Current Topics

Bioceramic Research on Intelligent Implants and Drug Delivery System

Coral Exoskeletons as a Precursor Material for the Development of a Calcium Phosphate Drug Delivery System for Bone Tissue Engineering

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With the global rise in aging of populations, the occurrence of osteoporosis will continue to increase. Biomaterial and pharmaceutical scientists continue to develop innovative strategies and materials to address this disease. In this article, we describe a new perspective and approach into the use of coral exoskeletons as a precursor material to synthesize a calcium phosphate-based drug delivery system. Studies detailing the methodology of the conversion methods and the strategies and approach for the development of these novel drug delivery systems are described. Furthermore, *in vivo* studies in osteoporotic mice using a drug loaded and chemically modified version of the biomimetic delivery system showed significant cortical and cancellous bone increases. These studies support the notion and the rationale for future research and development of the use of coral exoskeletons as materials for drug delivery applications.

Key words biomimetic; beta-tricalcium phosphate; osteoporosis; coral; drug delivery system

1. INTRODUCTION

The global population is entering an era of increased longevity owing to improvements and advancements in healthcare technologies. This will significantly increase the aging population which will in effect add to the already overstretched healthcare costs associated with care of the elderly. Furthermore, society as a whole expects continued biomedical breakthroughs as scientific innovation continues to advance. Part of the aging process is the increase in the number of ailments an individual faces. Amongst these, bone fractures as a result of breakage or from a disease like osteoporosis is one of many challenges that can significantly impact a patient's life as well as increase socioeconomic costs. This has continued to inspire biomaterials scientists to develop innovative and novel materials for fulfilling these ongoing demands. The last decade has witnessed the rise and advancement of the multidisciplinary field of tissue engineering and regenerative medicine and many exciting innovations have been presented. The increase in therapeutic efficacy of pharmaceutics has provided patients with more potent treatment and therapies. However, the treatment of osteoporosis requires a continued and prolonged therapy that needs an alternative perspective and system to current oral tablet intake and/or direct injections. The development and application of controlled drug delivery system is not new and many of these systems are able to treat diseases that require long-term release of pharmaceuticals. Depending on the application, the key-defining factor that impacts the success of a drug delivery system is dependent on the selection of the scaffold material and the pharmaceutics that are incorporated or loaded into the material. Many of these materials are developed based on synthetic production and optimization over time, and their effectiveness is still quite limited. This has led to the development of a new field in biomimetics, where scientists turn to nature for inspiration and as a source of new material. For biomaterial scientists, this new source of undiscovered and innovative material is found in the marine environment. Coral exoskeletons, which are composed of intricate and unique architectures made up of chambers of interconnected pores, provide the ideal carrier material for drug delivery. The complexity of these porous structures and networks is beyond what can be synthetically produced or replicated. The uniformity of these pores will allow a more predictable drug loading and subsequent release. Furthermore, these exoskeletons possess macro, micro and nano-pores that allow different diffusion rates for pharmaceutics.[1)] This article will discuss the potential application of using coral exoskeletons as a novel material for the development of future drug delivery systems.

2. CORAL EXOSKELETONS TO CALCIUM PHOSPHATE

The marine environment has been an attractive source of material for many biomedical and pharmaceutical applications owing to the vast diversity in macro-organisms. Similarly, the diversity of coral species is equally complex and requires continued research into categorizing the structures of these species. The structures of each coral species remain relatively similar but can sometimes be distinguished in response to

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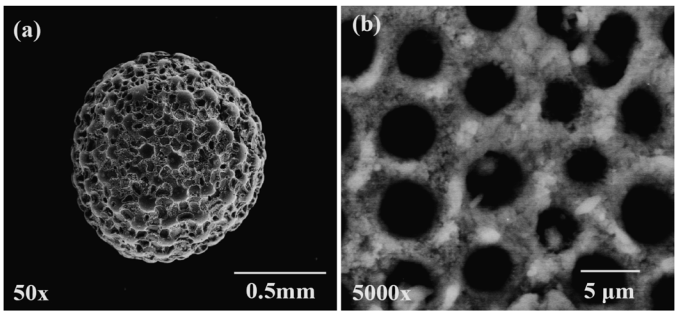

Fig. 1. Scanning Electron Micrographs at (a) Low Magnification (50×) Showing the Spherical Structure of the Coral Exoskeleton and the Pore Distribution throughout the Surface; (b) High Magnification (5000×) Image Showing the Uniformity of the Pores Averaging *ca.* 5 μm

their local environment. Our group has identified the species *foraminifera*, which can be found in many parts of the world, to possess an ideal structure as a carrier material for drugs. *Foraminifera* are distinguished from other species by its unique interconnected porous network, as previously discussed, and the naturally spherical structure of the material.[1] Macrospheres are particularly interesting with added potential as they have a higher surface area and can conform better to irregular sites. *Foraminifera* exoskeletons are generally found as fossilized calcium carbonate in the form or aragonite or calcite. They can then be hydrothermally converted by chemical replacement of the carbonate with phosphate to derivatives of calcium phosphates depending on the Ca/P ratio, which can be controlled.

