Development of Ti-Al intermetallic compounds porous material with reactively synthesizing and its biological application

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Abstract. Bacterial infection is one of the most common problems after orthopedic implant surgery. If not prevented, bacterial infection can result in serious and life threatening conditions such as osteomyelitis. Thus, in order to reduce chances of such serious complication, patients are often subjected to antibiotic drug therapy for 6-8 weeks after initial surgery. The antibiotics are systemically delivered either intravenously, intramuscularly or topically. Systemic antibiotic delivery entails certain drawbacks such as systemic toxicity and limited bioavailability. Further, in order for the drug to be effective at the site of implantation, high doses are required, which can result in undesired side effects in patients. Thus, local antibiotic therapy is the preferred way of administering drugs. To that end, we have developed Ti-Al intermetallic compounds porous material for local delivery of antibiotics off-implant at the site of implantation. The Ti-Al intermetallic compounds porous materials were fabricated with reactively synthesizing. The fabrication strategies allow us to precisely control the dimension of aperture, thus enabling us to load different amounts of drugs and control the release rates. In this work we have fabricated Ti-Al intermetallic compounds porous material with 40 ~ 200 nm and 2 ~ 5 μm apertures. We have loaded these tubes with 180, 360 and 540 μg of penicillin G. The penicillin G release kinetics from the apertures and its effect on *Staphylococcus aureus ssp. anaerobius* (S. *aureus*) adhesion were investigated. Further, an osteosarcoma cell line called MG-63 was cultured on penicillin G-loaded aperture to evaluate the effect of Ti-Al intermetallic compounds porous material on cell functionality. Our results indicate that we can effectively fill the apertures with the drug and the drug eluting apertures significantly reduced bacterial adhesion on the surface. Also, there is enhanced MG-63 differentiation on apertures filled with penicillin G.

Introduction

The number of patients in need of an internal fixation device or artificial joint in the United States has grown rapidly in last decade. It is expected that by 2010 more than 4.4 million people will have at least one internal fixation device and more than 1.3 million people will have an artificial joint. The success of these implants depends not only on the bone–implant integration, but also on the presence of a sterile environment around the implant, which will prevent bacterial infection. Infection is one of the most serious complications that may arise after orthopedic implant surgery. Acute infection or chronic osteomyelitis develops in as many as 5-33 % of implant surgeries despite the use of strict antiseptic operative procedures employed[1]. Although infection is not a common reason for implant failure, antibiotic treatment is usually prescribed to patients to prevent any complications that may arise after implant surgery. Our initial concern is with *Staphylococcus aureus ssp. Anaerobius* (*S. aureus*), a bacterium responsible for tremendous morbidity and mortality that exists in approximately 25% of humans[2].

In this paper we investigate the ability to control antibiotic release from the Ti-Al intermetallic compounds porous material to prevent bacterial adhesion while maintaining the osseointegrative properties of the nanostructured surface.

Experimental section

Fabrication of Ti-Al intermetallic compounds. Ti-Al porous alloy was prepared through reaction synthesis, Ti, Al element powder and conventional powder metallurgy (P/M) process. Basised on different heating technology during sintering process, the porosity formation mechanism of porous material was divided into two completely different mechanisms. The heating up road could be described as: heating green body slowly to a certain temperature under melting point of Al, and preserving heat adequately, carrying out reaction of Ti and Al through solid phase diffusion, and Ti, Al element powders were vastly consumed but Al keep in solid phase, then enough thickness pre-reaction layer was being made up among grains. During the course of heating, a great lot of Kirkendall pores were formed in alloy because of difference of diffusion speed in Ti Al elements and Kirkendall effect was initiated by partial diffusion of Al. Finally, porous stable phase structure was gained when it heating to high temperature, as it being shown in Fig. 1. The porous materials were prepared by reactively synthesizing technics, whose pore forming mechanism was mostly chemistry reactively mechanism. The pore structure could be strictly control through modulating preparation parameter and preserving similar figures of green body, then taking on technical characteristic of near net forming, having production prospects in biomedicine with Ti-Al's good biocompatibility.

