Original Article

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Sustained release vancomycin-coated titanium alloy using a novel electrostatic dry powder coating technique may be a potential strategy to reduce implant-related infection

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Summary **In order to tackle the implant-related infection, a novel way was developed in this study to coat vancomycin particles mixed with controlled release coating materials onto the surface of titanium alloy by using an electrostatic dry powder coating technique. To characterize this sustained release antibacterial coating, surface morphology, *in vitro* and *in vivo* drug release were sequentially evaluated. *In vitro* cytotoxicity was tested by Cell Counting Kit-8 (CCK-8) assay and cytological changes were observed by inverted microscope. The antibacterial properties against MRSA, including a bacterial growth inhibition assay and a colony-counting test by spread plate method were performed. Results indicated that the vancomycin-coated sample was biocompatible for Human osteoblast cell line MG-63 and displayed effective antibacterial ability against MRSA. The coating film was revealed uniform by scanning electron microscopy. Both the *in vitro* and *in vivo* drug release kinetics showed an initially high release rate, followed by an extended period of sustained drug release over 7 days. These results suggest that with good biocompatibility and antibacterial ability, the sustained release antibacterial coating of titanium alloy using our novel electrostatic dry powder coating process may provide a promising candidate for the treatment of orthopedic implant-related infection.**

Keywords: Implant-related infection, antibacterial coating, drug delivery system, electrostatic coating, surface modification

1. Introduction

With the increasing incidence of trauma, joint degeneration and bone tumor, more and more implants are applied in orthopedic surgeries, playing a critical role in improving the stability and rebuilding the anatomical structure of bone and joint. However, the treatment of implant-related infections still remains a huge challenge for orthopedic surgeons. Incidence of postoperative infection after arthroplasty or closed fracture fixation ranges from 0.3 to 3% (*1-4*) and reaches nearly 30% in open fracture. The consequences for patients, with no doubt, are tremendous including prolonged hospitalization with pain and immobilization, revision surgery and long-term antibiotic therapy, and the huge costs during the entire treatment (*5,6*). The conventional treatment for implant-associated infections is limited to systemic administration of antibiotics, which is usually difficult to achieve the required local delivery of medicine and ineffective once the bacteria biofilm on the surface of implant is formed. Fortunately, the application of local drug delivery system (LDDS) in orthopedics, like modification of the titanium surface or antibiotics delivery coating of implants, which can effectively raise the antibiotic concentrations around bones and reduce the risk of toxicity from large systemic administration, has brought us a new way to tackle this problem (*7*). Though

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great progress has been made, there still remain some technical difficulties like limited antimicrobial spectrum, short maintenance of effective antibiotic concentration due to the burst release of loaded drugs, relatively complicated and high energy consuming coating process *etc*.

In this study, we developed a novel way to coat vancomycin particles mixed with controlled release coating materials onto the surface of titanium alloy by using an electrostatic dry powder coating technology, which had formerly been applied for pharmaceutical usage (*8*). The surface morphology, *in vitro* antibacterial properties, biocompatibility and drug releasing kinetics of the vancomycin-coated titanium alloy were evaluated.

2. Materials and Methods

2.1. *Preparation process of vancomycin-coated titanium alloy*

2.1.1. *Preparation of titanium alloy*

Plain titanium alloy discs (Ti6Al4V) were custom made by Shanghai Institute of Ceramics, Chinese Academy of Sciences, 6 mm in diameter and 2 mm in height (Figure 1A). Before the coating process, the matrix surface is cleaned by 1 mol/L hydrochloric acid, methanol, 70% ethanol and ultrasonic vibration of distilled water, each for 10 min, and then air-dried.

2.1.2. *Preparation of coating materials*

Eudragit RS and Eudragit RL (Evonik Degussa Corporation, Germany), as sustained release control agents, are copolymers derived from esters of acrylic and methacrylic acid usually used for sustained release coating of pharmaceutical dosage forms. Triethyl citrate (TEC), as a liquid plasticizer, was purchased from Caledon Laboratories Ltd. (Ontario, Canada). Talc was purchased from Mallinickrodt Baker Inc (Canada). Vancomycin were provided (Zhejiang Medicine Co, Ltd Xinchang Pharmaceutical factory, China) as antibiotics.

Particle size reduction of Eudragit RL, Eudragit RS, talc and vancomycin were conducted separately by a jet mill, prior to use. Particle size of the powder was confirmed by a Particle Size Distribution Analyzer (TSI Corporation, Model 3603, Shoreview, MN, USA). The particle size at 50% of total weight fraction was used as average particle size. The average particle size of Eudragit RL, Eudragit RS, talc and vancomycin was 18.4 μm, 16.5 μm, 28.9 μm, and 29.0 μm respectively.