$$10CaCO_3 + 6(NH_4)_2HPO_4 + 2H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 6(NH_4)_2CO_3 + 4H_2CO_3 \quad (1)$$

For a fast-acting drug delivery system, the material might not require conversion as calcium carbonate degrades faster than calcium phosphates and may be appropriate for the intended purpose. For long-term drug release systems, a more stable form of calcium phosphate like tricalcium phosphate (Ca/p=1.5) is more ideal. Roy and Linnehan[2] were the first to present this conversion method when they converted coral to hydroxyapatite. Since then, the process has been refined but generally involves placing the exoskeleton in phosphatic solution under 15000 psi for 24–48 h at 220–250°C. This conversion strategy allows for the preservation of the structure of the original material while transforming the chemical structure.

3. DEVELOPMENT OF BIOMIMETIC DRUG DELIVERY SYSTEMS

To stimulate the bone formation and regeneration process, many bioactive agents are available for loading into carrier materials. These can include various types of bone morphogenetic proteins (BMPs), growth factors and pharmaceutical drugs. In previous studies, it was shown that an antibiotic (gentamicin sulfate),[3] bisphosphonate (pamidronate),[4] and simvastatin[5] could be loaded into beta-tricalcium phosphate (β-TCP) derived from *foraminifera*. The most common strategy is by direct adsorption of the drug compound in concentrated solution over a period of time. The loading efficiency is dependent on the type of drug, but from previous studies the antibiotic gentamicin and the bone-stimulating drugs bisphosphonate and simvastatin have yielded 70%, 95% and 80% loading, respectively.[3–5] Osteoporotic treatment would require a more prolonged therapy and as such the coating of the material would "slow" the release of the drugs and allow for a longer therapeutic release. Another common strategy for producing more bioactive materials is by the modification of key ions in the chemical composition of the material. Additional ions such as strontium, magnesium and zinc can be incorporated into the lattice structure to further control the degradation of the material.

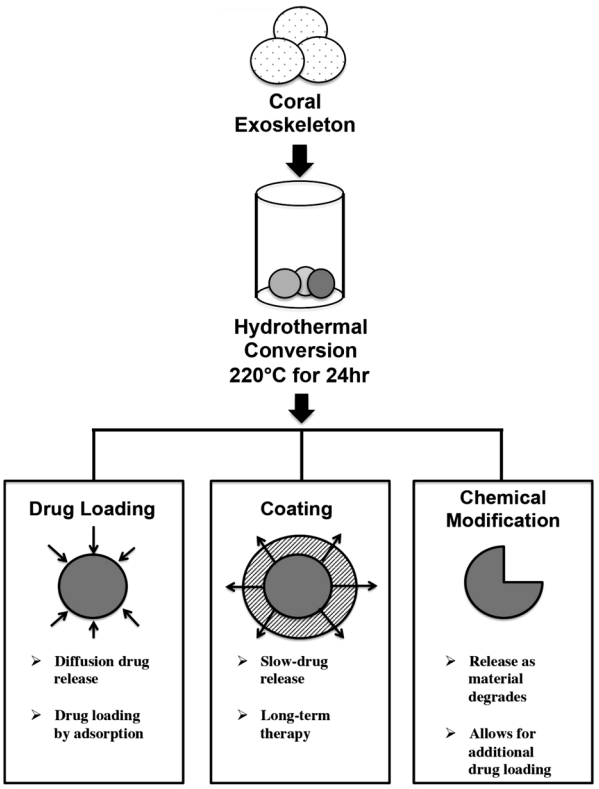

Fig. 2. A Schematic Diagram of the Different Strategies Aimed at Developing Drug Delivery Systems Based on Coral Exoskeletons

4. EFFECT ON BONE REPAIR FROM SIMVASTATIN LOADED DRUG DELIVERY SYSTEM

The therapeutic efficacy of a β-TCP carrier material was examined by loading the material with simvastatin, a known stimulant for bone formation, and comparing the effect of the system with and without an added apatite coating by implanting the device into the soft tissue in osteoporotic mice.[6] The results showed that both systems were able to achieve sig-

