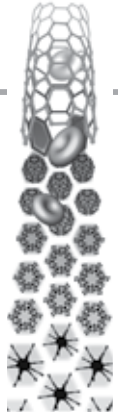

Antibiotic delivery potential of nano- and micro-porous marine structure-derived β-tricalcium phosphate spheres for medical applications

Aims: This study gives a detailed evaluation of the antibiotic potential of a marine structure-based new drug delivery system produced by hydrothermally converting *foraminifera* exoskeletons to β-tricalcium phosphate (β-TCP) to treat clinical strain *Staphylococcus aureus* (MW2). **Materials & methods:** *Foraminifera* precursor materials were hydrothermally converted at 250°C for 48 h to produce β-TCP and loaded with gentamicin sulfate by adsorption for 24 h. The physicochemical properties of the material were characterized by scanning electron microscopy, powder x-ray diffraction and for pore size distribution profiles. The antibacterial efficacy of the system was tested for inhibition of *S. aureus* growth and *in vitro* cellular behavior were tested with human osteoblast cells (MG63) for cell viability. **Discussion:** Pore size distribution profiles showed that the structure allows the uniform distribution of nanopores of 1.5 nm and micropores of approximately 5 µm. The *in vitro* release profile indicates an initial burst release of 5% of total incorporated gentamicin. A time-delayed antibacterial efficacy test was designed to introduce the bacteria at predetermined time intervals from 0 to 60 min and showed that gentamicin prevents *S. aureus* grown in the same culture within 30 min, with no evidence of bacterial regrowth within 24 h. Human osteoblast cell (MG63) studies showed no detrimental effect on cell viability. **Conclusion:** In the light of these results nano- and micro-pores containing β-TCP spheres show promise as potential bone void filler particles with antibacterial effects.

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KEYWORDS: biomimetic ■ calcium phosphate ■ drug delivery system ■ gentamicin ■ *Staphylococcus aureus*

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The management of bone infection associated with bone defect implants is a major challenge in maxillofacial and orthopedic surgery. Systemic administration of antibiotics alone is generally inefficient in eradicating bacteria, especially if infection occurs in the bone, due to poor antibiotic penetration. As antibiotics cannot be intravenously delivered directly to an infected bone at sufficiently high concentrations without producing systemic toxic effects [1,2], local administration such as closed irrigation and suction, local injection and implantable pumps are widely used but are regarded as clinically inconvenient [3]. Furthermore, this can increase the prevalence of highly resistant pathogens such as methicillin-resistant *Staphylococcus aureus* [4]. Therefore, the challenge is to develop successful strategies that combat bacterial infections and, thus, minimize bacteria-induced bone damage. These effects can, in turn, ultimately reduce costs to both the patient and healthcare providers. As such, local application of antibiotics can provide the required amount of drug at the site of infection while avoiding systemic effects. Currently, the most extensively studied and commercially available material in orthopedics for local antibiotic delivery is polymethyl methacrylate (PMMA), which is typically combined with antibiotics such as gentamicin [5,6], tobramycin [7,8] and vancomycin [9]. The use of antibiotic-incorporated PMMA implants has been shown to be reasonably successful, but their use has been limited owing to: their low drug release ratio; and the fact that they are nonresorbable implants and will require revision surgery to remove, leaving empty spaces that have to be filled and possible thermal damage to the antibiotics if cured *in vivo* [10]. To overcome these problems, an antibiotic carrier that can provide a combined therapeutic approach of drug release and bone substitution with antibacterial efficacy against infections is needed [11]. To achieve the desired therapeutic effect without any of the side effects, it is necessary to ensure that initial release of an active drug should exceed the minimum effective concentration in the systemic circulation, but it should be less than the allowable toxic concentration. For slow drug release inside bone, porous calcium phosphate is considered more suitable as it closely resembles the inorganic constituent of bone. As the intended

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material is designed to act as a scaffold for drug delivery applications, the material composition that is most suitable is β-tricalcium phosphate (β-TCP), which has the appropriate biocompatibility and faster dissolution rate compared with hydroxyapatite and is one of the US FDA-approved biomaterials currently used in surgery. Inorganic biomatrices such as coral exoskeletons can be converted hydrothermally into different mineral types (e.g., hydroxyapatite and TCP), while retaining the pre-existing structural architecture to modulate their mechanical properties, dissolution properties and their bioresponsiveness [12]. In general, hydrothermal conversion simply involves the use of high pressure to replace the carbonate component of the material with phosphate. By controlling the calcium:phosphate ratio, one can control the specific resulting calcium phosphate structure and composition, and, therefore, the dissociation rate within the physiological environment. White *et al.* and Roy *et al.* were the first groups to fully utilize the properties of natural marine skeletons, namely corals, in the 1970s [13–15]. Since that time, hydrothermally converted coral skeletons have been in clinical use for specific non-load-bearing orthopedic and dental applications [13–16]. A marine exoskeleton species '*foraminifera*' was used in this study as it possesses a unique interconnected porous network with pore sizes ranging from nano- to micro-meters, and provides a larger surface area due to its unique architecture.

