HAp powders are usually prepared using a range of methods such as hydrothermal conversion, wet chemical synthesis, calcination of bone, and solid-state reaction.

Synthetic bone-graft substitutes can be classified as inert, bioactive, and resorbable substitutes based on observed tissue response. For bone defect filling applications, resorbable bone-graft substitutes are preferred as they can be replaced after implantation by new natural bone. As a result of their fast dissolution characteristics, β-tri-calcium phosphate (β-TCP) and biphasic calcium phosphate mixture (with HAp) is one of the most commonly used bone substitute materials. It has also been suggested that the presence of magnesium stabilizes the β-TCP structure [19] and increases the transition temperature from β-TCP to α-TCP and decreases the solubility of β-TCP [20].

Marine Skeletons

The primary source of natural skeletons for bone tissue engineering has been natural and calcium phosphate-converted coral because of its morphology and crystallographic, chemical, and structural compatibility to native human bone [3]. As a result of the hydrothermal processing, natural skeletons can be used in a direct manner as a scaffold for growing cells into tissue and ultimately in the formation of new bone tissue [21]. Since then, invertebrate marine skeletons of hydrozoans and cuttlefish [22], marine sponges [6], nacre seashell, echinoderm spines [23], and others have been utilized. The basic reasoning to use these structures as clinical bone-graft materials is based on their macro and micro structures with appropriate ranges of pore sizes, its structural interconnected networks, which allow easy pathways for organizing and sustaining bone tissue growth. Whole natural skeletons have been used as templates for transporting biomolecules, and diatom skeletons have been tried with an antibody to be utilized in immunodiagnostics [24].

Marine Sponges

Marine sponges share much in common with multicellular tissues. Similarities, from a biochemical and morphological perspective, exist between marine sponge and vertebrate extracellular matrix suggesting that the fundamental rules of organization

evolved initially by marine sponges. To date, three types of collagen have been identified from marine sponge. All sponges are comprised of collagen fibrils 22 nm thin with highly ordered periodic banding. These collagen fibrils are secreted in bundles in a similar fashion to vertebrates. The amino acid sequence and genome organization are similar even though the ultrastructure of collagen is relatively simple compared to vertebrate collagens. Correspondingly, collagen fibrils are closely associated with proteoglycans which, in mammalian tissue design, shape and form at long-range scale. Dermatopin, fibronectin, and tenascin polypeptides are also discovered in marine sponge collagen fibers and cross-react with antibodies raised against vertebrate analogies underlining their common origins. A number of sponge species possess an analogue of type IV collagen found in vertebrate basement membrane collagens [25]. The organization of collagen fibrils is analogous to collagen type XIII which sticks cells to surfaces [26]. It is with these properties (cell adherent collagens and fibronectin) that collagenous marine sponge represents a significant potential for future development as bioactive tissue engineering scaffolds.

Marine sponges are at the moment extensively exploited for novel biological compounds as potential treatments for leukemia, cancer tumors, and inflammation. They are also a source of collagen for cosmetics [27] and dermatological preparations [28]. Half of all marine-derived materials in total are sources from a wide spectrum of marine sponges. Collagenous marine sponge skeletons are extremely strong, soft, elastic, highly absorbent, and resistant to bacterial attack and high temperatures. They are very suitable for use in surgical procedures as a result of these properties. Investigations are being carried out by several researchers to examine feasibility and the exact conditions needed to commercially grow marine sponges on a large production scale. Some have established aquatic pilot forms for the cultivation of selected bath sponge species. An additional aim for cultivating marine sponges is the extraction of medically important secondary metabolites in much greater quantity than is possible compared to collections made by conventional bioprospecting.

It has been suggested that useful lessons in the construction of man-made frameworks with minimal starting materials for maximum strength have been provided by the superior optimized structural design of marine sponges [29, 30]. Consolidated silica spheres on the nanometer scale are arranged in well-defined microscopic concentric rings held together by organic matrix to form laminated spicules. Influenced by the laminated silica-based cement, the assembly of these spicules into bundles results in the formation of a macroscopic cylindrical lattice-like structure reinforced by diagonal ridges (Fig. 1). Hence, there is considerable mechanical benefit to specific arrangements of structural elements at many different hierarchies of scale.

The 3-D topology and specific surface features of hydrozoans have been suggested to initiate faster cell adhesion, proliferation, and differentiation [31]. Further work is needed to determine the exact mechanism of action between cell and material. The potential of a clinically relevant scaffold for a range of tissues such as the bone, cartilage, fat connective, liver, and kidney is accomplished by collagenous marine sponges. The fiber-bonded meshwork of sponges provides channels for cell

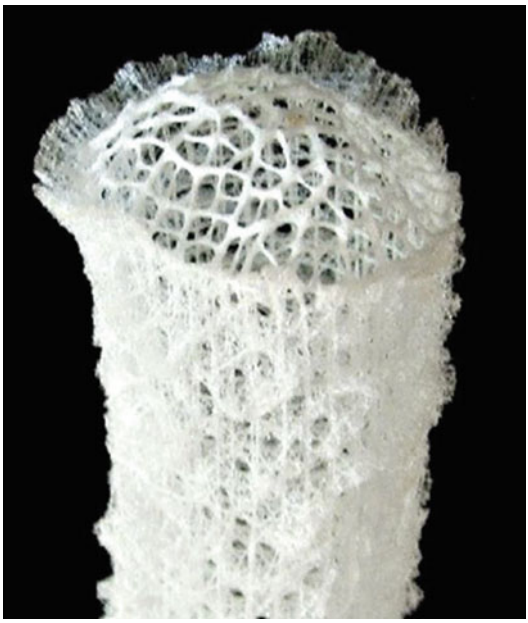

Fig. 1 Glass sponge

guidance along with spaces for rapid tissue infiltration and infilling. The collagenous composition of the fibers has been found to promote attachment of all types of human cells. The unique layered ultrastructure may explain the high wettability and adsorption of growth factors onto the collagen fibers which infuse into attached cells and promote their activities. It has been shown that the formation of tissue in vivo within 4 weeks to be both extensive (completely filling the entire sponge implant) and well developed with the quality and structure of tissue being equivalent to immature bone and neocartilage [32].

