Electric Supplementary Information (ESI)

Tuning the enzymatic hydrolysis of biodegradable polyesters and it's application to surface patterning

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1. Experimental procedure

1.1 Chemicals

PLLA and proteinase K were purchased from Polysciences Inc. (Warrington, PA) and Roche Diagnosis (Mannheim, Germany), respectively. PCL was obtained form Wako (Japan), and lipase from *Pseudomonas* sp. was purchased from Aldrich-Sigma. The number-average molecular weight (M_n) and polydispersity (M_w/M_n) of the PLLA and PCL was determined by gel permeation chromatography (GPC) at 40 °C, using a Shimazu 10A GPC system and a 6A refractive index detector with two joint columns of Shodex K-80M and K-802. Chloroform was used as eluent. Polystyrene standards of low polydispersity were used for the calibration of molecular weight. The M_n and M_w/M_n of PLLA were 420 kDa and 1.7, whereas those for PCL were 130 kDa and 1.4, respectively. The PHB was microbially synthesized, and the M_n and M_w/M_n were 240 kDa and 2.1, respectively.

1.2 Preparation of amorphous PLLA thin films

The PLLA was dissolved in chloroform (1.5 wt%), and spin-cast on either Si substrate for AFM or QCM oscillator at 3000rpm. The cast thin film of 200 nm in thickness was melt at 220 ˚C, and quenched immediately at 0 ˚C, resulting in the smooth amorphous PLLA thin films.[S1] The 100 nm thick PCL and PHB thin films were also spin-cast on Si substrate from the 1.0 wt% chloroform solution. The PCL thin film was melt at 100 ˚C, whereas the PHB thin film was melt at 180˚C. Then, both thin films were crystallized at room temperature (25 ˚C) for 1 week. After the above procedure, the thin films never peeled off from the substrates during the washing process using Milli Q water.

1.3 UV-ozone treatment and enzymatic lithography

The amorphous PLLA and PCL thin films were treated with UV-Ozone Cleaner (UV253, Filgen) for 30 min. For the enzymatic lithography, TEM grids (Fine mesh: hole size 43 μm, bar size 20 μm, Nisshin EM, Japan; Thin bar grid: hole size 7.5 μm, bar size 5 μm, Gilder, UK; hexagonal grid: hole size 30 μm, bar size 33 μm, VECO, The Netherland) or assembled colloidal nanoparticles (size: 500 nm, Polysciences Inc.) were placed on the PLLA film during the UV-ozone treatment. For the UV irradiation, the oxygen supply was just stopped to reduce the amount of ozone production. After the surface modification, enzymatic degradation was carried out for 5-30 min.

The colloidal monolayer composed of polystyrene spheres with 500 nm in diameter was prepared via self-assembly on a cleaned glass substrate (24 × 24 mm^2), and slowly dipped into water.[S2] Then, it was peeled off from the glass substrate and floated on the water surface.

The colloidal monolayer was picked up by the PLLA thin films on a Si support, and gently dried in air. After the UV-ozone treatment, the PS layer was removed by sonication. Then, enzymatic degradation was carried out.

1.4 Enzymatic degradation

Enzymatic degradation was performed for PLLA, PCL and PHB, which can be degraded by proteinase K (1 mg mL^{-1}), lipase (1 mg mL^{-1}), and PHB depolymerase (1 μg mL^{-1}), respectively. The PHB depolymerase was expressed according to the previous method,[S3] and purified from the soluble fraction of recombinant SHuffle express *E. coli* using HiTrap TALON crude (GE Healthcare) along the supplier's instructions. The purified enzyme was dialyzed against 10 mM phosphate buffer (pH 7.0), concentrated and stored at -30 ˚C.

1.5 QCM Measurements

Enzymatic degradation rate of PLLA thin films was measured by QCM (QCA-917, Seiko EG&G) at 25 ˚C controlled by circulating water. A polished 9 MHz AT-cut quartz crystal coated by Au (area: 0.189 cm^2) was used as the QCM sensor. A frequency shift of 1 Hz is corresponding to the mass change of 1 ng on the Au electrode (0.189 cm^2), according to the Sauerbrey's equation.[S4] The enzymatic reaction was started by replacing the 50 mM Tris-HCl buffer (pH 8.5) to proteinase K (1mg mL^{-1}). The resonance frequency and the admittance were monitored simultaneously to evaluate the influence of friction change.