The major aim of this research is based on this idea that naturally occurring marine structures contain nano-, meso- and micro-pores and intricate channels, which allow filtering in a sea environment, and can be loaded with pharmaceuticals, including antibiotics, which will induce controlled release within a physiological environment and can be used as slow drug delivery devices during surgery. It should be noted that these natural scaffolds with uniform interconnected pores are not yet synthetically producible.

It is envisaged that this new innovative regenerative medicine approach will provide an alternative form of treatment for bacterial infection, providing a therapy that will allow patients to recover quickly.

Materials & methods

Production of β-TCP micro- & macro-spheres & incorporation of antibiotics

β-TCP micro- and macro-spheres were produced by a previously described method [12,17]. For simplicity, we will describe them as macrospheres. Briefly, the *foraminifera* samples were cleansed with sodium hydrocholorite and the calcium:phosphate ratio (1:5) required to obtain β-TCP was calculated with diammonium hydrogenophosphate (Sigma-Aldrich, Sydney, Australia), which is used as the phosphate material to replace the carbonate composition of the *foraminifera*. The hydrothermal conversion was carried out in a Parr reactor at 250°C at 8.0-MPa pressure for a predetermined time based on the amount of material used. Scanning electron microscopy (SEM) images of the gentamicin sulfate powder and the gentamicin-loaded macrospheres were taken with a Philips (FEI) XL 30 ESEM (Philips, Eindhoven, The Netherlands). The microscope was operated in low vacuum mode at 0.8 Torr, 25 kV accelerating voltage and a working distance of 10 mm, using the back-scattered electron detector. Pore size and distributions were recorded using the Brunauer, Emmett and Teller (BET) Theory using a ChemBET automated analyzer (Quantachrome Instruments, FL, USA). Gentamicin is effective against *Staphylococci* and is commonly administered for implant-associated infections. Other antibiotics (i.e., vancomycin and linezolid) are also suitable drugs for combating bacterial infections, but in this research, we incorporated gentamicin sulfate (Sigma-Aldrich) and evaluated its effect on methicillin-resistant *Staphylococcus aureus*. Gentamicin sulfate were dissolved in distilled water at a concentration of 100 mg/ml. β-TCP macrospheres were immersed in the solution in a rotary evaporator (Büchi Rotavapor RT200; Flawil, Switzerland) until the solution was dried, and then placed in a 100% humidity vacuum seal. The actual amount of gentamicin loaded into each β-TCP macrosphere was calculated by taking the difference between the weight of the sample before and after loading in gentamicin solution. All measurements were performed with a sample number of six (n = 6). The macrospheres were initially autoclaved before use and were subjected to UV sterilization for 1 h after gentamicin loading.

Gentamicin *in vitro* release studies

The gentamicin release rate studies were conducted by placing each of the macrospheres into a vial with 10 ml of phosphate-buffered saline (PBS; 0.1 M, pH 7.4) at 37°C with constant shaking (100 rpm). The solution was replaced with fresh buffer solution every 24 h and gentamicin concentration was determined by using a UV-visible spectrophotometer (Ultrospec 2100 Pro; Biochrom, Cambridge, UK) at the maximum absorbance of gentamicin–O-phthaldialdehyde complex of

332 nm. O-phthaldialdehyde reagent was prepared by adding 2.5 g of O-phthaldialdehyde, 62.5 ml of methanol and 3 ml of 2-mercaptoethanol to 560 ml of 0.04 M sodium borate prepared in deionized water solution. The reagent was stored in a brown bottle in darkness and settled for at least 24 h prior to use. A calibration curve of gentamicin in PBS was plotted before each determination.

