Chapter 13
Marine Structures as Templates for Biomaterials

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Abstract During the last two decades, "learning from nature" has given us new directions for the use of natural organic and inorganic skeletons, drug delivery devices, new medical treatment methods initiating unique designs and devices ranging from nano- to macroscale. These materials and designs have been instrumental to introduce the simplest remedies to vital problems in regenerative medicine, providing frameworks and highly accessible sources of osteopromotive analogues, scaffolds and drug delivery device proteins. This is exemplified by the biological effectiveness of marine structures such as corals and shells and sponge skeletons, extracts of spongin and nacre, sea urchin, sea snails and *Foraminifera*. Organic matrix and inorganic marine skeletons possess a habitat suitable for proliferating added mesenchymal stem cell populations and promoting clinically acceptable bone formation. A wide range of applications of these marine structures and their conversion methods are covered by excellent review papers and chapters. In this chapter based on our research, published work and book chapters, we aim to cover the nature, morphology and the use of some of these structures for tissue engineering, bone grafts, drug delivery and specific extracts such as proteins for regenerative medicine.

Keywords Marine structures • Nacre • Coral • Sponges • Sea urchin • Protein • Bone grafts • Drug delivery

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13.1 Introduction

Just after the "general relativity theory" was published, Eduard, Einstein's younger son, asked "how come he was so famous". He smiled and remarked that "When a blind beetle crawls over the surface of a curved branch, it doesn't notice that the track it has covered is indeed curved"; he quietly added to that, "I was lucky enough to notice what the beetle didn't notice".

The term "learning from nature" or "biomimetics" has been a very promising and satisfactory concept that allows us to improve the efficiency and the functionality of an engineering design or device. Nature has been the most inspiring creative engineering source in the human history. Throughout history we have been trying to learn to mimic nature's designs to develop application for solving problems either in our daily life, in engineering, in science or most recently in medicine with unique designs and approaches.

Nature can teach us in many ways on how to build structures, design architectures and fabricate materials and substances with exemplary high performance for many functions. The study of biomimetics and marine structures has provided approaches for generating unique inorganic scaffolds and organic materials for use in regenerative medicine with the potential to outperform conventional advanced man-made functional materials. The way natural systems allocate energy for different functions can instruct us on how to best optimise the design of materials and structures with minimum energy use and with molecular or nano-level control. For example, both bone formation and resorption depend on the same principles.

The essence of better performing natural materials compared to their synthetic counterparts is to give more attention to design and optimisation, the property of self-assembly, approach to the problems with mixed laminated and/or mixed organic–inorganic composites and control and repair of fracture. The building functions of organisms make use of "bottom-up" chemical processes over many length scales.

By harnessing these processes including many nanoscale ones, we have the potential to generate "living materials" that adapt and respond to their surroundings [1]. There is now greater appreciation and understanding of the significance of bio-inspired and nanoscale approaches than ever before to the generation of new medical materials and devices. The structural component of tissue engineering is the most amenable to this type of analysis alongside the increased sophistication of materials chemistry and new nanofabrication methods to provide exceptional biomimetic solutions.

This review is based on many published book chapters and papers that cover only a small fraction of an enormous range and richness of marine structures. This chapter outlines the development and progress of biomimetic approaches in regenerative medicine and provides worked examples from our own research and fellow researchers specifically on inorganic natural nano- and microporous structures.

13.2 Biomimetics and Evolution

The translation of products from nature into technology is fundamental and the most powerful and successful way of resolving technological and scientific problems. Natural history collections are a unique and rich source of practical ideas and solutions for the initial stages of tissue reassembly in artificial culture. Studying the chemistry, the evolution of tissues and organs, their function and design is an undiscovered route to provide elements that can be used to reconstruct tissues in the simplest and most practical way possible. We can even use fossilised organisms as good models for providing new materials. While nature cannot produce the perfect designs, it can generate the most ideal, optimised and functional adaptive ones.

Biological structures and biomaterials have evolved by natural selection over many millions of years of strict conditioning, smoothing out trade-offs between conflicting demands and limitations of an environment in order to maximise fitness. These conflicts are omnipresent but can be partially resolved to generate extremely well-functioning materials. The end results are biomaterials with compromises that exhibit high levels of performance made with minimum use of energy.

The evolution of tissues by natural selection provides us with a view of how different strategies of development have been harnessed by organisms according to function. As a result we should be able to provide simplified assembly strategies to recreate functional approximations of every human tissue.

One of the most fascinating bio-inspired approaches is to directly use cells and organisms to grow biomaterials and grow them to our specifications and requirements literally in the beaker or test tube [2]. This can be achieved by judicious modulation of the growing environment. Single-celled organisms such as diatoms, Foraminifera and coccolithophores are a convenient starting point as they are the most rudimentary and elementary organisms to grow and support in artificial culture and provide enough utility for proving this approach as practically beneficial. Diatoms are of great interest for the development of new strategies in nanotechnology and molecular assembly as they provide modes of construction at these scales that could benefit the development of new generation biomedical devices such as miniature biosensors. Diatoms have even been described as “natural-born lithographers” in recognition of the technique [3]. Sussman is exploiting the mechanisms of patterning by diatoms to use in patterning microchips. Others have suggested using them as drug-eluting modules because of their beneficial microscopic size and reticulated internal pore structure [4]. Growing materials with living cells integrated during synthesis and construction is an attractive proposition. In this way the directed evolution may be possible with specific organisms that rapidly reproduce so that many thousands of generations are produced in short experimental periods. Protocols are well established now for the mass production of new proteins using a combination of site random mutagenesis followed by high-throughput screening [5].

Synthetic tissue biology is a newly emerging discipline which seeks to engineer tissues and form them into complex biological assemblages [6]. One approach in this endeavour is to reverse engineer biological materials, tissues, organs or systems to "decipher" how they are put together and how they operate at highest level of detail. It can be suggested that it can revolutionise the concepts and approach for re-engineering biological systems.

Synthetic biology for forming multicellular tissues uses the most advanced methods available for building extracellular environments to direct morphogenesis of cells and tissues. In another way cells are designed and constructed with novel functions and coaxed into multicellular organisations.

In the vast diversity of nature, there are countless identifiable "ground" plans about how to construct and organise cells and tissues into organs. Matter joins together in an evolution by natural selection which has consistently originated assembly rules and design solutions that have been conserved and reapplied in organisms throughout the gradual ascendency and emergence of new forms of life.

13.3 Biomaterial Synthesis and Production

A century ago, artificial devices were made from materials as diverse as gold and wood and were developed to a point where they could replace various components of the human body. These materials are capable of being in contact with bodily fluids and tissues for prolonged periods of time, "whilst eliciting little, if any, adverse reactions". When these synthetic materials are placed within the human body, the tissues react towards the implant in a variety of ways. The mechanism of tissue interaction at a nanoscale level is dependent on the response to the implant surface. As such, three terms for description of a biomaterial, representing the tissue responses, have been defined by Hench and West, in 1990, as bioinert, bioresorbable and bioactive [7].

Based on the acceptance of the importance of tissue–implant interactions on the nanoscale, quite extensive development of nanotechnology in science and engineering has taken place during the last two decades. This does not come as a surprise, considering that functional nanostructured materials have the capability of being adapted and integrated into a range of engineering and biomedical devices. This is because most biological systems, including viruses, membrane and protein complex, exhibit natural nanostructures. The microstructure and properties of these new generation nanostructured materials depend on the synthesis method as well as on the processing routes. Hence it is of extreme importance to select the most appropriate technique for the preparation of materials with desired designs and property combinations.

Synthesis techniques commonly used for the production of inorganic materials such as advanced ceramics include a range of solid state, liquid state and gaseous ionic state processing methods. Wet chemical processing techniques such as

co-precipitation and sol–gel have been commercially employed to obtain nanoparticles, nanocoatings and nanostructured solid blocks and shapes. In modern ceramic technology pressing is accomplished by placing the powder into a die and applying pressure to achieve compaction. Hot pressing (HP) and hot isostatic pressing (HIP) are the most commonly used methods for the production of bioceramics. HIP can induce the higher densities and small grain structures required for good mechanical properties, whereby heat and pressure are applied simultaneously and the pressure is applied from all directions via a pressurised gas such as helium or argon. Flat plates or blocks and non-uniform components are relatively easily produced using HP or HIP.