2.1.3. *Electrostatic powder coating process*

The powder coating process was conducted in a laboratory scale electrostatic dry powder pan coater system comprising of a heating oven, a liquid spray nozzle and an electrostatic spray gun (Figure 1C). The titanium alloy discs were suspended inside the oven and preheated at a certain temperature (30-60°C) for 10 min before the coating started. The liquid plasticizer (TEC) was regulated by a liquid metering pump (Fluid Metering Inc., USA) and sprayed onto the titanium surface through a liquid atomizing nozzle at a rate of 0.3 g/min (Table 1). Afterwards, the coating particles were sprayed by an electrostatic spray gun (Nordson Corporation, USA). After spraying the powder, the titanium alloy discs were further cured for 4-8 h to allow film formation. In order to achieve the sustained release effect of the drug, the antibiotics coating materials and polymer coating materials were coated onto the surface of the titanium alloy discs by a plurality of sets of spray. It sprayed a mixture of antibiotics and polymer materials for the first layer then sprayed the sustained release polymer materials for the second layer (Table 1). This step was repeated for several times. By improving the thickness and strength of the coating film, it achieved the effect of the drug release. The average weight gained after coating was up to 16%. The coated samples were then air dried for 3 days at 37°C (Figure 1B).

2.2. *Surface characterization*

The surface morphology of the coated sample at curing temperature 60°C and curing time (4, 8 h) were examined by scanning electron microscopy (SEM). The samples were sputter coated with gold for 120 s under argon atmosphere using EMITECH K550 sputter coater (Emitech Ltd, Ashford, UK), and then were observed with a scanning electron microscope (S-2600 N Hitachi, Ontario, Canada).

2.3. *In vitro drug release*

Release of vancomycin from coated samples was investigated in a PBS buffer (pH 7.4). The coated surfaces were immersed in 10 mL of PBS and incubated at 37°C with an orbital shaker at 70 rpm. The PBS was replaced fresh by the addition of the same volume at 4, 24, 48, 72, 120, 170 h. PBS samples (10 μL) were collected at a predetermined time (1, 4, 24, 48, 72, 120, 170 h) and then diluted 20 times. The content of vancomycin in the supernatant was measured with LC-MS/MS. The LC-MS/MS system consists of an AB Sciex Qtrap 5500 (AB SCIEX, USA) with a Waters Acquity UPLC (Waters Corporation, USA). The instrument conditions are shown in Table 2. The cumulative amount of vancomycin released from the samples was plotted against time.

2.4. *Antibacterial tests of vancomycin-coated sample*

2.4.1. *Bacterial zone of inhibition test (ZOL)*

To check for the antibacterial potency of the vancomycin-

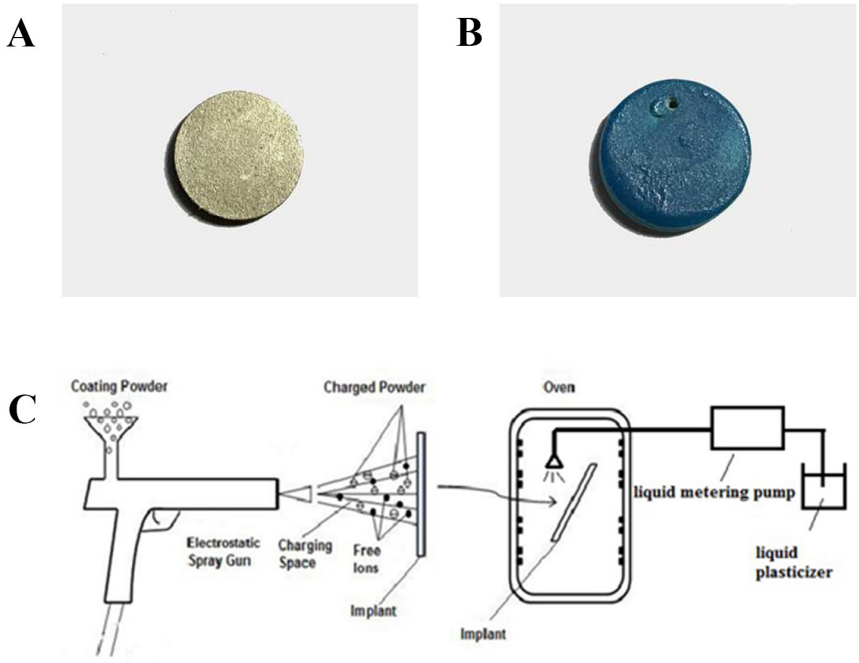

Figure 1. The appearance and coating process of the titanium alloy sample. (A), Uncoated sample; **(B)**, Vancomycin-coated samples; **(C)**, The electrostatic powder coating process.