Antibacterial efficacy test

The bacterial strain used in this study was methicillin-resistant *S. aureus* (MW2). The bacterial strain was tested for gentamicin susceptibility by a broth dilution method as recommended by the National Committee for Clinical Laboratory Standards (PA, USA). The MIC was defined as the lowest concentration of gentamicin that inhibited growth of the test bacteria. Briefly, serial dilutions of gentamicin (100, 10, 1, 0.1 and 0.001 mg/l) in Brain Heart Infusion (BHI; Merck, Frenchs Forest, Australia) broth were prepared. Bacterial suspensions were then added to each tube. Tubes containing growth media alone or bacterial culture without gentamicin were included as negative and positive controls, respectively. The cultures were inspected for bacterial growth after incubation at 37°C for 24 h and the MICs of the gentamicin for each were recorded. *S. aureus* (MW2) was grown overnight in shaking cultures at 37°C in BHI broth and then subcultured for 3 h before introducing a single gentamicin-loaded β-TCP macrosphere. Every 30 min for up to 350 min, 1 ml of the bacterial media was extracted to determine the optical density and used to generate a curve representing bacterial growth.

Bacterial adhesion test

The bacterial adhesion test was conducted with an overnight culture (16 h) of *S. aureus* prepared in 10 ml of BHI broth. By reference to a standard optical density calibration curve, the cells were resuspended at a concentration of approximately 10^4 cells/ml. The samples included β-TCP macrospheres as control and β-TCP macrospheres loaded with gentamicin. Next, 1 ml of the bacterial suspension was added to each well and the plate was incubated at 37°C for 24 h. On completion of incubation, the β-TCP samples were placed into a fresh 24-well plate and repeatedly washed with 2 ml of sterile PBS to remove any loosely adherent bacteria. Each material was then placed into 1 ml of fresh BHI broth and vortexed at maximum power for 3 min to remove bacteria that had adhered to the material. Aliquots (100 µl) of the vortex solutions were serially diluted (200 µl in 2 ml) and plated in triplicate BHI plates and incubated at 37°C for 17 h. The colonies formed from the incubation solutions and vortex solutions were subsequently counted.

Human osteoblast (MG63) cell proliferation & viability

Assessment of the antibiotic β-TCP macrospheres on osteoblast proliferation was carried out *in vitro* using cultures of MG63 human osteoblast cells (American Type Culture Collection, VA, USA). The cells were grown in 15% fetal bovine serum in Roswell Park Memorial Institute medium (Invitrogen, Mount Waverley, Australia) and incubated at 37°C, 5% CO_2. Cells were passaged using Trypsin–EDTA (Sigma-Aldrich). MG63 cells were cultured in 96-well plate in the presence of the β-TCP samples at a concentration of 3×10^4 cells/ml and incubated for 1, 3 and 7 days in controlled atmospheric conditions. Tissue culture-grade polystyrene was used as a control and replicates were prepared. The MG63 cells were directly observed using a Philips (FEI) XL 30 ESEM. The microscope was operated in low vacuum mode at 0.8 Torr, 20-kV accelerating voltage and a working distance of 10 mm, using a back-scatter electron detector. For the cell viability evaluation, MG63 cells were grown in 96-well plates for 1, 3 and 5 days. The medium was then removed from the wells containing cells and replaced with fresh medium. Fresh medium was also added to a sterile flask containing no cells to serve as a negative control. Next, 20 µl of AlamarBlue® (Life Technologies, Sydney, Australia) was added to each well. There was no immediate color change in any flasks upon addition. The plates were then reincubated at 37°C, 5% CO_2 for 4 h. Cell viability data were collected by measuring fluorescent intensity monitored at 570 nm.

Statistical analysis

All data were examined based on three to five different measurement values and standard deviation. Metric data were analyzed by one-way analysis of variance followed by Scheffe's *post hoc* test at a significance level of $p < 0.05$.

Results

Antibiotic loading of the macrospheres

β-TCP samples loaded with gentamicin were weighed before and after antibiotic loading to obtain the average loading per sample. The average antibiotic loading per sample was found to

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be 1.2 mg per macrosphere (for n = 10). FIGURE 1A shows the structure of the converted spheres by SEM. Following the incorporation of gentamicin into the macrospheres by means of a rotary evaporator with a concentrated gentamicin solution, it can be seen that the gentamicin uniformly deposited and coated the surface (FIGURE 1B). The phase composition of the *foraminifera* material before and after hydrothermal conversion was examined by x-ray powder diffraction analysis and matched with the Joint Committee on Powder Diffraction Standards (JCPDS) database. The x-ray diffraction (FIGURE 1C) pattern showed that *foraminifera* material mainly consists of calcium carbonate (JCPDS 5-0586) and, after hydrothermal conversion, the material matched the pattern for β-TCP (JCPDS 9-169). The pore size distribution of the material was analyzed, and presented in FIGURE 1D & E, which shows that the spheres contain both micro- (1–5 µm) and nano-pores (1.5 nm). The distribution profile also shows very uniform distribution of these pores in the material and the presence of smaller pores (<1 nm). The *in vitro* cumulative release profile of gentamicin in PBS solution, which closely mimics the physiological conditions, is presented in FIGURE 1F. The release of gentamicin reached equilibrium after 30 min, at which point, approximately only 5% of the total incorporated gentamicin had been released. The release study was observed for a total of 24 h, but as the trend remained constant this was not shown in the figure. The loading efficiency of gentamicin with β-TCP was calculated based on the amount of drug loaded/theoretical drug loading, and showed a 40% loading efficiency.