Sol–gel processing is unique in that it can be used to produce different forms, such as powders, platelets, coatings, fibres and monoliths of the same composition, merely by varying the chemistry, viscosity and other factors of a given solution [8]. The advantages of the sol–gel technique are numerous: it is of the nanoscale; it results in a stoichiometric, homogeneous and pure product, owing to mixing on the molecular scale; high purity can be maintained as grinding can be avoided; it allows reduced firing temperatures due to its small particle sizes with high surface areas; it has the ability to produce uniform fine-grained structures; it allows the use of different chemical routes (alkoxide or aqueous-based); and it is easily applied to complex shapes with a range of coating techniques. Shrinkage up to a number of coatings, depending on the chemistry, is fairly uniform perpendicular to the substrate and the coatings can dry rapidly without cracking. However, shrinkage becomes an important issue in monolith ceramic production [8].

Most biomaterials aimed to be used within the physiological environment require appropriate surface finish to allow soft or hard tissue attachment without any adverse reaction. In addition biomaterials for hard tissue attachment need similar chemical composition of bone. Hard tissues of nearly all animals include calcium and phosphate ion combinations as calcium phosphate compounds with a variety of minerals. The structure of the bone consists of nano-to-micron range interconnected pores. To emulate and produce these intricate designs synthetically is difficult or in some instances nearly impossible due to the restrictions in resolution of the currently used production techniques although new generation 3D printing techniques might be one of the most recent techniques to achieve this difficult task in the near future.

On the other hand, nature has the answer to these intricately fine porous structures: some marine structures by their virtue of natural need contain excellent interconnected pores and architectures that can encounter and solve the problems mentioned above. They have very fine nanometre to a few hundred micron range interconnected pores and excellent mechanical properties. In addition most are made of or contain inorganic materials such as calcium carbonate and calcium phosphates with a range of minerals containing Mg, Sr and Si that helps to improve the properties of hard tissues after implantation. The organic matter within the marine skeletons (although small) contains a range of materials such as proteins with very promising possible medical applications [9].

13.4 The Inorganic Matter and Proteins

The marine environment is uniquely rich in structures with highly functional architectures with interconnected open pores, which offer high mechanical strength and chemical compositions suitable for use as is or converted to materials appropriate for human implantation.

Currently an increasing number of compounds and materials are being identified from marine organisms such as calcium carbonates to proteins and applied to medical applications. In this regard, marine species have been a valuable resource for the discovery of novel active pharmaceuticals [9].

In applications for tissue engineering, coral skeletons and converted coralline apatites are exquisite examples [10]. They have demonstrated substantial clinical success as templates for tissue reconstruction. This has spurred on researchers to look at other skeletons with better mechanical and/or biological properties. These unique 3-dimensional marine structures supported growth and enhanced differentiation of stem cell progenitors into bone cells unlike standard carbonate frameworks that do not induce stem cell differentiation.

These marine materials and designs have been instrumental to introduce the simplest remedies to vital problems in regenerative medicine, providing frameworks and highly accessible sources of osteopromotive analogues and mineralising proteins. This is exemplified by the biological effectiveness of marine structures (including corals and shells and sponge skeletons) to house self-sustaining musculoskeletal tissues and to the promotion of bone formation by extracts of spongin. Biological molecules that pivotal to the regulation and guidance of bone morphogenesis and particularly the events in mineral metabolism and deposition were the first molecular components established for calcification, morphogenesis and wound healing. It emerges that bone morphogenic protein (BMP) molecules, the main cluster of bone growth factors for human bone morphogenesis, are secreted by endodermal cells into the developing skeleton. Signalling proteins, TGF-β and Wnt-prime targets in bone therapeutics are present in early marine sponge development. Furthermore, ready-made organic and inorganic marine skeletons possess a habitat suitable for proliferating added mesenchymal stem cell (MSC) populations and promoting clinically acceptable bone formation [11].

13.5 Marine Skeletons

Using natural skeletons in a direct way as a scaffold for growing cells into tissue emerged for making new bone tissue, as a product of hydrothermal processing [12]. Transformed coral (converted to calcium phosphates) has been the primary source of natural skeletons for bone tissue engineering because of its chemical, crystallographic and structural complementarity to native human bone [13]. For the same reasons, since then, researchers have made use of invertebrate marine skeletons of

hydrozoans, cuttlefish [14], marine sponges [1], nacre sea shell and echinoderm spines [15] as templates with optimal ranges of pore sizes, channels and structural networks for organising and nourishing the growth of human tissues as a prelude to transplantation into the patient. In other developments whole natural skeletons (without conversion to HAp) have been used as templates for carrying biomolecules. Accordingly diatom skeletons have been tethered with active biomolecules such as an antibody to be used in immunodiagnostics [16]. Mollusc shells are a fascinating model for understanding the complexities of biomineralisation such as the control and regulation of protein–mineral interactions [17].

Considerable research efforts have been focused on the development of efficient and cost-effective methods to produce calcium phosphate with apatite structure from biogenic natural materials. These include hydroxyapatite (HAp), tricalcium phosphate (both α- and β-TCP), tetracalcium phosphate (TTCP) and octacalcium phosphate (OCP). Different natural materials composed of calcium carbonate and possessing unique architecture like coral [10, 18–20], sea urchins [21, 22], pearl [23], nacre [24], Mediterranean mussel [25], sea and land snails [26] and cuttlefish [14] have been reported to have potential to be used or produced as calcium phosphate materials for biomedical applications. Past research shows that we have so far identified candidate biomatrices in nature, with varied chemical homologies and structural analogies to human extracellular matrices and whole tissues. The utility of selected species of these marine animals has been applied to the regeneration of human bone and cartilage. However, their full utility in these tissues and other tissues has yet to be harnessed and fully exploited.

13.6 Marine Shells (Nacre)

Unlike any other biomaterial, nacre from the pearl oyster, *Pinctada maxima*, is able to induce osteogenesis and bone formation from latent osteoprogenitors along an endochondral pathway, consisting of a cartilage tissue intermediary phase [27].

The outer nacreous layer of a particular species of mollusc shell is an unlikely and unexpected source of biomaterial for engineering new bone. In the absence of synthetic chemistry, natural biomaterials were widely used by physicians from ancient civilisations of India, China, Egypt and Central America, and it was the ancient Mayans who discovered the unique property of nacre to heal seamlessly onto living human bone without causing harm. The scientific basis of fusion with bone was first uncovered by Lamghari and Lopez. Under closer scrutiny nacre was found to activate skeletal cells, induce bone formation and provide structural support in a clinical trial [28, 29].

Nacre has been tested in human, sheep and rabbit models [28, 29]. In human patients fresh woven bone bonds itself throughout the nacre implant, augmented by the heightened activities of osteoblasts and osteoclasts. While nacre is stably tolerated in vivo, its degradation and resorption is limited, and this could hinder its use within calcified tissue requiring rapid self-renewal [28, 29]. Although somehow

controversial in definition according to nacre researchers, the "water-soluble matrix fraction" (WSM) of nacre directly induces bone formation [30]. Molecules from nacre matrix have been shown to decrease bone resorption by acting on osteoclast metabolism [31]. The available evidence suggests that mobile signal transmitters involved in the biological control of mineralisation (as an initiator and inhibitor of calcium carbonate crystallisation at the mineralising growing front) dissolved into solution induced differentiation of surrounding latent osteoprogenitor cells [32]. The reason why nacre directly induces human cells to form new bone is best explained by the idea that "a signalling" biomolecule involved in regulating cell-mediated biomineralisation is common to both vertebrate bone tissue and nacre. These biomolecules must have been, therefore, conserved by evolutionary selection pressures.