Marine Shells

The outer nacreous layer of a certain species of mollusk shell is an unlikely and unexpected source of biomaterial for engineering new bone. Mollusk shells (Fig. 2) are an interesting model for understanding the complexities of biomineralization such as controlling and regulating protein-mineral interactions. *Pinctada maxima*, nacre from the pearl oyster, are different from any other biomaterial that is able to stimulate osteogenesis and bone formation from latent osteoprogenitors along an endochondral pathway, consisting of a cartilage tissue intermediary phase [33].

Testing of nacre has been carried out on human, rabbit, and sheep models [34, 35]. In human patients, fresh woven bone bonded itself all the way through the nacre implant, augmented by the heightened activities of osteoblasts and osteoclasts. Its degradation and resorption are limited even though nacre is stably tolerated in vivo, and this could impede its use within calcified tissue requiring

Fig. 2 Mollusk shell (*Nautilus pompilius*)

rapid self-renewal [34, 35]. While in some way controversial in definition, according to nacre researchers the "water-soluble matrix fraction" (WSM) of nacre directly induces bone formation [36]. Molecules from nacre matrix have been shown to reduce bone resorption by acting on osteoclast metabolism [37]. Evidence available suggests that mobile signal transmitters, involved in the biological control of mineralization (as an initiator and inhibitor of calcium carbonate crystallization at the mineralizing growing front), dissolve into solution-induced differentiation of surrounding latent osteoprogenitor cells [38]. The reason behind exactly how nacre encourages human cells to form new bone can be best explained by the idea that a "signaling" biomolecule is involved in the regulation of cell-mediated biomineralization which is common to both vertebrate bone tissue and nacre. Thus, these biomolecules must have been conserved by the burdens of evolutionary selection.

The supposed osteopromotive effect of nacre as determined by ALP expression is also proportionate with dexamethasone treatment, at least in fibroblasts. Size-exclusion HPLC of the water-soluble matrix has exposed protein fractions rich in alanine and glycine, with specific biochemical effects on human fibroblasts that modulate cell differentiation and proliferation [39]. In the nacre matrix, peptides are prevalent. Specific individual fractions have been revealed to give rise to certain responses from cultured osteoblast cells. The secretion of ALP is increased by protein fractions with low molecular weight (less than 1 kDA), while on the other hand protein fractions with high molecular weight leads to a reduction in ALP secretion. Detailed sequencing of water-soluble proteins using proteomics offers improved characterization of nacre matrix proteins. It has also been discovered that nacre WSM increases the secretion of cytoplasmic Bcl-2, a key inhibitor of apoptosis, as well as having an influence on rat calvarial osteoblast maintenance and survival. Low molecular weight fractions were recently found to increase expression of collagen type I and the ostrogenic-associated mRNA expression of osteopontin and Runx-2 [40].

A further 110 molecules in the 100–70 Da range comprising of glycine-enriched peptides with structural similarities and high affinities for each other were discovered after detailed characterization of the bioactive low molecular weight molecules.

A highly defined matrix protein with a 10 kDa size called p10 has specifically demonstrated an increase in human fibroblast cell ALP expression [41] providing greater expectation that the osteogenic signal molecules can be isolated in their vital functional form. A soluble p60 protein conglomerate extracted from decalcified nacre possesses sufficient bioactivity on 3T3 and MSc to induce the secretion of mineral nodules. Some of the specific biomolecular mechanisms and associations between the signal molecules and cellular processes are being uncovered gradually [1].

Uncertainty arose among biomineralization researchers regarding the fact that nacre proteins are the primary cause of osteoinduction. A study revealed nacre failed to stimulate an in vivo osteogenic response even though bone-to-nacre apposition and bonding did occur directly [42]. The authors suggested that nacre provided a favorable surface chemistry to the recruitment of osteoclasts and osteoblasts. Surface-modified nacre was found not to be osteoinductive within demineralized bone matrix in an in vivo ectopic bone environment; however, its integration and fusion with bone were better than non-nacre controls. In another study, the relationship between interfacial properties and the biocompatibility of nacre and specifically its unique bone-bonding ability was investigated by Kim et al. [43]. They concluded that the organic matrix is the reason behind the excellent bonding that occurs between nacre and bone and is due to the creation of a favorable surface charge for optimal biological associations. The organic matrix of nacre is believed to create a new interfacial microenvironment when implanted that forms many functional associations with the surrounding tissue resulting in a better bone bonding than bioceramic implants without an organic matrix.

The osteogenic responses to nacre particles and pearl preceded much faster following soaking in a simulated body fluid (SBF) that generates a HAp-rich layer on the particles according to a study by Shen et al. [44]. The WSM was implicated in the formation of this HAp layer and the augmented cell responses [36, 40]. Combining all the facts, it can be concluded that nacre provides an appropriate tissue-compatible physical platform that shows unique peptides which initiate and drive bone formation.

