

Environmental Digital Pulsed Force Mode AFM and Confocal Raman Microscopy in Biomedical Coatings Research

G. Haugstad* and K. Wormuth**

*University of Minnesota, Minneapolis, MN, USA, haugs001@umn.edu

**Surmodics, Eden Prairie, MN, USA, kwormuth@surmodics.com

ABSTRACT

Controlled release of amorphous drug from a polymer matrix depends intimately upon the degree of mixing of drug and polymer, the susceptibility of the drug to crystallization, and the ability of the drug to dissolve and diffuse through water-swollen polymer. Ideally, characterization methods would follow these processes on the molecular level *in situ* and in real time. We move closer to this ideal state of characterization through application of two imaging methods: digital pulsed force mode atomic force microscopy (D-PFM AFM) and confocal Raman microscopy (CRM). We examine model spin-coated films (~1 μm thick) containing the drug dexamethasone dispersed in poly(n-alkyl methacrylate) homopolymer and blend coatings. We report aqueous-immersion studies of surface and subsurface structural changes due to drug elution over time frames ranging from very fast (a few minutes) to slow (tens of hours).

Keywords: atomic force microscopy, drug elution, biomedical coating, polymer, surface morphology

1 INTRODUCTION

One particularly successful application of amorphous drug/polymer mixtures is as coatings on drug eluting stents. Controlled release of an antiproliferative drug from the coating minimizes restenosis after a stent is placed to prop open a blocked artery. Despite successful application, the nature of amorphous drug elution from stent coatings remains incompletely understood. Open questions revolve around potential crystallization of the drug upon exposure to body fluids, diffusion of water and salts into the coating, and migration of drug out of the coating. Upon exposure of amorphous drug/polymer mixtures to an aqueous medium, water likely plasticizes the drug/polymer mixture and solubilizes the drug. Upon plasticization, drug mobility increases and the probability for drug crystallization rises. However, the presence of polymer may arrest crystallization or kinetically stabilize amorphous drug.[1,2]

Here we directly examine drug/polymer coatings immersed in water and watch drug elution and crystallization *in situ* employing atomic force microscopy for high-resolution (~1-10 nm) surface sensitivity and confocal Raman microscopy for somewhat lower resolution (~250-500 nm) imaging but with 3D spatial sensitivity.

2 EXPERIMENTAL METHODS

Coatings of drug/polymer mixtures containing dexamethasone drug in (a) poly(butyl methacrylate) (PBMA), a 50:50 ratio, or (b) PBMA/poly(lauryl methacrylate) (PLMA) blends, a 43.5:43.5:13 ratio (minority PLMA, of rubbery character), were prepared by spin coating (50 mg solids per ml THF solvent) at 3000 rpm on silicon wafers (native oxide surface) with no further thermal or chemical treatment. Coated wafers were cleaved to provide pieces approximately 2x2-cm in size, large enough to comprise the floor of an open AFM liquid cell.

A Molecular Imaging PicoPlus (now Agilent Technologies 5500) environmentally controlled (humidity, sample temperature) scanning probe microscope driven by an attached digital pulsed force mode controller (D-PFM, WITec, GmbH) allowed the surfaces of the coatings to be probed under water immersion. For long experiments the PicoPlus' integrated environmental sample chamber was maintained at a relative humidity of >80% (using a ultrasonic humidifier powered by a feedback-driven humidity control, ETS Systems) to prevent evaporation from the liquid cell, which would change the concentration of the eluted drug. (Based on the total drug content in the film, the volume of the liquid cell, and the known solubility of dexamethasone in water, the concentration of released drug was determined to remain well below liquid-cell saturation throughout the experiment.) At each image pixel location the D-PFM controller monitors and optionally collects the entire force-Z curve to examine tip-sample interaction in detail (up to gigabyte data file regime). This necessarily rapid distance cycling was performed sinusoidally in time at a frequency of 2000 approach-retract cycles per second, using Z amplitudes of a few tens of nanometers, employing silicon tip/cantilevers of ~3 N/m spring constant and ~75 kHz resonant frequency (Applied Nanostructures). Real-time analysis of the force curves provides images related to tip-sample adhesion, contact stiffness and viscoelastic loss angle, in addition to surface topography. The latter is obtained via the usual feedback-driven Z scanner (DC) displacement so as to keep the maximum cantilever deflection within each cycle (force signal relative to that out of contact) constant from pixel to pixel. Thus the technique is quasistatic like contact mode AFM (force equilibrium driving the Z feedback circuit) yet involves a brief (~0.1 ms) intermittent contact during which

lateral stresses and sliding are minimal (akin to dynamic AFM, also known as AC, vibrating-tip or “tapping mode”).

