Polycaprolactone-Coated Alginate/β-Tricalcium Phosphate Beads to Locally Deliver Vancomycin: A Pilot Study

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Abstract: Orthopedic device-related infections (ODRI) are difficult to control and the management of ODRI most frequently includes surgery and long-term antimicrobial therapy. Local application of vancomycin through a biodegradable carrier like alginate would provide a valuable tool, although it is hard to control the drug-release for a prolonged period of time due to its permeability. Coating with hydrophobic polymer such as polycaprolactone (PCL) may sustain the vancomycin release. We fabricated four types of vancomycin containing alginate/β-TCP beads (uncoated, coated with 1.25 w/v%, 2.5 w/v%, and 5.0 w/v% PCL). Scanning electron microscope (SEM) revealed that β-TCP particles were uniformly distributed on the surface of the uncoated beads and the most homogenous coating layer was observed using 2.5 w/v% PCL. Vancomycin release and its bioactivity were measured at the designated time points (1, 4, 12, 24 hours, then every day until disintegration). Burst release occured on the first hour, day 1, 2 and 6 respectively. The beads without coating dissolved at day 3, and those with different coatings dissolved at day 5, 6, and 9. The minimum concentration of the vancomycin in the elution was approximately 5 mg/L, higher than the vancomycin's minimum inhibitory concentrations (MICs) for Methicillin-resistant Staphylococcus aureus (MRSA). PCL-coated alginate/β-TCP beads loaded with vancomycin may provide a potential local drug delivery device for the adjuvant antimicrobial therapy of the ODRI.

Keywords: Alginate/β-tricalcium phosphate, polycaprolactone, local delivery, vancomycin, orthopedic infection.

INTRODUCTION

Orthopaedic device-related infection (ODRI), which refers the infection of the orthopaedic implantations, remains a major complication after orthopaedic surgery resulting in physical, mental, and economic hazard [1-4]. These infections are difficult to control and easy to recur, even many years after the initial episode [3-7]. The management of ODRI most frequently includes debridement with or without implant removal and long-term systemic antimicrobial therapy [6, 8-10]. Free-floating (or planktonic) bacteria may be well eliminated by conventional systematic administration of antibiotics; however, the most obstinate pathogen in ODRI are not the planktonic bacteria but their sessile forms embedded in biofilms [11, 12]. Failure of systemic antibiotics to control ODRI is not only the result of poor penetration of drugs into biofilm [13], but also due to lower antimicrobial susceptibility of stationary bacteria in the biofilm environment [14, 15]. Methicillin-resistant Staphylococcus aureus (MRSA) is one of the most frequently associated bacteria with ODRI. MRSA can persist within the implant site by producing variant microcolonies and/or biofilm [11, 16-19]. Vancomycin in one of the antibiotics that shows superior bactericidal activity against biofilm-embedded MRSA compared to other antibiotics, such as clindamycin, linezolid, and tigecycline [20]. However, without a proper carrier, vancomycin whould not be able to provide high initial levels to penetrate glycocalices on the surface of bacteria rapidly. Ideal antibiotic delivery carriers should be biocompatible, degradable with bony replacement, osteoinductive and osteoconductive properties [21]. The local concentration of vancomycin should be subsequently kept above the critical level, which is estimated to be over 200 mg/L for a minimum of 72 hours [22].

Sustained drug delivery systems based on natural polymers are attractive due to their biocompatibility. Alginate is one of the most promising natural biodegradable materials [23]. Alginic acid is water soluble, and it can be ionically cross-linked with divalent cation, such as calcium ions, to form alginate gels [24, 25]. However, alginate is hydrophilic, and that makes it difficult to control the drug-release behavior for a prolonged period of time in hydrated microenvironment [26-28]. Being coated with other degradable hydrophobic polymers is one of the

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methods to delay the delivery of the antibiotics [29]. Polycaprolactone (PCL) is such a kind of biodegradable polyester with good biocompatibility and hydrophobicity [30-35]. And it is an ideal material for its valuable properties such as nontoxicity for organism and gradual resorption (5-6 months) after implantation. Food and Drug Administration (FDA) has approved several PCL containing medical and drug delivery devices [36, 37].

β-tricalcium phosphate (β-TCP), a synthetic calcium phosphate ceramic, is similar to the mineral phase of natural bone in chemical compositions [38]. In our previous study, β-TCP could be entrapped in the alginate gel network homogenously and be delivered together with the antibiotics to act as calcium resource in the bone defects repair, which is normal in ODRI.

In this study, PCL coated alginate/β-TCP beads loaded with vancomycin was prepared. The microstructures and the vancomycin release profiles *in vitro* were observed and compared. The aim of this study was to investigate whether the vancomycin delivery of drug-containing alginate/β-TCP beads could be retarded in a sustained and controlled manner through coating PCL layer.