Antibacterial action of the antibiotic-loaded macrospheres

Before testing the antibacterial efficacy, the MIC of gentamicin sulfate against *S. aureus* was determined and found to be 10 µg/ml. Growth curve graphs of *S. aureus* were created based on bacterial cell density. From the graph it can be seen that bacterial growth in the presence of the β-TCP was normal, but there was no bacterial growth in the presence of β-TCP macrospheres loaded with gentamicin (FIGURE 2A). In order for these biomaterials to be utilized as an efficient bacterial prevention delivery system, it was necessary to determine whether the release rate

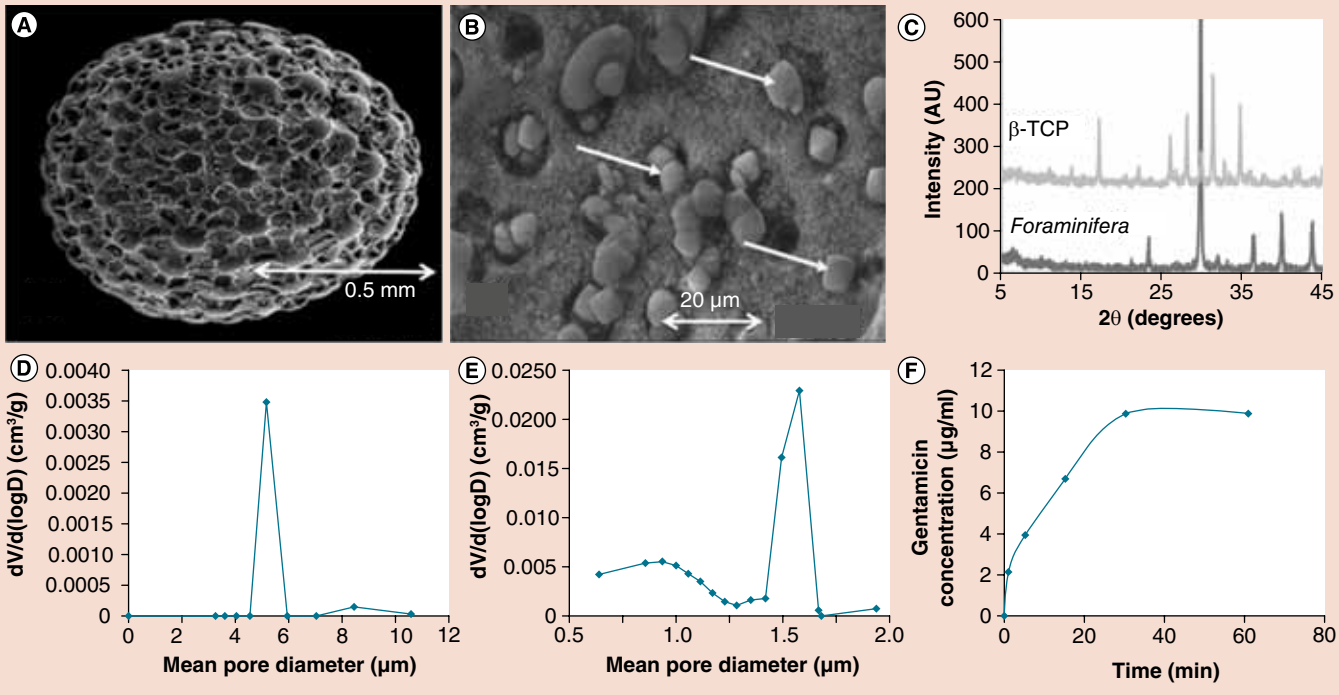

Figure 1. Physicochemical characterization of gentamicin-loaded β-tricalcium phosphate. (A) Scanning electron microscopy image showing the structure of the converted β-TCP macrosphere. **(B)** After loading, gentamicin scanning electron microscopy images show the attachment and distribution of gentamicin on the surface of the material. The arrows indicate gentamicin particles attached to the macrospheres. **(C)** The x-ray diffraction pattern showed peaks corresponding to β-TCP in accordance with the Joint Committee on Powder Diffraction Standards database. The **(D)** micro- and **(E)** nano-pore size distribution of the material. **(F)** The *in vitro* release profile of gentamicin in phosphate-buffered saline showing that the release reached equilibrium after approximately 30 min and remained constant for 24 h (standard deviation is included but error bars are too small to be seen).
β-TCP: β-tricalcium phosphate.