A WITec Alpha 300 confocal Raman microscope with 532 nm wavelength laser light and a water immersion objective provided Raman images, an entire Raman spectrum collected at each pixel. The images were deconvolved using an augmented classical least squares algorithm yielding images contrasting the spatial distribution of drug polymorphs and polymers.

3 RESULTS AND DISCUSSION

3.1 Raman microscopy

We determined the spatial distribution of PBMA, PLMA, and amorphous and crystalline dexamethasone within coatings by exploiting the significant Raman spectral differences between the polymers and drug polymorphs. In control experiments with confocal Raman imaging, thin coatings of dexamethasone in poly(butyl methacrylate) (PBMA) appear highly resistant to crystallization upon exposure to water. With the presence of a minority fraction of poly(lauryl methacrylate) (PLMA) in the coating, however, one hour of exposure to water produces stark effects. In Figure 1, Raman images show nucleation of dexamethasone crystals primarily in the proximity of PLMA domains, depth resolved to within $\sim 0.5 \mu\text{m}$ of the surface (roughly half the coating thickness).

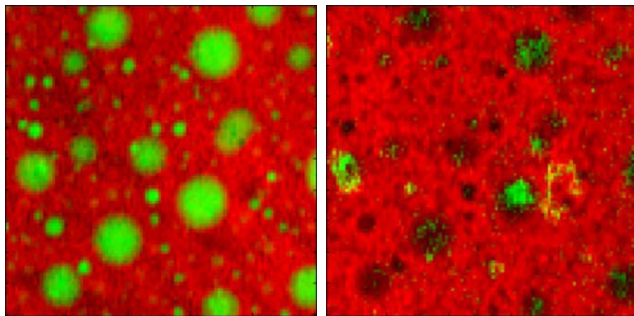


Figure 1: 25x25- μm Raman spectroscopy images of a coating of dexamethasone/PBMA/PLMA under water after exposure to water for 1 hour. *Left*: Red = PBMA, Green = PLMA. *Right*: Red = amorphous dexamethasone, Green = crystalline dexamethasone

3.2 AFM, dexamethasone in PBMA

Raman images of a dexamethasone/PBMA coating (i.e., no PLMA) show homogeneous mixing (within $\sim 270 \text{ nm}$ lateral resolution, i.e., half wavelength of the laser light). In contrast, the representative set of AFM images in Figure 2 reveals a nano-structured surface topography which dramatically evolves upon exposure to water. Prior to exposure to water, the surface contains small, mainly circular bumps $\approx 200 \text{ nm}$ in diameter and $\approx 20 \text{ nm}$ tall (Figure 2 top left, bright regions); these also exhibit

reduced adhesion with the AFM tip (not shown). Stiffness and adhesion images acquired on dry coatings at elevated temperatures at which PBMA softens (not shown) imply that the bumps are dexamethasone-rich and the intervening locations polymer-rich. After approximately 10 minutes of exposure to water, a significant number of circular pits or pores appear in place of bumps (Figure 2 top right, dark regions), which suggests that a *first* subpopulation of bumps rapidly releases drug. A *second*, lesser subpopulation of bumps bulge out vertically by as much as 50 nm (and are apparently “sharp” enough to reverse-image the pyramidal AFM tip shape). After 150 minutes exposure to water, the initial holes further deepen while remaining bumps decrease in height. After 23 hours in water, holes have further expanded while bumps have receded to near the baseline of the “web” of coating between the holes.