The so-called osteopromotive effect, as measured by ALP expression, of nacre is also commensurate with treatment with dexamethasone, at least in fibroblasts. Size exclusion HPLC of the water-soluble matrix has uncovered protein fractions rich in glycine and alanine, with specific biochemical effects on human fibroblasts that modulate cell differentiation and proliferation [33]. Peptides are prevalent in the nacre matrix. Particular individual fractions have been shown to give rise to specific responses from cultured osteoblast cells. Protein fractions with low molecular weight (less than 1 kDa), for example, upregulated ALP secretion, whereas high molecular weight fraction reduced ALP secretion. Detailed sequencing of water-soluble proteins using proteomics offers enhanced characterisation of nacre matrix proteins. Nacre WSM was also shown to increase the secretion of a key inhibitor of apoptosis, cytoplasmic Bcl-2, and has 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 osteogenic associated mRNA expression of osteopontin and Runx-2 [34].

Further detailed characterisation of the bioactive LMW molecules has led to the identification of 110 molecules in the 70–100 Da range comprising of glycine-enriched peptides with structural similarities and high affinities for each other. A highly defined matrix protein with a 10 kDa size named as p10 has specifically demonstrated an increase in human fibroblast cell ALP expression [35] lending greater hope 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 gradually uncovered. Some biomineralisation researchers are doubtful that nacre proteins are the primary cause of osteoinduction. In a study by Liao et al. [36], nacre failed to stimulate an in vivo osteogenic response, although bone-to-nacre apposition and bonding did occur directly. Liao et al. suggested that nacre provided a favourable surface chemistry, rich in phosphorus, favourable to osteoclast and osteoblast recruitment, attachment and matrix synthesis [36]. In an in vivo ectopic bone environment, surface modified nacre was found not to be osteoinductive and osteoconductive within demineralised bone matrix, but its integration and fusion with bone was

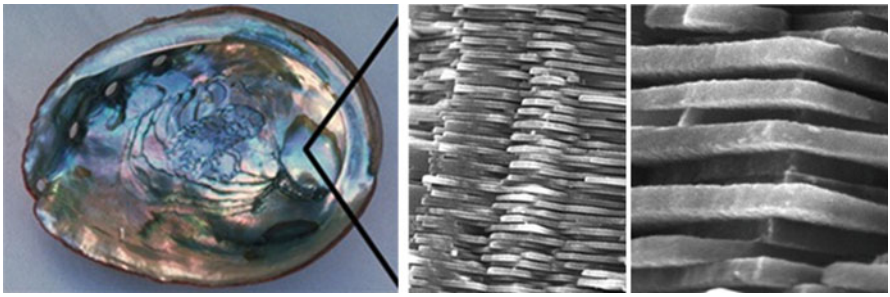

Fig. 13.1 Nacre microstructure showing platelike structure containing calcium carbonate platelets and protein interface helping good fracture toughness

better than non-nacre controls. In another study Kim et al. [37] investigated the role of interfacial properties on the biocompatibility of nacre and specifically its unique bone-bonding ability. Kim and colleagues concluded that the organic matrix is what makes nacre bond to bone so well, as it creates a favourable surface charge for optimal biological associations. When implanted the organic matrix of nacre is thought to generate a new interfacial microenvironment that forms many functional associations with the surrounding tissue leading to a better bone bonding than bioceramic implants without an organic matrix. According to Shen et al. the osteogenic responses to nacre particles and pearl proceeded much faster following soaking in a simulated body fluid (SBF) that generates a HAp-rich layer on the particles [23]. The WSM was implicated in the formation of this HAp layer and the augmented cell responses.

Taken altogether nacre provides an appropriate tissue-compatible physical platform which slowly elute unique peptides that initiate and drive bone formation. In addition nacre—due to its organic content and platelike design—is mechanically tough (fracture toughness equivalent to titanium), non-immunogenic and rapidly biodegradable, without eliciting detrimental physiological effects (Fig. 13.1). These characteristics of nacre offer us a unique substrate for delivery of a functional (possibly osteopromotive) agent to sites of bone loss in quantities that lead to rapid bone repair and regeneration.

13.7 Marine Skeletal Proteins in Regenerative Medicine

It has already been shown that coral and marine sponge skeletons can support self-sustaining musculoskeletal tissues and that extracts of spongin collagen and nacre seashell organic matrices promote bone mineralisation. Use of ready-made organic and inorganic marine skeletons is one of the simplest potential remedies to major problems hindering the future development of regenerative orthopaedics such as providing a richness of framework designs and now a potentially rich, accessible source of osteopromotive analogues and biomineralisation proteins. This should not

be surprising given that the pivotal biomineralisation proteins, which orchestrate bone morphogenesis, are also found in the earliest calcifying marine organisms. In support of this notion, 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 signalling proteins are also present in early marine sponge development and instrumental to stem cell activation in Cnidarians. Based on this match between vertebrate and invertebrate main developmental proteins, we have published the nature and extent of this evolutionary relatedness and use it to support the development of a new strategy, which is to my selected marine origin organic matrices for novel metabolic signalling and structural proteins/peptides and protein analogues to apply in regenerative orthopaedics, particularly when using adult stem cells [9, 38]. To support this we showed the early-stage evidence gathered in our own laboratory the presence of fibrinogen fragments and early osteopromotive effects of a coral organic matrix extract on stem cells [39]. In practice the discovery of new osteopromotive and osteo-accelerant protein analogues will require the use of traditional chromatography techniques, osteoactivity assays to hone in on potential proteins of significance and advanced proteomic tools to provide accurate sequencing, determine the mechanisms and molecular pathways involved in osteoactivation and determine the efficiency and effectiveness of marine skeleton-derived protein modulation of the stem cell (MSC) proteome. As more analogues are discovered using proteomic tools, skeletal organic matrices may have ever-increasing utility for regenerative orthopaedics [9].

13.7.1 Marine Sponge Skeletons

Marine sponges possess the most primitive form of extant tissue but share much in common with multicellular tissues which have apparently conserved many features evolved by these first multicellular organisms [40]. Morphological and biochemical similarities exist between marine sponge and vertebrate extracellular matrix (ECM) alluding to fundamental rules of organisation evolved first by marine sponges. Three collagen types have so far been identified from marine sponge. All sponges are composed of 22 nm thin collagen fibrils with highly ordered periodic banding. Although the collagen ultrastructure is relatively simple compared to vertebrate collagens, amino acid sequences and genome organisation are similar. Collagen fibrils are secreted in bundles in a similar manner to vertebrates. Similarly collagen fibrils are closely associated with proteoglycans which, in mammalian tissue blueprint, shape and form at long range scales. Fibronectin, dermatopontin and tenascin polypeptides are also found in marine sponge collagen fibres and cross-react with antibodies raised against vertebrate analogues highlighting their common origins. Some sponge species possess an analogue of type IV collagen found in vertebrate basement membrane collagens [41]. The organisation of collagen fibrils is analogous to collagen type XIII which sticks cells to surfaces. It is with

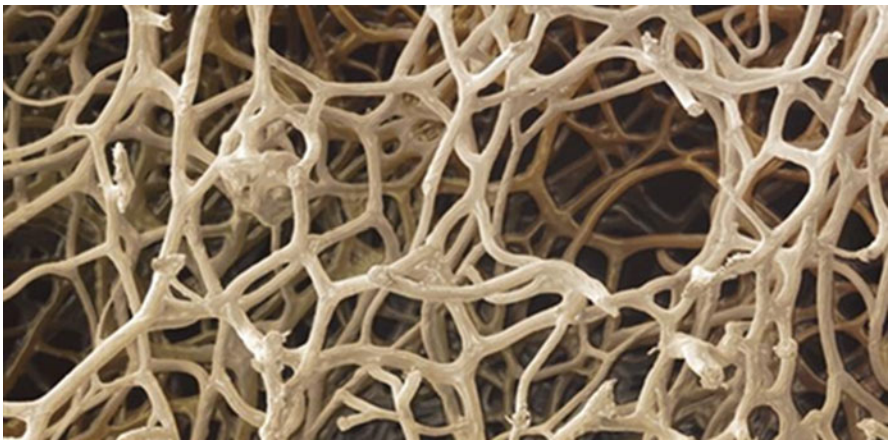

Fig. 13.2 Sponge interconnected lattice-like structure

these properties (fibronectin and cell adherent collagens) that collagenous marine sponges represent a significant potential for future development as bioactive tissue engineering scaffolds.

