Chapter 1
Thoughts and Tribulations on Bioceramics and Marine Structures

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Abstract Marine organisms are structured and constituted by materials with a vast range of properties and characteristics that may justify their potential application within the biomedical field. This is demonstrated by the biological effectiveness of marine structures such as corals and shells and sponge skeletons to house self-sustaining musculoskeletal tissues and their ability to promote bone formation though the use of extracts from sponging and nacre seashells. The design and composition of marine structures have been instrumental in the solving vital problems in regenerative medicine through the introduction of basic remedies that provides frameworks and highly accessible sources of osteopromotive analogues of bioceramic monoliths, nanofibres, micro and macrospheres. The clinical success of any future regenerative implants will be dependent on the production of highly proficient scaffolds that biologically operates at the nano-, micro- and macroscopic levels. Moreover, the implant will also need to coordinate, assemble, and organize cells into tissues as well as releasing encapsulated chemical signals in a targeted way and convey them into the body. As a result, an increasing number of different types of compounds are being isolated from aquatic organisms and transformed into products for health applications, including controlled drug delivery and tissue engineering devices. Despite the fact that they are extremely effective, the development of these materials has their drawbacks that needs be addressed. This chapter reviews the current bioceramics and natural marine structures including their structure, morphology, and applications in regenerative medicine, bone grafts, and drug delivery. In addition, the extraction of

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biological materials such as proteins from marine materials will also be discussed. An example of this specific biomimicry is provided by filtering the microskeleton of Foraminifera and coralline microspheres. New selected strategies based on our research as well as the works of others concerning the engineering of new bone tissues based on biomimicry will be also examined.

Keywords Hydroxyapatite · Coral · Sponge · Sea urchin · Nacre · Hydrothermal conversion · Bioceramics

1.1 Introduction

There is a constant and bountiful diversification of lipids, carbohydrates, pharmaceutical and therapeutic proteins. This has been stimulated by an increase in knowledge of molecular events associated with tissues and organs in healthy and diseased states. Yet, improvements are needed to boast their safety and clinical effectiveness. For instance, potent drugs generate unwanted and potentially damaging side effects as well as producing toxicity in otherwise healthy organs and tissues. Normal physiological functions of tissues and organs can also be damaged when there is an excessive concentration of any therapeutic biomolecules distributed to the incorrect and disease-free site.

These problems can only be addressed through accurate targeting of proteins, minerals, and pharmaceutics into specific tissues and cells delivered using small and highly mobile transplantation units with efficacy equivalent to lentivirus mediated gene-to-cell transfer. In general, the capsule is one of the most effective modules used in targeted drug delivery. Typically, they have dimensions ranging between 100 nm and 10 μm. For tissue modulation and high packing efficiencies for tissue repair, a capsule that is spherical in shape possesses the greatest encapsulation efficacies with corresponding surface areas as well as high capacities for therapeutic ingredients.

Molecules essential to the guidance and regulation of bone morphogenesis and in particular the events associated with mineral deposition and metabolism can also be discovered in the earliest marine organisms as they represent the first molecular components established for calcification, morphogenesis and wound healing. It appears 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. In addition, signaling proteins such as transforming growth factor (TGF) and Wnt, prime targets in bone therapeutics, are present during the early stages of marine sponge development. Likewise, ready-made organic and inorganic marine skeletons possess a habitat suitable for the proliferation of additional mesenchymal stem cell populations and promoting clinically acceptable bone formation.

To engineer bone tissues within a culture dish, the utilization of recombinant matrix and growth proteins are vital as they assist in speeding up the growth of cultivated tissues into quantities that is acceptable or sufficient for clinical applica-

tions. The skeletal organic matrices of calcifying marine invertebrates provide an unexplored source that can be potentially used in the extraction of growth-inducing proteins. They have the advantage of being ready-made and retain the native state of the original protein.

Examination techniques such as cell assays, chromatography and proteomic studies can be used to identify and evaluate proteins that can possibly be used in bone repair regardless of whether they are derived from cultivated tissues or extracted from marine skeletons. Given the evidence supporting bone matrix protein analogues in marine invertebrates at the moment along with the techniques established in the retrieval and production of proteins, there is an undisputable possibility that they can potentially be used to regenerate living bone in a clinical environment.

Significant evidence reveals that skeleton building BMP-2/4 and TGF-β can be found within various marine invertebrates such as corals. The latest innovations in long-term marine invertebrate cell cultivation in addition to the best practice maricultural can be implemented to ensure that these proteins are produced in a sustainable manner and that the supply is constant. This approach also ensures that coral reef habitats are not damaged during the collection of specimens.

Tissue engineering frameworks or scaffolds that are intended to imitate and mimic their native extracellular matrix counterparts will possess the best possibility of clinical acceptance and success. The creation of bioceramics that duplicate the inorganic nanocomponents of human bone and organizing these nanocomponents into a hierarchical three-dimensional (3-D) structure is required to translate this scaffold into bone tissue. A variation of this approach is to synthesize and deposit biomimetic nanostructured coatings onto existing implant materials and structures. Coatings and materials derived from sol-gel method can provide nanometric building blocks that mimic the components and structures similar to those observed in biological inorganic matter. Another biomimetic approach it to directly seize or capture already existing structures from natural sources and enhances their function so that they can be utilized to address specific clinical problems or challenges.

There are abundant sources of marine materials and structures as well as techniques that have undergone an evolution that resulted in new and different applications and functions to their original intended purpose. One of the simplest strategies is to select a pre-designed, pre-formed structure such as unique marine structures and to modify it in such a manner that the resultant product can be utilized for any new application in the future [1]. Furthermore, we can study nature and attempt to accurately replicate and reinventing vital components in the laboratory. In addition, we endeavor to learn more from nature the principle of low energy usage in the construction process, importance of structural organization and implementation of transformative self-assembly and non-equilibrium chemistry.

Throughout the past two decades, coral has been recognized as the one of the most successful natural skeletons that has been applied in the clinic. However, corals lack high compressive strength despite its bone emulating properties and in particular its morphology at different scales. Consequently, this limits their use in load bearing applications unless it is fixated with internal devices.