MATERIALS AND METHODS

Preparation of Vancomycin-Containing Alginate/β-TCP Beads

Five hundred microliters of vancomycin (Eli Lilly, Suzhou, China) solution (400 mg/mL) was added drop by drop into 10 mL 2.0 w/v% alginate (low viscosity; Sigma-Aldrich Co LLC, St Louis, MO, USA) solution. Subsequently, the mixture was magnetically stirred (400 rpm) keeping transparent without flocculent precipitation and allowed to equilibrate for another 5 minutes. β-TCP powder (Ensail Beijing Co, Ltd, Beijing, China) was prepared before hand by milling for 8 hours to a mean particle size of 5.4 μm (measured by a laser particle analyzer). Then 100 mg β-TCP powder was poured into the vancomycin/alginate mixture and vigorously stirred for 2 hours until β-TCP was uniformly dispersed in the mixture. Using a syringe, the mixture was added into a gently stirred 100 mL 1.0 w/v % calcium D-gluconate solution (Sigma-Aldrich Co LLC) in drops. Spherical white beads (1-2 mm in diameter) formed in the calcium D-gluconate solution and they were allowed to harden in the solution for 1 hour. The antibiotic-containing beads were then frozen at -20° C for 48 hours. Removal of the frozen samples was followed by placement into a freeze-dryer (LGS-4; Xing Zong Vacuum Technology Co, Ltd, Shanghai, China) at a preset temperature of -5° C and lyophilized at 0.5 mmHg for 72 hours to completely remove the water. The resulting vancomycin-containing alginate/β-TCP beads were 0.8 mm to 1.5 mm in diameter.

PCL Coating Process of the Alginate/β-TCP Beads

The vancomycin-containing alginate/β-TCP beads were coated with three different concentrations of PCL (1.25 w/v%, 2.5 w/v%, and 5.0 w/v%). Different amounts of PCL (molecular weight = 80,000; Bright China Industrial Co, Ltd, Shenzhen, China) were dissolved in 20 mL dichloromethane to prepare three PCL solutions with the concentration of 1.25 w/v%, 2.5 w/v%, and 5.0 w/v%. The lyophilized beads were dipped into 5 mL PCL solutions with different concentrations for 1 minute followed by vacuum drying for 24 hours at room temperature.

Weight Increment Measurement

Each group of beads was weighed on a precision weighing balance (CPA1003P; Sartorius Mechatronics, Beijing, China) before and after the coating process. After accurate weighing, the weight increments of the samples were recorded. Weight increment after coating can be considered as the total amount of the PCL that has been coated on the material. The measurement was done for five times and an average was then calculated.

Microstructure of the Beads

The microstructure of beads was examined by scanning electron microscope (SEM, Tescan-5136MM, Czech Republic). The samples were mounted on aluminum stubs with conductive paint and were sputter coated with gold (10 mA, 120 seconds). Ten beads from each group was measured and compared with uncoated alginate/β-TCP beads.

Drug Loading and Encapsulation Efficiency Measurements

To determine the drug loading and the encapsulation efficiency, approximately 50 mg of accurately weighed vancomycin-containing uncoated beads were immersed into 100 mL of phosphate buffer solution (pH 7.4) containing disodium ethylenediamine tetraacetate (EDTA). The resulting mixture was shaken on a mechanical shaker for 24 hours. After the solution was filtered (0.45-μm pore size), 1 mL of this solution was diluted using phosphate buffer solution (pH 7.4), and analyzed spectrophotometrically at 280 nm using a

UV spectrophotometry in a UV-Vis (Shimazu UV 2201, Shimadzu Corporaton, Kyoto, Japan). The precision of the method was assessed by contrasting repeat measures obtained by using the same analytic method and sample. A more appropriate measure of correspondence, also presented, is the concordance coefficient, which, in addition to measuring the agreement between two readings, also measures the departure from the 45° line through the origin. The accuracy was assessed by using the same statistical approach, but by comparing the method with the standard laboratory method based on the same samples.

Drug loading and encapsulation efficiency were calculated using Equations 1 and 2, respectively.

$$\text{Drug load} = W_1/W_2 \times 100\% \quad (1)$$

$$\text{Encapsulation efficiency} = (W_3 - W_4)/W_3 \times 100\% \quad (2)$$

W_1 is the weight of drug in the beads, W_2 is the gross weight of beads, W_3 is the total weight of drug, and W_4 is the weight of drug that remained in the liquid medium after encapsulation.

