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Elution characteristics of teicoplanin-loaded biodegradable borate glass/chitosan composite

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ABSTRACT

Local antibiotic delivery system has an advantage over systemic antibiotic for osteomyelitis treatment due to the delivery of high local antibiotic concentration while avoiding potential systemic toxicity. Composite biomaterials with multifunctional roles, consisting of a controlled antibiotic release, a mechanical (load-bearing) function, and the ability to promote bone regeneration, gradually become the most active area of investigation and development of local antibiotic delivery vehicles. In the present study, a composite of borate glass and chitosan (designated BG/C) was developed as teicoplanin delivery vehicle. The *in vitro* elution kinetics and antibacterial activity of teicoplanin released from BG/C composite as a function of immersion time were determined. Moreover, the pH changes of eluents and the bioactivity of the composite were characterized using scanning electron microscopy coupled with energy-dispersive spectroscopy and X-ray diffraction analysis.

Despite gradual advances in the surgical techniques and antimicrobial agents, the treatment of osteomyelitis remains a challenge for clinicians. Local antibiotic delivery systems have offered attractive alternatives to parenteral antibiotic for osteomyelitis treatment due to the ability to deliver high local antibiotic concentration, which potentially avoids systemic toxicity (Hanssen, 2005).

Polymethylmethacrylate (PMMA), a standard material for local antibiotic delivery, has been used in orthopaedic surgery for several decades. But some disadvantages associated with PMMA, such as low antibiotic release rate, low biocompatibility, thermal injury and an additional surgical removal limit its clinical application. Various biodegradable materials including hydroxyapatite, calcium phosphate, bioglass and polymers (e.g., collagen, chitosan, polylactic) have been explored to replace PMMA. However, some deficiency have been observed more or less with them including their transient cytotoxic effect leading to inflammatory reaction, insufficient osteoconductive capacity and quick elution *in vitro* (McLaren, 2004).

An ideal local antibiotic delivery system should have a combination of functions, including the ability to provide a controlled antibiotic release rates, the provision of physicochemical characteristics necessary for osteoconduction and osseous integration. In an attempt to attain this desirable combination of properties, a composite of borate glass and chitosan (designated BG/C) was developed in the present work as an antibiotic delivery vehicle. The borate glass has the same composition as conventional silicate-based 45S5 bioactive glass, but with SiO_2 replaced with B_2O_3, thus possesses high bioactivity than the latter due to the lower chemical durability and is potent in promoting bone formation quickly (Huang et al., 2006; Richard, 2000). Chitosan has favourable biocompatible, biodegradable and nontoxic properties, and is proven to be served as a useful vehicle for controlled release of drugs because of its complexation property (Cevhe et al., 2006). Teicoplanin was selected as the antibiotic because it has a long serum half-life and a broad-spectrum antibacterial activity against most Gram-positive aerobic and anaerobic organisms, such as methicillin-resistant *Staphylococcus aureus* (MRSA), the most frequent osteomyelitis-inducing microorganism clinically.

Therefore, the purpose of the present work was to investigate the *in vitro* elution kinetics of teicoplanin from the teicoplanin-loaded BG/C (designated T/BG/C) composite, and the changes of surface morphology and structural characteristics of T/BG/C pellets as a function of immersion time in PBS by using field-emission scanning electron microscope (FE-SEM, Quanta 200 FEG, The Netherlands) coupled with energy-dispersive spectroscopy (EDS), as well as X-ray diffraction analysis (XRD; Model D/max 2550 v, USA).

Borate glass with the composition (mol%) $6Na_2O{\cdot}8K_2O{\cdot}8MgO{\cdot}22CaO{\cdot}54B_2O_3{\cdot}2P_2O_5$ was prepared as described in

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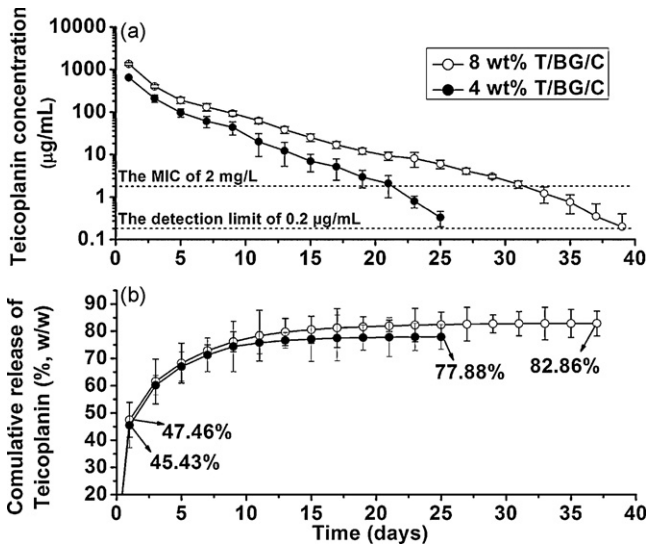

Fig. 1. Release of teicoplanin from T/BG/C pellets (a) and cumulative percentage (b) as a function of immersion time in PBS.