This chapter reviews the current bioceramics and natural marine structures including their structure, morphology, and applications in regenerative medicine, bone grafts, and drug delivery. In addition, the extraction of biological materials such as proteins from marine materials will also be discussed. An example of this specific biomimicry is provided by filtering the microskeleton of Foraminifera and coralline microspheres. New selected strategies based on our research as well as the works of others concerning the engineering of new bone tissues based on biomimicry will be also examined.

1.2 History and Classification of Bioceramics

Bioceramics were utilized as implants since the start of the 1970s to perform a single and biologically inert role. The inadequacies of these synthetic bioceramics as replacements for human tissues were emphasized with the growing awareness that human tissues and cells conduct many other essential metabolic and regulatory functions.

Since then the requirements of bioceramics have changed to providing a more positive interaction with the host from just essentially sustaining a physical function without causing a host response. This has been accompanied by increasing demands on medical devices that they not only improve the quality of life but also extend its duration. Above all, bioceramics may potentially be employed as body interactive materials intended to assist in the healing process or to promote the regeneration of tissues and therefore restore physiological functions.

Basically, a biomaterial is a non-drug material that is ideal to be incorporated into systems which replaces or enhances the role of human organs or tissues. Artificial devices a hundred years ago were manufactured and developed from materials such as wood and gold to a point where they potentially could replace different parts of the human body. Such materials are capable of causing little or no adverse reactions while being in contact with tissues and fluids of the body for an extended period of time.

The reaction of human tissues toward synthetic implants can be of several different ways once these materials are exposed to the body. The reaction observed at the implant surfaces will determine the nature of tissue interaction at a nanoscale level. Consequently, three definitions have been derived to describe the tissue responses of a biomaterial: bioactive, bioresorbable, and bioinert [2].

For any material, if it is classified as bioactive signifies that there are interactions between the material such as hydroxyapatite (HAp) and the surrounding bone and even with soft tissues in some cases once it is implanted into the human body. Bioresorbable for a material such as tri-calcium phosphate (TCP) implies that it will begin to dissolve or be resorbed and slowly replaced by developing tissues such as bone once it is placed inside the human body. Lastly, bioinert denotes that a material will have minimal interaction with its surrounding tissues once inserted into the body.

Examples of bioinert materials include stainless steel, titanium, alumina, partially stabilized zirconia, and ultra-high molecular weight polyethylene.

For any implant, their clinical successes are determined by factors such as biofunctionality, biocompatibility, the experience of the surgeon, the health conditions of the patients, the design of the implant and the interactions at the tissue-implant interface.

Over the last three decades, enhancements in interfacial bonding by nanoscale interactions based on biomimetics have been of great interests to many researchers worldwide. The process of biomimetics is based on the idea that information is processed and stored by any biological system at the molecular level. At the moment, this notion has been expanded to the development of nanocomposites for tissue engineering such as scaffolds for bone regeneration and biomedical devices, and a number of companies are at the early stages of commercializing new-generation implants modified at a nanoscale level intended for applications in soft and hard tissue engineering and for applications in ocular, orthopedic, and maxillofacial surgery [3–6]. Furthermore, numerous research teams have described the synthesis of novel bone nanocomposites composed of hydroxyapatite and gelatin, collagen, or chondroitin sulfate using a self-assembly approach.

1.3 Productions of Bioceramics and Nanobioceramics

A nanostructured material can be defined as a material that is composed of complex structures and dimensions that fall within the limits of 1–1000 nm. During the last thirty years or so, a vast development of nanotechnology has been witnessed because of this size in the areas of materials engineering and science. It is extremely vital to choose the most suitable technique in the synthesis of nanomaterials and nanocomposites with preferred properties or a combination of different properties. This is due to the fact that the properties and microstructure of nanostructured materials are governed by their structure and chemistry as well as how they were synthesized and processed.

In the fabrication of advanced ceramics, the most widely used method includes wet chemical processing approaches (for example sol-gel and co-precipitation) and pressing, all of which have been employed to synthesize nanoparticles, nanocoatings, and nanostructured solid blocks and shapes. Pressing, in modern ceramics technology, is achieved by placing the ceramic powder into a die and pressure is applied causing compaction. Similarly, high-density bioceramics are commonly produced using hot pressing and hot isostatic pressing. Using hot pressing, it is relatively easy to manufacture non-uniform components as well as flat plates or blocks. On the other hand, the smaller grain structures and higher densities required by bioceramics can be satisfactorily produced using hot isostatic pressing where heat and pressure are simultaneously applied from every direction using a pressurized gas such as argon or helium.

In addition to pressing, the unique process of sol-gel can also be applied to manufacture ceramics of various forms such as coatings, platelets, fibers, monoliths, and powders with identical composition simply by changing the viscosity, chemistry, and other features of a given solution. There are a number of advantages associated with the sol-gel method including the manufacture of a pure, stoichiometric and homogeneous product due to the mixing is being carried out at a molecular level, and this high purity can be maintained as grinding can be avoided. Furthermore, a reduction in the firing temperature can be achieved owing to its small particle sizes with high surface areas.

The sol-gel technique has the capability of producing uniform fine-grained nanostructures using either the aqueous-based or alkoxide chemical routes. Using the sol-gel approach to synthesize coatings have an additional benefit as the process only requires a small amount of precursor materials, resulting in their costs being relatively unimportant. Depending on the chemistry, shrinkage is considered relatively uniform perpendicular to the substrate for multi-layered coatings and the coatings can be dried rapidly without cracking. In spite of this, shrinkage is an important factor during the production of ceramic monoliths [3–6].

There is a potential to expand the design and production of new nanomaterials that are beneficial for medical applications using the combination of the unique ceramic production techniques previously discussed and advancements in new enabling technologies such as surface modification techniques, 3-D printing that utilizes both liquid initiated and solid powders, and micro- and nanoscale and biomimetic (bioinspired) fabrications at an unparalleled rate.

At present, the focus is on the manufacture of novel nanoceramics that are applicable for a wide range of functions such as cancer treatment, tissue engineering and regeneration with increased bioactivity, viral and bacterial infection treatment, materials used in minimally invasion surgery, deliveries of gene, drug, and oxygen to damaged tissues, and implantable medical devices that has been surface modifies to achieve improved hard and soft tissue attachments.