In Vitro Drug Release Studies

The antibiotics release profiles of the uncoated and coated alginate/β-TCP beads were evaluated. To analyze the vancomycin release behavior, five sets of samples (200mg) from each type were immersed in polyethylene vials with 10 mL of phosphate-buffered saline (PBS; 0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride, pH 7.4) for 7 days. The vials were sealed tightly and incubated at 37° C without stirring. All the medium was withdrawn at predetermined periods of time (1 hour, 4 hours, 12 hours, 24 hours, then every day until disintegration of the beads) and replaced with an equivalent amount of fresh PBS. The concentration of vancomycin released from the samples was determined by measuring the absorbance at λ = 280 nm using a UV spectrophotometry in a UV-Vis (Shimazu UV 2201, Shimadzu Corporaton, Kyoto, Japan). Each absorbance value was converted to the drug concentration using a standard curve, which was drawn by measuring the optical absorbance of the vancomycin dissolved in the PBS with concentrations in the range of 4 to 500 μg/mL. A linear relationship between the vancomycin concentration (x) and the optical absorbance (y) was obtained ($Y = 0.003X + 0.038$, $R^2 = 0.999$). The actual mass of drug released was calculated based on the measured concentration and actual collected sample volumes. The cumulative percentage release was calculated as the ratio of the mass released at each time point to the total amount of vancomycin embedded in the composites. The cumulative release data were fitted to a logarithmic function, which was differentiated with respect to time to calculate the daily percentage release [39]. All experiments were repeated three times. The experimental data were expressed as means ± SD.

Antimicrobial Activity of the Elusions

Antimicrobial activity of the elusions of the uncoated/coated beads were assessed on each predetermined time points against MRSA (strain, ATCC 33597) grown on agar plates (10^6 CFU/cm^3) at 37°C. Antimicrobial Susceptibility Test Discs (Oxoid Sensi-Disc; Basingstoke, Hampshire, UK) were used as the control. Inhibition zone diameters were measured and scaled. For the antimicrobial susceptibility test, zones that were 9 mm or less were considered resistant. Zones between 10 and 11 mm were considered intermediate and those that were 12 mm or greater were considered sensitive to MRSA [40].

Data Analysis

In all the experiments, five samples were tested. All the data was entered to STATA 10.1 (StataCorp, College Station, TX, USA) software and analyzed using the survey analysis statistics methods. The differences in the drug releasing behavior were determined among the vancomycin-containing alginate/β-TCP beads coated with different concentrations of PCL solutions using repeated-measures analysis of variance (ANOVA). The level of significance was set at 0.05.

RESULTS

Morphology and Microstructure of the Beads

Newly prepared vancomycin-loaded alginate/β-TCP beads before lyophilization were white, spherical, and elastic (Figure **1A-B**), whereas the lyophilized beads were smaller and inelastic (Figure **1C-D**). The representative alginate/β-TCP beads turned into polyhedron with the size ranging from 800 to 1100 μm after lyophilization (Figure **2A-B**). The β-TCP particles were approximately 5 μm and were uniformly distributed on the surface of the beads (Figure **2C**). Coated with 1.25 w/v% PCL, the beads had a large unsealed area and small amount of residual polyester on the lowest part of the beads (Figure **3A-B**). With 2.5 w/v% PCL-coated beads, we observed a more uniform

