Prolonged Antibiotic Release by PLGA Encapsulation on Titanium Alloy

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Received 25 Aug 2011; Accepted 15 Nov 2011; doi: 10.5405/jmbe.1001

Abstract

Deep infection after artificial joint arthroplasty is a devastating complication. Local antibiotic delivery in revision surgery is useful in preventing the recurrence of infection. The present study investigates whether encapsulated antibiotic-loaded plasma-sprayed titanium alloy hip prosthesis materials that use biodegradable poly(lactic-co-glycolic acid) (PLGA) copolymer extend the duration of steady antibiotic release without a burst release. Two kinds of antibiotic (vancomycin and cefuroxime) and various concentrations and number of layers of PLGA encapsulation are investigated to determine the optimal antibiotic release. The antibiotic release is quantified with an elution test. The extracts were collected at 1, 6, and 12 hours and daily after 24 hours for antibiotic concentration analysis using a spectrophotometer. The degradation of PLGA correlated with the antibiotic release rate is characterized by weight change, pH value, and morphology observation. The effective antibiotic release durations for single-layer 15% PLGA in the vancomycin group, single-layer 15% PLGA in the cefuroxime group, double-layer 15% PLGA in the cefuroxime group, and one layer of 30% PLGA over one layer of 15% PLGA are 5, 7, 10, and 17 days, respectively. PLGA encapsulation can provide an extended antibiotic release of up to 2.5 weeks without a burst release. The release can be further extended in vivo to reach clinical effectiveness.

Keywords: Deep infection, Poly(lactic-co-glycolic acid) (PLGA), Antibiotic release, Cementless hip prosthesis

1. Introduction

Arthroplasty is widely applied for the treatment of severe joint disorders. However, deep infection occurs in approximately 1% to 5% of cases [1]. Peri-prosthetic infection is associated with bacteria growing into biofilms and attaching onto the prosthesis. Staphylococcus aureus (S. aureus) is the most common bacteria identified from infection sites after arthroplasty [2]. It is also the most common bacteria found in catheters or other surgical implants such as artificial arteries and dialysis [3]. The bacteria produce coagulase, which induces blood clots that cover the bacteria, protecting them from attack by phagocytes [4].

Biofilm formation on infected arthroplasty prostheses is difficult to eradicate even with antibiotic therapy. The removal of implants and a thorough debridement are usually necessary, but there is still a risk of re-infection [5]. Re-implantation of the prosthesis with antibiotic-impregnated bone cement provides effective local antibiotic elution for an extended period and has been reported to reduce the rate of re-infection [6]. However, cement fixation may not be a good choice for some conditions, such as the acetabular cup or a sclerotic femoral canal. A cementless prosthesis with comparable resistance to bacteria may be ideal in these conditions.

Antibiotics can be simply classified as bactericidal and bacteriostatic, and their effectiveness is concentration- and time-dependent. Antibiotics kill bacteria at the same rate and to the same extent once the minimum inhibitory concentration (MIC) has been achieved [7]. Both vancomycin and cefuroxime have been proven effective against S. aureus [8,9]. Vancomycin is a broad-spectrum antibiotic effective against a wide range of Gram-positive and Gram-negative bacteria. Cefuroxime has significant activity against S. aureus and S. epidermidis. It has been proved to have no toxic or antiproliferative effects on human osteoblasts in in vitro experiments [10].

Biodegradable drug delivery systems have the ability to provide the extended release of therapeutic agents through the gradual degradation of polymer materials [11]. Poly(lactic-co-glycolic acid) (PLGA) has been widely used in a variety of

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biomedical devices, such as sutures, implants, micro and nanoparticles, drug delivery systems, and tissue engineering, due to its biocompatibility and biodegradability [12]. Local drug delivery systems can achieve high local concentrations of the drug in the target region, instead of an overall high serum concentration in the whole body system [13]. Bone cement loaded with antibiotic is the standard drug-eluting local delivery system for the treatment of prosthesis deep infection [13]. Nevertheless, the duration of the antibacterial capability and the high level of residual antibiotic inside the cement are still less than ideal for infection treatment.

The present study investigates the extension of the antibiotic release duration of antibiotic-loaded Ti6Al4V alloy encapsulated with biodegradable PLGA for future cementless total hip joint prostheses with a self anti-infection capability. Two kinds of antibiotic, vancomycin and cefuroxime, were used in this study. The influence of the concentration and number of coating layers of encapsulating PLGA on the antibiotic release rate and duration were temporally quantified using a spectrophotometer to determine the optimal design for long-term self anti-infection modification for prostheses.