1.3.1 Nanocomposites

A nanocomposite can be described as a heterogeneous mixture of two or more materials where at least one of those material should be a nanomaterial. Through the use and assistance of a secondary substitution material, it is feasible to engineer the mechanical properties of the composite (i.e. Young's modulus) to match those of natural bone through the use of the composite approach. At present, one such example is a composite composed of hydroxyapatite and polymeric material, which have been shown to possess a Young's modulus value similar to that of bone tissue.

Nanocomposite can be manufactured by either mixing physically or through the introduction of a new constituent into an already existing nanomaterial and this enables the properties of the nanostructured material to be altered and may even offer new functions or applications. For instance, it has been reported that certain

biomolecules or biopolymers such as poly(lactic acid) (PLA), poly(lactic-*co*-glycolic acid) (PLGA), polyamide, collagen, silk fibrin, chitosan, and alginate have been amalgamated with nano-hydroxyapatite systems.

Another type of nanocomposite is the gel system which has been developed for biomedical applications. With this system, a gel (essentially a three-dimensional network immersed in a fluid) is used to entrap or encapsulate nanomaterials. This results in improvements in the properties of the nanomaterials and can be adapted to meet the specific requirements of individual biomedical prosthetics or devices. An example of a gel system which can be utilized as a drug delivery vehicle is a nanogel. In essence, a nanogel is a nanosized flexible hydrophilic polymer gel and one of the key advantages with such system is that they permit for a high "payload" of macromolecules of up to 50 wt%, a value which normally cannot be approached with conventional nanodrug carriers [7, 8]. Through ionic interactions, these nanogels can spontaneously entrap and bind any kind of negatively charged oligonucleotide drugs. Additionally, an innovative intracellular biosensor has been developed by encapsulating indicator dyes into an acrylamide hydrogel [9, 10]. Moreover, an enzyme-based biosensor was developed using a carbon nanotube aqueous gel as they have been suggested to be an enzyme-friendly platform [11].

1.4 Liposome-Based Delivery Vehicles

It can be said that lipids and bioceramics complement each other. In nature, lipid vesicles are the primary template for controlled biomineralization into marine shells, teeth and bones. Liposomes are synthetic vehicles that can be used in conjunction with proteins and drugs to treat diseases, foreign body wounds and cancer [12]. They can effectively encapsulate and immobilize a wide-range of genes and drugs that are different in size and structure. One of the biggest advantages of liposome is that the hydrogen ion concentration, pH, as well as other ionic concentration can be controlled without affecting the core of the liposome.

In addition, liposomes can also offer protection for encapsulated biological materials such as peptides from being degraded and damaged. Furthermore, they possess higher loading capabilities in comparison to microemulsion, in particular when it comes to water-soluble additives. Stimulations for liposomes is gathered from cells and its intimate similarity to cell boundaries and delineated sacs. In general, single and multilayered spherical bilayer vesicles with thicknesses between 40 nm and 50 μm are synthesized by mixing amphipathic lipids in a polar solvent. Liquid-crystals created by lipids generate highly-ordered structures that can produce three-dimensional bicontinuous cubic organizations and they showed promise as sustained delivery vehicles for peptides and proteins [13].

For any man-made or synthetic delivery systems, they lack the presence of biorecognition molecules which allows specific targeting in cancer and gene therapies to occur unlike vesicles and cells. The addition of a lipid layer to those man-made replicates can enhance their functions. The deposition of a chitosan coating can also

result in such improvements, for instance a reduction in leakage of encapsulated substances as well as an increase in the stability of the delivery system. One important method of mobilizing and directing liposome-based vehicles to a pre-selected destination is to use selective liposome targeting to cell-surface receptors. At the moment, the most effective strategy of solving this problem is through the use of recombinant immunoglobulins. The combination of liposomes and a synthetic polyethylene glycol (PEG) can produce a composite delivery system with enhanced capabilities such as higher targeting potentials.

It has been documented that the use of liposomes has been reduced due to factors such as low encapsulation efficiency and inadequate storage capacity [14]. Liposome-based structures with greater stability and organizations can be produced using crystalline materials with relative ease. Drug encapsulation and elution properties can be controlled and regulated by altering the molecular structure.

1.5 Bioceramic-Based Delivery Vehicles

As previously mentioned, bioceramics represent an important class of biomaterials designed for the tissue engineering of bone, cartilage, and teeth. Biominerals, despite their characteristic crystallographic structure, can be remodeled through specialized biological regulations and controls of mineral deposition on organic membranes as well as within and between cells into an intricate three-dimensional morphologies and richly diverse collection of curved shapes [15–17]. In comparison to mineral crystals created from the laws of physics, a number of the morphologies which are produced by biology are more architecturally complex. On the other hand, silica in general precipitates as spherical colloidal particles, while unicellular organisms can manipulate silica minerals into “lace-like” structures.

Due to their bioresorption capacities and biocompatibility, hard mineralized materials and their derived structures are ideal as substitutes for calcified bone and joint tissues. In terms of bone replacement surgery, the most promising materials that can be applied as a drug delivery system in calcified tissues are biphasic calcium phosphate bone substitutes derived from a mixture of hydroxyapatite and β-tricalcium phosphate [6]. Studies are being carried out to enhance their biological activity using a number of different means, as these minerals by themselves are biologically inert.

In terms of bone repair and reconstruction that utilizes biomimetic drug delivery vehicles, an issue that has become increasingly vital and essential is the combined release of multiple biological molecules and therapeutic drugs from the same system. For example, the opinion concerning the procedure being used in the treatment of osteoarthritis is to achieve a co-operative balance between bone promoting and resorbing drugs and antibiotics. This is due to the fact that there is always a constant and recurrent threat of bacterial infection arising from the highly invasive nature of bone surgery [18, 19].

This belief was applied during the synthesis of biomimetic nanoapatite crystals engineered to release alendronate and anti-cancer and/or anti-metastatic drugs in a

combined manner via the controlled desorption on the crystal surface [20]. The rate of release was governed by the use of either plate-shaped or needle-shaped crystals with various surface areas and charges. The theories and concepts provided by biomimetic materials chemistry has generated a number of key benefits that can be applied in the regeneration of calcified tissues [17, 21].