Furthermore, due to its organic content and platelike design, nacre is mechanically tough with a fracture toughness equivalent to that of titanium, rapidly biodegradable, and non-immunogenic, without eliciting detrimental physiological effects. These characteristics of nacre offer us a unique substrate for the delivery of a functional and even possibly an osteopromotive agent to sites of bone loss in quantities that lead to rapid bone repair and regeneration.

Sea Urchin

The skeleton of sea urchin spines consisted of large single crystals of magnesium-rich calcite which have smooth, continuously curved surfaces and form a three-dimensional fenestrated mineral network. Through hydrothermal reaction, spines of the echinoids *Heterocentrotus trigonarius* and *Heterocentrotus mammillatus* can be

converted to bioresorbable magnesium-substituted tricalcium phosphate (β-TCMP) at 180 °C. Conversion to β-TCMP occurs preferentially to hydroxyapatite formation due to the presence of magnesium in the calcite lattice. The three-dimensional interconnected porous morphology of the original spine is nonetheless maintained by the converted β-TCMP. It is believed that the primary conversion mechanism is the ion-exchange reaction, even though a dissolution-reprecipitation process taking place that creates some calcium phosphate precipitates on the surfaces of the spine network [1]. Using a rat model, the in vivo studies demonstrated new bone growth up to and around the β-TCMP implants after installation in femoral defects for 6 weeks. Some new bone was found to migrate through the spine structural pores indicating good bioactivity and osteoconductivity of the porous β-TCMP implants [1].

The skeletal plates of sea urchin are perforated by a very regular series of pores. Approximately three quarters of the pores are exits for tube feet (200 μm pore diameters at the spine bases to 600 μm pore diameters for the tube feet in *Centrostephanus nitidus*), and one quarter are channels connected to the alimentary and reproductive systems and to a great extent larger for the accommodation of more fluids (1000–2000 μm per diameter). Echinoderm skeletons are made from a three-dimensional single crystalline meshwork that is both intricately shaped and unique with a topological structure in which every internal pore and channel is in direct contact with all others (periodic minimal surface). Mass transfer and tissue development are likely to be facilitated by this property [13]. Studies revealed that the replication of perforate echinoderm structural elements using the replamine form approach for hard tissue replacements to bone as well as candidate prostheses for the blood vessels and trachea is encouraging. In this context, the skeletal ossicles from the sea star (*Pisaster giganteus*) have been examined, and the results suggested they provide an ideal architecture along with chemical and physical properties beneficial to bone restoration [23].

Similar to bones and teeth, the sea urchin spicule is a composite of organic and inorganic materials that the animal produces using the most readily available elements in seawater. The fully formed spicule consisted of a single crystal with an unusual morphology in three dimensions (Fig. 3). It has no facets and forms a starlike shape. To achieve such unusual morphologies, sea urchin and other marine organisms initially deposit a disordered amorphous mineral phase and then allow it to transform slowly into a crystal aligned neatly into a lattice with a specific and regular orientation and at the same time maintaining their morphology. A unique transformation occurs from disordered amorphous to ordered crystalline structures. This amorphous-to-crystalline phase transformation from the liquid state can teach us to produce highly ordered and well-oriented synthetic nanomaterials and composites.

The sea urchin spicule is created inside a clump of specialized cells and begins as the animal sets down a single crystal of calcite, from which the rest of the spicule is generated. Three arms extend at 120° from each other starting from the crystalline center. The three radii are initially 40–100-nm-size amorphous calcium carbonate then gradually transform to well-organized and oriented calcite. The mechanism is

Fig. 3 Sea urchin with spicules

unclear but may possibly be through dissolution and ordered precipitation mechanisms at known crystallographic orientations of aragonite or calcite.

Marine Skeleton and Organic Matrices in Regenerative Medicine

It has previously been suggested that coral and marine sponge skeletons can support self-sustaining musculoskeletal tissues and that extracts of sponging collagen and nacre seashell organic matrices encourage bone mineralization. The use of ready-made organic and inorganic marine skeletons is one of the simplest possible answers to major problems hampering the future development of regenerative orthopedics such as providing a richness of framework designs and a potentially rich and accessible source of osteopromotive analogues and biomineralization proteins. This fact should not come as a surprise given that the crucial biomineralization proteins that orchestrate bone morphogenesis are also discovered in the earliest calcifying marine organisms. To further reinforce this concept, it has emerged that BMP molecules, the main cluster of bone growth factors for human bone morphogenesis, are secreted by endodermal cells into the developing skeleton. In addition, the regenerative signaling proteins are also present in early marine sponge development and instrumental to the activation of stem cells in cnidarians. Based on this partnership between the main developmental proteins of vertebrates and invertebrates, we have published the extent and nature of this evolutionary connection and used it to support the development of a new strategy which is to source certain marine-based organic matrices for novel metabolic signaling and structural proteins/peptides and protein analogues for applications in regenerative orthopedics, especially when using adult stem cells [13, 45]. Moreover, early-stage evidence based on our own findings revealed the presence of fibrinogen fragments and early osteopromotive effects of a coral organic matrix extract on stem cells [13].

In reality, the discovery of new osteopromotive and osteo-accelerant protein analogues will require the use of traditional chromatography techniques and osteoactivity assays to pinpoint potential protein of significance, while advanced proteomic tools will provide accurate sequencing and determine the mechanisms and molecular pathways involved in osteoactivation as well as the efficiency and effectiveness of marine skeleton-derived protein modulation of the stem cell proteome. Skeletal organic matrices may have ever-increasing function for regenerative orthopedics as more analogues are discovered using proteomic tools [13, 45].