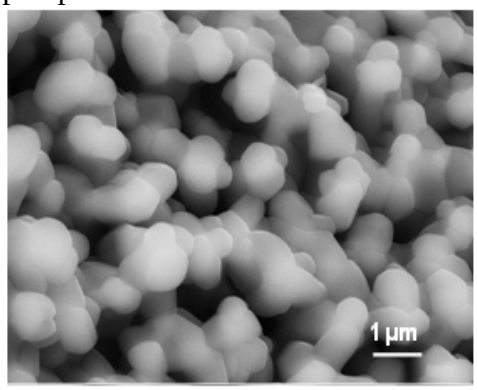

Fig. 1 SEM photos of pore structures of Ti-Al alloy porous membrane by reactively synthesized.

Filling of Ti-Al porous materials. The Ti-Al intermetallic compounds porous materials were filled via a simplified lyophilization method[3]. In brief, 1 200 μg ml^{-1} of solution of penicillin G (North China pharmaceutical Co., LTD) was prepared in PBS. Ti-Al alloy surfaces were cleaned with deionized water prior to loading of the antibiotic. Ten microliter of penicillin G solution was pipetted onto the Ti-Al intermetallic compounds porous material surface and gently spread to ensure even coverage. The surfaces were then allowed to dry in a laminar flow cabinet at room temperature for 2 h. After drying, the loading step was repeated until the appropriate amount of drug was present in the Ti-Al intermetallic compounds porous material. In this way the surfaces were loaded with 180, 360 and 540 μg of penicillin G. After the final drying step, the surfaces were rinsed quickly by pipetting 3 ml of PBS over the surface to remove any excess drug. The rinse solutions were collected and stored for further analysis.

Release from Ti-Al porous materials. In order to release penicillin G from the Ti-Al porous material, the surfaces were immersed in 3 ml of PBS in a 6-well plate at room temperature with orbital shaking at 70 rpm. 2 ml of samples were taken after specific intervals of time to determine the release kinetics. Samples were collected periodically for up to 180 min. The solution was replaced with 2 ml of fresh PBS every time the samples were taken. The samples were analyzed for drug content using a colorimetric assay described elsewhere. The concentrations were adjusted for dilutions due to replacement of fresh PBS. The hydroxylamine hydrochloride reacted with penicillin G and hydroxamic acid ramification products were obtained, whose absorbances were measured at 217 nm. A standard curve with known concentrations of penicillin G was used to determine the unknown concentrations.

Results and discussion

Loading efficiency of Penicillin G in Ti-Al porous material .There are many excellent performances in Ti-Al alloy material. For example: low density, good biocompatibility, high specific strength and high specific stiffness, excellent ant- environment corrosion resistance, good machinability, and so on.

The apertures were loaded with different amounts of penicillin G using the procedure described in the experimental section. Before the release studies were performed, it was important to evaluate the loading efficiency of the drug in the apertures. The concentrations of original and the rinse solutions were measured using the previously described colorimetric assay. The loading efficiency was expressed as percentage of loaded drug after washing:

$$\eta = \frac{\rho_0 - \rho_r}{\rho_0} \quad (1)$$

Where η is loading efficiency, ρ_0 is drug concentration in the original solution, ρ_r is drug concentration in the rinse solution.

Loading efficiencies for apertures loaded with 180, 360 and 540 μg of penicillin G were tested. The results indicate approximately 80-90% of the drug is retained in the apertures after an initial wash.

Fraction of total drug released from Ti-Al porous material. In order to release penicillin G from the Ti-Al porous materials, the surface were immersed in 3 ml of PBS in a 6-well plate at room temperature with orbital shaking at 70 rpm, 2 ml of samples were taken after specific intervals of time to determine the release kinetics. Samples were collected periodically for up to 180 min and the concentration was determined using a colorimetry assay. As the release data obtained from Ti-Al porous materials (40 ~ 200 nm to 2 ~ 5 μm apertures) loaded with 180, 360 and 540 μg of penicillin G, there was slower and sustained release from the Ti-Al porous materials loaded with a higher amount of drug compared to those loaded with lower amounts. The Ti-Al porous materials loaded with 180 μg of penicillin G, all the drug was eluted out within 60 min. Alternately, the Ti-Al porous materials loaded with 360 μg of penicillin G, all the drug was eluted out with 160 min. However, it took almost 180 min for all the drug to elute out of Ti-Al porous materials that were loaded with 540 μg of penicillin G.