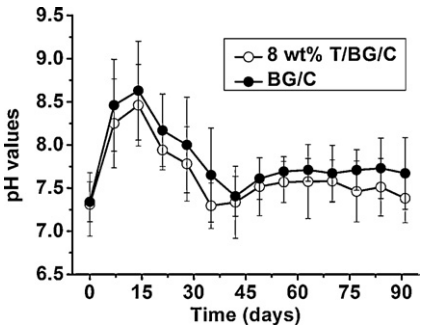

Fig. 2. pH of the PBS resulting from the immersion of the T/BG/C and BG/C pellets.

previous studies (Yao et al., 2007). Teicoplanin powder (Gruppo Lepetit S.P.A., Italy) was mixed with the glass powder to give two different concentrations (4 and 8 wt%). A solution was prepared by mixing chitosan (98% deacetylated; Sinopharm Chemical Reagent Co., Ltd., China), citric acid, and glucose in the ratio 1:10:20 (by weight), and mixed with the glass/teicoplanin powder in the ratio of 1:2 (by weight). The resulting mixture of T/BG/C was placed in polyethylene molds without compression to make 4 and 8 wt% of T/BG/C pellets. Pellets of BG/C composites were prepared using the same method but without the teicoplanin.

Triplicate samples of four pellets of each T/BG/C composite (4 and 8 wt% teicoplanin) were immersed into sterile polyethylene containers containing 10 ml of phosphate buffered solution (PBS, pH 7.4) and kept in a water bath incubator at 37 °C. The PBS solution was completely refreshed every 48 h and the teicoplanin level in sample eluent was determined using high performance liquid chromatography (HPLC) as described previously (McCann et al., 2002). The release rate of teicoplanin was obtained by dividing the theoretical amount by the actually released amount.

For exploring whether the antibacterial activity of teicoplanin would be influenced by the manufacturing process, an evaluation on the antibacterial activity of the teicoplanin released from T/BG/C pellets (eluents after 48 h) was conducted to compare with that of non-processed teicoplanin. The minimum inhibitory concentration (MIC) for teicoplanin against MRSA (ATCC43300) was determined using an antibiotic twofold tube-dilution method standardized by the National Committee for Clinical Laboratory Standards (NCCLS, 2000).

Fig. 1 showed the elution profile of the teicoplanin from the pellets with different teicoplanin concentrations over the immersion time. The measured initial released concentration was 648.81–1355.60 μg/ml on the first day of immersion, with approximately 45–47% of release rate, followed by a slowly stable release up to 25–37 days, with concentrations of 18.99–10.47 μg/ml. The total release rate of teicoplanin was 78.88–82.86%. The released quantity of teicoplanin depended on the initial concentration and the kinetic was parallel.

Compared with previous studies, the more sustained release in the present study could be attributed to the presence of chitosan since it has a cellulose-like structure. The free amine groups in the polymeric chains of chitosan provide reactive and polycationic groups that could take part in complexation reactions, enabling it to interact with negatively charged groups in polymers and with polyanions (Prabaharan, 2008). The addition of citric acid, as well as the deposition of PO_4^{3-} ions from PBS on the surface of composite pellets after immersion in PBS could further improve the crosslinking and increase the viscosity of liquid phase of chitosan. This could contribute to a further reduction in the initial release rate of teicoplanin and to a more a sustained release rate subsequently (Zhang and Zhang, 2002). Furthermore, the MIC of released teicoplanin from T/BG/C pellets against standard MRSA strain was similar to that of non-processed teicoplanin, indicating that the nature of the antibiotic was not altered by the production process.

Within the limits of experimental error, the presence of teicoplanin (8 wt%) in the pellets did not influence the pH of the solution (Fig. 2). The results showed a characteristic trend, in which the pH increased initially (starting value = 7.3), and after reaching a maximum value of ~8.5 after ~14 days, it decreased to a nearly steady value (~7.5) after ~35 days. The components in the glass such as Na_2O and B_2O_3 dissolved into the solution to form Na^+ and BO_3^{3-}, whereas Ca^{2+} ions from the glass react with PO_4^{3-} from the solution to precipitate HA. The strongly basic NaOH overwhelms the weak acidic tendency of $B(OH)_3$, so the pH increased (Huang et al., 2006). However, incorporation of chitosan–citric acid in the borate glass matrix reduced the pH increase and meanwhile the acidic degradation byproducts from chitosan were buffered too. Moreover, the gradual formation of HA on composite surface and neutralization of solution pH further reduced the dissolution of chitosan–citric acid and provide a more sustained release of teicoplanin.