Coral Skeletons in Tissue Engineering

Natural coral exoskeletons have been extensively used in dental, craniofacial, neurosurgery, and orthopedic as a bone replacement due to their combination of good mechanical properties, open porosity, and its ability to form chemical bonds with bone and soft tissues in vivo [3]. Corals in general have the best mechanical properties of all the porous calcium-based ceramics. The organic composition has an important part to play in the excellent mechanical properties and biocompatibility of coral. The abundance, conformation, and composition of the organic matrices are responsible for the successful biological integration of coral with human host [3, 46].

The start of the coral life cycle begins with the polyps which absorb the carbonic acid and calcium ion available in the seawater to generate calcium carbonate in the form of aragonite crystals. The remaining composition composed of trace elements of magnesium, strontium, fluorine, and phosphorous in the phosphate form [47]. These elements play a critical role in the bone mineralization process once implanted in the human body as well as in the activation of key enzymes associated with bone remodeling cells. Strontium has been revealed to play a role in the mineralization process by stimulating osteoblasts while inhibiting osteoclasts [48]. In a similar fashion, bone formation is facilitated by fluorine through similar stimulatory effect on osteoblast proliferation. It is also a well-known fact that magnesium is also beneficial in bone remodeling by improving the mechanical properties of newly formed bone [49].

The use of coral skeletons for general routine orthopedic surgery and tissue engineering has until now been restricted to external fixation devices and unsuitable for strictly load-bearing applications due to their calcium carbonate structure with high dissolution rates. However, sol-gel coating technologies can be utilized to improve the strength of corals, and this will allow them to be used more frequently at different skeletal locations [11].

Either in their natural or hybridized synthetic forms, corals offer great opportunities to tissue engineering of the bone. When combined with in vitro expanded human bone marrow stromal cells (HBMSC), coral skeleton increased osteogenesis greater than those obtained with scaffold only or scaffold with fresh marrow [13]. Orthopedic and maxillofacial surgical studies showed in vivo large animal segmental defect that led to complete re-corticalization and formation of medullary

canal with mature lamellar cortical bone and onlay graft for contour augmentation of the face giving rise to clinical union in a large number of cases [50, 51].

Ehrlich et al. [52] emphasized that, as a result of the unique nanoscale organization of organic tissue and mineral, biomineralization and structural studies of coral can be used to enlighten the development of new advanced functional materials. The deep-sea bamboo coral exhibits at a macrostructural level bone-like biochemical and mechanical properties. A specialized collagen matrix (acidic fibrillar) acts as a model for future potential tissue engineering applications. Opportunities in tissue engineering using coral skeletons have yet to be fully realized and exploited. The matrix supported the growth of both osteoblast and osteoclast, and the exceptional bio-elastomeric properties of the collagen matrix (gorgonin) of this coral make it potentially ideal for use as blood vessel implants. Collagenous gorgonin proteins can be hardened and cross-linked using quinones, and the final product closely resembles human keratin. The mechanism by which gorgonin is synthesized and interacts with the process of mineralization may provide lessons for the production of a synthetic collagen-like material [52].

Marine Structures in Drug Delivery Applications

Slow drug delivery system is a system which is capable of preloaded release of pharmaceutical agent to a site at a specific rate and most importantly at a therapeutically relevant concentration. The primary purpose of this kind of system compared with conventional drug intake such as injection or tablet is to enable the local and specific area delivery, dosage and duration control, and therefore appropriate active drug delivery at the same time causing minimal systemic side effects. The fundamental basis that defines the meaning of a drug delivery system remained unchanged even though technological advancement has produced innovative and refined drug delivery systems. The therapeutic advantages of these systems can be attributed to many underlining features such as the predictability of release rate and minimized drug concentration, thereby reducing any possible adverse systemic effect [53].

Prolonged duration of drug therapy such as the need for frequent re-dosing has been problematic in many global applications of drugs such as the treatment protocol in Africa for malaria. During the development of a drug delivery system, a number of factors are taken into consideration in accordance to the desired application. These include the administration route, the toxicity, the agent to be carried, the physical and chemical properties of the material, the rate of degradation, the loading efficiency, and the practicality for large-scale production, in addition to other parameters.

Various materials such as polymers, ceramics, polysaccharides, and alginate have shown potential advantages as drug delivery systems [54]. In spite of this, marine materials such as coral exoskeletons and marine shells show better potential due to their easy conversion to calcium phosphates, intricate interconnected pores, and their controllable dissolution rates. The interconnectivity and size of the coral pores are critical factors in the amount of coral used as a bone graft and slow drug delivery material (Fig. 4). Furthermore, the uniform porosity of the exoskeletons offers a

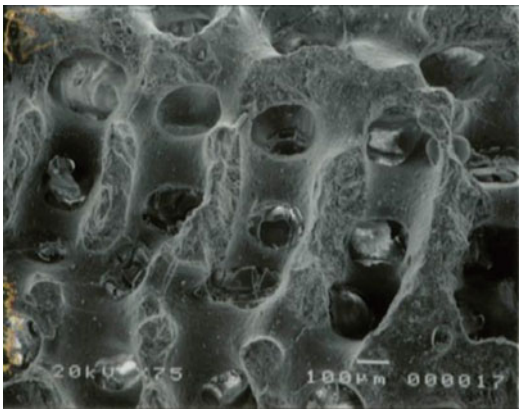

Fig. 4 Coral structure showing micropores

more constant drug loading and therefore providing a more predictable drug release rate of which both are vital factors that directly influence the effectiveness of the drug delivery system.

