Characterization of Real-time Drug Release from Engineered Biomedical Coatings

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Introduction:

Coronary stents are industrially produced and are a non-invasive alternative to heart bypass surgery. Biomedical thin-film coatings are currently applied to all arterial stents for the release of anti-inflammatory drugs. While drug release from these films is effective, the parameters affecting the release profile are not easily controlled. Nanocopoeia, Inc. of St. Paul, MN, has developed the ElectroNanospray™ process to create advanced composite biomedical coatings of drug and polymer. Previous research has demonstrated that the release profile varies with the process conditions. This suggests that a higher level of controlled drug-release is possible with this process than is currently available. However, the release of drugs from polymeric coatings is non-linear, suggesting complex elution mechanisms that are unknown.

We have characterized the structure and chemical composition of these composite coatings before and during the release process using complimentary high-resolution microscopy methods. The completed characterization work will be used by Nanocopoeia to better understand the release mechanisms of elution. These understandings will then be used to link ElectroNanospray™ parameters to highly-controlled—and predictable—release profiles, thereby allowing the creation of individually-tailored coatings for specific biologic needs.

Experimental Method:

Composite films of polyisobutylene-polystyrene polymer with 10% (wt/wt) dexamethasone or rapamycin generated on a stainless steel substrate using the ElectroNanosprayTM process at 3-12 μm thicknesses were obtained from Nanocopoeia, Inc. These coatings were then analyzed using atomic force microscopy (AFM) and confocal Raman spectroscopy imaging. When combined, these two complementary techniques provide data which can be used to identify drug location and observe the elution from the polymer matrix in real-time.

AFM imaging was chosen for the ability to image the coating surface at high resolution in both air and an aqueous environment. An Agilent Technologies 5500 scanning probe microscope with Witec digital pulsed force mode attachment was used. Images of topography, cantilever deflection, and tip-sample adhesion were generated to provide varied mechanical property information.

Raman spectroscopy imaging involves the collection of monochromatic light scattered by the sample. Characteristic peaks were chosen based on scans of pure component samples; a 1640-1680 cm^{-1} peak for C = O functional groups on drug molecules and a stronger 2970-3025 cm^{-1} peak for aliphatic C-H groups on the copolymer were selected. Scattered Ar-ion laser light was gathered with a Witec Alpha300 R confocal Raman microscope and sent to a spectroscopic detector for each image pixel. The chosen signal peaks were then integrated by the Witec control software to generate a color-contrasted image. This characterization technique allows for spatial resolution of chemical composition and density within the coating.

In-air images were first taken at varying scan sizes and resolutions from 2.5 to 25 μm square and 80 × 80 pixels (Raman) to 512 × 512 pixels (AFM). The samples were then immersed in a phosphate buffer saline (PBS) solution, and time-elapse imaging was similarly conducted in solution at ½ hour, 1, 2, 4, 6, and 24 hours. These images were then qualitatively analyzed for key features and patterns of change which point to observable trends in drug release.

Results:

Raman images generated from characteristic peaks in solution for dexamethasone samples indicated that the drug was primarily aggregated in vertical columnar domains of increasing diameter, between 2 and 5 μm in size, from 1 to 6.5 hours. Smaller particulate drug aggregates, 500 nm to 1 μm in size, were also observed interspersed between the columnar domains. The visible contrast between the larger aggregates and smaller particles increased over 17 hours, indicating that the drug peak intensity within the large domains decreased. Figure 1 is a characteristic image for the drug peak and Figure 2 is for the polymer peak, with selected peaks intensity indicated by lighter regions. Raman imaging on rapamycin samples has not yet been conducted systematically.

AFM imaging in air on rapamycin samples at 2.5 μm size showed no characteristic features on the surface, but the phase separation of the block copolymer was evident in the deflection and adhesion images. When placed into solution, large dome-shaped features of low tip adhesion and high deflection (deflection is the slope of the surface, dZ/dX) appeared that were not previously evident, indicating that the features were of a different composition than the surface and were protruding. These features are visible in Figure 3. These features increased in number through 2.5 hours, and decreased in size by 25 hours. An image at 25 hours is included as Figure 4; note with comparison to Figure 3. AFM imaging on dexamethasone samples showed no conclusive changes between samples in air and in solution, and no trends in surface changes were observed over time *in situ*.

Conclusions:

Raman imaging showed that the drug was aggregated in larger micron-sized domains, and that release was primarily due to the decay of these larger features. The decrease in contrast range between columns and particles over time indicates that the relative concentration of drug in the columns was decreasing over time; we believe this to be the main indicator of elution from the columns. The dome-shaped features appearing on the surface of the coating in AFM images are concluded to be the emerging heads of the columns of drug within the coating.