2. Materials and methods

2.1 Antibiotic loading on plasma-sprayed titanium alloy disc

Plasma-sprayed titanium alloy Ti6Al4V is widely used for the bone on-growth surface in total hip arthroplasties. Titanium alloy metal discs with a 7-mm diameter and a 2-mm thickness were processed with a vacuum-plasma-sprayed titanium coating to simulate the surface of a cementless prosthesis. The maximum amount of antibiotic solution the disc can hold is 50 μl. Vancomycin (Vancocin CP, Eli Lilly Japan K.K., Japan) or cefuroxime (Ucexafim, Bio Invigor, Taiwan) at 12 mg/ml was dropped directly onto a metal disc, which was then placed in a desiccator cabinet overnight to dry.

2.2 PLGA encapsulation

6 and 12 g of PLGA biomaterial with a copolymer ratio of 85/15 (lactide/glycolide%) at molecular weights of 35,000-65,000 g/mol (Bio Invigor, Taiwan) were dissolved in 40 ml of acetone to prepare 15% and 30% mg/ml PLGA-acetone solutions, respectively. To accelerate the PLGA dissolution in acetone, the PLGA was stirred for 1 hour. Subsequently, an antibiotic-loaded metal disc was immersed in the PLGA-acetone solution for 5 minutes, and then was quickly taken out and placed in a laminar flow hood for acetone evaporation. For the multiple-layers encapsulation group, the samples were re-loaded with 50 μl of antibiotic after the first encapsulation was dry, and then encapsulated with PLGA for a shorter time (30 seconds). Two concentrations, 15% and 30%, of PLGA solution were used for the second layer in different groups. The encapsulation parameters of the four groups (A, B, C, and D) and experimental sequences (1 to 4) are shown in Table 1. The amounts of PLGA on the discs (n = 5) were measured by the weight change among the groups using an electronic scale (XB120A, Precisa Instruments, Switzerland).

<table>
<thead>
<tr>
<th>Step</th>
<th>Group</th>
<th>First antibiotic loading</th>
<th>First PLGA encapsulation</th>
<th>Second antibiotic loading</th>
<th>Second PLGA encapsulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>Vancomycin 15%; 5 min</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>Cefuroxime 15%; 5 min</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>Cefuroxime (15%-15%) 15%; 5 min</td>
<td>Cefuroxime 15%; 30 s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>Cefuroxime (15%-30%) 15%; 5 min</td>
<td>Cefuroxime 30%; 30 s</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

V. Vancomycin; C. Cefuroxime; N/A, not available

2.3 Elution test

The elution test was used to evaluate the antibiotic release behavior of the encapsulations on the metal discs of the four groups. Phosphate buffered saline (PBS, pH 7.4) solution was used for the elution test to extract the antibiotic. Processed metal discs were first immersed in 2 ml of PBS in 15 ml of polypropylene conical tubes. The tubes were rotated continuously in a rotator (DSR 2800V, Digsystem Laboratory Instruments, Taiwan) at a frequency of 120 Hz at room temperature. Each metal disc was moved to a new tube with fresh PBS solution at 1, 6, 12, and 24 hours, and then every 24 hours. The remaining PBS solution was collected for antibiotic concentration analysis.

The vancomycin concentration determination followed a previously reported procedure [14]. Briefly, 120 μl of 2 mM benzocaine solution (2 mg/ml solution in 95% ethanol) (Sigma), 120 μl of 0.5 M sulphuric acid (Sigma), and 240 μl of 0.1% sodium nitrite solution (Sigma) were mixed, and then allowed to stand for 15 minutes; then, 1.5 ml of a PBS sample was added, and the mixture was allowed to stand for 5 minutes. Finally, 12 μl of triethylamine mixed with 40% ethanol solution was added and diluted to 3 ml with distilled water, and allowed to stand for 20 minutes. The wavelength of the spectrophotometer (GeneQuant 1300, Healthcare Bio-Science, UK) was set at 442 nm. For the cefuroxime concentration determination [15], 0.5 ml of 0.1 N sodium hydroxide (NaOH) was added into 1.5 ml of an undetermined solution. The measurement wavelength of the spectrophotometer was set at 292 nm.

