ACS APPLIED MATERIALS & INTERFACES

Research Article

www.acsami.org

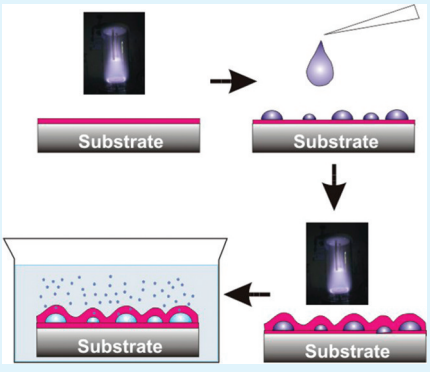
Controlled Release of Levofloxacin Sandwiched between Two Plasma Polymerized Layers on a Solid Carrier

Krasimir Vasilev,*[,†] Neil Poulter,[†,‡] Petr Martinek,[‡] and Hans J Griesser[‡]

[†]Mawson Institute and [‡]Ian Wark Research Institute, University of South Australia, Mawson Lakes, SA 5095, Adelaide, Australia

Supporting Information

ABSTRACT: Targeted delivery and controlled local release of drugs has a number of advantages over conventional systemic drug delivery approaches. Novel platforms for local delivery from solid drug carriers are needed to satisfy the requirements of various medical applications, in particular for the incorporation and release of hydrophilic drugs from a solid carrier material. We have utilized the plasma polymerization of n-heptylamine for the generation of two thin coated layers that serve two distinct purposes. First, an n-heptylamine plasma polymer layer is applied onto the surface of the solid carrier material in order to facilitate spreading of the drug, which is applied by solvent casting; levofloxacin in ethanol was used for this study. A second n-heptylamine plasma polymer coating then serves as a thin barrier coating to control the release. We show that the rate of release can be adjusted via the thickness of the plasma polymer overlayer. We also show that this modality of controlled release of levofloxacin completely inhibits Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization and biofilm formation on and near the coated biomaterial surface.



KEYWORDS: *drug delivery, controlled release, plasma polymerization, n-heptylamine, levofloxacin, Staphylococcus aureus, biofilm, antibacterial surface*

INTRODUCTION

The search for novel delivery platforms for the controlled release of pharmaceuticals has been a topic of extensive research efforts since the 1960s.[1−5] Targeted delivery and controlled local release of drugs locally has a number of advantages over conventional systemic drug delivery approaches. Examples are lower toxicity due to smaller doses, higher efficiency because of controlled kinetics of release, high selectivity, delivery of poorly soluble drugs, and others. Because of these benefits, there is a great deal of research focused on developing novel, more efficient drug delivery systems.[6−10] One area of interest is the delivery of drugs from solid support carriers, which can, for example, be implanted to achieve extended localized delivery. A strategy to achieve that is the development of drug-containing coatings that can be applied onto solid carrier materials, for example biomedical devices such as implants and stents, for spatially well-defined and controlled local release of pharmaceuticals. However, diffusive release from carrier polymeric coatings requires tailoring of several factors such as miscibility and diffusion rate; this entails that each drug/carrier system be designed and optimized individually,

Various platforms for the controlled delivery and release of drugs have been developed, with a particularly relevant new area being nanoporous materials.[8,9,11,12] More established systems are hydrogels and coatings prepared by layer-by-layer deposition (LBL) applied onto solid carrier materials.[5,7,10,13] However, some of such systems are complex, time-consuming, and costly to fabricate. For examples, nanoporous surfaces require prefabrication by electrochemical etching, which can be applied only to a few materials.[11] LBL requires a number of consecutive steps for building a multilayered system of desired thickness, it is limited by the necessity for charged polymers and surfaces, and when weak polyelectrolytes are used, the stability of the system is limited to certain pH and ionic strength ranges.[14]

A platform technology compatible with a wide range of drugs and solid carriers would be valuable for various biomedical applications. Here we report a platform technology that appears suitable for such wide use. In particular it addresses some of the difficulties associated with controlling the delivery of hydrophilic drugs from medical devices such as implants and stents. Such drugs are often poorly soluble/miscible in polymers and coatings used for biomedical devices. On the other hand, placing the bare drug on the surface of a device leads to excessively rapid dissolution and high local dosage.

