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Research paper

Vancomycin release from poly(D,L-lactic acid) spray-coated hydroxyapatite fibers

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ABSTRACT

The influence of the poly(D,L-lactic acid) (PDLLA) coating thickness on the *in vitro* vancomycin release from a hydroxyapatite (HA) carrier was studied. Microporous HA fibers with a porosity of 51 v% and an average pore diameter of 1.0 μm were fabricated by a diffusion-induced phase separation technique. They were loaded with 38 mg vancomycin hydrochloride (VH)/g HA, and their cylindrical shape enabled the application of the spray coating technique for the deposition of uniform PDLLA coating thicknesses, varying from 6.5 μm to 28 μm. The resulting *in vitro* VH release varied from a complete release within 14 days for 6.5 μm coatings to a release of 23% after 28 days for 28 μm coatings. It was clear that the VH release rate from a HA fiber can be adjusted by varying the PDLLA coating thickness. Microbiological tests of these fibers against a methicillin-resistant *Staphylococcus aureus* (MRSA) isolate pointed to the importance of the initial burst release and confirmed that the released antibiotics had the potential to interfere with *S. aureus* biofilm formation.

1. Introduction

Bacterial infection related to orthopedic implants still represents a major complication. Revision procedures are not always effective, and sometimes complete implant removal and replacement is required. These interventions imply a considerable impact in terms of morbidity, mortality, and medical costs [1]. Implant-associated infections are often biofilm related. Many micro-organisms are able to attach to implant surfaces and form a biofilm, which renders them extremely resistant to both the immune system and antimicrobial agents. Conventional systemic delivery of antibiotics often has little effect, because of poor penetration into ischemic and necrotic tissue and can cause systemic toxicity with associated renal and hepatic complications. Alternatively, local delivery of antibiotics from a medical device may enable the maintenance of a high local antibiotic concentration for an extended duration, causing an effect against sessile cells in a biofilm without exceeding systemic toxicity [2].

In evaluating novel orthopedic drug delivery systems, the used antibiotics must pass several tests, qualifying them for that purpose. Few antibiotics have been identified to meet those criteria. Among them, vancomycin seems to be suitable, since it is likely to penetrate the extracellular matrix very rapidly and shows superior bactericidal activity against biofilm-embedded staphylococci and especially methicillin-resistant *Staphylococcus aureus* (MRSA), compared with most other antibiotics. Vancomycin is less cytotoxic than all other commonly used antibiotics and is not likely to cause systemic side effects after local application. It shows very poor tissue penetration, which has been considered a disadvantage in intravenous application. However, this disadvantage turns into an advantage in local application, since there is also reduced penetration from the implanted site into the vascular system, keeping local tissue levels high and systemic levels low [3].

Poly(methyl methacrylate) (PMMA) has been the most widely studied carrier material for numerous antibiotics. However, PMMA enables only a small fraction of the loaded drug to be released and may possibly shelter resistant bacteria, thus causing treatment failure. Furthermore, PMMA is not biodegradable, and secondary surgery is necessary to remove the carrier material [4]. The drawbacks of PMMA stimulated the use of biodegradable carrier materials, including bioceramics, polymers, and ceramic/polymer composites, which eliminate the need for secondary surgery [5]. Calcium phosphate (CaP) ceramics, such as hydroxyapatite (HA), β-tricalcium phosphate (β-TCP), or dicalcium phosphate dihydrate

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(DCPD) have shown to be suitable carrier materials for local drug delivery in orthopedic applications. They exhibit osteoconductive properties, can easily be loaded with drugs, and the release profile can be tailored by the use of biodegradable polymers, such as poly(lactic acid) (PLA) or poly(lactic-*co*-glycolic acid) (PLGA) [6]. Baro et al. [7] demonstrated that a PLA coating delayed the gentamicin release from a HA/β-TCP/PLA composite. Similarly, Gbureck et al. [8] showed that the vancomycin release from microporous DCPD bioceramics can be sustained by dip coating in a PLGA solution.