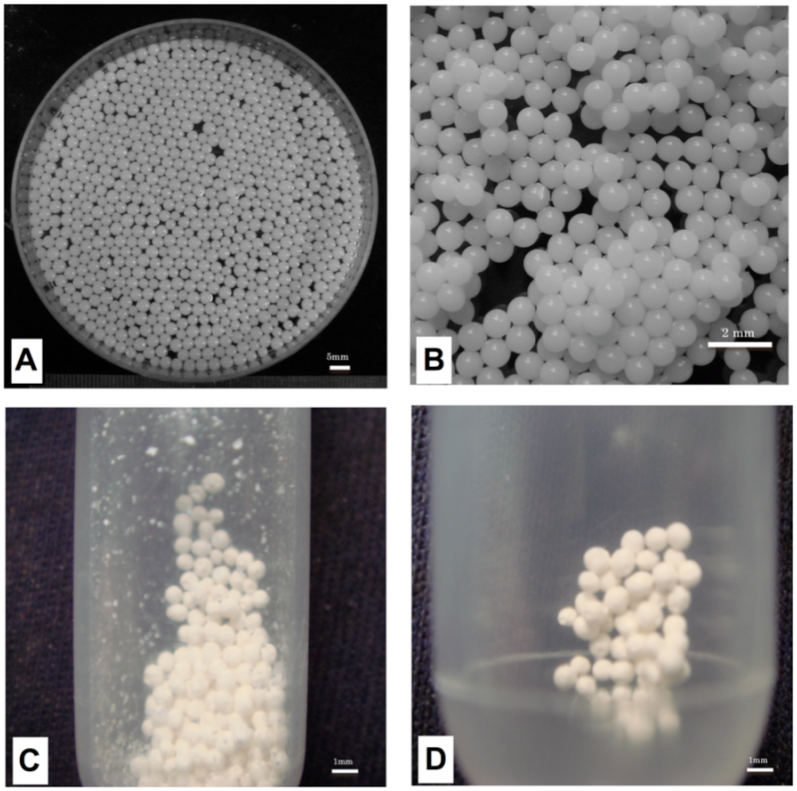

Figure 1: Photos of the newly prepared vancomycin-loaded alginate/β-TCP beads. (**A-B**) The vancomycin-loaded alginate/β-TCP beads before lyophilization. (**C**) The vancomycin-loaded alginate/β-TCP beads after lyophilization. (**D**) The vancomycin-loaded alginate/β-TCP beads after PCL coating.

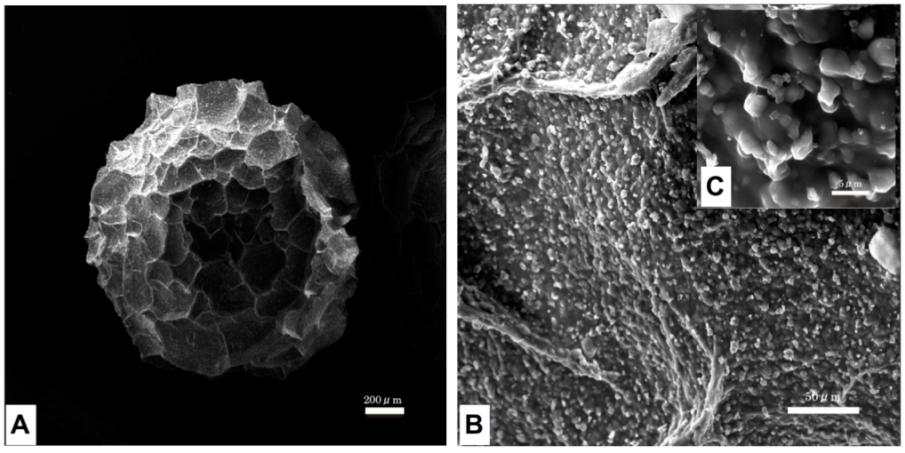

Figure 2: SEM of the vancomycin-loaded alginate/β-TCP beads before PCL coating. (**A**) The lyophilized beads turned into polyhedron; (**B-C**) The β-TCP particles distributed uniformly on the surface and the size of the β-TCP was approximately 5 μm.

coating layer on the surface with limited uncoated area (Figure **3C-D**). A shell-like layer of PCL was observed after being coated with 5.0 w/v% PCL covering almost all the β-TCP particles on the surface (Figure **3E-F**).

Weight Increment After Coating

The average weight increment ratio of the beads after being coated with 1.25 w/v% PCL was 11.8%± 8 .3%, and the ratios of the other two groups were 19.55 ± 6.6% (2.5 w/v% PCL) and 48.6% ± 13.4% (5.0 w/v% PCL), respectively (p<0.01) (Figure **4**).

Drug Loading and Encapsulation Efficiency of Alginate/β-TCP Beads

As calculated, before the coating process, the mean drug load of the beads was 39.5% ± 5.7%, and the encapsulation efficiency was 90.2% ± 8.5%.