1.5.1 Nano-hydroxyapatite Powders for Medical Applications

More accurately, bone mineral consists of nanoplatelets rather than nanocrystals initially described as hydroxyapatite, which bears the resemblance to the mineral dahllite. Presently, a consensus has been reached for a description which is more appropriate to describe bone apatite. They are now termed carbonate hydroxyapatite with a chemical formula similar to $(Ca,Mg,Na)_{10}(PO_4CO_3)_6(OH)_2$. The composition of commercially available carbonate hydroxyapatite is similar to that of bone mineral apatite.

Nanotechnology has created innovative methods for synthesizing man-made bone-like nanopowders. Nanopowders and nanoparticles of hydroxyapatite have created new opportunities in the development of nanocomposites for dental and orthopedic applications. They provide excellent bioactivity arising from their extremely high surface area for integration into bone [3–6]. Nanoplatelets and nanopowders of bone-like hydroxyapatite can be produced using a wide variety of synthesis techniques. One approach in particular that showed great promise in the synthesis of bone-like hydroxyapatite is the sol-gel approach. It should be mentioned that monophasic sol-gel hydroxyapatite coatings and powders are more difficult to synthesize despite that fact that previously published works revealed biphasic hydroxyapatite could be produced with relative ease.

At present, nanocomposites consist of hydroxyapatite macro- and nanoparticles and organic and/or biogenic materials such as synthetic peptides, growth factors, and collagen are being produced by a number of companies and research groups. The mechanical properties of the nanocomposite are enhanced through the combined use of macro and nanoparticles as this cannot be achieved simply by using nanoparticles. Increases in bioactivity and mechanical properties have been documented in dental and orthopedic applications with some of the nanocomposite materials as dental fillings and bone cements [22].

The production of porous hydroxyapatite scaffolds has been previously reported using two different approaches based on the manipulation of the hydroxyapatite slurries [16]. The first approach involves the infiltration of a polymeric sponge into the slurry until the inner walls of the polymer are completely covered by hydroxyapatite powders. The scaffold is subsequently fired to remove the polymeric sponge and the resultant ceramic skeleton is strengthened due to the sintering effect at high temperature. The second method involves the utilization of computer-driven rapid prototyping techniques such as robocasting to produce ceramic components with complex shapes and anisotropic microstructures. The process involves the extrusion

of a ceramic ink through a thin nozzle to construct a component one layer at a time following a computer-generated design. Sintering in air at temperatures ranging from 1100 to 1200 °C produces a dense ceramic with narrow grain-size distribution. Both methods are said to be capable of synthesizing ceramic scaffolds with suitable pore sizes to promote bone ingrowth [23].

In another study, a powder mixture composed of biodegradable fillers (β-tricalcium phosphate) and a reactive component (tetracalcium phosphate) was printed using an aqueous citric acid solution [24]. In order to significantly improve the mechanical properties of the printed components, two post-processing procedures, namely a sintering and a polymer infiltration process, were utilized. Samples of various shapes and sizes were printed to examine the feasibility of the developed three-dimensional printing process using a powder-binder system. Initial investigations including in vitro cytocompatibility examination revealed this innovative printing system could be an effective technique in the manufacture of patient-specific ceramic scaffolds and substitutes for bone-tissue engineering.

A powder-binder system was also applied in another study to examine the possibility of creating ordered tubular structures with open porosity using microextrusion free-forming technique [25]. The mixture consists of fine hydroxyapatite powder suspended in isopropyl alcohol with a polyvinyl butyral binder. Tubular lattice scaffolds were produced and sintered at 1250 °C to create a ceramic structure that could potentially be utilized as a bone scaffold capable of encapsulating and releasing growth promoters in a controlled manner.

1.5.2 Calcite and Calcium Phosphate

Highly accessible collections of structural designs are provided by the rich taxonomic assortment of intricate calcite structures throughout the lower orders of the animal kingdom. A number of these structures will offer application for which they had not been originally designed, for instance the reticulated filtration system makes an ideal structure for drug entrapment and delivery by chance.

Presently, natural invertebrate skeletons are almost impossible to replicate using artificial means even though how these structures are synthesized at a molecular level in addition to how they are intrinsically assembled is well-known. As an alternative, chemistry that imitates simple forming phases during the morphogenesis of shell structures has created materials and constructs with similar detailed morphology and function.

A network of submicron and nanoscale interconnected channels and pores are found within naturally occurring for aminiera shells. These networks also provide additional paths for the fluids to flow and could potentially enhance the capability of the shells to accept metabolites, waste products, and growth medium.

The differentiation of osteoprogenitors into cartilage tissues and osteoid can be accelerated once it is cultivated along with structures that mimic the morphology of calcite shells of microscopic planktons synthesized using an analogous process [26].

Furthermore, it was discovered that growth factors could be entrapped between and inside the crystal plates during the assembly of microsphere, and these growth factors are released into the surrounding environment as the calcite is slowly dissolved. In addition, in vivo regeneration of mature mineralized bone and neocartilage is possible by combining these bone conductive microporous spheres with human allograft and human bone marrow stromal cells, the precursors to osteoblasts [27].

Bio-templates are created through the specific arrangements of structural elements such as channels, pores, and struts. These templates can then be utilized to arrange and organize various cell types into anatomically coherent and accurate functional tissues. For instance, the estimated 70,000 different species of corals can provide sufficient structural diversity to match the varied textures found in human bone.

A number of different and unique filtration architectures have been identified from certain species of tropical coral sands. Their unique design, which is composed of macro- and microscopic pores, can be used to control the rate of drug release. More importantly, these coral sands can be completely converted into a variety of soluble calcium phosphates. As mentioned previously, it is extremely difficult and even impossible to replicate these small pores synthetically. As a result, such coral sand makes a novel and indispensable slow drug delivery vehicle. In addition, other pharmaceutic/therapeutic drugs and biological substances such as antibiotics, bone morphogenetic proteins, and stem cells can be incorporated within these structures for any imminent tissue engineering applications.