In general, PLA and PLGA degradation is accelerated by greater hydrophilicity, lesser crystallinity, lower average molecular weight, and smaller size of the finished device. The most important factor influencing the degradation rate is the composition of the polymer, which determines the hydrophilicity [9]. Li [10] reported that PLGA 75/25 (M_w = 111 kDa) exhibited a half-life of 10 weeks, compared with 20 weeks for PLGA 85/15 (M_w = 112 kDa). The glycolic units were hydrolyzed much faster than the lactic units, due to their higher hydrophilicity. Although the degradation of PLA and PLGA has already been studied in detail, the diffusion of antibiotics through such coatings remains unclear. In the present study, PLA was used instead of the copolymer PLGA, in order to minimize the effect of hydrolytic degradation.

The aim of this paper was to study the influence of the poly(D,L-lactic acid) (PDLLA) coating thickness on the *in vitro* vancomycin release from a HA carrier. Therefore, microporous HA fibers were prepared by a diffusion-induced phase separation technique. Their cylindrical shape enabled the application of the spray coating technique to deposit uniform PDLLA coatings of varying thicknesses. After loading with vancomycin and spray coating with PDLLA, the fibers were tested *in vitro* for their release profiles and their resulting antimicrobial activities against *S. aureus*.

2. Materials and methods

2.1. HA fibers

HA fibers were produced by a spinning technique based on diffusion-induced phase separation [11]. This involves the precipitation of a polymer after bringing a polymer solution into contact with a non-solvent. When solvent and non-solvent are miscible, the non-solvent diffuses into the solvent and vice versa and the polymer precipitates. In this way, not only polymeric but also ceramic fibers can be fabricated by suspending ceramic powder into the polymer solution. After diffusion-induced phase separation, the fiber is calcined to remove the polymer and sintered to develop neck growth between the ceramic particles, yielding densification of the structure and associated improvement of the structural integrity. Therefore, HA powder (Merck, Germany) was ball-milled in water and lyophilized. The particle size distribution was determined by laser diffraction in dry mode (LS230, Beckman Coulter, USA). Polysulfone (PSF; Udel, Solvay Advanced Polymers, USA) was dissolved in N-methyl-2-pyrrolidone (NMP; ISP, UK) (25% w/w), and HA was suspended in this solution, resulting in a PSF/HA 1/8 w/w ratio. The suspension was characterized by rheology (RS100, Haake, Germany) and extruded (0.1 ml/s) through a 2.6 mm nozzle. An air gap of 10 mm was applied before reaching the coagulation bath, filled with water to induce phase separation. The fibers were calcined at 600 °C at a heating rate of 10 °C/h and subsequently sintered during 3 h at 1200 °C. The CaP phase was identified by X-ray diffraction (XRD; X'pert, Philips, The Netherlands). The porosity and pore size distribution were measured by mercury porosimetry (Pascal 240, Thermo, USA). The microstructure was analysed by field emission scanning electron microscopy (FESEM; JSM-6340F, Jeol, Japan).

2.2. Drug loading and spray coating

The ceramic fibers were cut to a length of 55 mm for the release test and 45 mm for the microbiological test. They were subjected to vacuum impregnation during 5 min in an aqueous 10% w/v vancomycin hydrochloride (VH; Bufa, The Netherlands) solution. The drug loading was quantified gravimetrically after drying in air for 24 h. In the production of drug-eluting stents, the spray coating method has shown to deposit more uniform coatings than the dip coating method [12]. The cylindrical shape of the HA fibers enabled the application of the spray coating technique. Therefore, the drug-loaded fibers were taped over a length of 15 mm and clamped into a hydraulic-controlled turning lathe (125/600 U, Kellenberger, Switzerland). They were spray-coated for 10, 20, 30 or 40 translations with a 2% w/v solution of PDLLA (M_w = 15–20 kDa; η_{inh} = 0.20 dl/g) (Purasorb PDL 02, Purac Biomaterials, The Netherlands) in dichloromethane, using an airbrush (175-7, Badger Air-Brush, USA). The spray regulator was set to minimum flow rate. The operating air pressure was 0.7 bar, the distance between nozzle and fiber 65 mm, the translation speed 1.5 m/min, and the rotation speed 500 rpm. After drying in air for 24 h, the fibers were cut to a length of 40 mm for the release test and 30 mm for the microbiological test. The ends were sealed by dipping in a 10% w/v solution of ethylcellulose (EC; 10 cP in 5% w/v toluene/ethanol v/v, Acros Organics, Belgium) in dichloromethane with 0.1% w/v dibutyl sebacate (DBS; Acros Organics, Belgium) as plasticizer. The average PDLLA coating thickness, measured by FESEM (JSM-6340F, Jeol, Japan), was calculated from six values for each type of fiber.