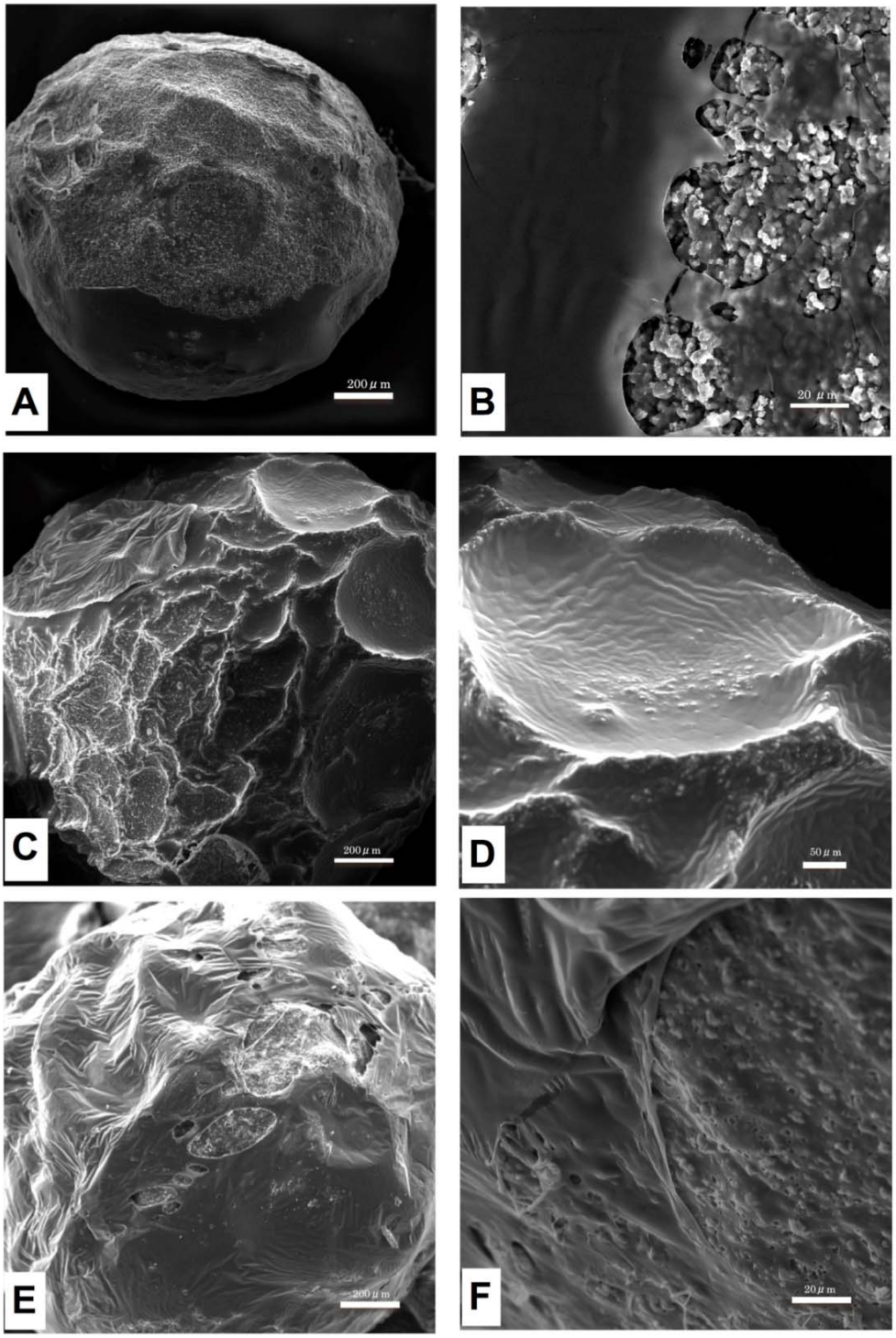

Figure 3: SEM of the vancomycin-loaded alginate/β-TCP beads after coated with PCL. (**A**) The gross view of alginate/β-TCP beads coated with 1.25 w/v% PCL; (**B**) in the bottom coated area of alginate/β-TCP beads coated with 1.25 w/v%, a thin layer of PCL coating could be observed with cracks and uncoated area; (**C**) the gross view of alginate/β-TCP beads coated with 2.5 w/v% PCL; (**D**) uniform coating could be seen on the surface of alginate/β-TCP beads coated with 2.5 w/v% PCL; (**E**) the gross view of alginate/β-TCP beads coated with 5.0 w/v% PCL; (**F**) a thick shell-like layer of PCL could be seen on the surface of alginate/β-TCP beads coated with 5.0 w/v% PCL.

In Vitro Drug Release

Almost all vancomycin from uncoated and PCL-coated beads was released into the PBS (pH 7.4) for the first 3 days. The vancomycin concentration of the PBS elusions of the uncoated beads on the first hour was 680 mg/L, and 16.2, 12.8, 10.0 mg/L on day 1, 2, and 3, respectively. The mean vancomycin

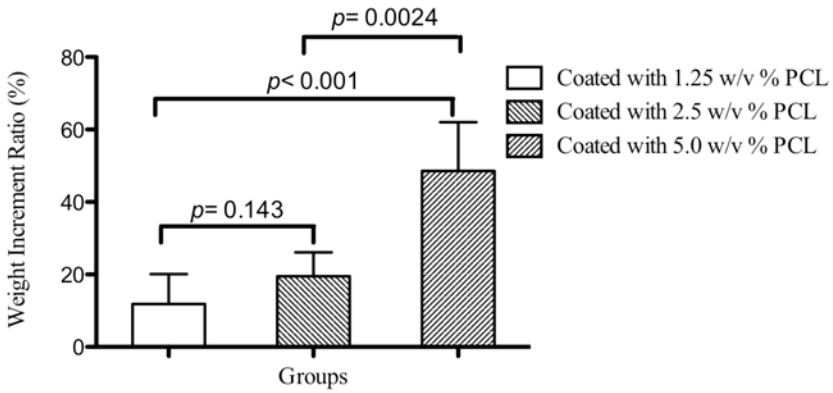

Figure 4: Weight increment of the alginate/β-TCP beads after being coated with different concentrations of PCL.