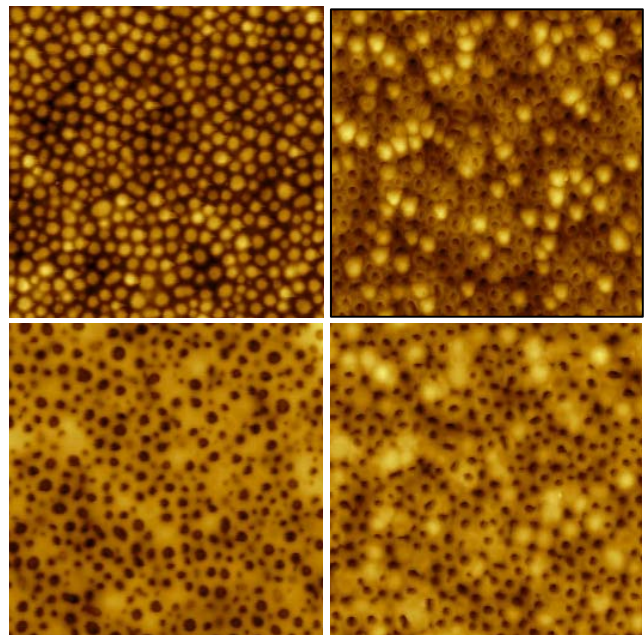


Figure 2: Representative 5x5- μm AFM height images of dexamethasone/PBMA coating in initial dry state (*upper left*) and at three time stages under water as follows. *Upper right*: ~ 10 minutes. *Lower right*: 150 minutes (identical region as upper right). *Lower left*: 23 hours.

Closer inspection of images of dexamethasone/PBMA reveals that a *third*, even lesser subpopulation of bumps observed during the first few minutes of water exposure that convert to holes after a few tens of minutes. Five examples are denoted with circles in Figure 3. This suggests the presence of a very thin skin of PBMA around some $\sim 100\text{-nm}$ drug domains that may later rupture to enable drug elution, whereas the first subpopulation of bumps seem to suddenly release drug upon water exposure, as if there is either no PBMA skin or a PBMA skin that is immediately perforated. Finally, the vertical growth then shrinkage of the second subpopulation of protrusions suggests PBMA-encapsulated drug domains that are

penetrated and expanded by water faster than drug can diffuse out in three dimensions; but ultimately the latter process causes the protrusions to collapse. Characterized over a full day, the vast majority of circular, drug-rich domains either converted to holes immediately or expanded then contracted to baseline. Continued studies should further elucidate these behaviors. We anticipate that there is a *continuum* of behaviors, with a range of drug elution times spanning a logarithmic scale, rather than three distinct rates as suggested above for expository purposes.

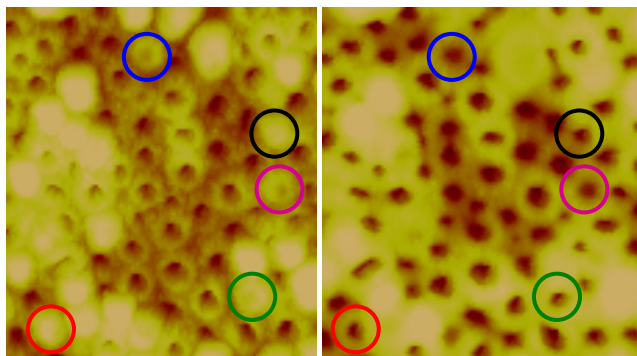


Figure 3: 2.2x2.4- μm AFM height images of a dexamethasone/PBMA coating under water. *Left*: after ~ 10 minutes in water. *Right*: same region after 150 minutes in water. Circles locate protrusion that become holes.

3.3 AFM, dexamethasone in PBMA/PLMA

AFM images of coatings containing the two-polymer blend exhibit rich morphologies spanning nano- to micro-scales. Figure 4 contains images of height and tip-sample adhesion (pull-off force) on a dexamethasone/PBMA/PLMA coating. Similar to the dexamethasone/PBMA case there are small, nearly circular bumps ≈ 200 nm in diameter, which exhibit relatively low adhesion with the AFM tip. But unlike the dexamethasone/PBMA case there are intermediate-size domains (≈ 500 -nm diameter) that exhibit high adhesion, plus large domains (diameters ranging from 1-4 μm as compiled in 50- μm survey images) that are either similar in surface height to the intermediate domains or bowl-shaped depressions, tens of nanometers and in some cases more than 100-nm deep. The large domains exhibit adhesion that is high compared to the small bumps but not quite as high as the intermediate-sized domains. Both the intermediate and large domains exhibit equally low contact stiffness (not shown), indicating primarily PLMA content, as this polymer is rubbery at room temperature, whereas PBMA is glassy. Comparison with Raman micrographs as in Figure 1 confirms this assignment for the large domains. AFM images on drugless (control) PBMA-PLMA coatings (not shown) also contain both the intermediate and large-sized PLMA domains, and with similar slight differences in adhesion, but in both cases lower than the adhesion measured on PBMA for similar maximum loading forces (~ 10 nN; whereas PLMA exhibits greater adhesion for

substantially larger pushing forces at turnaround, i.e., greater viscoelastic adhesion memory).