The unicellular organisms that created these microscopic shells could potentially be cultured and reproduced with high precision inside fermentation containers. This also implies that we are not restricted by any structural designs governed by factors such as the environment and evolution. Accordingly, it is possible for humanity to harness the highly efficient production methods offered by nature for applications such as tissue engineering and drug delivery [28]. By regulating the culturing conditions, growth patterns can be controlled and ultimately the structures being synthesized as demonstrated in a study by Townlet et al. [29].

At the moment, the conversion of coral sand particles that have distinctive filtration structures such as *Schlumbergera floresianus* and *Baculogypsina sphaerulata* into calcium phosphates with controlled solubilities and encapsulating these sand particles with a variety of drugs and biological materials for slow dissolution and targeted delivery intended for applications in tissue repair and regeneration is a key area of research being pursued [4, 6, 19].

Conversely, marine-derived hydrogels have been proven extremely adaptable in experimental biomedicine as a delivery vehicle for growth factors, drugs and genes [30, 31]. Naturally occurring polysaccharides are highly biocompatible with a LD50 equal to table salt [31] as well as being bioresorbable. Alginate polysaccharides derived from chitosan, seaweed, and crab shells are one of the most adaptable substances in use for applications in biomedicine such as gene and drug delivery and tissue engineering [32–35].

Despite their advantages, it is essential for polysaccharides to undergo physical and chemical modifications so that their functions within the human body can be maximized. By far the most useful system for therapeutic intracellular delivery is

chitosan nanoparticles. These nanoparticles are labeled as smart delivery vehicles due to the fact that chitosan is extremely responsive to changes in temperature and environment pH (within a limited range). In addition to polysaccharides, alginates are equally important as versatile vehicles that can be used to encapsulate and deliver genes and proteins in a sustained manner by virtue of their chemical structure; for example, gelling can be the result of changes in the pH level or through the actions of ionic substitutions [36, 37].

1.5.3 Mineral-Coated Polysaccharide Microspheres and Nanospheres

A technique that has been widely applied to increase the number of functions that any biomaterial can perform is the creation of a composite material, through either amalgamation or the deposition of a coating on its surface with another biocompatible material. This necessity arose from the realization that a single biomaterial often does not acquire enough functional properties for a specific application [38]. The resultant composite materials permit the combination of various functional characteristics.

A simplistic technique of adding multiple coatings to an implant is through the use of layer-by-layer assembly [39, 40]. There are a number of advantages associated with this manufacturing process such as the ability to construct intricate hierarchical components from molecular-based units. In addition, this technique can be carried out with relative ease on three-dimensional and flat substrates and an array of substrates can be used.

In comparison to structures at the microscale, numerous unique advantages are offered by nanostructures and some of the most important and distinct features include their ability to load pharmaceutics in a more efficient manner and their increased likelihood of penetrating into cells and passes directly through basement membranes. A common layer-by-layer manufacturing strategy being developed at the moment is centered in the electrostatic association between highly-charged polysaccharides such as highly deacetylated chitosan to produce different composites [41].

The development of better tissue engineering scaffolds that contributes to the natural processes of regeneration through increasing the quantity of natural bio-responsive molecular associations are clearly needed at the present moment. These regenerative processes are extremely dynamic and delicate in space and time. It has been theorized that bio-adhesive building blocks and modules can be produced from unique polysaccharide assemblies combined with functional biomolecules responsible for cell-cell and cell-matrix interactions for active involvement in the re-assembly of cell-mediated extracellular matrix, which are guided and instructed by the cells themselves (both host cells and cells that are being introduced). Moreover, the re-engineering of those bio-adhesive modules will permit the release of soluble proliferation and differentiation elements and to provide support for the formation of tissues within the newly cell-assembled polysaccharide matrices.

1.5.3.1 Production of Tissue Assembly Modules

The creation of tissues that are not only anatomically accurate but also identical in terms of biology and function is the central objective of all tissue engineers. Normally, a suitable environment that allows the organization of cells into functional tissues through self-assembly is created by the engineer [42]. This approach, on the other hand, does not always generate functional tissues with prominent quality. Consequently, the presence of a biological blueprint is essential in the design of the architecture and in organizing the vascular arrangements. One such method that can be used to resolve this issue is to construct building blocks of tissues and guiding the organized assembly into a product that has a high degree of accuracy from an anatomical perspective. The assembly process may be encouraged by factors such as chemical bonding, receptor-ligand binding, or physical forces.

Another approach that displays high potential is the utilization of interlocking elements that have a tendency to amass into complex structures as the procedures that determine how these elements are constructed are written into them, resulting in the production of structures that are highly functional and organized.

There are universal and fundamental design procedures that regulate the construction of biological materials at various dimensions, i.e. molecular and nanoscale [43]. Examples of building blocks that shows promise due to their ability to form specific associations and are high functional include microtubules, de-oxyribo nucleic acid strands, and peptide amphiphiles [44]. The manufacture of these components synthetically inside a laboratory can enhance their ability to control biological outcomes.

A number of functional applications are provided by microtubules including a fixture point for intracellular components, transportation link for moving materials, and most importantly providing structural integrity for cells. Newly constructed microtubules can be re-arranged from simple tubulin monomers by means of chemical stimulation followed by the application of a direct mechanical force [45, 46]. Sufficient amounts of energy are provided by this combined action to produce the bonds between the tubulin monomers. Conversely, the combination of mechanical interaction and chemical stimulation can also be used to dismantle this bond. In the same way as tubulin, de-oxyribose nuclei acids can also be used in the engineering of new structural frameworks with high integrity as they are categorized as self-assembly building blocks.

Significant amount of progress has been made on the synthesis of new biomaterials using molecular-engineered proteins and peptides [44]. The creation of new frameworks together with the supervision and involvement of living cells applied to the re-development of tissues is an attractive objective to be pursued as extracellular framework with desired functional and structural properties can be regenerated by accessing the genetic programming of the cell. A relationship exists between every cells and simplified building blocks and modules that represents an element of the extracellular matrix displaying certain molecular motifs, for example sulphation codes on glycosaminoglycans (GAG's) [47]. These components are initially synthesized followed by the secretion of cells they are related to.