One potential application of our platform technology is for the release of antibiotics from the surface of an implant or a device such as a catheter. The particular medical problem we tackle in this work is infections associated with medical implants and catheters. Today this problem is addressed by systemic administration of antibiotics, which in many cases

Received: September 27, 2011
Accepted: November 21, 2011
Published: November 21, 2011

does not lead to the desired outcome, and often requires revision surgery. In addition, administration of antibiotics may contribute to bacteria developing antibiotic resistance. A solution to the problem of device-associated infections may lie in an antibiotic release platform applied to the surface of devices.[10,15−17] This alternative method of drug delivery has inspired increasing research efforts over the past decade. Various antibacterial strategies have been proposed including nonfouling surfaces,[18] contact killing coatings,[19] silver nanoparticles based coating,[20] antimicrobial peptides,[21] LbL systems,[22] antibiotics releasing systems, and responsive surfaces.[23]

The danger of infections is greatest in the first six hours after medical intervention because the immune system of the patient is weakened at the site of tissue damage.[15,24,25] Thus, controlled delivery of antibiotics (many of which are hydrophilic molecules) over six hours and perhaps longer (depending on age, individual immune system, etc.) would be highly beneficial.[15] Here we report a facile strategy for the controlled release of hydrophilic antibiotics, which can be applied to the surface of any medical device. The technology is based on plasma polymerization, which enables the deposition of coatings in the nanometer thickness range onto most types of materials surfaces without the need for premodification.[26] In addition, plasma polymerization is a one-step, rather fast coating process and films of various physicochemical properties can be achieved by appropriate choice of precursor and deposition conditions. To control and tailor the release kinetics of drugs placed on the surface of solid carrier supports, we use thin film overlayers prepared by plasma polymerization as controllable barriers for the diffusive release of hydrophilic drug molecules off the solid support surface. Our hypothesis was that plasma polymer films of predetermined thickness may be used to control the release kinetics of antibiotics deposited on a biomedical device surface. This hypothesis originates from earlier research of our group, which demonstrated that when prepared under appropriate conditions, plasma polymer films based on n-heptylamine (HApp) adopt a porous morphology, with the effective diameter of the pores controlled by the conditions of deposition.[27] We used such pores previously for the dissolution of encapsulated gold nanoparticles.[28] The choice of HApp is also supported by its reported biocompatibility in terms of eukaryotic cell adhesion and proliferation.[20,31] However, challenges arising from the organic nature and the size of drug molecules needed to be overcome in this work.

EXPERIMENTAL SECTION

Materials. n-Heptylamine (HA), levofloxacin and phosphate buffered saline tablets (PBS) were purchased from Sigma-Aldrich and used without further purification.

Plasma Polymerization. Plasma polymerization was carried out in a custom-built reactor described elsewhere[29] using a commercial 13.56 MHz plasma generator (Advanced Energy). The depositions were carried out in an atmosphere of pure n-heptylamine at a pressure of 0.2 Torr. An input power setting of 20 W was used in combination with a matching network to minimize reflected power. HApp was deposited onto quartz substrates for drug release studies and onto silicon wafers for ellipsometry thickness determination. The thickness of the coatings was controlled by the time of application of the plasma power. The deposition rate at power input of 20 W and HA pressure of 0.2 Torr is ca. 0.5 nm/s.[27] The static air/water contact angle of these films is ∼70°.

Drug Loading. Levofloxacin was dissolved in ethanol at concentrations of 0.5, 1, 2, and 3 mg/mL. 12.5 μL of the solution was dropped onto quartz slides precoated with HApp and allowed to dry at room temperature. The size of the glass substrates was 1 cm × 2.5 cm and the polystyrene discs (Thermonex) for diffusion assays were 13 mm in diameter.

Drug Release. Quartz slides loaded with levofloxacin were immersed in 10 mL of PBS at 37 °C for a predetermined time period. The absorbance of the sample was measured at a wavelength of 287 nm before and after immersion in PBS using a Cary 5 UV−vis spectrometer. A calibration curve was constructed by measuring the absorbance of levofloxacin dissolved in PBS in the concentration range 25 ng/mL to 50 μg/mL.