concentration in the PBS of 1.25 w/v% PCL-coated beads on days 1, 2, 3, 4, and 5 was 560, 15.1, 17.2, 14.2, and 5.5 mg/L. The vancomycin concentration in the PBS of 2.5 w/v% PCL-coated beads in PBS on days 1, 2, 3, 4, 5, and 6 was 17.6, 380, 180, 100.2, 18.2, and 10.5 mg/L; The vancomycin concentration in the PBS of 5.0 w/v% PCL-coated beads on days 1, 2, 3, 4, 5, 6, 7, 8, and 9 was 5.8, 5.2, 8.5, 10.5, 55, 620, 25,10.2, and 5.2 mg/L (Figure **5A**).

The initial burst release (85.0%) of vancomycin from uncoated alginate/β-TCP beads occurred in the first hour followed by release of 98.8% in 2 days (Figure **5B**). The beads morphologically disintegrated by the day 3. The 1.25w/v % PCL-coated alginate/β-TCP beads got a similar initial burst release of vancomycin with the uncoated beads, but the burst release did not occur until 24 hours followed by release of 95.4% in 48 hours. The beads disintegrated on day 5. Being coated with 2.5 w/v% PCL could increase the stability of the beads in the PBS at 37° C, which resulted in extended release of vancomycin. Only 7.3% of vancomycin was released in 24 hours, and there was still more than 40% of vancomycin retained in the coated beads after 48 hours. The beads disintegrated on day 6. As to 5.0 w/v% PCL-coated beads, the drug release rate of day 1 was 5.1%. The burst release appeared on day 6, and the cumulative percentage release was 94.6% (Table **1**). The shell-like coatings of the beads still existed in the PBS after all the loaded vancomycin had been delivered on day 9. The peak concentration appeared on the first hour, day 1, day 2, and day 6 in uncoated, 1.25 w/v% PCL coated, 2.5% w/v% PCL coated, and 5.0 w/v% PCL coated beads, respectively.

Antimicrobial Activity of the Elusions

For the antimicrobial susceptibility test, the elusion discs on the end point of uncoated, 1.25w/v% PCL coated, 2.5%w/v PCL coated, 5.0w/v% PCL coated

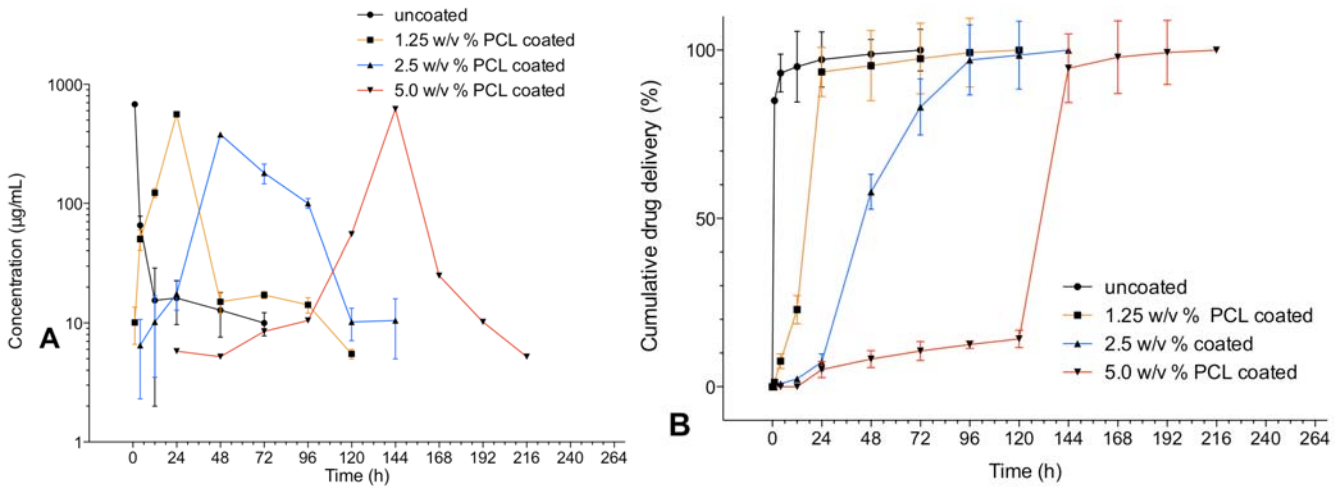

Figure 5: *In vitro* release profile of vancomycin from the vancomycin-loaded alginate/β-TCP beads uncoated/coated with different concentrations of PCL in PBS solution (pH 7.4) at 37° C. (**A**) The concentration of the elution of the vancomycin-loaded alginate/β-TCP beads coated/uncoated with different concentrations of PCL; (**B**) the cumulative vancomycin release from the vancomycin-loaded alginate/β-TCP beads uncoated/coated with different concentrations of PCL.