2.3. Release tests

The VH release from the spray-coated samples was assayed in triplicate under sink conditions. The samples were immersed in 20 ml phosphate-buffered saline (PBS) (pH 7.4, Sigma–Aldrich, USA) and shaken horizontally in a shaking water bath (OLS200, Grant, UK) at 37 °C and 50 rpm. At 1, 2, 3, 7, 14, 21, and 28 days, the fibers were replaced in 20 ml fresh PBS. The VH concentrations were determined spectrophotometrically by measuring the absorbance at 280.1 nm (Lambda 900, PerkinElmer, USA). The release profiles were recorded for each type of fiber, and the initial VH release rate during the first 3 days of immersion was plotted as a function of PDLLA coating thickness.

2.4. Microbiological tests

S. aureus strain Mu50 (MRSA) was grown on Tryptic Soy Agar (TSA; Oxoid, Belgium) at 37 °C. Biofilms were formed on fibers in 6-well microtiter plates (MTP; TPP, Switzerland). To this end, loaded and unloaded fibers were placed in 4 ml MRSA suspensions with a density of approximately 10^6 colony-forming units (CFU)/ml Tryptic Soy Broth (TSB; Oxoid, Belgium) for 1 h. Subsequently, fibers were transferred to 4 ml TSB and were incubated at 37 °C for an additional 24 or 48 h. The same approach was used for fibers that were incubated for 7 days, but growth medium was replaced every 48 h. To quantify the biofilm formation, each fiber was transferred to test tubes with 10 ml aqueous 0.9% w/v NaCl solution and the tubes were subjected three times to 30 s of sonication (Bransonic 3510, 42 kHz, 100 W, Branson Ultrasonics, USA) and 30 s of vortex mixing to detach the biofilm from the fibers. Using this procedure, all cells were removed from the fibers and clumps of cells were broken apart. Sessile *S. aureus* cells were plated on TSA and incubated at 37 °C for 48 h. Finally, the number of CFU per fiber was calculated by counting colonies on the plates. All experiments were carried out on at least three fibers for each composition. Statistical analysis on the log-transformed data (one-sided *t*-test) was performed using SPSS 15.0 software (SPSS, USA).

3. Results and discussion

3.1. HA fibers

The particle size distribution of the ball-milled and lyophilized HA powder showed a d_{10}, d_{50}, and d_{90} of 0.3 μm, 1.8 μm, and 4.8 μm, respectively. The HA suspension exhibited a shear-thinning behaviour with a viscosity of 28 Pa s at a shear rate of 10 s^{-1}. After sintering, fibers showed a length shrinkage of 8% and a diameter of 1.3 mm. The XRD diffractogram of a sintered fiber identified the CaP phase as HA (Fig. 1). Mercury porosimetry of sintered fibers measured a porosity of 51 v% and an average pore diameter of 1.0 μm. FESEM analysis of the surface (Fig. 2A–C) and the cross section (Fig. 2D–F) of a sintered fiber revealed a homogeneously porous microstructure.

3.2. Drug loading and spray coating

After impregnation and drying, the fibers contained 38 ± 2 mg VH/g HA [average ± standard deviation (SD)], in agreement with the expected value, based on the pore volume of the fibers and the concentration of the impregnated antibiotic solution. After drug loading, the fibers were spray-coated for 10, 20, 30, or 40 translations with PDLLA solution, resulting in average coating thicknesses (±SD) of 6.5 ± 0.5 μm, 14 ± 1 μm, 21 ± 2 μm, or 28 ± 3 μm, respectively (Fig. 3). Uniform coatings of varying thicknesses were successfully deposited by the use of the spray coating technique.