A hypothesis based on this phenomenon worthy of exploring relates to the deposition of biorecognition ligand molecules suitable for the integrin protein discovered on the surface of the selected cell to facilitate the possible establishment of new environment once contact is made between individual cells. In comparison to peptide-free surfaces, higher cell recruitment can be theoretically achieved by binding the polysaccharide capsules with a universal cell adhesion tripeptide such as arg-gly-asp-RGD after 24 h of incubation. However, it is vital to determine the size, length, surface density, and distribution pattern of peptides as these factors are known to have profound effects on cell migration and adhesion [48, 49]. In addition, a study has revealed the stimulation of myoblast cell phenotype can be intensified if the densities of RGD attached to alginate is in the range of 1–100 fmol/cm^2 [36].

1.5.3.2 Multi-layered Mineral-Coated Polysaccharide Spheres

Polyelectrolytes have been utilized to govern and control the release profile of the encapsulated substance during the manufacturing of the vehicle used in controlled drug delivery [40]. A number of advantages are offered by polyelectrolytes including their ability to assemble rapidly and effortlessly in mild conditions and with high functional efficacy [39].

Multiple concentric layers of biomaterials can be deposited onto polysaccharide spheres by electrostatic attraction between individual oppositely charged substrate layers. Entrapments of drugs and growth factors inside the water-filled space between membranes can be achieved using hydrogels consisting of multi-layered membrane, similar to the construction of an onion. More importantly, the intended application will determine the number of layers used to produce the hydrogel [50]. Alginate, β-chitin [32, 51–53], hyaluronate [54], chitosan [41, 55] have been identified as potential candidates in the production of multi-layered hydrogels with the aim of compartmentalizing a variety of therapeutic and/or pharmaceutic substances at different concentrations within the series of concentric shells. Furthermore, the individual layers of the concentric shell can be manufactured with customized properties such as degradation and permeability. Physical entrapments of cells and therapeutic proteins such as TGF-β3, BMP-2, and chito-oligomers for bone tissue regeneration at the interface between two concentric shells have been shown to be viable. Likewise, through the application of carbodiimide chemistry, proteins can be chemically conjugated and adsorbed into the polysaccharide substrate [36, 56, 57]. In addition, an increase in the resistance to mechanical forces and stability of chitosan shells can possibly be achieved through the addition of amorphous calcium phosphate [58].

The layers can be unwrapped one layer at a time towards the core of the concentric capsule and the encapsulated proteins are released during peeling process. This process also ensures the rupture of the entire capsule is belated. The calcium and phosphate ions within the crab shell chitosan and sodium alginate forming solutions are used to create an outer shell encasing the capsule and the concentrations of the ions are used to program and adjust the fracture and swelling rates of this outer shell.

Furthermore, a relationship exists between the thickness of the outer shell and the concentrations of the calcium and phosphate ions as the concentric capsule hardens. A thicker outer shell is produced by increasing the concentrations of the calcium and phosphate ions, and this in turn increases the time needed to rupture the capsule. On the other hand, the shell will become extremely brittle if the concentration of calcium ion exceeds 50 mM and phosphate ions is greater than 300 mM.

Similarly, entrapment of cells between individual layers is also possible, and the creation of concentration gradients of growth factors, proteins, and genes within every individual polysaccharide capsule that is significant physiologically from the exterior to the core is feasible with such assemblies.

1.5.3.3 Biomimetic Cell Assembly Mimics

Tissue regeneration operates alongside drug therapy and discussions have been raised concerning the possibility of harnessing certain cells to replace drug compounds by genetically engineering these cells to release therapeutic proteins in conjunction with local physiology. Furthermore, the same role may also be undertaken by harnessing the functional unit of cells. Cellular assemblies can be considered as functional components surrounded by an ordered arrangement of cells of various types with distinct roles in the reconstruction processes of tissues. The ability to synthesize basic units of tissues followed by their construction into multiple hierarchies and layers in the laboratory will be regarded as highly advantageous. In simple terms, this means that synthetic building blocks are used by the selected cells to create their own unique structures under suitable environments.

Several factors based on human biomechanics, partition chemistry, and biology governs the formation of mineralized tissues and regulates their assembly into defined spaces. The assemblies of cells are limited by borders comprised of impermeable substrates that encompass the mineralization in addition to regulating the chemistry and structure of the fluid-occupied environment [17, 21]. Cells arrange themselves into groups within the bone during the mineralization process. The collaborations of osteoblasts to produce fluid-filled compartments that separates from blood and other mineralized tissue is one of the hypotheses that has been proposed to describe bone growth. Moreover, fluid-filled regions are isolated from already existing mineral wall and organic polymer sheet [59].

The use of modular self-assembly to synthesize novel biomaterials is a process that has been well-established. The design and fabrication of, via biology in real-time, a new category of biomaterials can be achieved using the cell-mediated approach. The re-assembly of the matrix environment of human progenitor cells using synthetic and engineered multi-component biomaterial segments or “building blocks” is an idea that is still progressing. Cell-engineered biomaterials can be re-designed with optimized properties accurately with this approach [60].

Pre-fabricated scaffolds are currently being developed with integrated bio-recognition motifs or ligands by tissue engineers that are regulated by activities that are naturally occurring as well as responses by progenitor cells observed during the

early development ad regeneration of tissues in adults. Simulating the complex time-dependent interactions amongst individual cells and between cells and its matrix has been attempted. For instance, the fabrication of polymeric scaffolds stabilized with cross-links and ligands that can only be destroyed by matrix metalloproteinase-3 (MMP-3) has been achieved and their structure is altered once metalloproteinases are secreted by cells locally [61–63].

This alteration is designed to stimulate matrix events that provide re-enforcements to the regeneration process. The development of cell-independent self-assembling biomaterial structures that continuously simulate their natural counterparts has been the objective amongst different researchers. Improvements in both the quality and rate of tissue regeneration in addition to maximizing biological responses are provided by both techniques.

Microsponges composed of calcium carbonate with dimensions at or below that of a single cell were controlled in past studies by individual cells and organized favorably by co-cultured human bone marrow stromal cells into mono-layered aggregates with various cell densities [26]. The feasibility of coating the external surfaces of polysaccharide spheres with GGGRGD (Gl-gly-gly-Arg-gly-asp) peptides has previously been demonstrated which allows the preferential attachments of cells to the outer surfaces [56]. Furthermore, the amalgamation of crab shell chitosan with RGD tri-peptides in solution was carried out before the deposition and polymerization onto an alginate droplet [30].