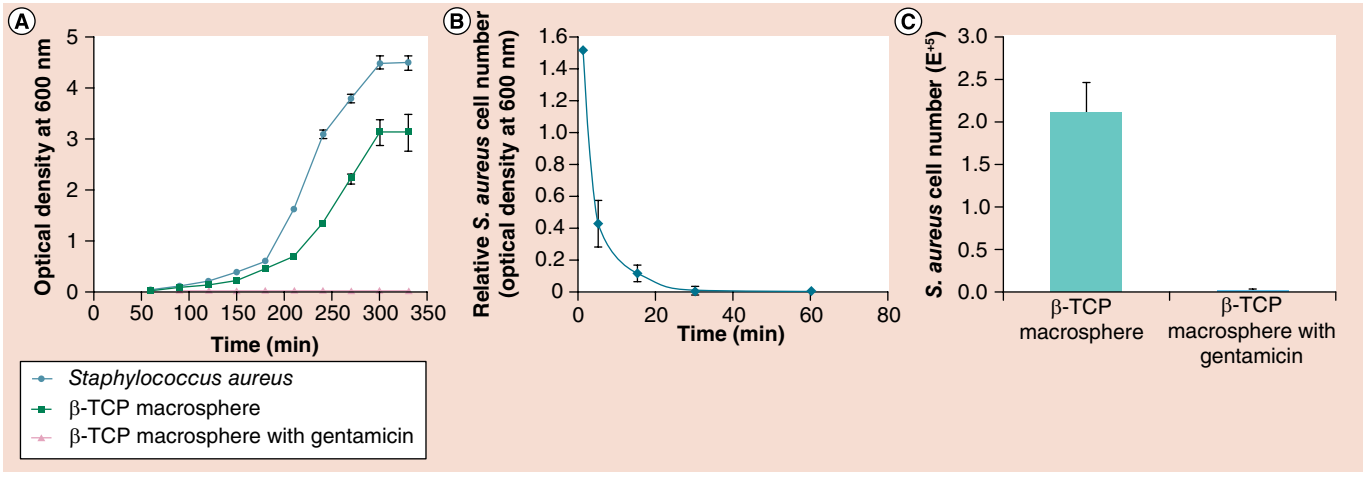

Figure 2. Efficacy of gentamicin-loaded β-tricalcium phosphate on *Staphylococcus aureus*. (A) Bacterial growth curve showing the inhibition of the growth of *Staphylococcus aureus* when exposed to β-TCP macrospheres loaded with gentamicin. **(B)** Time-delayed *S. aureus* growth curve revealing that after 30 min the bacteria are eliminated from culture media containing β-TCP macrospheres loaded with gentamicin. **(C)** The number of *S. aureus* adhered onto β-TCP macrospheres compared with β-TCP macrospheres loaded with gentamicin at 24 h. Error bars indicate the standard deviation of the three samples.
β-TCP: β-tricalcium phosphate.

of gentamicin is at an effective concentration against the growth of *S. aureus*. A time-delayed antibacterial efficacy test was designed to introduce the bacteria to the β-TCP macrospheres suspended in media at predetermined time intervals from 0–60 min. Figure 2B shows the *S. aureus* growth response following exposure to the gentamicin-loaded materials over these time intervals. As discussed previously, there was an initial burst release of the gentamicin from the spheres, which is reflected by a drop in bacterial optical density for the 1-min sample, followed by a dramatic decrease to zero for the 30-min sample. The test was carried out for a total of 24 h and, as expected, subsequent readings showed no change in the bacterial optical density compared with the first 30-min sample.

Furthermore, it is equally crucial to determine whether *S. aureus* adheres to the surface of the material as a result of the presence of phosphate on the spheres, which is described in more detail in the 'Discussion' section. Therefore, it was necessary to demonstrate that with the gentamicin incorporated onto the nano- and micro-pores and the general surface area, bacterial adhesion can be prevented. This can be clearly seen in Figure 2C, which demonstrates that negligible bacterial cell attachment occurred on the gentamicin-loaded samples.

