ElectroNanospray® to Engineer Nanocomposite Biomedical Coatings with Controlled Drug Release

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INTRODUCTION

The ElectroNanospray™ (ENS) process employs a high voltage electrical field to break the liquid flowing out of a capillary tip into nanoscale charged particles comprised of solvent carrier and active agent(s). The solvent evaporates rapidly from the particles in the spray stream, leaving even smaller but more highly charged particles that deposit on the surface of the oppositely charged or grounded target electrode [1], [2]. This enabling technology platform allows application of polymers and drugs to the surface of medical devices such as coronary stents in a single-stage process. Modification of the ENS process parameters resulted in surface coatings with rich morphologies ranging in appearance from smooth and heterogeneous to highly porous and rough (open matrix). Figure 1 shows an example of smooth film versus open matrix composite coatings generated by ENS.

Drug elution from polymeric coatings is a highly dynamic and complex process [3]. The dynamics of drug release and the evolution of surface morphology during release are believed to have a direct impact on the performance of the coated device [4], [5]. Commonly used techniques to study this process provide only limited understanding. For instance, High Performance Liquid Chromatography (HPLC) and other traditional analytical techniques allow researchers to monitor aggregate release over time, but provide little or no insight into how coatings can be engineered to "tune" drug release for specific biologic needs [6]. In order to engineer specific release time-profiles, it is necessary to understand how the drug is sequestered within a film and determine how it mobilizes and releases when immersed in an aqueous environment.

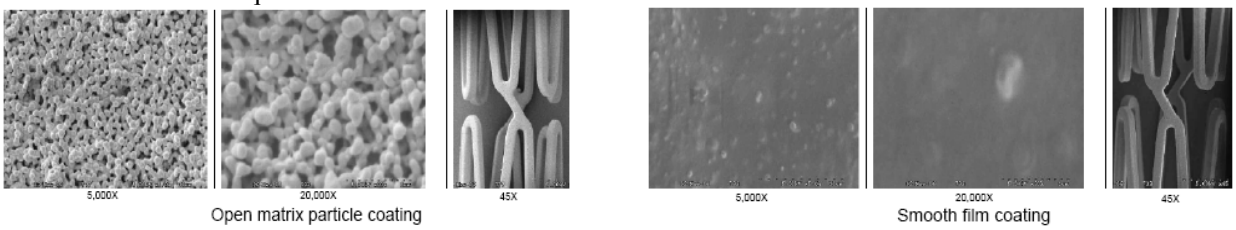

Fig. 1. SEM images of nanocomposite coatings generated by the ElectroNanospray process. Left row is the open matrix coating and the right row is the smooth film coating. The third image of each group shows the respective coatings applied to coronary stents.

In this research, an arborescent polyisobutylene-polystyrene (*arb*IBS) block copolymer and the drug rapamycin (sirolimus) were sprayed to the stainless steel surface by ENS. A smooth film and two open matrix particle coatings with different particle sizes were generated by adjusting the spraying parameters. To study the details of the drug release process, we employed high resolution Raman imaging to examine drug mobilization from the polymer matrix after immersion in phosphate buffered saline. This represents an important advance, because real-time sampling makes it possible to generate four-dimensional information about the drug release process (three spatial dimensions over time). To reveal the ultrastructural changes, other high-resolution imaging techniques such as in-liquid atomic force microscopy (AFM) with Digital Pulsed Force Mode (DPFM) and scanning electron microscopy (SEM) have also been employed to characterize the surface morphology, with dynamic, *in situ* imaging during the

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drug-release process with AFM and static *ex situ* imaging at specific time points with SEM. The combination of approaches enables the examination of drug sequestration, mobilization and release with a level of detail not available using any individual approach. These methodological understandings should broadly aid the engineering of controlled drug release from medical device coatings.

EXPERIMENTAL METHODS

Materials

Arborescent polyisobutylene-block-polystyrene (arbIBS), with 32.8 w/w% PS segments (TPE-4) was synthesized by living carbocationic polymerization at University of Akron[7]. Rapamycin was purchased from LC Laboratories and was used as received.