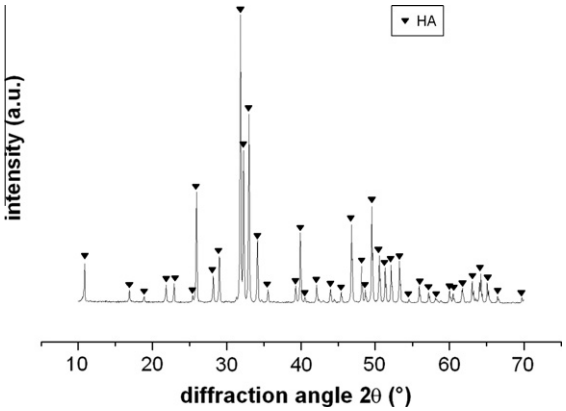

Fig. 1. XRD diffractogram of a sintered hydroxyapatite fiber.

3.3. Release tests

The uncoated fibers showed a complete VH release within the first day of immersion (Fig. 4). This indicates that VH was only physically adsorbed on the HA surface, and no strong chemical interactions occurred. The initial burst release was clearly reduced by the PDLLA coatings. After 1 day, the 6.5 μm and 14 μm coated fibers showed a VH release of only 13% and 9%, and their release was completed after 14 days and 28 days, respectively. The 21 μm and 28 μm coated fibers exhibited after 28 days a VH release of 71% and 23%, respectively. Unlike the release profiles of VH from PLGA-dip coated bioceramics, in which an initial burst release of 40% was observed within the first 2 days [8], the initial burst was lower for the spray-coated fibers. The spray coating technique allowed to seal the surface more effectively and to deposit uniform coatings to study the effect of the coating thickness on the release kinetics. The initial VH release rate showed a linear relationship with the PDLLA coating thickness (Fig. 5). This clearly indicated that the VH release rate from a HA fiber can be adjusted by varying the PDLLA coating thickness.

The sustained release of VH from this reservoir system can be based on three phenomena, namely (a) diffusion through the coating, (b) solubilization, and then diffusion via connected channels in the coating, and (c) liberation because of the degradation of the coating [13]. The VH release from the PDLLA-coated fibers can be expected to be mainly diffusion-controlled during the first days, since the full degradation of PDLLA microspheres with a similar M_w of 17 kDa lasts 53 days [14]. From day 12 until day 48, a polymer degradation phase was observed in the release profile of VH from PLGA-coated bioceramics [8]. In the present study, PLA was used instead of the copolymer PLGA, in order to minimize the effect of hydrolytic degradation. Although no degradation phase with an accelerated VH release could be detected in the release profiles (Fig. 4), from a certain moment, the polymer degradation can have influenced the VH release.

The effectiveness of antibiotic delivery systems for the local prevention of bacterial infections related to orthopedic implants is strongly dependent on the drug release profile [2,5,15]. If the drug is released quickly, the entire dose could be released before the infection is suppressed. On the other hand, if the release is delayed, the infection can develop further. Moreover, the release of antibiotics at levels below the minimum inhibitory concentration (MIC) can evoke bacterial resistance at the release site and intensify infectious complications. Immediately after implantation, the device is susceptible to surface colonization, and certain species of adhered bacteria are capable of forming a biofilm at the im-

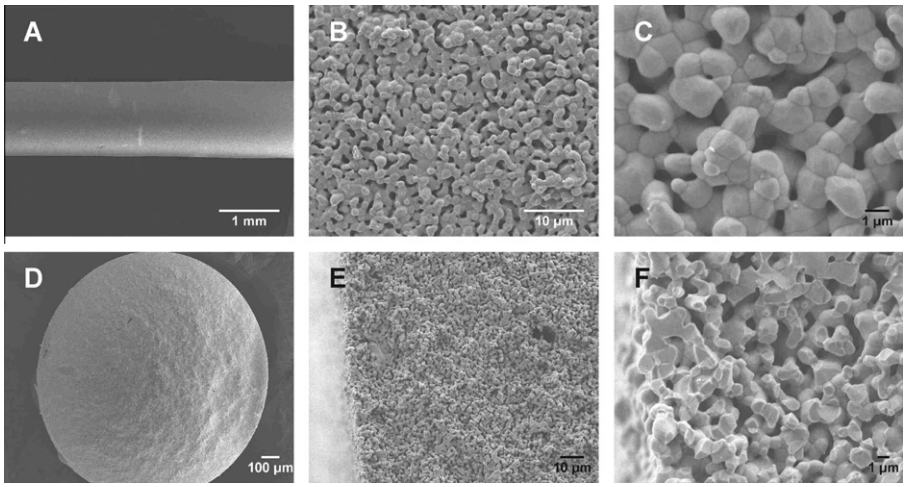

Fig. 2. (A–C) Surface and (D–F) cross section FESEM images of a sintered hydroxyapatite fiber.