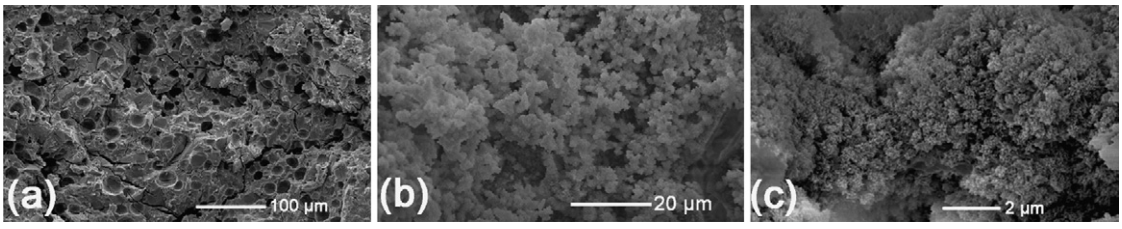

Fig. 3. SEM micrographs of the surfaces of T/BG/C pellet before (a) and after immersion in PBS for 120 days (b and c).

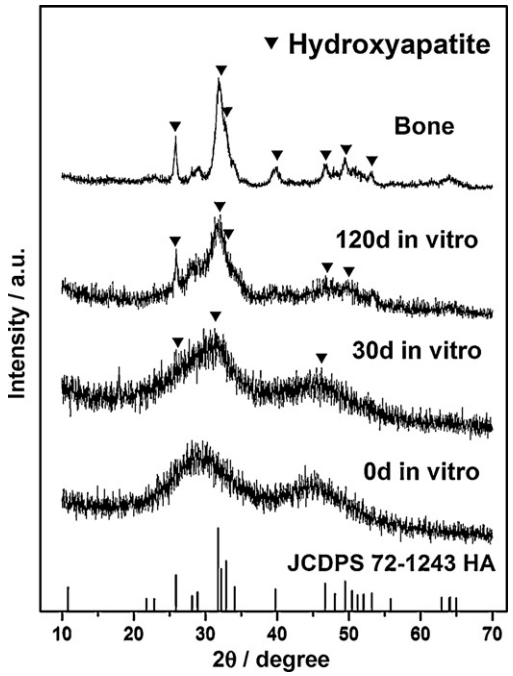

Fig. 4. XRD patterns of T/BG/C (8 wt% teicoplanin) pellets before and after immersion in PBS for different times. For comparison, XRD patterns of dry rabbit bone, and a reference hydroxyapatite (HA) are also shown.

FE-SEM (Fig. 3) results showed that the as-prepared pellets had a heterogeneous surface microstructure with a number of large pores (Fig. 3a). As the immersion time, a layer of spherical particles enriched in Ca and P (determined by EDS analysis) formed on the surface of the pellets with the degradation of pellets and gradually became denser and more uniform until it covered the whole surface of the pellets (Fig. 3b). High magnification SEM images showed that the surface layer consisted of fine, flake-like particles (Fig. 3c). EDS analysis revealed that the Ca/P atomic ratio of this surface layer was 1.62, which is close to the Ca/P atomic ratio (1.67) for stoichiometric hydroxyapatite. The XRD patterns (Fig. 4) show that the broad bands in the pattern of the as-prepared T/BG/C pellets before immersion are typical of an amorphous glass. Over the immersion time, the pattern started to show peaks characteristic of a reference hydroxyapatite (JCPDS 72-1243). The peaks are broad after 120 day of immersion, indicating that the as-formed HA was poorly crystallized or that the crystallite size of the HA was on a nanometer scale, or a combination of both. The formation of this HA material in the T/BG/C pellets is attributed to the conversion of the borate bioactive glass in the presence of the PO_4^{3-} ions in the PBS, as described in detail elsewhere (Huang et al., 2006), which indicates the favourable osteoconductivity of the composite *in vivo* (Wojick, 1999).

In conclusion, the composite of BG/C showed ability to serve as a satisfactory carrier vehicle for sustained and effective release of teicoplanin, as well as favourable mechanical property, biodegradability and osteoconductivity. This multifunctional role makes T/BG/C composite an desirable local antibiotic delivery system for osteomyelitis treatment. Our further study will investigate the *in vivo* efficacy of the T/BG/C for osteomyelitis treatment in animal model.

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