The currently proposed mechanism is a combination of two main transport mechanisms within the coating. Lateral motion of smaller particles within the coating is proposed to be driven by both diffusion and internal stresses. From this motion, particles move to the large columnar domains, and they are subsequently driven to the surface by the concentration gradient in the column. However, further systematic characterization with both techniques is needed to confirm these conclusions. These results demonstrate the power of these complementary techniques for understanding drug release from biomedical coatings.

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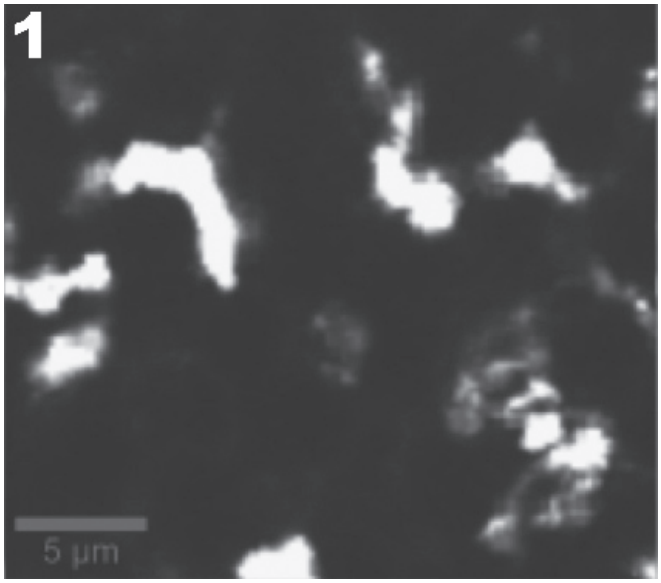

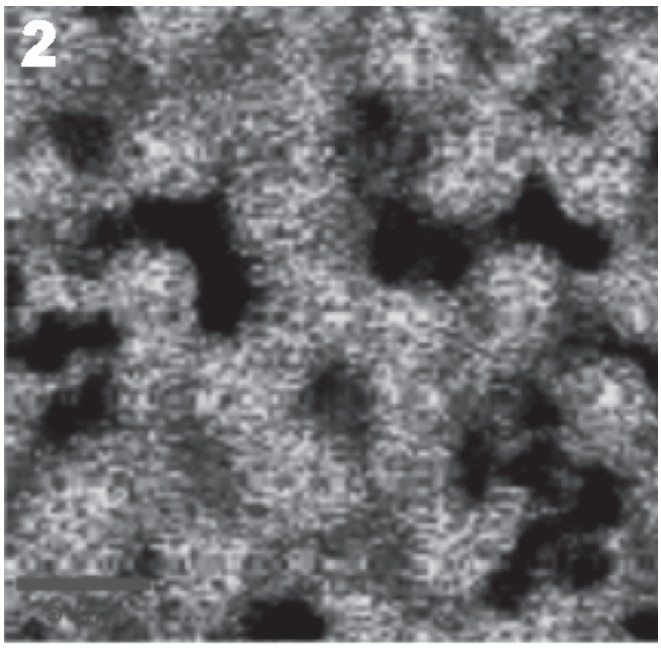

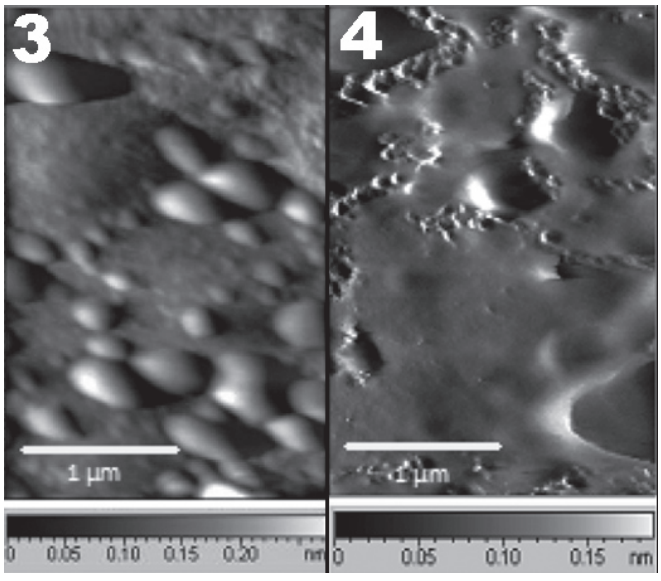

Figure 1, top: Drug Raman peak intensity, 25 × 25 µm, 6.5 hr in PBS.

Figure 2, middle: Polymer Raman peak intensity, 25 × 25 µm, 6.5 hr in PBS.

Figure 3, bottom left: Deflection AFM image, 5 × 5 µm, 1 hr in PBS.

Figure 4, bottom right: Deflection AFM image, 5 × 5 µm, 25 hr in PBS.