The creation of cellular assembly mimics has also been hypothesized, and in essence they are biocomposites consists of human cells and polysaccharide that imitate the conversion process to discrete mineralized modules from mature bone cells that occurred naturally. This transformation signifies the precursors to bone reconstruction [59, 64, 65]. The manufacturing of cellular assembly mimics utilizing polysaccharide modulus as starting materials applied in bone reconstruction is one of the primary capabilities that are being developed. The other is to create cell-recognized matrix elements that can be re-organized into macromolecular-scaled cellular niches.

The transportation of regenerative factors in the correct amount to treatment site in a chronological order has proven to be rather intangible. The difficulty stems from technical issues such as the synchronized release in collaboration with host physiology and the number of biological responses which can occur accordingly [66, 67]. The stability of proteins is limited as they degrade too rapidly. In comparison to the delivery of proteins to cells directly, strategies based on gene correction are intended to enhance the potency and effectiveness of protein synthesis and secretion form the cells [68, 69].

Factors such as selecting the appropriate synthetic biomaterials and lipids and the physical disruption of the cell membrane to permit the infiltration of new and prominent genes and transcription factors determine the success rate of cell-mediated gene therapy that utilizes non-viral transduction agents. Unproductive inefficiencies in gene expression levels and targeting along with inappropriate gene integration are associated with all of the aforementioned processes. A method has been reported in which the transfection efficiencies increased to more than 65% and a toxicity level

is reduced to less than 5% [70]. Furthermore, an increase in transfection efficiencies can be achieved through the use of lipid DNA polycondensates and cationic polymer, while at the same time eliminating issue of lysosome degradation [71, 72].

The release of encapsulated bioactive compounds in synchronization with the body's own biochemistry at dosage levels beneficial for cells in precise orders and for certain periods of time is of vital significance. Such approach will ensure maximum potency and efficacy is provided by the encapsulated compounds.

One of the key challenges concerns the release of individual compounds for extended periods in a slow and sustained manner aimed at permanently restoring the functions of tissues. Two methods can be utilized to control the release rate of the encapsulated substance. The first approach is based on the alteration of the shell of the delivery capsule in which the thickness and composition is adjusted, and in view of that, this technique exploits the theory of diffusion to slow the rate of release of the uploaded content. This approach has been proven effective in slowing down the release of plasmid de-oxyribose nuclei acid.

The second technique is centered on the fabrication of capsules one inside another to produce a nested arrangement [30]. This "host-guest" combination of capsules is somewhat effective in the release of encapsulate into the surrounding medium in a timely order. Under experimental conditions, capsules containing tyrosinase were synthesized and inserted into a vacant host capsule. This process was repeated twice, and all the capsules contain identical volumes of tyrosinase. The volume of enzyme that must diffuse through is increased through this repeated nesting process, and subsequently the release rate to the surrounding environment is decreased.

1.6 Concluding Remarks

Biomimesis is an idea with rising importance and relevance to a diverse range of sciences and technologies such as biology, materials, nanotechnology, and medicine. Gaining deeper insights into how nature works is vital to the research and development of smart materials, structures, and processes with self-actuating, self-stabilizing, and self-assembling properties. Tissue engineers are confronted with challenges related to the manufacturing of scaffolds with numerous functions (these functions often contradict with one another) that must be bio-responsive and evolve to a dynamic host environment in real-time.

The principles of bioinspired approach used in the design and production of tissue engineering scaffolds have been well-documented and emphasized through BioTriz methodology. There are at the moment additional techniques that are centered on nanotechnology and the synthesis of materials and structures using nanoparticles and nanomaterials. Several recent examples are displaying how biomimesis can be used to create innovative functional tissue engineering frameworks with morphologies and structures (both intricate and complex) unachievable using conventional production techniques.

Regenerative medicine is confronted with problems related to a shortage in clinically relevant scaffold designs and biological factors that promote the natural cycle of regeneration. Obtaining a greater understanding into hierarchical design in nature and harnessing the chemical properties of natural structures at all dimensional levels such as macro, micro, and nano will be instrumental in the re-assembly of functional structures that mimics natural skeletal design. We believe in utilizing bio-inspired and nanoscale materials chemistry to achieve this goal. Such knowledge will also enable tissue engineers to synthesize new and advanced bio-structures and materials that are truly patient-ready as well as being capable of responding to the functional demands imposed during the regeneration of native human tissues. Moreover, the internal microenvironments for embedded cells can be modulated to recreate elements of a native extracellular matrix adding an additional biomimetic element to this unique system.

Bioevaluation delivered convincing evidence that these scaffolds may offer clinical success as novel scaffolds or gene/bio-factor delivery vehicles for the engineering of both mineralized and soft human tissues.

In this chapter, we have provided an example of a self-assembling organic scaffold in which spontaneous mineralization of calcium phosphate can occur. This same procedure is comparable to the template-mediated mineralization observed in nature such as the mineralization of eggshells.

There is a rapidly expanding and ongoing diversification of pharmaceutics and therapeutic proteins, carbohydrates, and lipids. This has been stimulated by the increased knowledge of molecular events in both healthy and diseased organs and tissues. On the other hand, improvements are needed in order for these substances to be used safely and effectively under clinical conditions. For example, potent drugs produce unwanted and potentially damaging side effects and toxicity in otherwise healthy tissues and organs. Excessive concentration of any therapeutic biomolecule distributed to the incorrect or diseased-free locations can also degrade normal physiological function. Such problems can only be addressed through accurate targeting of proteins to the specific tissue and cells delivered using small and highly mobile transplantation vehicles with efficiency equivalent to lentivirus-mediated gene-to-cell transfer.

Biomedical engineers are continually motivated to improve and transform therapeutic medical treatments in an attempt to reduce the invasiveness of surgery in addition to the amount of pain, inflammation, and surgery time needed with drug and protein delivery vehicles. However, delivery systems currently utilized are deficient in two critical ways: inaccurate targeting and delivery is not sufficiently well regulated.