At present, marine sponges are extensively exploited for novel biological compounds as potential treatments for cancer tumours, leukaemia and inflammation. Marine sponges are also a source of collagen for cosmetics [40] and dermatological preparations [42]. In total 50 % of all marine-derived materials are sourced from a wide spectrum of marine sponges. Collagenous marine sponge skeletons are incredibly soft, strong, highly absorbent and elastic, resistant to high temperatures and bacterial attack. Such properties make them highly suited for surgical procedures. The exact conditions to grow marine sponges at a large enough scale for commerce are being investigated by a number of researchers. Some have established aquatic pilot farms for the cultivation of selected bath sponge species. Marine sponges are sufficiently adaptable for commercial scale production. Another aim for cultivating marine sponges is to extract medically important secondary metabolites in much larger quantities than is possible from collections made by conventional bio-prospecting. The superior optimised structural design of silica marine sponges has been alluded to and which provides useful lessons for construction of man-made frameworks with minimal starting materials for maximum strength [43, 44]. They reported on the structural properties of biosilica observed in the hexactinellid sponge Euplectella sp. Consolidated, nanometre-scaled silica spheres are arranged in well-defined microscopic concentric rings glued together by organic matrix to form laminated spicules. The assembly of these spicules into bundles, effected by the laminated silica-based cement, results in the formation of a macroscopic cylindrical lattice-like structure reinforced by diagonal ridges. It can be added that there is, therefore, considerable mechanical benefit to specific arrangements of structural elements at many different hierarchies of scale (Fig. 13.2).

It has been suggested that the 3D topology and specific surface features of hydrozoans instigated faster cell adhesion, proliferation and differentiation [45]. More needs to be done to determine the exact mechanism of action between material and cell. Collagenous marine sponges fulfil the potential of a clinically relevant scaffold for a range of tissues including bone and cartilage. The fibre-bonded meshwork of sponges provides conduits for cell guidance alongside spaces for rapid tissue infiltration and infilling. It has been discovered that the collagenous composition of the fibres promotes attachment of all human cell types. The unique layered ultrastructure may explain the high wettability and adsorption of growth factors onto the collagen fibres which infuse into attached cells and promote their activities.

Collagenous marine sponges fulfil the potential of a clinically relevant scaffold for a range of tissues including bone, cartilage, fat connective, liver and kidney. Tissue formation within 4 weeks in vivo was shown 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 [13].

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13.7.2 Echinoderm Skeletal Elements

Sea urchin skeletal plates are punctured 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), while the remainder are channels connected to the reproductive and alimentary systems and are very much larger to accommodate larger throughputs of fluid (1,000–2,000 μm pore diameter). Hydrothermal processing of echinoderm structures transforms the chemical and mechanical properties with equivalence to human bone. Echinoderm skeletons are constructed from a unique, intricately shaped, 3D, single crystalline meshwork with a topological structure in which every internal pore and channel is in direct contact with all others (periodic minimal surface). This property is likely to facilitate mass transfer and tissue development [46]. Studies using the replamine form technique for replicating perforate echinoderm structural elements generate promising hard tissue replacements to bone, as well as candidate prostheses for blood vessels and trachea. In this context the skeletal ossicles from the sea star (Pisaster giganteus) have been investigated. They provide an ideal architecture together with physical and chemical properties conducive to bone restoration [15].

The sea urchin spicule is a composite of organic and inorganic materials that the animal synthesises using the most readily available elements in seawater. The fully formed spicule is composed of a single crystal with an unusual morphology in 3D.

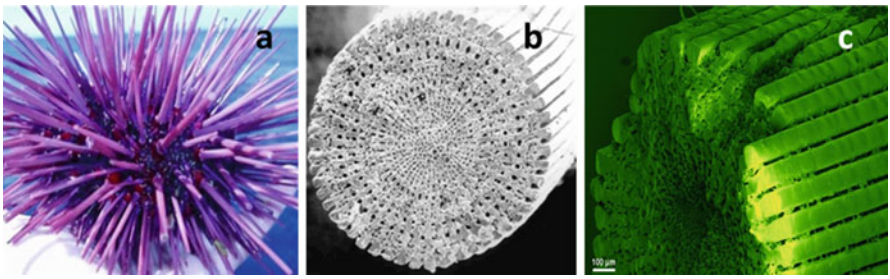

Fig. 13.3 Sea urchin structure showing the arrangements of the spikes: (**a**) general overall structure, (**b**) cross section of a single spike (spicule), and (**c**) enlarged spicule surface

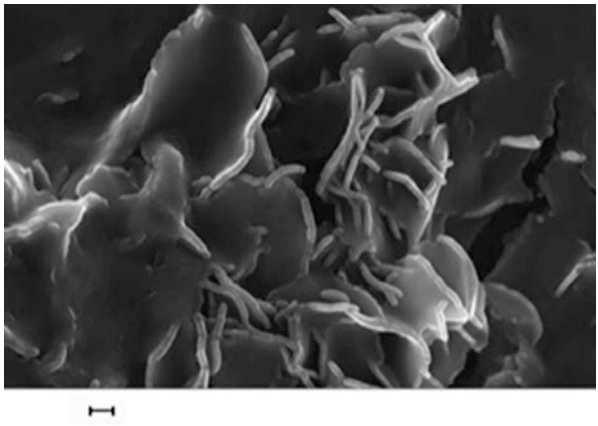

Fig. 13.4 Pseudo-platelike structure of amorphous (sol–gel-developed) calcium phosphate that transforms to fully crystalline plate nano-hydroxyapatite above 300 °C (scale 200 nm)

It has no facets and forms a starlike shape. To achieve such unusual morphologies, sea urchin and other marine organisms deposit a disordered amorphous mineral phase first and then let it slowly transform into a crystal with neatly aligned into a lattice with a specific and regular orientation while maintaining their general morphology. This is a unique transformation from amorphous to ordered crystalline structure—at room temperature—that needs to be clearly observed and understood for directional growth in future biomaterials. The sea urchin spicule is formed inside a clump of specialised cells and begins as the animal lays down a single crystal of calcite, from which the rest of the spicule is formed (Fig. 13.3). Starting from the crystalline centre, three arms extend at 120° from each other. The three radii are initially 40–100 nm-sized amorphous calcium carbonate but slowly convert to calcite. Mechanism as yet not clearly understood but might be through ordered precipitation and growth mechanism at known crystallographic orientations of calcite or aragonite.

Similar transformation from amorphous structure to crystalline form can be observed in a sol–gel-developed hydroxyapatite where the amorphous hydroxyapatite transforms to crystalline nanoplatelets by a thermally activated process (Fig. 13.4) without any large morphological change. In order to simulate the

amorphous to crystalline transformation, we have developed a novel method to produce single-phase, nano-sized, platelike, mixed A–B type carbonate-containing apatite (CAH) similar to bone apatite for effective bone tissue integration [47]. The methodology emulates biomineralisation, where topotactic transition from OCP to HAp.

The synthetic process developed involves formation of thin (1–1.4 nm) layered calcium phosphonate by a self-assembly process. At the early stages, the phosphonate-derived apatite shows slightly curved platelike-shaped amorphous apatite which converts to crystalline form with significant changes to both a- and c-axes as function of temperature. The thermal decomposition of these layered amorphous structures leads to formation of highly crystalline well-organised platelike carbonated apatite [47, 48]. The overall carbonate content varies from 4 to 6.4 wt%, within the temperature range of 500–700 °C. This carbonate content corresponds well with the amount found in mammalian hard tissues.