Effect of gentamicin-loaded macrospheres on the growth of MG63

It was important to ascertain that exposure of human osteoblast (MG63) cells to the gentamicin-loaded materials would not be detrimental to their survival. This was assessed by growing the MG63 cells in the presence of the gentamicin-loaded materials, which showed that there were no detrimental effects on osteoblast cell proliferation (Figure 3A). SEM imaging of the gentamicin-loaded β-TCP macrospheres also revealed osteoblast cells attached to the surface of the macrospheres (Figure 3B). On close examination of the attached osteoblast cells, a number of adhesion points (Figure 3C) to the surfaces were also observed, indicating a strong affinity. The osteoblast cells were observed throughout the surfaces of the materials, which suggests a high degree of *in vitro* biocompatibility between the spheres. This is crucial to facilitate bone in-growth and integration.

Discussion

Gentamicin, along with other antibiotics, has a short active lifespan in the body. This makes it necessary to administer consecutive series of antibiotic dosages to patients. The use of excessive amounts can lead to antibiotic resistance and toxicity. Unique ways must be found to deliver this antibiotic almost instantaneously to the infected site in fixed quantities and in a controlled manner, to achieve a dose above the MIC, over prolonged periods. One of the most effective ways of achieving targeted delivery of antibiotics is to use a carrier device that can be placed into the appropriate sections of the body and localize itself before slowly releasing the correct dosage locally, at the correct site for extended time periods. In search of more effective treatments with calcium phosphate materials, and knowing

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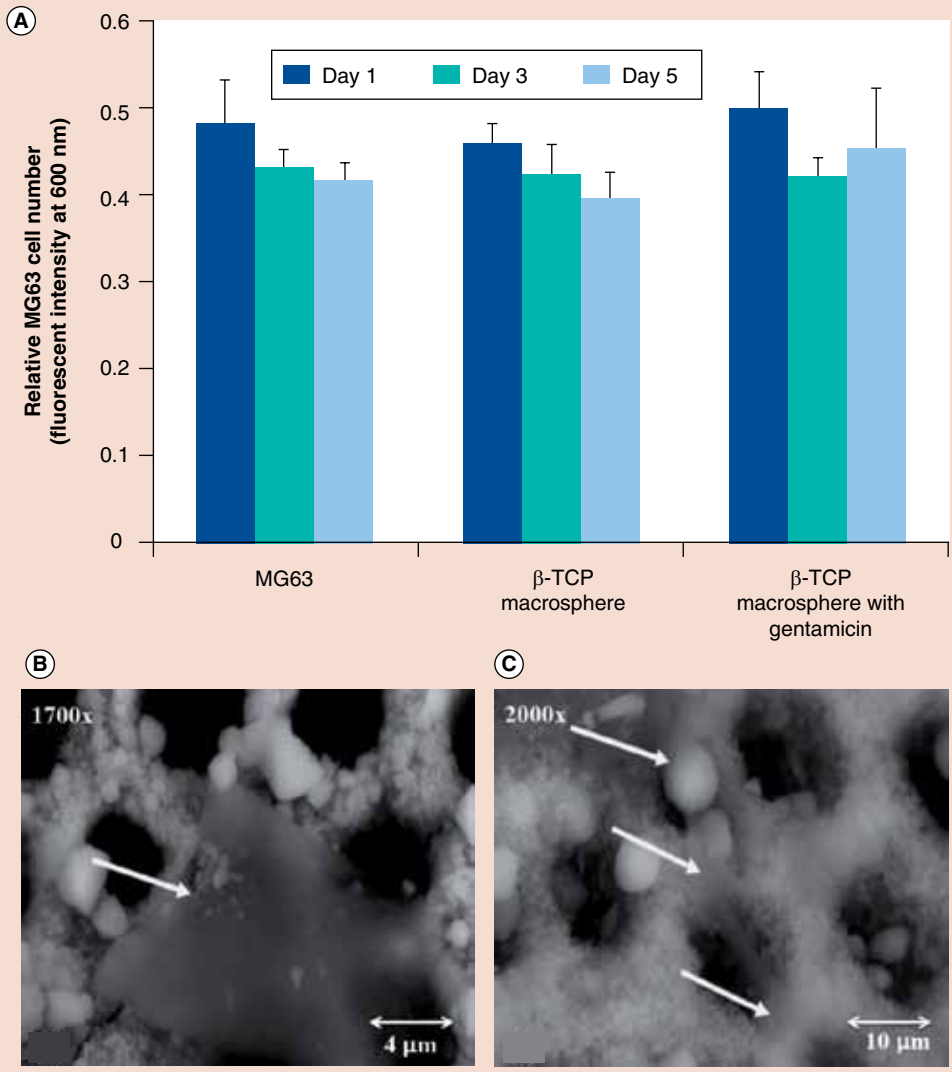