2.4 Concentration of residual antibiotic

After termination of the elution test, the residual antibiotic on the metal disc was measured to evaluate the efficiency and accuracy of the elution test. To investigate the residual antibiotic encapsulated by the PLGA matrix after the elution test, a metal disc was immersed in 30 ml of acetone overnight, and then sonicated with ultrasound (Transssonic TP 695/H, Elma, Switzerland) for 40 minutes. Then, an additional 10 ml of PBS solution was added, followed by 10 more minutes of sonication. After the acetone in solution had evaporated, the solution was centrifuged (Minispin 2323, Eppendorf AG, Germany) at 1500 rpm for 5 minutes. The supernatant was extracted for subsequent antibiotic analysis using a spectrophotometer.
2.5 PLGA in vitro degradation test

The temporal weight change was measured to evaluate the PLGA degradation. The metal discs were taken out every time PBS solution was exchanged to assess the antibiotic concentration; they were then dried in a desiccator overnight and weighed. The degradation of PLGA on the metal discs was quantified by the weight difference between time points. In addition, it was necessary to confirm the pH value of the solution, and whether the PLGA degradation product caused toxic-level acidity in the normal cells; an acidic environment is thought to inhibit normal osteoblast bone formation [16-18]. The pH value of the eluant from the previous elution test was measured with a pH meter (Delta 320, Mettler-Toledo Group).

2.6 Surface morphology

The surface morphology of the metal discs after PLGA degradation was investigated by metallographic microscopy (Eclipse LV150, Nikon, Japan) after 5 days’ elution. The metal disc samples were directly observed without grinding and polishing. The microscopy images of the surfaces indicated the progress of PLGA degradation.

2.7 Anti-bacteria test

In order to verify the efficacy of the antibiotic used in this study, the agar diffusion test was performed. ATCC25923 S. aureus, obtained from National Cheng Kung University Hospital, was cultured on agar plates (Laboratorios Conda, S.A., Spain) overnight. 25 μl of antibiotic solution was dropped onto a standard paper disc (6 mm in diameter) (Becton, Dickinson, USA) for antibiotic loading, and then the antibiotic-loaded paper discs were directly pressed down on the agar and incubated at 37 °C overnight. The action of the antibiotic appears as a circle around the paper disc, called an anti-bacteria zone, which indirectly indicates the activity of the antibiotic solution against bacteria growth. The MIC values of vancomycin at 8 μg/ml and cefuroxime at 2 μg/ml were determined in this agar diffusion test (n = 9).

2.8 Statistical analysis

All the data presented are mean values with error bars of standard deviations obtained using SPSS 13.0. The normal distribution of data was confirmed by the Shapiro-Wilk test before statistical analysis. Statistical differences between groups were analyzed by one-way analysis of variance (ANOVA) with the Tukey post hoc test. A value of p < 0.05 was defined as indicating a significant difference.

3. Results

3.1 Encapsulating PLGA weight

Encapsulating PLGA weights for groups B, C, and D are shown in Fig. 1. All three groups exhibit a weight increase after encapsulation. The statistical results show no difference between groups B and C; however, group D, with a high-concentration two-layer PLGA encapsulation, had a significantly greater weight increase than those of groups B and C (p < 0.001).

![Figure 1. Weight increases after PLGA encapsulation for groups B, C, and D. After encapsulation, all three groups had a proven weight increase. The statistical results show that the weight increases of groups B and C were different from that of group D; however, no difference was found between group B and group C. The data are mean ± standard deviation (SD) (n = 5 each). * P < 0.001 versus group D.](image)

3.2 Antibiotic release

All four groups of samples had a higher antibiotic release during the early hours (Fig. 2). Group C had the most severe burst effect; however, group D, also with a two-layer encapsulation, did not appear to have an obvious burst effect. All four groups had antibiotic release concentrations higher than the MIC of S. aureus suggested in other research [8,9]. The antibiotic release concentrations of some of the groups at a later period were below MIC (Fig. 3). The effective antibiotic release durations of the four groups were 5-17 days (Table 2). The ratio of accumulated antibiotic release to the amount of initially loaded antibiotic was calculated (Fig. 4). Group A presented the highest accumulated antibiotic release at 95% at the end of the effective antibiotic release period; group B had released only 64% of the antibiotic by that time. For two-layer encapsulation, group C had a higher antibiotic release than that of group B; however, group D had the total antibiotic release reduced to nearly 37% of the total amount during the effective period. More than 60% of the antibiotic was not eluted or was lost during the process in group D. The amount of residual antibiotic on the metal discs of group D was 482.7 ± 19.2 μg. The average accumulated released antibiotic from the elution test of group D was 441.2 μg. The initial antibiotic loaded on the surface of the metal discs for group D was 1200 μg. About 276 μg of antibiotic was lost with double PLGA encapsulation.