Confocal Raman Microscopy

Combining high resolution confocal optical microscopy and Raman spectroscopy, 3-D chemical map can be obtained from a material with the ultimate resolution of 200-300 nm laterally and ~500 nm vertically. (Thus "surface-focused" means the topmost several hundred nanometers.) Raman spectroscopy and Raman chemical imaging have been extensively employed to characterize drug polymorphs and the drug distribution in biomedical coatings [8]. In this research, a Witec (Ulm, Germany) Confocal Raman microscope was used to elucidate the spatial distribution of a steroid drug within the *arb*IBS polymer matrix. Raman imaging was performed by raster scanning a sample under the microscope objective. An array of spectra (i.e. 80×80 for all images presented here) was collected with the same integration time at each pixel location. Raman images were generated by integrating one or more characteristic peaks from each component for all spectra and rendering the peak intensity as brightness at each pixel location. The confocal capability allows imaging in both cross-sectional (i.e., Z vs. X) and lateral modes (i.e., X-Y at a given Z focal plane).

Atomic Force Microscopy

AFM was carried out using a Molecular Imaging PicoPlus/PicoScan 3000 system (now Agilent Technologies, Santa Clara, CA, model 5500) with environmental control (humidity, sample temperature) and open liquid cell, and employing a Digital Pulsed Force Mode attachment (WITec GmbH, Ulm, Germany) [9] to obtain images of height, tip-sample adhesion, stiffness and viscoelastic character, using silicon tip/cantilevers (rectangular, nominal spring constant 3 N/m, Applied Nanostructures).

Scanning Electron Microscopy

SEM analysis was carried out at 1.0 kV accelerating voltage using a Cold Field Emission Gun Scanning Electron Microscope (Hitachi S-4700, Pleasanton, CA). The sample was positioned at the appropriate lens-to-sample working distance tilted 45 degrees relative to the electron beam to optimize the contributions by both secondary and backscattered electrons for topographic contrast.

High Performance Liquid Chromatography (HPLC)

The coated coupons were placed into vials with 10 ml of phosphate buffered saline (PBS) and shaken at 120 RPM at 37°C. The incubation medium was removed from the vials at different time points and analyzed by High Pressure Liquid Chromatography (HPLC) on a Hewlett Packard 1090 HPLC system fitted with a 150 mm x 2.1 mm i.d., SB-C18 (3.5 micron particle size) column under the following conditions: flow rate, 1.0 mL/min; temperature, 75 ^{o}C; injection volume, 250 µL; eluent A, 10 mM phosphoric acid in water; eluent B,acetonitrile; elution conditions, gradient from 20-100-100-20-20 %B from 0-2.5-3.25-3.26-3.5 minutes. Calibration standards were prepared at rapamycin concentrations of 10, 50, 100, 500 and 1,000 ng/mL. All samples (calibration and release samples) contained at least 20% acetonitrile (v/v), and prednisone was added as an internal standard at approximately 500 ng/mL by dilution from a stock solution in acetonitrile.

RESULTS AND DISCUSSION

Drug Release by HPLC

Figure 2 shows the drug release profiles obtained by HPLC for the three films. The smooth film shows a "burst" release with a 0.5-1 hour, and it is followed by a slow and gradual release throughout the remainder of the testing

period. The release from the two open matrix samples remains slow over the whole testing period.

Films in Ambient Condition

Figure 3 displays elevated drug signal (bright) in the three films by (surface-focused) Raman imaging, and the surface morphology by AFM and SEM. The smooth film looks almost featureless by SEM, but Raman imaging reveals that the drug segregates into micron-sized domains (2-4 μm). The small scale AFM image shows that even smaller drug domains (60-100 nm) are evenly distributed through the whole film. Both open matrix films show rich surface morphology. Within the resolution of Raman, no distinct drug domains were identified, indicating significant mixing of the drug and polymer within each particle.

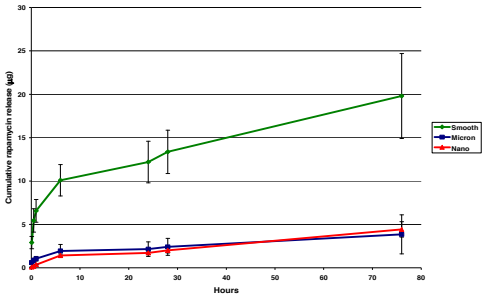

Fig. 2. 72-hour release profiles from the three films. Drug released more rapidly from the smooth films (green) and at the same rate from both the microscale and nanoscale particulate films (blue and red, respectively).