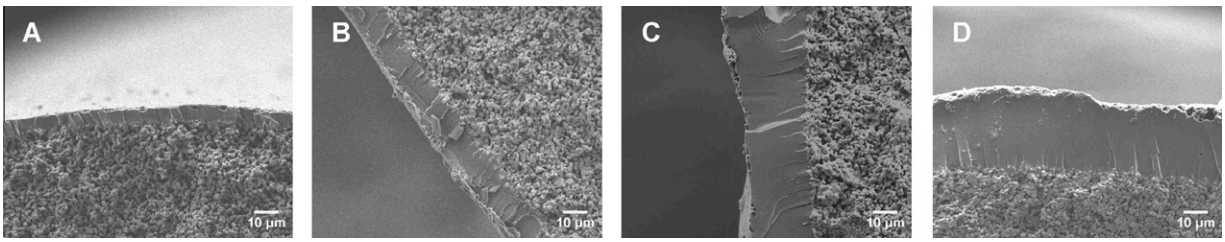

Fig. 3. Cross section FESEM images of hydroxyapatite fibers with (A) 6.5 µm; (B) 14 µm; (C) 21 µm; (D) 28 µm poly(D,L-lactic acid) spray coatings.

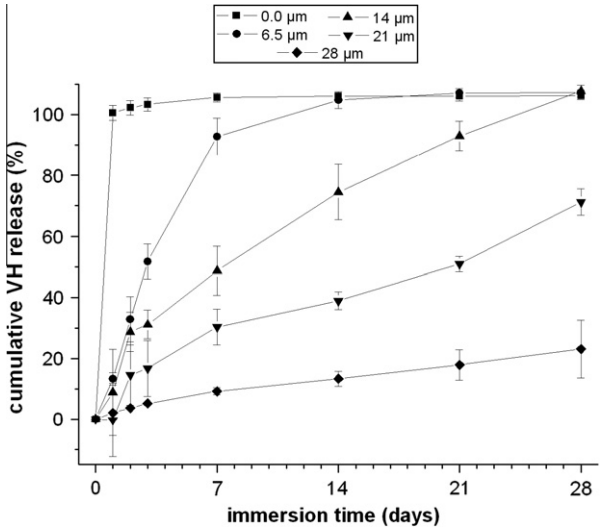

Fig. 4. Vancomycin release from poly(D,L-lactic acid) spray-coated hydroxyapatite fibers with varying coating thicknesses. Error bars represent standard deviation.

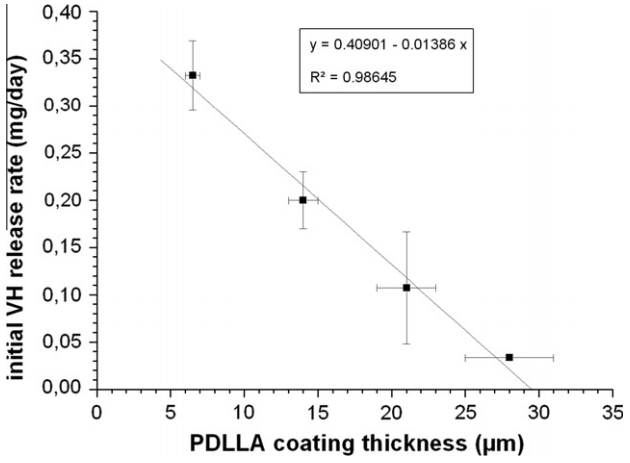

Fig. 5. Initial vancomycin release rate as a function of poly(D,L-lactic acid) coating thickness. Error bars represent standard deviation.