3.3 PLGA degradation

The temporal PLGA degradation ratio correlated with the antibiotic release, as shown in Fig. 5. Group C had the highest degradation ratio at each time point, followed by group B and group D. The high percentage of PLGA in group C matched the high accumulated antibiotic release in the group. The pH value of the eluting solution from group D at each time point did not show an obvious fluctuation within 17 days.
Figure 2. Early antibiotic release concentrations in extracts from 1 hour to 5 days. All four groups had antibiotic release concentrations higher than the MIC of S. aureus suggested in other research. Group C had the most severe burst effect; group D, also with a two-layer encapsulation, did not appear to have an obvious burst effect. The data are mean ± SD (n = 9 each).

Figure 3. Evaluation of antibiotic release concentrations from one day to 17 days. The antibiotic release concentrations of some groups at a later period were below MIC. The data are mean ± SD (n = 9 each).

Figure 4. Ratio of accumulated antibiotic release to the initially loaded antibiotic. More than 60% of the antibiotic remained in the encapsulation or was lost during the process in group D within 17 days. The data are mean ± SD (n = 9 each).

Table 2. Effective antibiotic release durations

<table>
<thead>
<tr>
<th>Group</th>
<th>Duration (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Vancomycin-15%</td>
<td>5</td>
</tr>
<tr>
<td>B: Cefuroxime-15%</td>
<td>7</td>
</tr>
<tr>
<td>C: Cefuroxime-15%-15%</td>
<td>10</td>
</tr>
<tr>
<td>D: Cefuroxime-15%-30%</td>
<td>17</td>
</tr>
</tbody>
</table>

3.4 Surface morphology

The surface morphologies of the samples were observed after PLGA encapsulation and 5 days of elution. A porous morphology on the PLGA matrix was found in groups A, B, and C; group C showed the most abundant pores. In contrast, group D did not show this porous morphology (Fig. 6).

Figure 5. Temporal accumulated PLGA degradation ratios. Group C had the highest degradation ratio at each time point, followed by group B and group D. The high percentage of PLGA in group C matched the high accumulated antibiotic release in the group (n = 3 each).

3.5 Anti-bacteria test

The average diameter of the anti-bacteria zone of the agar diffusion test for the MIC of vancomycin and cefuroxime is shown in Fig. 7. The antibiotic concentrations chosen in this test, vancomycin at 8 μg/ml and cefuroxime at 2 μg/ml, were proven to be effective against bacteria growth.

Figure 6. Representative surface morphology images of PLGA encapsulation after a 5-day elution test for groups A, B, C, and D. The surface morphologies of the samples were observed after PLGA encapsulation and 5 days of elution. Porous morphology on the PLGA matrix was observed in groups A, B, and C. Group C showed the highest porosity ratio. In contrast, group D did not show a porous morphology.

Figure 7. Diameters of bacteria inhibition zone for two antibiotic solutions at minimal inhibition concentration. The antibiotic concentrations chosen in this test, vancomycin at 8 μg/ml and cefuroxime at 2 μg/ml, were proven to be effective against bacteria growth. The data are mean ± SD (n = 9 each).

4. Discussion

In the present study, all groups of samples had effective anti-infection antibiotic concentration releases for 5 to 17 days. The double-layer encapsulation in group D, with a high-concentration PLGA second-layer coating, maintained an effective antibiotic release for up to 17 days, which is a longer
antibiotic release period than those of chitosan film (72 hours only) [19] and poly(methyl methacrylate) cement (8 days) [20]. Not only did group D have a long antibiotic release, it also had the least burst release early during the elution. Our previous study used the same single-layer PLGA encapsulation approach to extend the release of antibiotic from a metal disc surface by the direct contact method [21]. The metal disc still had an effective inhibitory zone for 5 to 7 days on an LB broth bacteria plate. The quantification of the extract of the elution test from group A and group B, both with single-layer encapsulation, indicates similar effective durations for the antibiotic release concentrations. The gradual degradation of PLGA prolongs the antibiotic release from the metal surface and could be an effective method for anti-infection joint prostheses.

The antibiotic release duration should increase with the degradation time and thickness of the PLGA. The thickness of the encapsulating PLGA should be directly correlated to its weight gain. In this study, the mass increase of PLGA encapsulation (Fig. 1) was directly proportional to the effective antibiotic release duration (Table 2).

