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Short communication

Reflectometric interference spectroscopy-based sensing for evaluating biodegradability of polymeric thin films

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ABSTRACT

Enzymatic degradation of poly(ε-caprolactone) (PCL) thin films was analyzed by reflectometric interference spectroscopy (RIfS)-based sensing system, and validated by attenuated total reflection infrared spectroscopy (ATR-IR) imaging. The degradation of the PCL thin film spin-coated on the silicon substrate on which 65-nm silicon nitride layer was deposited as an interference layer was easily monitored by shifting the peak bottom of reflectance spectra ($\Delta\lambda$) that is known to be proportional to the thickness of thin films. The $\Delta\lambda$ values decreased with increasing the concentration of lipase from *Pseudomonas cepacia*, and the obtained sensorgrams were applied for kinetic analysis using a curve fitting software. ATR-IR spectra and imaging analysis on the surface of the PCL film revealed that carbonyl groups on the surface decreased with time, resulting from proceeding with the enzymatic hydrolysis, and importantly, extinction of the carbonyl group was declined with proportional to the decrease in the film thickness measured by the RIfS system. Consequently, the present RIfS-based label-free monitoring system can provide a simple and reliable way for evaluating biodegradability on synthetic materials.

Statement of Significance

A RIfS-based sensing system in combination with ATR-IR measurements can be an analytical method for evaluation of biodegradability of polymeric thin films. This study demonstrates the utility of the RIfS-based sensing approach for analyzing the lipase-catalyzed degradation of PCL. Despite the RIfS is known as an inexpensive label-free detection method for biological interaction, the RIfS applications as monitoring methods for enzymatic degradation of biodegradable polymers had not been systematically explored. This study additionally demonstrated the capability of combined analysis of the biodegradation with ATR-IR spectra/imaging and RIfS measurements, which could be broadly applied towards evaluating biodegradability of various biodegradable polymers in environmental protection research.

1. Introduction

Biodegradable polymers such as poly(lactide), poly (ε-caprolactone) (PCL), proteins and polysaccharides have been extensively studied for biomedical applications [1,2]. Especially, enzymatic degradation of biodegradable polymers has been much paid attention to regulate degradation kinetics to achieve controlled release of drugs [3], drug targeting [4], and tissue engineering [5]. In order to understand the degradation mechanism of biodegradable polymers, both theoretical and experimental approaches have been studied; for example, Grozev et al. and Kulkarni et al. studied enzymatic chain scission kinetics of PCL by means of Langmuir monolayer method that is able to analyze progressive fragmentation of the PCL molecules [6,7] based on random fragmentation theory [8]. Yamashita et al. showed that combination of the techniques of quartz crystal microbalance (QCM) and atomic force microscopy (AFM) could be a powerful tool for analysis of enzymatic degradation of amorphous poly(L-lactide) film [9]. Also, Hou et al. reported that QCM with dissipation (QCM-D) and surface plasmon resonance (SPR) techniques could be applied for the enzymatic degradation of PCL film in relation to its crystallinity [10]. Recently, matrix assisted laser desorption ionization time of flight mass spectroscopy (MALDI TOF MS) and electrospray mass spectrometry (ESI MS) are focused on the determination of the structural architecture of biodegradable polymers in relation to their topology and chemical structure of the end groups [11].

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Reflectometric interference spectroscopy (RIfS) is a potential technique as an inexpensive label-free detection method for biological interaction including antigen-antibody interaction [12–18], glycoprotein detection [19], high-through-put screening of thrombin inhibitors [20], cell-membrane proteins detection [21,22], and whole cells detection [23,24]. The principle of *in situ* detection of biological events by RIfS is based on the measurements of spectral reflectance shift due to the change in optical thickness, defined by multiplying physical thickness (d) and refractive index (n), of the interference layer, where a part of the white light is reflected at each boundary, resulting in reflectometric interference phenomena [25]. Since optical thickness is given by multiplying physical thickness and reflective index of the interference layer, it is possible to estimate the interference layer thickness change from the RIfS response, meaning that change in physical thickness of a biodegradable polymeric thin film coated on the interference layer could be monitored by RIfS. Similarly, interferometric reflectance spectroscopy (IRS) using porous anodized aluminum oxide (pAAO) and silicon has been reported as an optical biosensor [26–28]. For example, a cationic biodegradable polymer deposition by a layer-by-layer technique on the pAAO results in increasing the net refractive index, thus red-shifting the effective optical thickness (EOT). Enzymatic degradation of the deposited film is performed, and a blue shift of the EOT is observed, indicating the dissolution of film upon the enzymatic degradation [28]. However, to the best of our knowledge, there are no reports regarding RIfS applications as monitoring methods for enzymatic degradation of biodegradable polymers; only gas sensing on spin-coated polymeric thin films was reported in conjunction with RIfS and IR absorption [29].

