

Surface Characterization of PLGA Particles by Atomic Force Microscope

Relevant for: Pharmaceuticals, PLGA, AFM, particles, generic drugs

The Tosca AFM is used to characterize PLGA polymer in qualitative and quantitative manner for an FDA approval for a generic injectable PLGA formulation



1 Introduction^[1]

Poly (lactide-co-glycolide) (PLGA) is a copolymer, containing polylactic acid (PLA) and polyglycolic acid (PEG). Due to its biocompatibility and biodegradability, it has been used for making injectable, long term formulations in the last three decades. An in depth understanding of PLGA polymers is critical for development of depot formulations as their properties control drug release kinetics. To date, about 20 PLGA-based formulations have been approved by the U.S. Food and Drug Administration (FDA) through new drug applications. However, none of these FDA approved formulations have generic counterparts on the market yet. A generic injectable PLGA product needs to establish qualitative and quantitative sameness of PLGA to that of a reference listed drug to obtain approval from FDA. Conventional characterizations of PLGA used in a formulation rely on measuring the molecular weight by gel permeation chromatography (GPC) based on polystyrene molecular weight standards, and determining the lactide: glycolide (L: G) ratio by ¹H NMR and the end-group by ¹³C NMR. These approaches, however, do not provide detail on microstructural arrangement of the PLGAs in a

particle. Atomic force microscope (AFM) was utilized

for structural characterization of the PLGA surface. AFM utilizes a sharp probe with the tip radius down to the nanometer scale to scan along the sample surface to acquire images. For a copolymer containing components with different mechanical properties, the surface distribution of polymer components can be distinguished by tapping mode phase image. Furthermore, the localized adhesion and Young's modulus can be quantitatively determined using force distance curve.

2 Experimental

The PLGA particles were made by a conventional emulsion technique as described in the publication by Skidmore.et.al ^[1]. The fabricated dry particles were filtered through a 150 μ m mesh sieve to remove large particles.

For AFM measurements, PLGA particles were spread over a clean silicon wafer as shown in figure 1 (200 % zoom). Three particles A, B and C were selected to characterize the surface morphology.



Figure 1. Optical views of particles, overview camera view (200 % zoom) of entire sample area.

The video microscope view of particles A, B and C are shown in figure 2. Particles with different sizes can be





Figure 2. Optical views of particles, video microscope views of three particles with different size, A (300 % zoom), B (450 % zoom) and C (600 % zoom).

clearly observed by using zoom function in video microscope. 300 %, 450 % and 600 % zoom were used for particles A, B and C, respectively. With the combination of overview camera and video microscope, particles to be measured can be easily selected. The tip engages safely and quickly onto the surface of a particle.

First AFM images were acquired by tapping mode at several random locations at the surface of particles A, B and C, using an Arrow NCR tapping mode cantilever. A few positions were then selected on the image for force curve measurements, using an Arrow FMR cantilever.

3 Results and discussion

A few images $2 \times 2 \mu m$ were acquired on particles A, B and C in tapping mode as shown in Figure 3. The surface morphology changes significantly at different locations although particles were fabricated in the same batch. The surface roughness of particles varies. The root-mean-square surface roughness Sq calculated from the height images according to ISO 25178 is 4.4 nm, 4.5 nm and 12.6 nm for particles A, B and C, respectively. On the surface of particle A and B, many small "grains" above the surrounding can be clearly observed. In order to make further investigation, we used force distance curves to characterize nanomechanical properties locally. A soft cantilever Arrow FMR (spring constant k = 2.8 N/m) was used. Figure 4 shows the surface topography and the corresponded phase image. There are many small "grains" spread over the surface plane. A phase contrast between "grains" and the surroundings can be observed in the corresponding phase image, indicating the possible difference of surface mechanical properties at these two distinct areas.

Several force curves were performed at positions indicated on the topography image in Figure 4. Red crosses are force curves recorded on the surrounding and blue crosses are force curves recorded on "grains". An illustration of the determination Young's modulus and of adhesion is shown in Figure 5 and 6. By knowing the system sensitivity and the spring constant of the cantilever, Young's modulus and maximum adhesion force can be quantitatively determined. The calculated Young's modulus and adhesion on three different locations are shown in Table 1. The maximum adhesion force measured on



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the "grains" and their surrounding is very similar, showing no significant difference. The measured Young's modulus on the "grains" is much smaller (around 50 % less) compared to that measured on the surrounding. It explains the cause of the contrast that is observed in the phase image. The surrounding is possibly PLA rich region and the "grains" are possibly PGA rich area.



Figure 4. Surface topography (top) and phase (bottom) of particle B, locations for force distance curve measurement were indicated by red and blue cross



Figure 5. An illustration of the Young's modulus calculation



Force curve parameters	Value	Unit					
Spring constant	2.80	N/m					
Sensitivity constant	620	nm/V					
Individual cursors	Unit	R1	R2	R3			
Force	nN	-2.16e-14	-13.9	-7.54			
Cursor pairs	Unit	R1-R2	R2-R3				
Force difference	nN	-13.9	6.34				

Figure 6. An illustration of the adhesion calculation

	Adhesion (nN)		Young's modulus (GPa)			
	"Grain" (blue cross)	Surrounding (red cross)	"Grain" (blue cross)	Surrounding (red cross)		
P1	12.5	12.8	1.01	1.57		
P2	13.9	11.6	1.03	2.30		
P3	10.5	15.5	0.63	1.58		
Average	12.3 ± 1.7	13.3 ± 2.0	0.89 ± 0.23	1.82 ± 0.42		

Table 1 Adhesion and Young's modulus on "grains" (blue cross) and the surrounding (red cross) shown in Figure 5 and 6

4 Summary

We have used Tosca atomic force microscope to characterize the surface morphology, surface roughness and localized nanomechanical properties of PLGA particles. The qualitative and quantitative characterization of PLGA particles by Tosca AFM provides an effective way to be used to determine the sameness of PLGA to that of a reference listed drug to obtain approval from FDA. It facilitates the development of generic PLGA products.

5 Reference

[1] S. Skidmore, J. Hadar, J. Garner, H. Park, K. Park, Y. Wang und X. Jiang, "Complex sameness: Separation of mixed poly(lactide-co-glycolide)s based on the lactide: glycolide ratio," *Journal of Controlled Release*, pp. 174-1784, 2019.

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