Thickness Characterization. A commercial imaging ellipsometer (Beaglehole Instruments, New Zealand) was used to analyze polymer film thickness. The measurements were carried out at a constant wavelength of 600 nm as the angles of incidence and reflected light detection were varied between 40 and 85 degrees. A refractive index of 1.55 was used for fitting for all samples.[27,18] The natural oxide layer on the silicon wafers was measured independently to be 1.57 nm thick, and this was accounted for in the polymer film thickness evaluation.

Bacterial Studies. Overnight cultures of methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC 43300) and methicillin susceptible *Staphylococcus aureus* (MSSA, ATCC 29213) bacteria were centrifuged, washed with tryptic soy broth (TSB) and resuspended in fresh TSB. Resuspended cultures were adjusted to 1 × 10^6 CFU mL^{-1} using predefined optical density calibrations. For the diffusion assay, 100 μL of adjusted bacterial culture was spread onto an agar plate to create a thin film of bacteria. Control and levofloxacin-loaded samples were placed onto the agar plates face-down and incubated at 35 °C for 18 h. After this time, the diameter of the zone of inhibition around the sample where no bacteria had grown was measured. For determining the effectiveness of coated surfaces against bacterial attachment, we applied 200 μL of adjusted bacterial culture to the surface of control and levofloxacin-loaded samples in six-well plates and incubated them at 35 °C for 18 h. The sample surface from which the supernatant had been removed was lightly rinsed with PBS to remove loosely bound material and placed into a 10 mL solution of saline, which was vortexed for 30s to detach surface-bound cells into solution. The resultant solution was serially diluted in saline, the resulting solutions spread onto nutrient agar and grown at 35 °C for 18 h. Appropriate levels of bacterial growth were selected for counting and conversion to colony forming units per milliliter.

RESULTS AND DISCUSSION

The experimental strategy implemented in this work is shown in Scheme 1. For wide applicability of our approach to many materials, first a 20 nm thick layer of HApp is deposited. The role of this layer is to provide defined wetting properties of the surface onto which the drug solution is applied, as on some materials insufficient wetting might cause inhomogeneous spreading. For the medical implant materials titanium and polyethylene for example, drying of the applied drop of drug solution would likely result in different shapes and sizes of the drug particles owing to different capillary forces acting on surfaces with different surface energies. As we will show below, particle size is an important characteristic of the system and plays a role in defining drug release rates. Thus, this first coating step is essential for extending our approach to surfaces of a wide range of biomaterials and devices.

The drug is applied by drop casting from a solution of a given concentration and dried. We selected levofloxacin as a model drug based on the following considerations. Levofloxacin is hydrophilic and a broad spectrum antibiotic of interest for medical devices applications.[21] It has an absorbance maximum at ∼300 nm, which makes it easy to detect spectrophotometrically.

Next, the drug particles formed upon drying are encapsulated on the surface by applying another layer of HApp of

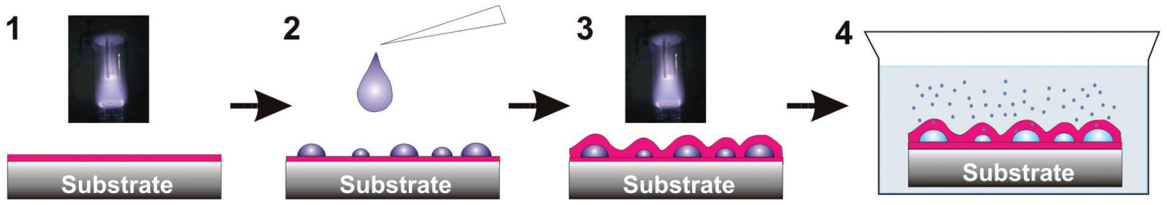


Figure 1. Schematic representation of the experimental strategy. (1) Deposition of an underlayer of HApp; (2) drug loading via dropcasting and drying; (3) deposition of an overlayer of HApp of adjustable thickness; (4) drug release into PBS.