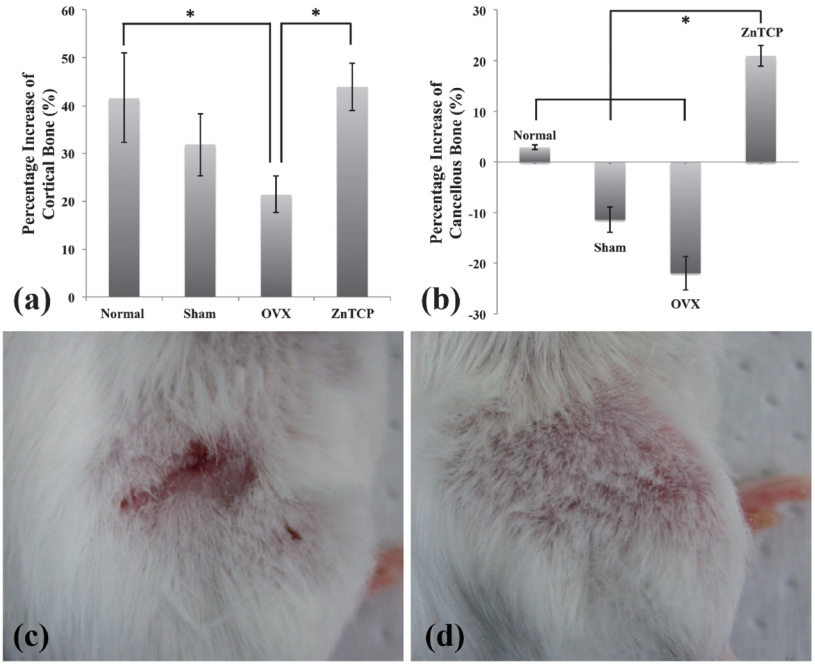

Fig. 3. The Therapeutic Effect of Zn-TCP on (a) Cortical and (b) Cancellous Bone Formation over 4 Weeks Showing Significant Bone Growth Matching or Surpassing Those of Normal Healthy Mice

Adverse muscle deterioration by (c) direct injection of zinc is obvious compared with the use of (d) Zn-TCP drug delivery system.

nificant localized cortical and cancellous bone growth over a 6-week period. However, the presence of the additional apatite coating was able to induce significantly stronger bone growth compared with no coating. This was attributed to the effect of delayed release of simvastatin. Furthermore, a comparison with direct injection of simvastatin showed adverse side effects of muscle inflammation at the site of injection compared with normal muscle function for the delivery system.[7] This study provides a demonstration of the healing abilities of the different drug release strategies available.

5. BONE REPAIR BY SYNTHETIC MODIFICATION OF KEY IONS

Apart from the unique structural characteristics of coral exoskeletons, their chemical composition is also of significant interest. As part of the natural growth of corals in the marine environment, key ions are required and absorbed to become part of the structure. These include trace elements consisting of strontium and magnesium.[8] Interestingly enough, both of these elements have been shown to be beneficial in the stimulation of bone regeneration and formation by inducing osteoblast proliferation while inhibiting osteoclast resorption.[9,10] *In vitro* cell culture assays exposing a human osteoblast cell line (MG63) and monocytes (U937) to the β-TCP samples confirm these properties. In essence, this indicates these key ions will be released as part of the natural degradation of the material. Furthermore, amongst the key ions involved in bone remodeling, zinc is also an important element that is required in cell regulation and zinc deficiency has been shown to be linked to osteoporosis. Zinc is also biologically active in osteoblasts and osteoclasts, similar to strontium.[11] This has motivated the syntheses of zinc-tricalcium phosphates (Zn-TCP), which in appropriate concentration and dosage, have been shown to have a stimulatory effect in the treatment of osteoporosis.[12–14] Traditionally, the synthesis of Zn-TCP requires high temperature sintering at around 800°C. By employing an additional hydrothermal treatment after the conversion of the β-TCP, Zn-TCP can be synthesized at relatively lower temperature (220°C). Recent studies have shown that the biomimetic Zn-TCP possesses therapeutic potential in bone repair in an osteoporotic mice model. In the span of 4 weeks the percentage increase in cortical bone was significantly different and in par with normal healthy mice. For the treatment of osteoporosis, the ability to stimulate cancellous bone is crucial as the lack of cancellous bone causes lower mechanical strength of the bone leading to susceptibility to breakage. The study showed that the control groups, with the exception of the normal healthy mice, all displayed negative cancellous bone growth while Zn-TCP exhibited a significant cancellous bone growth percentage of 20%. Furthermore, a comparative study was conducted to examine the effect of the Zn-TCP drug delivery system with direct injection of zInc. The results showed that direct injection, due to a high-localized concentration of zinc, led to severe muscle deterioration and eventually led to the unfortunate death of the animals. However, the mice in the Zn-TCP group showed no signs of any side effects and its effect on bone repair was evident. These results support the need for continued study into the development of biomimetic drug delivery systems.

6. CONCLUSION AND FUTURE PROSPECTIVE

This article summarizes the potential application of coral exoskeletons as a precursor material for the synthesis of calcium phosphate drug delivery systems. While past studies have been limited to the area of bone tissue engineering, it is envisaged that future developments will be expanded to other biomedical applications where appropriate. The studies described here have shown potential therapeutic benefits with this type

of system, and further research, optimization and development are still required before they can be clinically applied. However, it is exciting to know that such a small exoskeleton holds so much potential, and with such a vast ocean it is safe to believe that many more biomedically relevant materials and structures exist and are awaiting discovery by scientists.

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