Although sea urchin transformation is carried out at ambient temperatures, this analogue transition in sol–gel-derived synthetic HAp shows a platelike morphological change of amorphous but near platelike-shaped apatite to perfectly crystalline platelike apatite with thermal activation from room temperature to 300 °C (Fig. 13.4).

13.7.3 Coral Skeletons

Natural coral exoskeletons have been used widely as a bone replacement in orthopaedic, maxillofacial, dental and neurosurgery owing to their combination of good mechanical properties, open and interconnected porosity and ability to form chemical bonds with bone and soft tissues in vivo [49, 50]. Infect corals have the best mechanical properties of the porous calcium-based ceramics and resorb at a rate equivalent to host bone formation (Fig. 13.5).

The beginning of the coral life cycle starts with the polyps which absorbs the calcium ions and carbonic acid present in the seawater to produce the calcium carbonate in the form of aragonite crystals. The remaining composition consists of trace elements of magnesium, strontium, fluorine and phosphorus in the phosphate form [51]. Once implanted in the human body, these elements play a critical role in the bone mineralisation process and in the activation of key enzymes associated with bone remodelling cells. Strontium has been shown to contribute to the mineralisation process by stimulating osteoblasts while inhibiting osteoclasts [52]. Similarly, fluorine helps bone formation through similar stimulatory effect on osteoblast proliferation. Magnesium is also long known to be beneficial in bone remodelling as it has been shown to increase the mechanical properties of newly formed bone [53].

The organic composition has an important part to play in coral biocompatibility. The abundance, conformation and composition of the organic matrices are responsible for successful biological integration of natural coral with human host [38].

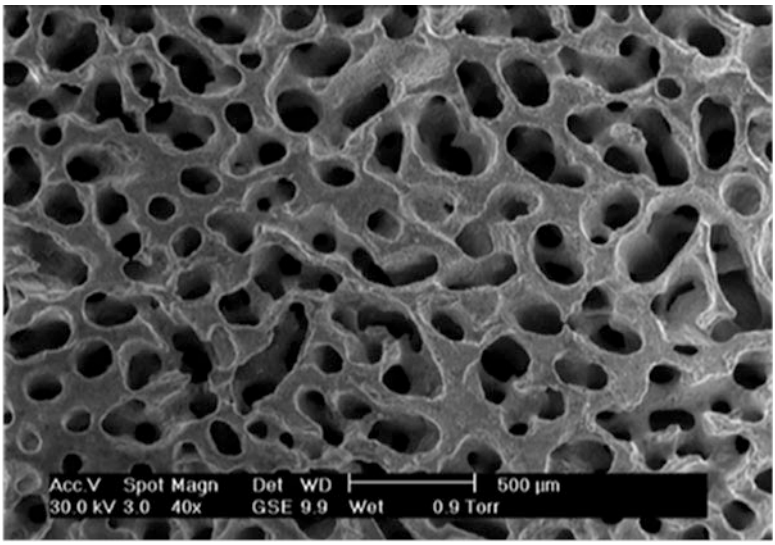

Fig. 13.5 Coralline apatite converted by hydrothermal process showing interconnected porous structure

Use of coral skeletons for general routine orthopaedic surgery and tissue engineering has been so far limited to external fixation devices as they are inappropriate for strictly load-bearing applications due to their calcium carbonate structure with high dissolution rates. Sol–gel coating technologies can be used to enhance the strength of corals, and this enables them to be used at more skeletal locations [50, 54]. Corals offer great opportunities to tissue engineering of bone either in their natural form or as hybridised synthetic forms. Coral skeleton combined with in vitro expanded HBMSC increased osteogenesis more than those obtained with scaffold alone or scaffold with fresh marrow [39]. In vivo large animal segmental defect in both orthopaedic and maxillofacial surgery led to complete recorticalisation 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 high number of cases [55, 56]. Structural and biomineralisation studies of coral can be used to inform the development of new advanced functional materials because of the unique nanoscale organisation of organic tissue and mineral as highlighted by Ehrlich et al. [20]. At a macrostructural level, the deep-sea bamboo coral exhibited bone-like biochemical and mechanical properties. A specialised collagen matrix (acidic fibrillar) serves as a model for future potential tissue engineering applications. The matrix supported both osteoblast and osteoclast growth, and the exceptional bio-elastomeric properties of the collagen matrix (gorgonin) of this coral make it potentially suitable for blood vessel implants. Quinones cross-link and harden the collagenous gorgonin proteins and closely resemble human keratin. The mechanism by which gorgonin is synthesised and interacts with the process of mineralisation may provide lessons for the generation of a synthetic-collagen-like material [20].

13.8 Drug Delivery and Marine Structures

Slow or targeted drug delivery system is a system that is capable of releasing a preloaded pharmaceutical agent to a targeted site at a specific rate and most importantly at a therapeutically relevant concentration. The main aim of this type of system compared with conventional drug intake (injection or tablet) is to facilitate the local and specific area delivery, dosage and duration control and hence appropriate active drug delivery while causing minimal side effects. While technological advancement has produced innovative and refined drug delivery systems, the fundamental basis that defines what a drug delivery system remains unchanged. The therapeutic advantages of these systems can be attributed to many underlining factors: predictability of release rate and minimised drug concentration, thereby reducing any possible adverse systemic effect. 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 of malaria in Africa. Many factors are considered in the development of drug delivery systems in accordance to the desired application. This includes the agent to be carried, the administration route, the material used, the degradation rate, the loading efficiency, the physical and chemical properties of the material, the practicality for large-scale production, toxicity, among other parameters.

Many materials such as ceramics, polymers, alginate and polysaccharides have shown potential advantages as drug delivery systems [57]. However, marine materials such as coral exoskeletons and marine shells show a better promise due to their easy conversion to calcium phosphates, intricate interconnected pores and their controllable dissolution rates.

The pore size and interconnectivity of the coral pores are a critical factor in the rate of coral as a bone graft and slow drug delivery material. Moreover, the uniform porosity of the exoskeletons provides a more constant drug loading and therefore providing a more predictable drug release rate of which both are crucial factors that directly impact the effectiveness of the drug delivery system. Natural structures although not perfect often exhibit intricate morphologies that justify the efforts for biomimetic approach.

13.9 Foraminifera and Drug Delivery

During an overseas summer break, I have noticed that the sand that I was walking on the beach looked as perfect spheres and showed intricate structure (Fig. 13.6). I brought some to our laboratories, and after XRD analysis I noticed that it was not silica sand but calcium carbonate. After proper characterisation, we identified it as a marine structure belonging to the *Foraminifera* family. *Foraminifera* are abundant and are found in all marine environments, but different species exist with different

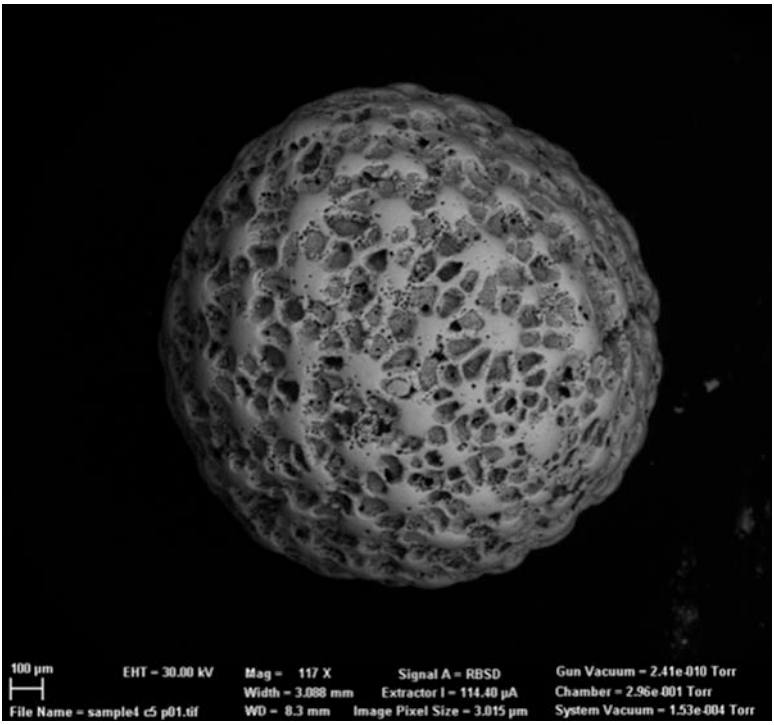

Fig. 13.6 Foraminifera macrostructure

shapes depending on their environment. *Foraminifera* are single-celled organisms with shells consisting of multilayer inner chambers commonly divided and added during its growth.