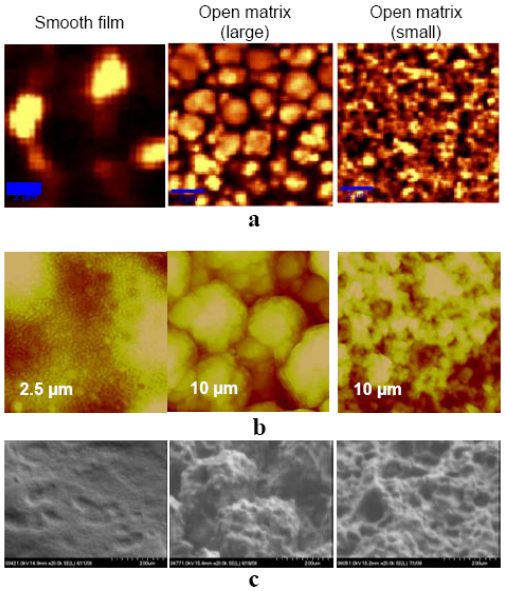

Fig. 3. Raman (a) AFM (b) and SEM (c) images on three films.

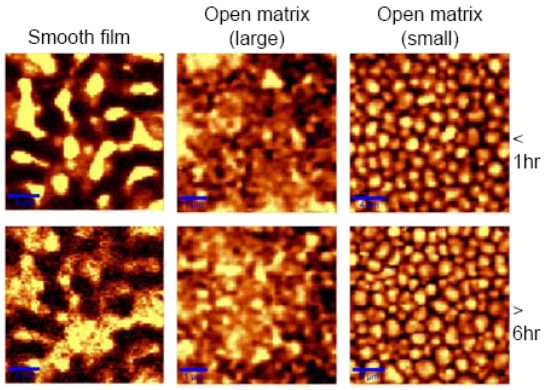

Fig. 4. Raman imaging on three films in PBS solution.

Drug Release Imaging in Real-Time

Figure 4 shows the Raman images (drug bright, surface-focused) captured in PBS solution after the films were immersed for different lengths of time. The smooth film showed a significant change in drug distribution in images taken after less than an hour of immersion in PBS. Some more diffuse drug signal in the image at bottom left was shown in the areas between the more concentrated drug domains, indicating the mobilization of the drug into the intervening polymer matrix. After a longer time of immersion in PBS, the micron-sized drug domains start to lose their previously sharp contrast in this surface-focused image. *In-situ* AFM images (data not shown) revealed nano-sized holes (~60-100nm) on the surface after more than 10 hours of elution, corresponding to the mobilized drug domains observed in Raman. Micron-sized pits were also found on the surface, consistent in size and shape with the drug domains revealed in Raman imaging after more than 6 hours. Both open matrix samples did not show a significant change of the drug signal distribution over a period of more than 6 hours, suggesting that the drug is more

tightly sequestered within the polymer.

Ex-situ SEM Imaging after Elution

Figure 5 shows SEM images of the three film surfaces after 10 hours elution in PBS. Smooth film shows micron-sized pits, with nanoscale hole inside the pits, which conforms the observation in real-time. Due to already porous nature of the surface, neither of the open matrix coatings show similar nanoscale changes, although closer examination of SEM images at higher magnification reveals small holes in the particles, probably left by the drug after elution.

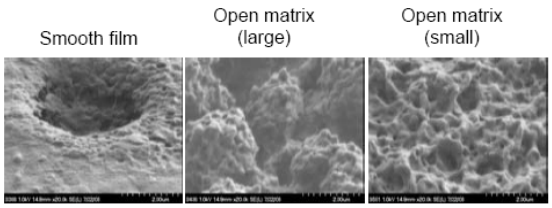

Fig. 5. *Ex-situ* imaging of the coating films by SEM

CONCLUSION

The combination of multiple imaging techniques allows us to probe the drug release process in real-time. Coatings created by ENS showed distinct surface morphology and drug release profiles. It was found from the real-time imaging that the smooth film has a highly mobile drug phase consistent with nano-sized particles. The fast erosion of these nanoparticles from domains with higher drug concentrations may contribute to the initial burst release seen in the drug elution profiles measured by HPLC. These larger domains likely form by phase segregation of the drug and polymer, enabled by more residual solvent in the smooth coatings and correspondingly longer drying times. These large domains allow for rapid elution of the drug columns from the film. It was not expected that the two particulate films would show almost identical release profiles. SEM and AFM suggest that the microscale particles seen in coarser open matrix coating may be aggregates of much smaller particles. Overall, the slow drug release from the small particles may be due to the higher degree of mixing between the drug and polymer that was maintained by the more rapid drying time, with less time for phase segregation. Thus, we conclude that the way the drug is sequestered in the films has a direct impact on the release profiles.

A better understanding of the mechanisms of drug sequestration, mobilization and release from polymeric compounds is critical for engineering drug release profiles to meet biologic needs. Our demonstration of engineering nanocomposite biomedical coatings with controlled release profiles may point to some new applications of these films.

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