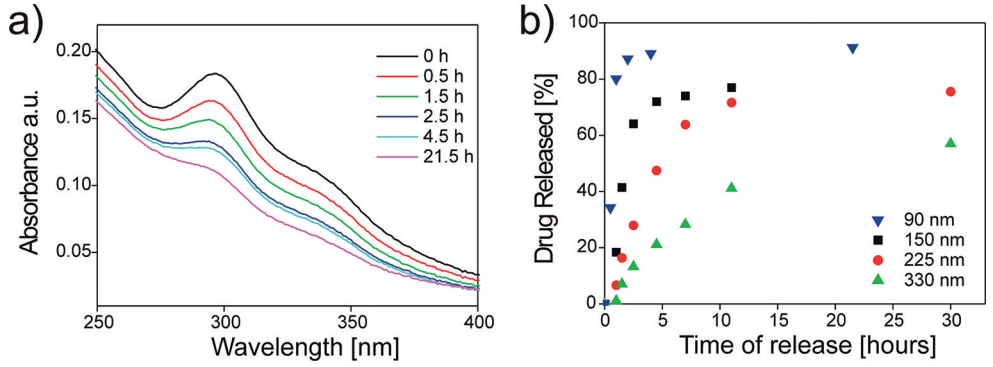


Figure 2. (a) Spectrophotometric detection of release of levofloxacin into PBS from underneath a plasma polymer film of thickness 150 nm for various time points: 0s, 0.5 h, 1.5 h, 2.5 h, 4.5 h and 21.5 h. The amount of loaded drug was 12.5 μg. (b) Release profiles of levofloxacin within 30 h for various thicknesses of the HApp overlayer. The amount of drug loaded was 25 μg.

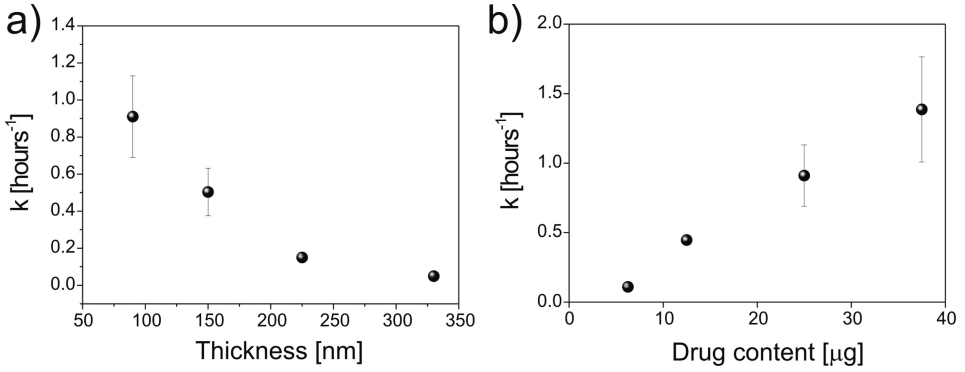


Figure 3. (a) Dependence of the kinetic parameter k on the thickness of the HApp overlayer, determined from the release data. The amount of drug loaded in the coating was 25 μg. (b) Dependence of the kinetic parameter k on the amount of loaded levofloxacin for a polymer overlayer thickness of 90 nm. The error bars were generated as the standard deviation from at least three measurements.

predetermined thickness. Plasma polymer thin films can be prepared with various densities of cross-links and water uptake, which is useful for adjusting the kinetics of diffusion of the drug through the plasma polymer barrier layer. A similar approach of using controllable barriers to the diffusive release of drug molecules off a solid support surface was reported by Susut and Timmons[30] with aspirin crystals, though they did not use a plasma polymer underlayer to facilitate the application of drug solutions onto the solid carrier; this underlayer makes our approach versatile and generically transferable to a wide range of solid carriers. In terms of medical device applications, a particular benefit in using HApp is that this plasma polymer film has been shown to facilitate good adhesion and spreading of mammalian cells.[31] The release of levofloxacin from the system is then studied spectrophotometrically.

An example of the spectrophotometric determination of release of levofloxacin is shown in Figure 2a. The absorbance at 285 nm was used to quantify the amount of levofloxacin in coatings deposited on quartz before and after a given time of immersion in PBS. In the example shown in Figure 2, the thickness of the plasma polymer overlayer was 150 nm and the amount of loaded drug was 12.5 μg. The plasma polymer itself (without levofloxacin) gives rise to a broad, structureless absorption spectrum (not shown) onto which the absorption bands of levofloxacin are superposed. It is worth noting that after encapsulation of the drug by the plasma polymer overlayer we can record the same absorption spectrum with a maximum at 285 nm, which implies that the drug molecules were not damaged during exposure to the plasma environment. Upon immersion in PBS, a decrease in the measured absorbance is evident.