Table 1: The *In Vitro* Vancomycin Release Profiles of Alginate/ β-TCP Beads Coated with Different Concentrations of PCL Solutions

Groups	Cumulative drug release after the first 24 hours	Cumulative drug release after the first 48 hours	Cumulative drug release rate after burst release	Burst release time
Uncoated	97.2% ± 8.2%	98.8% ± 4.4%	85.0% ± 0.5%	0~1 hour
1.25w/v%	93.5% ± 7.3%	95.4%± 10.4%	93.5% ± 7.3%	12~24 hour
2.5w/v%	7.3% ± 2.5%	57.9% ± 5.2%	57.9% ± 5.2%	48~72 hour
5.0w/v%	5.1% ± 2.4%	8.2% ± 2.5%	94.6%± 10.2%	120~144 hour

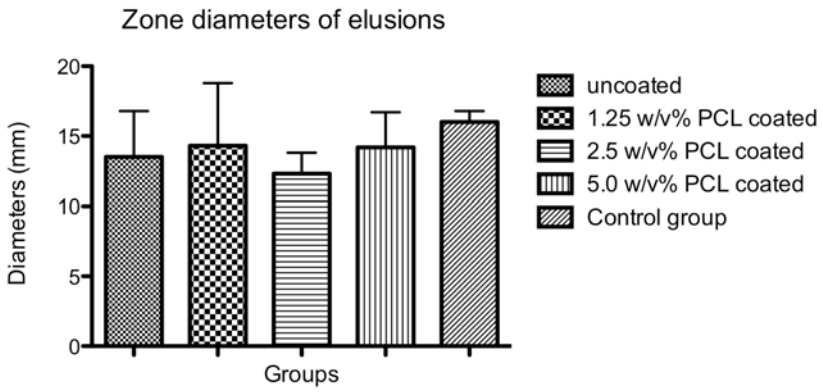

Figure 6: Inhibition zone diameters of elution (p>0.05).

developed inhibition zones with the average diameters of 13.5±3.3 mm, 14.3±4.5 mm, 12.3±1.5 mm, 14.2±2.5 mm, respectively (Figure **6**).

DISCUSSION

Based on *in vitro* experimental models, biofilm formation is classically viewed as a four-step process: 1) initial attachment of bacterial cells; 2) cell aggregation and accumulation in multiple cell layers; 3) biofilm maturation and 4) detachment of cells from the biofilm into a planktonic state to initiate a new cycle of biofilm formation elsewhere [41]. To maintain antibiotics at the therapeutic concentration at the implantation site for an extended period of time to avoid the initial attachment is essential to eradicate planktonic bacteria as much as possible [42], to sterilize sites contaminated with sessile bacteria, and to provide protection against biofilm colonization [43-45]. We sought to fabricate a kind of biodegradable beads, capable of eluting vancomycin and consequently keeping the local concentrations above the critical level for more than 72 hours.

This study is limited by several factors. First, the cytotoxicity test was not performed for the antibiotic beads, although all the components of the beads such as sodium alginate, β-TCP, vancomycin, and PCL are reportedly biocompatible and nontoxic [30-33]. These materials are resorbed very slowly and have been reported to cause foreign body reactions if left for prolonged periods in tissues [46, 47]. Second, as only three different concentrations of PCL were tested as coating solutions, there's possibility that other concentrations of PCL would work better than the 2.5w/v%, which might needs further investigation.

All of the existing systems release antibiotics at concentrations exceeding those of the MICs for the most common pathogens of chronic infection without releasing any antibiotic in the systemic circulation and without producing adverse effects [48]. Acrylic bone cement has been a common delivery vehicle for local antimicrobials in the form of antimicrobial-loaded bone cement (ALBC) [49]. Adams reported that high-dose ALBC delivers over 4 weeks for a variety of antimicrobials [50]. The use of ALBC is recommended by most authors for joint arthroplasty revisions [51]. Yet the high-dose ALBC is not commercially available and requires surgeon directed formulation, and performance of high-dose ALBC was affected by mixing method [52]. The prefabricated PCL-coated alginate/β-TCP beads carrying vancomycin would also be promising, since they are characterized by prolonged duration of release at concentrations 100 times the MICs (2 μg/mL) of the MRSA implicated in bone infections and resorbable in a certain period [53].