plant-tissue interface. Therefore, the local antibiotic release profile should exhibit a high initial release rate, in order to respond to the elevated risk of infection from bacteria introduced during implantation, followed by a second phase with a sustained release at an effective level for inhibiting the occurrence of a latent infection from systemically introduced bacteria. To prevent or treat bone infections, the initial burst release should be followed by a sustained release for 4–6 weeks [7,16,17]. For VH, this optimal release profile could be achieved by the combination of fibers without coating exhibiting an initial burst release within the first day and fibers with a 14 µm PDLLA coating, providing a sustained release,

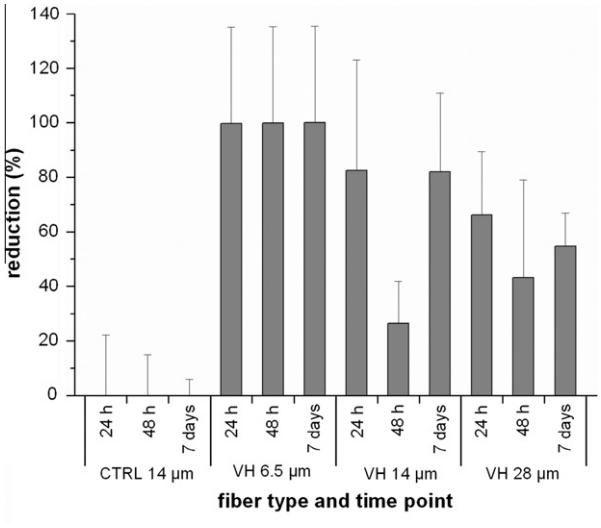

Fig. 6. Reduction in number of culturable cells retrieved from loaded fibers (VH) compared to unloaded control fibers (CTRL). Error bars represent standard error.

which is completed after 4 weeks. In this way, a slow residual release of VH in suboptimal concentrations could be prevented.

There are a number of unknown factors, such as perfusion, which make it difficult to predict the effective *in vivo* release behaviour. Baro et al. [7] demonstrated a faster gentamicin release from a PLA-coated HA/β-TCP/PLA composite *in vivo* than *in vitro*. This was attributed to a greater degree of PLA matrix and coating degradation and to the vascular network formed inside the implant.

When considering the use of PDLLA-coated HA fibers in load-bearing orthopedic applications, attention should be paid to the mechanical strength of the implant. If the integrity of the drug delivery system got affected, this would highly influence the release profile. PDLLA coatings on metallic implants have demonstrated satisfactory wear and adhesion properties [18,19]. On the other hand, the HA fibers cannot be applied as load-bearing implant materials, due to the intrinsic brittleness of ceramics [20]. For this kind of application, the PDLLA-coated HA fibers should be caged into porous metallic implants.

3.4. Microbiological tests

Unloaded 14 µm spray-coated HA fibers served as control. After 24 h, these fibers contained on average 7.4×10^7 CFU/fiber. This number increased with prolonged incubation (9.6×10^7 CFU after 48 h and 1.2×10^8 CFU after 7 days). Use of fibers loaded with VH resulted in a considerable reduction in number of culturable cells retrieved from the fibers (Fig. 6). Reductions were most pronounced for the 6.5 µm coated fibers (reductions >99.99% for all time points). This points to the importance of the initial burst release from these fibers (Fig. 4), although it should be noted that the reductions showed high variation (Fig. 6).

For the 14 μm coated fibers, considerable reductions (82%) were observed for 24 h and 7 days, but at 48 h only a reduction of 26% was observed. Similarly, also for the 28 μm coated fibers, reductions were most pronounced after 24 h and 7 days (66% and 55%, respectively) and somewhat lower after 48 h (43%). These different reductions between the different time points of the 14 μm and 28 μm coated fibers were not statistically significant ($p > 0.01$). Nevertheless, reductions were statistically significant ($p < 0.05$) after 7 days for all fibers investigated. These data confirm that released antibiotics had the potential to interfere with *S. aureus* biofilm formation.

4. Conclusions

Microporous HA fibers allow a sufficient loading with VH, and their cylindrical shape enables the application of the spray coating technique to deposit uniform PDLLA coatings of varying thicknesses. The release profile is controlled by the coating thickness, and the released antibiotics have the potential to interfere with *S. aureus* biofilm formation. An initial burst release is essential in reducing the number of culturable cells. In addition, thicker coatings are necessary to provide a sustained release at an effective level for inhibiting the occurrence of a latent infection.

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