Prior to any marine material can be used as a drug delivery material, it must first undergo a rigorous process to test the composition, purity, morphology and suitability for drug loading and its slow dissolution without any adverse effect to the patient. Unless specifically protein and organic matter is required to be extracted, prior to sterilisation of calcium carbonate material, any residual organic constituents are removed by immersing in solution of sodium hypochlorite and then drying at about 100 °C [58].

During slow drug delivery studies, spherical and star-shaped shells floresianus (Foraminifera) from the coral beach sand of the Great Barrier Reef, Australia, and Okinawa, Japan, were collected. The microspherical samples were intact and measured 0.5–1.5 mm in diameter (Fig. 13.6). These shells or more appropriately microspheres possess unique interconnected porous structures that have evolved to circulate seawater and collect light for the mutual benefit of symbiotic algal cells that reside inside the shell. Microcomputed tomography (µ-CT) and scanning electron microscopy (SEM) imaging confirmed that shells were internally permeated by a 3D

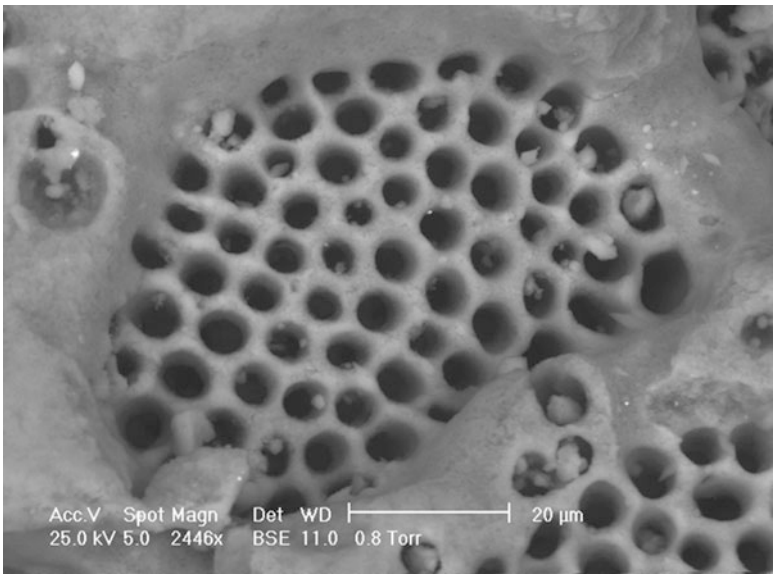

Fig. 13.7 Enlarged microstructure showing interconnected 5 μm porous structure

network of microscopic interconnected channels measuring 1–10 μm in diameter. Between the micropores surface of the solid spine area was made of calcium carbonate platelets consisting nano- and meso-pores (Fig. 13.7).

It was necessary to hydrothermally convert these microsphere shells into more stable, highly crystalline β-tricalcium phosphate (β-TCP) and/or HAp using the methods developed and published earlier [10]. In certain circumstances of drug delivery applications, TCP presents the more ideal composition compared with other calcium phosphates.

Chemical conversion of foraminifer's shells to HAp (microspheres) did not change the original untransformed structure making it available for adsorption of candidate drug compounds and to allow new bone cell penetration into the micropores. This was demonstrated in animal trials [59–61].

Constructs that are generated in this manner provide many distinct advantages for tissue engineering as a physical template and devices for controlled release of BMP, water-soluble proteins (WSP), genes and growth factors. Since the chemistry occurs at room temperature and consists of an aqueous phase, biological molecules can be safely incorporated during synthesis.

Natural spheres loaded with drugs can spontaneously degrade and progressively release entrapped biological contents introduced during synthesis. The release profile shows relatively slow, local release of drugs such as bisphosphonate, gentamicin and simvastatin from micro- and macrospheres for extended periods [62, 63].

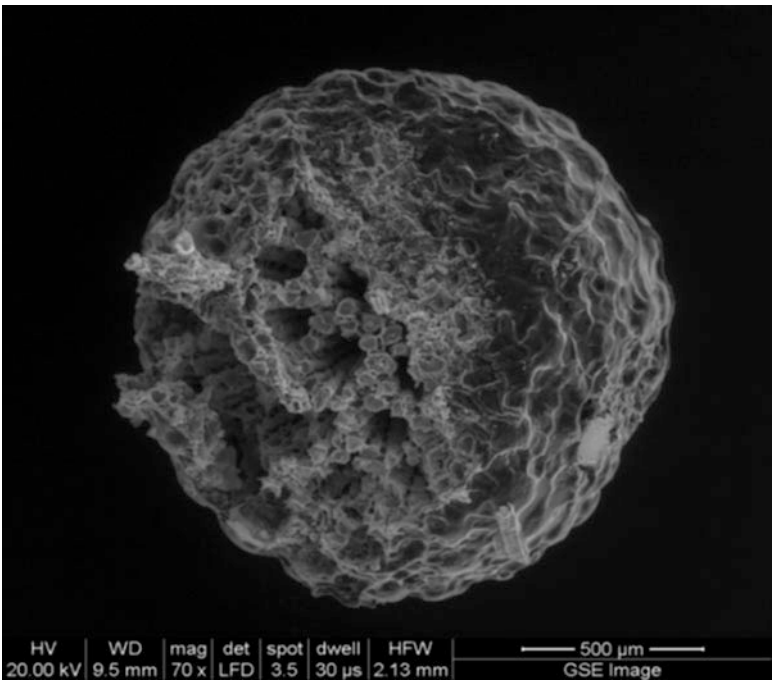

Fig. 13.8 Foraminifera microsphere during dissolution studies showing dissociation of the calcium phosphate and other loaded pharmaceuticals and minerals

Due to the pore architecture, these microspheres within the physiological environment can dissolve and supply both calcium and phosphate ions and any other beneficial ions and the drugs incorporated to the immediate tissues. Foraminifera after dissolution is shown in Fig. 13.8.

13.10 Stem Cell Regulation

There is also growing realisation that the composition of a scaffold material is vitally important to organise stem cell activities as they are dependent on the extracellular fabric for life support and to guide their subsequent evolution and development [38, 59]. Re-creation of the native stem cell environment where stem cells normally reside (such as bone marrow) and are protected, managed and stabilised as self-renewing undifferentiated cells and given instruction on how to regulate the rate of progenitor and successor cell production is an active research area.

Marine structures such as Foraminifera, coral skeletons and converted coralline apatites have demonstrated substantial clinical success as a template for tissue reconstruction. This has spurred on researchers to look at other skeletons with

better mechanical properties such as hydrozoans as unique potential candidates for tissue engineering of mineralised tissues [45]. The unique 3-dimensional structure supported growth and enhanced differentiation of stem cell progenitors into bone cells unlike standard carbonate frameworks that do not induce stem cell differentiation. It can be suggested that the 3D topology and specific surface features of hydrozoans induce faster cell adhesion, proliferation and differentiation.