In addition, alginate systems have shown interesting results within bone regeneration when used as a delivery vehicle and/or to guide tissue repair providing a temporary extracellular matrix (ECM) for cells to infiltrate and migrate while depositing new bone tissue [54]. Examples of other alginate systems providing signaling cues promoting osteogenesis in addition to scaffold degradation, includes controlled matrix rigidity for differentiation of stem cells, use of alginates with covalently bound cell attachment peptides and delivery of growth factors and genes [54-58].

Being a drug carrier, the alginate bead is biodegradable, non-immunogenic, biocompatible and reportedly does not induce any lasting inflammatory processes [59]. Gelation of alginate occurs when divalent cations take part in the interchain binding between G-blocks giving rise to a three-dimensional network in the form of a gel. The uncoated alginate beads were used as drug delivery systems (DDSs) for antibiotic treatment of osteomyelitis [26, 60-63]. In our study, the elusions of uncoated or coated alginate/β-TCP beads had the similar bactericidal activities with the control discs (16±0.8 mm), which demonstrated there was no influence to the activity of vancomycin during the coating preparation of the antibiotic-containing beads.

As to the PCL coating process of this study, the alginate/β-TCP beads had the most homogenous coating layer by using 2.5 w/v% PCL as coating solution. The viscosity of 1.25% PCL was relatively lower and its adhesion on the material would be low; after the coating process, most of the PCL solution could not stay on the beads, which might lead to insufficient coating of the beads. The 5.0% PCL was of high viscosity and would form a relatively thick and uneven shell-like layer on the surface after the coating process.

However the burst release happened in the early phase for the uncoated alginate beads in this study. This might be due to the fact that vancomycin macromolecules were loosely bound onto calcium alginate by ionic interaction. The alginate chains themselves are relatively stable under physiological conditions (pH 7.4, 37°C). The rate constant (*k*) for cleavage of the glycosidic linkages has been estimated to about 10^{-6} h^{-1}.[64] With the rate constant given above, an alginate with M_w of 200.000 g/mol will degrade to 100.000 g/mol in about 80 days at pH 7.4 and 37°C [64]. The major mechanism for degradation of alginate-based biomaterials *in vivo* is disintegration of the material due to the gradual exchange of gelling calcium ions with sodium [64]. The composite delivery system in this study has the advantage of being degradable because alginate and PCL can degrade *in vivo* and are easily handled by the metabolic pathways [65].

Previous study indicated that vancomycin-loaded alginate beads coated with poly-L-lysine (PLL) dissolved on days 17 to 21 with a maximum elution concentration of 50 mg/L [62]. In this study, vancomycin release was sustained to days 3, 5, 6, and 9 in the uncoated, 1.25w/v%, 2.5w/v% and 5.0w/v% PCL-coated groups respectively, with the elution concentration ranged from 5.2 mg/L to 680 mg/L, which were higher than the MIC of MRSA. The concentration of the elution was higher in PCL-coated beads of our study than the PLL-coated alginate beads, although it dissolved (day 5 and 6) earlier than PLL-coated (day17~21).

β-TCP was selected as our model calcium phosphate, because it is more resorbable than hydroxyapatite in a biological environment [66]. β-TCP has good biodegradability and osteoconductivity [67]. An ideal material is able to oppose biofilm formation and, support bone repair as well [68]. We estimate that the β-TCP suspended in the PBS would be resorbed by osteoclast through macrophage and be a source of calcium for repair of the bone defect *in vivo*. Similar to other degradable drug delivery systems, a burst release of antibiotic was observed during the first few hours of testing in uncoated alginate/β-TCP beads [69-71]. Furthermore, the fast disintegration of the beads may due to the incorporation of the β-TCP, which might compromise the inner structure of the alginate beads and allow them to dissolve easier. The other reason of the fast disintegration of the beads might be the insufficient exchange between sodium ions (Na^+) and calcium ions (Ca^{2+}) during the harden process of the beads, which made the alginate polymer less cross-linked. The reason for retarded drug release of the PCL- coated beads might be that the hydrophobic PCL could hinder water from intrusion into the material to contact with hydrophilic alginate. As to the 5.0w/v% PCL-coated material, polyester sealed most of the surface so that water has difficulty entering the inner part. On the other hand, with that hydrophobic coating, the PBS could not intrude into the material freely, which would hinder the diffusion of the hydrophilic vancomycin at the early stage. Large amount of drug embedded in the beads could not burst out until the PCL coating was lifted up. In conclusion, the presence

of a PCL layer could improve the stability of alginate/β-TCP beads in PBS and slow down the diffusion of vancomycin from the alginate/β-TCP beads. As a result of the retarded diffusion and disintegration of the bead, the vancomycin might be released from the alginate/β-TCP formulation in an extended profile. It was proposed that combining groups of these beads without or with different thickness of coating would provide a composite vancomycin elution profiles for sustained delivery. The proposed local delivery approach will require further laboratory and pre-clinical investigations.

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