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Photoinitiator-free micro/nano fabrication of biomaterials with nonlinear deep UV excitation

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ABSTRACT

Two-photon fabrication is expected to be a technique for fabricating biological tissues for regenerative medicine and drug discovery because of its capability of fabricating 3D structures on a subcellular scale. In this study, we conducted two-photon fabrication of biocompatible materials without photoinitiators. By using a visible-wavelength femtosecond pulsed laser as excitation light, two-photon polymerization is induced in deep UV absorbing moieties without the use of photo-initiators. We performed 3D micro/nanofabrication of a biocompatible hydrogel material. By using Raman spectral change, we investigated the photo-chemical process of the biocompatible upon the irradiation of visible pulsed laser light.

Keywords: two-photon fabrication, deep-UV, 3D, biomaterial

1. INTRODUCTION

Two-photon fabrication (TPF), which utilizes photopolymerization reaction induced by two-photon absorption, has been considered as a technique suitable for the three-dimensional fabrication of micro- to nano-sized structures [1, 2]. The application to the biotechnology field has been attracting attention in recent years because of precise 3D fabrication with a feature size much smaller than cells. It has been used to create microstructures for cell culturing [3], tissue engineering [4], and microfluidics [5].

One of the issues for bio-applications of TPF is the development of photoinitiators to be added to a photopolymerizable resin. Photoinitiators absorb two photons of near-infrared light efficiently to start a photopolymerization reaction. Photoinitiators in bio-applications are required to have high biocompatibility and high water solubility in addition to the general requirement of a high two-photon absorption cross-section. However, commonly used photoinitiators decrease the viability of cells that are cultured on fabricated structures [6].

Recently, TPF without photoinitiators has been proposed by using visible femtosecond pulsed laser light [7,8]. Two-photon excitation with visible light can access one-photon absorption in the deep UV (DUV) region. In the DUV region, monomers have strong one-photon absorption coming from the typical functional groups such as C=C bond, C=O bond, and thiol. We have demonstrated photoinitiator-free TPF using nonlinear DUV excitation (DUV-TPP) by using a femtosecond pulsed laser with a wavelength of 400 nm [8]. We succeeded in 3D nano-fabrication of acrylate resin, and inorganic materials without photoinitiators. The shortened wavelength for TPF led to a smaller feature size due to the smaller focal spot size and also realized the decrease of the required excitation intensity due to the direct excitation of monomer molecules occupying all in resin. We expect the application of DUV-TPP to a biomaterial enables 3D fabrication of microstructures without loss of its biocompatibility.

In this research, we demonstrated photo-initiator TPF of a biocompatible material by using DUV-TPP. We used a hydrogel material of PEG-diacrylate (PEGda), which has been used in various biological applications [3, 9, 10]. We performed 3D fabrication of a PEGda micro-structure without any addition of photoinitiators. We measured a feature size in the fabricated structure. To confirm whether the curing of PEGda was due to a polymerization reaction, the curing

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process was investigated by Raman microscopy. By examining Raman spectral change in between before and after the irradiation of visible pulsed laser beam, we considered a photochemical reaction of PEGda upon DUV-TPP.

2. RESULTS AND DISCUSSION

In this study, a light source for DUV-TPP was a near-infrared (NIR) femtosecond pulsed laser with a center wavelength of 800 nm, a pulse width of 80 fs, and repetition of 82 MHz. The NIR pulsed laser light was focused on a β-BaB_2O_4 (BBO) crystal to obtain a visible pulsed laser light with a center wavelength of 400 nm via second-harmonic generation. The generated visible pulsed light was collimated and passed through a bandpass filter with a center wavelength of 400 nm to remove the NIR pulsed laser light. The collimated visible pulsed light was focused into resin on a coverslip by using an oil-immersion objective lens with a magnification of 100 and NA of 1.45. To fabricate a three-dimensional microstructure, the coverslip on a piezo stage was moved in three dimensions. To extract the fabricated structure in the air, the coverslip was immersed into ethanol for 15 min. and is dried with nitrogen gas. The developed 3D structure was coated with platinum with a thickness of 2 nm for scanning electron microscopy (SEM) observation.

In this study. we used pure PEGda (700 Mn). Fig.1(A) shows an SEM image of a fabricated micro-structure. We fabricated a micro-woodpile structure with a side of 5 μm. Exposure time are 500 kW/cm^2 and 4 ms/voxel. Fig.1(B) is a magnified image of the part shown with the yellow rectangle in Fig.1(A). We observed the linewidth of 120 nm. Some lines on the right part of the top surface are not drawn well. One of the possible reasons is the mismatch of refractive indices between of oil immersion (1.51) and of PEGda (1.47) [11], leading to the spherical aberration.