To examine the influence of the HApp overlayer thickness on the rate of release of levofloxacin, we applied overlayers with thicknesses of 90, 150, 225, and 330 nm. Typical release profiles within 30 h for samples loaded with 25 μg of levofloxacin are shown in Figure 2b. With a 90 nm thick overlayer 90% of the drug is released within 5 h, whereas for an overlayer of 330 nm, less than 60% of the drug is released

within 30 h. The absorbance measurements suggest that even for the thinnest coating there is still a small amount of remaining drug at 70 h. These data show that by careful adjustment of the thickness of the HApp overlayer we can tune the release rate of the system. It is common for all release curves that in the first few hours the release is fast, followed by a slower release. Such a release profile is desirable for the release of antibiotics in implant applications.[15] An initial large amount of antibiotic is important to combat bacteria introduced during implantation, followed by sustained release to combat bacteria arriving systemically afterward. These release kinetics data also show that the target release time of 6 h or longer can be achieved. Using thicker coatings, the release can be extended beyond this period, which may be a necessity for elderly patients or patients with a weaker immune system.

Analysis of the release data was conducted in terms of first order kinetics, which gave the best fit to the experimental data. The kinetic parameter k was derived using the following equation: $\ln (L_r/L_0) = -kt$, where the ratio (L_r/L_0) denotes the fraction of levofloxacin remaining in the coating at time t. Figure 3a shows the kinetic parameter k as a function of the thickness of the HApp overlayer. In accord with the data shown in Figure 2b, the release rate decreases with increasing thickness of the overlayer.

The ability to control the amount of loaded drug in the coating is an important feature. To examine the effect of the amount of loaded levofloxacin on the release kinetics, we deposited it from solutions of concentrations of 0.5, 1, 2, and 3 mg/mL, which resulted in total amounts of drug of 6.25, 12.5, 25, and 37.5 μg, as the volume of the casting solution was kept the same. The kinetic parameter k obtained upon analysis of the release data is plotted in Figure 3b as a function of the amount of drug loaded for a thickness of the plasma polymer film of 90 nm. Increasing the loading leads to an increase in k. The dependence appears to be almost linear.

This observation can be rationalized by the manner the plasma polymer coats the drug particles. Optical microscopy images of the surface of samples loaded with different amounts of levofloxacin showed that higher concentrations of drug lead to the formation of larger particles (images and particle size distributions in the Supporting Information). Optical microscopy imaging also revealed that the larger particles dissolved faster, which correlates with the measured release rates (see the Supporting Information). Putatively, when the size of the drug particles is much larger than the thickness of the plasma polymer overlayer, all sides of the drug particles are not coated equally uniformly by the plasma polymer. The plasma polymer coating on the top of the drug particles probably has the same thickness as on a flat surface, but it may be expected that there is a thinner coating on the sides of the particles. Such a scenario may be anticipated taking into account the main plasma species—ions and radicals—that contribute to film deposition. Passing through the plasma sheath over the sample, ions are accelerated and directional, as the substrate has a slightly negative potential compared to the plasma, with the highest probability of landing on top of a drug particle. Radicals, on the other hand, travel randomly and can deposit anywhere including the top and sides of a drug particle. There is no published work on how ions and radicals contribute to HApp film deposition. However, such studies have been carried out for allylamine[23] and it was shown that ions make a significant contribution to film growth. Thus, it is reasonable to expect a thinner coating on the sides of the particles since mainly radicals would contribute to film growth there. A thinner coating on the side will allow the solvent to access more rapidly the drug particles and also cause faster diffusion. For small particles this is less the case. We have previously demonstrated that nanoparticles up to 70 nm can be homogeneously coated and eluted from HApp films.[28]

Ultimately, our aim was to examine the efficiency of this antibiotic release system against *Staphylococcus aureus*. It is well-documented that this bacterium causes the majority of infections associated with biomedical implants.[32] Figure 4

