Enzymatic Surface Erosion of Poly(trimethylene carbonate) Films Studied by Atomic Force Microscopy

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Statement of Purpose: Biodegradable polymers are widely used as medical implants, and in drug delivery and tissue engineering applications. Most polymers used in these applications degrade by a bulk erosion process. Only few polymers show surface-eroding characteristics, examples are polyanhydrides, poly(ethylene carbonate) and poly(ortho esters). Recently we found that *in vivo* poly(trimethylene carbonate) (PTMC) degraded rapidly by surface erosion. This behaviour could also be observed when PTMC was incubated in lipase solutions. [1]

In this study, the rapid surface erosion of spin-coated PTMC films by lipase solutions was investigated by atomic force microscopy (AFM). To illustrate a potential use of this enzymatic surface erosion process, micro-contact printing (μCP) of a PTMC surface was performed by impregnating a patterned PDMS stamp with the lipase solutions and subsequently contacting the surfaces with the stamp.

Methods: PTMC (M_n=2.9×10^5) was synthesized by ring-opening polymerization. It is an amorphous polymer with a glass transition temperature of approximately –20 °C. Lipase solutions from *Thermomyces lanuginosus* (Sigma, EC3.1.1.3, minimum 50000 units/g) were used as received. PTMC films with a thickness of 23 to 48 nm were prepared by spin-coating technique. μCP of PTMC films with lipase solutions was performed using PDMS stamps, which consisted of 2-μm wide recessed lines spaced by 5-μm wide protruding lines. AFM experiments were carried out in air using a NanoScope IIIa instrument (Digital Instruments).

Results/Discussion: Figure 1 shows tapping mode AFM height and phase images of PTMC film surfaces before and after immersion in lipase solution for 1 min at 37 °C. Initially the surface of the spin-coated film was very smooth. From the height image, a mean roughness (R_a) of 0.7 nm was determined; the corresponding phase image shows a homogenous polymer phase (Figure 1A). These PTMC films are stable in water for at least more than 1 d.

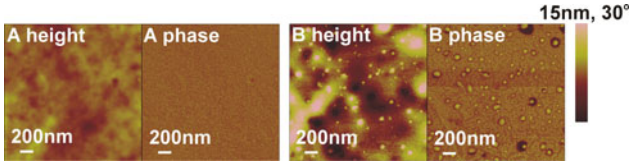

Figure 1. Tapping mode AFM height and phase images of PTMC films before treatment (A), and after immersion in lipase solution at 37 °C for 1 min (B).

After immersion in the lipase solution for only 1 min, the surface of the PTMC film had become significantly rougher (Figure 1B). R_a had increased to 2.5 nm. Pits and particles, with depths and heights of respectively 5 to 10 nm and 1 to 20 nm, appeared. Their diameters were 20 to 160 nm. The corresponding phase image shows that regions that are pits in the height image have a phase that equals the background. These pits are likely formed upon dissolution of the water-soluble degradation products.

The regions that are particles in the height image have a phase that differs from the background. Large particles could be aggregations of non-soluble PTMC oligomers and/or enzyme molecules. The sizes of the smallest particles approach the size of individual lipase molecules. By decreasing the enzyme treatment time to less than 5 s, enzymatic degradation was limited and individual lipase molecules could be discerned in the AFM height and phase images. (Average size of 200 particles obtained from height images are: d=12.4±6.0 nm, h=2.3±0.7 nm.)

The enzymatic surface erosion resulted in a decrease in film thickness. The surface erosion rate of the PTMC films was 11.0±3.7 nm/min. This is comparable to that of much thicker, compression-molded discs (4.7 nm/min).[1]

Patterned structures on PTMC films were obtained by μCP of lipase solutions on PTMC film surfaces for 30 s using PDMS stamps. After μCP, the resulting pattern on the surface of the PTMC films was probed using contact mode AFM to obtain height and friction images (Figure 2). From the images, alternating parallel lines, which were approximately 2- and 5-μm wide, could be distinguished. Compared to the 2-μm wide lines, the 5-μm wide lines that had been in contact with the lipase solution on the PDMS stamp were approximately 5 nm lower, and were of higher friction. Their higher friction is probably due to increased hydrophilicity.[2] This is a novel strategy to form predefined micro-patterned structure on a biocompatible and biodegradable polymer surface.

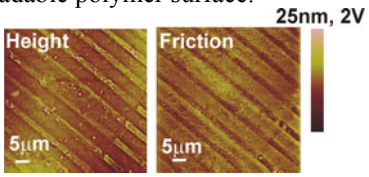

Figure 2. Contact mode AFM height and friction images of PTMC films after μCP using lipase solutions.

Conclusions: The enzymatic surface erosion of spin-coated PTMC films was studied using AFM. After immersion in lipase solutions, the roughness of the films increased and their average thickness decreased.

μCP of PTMC films using a PDMS stamp and lipase solutions allowed simultaneously patterning of the surface with predefined microstructures of varying heights and surface properties. Such surfaces are interesting for applications where cell patterning is required.

References:

[1]Zhang Z. et al. Biomaterials. In press (2005)
[2]Wang R. et al. Nature. 1997;388:431-432.