Foraminifera

Foraminifera are single-celled organisms with shells consisting of multilayer inner chambers commonly divided and added during its growth (Fig. 5). Depending on their environment, different species with different shapes can exist. The microstructure of Foraminifera includes meso- and micro-interconnected pores to assist filtration within marine environment. These observations created a novel idea in drug delivery, and Ben-Nissan and his coworkers applied these structures as slow drug delivery devices incorporating a range of mineral and therapeutic drugs including antibiotics [53]. Natural spheres loaded with drugs can spontaneously degrade and progressively release entrapped biological contents introduced during synthesis.

One significant and successful example of biomimetic materials chemistry applied to the delivery of drugs and/or genes utilizes template-mediated mineralization chemistry within a complex organized 3-D reaction field with patterning that mimics plankton shells. The design of this chemical system is to integrate processes of "self-organization" and "self-assembly" in space. In this manner, constructs that are created provide many clear advantages for tissue engineering as a physical template and a device for controlled release of a variety of drugs, proteins, genes, BMP, and growth factors. Biological molecules can be safely incorporated during synthesis as the chemistry consists of an aqueous phase and occurs at room temperature.

Another functional success has been the possibility for drug incorporation and delivery from nano-, micro-, and macrospheres. It was during an overseas summer break where a discovery was made by one of the authors of this chapter. After a close inspection, the sand on the beach appeared as perfect spheres and showed quite

Fig. 5 Star Foraminifera structure from Australia

unique intricate structures. A small sample was taken back to our laboratory, and it was revealed the sand was in fact calcium carbonate marine shells instead of silica sand after scanning electron microscopy (SEM) and X-ray diffraction (XRD) analysis. After proper characterization, the marine structure was identified and it belongs to the Foraminifera family.

Spherical fossilized shells *Floresianus* (Foraminifera) from coral beach sand from the Great Barrier Reef in Australia were also collected. The samples were intact, lacked spines, and measured 0.5–1.5 mm in diameter. These "shells" or a more appropriate term "microspheres" possess unique fenestrated structures that have evolved to collect sunlight and circulate seawater for the mutual benefit of symbiotic algal cells that reside inside the shell. SEM and microcomputed tomography (μ-CT) imaging confirmed that these shells were internally permeated by a 3-D network of microscopic interconnected channels measuring 1–10 μm in diameter. A separate chapter in this book authored by Chou et al. will cover the details of some of the work on Foraminifera as drug delivery devices.

Before any marine material can be utilized as a graft or a drug delivery vehicle, they must first go through a laborious process to investigate the composition, morphology, purity, and suitability for drug loading and its slow dissolution without causing any adverse effect to the patient. Except in special cases, protein and organic matter are required to be extracted prior to the sterilization of calcium carbonate material. Any residual organic constituents are removed by immersing in sodium hypochlorite solution and then dried at a temperature of approximately 100 °C [13].

It is essential to hydrothermally convert these microsphere shells into β-TCP and/or HAp which is more stable and highly crystalline using the methods developed and published earlier [3]. In certain situations of drug delivery applications, β-TCP exhibits a more ideal composition compared with other calcium phosphates.

The original structure of the microspheres is not altered during the chemical conversion to calcium phosphate making the adsorption of selected drug compounds

possible as well as allowing new bone cells to penetrate into the micropores after bone-graft implantation. In the physiological environment, these microspheres can dissolve and supply calcium and phosphate ions in addition to the drugs incorporated to the immediate bone structure as a result of their pore architecture. The release profile shows relatively slow, local release of drugs such as bisphosphonate, gentamicin, and simvastatin from micro- and macrospheres for extended periods [16, 53].

Marine Structures in the Regulation of Stem Cells

Similarly, there is an increase in awareness that the composition of a scaffold material is critically important for the establishment of stem cell activities as they are dependent on the extracellular fabric for life support and to guide their subsequent evolution and development [2]. This can be accomplished through the use of natural structural biomaterials as well as their derivatives and reconstituted forms. Vital aspects of regenerative medicine are to improve stem cell processing, provide microenvironments that are better at regulating tissue formation and development, and manufacture less invasive transplantation modules with site-specific targeting properties. These can be demonstrated with the recreation of a mesenchymal stem/progenitor cell niche for regulating cell proliferation and outcome.

At the moment, an active area of research is the recreation of a native stem cell environment in which stem cells such as those of the bone marrow are protected, managed, and stabilized as self-renewing undifferentiated cells and instructions on how to regulate the rate of progenitor and replacement cells produced are provided. Nurcombe and Cool [55] suggested the glycosaminoglycan heparin sulfate is the master molecule controlling the dynamics of almost all stem cell functions.

Creating a unique and customized environment which is capable of regulating the differentiation and proliferation of stem cells is a fundamental goal intended at preserving their characteristics in artificial culture and for subsequent transplantation. Research groups from around the world have coordinated stem cell activities within microcapsules using a number of templates including polysaccharides. Cells are immobilized by the polysaccharide hydrogels in three-dimensional configurations lightly controlled by the substrate viscosity, additives used, and initial cell seeding density. Their broad applicability and versatility are significantly better compared to typical synthetic materials for most tissues within the human body. It has been reported that some of the most significant tissue responses have been observed when native extracellular matrix fragments of high molecular weight are embedded within polysaccharide frameworks. For instance, the addition of human aggrecan glycosaminoglycan to biomineral-coated chitosan/alginate microcapsules accelerated the endogenous production of cartilage matrix from embedded chondroprogenitors. However, it is important that proper modifications of chitosan and alginate are required to optimize their biological qualities as this will play a crucial role in the clinical outcomes. For example, it is now necessary to partially oxidize sodium alginate with sodium periodate to ensure a smooth and consistent

degradation which does not otherwise occur. The human body does not naturally produce enzymes that can promptly degrade alginate in its polymeric form.