1.6 AFM Observations

Surface morphologies of PLLA thin films were visualized by a dynamic force (tapping) mode AFM (SPI4000/SPA400, SII Nanotechnology Inc., Japan) with PZT FS-20N and PZT FS-150N scanners. For the AFM observations in air (25 °C), two types of silicon cantilevers were used such as OMCL-AC240TS-W2 (Olympus, Japan) and SI-DF20 (SII Nanotechnology, Japan). Simultaneous registration was performed for height and phase images.

1.7 Contact angle measurement

The static contact angle was measured by using the Drop Master 400AK contact angle meter (Kyowa Interface Science, Japan). A 1μL of Milli Q water was dropped onto the pristine and UV-ozone treated PLLA surface, and the contact angle was measured at an ambient condition (25 °C).

2. Additional data

2.1 Effect of UV-ozone exposure time on the enzymatic degradation rate

The vertical erosion rate of the PLLA thin film treated with UV-ozone for 15 min was also measured by using AFM in order to examine the effect of UV-ozone exposure time on the enzymatic degradation rate. The vertical erosion rate was ca. 6.3 nm min^{-1}. As shown in the text, the erosion rate of the pristine PLLA thin film was ca. 10.0 nm min^{-1}, whereas that of UV-ozone treated thin film for 30 min was ca. 5.2 nm min^{-1}. Note that longer UV-exposure time than 60 min strongly damaged the PLLA thin film possibly due to the photodegradation of the polymer chains (UV effect overcomes the UV-ozone treatment). These results indicate that there is an optimal UV-ozone treatment time for the retardation of enzymatic degradation rate. Although the UV-ozone treatment is active even for 15 min, the degradation rate was only retarded to be the 60% of pristine PLLA. Then, we fixed the treatment time of 30 min for the effective surface modification of the PLLA thin films in the present study.

2.2 Contact angle

The averaged contact angle against Milli Q water was measured on PLLA thin films before and after the UV-ozone treatment, and revealed that the UV-ozone treated PLLA surface was more hydrophilic than that of pristine one, as shown in Fig. S1.

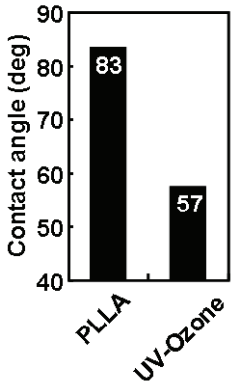

Fig. S1 Averaged contact angle of pristine and UV-ozone treated PLLA thin films.

2.3 Pattern formation after the UV-ozone treatment and enzymatic degradation

In addition to the Figs. 2(B) and 2(C), the UV-ozone treatment and UV irradiation could be used for the fabrication of different micro-patterns, which were dependent on the mask shape, as shown in Fig. S2. Various patterns could be formed on the basis of the UV-ozone treatment/UV irradiation.

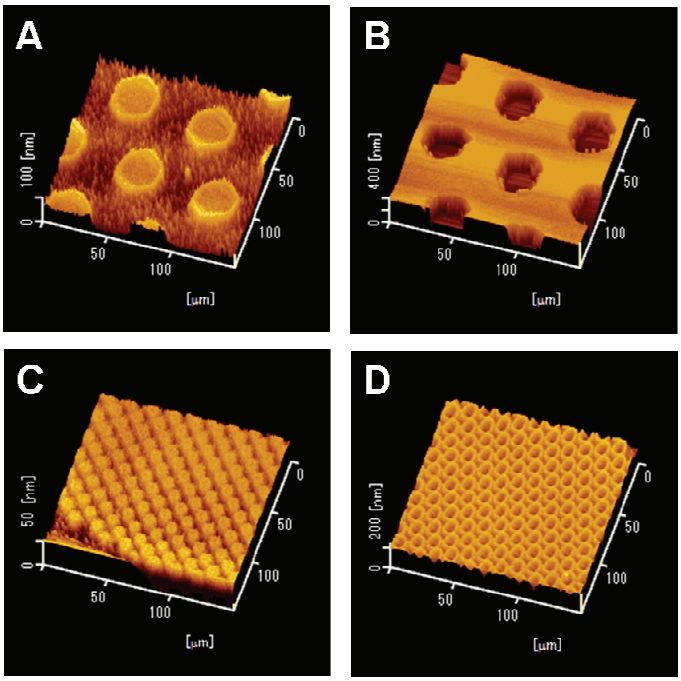

Fig. S2 AFM images of UV-ozone treated (A,C) or UV irradiated (B,D) PLLA thin films after the enzymatic degradation by proteinase K.