Table 1. Compositions and processing condition of coating

Antibiotics coating materials	65% Eudragit RS/Eudragit RL (2:1) + 35% Vancomycin
Sustained release coating materials	80% Eudragit RS/Eudragit RL (2:1) + 19.9% Talc + 0.1% TiO_2
Plasticizer	TEC
Temperature (°C)	60
Curing time (h)	4 to 8
Air flow rate (SCFH)	2.5
Atomizing pressure (psi)	54
Carrier air pressure (psi)	54
Voltage of spray gun (KV)	50

Table 2. The LC-MS/MS conditions

Column	Waters CORTECS UPLC, 2.1 × 50 mm, 1.6 μm 0.4 mL/min		
Flow rate	45°C		
Column temperature	10 μL		
Injection volume	Water (0.1%FA, A), acetonitrile (0.1%FA, B)		
Mobile phase	Time (min)	%A	%B
Gradient	1. Initial	99.0	1.0
	2. 1.50	85.0	15.0
	3. 1.60	5.0	95.0
	4. 2.60	5.0	95.0
	5. 2.70	99.0	1.0
The MS/MS conditions	Analyte: Vancomycin		
	Precursor ion: 724.5		
	Product ion: 144.1		
	DP (Volts): 100		
	EP (Volts): 10		
	CE (Volts): 20		
	CXP (Volts): 13		
Ion source	ESI		
Curtain Gas, Ion Source Gas 1, Ion Source Gas 2	45.0 psi		
Temperature	550°C		
Ion Spray Voltage	5500V		

coated sample, bacterial growth inhibition assay was performed. MRSA as the experimental bacteria, was offered by the Microbiology Laboratory of Zhongshan Hospital Affiliated to Fudan University. The bacterial suspension was diluted to 10^7 CFU/mL and inoculated on an agar plate. Then the sterile coated sample was put gently on the MRSA spreaded agar plate and incubated at 36.5°C. Without replacing the coated sample, the

MRSA spreaded agar plate was replaced every three days and the zone of inhibition was measured. The uncoated sample was treated with the same methods.

2.4.2. *Colony-counting test by spread plate method*

MRSA offered by the Microbiology Laboratory of Zhongshan Hospital Affiliated to Fudan University was incubated in a sterile Brain Heart Infusion broth (BHI) for 24 h. The concentration of MRSA was then adjusted to 10^7 CFU/mL and 100 μL of the suspension was added onto the surface of a coated sample. The sample surface was then covered by a plastic film to maintain the humidity above 90%, and incubated at 36.5°C for 48 h. After that, the sample was collected, washed with 2 mL of NS to remove loosely adherent cells on both sample surface and plastic film, and placed on a sterile plate. 100 μL of the suspension was then plated onto the agar plates and incubated at 36.5°C for another 48h. Mean numbers of colony-forming units (CFU) were quantified as a measure of antibacterial activity for each sample. The uncoated samples were treated with the same methods and served as a control.

2.5. *In vitro biocompatibility evaluation*

2.5.1. *Cell cultivation*

Human osteoblast cell line (MG-63, Cells Resource Center, Shanghai Institutes of Biological Science, Shanghai, China) was used for study. The MG-63 cells were cultured in the dulbecco's modified eagle medium (DMEM; Gibco, Invitrogen, Inc, USA) with 10% fetal bovine serum (FBS; Gibco, Invitrogen, Inc, USA), 1% antimicrobial of penicillin-streptomycin (Antibiotic-Antimycotic; Gibco, Invitrogen, Inc, USA) at 37°C in a humidified atmosphere of 5% CO_2. The DMEM was refreshed every 3 days during cell culturing. Confluent cells were harvested after trypsinization (0.25% trypsin, EDTA) and then placed in 96-well plates with the cell density of 1.0×10^4 cell/mL, prepared for further experiments.

2.5.2. *Preparation of the leaching solution*

The coated samples, with uncoated samples serving as a control were separately put in a centrifuge tube and completely soaked in a-MEM medium supplemented with 10% FBS. Then the centrifuge tubes were placed onto an automatic shaking bed with an appropriate speed and swing trace, allowing a full contact between the sample and culture medium. The culture supernatant of both coated and uncoated samples were filtered at predetermined times (2, 5, 8 days) as the leaching solution and stored at 4°C for further use.

2.5.3. *Cytotoxicity evaluation with the CCK-8 assay and the inverted microscope*

The leaching solution collected at 2, 5, 8 days were separately added to a 96-well plate where the prepared MG-63 cells were seeded in advance. The CCK-8 (Cell Counting Kit-8) assay was then tested to evaluate the cells proliferation at predetermined times (12 h, 24 h, 3 d, 6 d, 8 d) and the cytological changes of MG-63 was also observed by the inverted microscope. At each time point, 1 mL medium was added to each well with 100 μL CCK-8 reagent. After a 4 h incubation at 37°C, the optional density was read as 450 nm using an automated plate reader (ELX800, Bio-Tek, USA).