Consequently, ongoing research and discovery of new and more clinically acceptable technologies that can remotely arrive at a designated tissue site and deliver drugs, growth factors, or genes continuously in any specific three-dimensional and chronological patterns. Spheres (with low dimensions) can be applied effectively to transport these biological modules and protect them from physiological degradation. Furthermore, they conform to the shape of the cell during transportation.

Universally, transportation capsules with dimensions that fall within 10 μm–100 nm are one of the most effective unit used in targeted delivery. Capsules in the shape of a sphere possess minimal surfaces areas and a volume where a large amount of therapeutic constituents can be encapsulated. Spherical capsules are also known for their high encapsulation efficacies for tissue modulation, and high packing efficiencies for tissue repair.

Applying the lessons learnt from basic versions in the natural world and from functional replicas in human biology could provide unique answers to these challenges. The first and most difficult step is the discovery of biological analogue that is best suited for carrying out the function we want to develop. For instance, in human biology, studying matrix vesicles will provide us valuable insights into how proteins are captured and coated in addition to how these vesicles is able to dock and fused to their target. A second role model for biomimicry is the filtering microskeleton of foraminifera.

We have selectively highlighted biomimicry approaches to produce new devices that may potentially deliver drugs and genes to their intended destination in correct dosages. Biomimicry, in this chapter, involves the selection of suitable analogy from nature that solves similar problems to the one under examination. Often, we noticed that non-human biology provides simpler and more convenient solutions. We have also examined how lipid vesicles can be routinely self-made to mimic their natural counterparts. We have also showed how bioceramic spheres were made synthetically using biomimicry chemistry for capturing and delivering growth factors to osteoprogenitors. Equally and perhaps more advantageous is the direct use of natural skeletons suited to deliver bone promoting drugs and antibiotics in tandem.

In the future, cells may also play a part in the delivery of beneficial therapeutic proteins. In all probability, this might be the best way forward since cells can be genetically programmed to secrete proteins in synchronization with the host. Future work may also encompass ways of detecting the status and position of integrated devices.

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During in his formative years Prof. Ben-Nissan worked on Titanium and its alloys and Magnesium alloy development and casting technologies and their properties for both engineering and medical applications. Over the last four decades, Professor Ben-Nissan has worked and contributed to the biomedical materials, implant design, production and analysis of various advanced ceramics, nano-coated sol-gel developed thin films, coated orthopedic and dental implants, anti-microbial slow drug delivery devices and methods, marine structures for clinical

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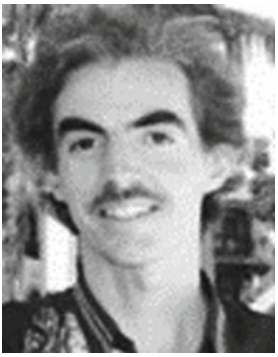
He has successfully developed materials for implant technology such as ceramic knee prosthesis, calcium phosphate based bioactive materials, bone graft production and bio-composites, and conducted research on biomechanics and modelling such as jaw bone, knee and hip joints, reliability and implant design modular zirconia ceramic knee prosthesis, femoral head and taper stresses and artificial ocular implants and bionic eye and recently on 3D printing of bioceramics and metallic implants and anti-microbial multifunctional coatings for drug delivery which are supported by the European Commission and the Australian Academy of Science research grants.

Since year 2000 he has published over 200 fully refereed papers in journals, and a book and 43 book chapters. He edited a book on Calcium phosphates and working on a second one on the use of Marine Structures in the Biomedical field. He is the editor of the Journal of the Australasian Ceramic Society. He was awarded by the Australian Ceramic Society's prestigious award for his contributions to the "Ceramics Research & Development and Education in Australia". For his research on multifunctional nanocoatings he also received "The Future Materials Award".

Andy H. Choi Dr. Andy Choi is an early career researcher who received his Ph.D. from the University of Technology Sydney (UTS) in Australia in 2004 on the use of computer modelling and simulation known as finite element analysis (FEA) to examine the biomechanical behavior of implants installed into a human mandible. After completing his Ph.D., he expanded his research focus from FEA to sol-gel synthesis of multifunctional calcium phosphate nano coatings and nano composite coatings for dental and biomedical applications.

In late 2010, Dr. Choi was successfully awarded the internationally competitive Endeavour Australia Cheung Kong Research Fellowship Award and undertook post-doctoral training at the Faculty of Dentistry of the University of Hong Kong focusing on the application of FEA in dentistry and the development of calcium phosphate nano-bioceramics.

He is served as an associate editor for the Journal of the Australian Ceramic Society and on the editorial boards for a number of dentistry, nanotechnology, and orthopedics journals. To date, Dr. Choi has authored over 50 publications including 3 books and 26 book chapters on calcium phosphate, nano-biomaterial coatings, sol-gel technology, marine structures, drug delivery, tissue engineering, and finite element analysis in nanomedicine and dentistry.



David W. Green Dr. Greem is working at the moment the bioengineering interface between physical, chemical and biological phenomena; He is attempting integration of non-living matter with living matter for manufacture of bioinspired systems. Consequently, to revitalize cells and tissues into novel regenerative therapies. Accordingly, he is guided by biomimetics and bioinspiration philosophies to create new innovations for healthcare. Presently, Dr. Green is focused on biomimetic development of anti-biofilm materials and stem cell niches.

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Sophie Cazalbou Assoc. Prof. Cazalbou's research activities mainly concern the formulation, shaping and characterization of new bioactive biomaterials mainly used as bone substitutes and capable of releasing in vivo active substances such as ions, molecules, and proteins. She is currently interested in developing new minerals and composite biomaterials in supercritical CO_2. This process of "green chemistry" opens new perspectives in the synthesis and development of highly reactive ceramic with controlled architecture. She is working on the following areas: (1) Formulation of biologically active biomaterials (such as coatings, ceramics, cements, composites); (2) Formulation of biomaterials used as delivery systems for active substances (such as antibiotics, anti-inflammatories, growth factors, biologically active ions, bisphosphonates); (3) Influence of microstructure on the properties of transport through the pore space (transport of active species, biological fluids and cells); and (4) Theory of percolation used as pre-formulation element.