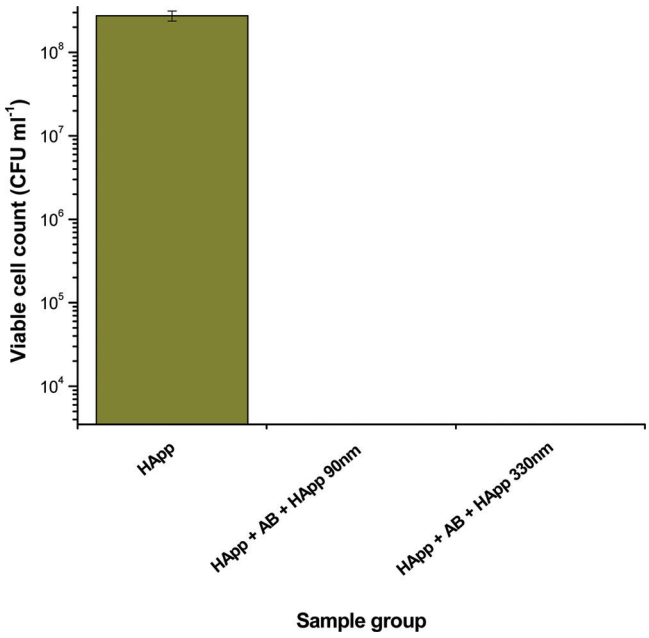


Figure 4. *Staphylococcus aureus* (MSSA) adhesion and biofilm formation over 18 h on control samples and samples loaded with 12.5 μg of Levofloxacin. The overlayer thickness of the HApp overlayer was 90 and 330 nm, respectively. The error bars were generated as the standard deviation from at least three measurements.

shows results (from triplicate testing) using samples with 12.5 μg of levofloxacin and a control without antibiotic over 18 h. Clearly, bacteria readily grow and form biofims on samples coated with HA plasma polymer. In contrast, samples loaded with levofloxacin showed complete inhibition of bacterial growth. This was the case regardless of the thickness of the plasma polymer overlayer; even the slowest release rate was sufficient to prevent bacterial surface colonization and biofilm formation.

Another test aiming to study the bacterial growth in the area surrounding the sample is shown in Figure 5. When the sample is not loaded with levofloxacin (a) a bacterial lawn extends to the edge of the sample. However, when levofloxacin is loaded, there is a clearly visible area around the sample where the growth of bacteria is inhibited. The diameter of the area was measured to be 38±3 mm. Quantitiative evaluation of the inhibition area (Figure 5d) showed no clear dependence on the release rate. The examples in Figure 5b and 5c show samples coated with 90 and 330 nm thick HApp overlayers, which represents the fastest and slowest release rates as a function of the overlayer film thickness. The zone of inhibition of these samples is <15% smaller compared to a sample without an overlayer (Figure 5d). These results suggest that throughout

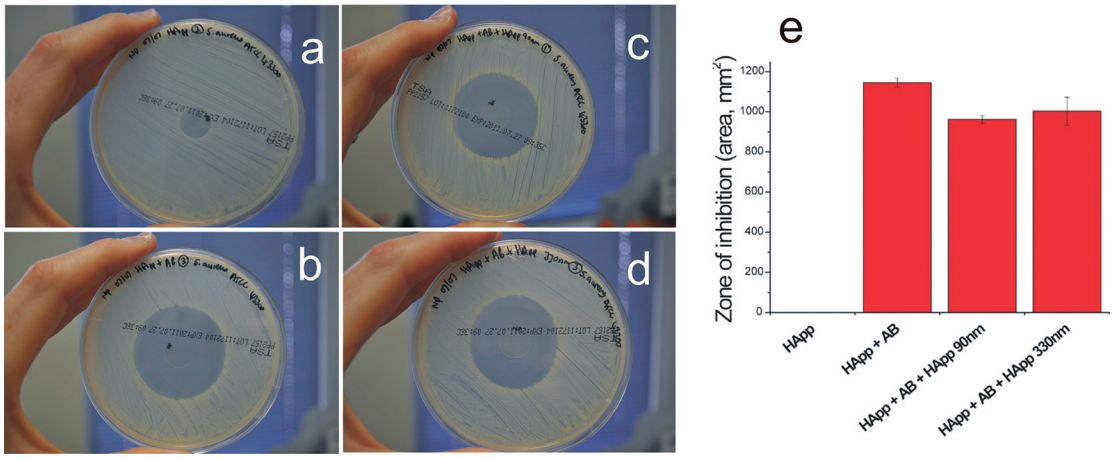


Figure 5. Diffusion assay showing inhibition of *Staphylococcus aureus* (MRSA) growth in the area surrounding samples of diameter 10 mm: (a) control sample (HApp, no levofloxacin); (b) levofloxacin loaded with no overlayer; (b) 90 nm thick HApp overlayer; (d) 330 nm thick overlayer; and (e) quantification of inhibition area.