2.6. *In vivo drug release in animal studies*

The 10-week-old male SD rats (Shanghai Sippr-BK laboratory animal Co. Ltd, Animal license No. 2013-0016) were kept in cages with individual filtered aeration and fed with a standard diet with water ad libitum. Before implantation, the animals were anesthetized by intraperitoneal injection of 10% chloral hydrate (0.3 mL/100 g). The back of the mice, which was chosen as the implantation site, was shaved and sterilized with 70% ethanol. Then, the coated sample was buried subcutaneously and the wound was closed by simple interrupted wound sutures. The venous bloods from the tails were collected at different time points after surgery and centrifugated to plasma, frozen at -20°C. Plasma samples (20 μL) were collected and added 100 μL 8% $HClO_4$ as the protein precipitation agent, and then centrifugated under 20,000g for 10 min. Since the accumulative amount of released drug can't be achieved because of the metabolic processes of drugs in rats, the concentration of vancomycin at different time points in the supernatant was then measured instead with LC-MS/MS. The instrument and testing conditions were the same as the *in vitro* drug release part (Table 2). All animal experiments were done in accordance with the regulations and with the approval from Laboratory Animal Ethics Committee of Zhongshan Hospital Affiliated to Fudan University.

2.7. *Statistical analysis*

Data were presented as the mean ± standard deviation from at least three independent experiments and each experiment was performed twice. Statistical analyses were performed using a one-way analysis of variance (ANOVA). $p < 0.05$ was considered significant.

3. Results

3.1. *Surface morphology of vancomycin-coated sample*

Figure 2 (A, B) shows SEM images (1.8 k×) of the surface of coated sample after different curing times.

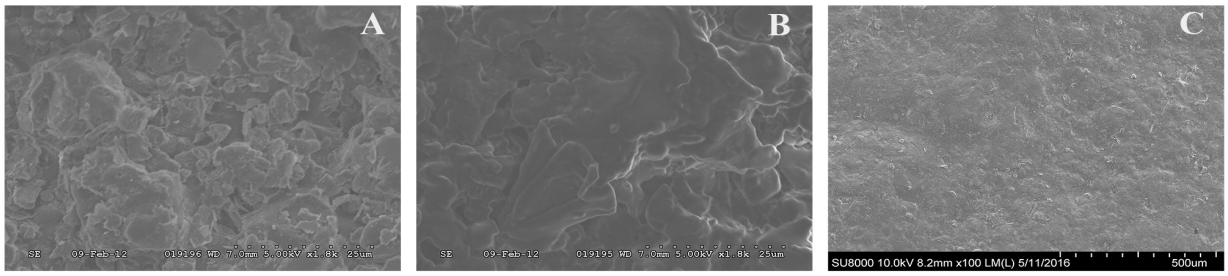

Figure 2. SEM micrographs of vancomycin-coated sample surface curing at 60°C for different time intervals. (A), After 4 h, the film was partly formed and the boundaries between particles were still obvious. **(B)**, After 8 h, the boundaries became smaller and the film was relatively uniform. **(C)**, SEM image (100×) shows the overall surface morphology of the coating and its compact granular structure of different sizes from 10 to 30 μm.

The film was partly formed after the sample was cured for 4 h and the boundaries between particles were still obvious (Figure 2A). After being cured for 8 h, the boundaries became smaller and the film was relatively uniform (Figure 2B). Another SEM image (100×) shows the overall surface morphology of the coating and its compact granular structure of different sizes from 10 μm to 30 μm (Figure 2C).

3.2. *In vitro and in vivo drug release kinetics of vancomycin*

In vitro drug release from vancomycin-coated samples was observed and the average cumulative amount of drug at 1, 4, 24, 48, 72, 120 and 170 h was 47.077 ± 3.943, 78.167 ± 21.915, 178.301 ± 70.127, 228.992 ± 93.012, 274.898 ± 113.220, 302.003 ± 131.446, and 312.084 ± 137.919 μg respectively. The data were then plotted against time and as is presented in the drug release profile (Figure 3A), the vancomycin-coated samples show a sustained release over 7 days, followed by the elution of 80% of the drug within the first 72 h and the antibiotic release rate gradually declines over time.