13.11 Concluding Remarks

In nature, structures possess enviable properties such as complexity, sophistication and miniaturisation that are not (as yet) possible to fabricate in the laboratory. However, we are gradually inventing ways of replicating nature to produce similar levels of sophistication albeit to a limited extent. We are only able to recreate microscopic structures and surfaces with some level of biomimetic detail. This has been particularly true for the replication of both bio-organic and inorganic structures. One versatile approach has been to use biological microstructures as templates for the reproduction of inorganic structures with identical features. They have clear significance to the production of replacements for calcified tissues. This is achieved by using techniques in biomineral inspired materials chemistry. The concept is to exploit the consecutive developmental pathway of systems that nature employs to make skeletons from molecules into micro- and macroscopic structures. The process in inorganic fabrication is analogous to building a house. It begins with supramolecular pre-organisation, interfacial recognition, vectorial regulation and chemical transformation lending to multilevel processing. These processes are developed within confined reaction spaces directed in their formation by the templates themselves. The continual multiplication of these nano-assemblies builds up into the emergence of morphology and macroscale biomimetic forms.

In the near future, studies of the way natural materials are constructed and the way they adapt to their environment will enable us to produce an exciting array of self-responsive structures and materials for regenerative medicine and structural engineering applications. In nature biomaterials are made with immaculate resource and energy efficiency using common, readily available substrates through self-assembly into highly organised and structurally optimised hierarchies. This gives us the opportunity to produce structures with intricate shapes and architectures that are tailored to their functions and do not break down. The science and engineering have shown us how biomimetic approaches can yield promising outcomes for application in tissue engineering of skeletal tissues. Our work along is a part of ongoing research towards the design of clinically relevant scaffolds for regenerative medicine using a unique set of self-organising hierarchical structures including marine structures that are designed and synthesised according to biological principles of design.

There is a clear and present need for better tissue engineering scaffolds that possess more natural bio-responsive environments conducive to guiding the natural processes of regeneration which can be highly intricate and dynamic in space

and time. Thus scaffolds must have intelligence designed into them to meet this biological challenge. We contend that there needs to be a step change to scaffold environments that are responsive whereby the synthesised biomatrix evolves in real time to meet the demands and optimisations of adaptive growth and regeneration of human tissues. As cells proliferate and differentiate, they alter their environment. Future advanced biomimetic scaffolds must be able to adapt to these changes and meet the ever-changing needs of developing tissues. Nature—although not perfect—uses simple chemistries, sound biological principles and formation of adequate structures with unique morphologies to distribute functional stress. Nanofabrication using combined biochemical, biological and biomechanical principles of assembly and design is still in its infancy. Use of this bio-inspired nanofabrication for tissue engineering is a unique approach that has enormous potential to improve scaffold design and tailor physico-chemical environments with an ability to micro-evolve. This is the next challenge: to grow materials with cells and promote their regulation of material synthesis.

Marine structures constantly adapt their composition, growth and hence function to dynamically changing environmental conditions and have the ability for self-repair when adaptation fails or is too slow. Advanced functional materials with these characteristics would see enormous benefit to engineering industries and biomedicine. At a time when we are concerned about air pollution, global climate change, consuming less energy and using fewer and sustainable resources, the design and fabrication of a new technology based on biomimetics is such an imperative.

Acknowledgments To write a chapter that involves a range of natural and synthetic materials and their discovery and development can only be achieved by taking into account a large number of research studies and related published papers. During the last two decades, a number of colleagues and past and present students contributed enormously in both research and their dissemination through publications. This chapter has been the collation of the ideas and published work that only touches the surface of the large contributions made during the years. There are a number of colleagues that we would like to thank: Dr. Joshua Chou who has been a very resourceful young researcher and contributed well in the drug delivery work. We would also like to acknowledge the contributions of Professors R. Vago, F.N. Oktar and R.M Conway and Drs. A. Milev, A. Choi, C. Chai, J. Russel and J. Hu and Mr I.J. Macha, which, without their contribution and timely visions, this chapter could not have been written.

References

1. Green D, Howard D, Yang XB, Kelly M, Oreffo ROC (2003) Natural marine sponge fibre skeleton: a biomimetic scaffold for human osteoprogenitor cell attachment, growth and differentiation. Tissue Eng 9:1159–1166
2. Parker AR, Martini N (2006) Structural colour in animals-simple to complex optics. Opt Laser Technol 38:315–322
3. Mock T, Samanta MP, Iverson V, Berthiaume C, Robison M, Holtermann K 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(5):1579–1584

4. Green D, Ben-Nissan B (2008) Biomimetics: bio-inspired engineering of human tissue scaffolding for regenerative medicine. In: Tateishi T (ed) Biomaterials in Asia: in commemoration of the 1st Asian Biomaterials Congress. Tsukuba, Japan, pp 364–385
5. Kim ES (2008) Directed evolution: a historical exploration into an evolutionary experimental system of nanobiotechnology, 1965–2006. Minerva 46:463–484
6. Sia SK, Gillette BM, Yang GJ (2007) Synthetic tissue biology: tissue engineering meets synthetic biology. Birth Defects Res C 81(4):354–361
7. Hench LL, West JK (1990) The sol-gel process. Chem Rev 90:33–72
8. Ben-Nissan B, Choi AH (2006) Sol-gel production of bioactive nanocoatings for medical applications. Part I: an introduction. Nanomedicine 1(3):311–319
9. Green DW, Li G, Milthorpe B, Ben-Nissan B (2012) Adult stem cell coatings using biomaterials for regenerative medicine. Mater Today 15(1–2):61–68
10. Ben-Nissan B (2003) Natural bioceramic: from coral to bone and beyond. Curr Opin Solid State Mater Sci 7(4–5):283–288
11. 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 SK (ed) Marine biomaterials. Taylor & Francis/CSR Books. Boca Raton, FL, USA
12. Roy D, Linnehan S (1974) Hydroxyapatite formed from coral skeletal carbonate by hydrothermal exchange. Nature 247(438):220–222
13. Vago R, Plotquin D, Bunin A, Sinelnikov I, Atar D, Itzhak D (2002) Hard tissue remodeling using biofabricated coralline biomaterials. J Biochem Biophys Methods 50:253–259
14. Rocha JHG, Lemos AF, Agathopoulos S, Valero P, Kannan S, Oktar FN et al (2005) Scaffolds for bone restoration from cuttlefish. Bone 37(6):850–857
15. Martina M, Subramanyam G, Weaver JC, Hutmacher DW, Morse DE, Valiyaveettil S (2005) Developing macroporous bicontinuous materials as scaffolds for tissue engineering. Biomaterials 26:5609–5616
16. Townley HE, Parker AR, White-Cooper H (2008) Exploitation of diatom frustules for nanotechnology: tethering active biomolecules. Adv Funct Mater 18(2):369–374
17. Marin F, Luquet G (2004) Molluscan shell proteins. Comptes Rendus Palevol 3(6–7):469
18. Demers C, Hamdy C, Corsi K, Chellat F, Tabrizian M, Yahia L (2002) Natural coral exoskeleton as a bone graft substitute: a review. Biomed Mater Eng 12:15–35
19. Nowak D, Florek M, Nowak J, Kwiatek W, Lekki J, Zieba E et al (2005) Micro-spectrometric investigations of inorganic components of the black corals for biomedical applications. Key Eng Mater 284–286:297–300
20. Ehrlich H, Etnoyer P, Litvinov SD, Olennikova MM, Domaschke H, Hanke T et al (2006) Biomaterial structure in deep-sea bamboo coral (Anthozoa: Gorgonacea: Isididae): perspectives for the development of bone implants and templates for tissue engineering. Mater Werkst 37(6):552–557
21. Agaogullaria D, Kelb D, Gökçea H, Dumana I, Öveçoglua ML, Akarsubasic A et al (2012) Bioceramic production from sea urchins. Acta Phys Pol A 121(1):23–26
22. Samur R, Ozyegin L, Agaogullari N, Oktar FN, Agatopoulos S, Kalkandelen C et al (2013) Calcium phosphate formation from sea urchin (Brissus latecarinatus) via modified mechano chemical (ultrasonic) conversion method. Metallurgia 52(3):375–378
23. Shen Y, Zhu J, Zhang H, Zhao F (2006) In vitro osteogenic activity of pearl. Biomaterials 27:281–287
24. Bahar H, Yaffe A, Binderman I (2003) The influence of nacre surface and its modification on bone apposition: a bone development model in rats. J Periodontol 74:366–371
25. Macha IJ, Ozyegin LS, Chou J, Samur R, Oktar FN, Ben-Nissan B (2013) An alternative synthesis method for di calcium phosphate (monetite) powders from Mediterranean mussel (Mytilus galloprovincialis) shells. J Aust Ceram Soc 49(2):122–128
26. Kel D, Gökçe H, Bilgiç D, Ağaoğulları D, Duman I, Öveçoğlu ML et al (2012) Production of natural HA from land snails. Key Eng Mater 493:287–292