Fluorescence microscope images of bacteria. Growth of organisms in direct contact with the biomaterial surface plays a pivotal role in biofilm formation as they link the entire biofilm to the biomaterials surface[4]. Thus, in this work we have investigated bacterial adhesion on Ti-Al porous materials loaded with 540 μg penicillin G (Ti-Al-P) to evaluate the efficacy of eluting drug in preventing bacteria adhesion and subsequent colony and biofilm formation. Staphylococcus epidermis bacteria strain was cultured on different surfaces and stained with Ho. 33342 and propidium iodide. Bacteria cells were then observed using a fluorescence microscope magnified by ×100 under green light irradiation. We had got the fluorescence microscopy images of bacteria colonies formed on Ti, Ti-Al porous materials and Ti-Al-P after 26 h of culture. These images suggest that Ti-Al-P has fewer and smaller bacteria colonies present on the surface compared to Ti and Ti-Al porous materials.

Proliferation and viability of MG-63 on Ti-Al-P. Drug eluting Ti-Al porous material as coatings for implants have a variety of biological applications. However, in this study we have focused on the use of these Ti-Al porous materials as coatings for orthopedic implants. Thus, we have investigated MG-63 adhesion on these surfaces.

Significant MG-63 attachment to a surface is necessary in order for them to spread and differentiate. By determining the initial attachment of MG-63 onto the Ti-Al porous material surfaces, we can examine the correlation of cell attachment and physical and mechanical properties of the scaffold. Contact and interactions between cells will eventually affect the differentiation process. The experiment shows the fluorescence microscope images of MG-63 cells attached to the Ti, Ti-Al and Ti-Al-P films stained with acridine orange when cells had been seeded for 1 h. The viability of MG-63 cells cultured on Ti, Ti-Al and Ti-Al-P films at 24 and 48 h after cell seeding was tested by

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, and the corresponding results are compared. The results were compared to that from Ti and Ti-Al without penicillin G to investigate the effect of drug release on cell adhesion and proliferation. Ideally the drug should not interfere with the cellular processes if releasing at physiologically relevant rates. It can be seen that an approximate 70% increase in the number of cells present on Ti-Al porous material (both Ti-Al and Ti-Al-P) surfaces compared to flat titanium surfaces (Ti) after 1 h of culture. Similarly, after 24 h and 48 h of culture, there were approximately 30% more cells present on Ti-Al porous material (both Ti-Al and Ti-Al-P) surfaces compared to titanium surfaces (Ti) ($p<0.05$).

It can be observed from the experiment that the relative cell growth rate on the Ti-Al film is higher than on Ti, which indicates the excellent cytocompatibility of the Ti-Al film for MG-63 cells. Therefore, the Ti-Al film can promote both the attachment and viability of MG-63 cells and thus results in higher adhesion of MG-63 cells, which is beneficial for implants. This suggests that topographical cues at porous nanostructure promote cell adhesion and proliferation. These results are strongly supported by results obtained by several other researchers with different types of nanostructured surfaces[4-7]. These results suggest that porous surfaces provide a favorable interface for MG-63 differentiation and matrix production. Further, there is no difference in results obtained from Ti-Al porous material with (Ti-Al-P) and without penicillin G (Ti-Al) suggesting that the drug does not negatively affect cellular functionality.

Conclusion

In this work, Ti-Al intermetallic compounds porous material was prepared and applied in biomedicine. The Ti-Al material possesses good stability and excellent biocompatibility, and promotes adhesion and proliferation of MG-63 cells. Therefore, the Ti-Al porous materials can be used as a material for in vitro cell culture and can also be used as the implant materials in biomedicine.

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