During the *in vivo* experiment, all animals tolerated the surgery without death, local infection or other complications. The average vancomycin concentration was measured to be 86.940 ± 22.775, 269.780 ± 58.983, 512.440 ± 45.349, 534.160 ± 93.924, 401.000 ± 113.225, 197.100 ± 125.807, 72.300 ± 32.604, 34.740 ± 15.099, 18.020 ± 7.986, 14.660 ± 7.545, 11.600 ± 6.280, and 10.000 ± 4.288 μg/L at 0.5, 1, 2, 3, 6, 24, 50, 72, 97, 120, 148, and 168 h respectively using LC-MS/MS and then plotted against time (Figure 3B). As is presented in the drug release profile (C-T), the drug concentration was detected at 0.5 h after the implantation of the vancomycin-coated sample and increased rapidly until reaching the peak at 2 h, and then followed by a sustained slow release. The drug concentration could be still detected seven days after the surgery.

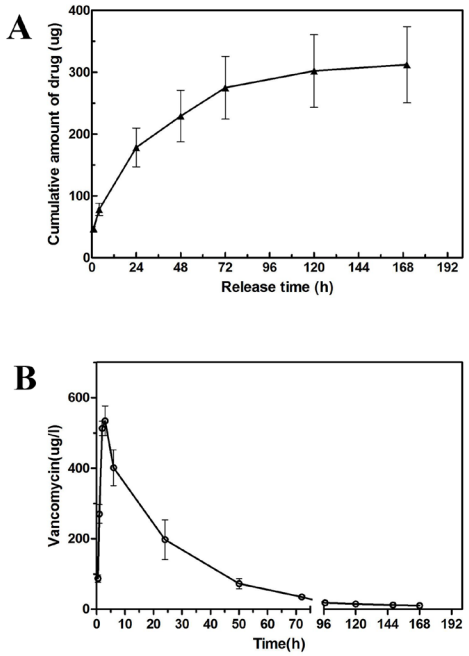

Figure 3. *In vitro* and *in vivo* drug release profile from vancomycin-coated samples. (A), *In vitro* drug release from vancomycin-coated samples were observed and the average cumulative amount of drug at 1, 4, 24, 48, 72, 120, and 170 h were recorded respectively and plotted against time. **(B)**, During the *in vivo* experiment, the average vancomycin concentration was measured at 0.5, 1, 2, 3, 6, 24, 50, 72, 97, 120, 148, and 168 h respectively using LC-MS/MS and then plotted against time (C-T profile).

3.3. *Analysis of in vitro antibacterial ability*

The antibacterial property of the coated sample can be directly visualized through the image of bacterial inhibition zone after 3 days incubation (Figure 4A). Bacterial zone of inhibition depends on the amount of released vancomycin and directly proportionate to the amount of eradicated bacteria. The average diameter of inhibition zone at 3, 6, 9, 12, 15, and 18 d were 16.48 ± 2.74, 10.98 ± 1.14, 6.12 ± 1.47, 4.15 ± 1.19, 2.55 ± 1.15, 0.30 ± 0.27 mm and then plotted against time. As is presented in Figure 4C, the zone of inhibition showed the highest diameter on the third day. Then with the incubation time prolonged, the size of the antibacterial inhibition zone gradually decreased and became nearly undetectable after 18 days. As was expected, the

uncoated sample did not generate the zone of inhibition under the same conditions (Figure 4B).

The MRSA adhesion was examined by the spread plate method, with the number of viable bacteria expressed relative to that of bacteria grown on uncoated Ti6Al4V. As is showed in Figure 4D, the uncoated sample had no effect on MRSA adhesion after 48 h and abundant bacteria had grown all over the plate. However, no bacterial colony was observed on the coated sample (Figure 4E), indicating that the vancomycin-coated Ti6Al4V has an effective antibacterial ability against MRSA.