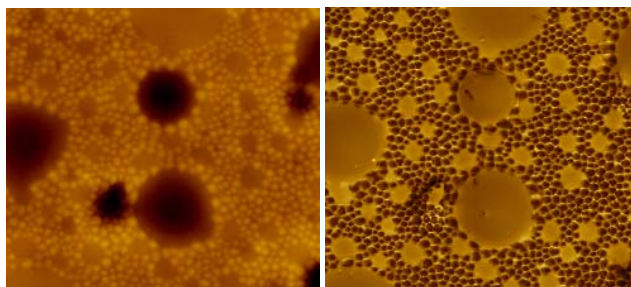


Figure 4: Representative 10x9- μm AFM height (*left*) and tip-sample adhesion (*right*) images of dexamethasone-in-PBMA/PLMA coating in its initial dry state.

Upon immersion in water, dramatic changes in both morphology and adhesion are immediately apparent. As with the simpler dexamethasone/PBMA coatings, many of the small bumps are replaced by small holes on the ~ 10 -minute time frame, then grow in depth over times spanning hours. Another similarity is the outward bulging of a small subpopulation of the original small bumps. The intermediate-sized, PLMA-rich domains more significantly bulge outwards, by ≈ 200 nm, suggesting local penetration of water and/or subsurface aggregation of drug; whereas the large PLMA domains seemingly remain unchanged in relative surface height. With passing time the adhesion signatures also evolve, generally towards lower adhesion, except for the large PLMA domains which remain remarkably constant in adhesion.

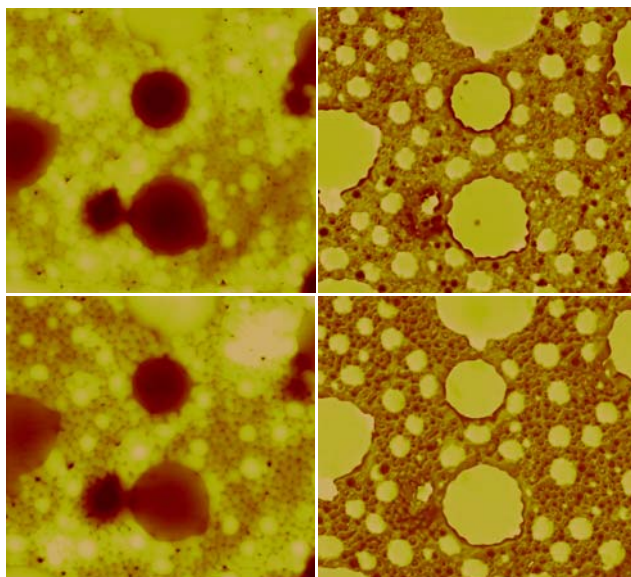


Figure 5: 10x10- μm AFM height (*left*) and tip-sample adhesion (*right*) images of dexamethasone-in-PBMA/PLMA coating in water after 22 (*top*) and 230 (*bottom*) minutes immersion time. Same region as Figure 4.

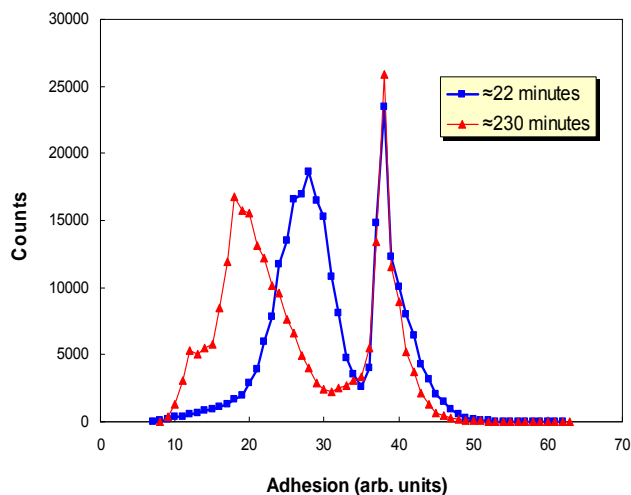


Figure 6. Histograms of the adhesion images in Figure 5.