27. Lopez E, Vidal B, Berland S, Camprasse S, Camprasse G, Silve C (1992) Demonstration of the capacity of nacre to induce bone formation by human osteoblasts maintained in vitro. Tissue Cell 24:667–679
28. Lamghari M, Antonietti P, Berland S, Laurent A, Lopez E (2001) Arthrodesis of lumbar spine transverse processes using nacre in rabbit. J Bone Miner Res 16(12):2232–2237
29. Lamghari M, Berland S, Laurent A, Huet H, Lopez E (2001) Bone reactions to nacre injected percutaneously into the vertebrae of sheep. Biomaterials 22:555–562
30. Rousseau M, Lucilia PM, Almeida MJ, Milet C, Lopez E (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
31. Duplat D, Chabadel A, Gallet M, Berland S, Bedouet L, Rousseau M et al (2007) The in vitro osteoclastic degradation of nacre. Biomaterials 28(12):2155–2162
32. Westbroek P, Marin F (1998) A marriage of bone and nacre. Nature 392:861–862
33. Almeida MJ, Pereira L, Milet C, Haigle J, Barbosa M, Lopez E (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
34. Rousseau M, Boulzaguet H, Biagianti J, Duplat D, Milet C, Lopez E et al (2007) Low molecular weight molecules of oyster nacre induce mineralization of the MC3T3-E1. J Biomed Mater Res A 85A:487–497
35. Zhang C, Li S, Ma Z, Xie L, Zhang R (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. Mar Biotechnol 8:624–633
36. Liao H, Mutvei H, Hammarstrom L, Wurtz T, Li J (2002) Tissue responses to nacreous implants in rat femur: an in situ hybridization and histochemical study. Biomaterials 23:2693–2701
37. Kim YM, Kim JJ, Kim YH, Rho JY (2000) Effects of organic matrix proteins on the interfacial structures at the bone-biocompatible nacre interface in vitro. Biomaterials 23:2089–2096
38. Green DW, Padula MP, Santos J, Chou J, Milthorpe B, Ben-Nissan B (2013) A therapeutic potential for marine skeletal proteins in bone regeneration. Mar Drugs 11(4):1203–1220
39. Green DW, Ben-Nissan B (2010) Biomimetic applications in regenerative medicine: scaffolds, transplantation modules, tissue homing device and stem cells. In: Amiji M, Tochilin V (eds) Handbook of materials for nanomedicine. Pan Stanford Publishing, Singapore
40. Swatschek D, Schatton W, Kellermann J, Muller WEG, Kreuter J (2002) Marine sponge collagen: isolation, characterization and effects on the skin parameters surface pH, moisture and sebum. Eur J Pharm Biopharm 53:107–113
41. Boute N, Exposito J-Y, Boury-Esnault N, Vacelet J, Noro N, Miyazaki K et al (1996) Type IV collagen in sponges, the missing link in basement membrane ubiquity. Biol Cell 88:37–44
42. Nicklas M, Schatton W, Heinemann S, Hanke T, Kreuter J (2009) Preparation and characterization of marine sponge collagen nanoparticles and employment for the transdermal delivery of 17b-estradiol-hemihydrate. Drug Dev Ind Pharm 35(9):1035–1042
43. Aizenberg J, Weaver JC, Thanawala MS, Sundar VC, Morse DE, Fratzl P (2005) Skeleton of Euplectella sp structural hierarchy from the nanoscale to the macroscale. Science 309(5732):275–278
44. Miserez A, Weaver JC, Thurner PJ, Aizenberg J, Dauphin Y, Fratzl P et al (2008) Effects of laminate architecture on fracture resistance of sponge biosilica: lessons from nature. Adv Funct Mater 18(8):1241–1248
45. Abramovitch-Gottlib L, Geresh S, Vago R (2006) Biofabricated marine hydrozoan: a bioactive crystalline material promoting ossification of mesenchymal stem cells. Tissue Eng 12(4):729–739
46. Aizenberg J, Hendler G (2004) Designing efficient microlens arrays: lessons from nature. J Mater Chem 14:2066–2072
47. Milev AS, Kannagara GSK, Ben-Nissan B, Wilson M (2004) Template directed synthesis of nanosized bone-like apatite. In: Schulte J (ed) Proceedings of the Asia Pacific nanotechnology forum 2003. World Scientific Publishing Co, Singapore, pp 87–92

48. Milev AS, Kannangara GSK, Ben-Nissan B (2003) Morphological stability of plate-like carbonated hydroxyapatite. Mater Lett 57(13–14):1960–1965
49. Vago R (2008) Beyond the skeleton. Cnidarian biomaterials as bioactive extracellular microenvironments for tissue engineering. Organogenesis 4(1):18–22
50. Ben-Nissan B (2004) Biomimetics and bioceramics. In: Reis RL, Weiner S (eds) Learning from nature how to design new implantable biomaterials: from biomineralization fundamentals to biomimetic materials and processing routes. Nato science series, vol 171, Springer, Dordrecht, pp 89–103
51. Stanley G (2003) The evolution of modern corals and their early history. Earth Sci Rev 60(3–4):195–225
52. Bonnelye E, Chabadel A, Saltel F, Jurdic P (2008) Dual effect of strontium ranelate: stimulation of osteoblast differentiation and inhibition of osteoclast formation and resorption in vitro. Bone 42(1):129–138
53. LeGeros R (1981) Apatites in biological systems. Prog Cryst Growth Charact Mater 41(1–2): 1–45
54. Choi AH, Ben-Nissan B (2007) Sol-gel production of bioactive nanocoatings for medical applications. Part II: current research and development. Nanomedicine 2(1):51–61
55. 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(5):260–264
56. Leupold J, Barfield W, An Y, Hartsock L (2006) A comparison of ProOsteon, DBX, and collagraft in a rabbit model. J Biomed Mater Res B Appl Biomater 79(2):292–297
57. Bose S, Tarafder S (2012) Calcium phosphate ceramic systems in growth factor and drug delivery for bone tissue engineering: a review. Acta Biomater 8(4):1401–1421
58. Hu J, Russell JJ, Ben-Nissan B, Vago R (2001) Production and analysis of hydroxyapatite from Australian corals via hydrothermal process. J Mater Sci Lett 20(1):85–87
59. Chou J, Ito T, Bishop D, Otsuka M, Ben-Nissan B, Milthorpe B (2013) Controlled release of simvastatin from biomimetic β-TCP drug delivery system. PLoS One 8(1):e54676
60. Chou J, Ito T, Otsuka M, Ben-Nissan B, Milthorpe B (2013) Simvastatin-loaded β-TCP drug delivery system induces bone formation and prevents rhabdomyolysis in OVX mice. Adv Healthc Mater 2(5):678–681
61. Chou J, Green DW, Singh K, Hao J, Ben-Nissan B, Milthorpe B (2013) Adipose stem cell coating of biomimetic β-TCP macrospheres by use of laboratory centrifuge. Biores Open Access 2(1):67–71
62. Chou J, Ben-Nissan B, Green DW, Valenzuela SM, Kohan L (2011) Targeting and dissolution characteristics of bone forming and antibacterial drugs by harnessing the structure of microspherical shells from coral beach sand. Adv Eng Mater 13(1–2):93–99
63. Chou J, Valenzuela S, Bishop D, Ben-Nissan B, Milthorpe B (2012) Strontium- and magnesium-enriched biomimetic beta-TCP macrospheres with potential for bone tissue morphogenesis. J Tissue Eng Regen Med. doi:10.1002/term.1576