Figure 3. *In vitro* cellular response to gentamicin loaded β-tricalcium phosphate. (A) Human osteoblast continuous cell line (MG63) proliferation in response to β-TCP samples and β-TCP spheres loaded with gentamicin at 1, 3 and 5 days, showing that there is no cytotoxicity. **(B)** Scanning electron microscopy image of β-TCP macrosphere loaded with gentamicin showing the attachment of a human osteoblast (MG63) cell and **(C)** at a higher magnification the multiple attachment point over multiple pores can be seen.
β-TCP: β-tricalcium phosphate.

the restrictions of systemic global delivery, this study was designed to evaluate, *in vitro*, the use of biodegradable β-TCP macrospheres containing nano- and micro-pores for the loading and release of gentamicin sulfate for potential applications as a prevention or treatment for invasive bacterial local infections for many different medical applications.

From the presented results, it was also shown that enough gentamicin was released at a sufficient concentration to inhibit the growth of *S. aureus*. As bacterial infection does not always occur immediately after a surgical operation, a time-delayed experiment was performed to mimic the introduction of *S. aureus* over a period of 24 h to determine whether the gentamicin released was still active. The results showed that after 30 min of introducing the gentamicin-incorporated samples, complete inhibition of *S. aureus* growth over 24 h was observed. The MIC of *S. aureus* was determined to be 10 µg/ml and from the gentamicin release profile it was observed that the initial burst release of the antibiotic peaked at approximately 50 µg after 24 h, indicating that one of these spheres is capable of releasing an initial concentration capable of preventing *S. aureus* infection and can continue to deliver gentamicin for an

extended time based on the dissolution rate, which can be controlled by the size and chemistry of the sphere. The total concentration of gentamicin incorporated in an average-sized macrosphere was calculated to be 1.2 mg of gentamicin per β-TCP sample, suggesting that only approximately 5% of the total gentamicin was released.

When the macrospheres are first introduced into the solution environment there will always be a burst release because the pores are filled with the medium, inducing this initial surge. After the surface release reaches an equilibrium, the gentamicin that remains in the micro- and nano-pores will be slowly released over time in this steady-state period. This indicates that 95% of the gentamicin is still retained owing to the unique architecture of the nanopores, struts and channels, which amplifies physiological degradation and natural β-TCP dissolution and helps to release attached drugs in a controlled manner. An average-sized β-TCP macrosphere is anticipated to degrade within 1 year; however, owing to the limitations of the current release study, future studies should be carried out to study the long-term release profile of gentamicin with different size spheres. In addition, the dissolution rate can be further controlled with the pore size, pore distribution and by the calcium phosphate chemical composition adjustment. Control of release rates with different calcium phosphates is well known, for example biphasic apatite, which is currently used as bone grafts.

In orthopedic applications the use of these next-generation calcium phosphate materials with unique architectures is significant as these new bone graft spheres would not require any revision surgery for removal compared with similar PMMA antibiotic delivery systems that are currently used.

The adhesion of *S. aureus* to some surfaces has been shown to be due to its ability to bind to specific host matrix proteins [18]. The adhesions that mediate the binding to host proteins, termed microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), are microbial surface components that recognize adhesive matrix molecules. It was reported that these MSCRAMMs could mediate adhesion of the bacteria onto prostheses by binding to host proteins that cover the implant surface *in vivo* [19]. This was thought to occur via the cationic amino group of the phosphate chemical structure, which acts by attracting bacteria by either direct electrostatic interaction or through direct surface protein interactions. As the surface of the β-TCP contains phosphate ions, it is possible that this could interact with the MSCRAMM of the bacteria. It, therefore, recognizes the simulated host protein on the surface of the material and mediates increased bacterial adhesion to the surfaces of the β-TCP. In order to quantify the number of adherent bacteria on the macrospheres, an adapted modified vortex device method, which relies on a whirlpool-type force to remove bacteria from a solid matrix, was used [20,21].

It was shown that *S. aureus* does not attach to the gentamicin macrospheres *in vitro*. It is possible that in the early stages *in vivo* serum proteins coat the macrosphere surfaces and cause bacterial adherence, as reported in the published work [19]. These protein-coated surfaces will be further exposed by the natural degradation of the calcium phosphate ions from the macrospheres where the newly exposed area would release further antibiotics to challenge bacterial adherence.