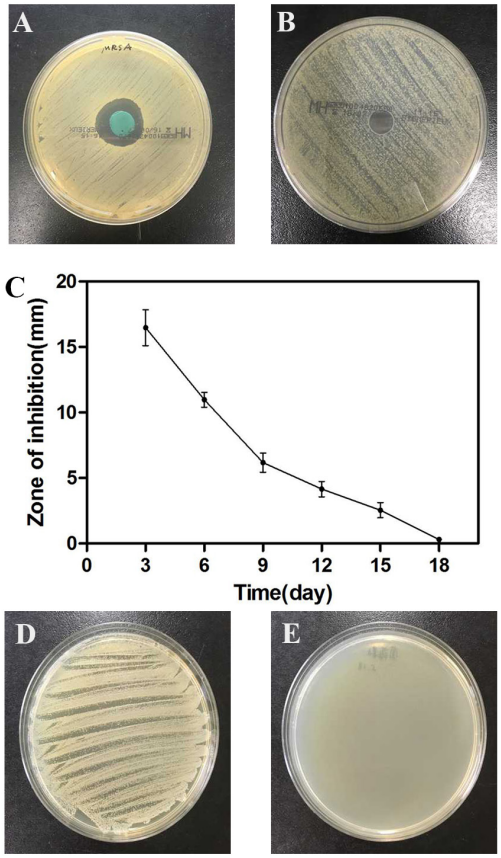

Figure 4. Antibacterial tests of vancomycin-coated samples. **(A)**, Bacterial zone of inhibition was shown by the amount of released vancomycin from coated sample and directly proportional to the amount of eradicated bacteria. **(B)**, The uncoated sample did not generate the zone of inhibition under the same conditions. **(C)**, The ZOI diameters of coated samples at different times were measured and plotted against time. **(D)**, In the colony-counting test by spread plate method, the uncoated sample showed no effect on MRSA adhesion after 48 h and abundant bacteria had grown all over the plate. **(E)**, No bacterial colony was observed on the coated sample.

3.4. *Cytotoxicity test and cytological changes of MG-63*

The cyto-compatibility of coated and uncoated samples were evaluated with the CCK-8 assay using MG-63 as a model. The OD of MG-63 cells in coated and uncoated groups at different times as measured by after the CCK-8 assay were presented in Table 3, and then plotted against time (Figure 5A-C), suggesting the proliferation behaviors of MG-63 cells grown in the leaching solution (2, 5, 8 days) of both coated and uncoated samples. A twofold increase in absorbance from day 1 to day 3 was recorded, which indicated cell viability with both coated and uncoated samples. Both numbers then decreased after that. The proliferation behaviors of MG-63 cells in both coated and uncoated groups were very similar to each other in every measurement point and no significant statistical differences of OD were found between two groups ($p > 0.05$).

The cells in both groups were clustered, confluent, multi-layered, mature, clearly fusiform with increased volume, showed similar distribution, well spreading and cell-cell contact by inverted microscope after a 3 days cultivation (Figure 5D), indicating that the vancomycin-coated Ti6Al4V presented no obvious cytotoxicity.

4. Discussion

The implant-related infection, which often leads to prolonged patient pain, functional losses and causes huge treatment costs, is a catastrophic complication of orthopedic surgery. In the past forty years, a new method of local drug delivery system (LDDS) has been developed based on a series of original inorganic materials and organic-polymers with fine biocompatibility. Compared with traditional systemic drug delivery, it can directly raise the local antibiotic concentration, allow greater control over toxicity of dose and reduce the risk of promoting antibiotic resistance.

A series of challenges are involved when it comes to the developing process of the antibacterial coating

Table 3. OD of MG-63 cells in coated and uncoated groups at different times with the CCK-8 assay

Items	Vancomycin-coated group			Uncoated group		
	2 days LS*	5 days LS	8 days LS	2 days LS	5 days LS	8 days LS
0.5 day	0.489 ± 0.048	0.573 ± 0.049	0.555 ± 0.038	0.448 ± 0.020	0.472 ± 0.043	0.455 ± 0.038
1 day	0.517 ± 0.076	0.567 ± 0.024	0.547 ± 0.072	0.474 ± 0.058	0.496 ± 0.051	0.481 ± 0.026
3 days	1.174 ± 0.211	1.168 ± 0.216	1.140 ± 0.156	1.140 ± 0.302	1.057 ± 0.194	1.123 ± 0.093
6 days	1.076 ± 0.237	0.822 ± 0.107	0.721 ± 0.138	1.090 ± 0.106	0.771 ± 0.166	0.743 ± 0.200
8 days	0.479 ± 0.036	0.609 ± 0.105	0.427 ± 0.041	0.465 ± 0.085	0.499 ± 0.118	0.497 ± 0.081

*LS, leaching solution.

