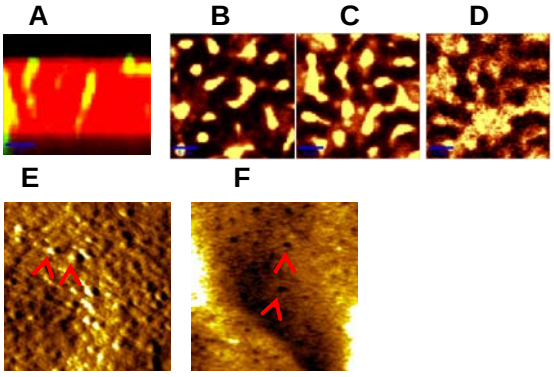
A Comprehensive Approach for Real-time Drug Release Imaging from Polymeric Coatings

John Foley[1], Robert Hoerr[1], Mike Matuszewski[1], Judit Puskas[2], Greg Haugstad[3], Jinping Dong[3], Chris Frethem[3]
[1]Nanocopoeia, Inc, St. Paul, MN, [2]University of Akron, Akron, OH,
[3]University of Minnesota Characterization Facility, Minneapolis, MN.

Statement of Purpose: Drug release from polymeric compounds is a highly complex and poorly understood process. Release from samples is usually non-linear and the mechanisms that control release are not known. The traditional approach of measuring percent release over time provides researchers with only a grainy snapshot of the process. The purpose of this study was to take a comprehensive approach to examine drug sequestration, mobilization and release from a polymer immersed in PBS to better understand how to engineer coatings to tailor release profiles to meet biological objectives.

Methods: An arborescent polyisobutylene-polystyrene (*arb*IBS) block copolymer was created by living carbocationic polymerization[1]. Coatings were applied to 0.2"x0.4" stainless steel coupons by ElectroNanospray (Nanocopoeia, Inc., St. Paul, MN) of a 1%/0.1% polymer/drug solution. The final coating contained 10 wt% drug (rapamycin) in a *arb*IBS polymer matrix. High pressure liquid chromatography (HPLC) analysis of rapamycin release was performed on a Hewlett Packard 1090 HPLC system. SEM analysis of uncoated samples was carried out at 1.0 kV accelerating voltage using a Cold Field Emission Gun Scanning Electron Microscope (Hitachi S-4700; Pleasanton, CA). 4D confocal Raman spectroscopy was carried out with a Witec confocal Raman microscope (Ulm, Germany) fitted with an Omnichrome Argon ion laser with 514.5nm excitation and 50mW maximum output power. Atomic force microscopy was carried out using a Molecular Imaging PicoPlus/PicoScan 3000 system (now Agilent 5500; Santa Clara, CA) with environmental control (RH, T) and liquid cell, and the Witec Digital Pulsed Force Mode attachment to obtain images of height, tip-sample adhesion, stiffness and viscoelastic character.

Results: *arb*IBS/rapamycin films were sprayed onto stainless steel coupons; coating morphology was found to be a smooth, closed matrix by SEM. Identical samples were incubated in phosphate-buffered saline and drug release measured by HPLC. Post-release films were dried and uncoated samples were examined by SEM. The morphology contained evenly distributed micron-scale (~2-3 μm) pits which contained smaller, ~100 nm scale pitting. Identical samples were next examined by 4D confocal Raman microscopy. 4D Raman imaging revealed that the drug segregates into discrete micron sized columns that can span the bulk of the polymer depthwise. These columns are similar in size and distribution to the surface pitting that was observed by SEM. The drug was found to rapidly mobilize (< 5 min.) after immersion in PBS. Imaging captured over the 12 hour release period showed that the drug diffuses out of the concentrated columns and migrates upward towards the surface of the polymer. Raman images are diffraction limited and thus, the complementary approach of AFM was used to examine drug distribution at nanoscale level. AFM data obtained before immersion showed discrete, 80 – 120 nm domains of decreased adhesion suggesting that the drug is sequestered in nanoscale domains throughout the polymer as well as in clusters within the larger microscale domains. In-liquid AFM after 18 hours showed discrete holes of similar dimensions.



***In-situ* drug release by confocal Raman microscopy and Atomic Force Microscopy.** The top four images are chemical maps from Raman microscopy. Image A) is a cross section scan showing the contrast between the drug, rapamycin (yellow), and the *arb*IBS polymer (red); the dark region at the top is air and the dark region at the bottom is substrate. In this smooth coating, the drug segregates into discrete columns that span the bulk of the polymer depthwise. B) shows a lateral scan of the surface of the coating in ambient conditions. Drug particles with sizes of 2-5 μm were observed representing the tops of the drug columns. Image C) was captured after immersing the coating in water for 5 minutes. The diffuse signal around the drug columns appears as the drug begins to migrate in the polymer. Image D) was captured after 8 hours of immersion (Blue bars = 5 μm). The contrast of large drug particles fades as the diffuse phase gets even concentrated. E) and F) are AFM images showing some much smaller drug particles (red arrows, 80-120 nm) densely imbedded in the polymer matrix in air (E), and after being immersed in water for 18 hours (F), small holes, corresponding to the fast release of a second, mobile phase of drug observed by Raman.

Conclusions: In order to better engineer drug eluting materials it is essential to understand how the drug and polymers behave in aqueous environments. SEM, 4D confocal Raman microscopy and in-liquid AFM are powerful, complementary tools that can help to deepen our understanding of this important process.

References:

1) Puskas, J Journal of Polymer Science: Part A: Polymer Chemistry. 2005; Vol. 43, 1811–1826.