We obtained Raman spectra of PEGda before and after DUV-TPP. We used a continuous wave laser beam with a wavelength of 532 nm as a Raman excitation, with which PEGda shows no one-photon absorption. We used the same objective lens as DUV-TPP. Blue and red lines in Fig.2 show Raman spectrum of PEGda before and after DUV-TPP, respectively. We assigned Raman peaks in PEGda by using previous research [12]. Raman spectra are normalized with Raman peak of the O-CH_2 bond at 1469 cm^{-1}, which is not a chemical bond related to polymerization. It was found that the decrease of Raman peaks related to the C=C double bond and increase of those related to the C-C bond. Since CH_2 in-plane is connected to C=C, the planarity is lost and the Raman intensity also decreased when the C=C bond is cleaved. These results indicate C-C bonds are formed by breaking C=C bonds in monomer without photo-initiators.

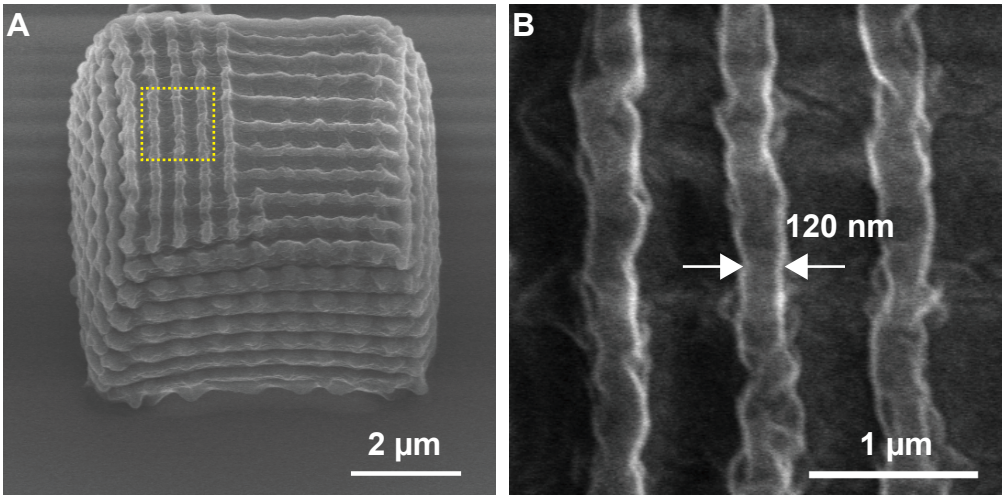

Fig.1: SEM image of a wood-pile structure fabricated by using PEGda without a photo-initiator.

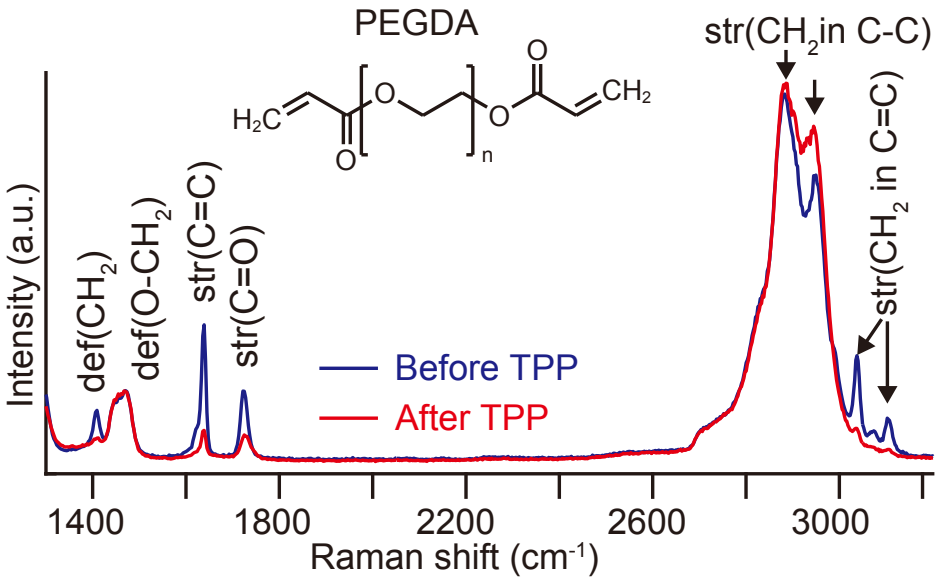

Fig.2: Raman spectra of PEGda before and after DUV-TPP using a femtosecond pulsed laser at 400 nm. The excitation for Raman spectrum was 532 nm. The objective lens (100x, N.A. 1.45) was used for both of DUV-TPP and Raman. The exposure time and excitation intensity for Raman spectrum were 10 s, and 170 mW/μm^2, respectively.

3. CO CLUSION

In this research, we performed TPF of a biocompatible hydrogel, PEGda, without photoinitiators, by using DUV-TPP with a femtosecond pulsed laser with a center wavelength of 400 nm. We achieved the 3D fabrication of a micro-scaled woodpile structure from pure PEGda. We confirmed a linewidth of 120 nm on the 3D fabricated structure. By comparing Raman spectra of PEGda before and after DUV-TPP, it is indicated that photopolymerization of PEGda happened through the cleavage of C=C bonds and the formation of C-C bonds.

4. ACKNOWLEDGEMENT

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