the entire range of the release kinetics established in this study, there is always sufficient amounts of antibiotic released into the surrounding area for inhibition of bacterial growth.

The antibiotic delivery and release platform developed in this work protects from infection not only the surface of the sample but also the surrounding area, which may be beneficial for combating bacteria located in injured tissue near the implant. This is important in some clinical scenarios because bacteria may be introduced into the site of surgical intervention not only through the implanted device, but they may also already be present, for example, after an accident trauma, or they can contaminate an open wound from other sources such as medical devices used in surgery, clothing, etc. The ability to deliver and release a controlled amount of antibiotic in a targeted manner to a specific body site may solve some significant medical problems. First, it prevents the systemic toxicity of some of the antibiotics to organs such as kidney and liver, which presents a concern in traditional oral delivery. Second, this strategy may solve the problems with antibiotic resistance of MRSA and other pathogens because bacteria are exposed directly to a high local dose of antibiotic before they have time to form a biofilm or undergo genetic mutation necessary to develop resistance. In addition, our antibiotic delivery strategy can be transferable to various biomedical products such as bandages and wound dressings, because it is not limited to a particular substrate material.

CONCLUSIONS

In summary, we present a novel and facile approach for controlled drug release by embedding drug particles in-between two thin film coatings deposited by plasma polymerization. We have demonstrated that the drug release rate can be controlled by the thickness of the plasma polymer overlayer and that the concentration of the drug solution used for loading is important in determining the final release rate. We also show that biofilm development by *Staphylococcus aureus* is inhibited on the surface of such samples and out-diffusion of levofloxacin also creates a substantial inhibition area for bacterial growth in the vicinity of samples. The ability to deposit plasma polymer layers readily on most solids used as biomaterials (highly hydrated hydrogels, however, being challenging) and the flexibility of the approach in terms of a wide range of permeability and hydrophilicity achievable with plasma polymer films of various chemical compositions, are attractive features of our approach, which should lend itself to utilization for a wide range of controlled local release applications off solid carrier materials and nanoparticles.

ASSOCIATED CONTENT

Supporting Information

Optical microscopy images of drug loaded samples before and during release. Drug particles size distribution before and after release. XPS survey spectrum and chemical composition of a typical HApp film. This material is available free of charge via the Internet at http://pubs.acs.org/.

AUTHOR INFORMATION

Corresponding Author

*Tel: +61 8 83025697. Fax: +61 8 83025689. E-mail: krasimir.vasilev@unisa.edu.au.

ACKNOWLEDGMENTS

This work was supported in part by the Australian Government under NHMRC grant 1000737. KV acknowledges the Australian Research Council for support through fellowship FT100100292.

REFERENCES

(1) Hoffman, A. S. *J. Controlled Release* **2008**, *132*, 153.
(2) Husseini, G. A.; Pitt, W. G. *Adv. Drug Delivery Rev.* **2008**, *60*, 1137.
(3) Jewell, C. M.; Lynn, D. M. *Adv. Drug Delivery Rev.* **2008**, *60*, 979.
(4) Uhrich, K. E.; Cannizzaro, S. M.; Langer, R. S.; Shakesheff, K. M. *Chem. Rev.* **1999**, *99*, 3181.
(5) Wu, P.; Grainger, D. W. *Biomaterials* **2006**, *27*, 2450.
(6) Degim, I. T.; Celebi, N. *Curr. Pharm. Des.* **2007**, *13*, 99.
(7) Biondi, M.; Ungaro, F.; Quaglia, F.; Netti, P. A. *Adv. Drug Delivery Rev.* **2008**, *60*, 229.
(8) Colilla, M.; Manzano, M.; Vallet-Regi, M. *Int. J. Nanomed.* **2008**, *3*, 403.
(9) Vallet-Regi, M.; Balas, F.; Arcos, D. *Angew. Chem., Int. Ed.* **2007**, *46*, 7548.
(10) Zilberman, M.; Elsner, J. J. *J. Controlled Release* **2008**, *130*, 202.
(11) Vasilev, K.; Poh, Z.; Kant, K.; Chan, J.; Michelmore, A.; Losic, D. *Biomaterials* **2010**, *31*, 532.
(12) Simovic, S.; Losic, D.; Vasilev, K. *Chem. Commun.* **2010**, *46*, 1317.