These results confirm that incorporation of gentamicin onto the β-TCP macrospheres does not affect gentamicin's antibiotic action, which has been demonstrated by the inhibition of *S. aureus* when grown in the presence of the antibiotic-loaded macrospheres.

It is important for bone regeneration that bone cells are unaffected by antibiotic treatment. In our tests combining gentamicin spheres and cultured human osteoblasts, the *in vitro* cell growth was found to be normal and the cells were seen to adhere and spread onto the surface of all macrospheres.

The key strengths of this material are: a pore size distribution ranging from nano- to macro-size, with micropores suited to cellular ingrowth, high encapsulation and retention efficiency of gentamicin, controlled release kinetics that can be further optimized over extended periods, and the release of antibiotics at nontoxic levels.

In terms of clinical practicality, this delivery system can be easily upscaled for wide-spread production. High-purity natural and artificially grown marine exoskeletons are available and abundant. Large-scale commercial hydrothermal equipment is also available and, most importantly, the retention of antibiotics can occur in a controlled manner. As such, it is envisaged that this system can be applied in clinical settings as a treatment for bacterial infection in a number of medical applications for both hard and soft tissue systems.

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Conclusion & future perspective

This study has shown that nano- and micro-pores containing converted marine structures are good candidates for antibacterial drug delivery with controlled local gentamicin release. They can be stably loaded with sufficient quantities of the antibiotic gentamicin to kill *S. aureus* bacteria in the same culture in 30 min, and no bacteria growth within 24 h was demonstrated after adding macrospheres containing both nano- and micro-pores. It was additionally shown that *S. aureus* do not attach to the gentamicin-containing spheres. As the microstructures degrade there is an initial burst release of gentamicin within 30 min of administration followed by a constant steady elution as a steady-state period is reached. From this point, the continuing degradation controls the release of antibiotic that remains encapsulated. The nano-, meso- and micro-pores are pertinent during the drug loading and dissolution process.

As a proof-of-concept, this study showed that antibiotics can be successfully incorporated into hydrothermally converted β-TCP macrospheres derived from marine structures, and *in vitro* results suggests biocompatibility and the capacity to eliminate clinical strain *S. aureus* (MW2).

The main outcome of this study could provide an alternative therapeutic method for the prevention and possible treatment of *S. aureus* infection for bone-related procedures.

This potential treatment for *S. aureus* using a biomimetic scaffold to delivery therapeutic concentration of antibiotics has the potential to significantly impact on and reduce the adverse effects of current practice for maxillofacial and orthopedics patients suffering from bacterial infection after surgery.

For specific hard and soft tissue applications, it is important that cells are unaffected by antibiotic treatment. In tests combining gentamicin-loaded spheres and cultured human osteoblasts, the *in vitro* cell viability was normal and they adhered and spread onto the surface of all macrospheres.

This study aimed to characterize the β-TCP macrospheres *in vitro* and long term *in vivo* to determine their potential for clinical applications.

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Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Production of α-TCP macrospheres & incorporation of antibiotics

- Calcium carbonate precursor material based on *foraminifera* exoskeleton can be hydrothermally converted to β-tricalcium phosphate (β-TCP) while retaining the fidelity of the original structure, thereby allowing successful loading of gentamicin sulfate within the interconnected porous network of the material. It was found that the material possesses both micro- and nano-pores.

Gentamicin* in vitro *release studies

- The initial burst release of gentamicin reached a constant equilibrium after 30 min and remained constant for 24 h constituting only 5% of the total gentamicin loaded being released. Future studies will examine the release of gentamicin in other biologically relevant media (e.g., simulated body fluid and acetate buffer – pH 4.5).

Antibacterial action of the antibiotic-loaded micro- & macro-spheres

- The gentamicin released from the β-TCP macrospheres was able to inhibit the growth of *Staphylococcus aureus* (MW2).
- In a time-delayed study, the delivery system prevented the growth of *S. aureus* after 30 min.
- Bacterial adhesion assays showed no *S. aureus* attached to the material, which is notable because studies have shown *S. aureus* to have an affinity for phosphate-related material.

Effect of gentamicin-loaded macrospheres on the growth of human osteoblast cell line, MG63

- A human osteoblast cell line (MG63) proliferation assay showed that the β-TCP macrospheres loaded with gentamicin did not affect the overall growth of the cells over 5 days.

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