2.4 Effect of UV-ozone treatment on the enzymatic degradation of PCL and PHB

To reveal the versatility of a UV-ozone treatment, representative biodegradable polyesters of PCL and PHB were selected for the further experiments. Both materials were treated by the UV-zone, and enzymatic degradation behavior was compared to the pristine ones. Fig. S3 shows the time-dependent frequency change during the enzymatic degradation of the PCL and PHB thin films on a QCM sensor. In both cases, the degradation was retarded at ca. 50 %, suggesting that the UV-ozone treatment can also be applied for both polyesters.

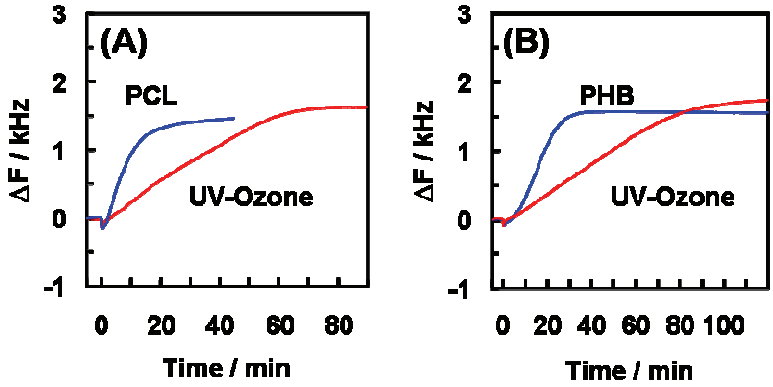

Fig. S3 Time course of enzymatic degradation in PCL (A) and PHB (B) thin films by lipase and PHB depolymerase, respectively. Blue and red curves indicate the pristine and UV-ozone treated materials, respectively.

2.5 Enzymatic lithography of PCL

In the case of PCL, the erosion depth during the enzymatic degradation by lipase was also checked, as shown in Fig. S4(A). The erosion rate was strongly retarded, as similar to the QCM results. Then, the UV-ozone treatment/UV irradiation was applied to the PCL thin films, and the optical micrographs and AFM images are shown in Figs. S4(B-E). The erosion pattern was opposite in comparison to the UV-ozone treated samples, as similar to the PLLA case.

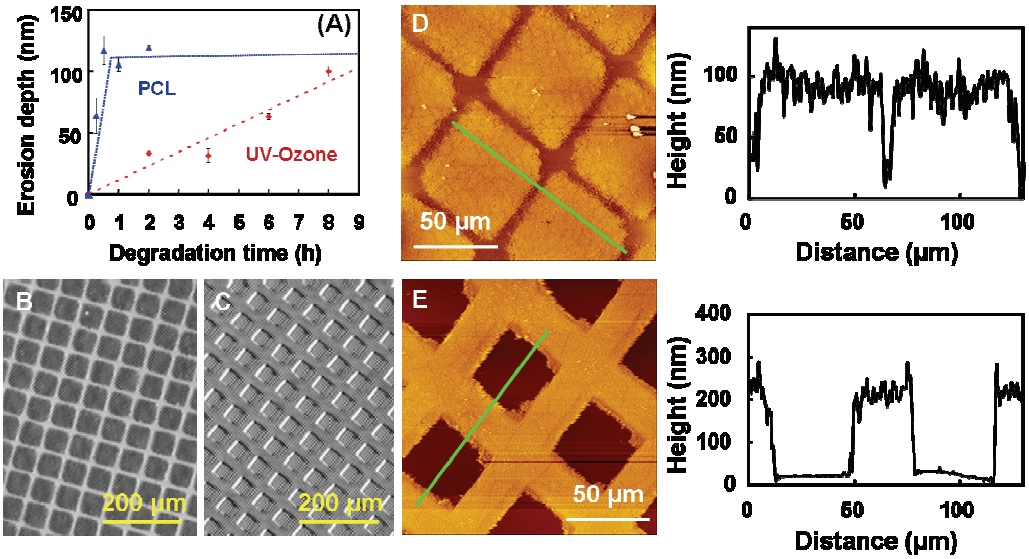

Fig. S4 Time-dependent erosion depth of PCL during the enzymatic degradation by lipase (A). The depth was measured by using AFM. Optical micrographs and AFM images of the UV-ozone treated (B,D) and UV irradiated PCL thin films (C,E). The graphs next to the AFM images are cross sectional data that the green lined region.

References

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