Another significant aspect regarding the precise chemistry of alginate polymer chains is the composition and arrangement of the component sugar units. These properties have important effects on their relative bio-effectiveness. Due to their hydrophilic nature, protein deposition is more selective than on hydrophobic surfaces and more likely to recruit adhesion proteins. This can detrimentally reduce cell anchorage/attachment which has important consequences for cell survival as well as the regulation of migration, differentiation, and proliferation [56].

Therefore, it is important to increase cell attachment through blending candidate proteins with alginate or binding peptide sequences to the alginate biopolymer. The bio-responsiveness is characterized and directed by the sequential pattern of sugar residues along the alginate polymer chain. Higher mannuronic/glucoronic ratios resulted in smaller pore sizes. Macrophages and lymphocytes are stimulated by mannuronic-rich alginates. However, high-glucoronic alginates are preferred as they are more immune-suppressive in nature.

The sustained delivery of gene and protein therapeutics is a new and promising area of interest specifically in cancer research. It has been proven somewhat elusive when it comes to providing the correct dosage of regenerative factors in appropriate temporal sequence. Gene correction strategies have evolved to overcome this significant issue. Stem cell-mediated gene therapy using non-viral transduction agents is dependent on synthetic biomaterials, lipids, and physical disruption of the cell membrane to permit the entry of foreign genes. On the other hand, significant cell toxicity can be associated with these approaches. Alginate-chitosan matrices have been found to yield minimal cell toxicity of less than 5 % [13].

It is essential that the added bioactive factors are released in precise sequences at cell-instructive doses and at specific time frames in synchronization with the body's own biochemistry. Such actions will ensure that the added biological factors provide their maximum effect and potency. One of the primary issues is the release of individual factor in a slow and sustained manner for long periods of time to permanently restore tissue function. The release of encapsulates can be regulated in one of two ways. The first approach is to modify the composition and thickness of the capsule shell resulting in slow diffusion. This approach has been proven effective at delaying the release of plasmid deoxyribose nucleic acid.

On the other hand, it is also possible to create nested arrangements of bead within another bead. The host-guest arrangement of capsules can be an effective mechanism for temporal control of encapsulates. This idea was verified when the exogenous release of tyrosinase from embedded guest capsules was significantly delayed when compared to tyrosinase release from the surrounding host capsule. An additional mechanism for regulating encapsulates is to entrap them between shell layers or within one of a succession of layers such as entrapping BMP-2 at the interface between alginate and chitosan. Furthermore, it is also possible to coat successive alternating layers of positively and negatively charged polysaccharides around the original germinal core. The adaptability and versatility of this simple-to-construct delivery system are potentially suited to a wide range of applications. This can be

demonstrated by adding a series of bioactive proteins individually inside each, and later, as the layers peel away from the outer surface toward the core over time, the encapsulated protein is released in a sequential manner. Similarly, engineering an intricate series of protected domains within each shell is possible by laminating each applied shell layer with calcium phosphate. Then again, cells can be entrapped inside the individual layers. There is the capability with such assemblies of creating physiologically significant concentration gradients of genes, growth factors, and proteins within each capsule.

Concluding Remarks

Marine structures have been widely explored during the past decade from the imitation of efficient designs of nature or biomimetics to soft and hard tissue engineering as well as from slow or controlled drug delivery to biosensors and bioreactor applications. The new approaches include the use of natural organic and inorganic skeletons, micro- and nanoscale slow drug delivery devices, new medical treatment protocols inspired by unique designs, and devices incorporating stem cells, proteins, and peptides.

In nature, structures possess desirable properties, and gradually we are discovering new ways of reproducing nature to create similar levels of sophistication even though only to a limited extent. One versatile approach is to use biological microstructures as templates for the reproduction of inorganic structures with identical features. They have a distinct consequence to the production of replacements for calcified tissues. This is achieved by using techniques in biomineral-inspired materials chemistry. The concept is to utilize the consecutive developmental pathway of systems that nature employs to make skeletons from molecules into micro- and macroscopic structures.

Additional studies of the manner of natural materials are constructed, and the condition they adapt to their environment will allow us to produce a breathtaking array of self-responsive structures and materials for regenerative medicine, structural applications, and applied engineering materials. In nature, biomaterials are composed of perfect resource and energy efficiency using common and readily available substrates through self-assembly into highly organized hierarchies. Examining the synthesis and design methods from these natural as well as marine structures will provide us with an opportunity to create structures with complex shapes and architectures that are customized to their functions and do not break down. Previously, science and engineering have shown us in what way biomimetic approaches can yield promising outcomes for application in tissue engineering of skeletal tissues. Current work is part of the continuing research toward the design of scaffolds that are clinically relevant for regenerative medicine using a unique set of self-organizing hierarchical structures invented and produced according to biological principles of design is very promising.

At present, there is a clear need for better tissue engineering scaffolds that possess more natural bio-responsive environments favorable to guiding the natural

procedures of regeneration which can be extremely intricate and dynamic in time and space. Therefore, intelligence must be incorporated into the scaffold designs to meet this biological challenge. We argue that there needs to be a transformation to scaffold environments that are responsive whereby the synthesized biomatrix evolves in real time to meet the demands and optimize for the adaptive growth and regeneration of human tissues. The environments of the scaffolds are adjusted as cells proliferate and differentiate.