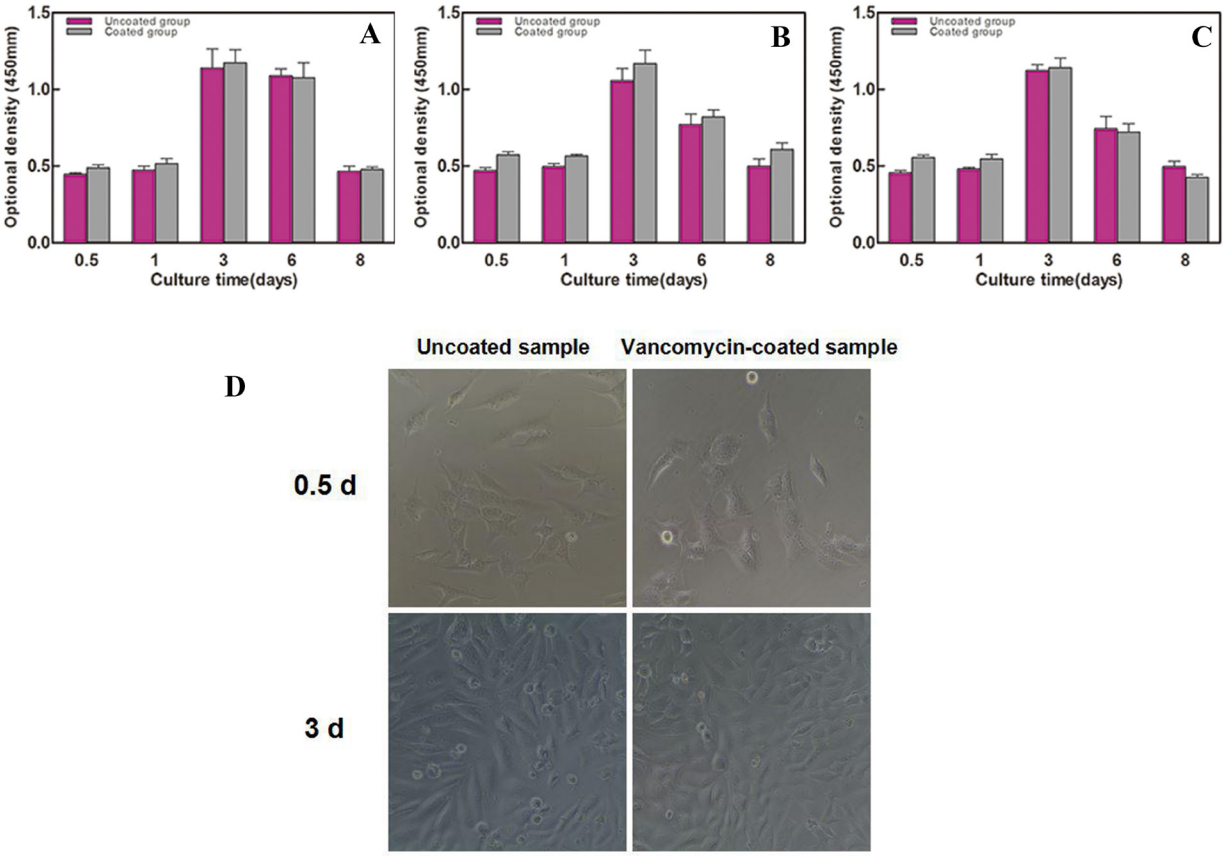

Figure 5. Proliferation behaviors of MG-63 cells. The OD of MG-63 cells in **(A)**, 2 days, **(B)**, 5 days, **(C)**, 8 days leaching solution of coated and uncoated groups at different times as measured by after the CCK-8 assay were plotted against time. **(D)**, Morphological changes of MG-63 cells cultured in 5 days leaching solution of coated and uncoated sample for 0.5 d and 3 d were observed by inverted microscopy.

for metals. For example, the antibiotics usually would no longer stay active when experiencing dramatic changes of the environmental temperature or their own physicochemical properties. Also, an effective binding pathway or interface between antibacterial agents and the implants remains to be found. Furthermore, the whole coating process of the antibacterial coating should be carried out on an environmental and economical base.

To overcome those challenges, efforts have been made throughout the world. In some researches, the titanium alloy was simply immersed in antibacterial solution and then dried for subsequent use, which is effective and easy to operate. But the relatively long processing time, less drug loading, burst release of drug and short maintenance time limit the application of this method. It is also reported that huge progress has been made in antibacterial property and osteointegration of titanium alloy through nanoscale surface patterning methods (*9-11*) or subsequently in conjunction with some antibacterial ions like silver ion (*12,13*). Nevertheless, disadvantages still exist in many aspects of this surface modification method such as the narrow antibacterial spectrum and inadequate maintenance of effective antibiotic concentration. Additionally, it should be cautioned that the dendritic cells and macrophages activated by nanotechnologies can also lead to unintended inflammation which is definitely harmful (*14*). Meanwhile, the mechanical property of titanium nanocoatings is also a concern since damages may occur during surgical implantation. Some other researchers tried to modify the implant's surface by grafting bioactive molecules like protein or polypeptide, together with antibacterial agents onto them (*15,16*). It's not yet well accepted because of the unstable antibacterial properties after a multiple organic processing and the harsh requirements for preservation.

The trends toward "green" manufacturing and cost effectiveness have further spurred development of solvent-free coating techniques in both pharmaceutical and medical products. Some coating processes like hot-melting coating (*17*), compression coating (*18,19*) and dry powder coating (*20,21*) have been investigated and widely used within the paint and automobile industries.