In this study, a RIfS-based sensing system was applied for *in-situ* monitoring of enzymatic degradation for a biodegradable polymeric thin film. Here, we used PCL as a biodegradable polymer and spin-coated it on a silicon nitride (SiN)-deposited silicon substrate on which the SiN layer (65 nm) works as an interference layer (Fig. 1(a)). The optical thickness change was monitored by using a RIfS system with upright episcopic illumination (Fig. 1 (b)) [19]. Furthermore, we applied attenuated total reflection infrared spectroscopy (ATR-IR) imaging of the PCL-coated substrate during the degradation, in order to discuss a relationship between the optical thickness change and enzymatic ester bonds cleavage.

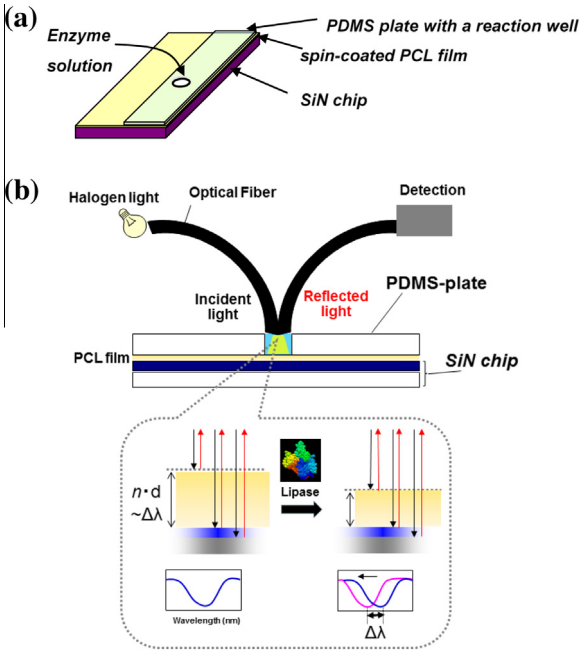

Fig. 1. Illustration of the PCL spin-coated silicon nitride deposited silicon substrates (SiN chips) covered with a PDMS plate with a reaction well for enzymatic degradation experiments (a), and whole images of the RIfS-based monitoring of enzymatic degradation of the PCL film (b).

2. Experimental section

2.1. Materials

Poly (ε-caprolactone) (PCL) (M_n = 70,000 ~ 100,000) and tetrahydrofuran (THF) were purchased from Wako Pure Chemical Co. (Osaka, Japan). Lipase from *P. cepacia* (LPS) was purchased from Sigma-Aldrich Japan (Tokyo, Japan). Silicon nitride-deposited silicon substrates (L26 × W18 × H0.725 mm or L26 × W9 x H0.725 mm) as RIfS sensor chips (SiN chips) and a RIfS-based sensor (MI-Affinity LCR-01) were purchased from Konica Minolta Technology Center, Inc. (Tokyo, Japan). An open well was fabricated on the SiN chip by placing a PDMS plate with a hall (3 mm I.D.) located right under the light source when the chip was set on the RIfS-based sensor.

2.2. Spin-coating on a SiN-based chip with PCL

PCL (17.7 mg) dissolved in THF (0.2 mL) was dropped on the SiN chip, followed by setting in a spin coater (SPINCOATER ZH-DX II, Mikasa, Co. Ltd., Tokyo, Japan). Spin coating was carried out with a rotation speed of 3000 rpm for 30 s at room temperature. In detail, the starting condition was 300 rpm for 10 s, and then the speed was changed to 2000 rpm with a slope of 540 rpm/s and kept for 5 s, followed by elevating the speed 3000 rpm with a slope of 333 rpm/s. After 20 s rotation, the speed was down to zero with a slope of 1000 rpm/s. The obtained PCL-coated SiN chip was placed in an oven for 5 h at 40 °C.

2.3. Degradation experiments using RIfS and ATR-IR

LPS was dissolved in 25 mM phosphate buffer (pH 7.0) to be 0.10, 0.25, 0.50 and 1.00 mg mL^{-1}. Each lipase solution (7 μL) was loaded into the open well fabricated on the PCL-spin coated SiN chip (Fig. 1(a)) and set in the RIfS-based sensor. After covering the well with a transparent film to prevent vaporization of the buffer, the PCL degradation was monitored by measuring shift of the peak bottom of reflectance spectra (Δλ) that is related to optical thickness continuously or at appropriate intervals.

The degradation was also monitored by ATR-IR using an FT-IR microscope and imaging system (Agilent 660-IR equipped with a Slide-on ATR attachment (ATR crystal: Germanium, light penetration depth is 0.7 μm), 620-IR microscope, and 32 × 32 multichannel MCT detector, Agilent Technologies, CA, USA). Six open wells were fabricated on a PCL-spin coated SiN chip with the PDMS plates, and 7 μL of LPS solution (0.5 mg mL^{-1}) was dropped on each well at an interval of 30 min (LPS-exposed time: 0, 30, 60, 90, and 120 min). After removing all the PDMS open wells and washing with pure water, the chip was dried and ATR-IR imaging was obtained (wavenumber range: 4000–900 cm^{-1}, wavenumber resolution: 8 cm^{-1}, integration number: 256, measurement area: 35 × 35 μm,pixel resolution: 1.1 × 1.1 μm).

2.4. Statistical analysis

Unless otherwise noted, all statistics are based on a Kruskal-Wallis one way analysis of variance on ranks. The a priori alpha