Advanced biomimetic scaffolds in the future must be capable of adapting to these changes and undergo the ever-changing needs of developing tissues. It is anticipated the synthesis of biomaterial scaffolds with functional cross-links and pendant side groups that interact with surrounding cell population at three different levels: nano/meso (at the contact interface), micro (at the architectural level), and macro (at the bio-functional level). Nanofabrication utilizing biological principles of assembly and design is still in its infancy. The application of this bio-inspired nanofabrication for tissue engineering is a unique approach that has vast potential to improve scaffold design and shape physicochemical environments with the ability to micro-evolve. To learn from nature and grow materials with cells and promote the regulation of material synthesis with biogenics including antibodies, proteins and peptides are the most pertinent challenges.

References

1. Ben-Nissan B, Green DW (2013) Marine materials in drug delivery and tissue engineering: from natural role models, to bone regeneration and repair and slow delivery of therapeutic drugs, proteins and genes. In: Kim S-K (ed) Marine biomaterials. Taylor and Francis/CSR Books, Boca Raton, pp 575–602
2. Green DW, Li G, Milthrope B, Ben-Nissan B (2012) Adult stem cell coatings using biomaterials for regenerative medicine. Mater Today 15:61–68
3. Ben-Nissan B (2003) Natural bioceramic: from coral to bone and beyond. Curr Opin Solid State Mater Sci 7:283–288
4. Mann S (1983) Mineralization in biological systems. Struct Bond 54:125
5. Gonzalez-McQuire R, Green D, Walsh D et al (2005) Fabrication of hydroxyapatite sponges by dextran sulfate/amino acid templating. Biomaterials 26:6652–6656
6. Green D, Walsh D, Yang X et al (2004) Stimulation of human bone marrow stromal cells using growth factor-encapsulated calcium carbonate porous microspheres. J Mater Chem 14:2206–2212
7. Walsh D, Mann S (1996) Feigning nature's sculptures. Chem Br 32:31–34
8. Walsh D, Boanini E, Tanaka J et al (2005) Synthesis of tri-calcium phosphate sponges by interfacial deposition and thermal transformation of self-supporting inorganic films. J Mater Chem 15:1043–1048
9. Hall SR, Swinerd VM, Newby FN et al (2006) Fabrication of porous titania (brookite) microparticles with complex morphology by sol-gel replication of pollen grains. Chem Mater 18:598–600
10. Green DW (2004) Bio-inspired ceramic structures: from invertebrate marine skeletons to biomimetic crystal engineering. J Aust Ceram Soc 40:1–7
11. Ben-Nissan B, Choi AH (2006) Sol-gel production of bioactive nanocoatings for medical applications. Part I: an introduction. Nanomedicine 1:311–319

12. Parker AR, Martini N (2006) Structural color in animals-simple to complex optics. Opt Laser Technol 38:315–322
13. Ben-Nissan B, Green DW (2014) Marine structures as templates for biomaterials. In: Ben-Nissan B (ed) Advances in calcium phosphate biomaterials, Springer series in biomaterials science and engineering (SSBSE). Springer, Berlin, pp 391–414
14. Mock T, Samanta MP, Iverson V et al (2008) Whole-genome expression profiling of the marine diatom *Thalassiosira pseudonana* identifies genes involved in silicon bioprocesses. Proc Natl Acad Sci U S A 105:1579–1584
15. Belegratis MR, Schmidt V, Nees D et al (2014) Diatom-inspired templates for 3D replication: natural diatoms versus laser written artificial diatoms. Bioinspir Biomim 9:016004
16. Kim ES (2008) Directed evolution: a historical exploration into an evolutionary experimental system of nanobiotechnology, 1965–2006. Minerva 46:463–484
17. Sia SK, Gillette BM, Yang GJ (2007) Synthetic tissue biology: tissue engineering meets synthetic biology. Birth Defects Res C Embryo Today 81:354–361
18. LeGeros RZ (1993) Biodegradation and bioresorption of calcium phosphate ceramics. Clin Mater 4:65–88
19. LeGeros RZ, Gatti AM, Kijkowska R et al (2004) Magnesium tricalcium phosphate: formation and properties. Key Eng Mater 254–256:127–130
20. Ando J (1958) Tricalcium phosphate and its variation. Bull Chem Soc Jpn 31:196–201
21. Roy DM, Linnehan S (1974) Hydroxyapatite formed from coral skeleton carbonate by hydrothermal exchange. Nature 247:220–222
22. Rocha JHG, Lemos AF, Agathopoulos S et al (2005) Scaffolds for bone restoration from cuttlefish. Bone 37:850–857
23. Martina M, Subramanyam G, Weaver JC et al (2005) Developing macroporous bicontinuous materials as scaffolds for tissue engineering. Biomaterials 26:5609–5616
24. Townley HE, Parker AR, White-Cooper H (2008) Exploitation of diatom frustules for nanotechnology: tethering active biomolecules. Adv Funct Mater 18:369–374
25. Boute N, Exposito JY, Boury-Esnault N et al (1996) Type IV collagen in sponges, the missing link in basement membrane ubiquity. Biol Cell 88:37–44
26. Exposito JY, Cluzel C, Garrone R et al (2002) Evolution of collagens. Anat Rec 268:302–316
27. Swatschek D, Schatton W, Kellermann J et al (2002) Marine sponge collagen: isolation, characterization and effects on the skin parameters surface pH, moisture and sebum. Eur J Pharm Biopharm 53:107–113
28. Nicklas M, Schatton W, Heinemann S et al (2009) Preparation and characterization of marine sponge collagen nanoparticles and employment for the transdermal delivery of 17b-estradiol-hemihydrate. Drug Dev Ind Pharm 35:1035–1042
29. Aizenberg J, Weaver JC, Thanawala MS et al (2005) Skeleton of *Euplectella* sp structural hierarchy from the nanoscale to the macroscale. Science 309:275–278
30. Miserez A, Weaver JC, Thurner PJ et al (2008) Effects of laminate architecture on fracture resistance of sponge biosilica: lessons from nature. Adv Funct Mater 18:1241–1248
31. Abramovitch-Gottlib L, Geresh S, Vago R (2006) Biofabricated marine hydrozoan: a bioactive crystalline material promoting ossification of mesenchymal stem cells. Tissue Eng 12:729–739
32. Vago R, Plotquin D, Bunin A et al (2002) Hard tissue remodeling using biofabricated coralline biomaterials. J Biochem Biophys Methods 50:253–259
33. Lopez E, Vidal B, Berland S et al (1992) Demonstration of the capacity of nacre to induce bone formation by human osteoblasts maintained in vitro. Tissue Cell 24:667–679
34. Lamghari M, Berland S, Laurent A et al (2001) Bone reactions to nacre injected percutaneously into the vertebrae of sheep. Biomaterials 22:555–562
35. Lamghari M, Antonietti P, Berland S et al (2001) Arthrodesis of lumbar spine transverse processes using nacre in rabbit. J Bone Miner Res 16:2232–2237
36. Rousseau M, Lucilia PM, Almeida MJ et al (2003) The water-soluble matrix fraction from the nacre of *Pinctada maxima* produces earlier mineralization of MC3T3-E1 mouse pre-osteoblasts. Comp Biochem Physiol B 135:1–7