These latter observations are quantified as histograms of adhesion images, presented in Figure 6. The tall, sharp peak is the signal derived from the large PLMA domains. Its constancy indicates a lack of surface changes within these domains, which at first seems inconsistent with the appearance of a strong crystallized drug signal observed in the immersion Raman images (Figure 1). Thus we interpret this crystallization Raman signal as originating *at depth*, whether buried inside the PLMA domains or below them; but most likely at their peripheries given that Raman experiments show strong preferential partitioning of dexamethasone into PBMA, with little dexamethasone signal found in PLMA domains. We suggest that it is the presence of *interfaces* with PLMA that triggers the crystallization of dexamethasone.

The reduction of adhesion with passing time seen in Figure 6 for all other surface constituents is intriguing. The adhesion measurement is fundamentally a probe of contact hysteresis, in turn a gauge of molecular mobility during the formation and break of tip-sample contact. Molecular rearrangements during contact allow the interfacial system to achieve a lower-energy state that requires a pulling force to disrupt.[3] Our observations suggest a decrease in surface molecular mobility as drug is eluting from the coating. Certainly the dexamethasone molecules are mobile, and as they evacuate from the near-surface region one might expect a decrease in overall molecular mobility of the matrix; this is especially likely because the presence of dexamethasone in PBMA should plasticize the polymer. The shifting of what appear to be at least three distinct surface components – both the low adhesion, broad peak and shoulder, and a high-adhesion shoulder – seem to suggest that the removal of dexamethasone reduces PBMA conformational mobility, and perhaps PLMA mobility as well (in the ~500-nm domains, perhaps near the PLMA-PBMA interface). Control immersion experiments on drugless coatings show no changes in PBMA or PLMA.

Further AFM experiments could examine force-distance profiles in detail to assess steric and bridging forces between tip and PBMA/PLMA,[4] with and without drug present in the initial coating, to better understand conformational states at the tip-sample interface.

4 CONCLUSIONS

Digital pulsed force mode AFM and confocal Raman microscopy were successfully employed, together with water immersion, to elucidate the structure and behavior of ~1- μm thick films of dexamethasone/PBMA (50:50) and dexamethasone/PBMA/PLMA (43.5:43.5:13).

AFM findings include (a) the rapid formation (burst) of ~200-nm diameter nanoscale surface holes in place of a majority subpopulation of ~200-nm diameter bumps present in dry, as-cast films, due to rapid elution of dexamethasone; (b) the slower appearance of a small number of additional holes in place of the original bumps, and the growth then collapse of the larger number of remaining original bumps over a period of many hours, due to much slower elution of dexamethasone. The presence of an unbreached PBMA surface barrier is proposed to result in a slower, diffusional release of drug (process b), whereas molecular-scale breaks in this barrier (likely below AFM resolution) may rapidly evacuate dexamethasone from pockets (process a).

Raman microscopy findings include the immersion-induced formation of dexamethasone microcrystallites in the vicinity of PLMA. The lack of surface crystallites in AFM images indicates that Raman is sensing objects below the surface, likely interfacing with PLMA domains.

We conclude that complementary D-PFM AFM and confocal Raman microscopy provide insights into the mechanisms of drug elution from, and crystallization within, biomedical coatings. Revealed relationships between coating structure and release kinetics are useful to the development of drug-eluting coatings formulations.

REFERENCES

- [1] H. Konno and L. Taylor, *J. Pharm. Sci.* 95, 2692, 2006.
- [2] P. Marsac, S. Shamblin, L. Taylor, *Pharm. Res.* 23, 2417, 2006.
- [3] J. N. Israelachvili, “Intermolecular and Surface Forces”, Academic Press, 1991.
- [4] H. –J. Butt, B. Cappella, and M. Kappl, *Surf. Sci. Rep.* 59, 1, 2005

ACKNOWLEDGMENTS

Support was provided by Surmodics, Inc., in the context of the Industrial Partnership for Research in Interfacial and Materials Engineering at the University of Minnesota. Parts of this work were carried out in the University’s Institute of Technology Characterization Facility, which received partial support from NSF through the NNIN program.