The degradation rate corresponding with the antibiotic release rate also depends on the molar ratio of the copolymer. PLGA is a copolymer of hydrophobic poly lactic acid (PLA) and hydrophilic poly glycolic acid (PGA). When PLGA is immersed in PBS solution, the PGA quickly begins to degrade due to its hydrophilicity. This fast PGA degradation caused a burst antibiotic release at the beginning of elution in all four groups. The early glycolic acid degradation created pores within the PLGA matrix. In the later period of antibiotic release, the antibiotic molecules diffuse through the pores created by the early PGA degradation, leading to a more stable antibiotic release rate. The burst release of groups A, B, and C was more obvious than that of group D (Fig. 2). The high concentration of the second-layer PLGA encapsulation has denser PLGA on the outer layer, which reduces the early quick degradation of PGA. This slow release caused the high residual antibiotic amount for group D. The encapsulating PLGA usually degrades in several months, so the residual antibiotics are released at a later time.

The plasma-sprayed surface enhances bone on-growth during healing. The PLGA encapsulation could potentially interfere with the bone integration between the bone and the prosthesis. Willie et al. indicated that remodeling rates in sheep were similar to those in human bone in-growth [22]. It has been reported that bone in-growth progressed from 20.1 ± 8.2% at 6 weeks in situ to 23.8% ± 7.9% at 12 weeks and 30 ± 5.1% at 24 weeks in an ovine model. Hence, bone attachment and ingrowth on a biomaterial-coated metal prosthesis might be achieved by controlling the properties of the degradable PLGA. The early degradation of hydrophilic PGA from PLGA should provide initial space for osteoblasts to migrate into the encapsulation. In the later stage, the degradation rate of the remaining PLA should match the progress of bony in-growth. The degradation of PLGA with bone in-growth and local chemostasis requires further study.

The diverse molecular structures and properties of antibiotics result in different release mechanisms. In this study, the total amount of antibiotic of samples from group A was about 95% of the total initially loaded vancomycin. Nevertheless, the same single-layer 15% PLGA encapsulating cefuroxime samples in group B released only about 65% of the total initially loaded antibiotic. Since the vancomycin molecule contains abundant hydrophilic functional groups, this hydrophilicity reduced the affinity of vancomycin to more hydrophobic PLGA encapsulation after the initial hydrophilic PGA degradation. This hydrophilic property also enhances dissolution in PBS solution and speeds vancomycin release from the PLGA matrix.

The mechanism of antibiotic release from the PLGA matrix mostly depends on the degradation of PLGA. For group C, the first layer of encapsulating PLGA re-dissolved during the second 15% PLGA encapsulation process, which may have accelerated the degradation of PLGA. Because a hydrophobic solvent was used to dissolve PLGA, the hydrophobic PLA was re-dissolved more than the PGA, leaving the remaining PLGA matrix with more hydrophilic PGA. The higher hydrophilic PGA in the PLGA matrix may have accelerated the total PLGA degradation rate. Unlike group C, group D showed a significant increase in the final encapsulating PLGA weight (Fig. 1) because of the doubled PLGA concentration in the second layer. The thicker outer PLGA matrix in the second-layer 30% PLGA encapsulation reduced the overall PLGA degradation.

The morphology of PLGA encapsulation after the elution test reflects the severity of its degradation. The morphological differences among groups in the encapsulating PLGA matrix after 5 days of the elution test proved that the degree of degradation of PLGA is influenced by the encapsulation process (Fig. 6). The porous structure of groups A and B could correlate with their higher degradation rate. The visibly higher porosity of group C may explain the rapid PLGA degradation rate. The rough surface without apparent surface porosity of group D proved that the 15%-30% double encapsulation process forms a stronger composition to retard matrix degradation.

5. Conclusion

Two kinds of antibiotic and three PLGA encapsulation processes were investigated in this study. The longest effective antibiotic release period was in the 15% PLGA and 30% PLGA double encapsulation group, which had an antibiotic release period of up to 17 days without a severe early burst effect. The results show that the processes developed in this study have clinical application potential.

Acknowledgments

The authors would like to acknowledge the financial support for this study from the National Science Council (NSC 96-2221-E-006-274) in Taiwan. We are also grateful for the assistance of Prof. Tzer-Min Lee from the Institute of Oral Medicine at National Cheng Kung University (NCKU) in providing the titanium discs and plasma treatment, Dr. Jer-Chia Hsu from the Department of Orthopedic Surgery at NCKU
Hospital and Dr. Meng-Yi Chen for assistance in the clinical relevance and preparation for the elution test, and thank Prof. Chuan-Fa Chang from the Department of Medical Technology at NCKU for assistance in using the spectrophotometer.

References