37. Duplat D, Chabadel A, Gallet M et al (2007) The in vitro osteoclastic degradation of nacre. Biomaterials 28:2155–2162
38. Westbroek P, Marin F (1998) A marriage of bone and nacre. Nature 392:861–862
39. Almeida MJ, Pereira L, Milet C et al (2001) Comparative effects of nacre water-soluble matrix and dexamethasone on the alkaline phosphatase activity of MRC-5 fibroblasts. J Biomed Mater Res 57:306–312
40. Rousseau M, Boulzaguet H, Biagianti J et al (2007) Low molecular weight molecules of oyster nacre induce mineralization of the MC3T3-E1. J Biomed Mater Res A 85:487–497
41. Zhang C, Li S, Ma Z et al (2006) A novel matrix protein p10 from the nacre of pearl oyster (*Pinctada fucata*) and its effects on both $CaCO_3$ crystal formation and mineralogic cells. Marine Biotechnol 8:624–633
42. Liao H, Mutvei H, Hammarstrom L et al (2002) Tissue responses to nacreous implants in rat femur: an in situ hybridization and histochemical study. Biomaterials 23:2693–2701
43. Kim YM, Kim JJ, Kim YH et al (2000) Effects of organic matrix proteins on the interfacial structures at the bone-biocompatible nacre interface in vitro. Biomaterials 23:2089–2096
44. Shen Y, Zhu J, Zhang H et al (2006) In vitro osteogenic activity of pearl. Biomaterials 27:281–287
45. Green DW, Padula MP, Santos J et al (2013) A therapeutic potential for marine skeletal proteins in bone regeneration. Mar Drugs 11:1203–1220
46. Vago R (2008) Beyond the skeleton. Cnidarian biomaterials as bioactive extracellular micro-environments for tissue engineering. Organogenesis 4:18–22
47. Stanley G (2003) The evolution of modern corals and their early history. Earth Sci Rev 60:195–225
48. Bonnelye E, Chabadel A, Saltel F et al (2008) Dual effect of strontium ranelate: stimulation of osteoblast differentiation and inhibition of osteoclast formation and resorption in vitro. Bone 42:129–138
49. LeGeros R (1981) Apatites in biological systems. Prog Cryst Growth Charact Mater 41:1–45
50. Papacharalambous S, Anastasoff K (1993) Natural coral skeleton used as onlay graft for contour augmentation of the face. A preliminary report. Int J Oral Maxillofac Surg 22:260–264
51. Leupold J, Barfield W, An Y et al (2006) A comparison of ProOsteon, DBX, and collagraft in a rabbit model. J Biomed Mater Res B Appl Biomater 79:292–297
52. Ehrlich H, Etnoyer P, Litvinov SD et al (2006) Biomaterial structure in deep-sea bamboo coral (Anthozoa: Gorgonacea: Isidiae): perspectives for the development of bone implants and templates for tissue engineering. Mater Werkst 37:552–557
53. Chou J, Valenzuela SM, Green DW et al (2014) Antibiotic delivery potential of nano and micro porous marine structures derived β-TCP spheres for medical applications. Nanomedicine 9:1131–1138
54. Bose S, Tarafder S (2012) Calcium phosphate ceramic systems in growth factor and drug delivery for bone tissue engineering: a review. Acta Biomater 8:1401–1421
55. Nurcombe V, Cool SM (2007) Heparan sulfate control of proliferation and differentiation in the stem cell niche. Crit Rev Eukaryot Gene Expr 17:159–171
56. Rowley JA, Madlambayan G, Mooney DJ (1999) Alginate hydrogels as synthetic extracellular matrix materials. Biomaterials 20:45–53