In the last few years, a low-temperature electrostatic dry powder coating process has been developed and successfully applied to make a sustained release coating of tablets (*8*). On the basis of that, we made a series of preliminary experiments, and first applied this electrostatic dry powder coating technology to

coat vancomycin particles mixed with control release coating materials onto the surface of titanium alloy. Different from the traditional dry powder coating technology, it forms an electrical field created by an electrostatic charging gun and grounded substrate to direct the charged powder flow and assist charged powder particle deposition. Also the repulsive force among the charged particles can cause a better distribution of deposited particles so that a more uniform coating film is achieved. Massive production can be done and no complicated physicochemical treatment is needed during the coating process. Besides, certain amount of liquid plasticizer is sprayed onto the surface of the implants through a liquid atomizing nozzle before the coating process, which greatly increases the surface electrical conductivity and reduces the transition temperature (Tg) of the coating polymers, thus facilitating the coalescence of charged polymeric particles into film and ensuring the whole coating process is done at a relatively low temperature (30-60°C). Since the vancomycin powder, usually mixed with bone cement, is commonly taken as an effective antibiotic in a LDDS, it can tolerate the high temperature (70-100°C) during the polymerizing process of PMMA and is absolutely applicable for the antibacterial coating of titanium alloy. Also, due to the solvent-free and low-temperature coating process, there will be no effect on the physicochemical property of coating particles and no acid metabolites degraded by polymers.

An ideal drug delivery system, especially an antibacterial coating in orthopedics, should be first biocompatible and exhibit initially high release rates (*i.e.* burst release) to counter increased infection risk immediately following surgery, followed by an extended period of sustained drug release conforming to therapeutic efficacious dose to prevent latent infection (*22*). The *in vitro* biocompatibilities of coated and uncoated samples were evaluated with the CCK-8 assay using MG-63 as a model. The cytological changes and proliferation behaviors of viable MG-63 cells showed that both coated and uncoated group had almost the same cells growth trend ($p > 0.05$) (Figure 5A). From our results, we also found that the number of viable cells was slightly higher in coated group than uncoated group for the most of time. We hypothesized that maybe it was the relatively high concentration of metal ions in uncoated group that inhibited the cell growth. In contrast, the leaching solution of vancomycin-coated group might not only provide a relatively sterile environment for cells, but also slow down the release of toxic metal ions from the implant itself. Further study is needed to determine the exact causes. Therefore, the data suggested that the vancomycin-coated Ti6Al4V had no inhibitory effect on the proliferation of MG-63 cells than uncoated one and proved its good biocompatibility.

As is presented in the *in vitro* drug release profile (Figure 3A), the vancomycin-coated sample shows an elution of 80% of the drug within the first 72 h, then followed by a sustained release over 7 days and the antibiotic release rate gradually declines over time, which directly indicates its property of *in vitro* sustained drug release. On the other hand, after the vancomycin-coated sample was buried inside the rats and went through the process of drug absorption, distribution and metabolism, the drug concentration was detected at 0.5 h and increased rapidly until reaching the peak at 2 h, and then followed by a sustained slow release (Figure 3B), which in another way indicates its property of *in vivo* sustained drug release. Therefore, from the point of drug release kinetics, the sustained release vancomycin-coated sample using a novel electrostatic dry powder coating process meets the conditions required for an ideal orthopedic implant.

The *in vitro* antibacterial property of the vancomycin-coated sample can be directly visualized through the bacterial inhibition zone and the colony-counting tests. As is presented in Figure 4C, the zone of inhibition showed the highest diameter at the third day. Then with the incubation time prolonged, the size of the antibacterial inhibition zone gradually decreased and became undetectable after 18 days. This result also supported the drug release profiles (Figure 3). It could be found that the existence time of bacterial inhibition zone (18 d) lagged behind the time of *in vitro* drug release (7 d). That was because the viscosity of agar is much greater than the PBS solution, which might hinder the drug release and extend the release time. During the colony-counting test, no bacterial colony was observed on coated sample (Figure 4E), indicating that the vancomycin-coated Ti6Al4V has effective antibacterial ability of MRSA.

5. Conclusion

In this study, a sustained release antibacterial coating of titanium alloy was produced by using a novel electrostatic dry powder coating process. This coating technique overcomes the limitations associated with some traditional coating techniques and offers many advantages like good antibacterial ability, ideal sustained drug release, low energy consuming and processing temperature, being environmental-friendly and economical, no complicated physicochemical treatment required, no acid metabolites degraded by polymers and availability for massive production. The clinical feasibility of this method awaits the results of further studies.

In conclusion, with good biocompatibility and antibacterial ability, the sustained release antibacterial coating of titanium alloy using a novel electrostatic dry powder coating process will provide a promising candidate for the treatment of orthopedic implant-related infections.

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