(13) Lin, C. C.; Anseth, K. S. *Pharm. Res.* **2009**, *26*, 631.
(14) Lebedeva, O. V.; Kim, B. S.; Vasilev, K.; Vinogradova, O. I. *J. Colloid Interface Sci.* **2005**, *284*, 455.
(15) Hetrick, E. M.; Schoenfisch, M. H. *Chem. Soc. Rev.* **2006**, *35*, 780.
(16) Mack, D.; Rohde, H.; Harris, L. G.; Davies, A. P.; Horstkotte, M. A.; Knobloch, J. K. M. *Int. J. Artif. Organs* **2006**, *29*, 343.
(17) Vandijck, D. M.; Depaemelaere, M.; Labeau, S. O.; Depuydt, P. O.; Annemans, L.; Buyle, F. M.; Oeyen, S.; Colpaert, K. E.; Peeman, R. P.; Blot, S. I.; Decruyenaere, J. A. *Int. J. Antimicrob. Ag.* **2008**, *31*, 161.
(18) Baveja, J. K.; Willcox, M. D. P.; Hume, E. B. H.; Kumar, N.; Odell, R.; Poole-Warren, L. A. *Biomaterials* **2004**, *25*, 5003.
(19) Gottenbos, B.; van der Mei, H. C.; Klatter, F.; Nieuwenhuis, P.; Busscher, H. J. *Biomaterials* **2002**, *23*, 1417.
(20) Vasilev, K.; Sah, V.; Anselme, K.; Ndi, C.; Mateescu, M.; Dollmann, B.; Martinek, P.; Ys, H.; Ploux, L.; Griesser, H. J. *Nano Lett.* **2010**, *10*, 202.
(21) Costa, F.; Carvalho, I. F.; Montelaro, R. C.; Gomes, P.; Martins, M. C. L. *Acta Biomaterialia* **2011**, *7*, 1431.
(22) Wong, S. Y.; Moskowitz, J. S.; Veselinovic, J.; Rosario, R. A.; Timachova, K.; Blaisse, M. R.; Fuller, R. C.; Klibanov, A. M.; Hammond, P. T. *J. Am. Chem. Soc.* **2010**, *132*, 17840.
(23) Zhou, J.; Loftus, A. L.; Mulley, G.; Jenkins, A. T. A. *J. Am. Chem. Soc.* **2010**, *132*, 6566.
(24) Anderson, E. M.; Noble, M. L.; Garty, S.; Ma, H.; Bryers, J. D.; Shen, T. T.; Ratner, B. D. *Biomaterials* **2009**, *30*, 5675.
(25) Busscher, H. J.; Ploeg, R. J.; van der Mei, H. C. *Biomaterials* **2009**, *30*, 4247.
(26) Vasilev, K.; Michelmore, A.; Griesser, H. J.; Short, R. D. *Chem. Commun.* **2009**, 3600.
(27) Vasilev, K.; Britcher, L.; Casanal, A.; Griesser, H. J. *J. Phys. Chem. B* **2008**, *112*, 10915.
(28) Vasilev, K.; Casanal, A.; Challougui, H.; Griesser, H. J. *J. Phys. Chem. B* **2009**, *113*, 7059.
(29) Griesser, H. J. *Vacuum* **1989**, *39*, 485.
(30) Susut, C.; Timmons, R. B. *Int. J. Pharm.* **2005**, *288*, 253.
(31) Griesser, H. J.; Chatelier, R. C.; Gengenbach, T. R.; Johnson, G.; Steele, J. G. *J. Biomater. Sci., Polym. Ed.* **1994**, *5*, 531.
(32) Harris, L. G.; Richards, R. G. *Injury* **2006**, *37*, S3.