Journal of Applied Science and Engineering Innovation, Vol.7, No.1, 2020, pp. 8-12
ISSN (Print): 2331-9062
ISSN (Online): 2331-9070

The Synthesis and Characterizations of Water-soluble Two-photon Polymerization Photoinitiator and Its Applications for 3D Hydrogel Microfabrication

Lili Huang[1,2], Zhong Xiong[1,2], Xianzhi Zeng[3], Zhimin Zhao[1], Yaoyu Cao[3], Yanzhi Xia[2]

[1] *College of Chemistry and Chemical Engineering, Qingdao University, Qingdao 266071, China*
[2] *State Key Laboratory of Bio-fibers and Eco-textiles, Institute of Marine Biobased Material, Collaborative Innovation Center of Shandong Marine Biobased Fibers and Ecological textiles, Qingdao University, Qingdao 266071, China*
[3] *Guangdong Provincial Key Laboratory of Optical Fiber Sensing and Communications, Institute of Photonics Technology, Jinan University, Guangzhou 510632, China*

Corresponding Author: Zhong Xiong (E-mail: xiongzhong22@163.com), Yaoyu Cao (E-mail: yaoyucao@jnu.edu.cn)

Abstract: Two-photon polymerization (TPP) microfabrication (TPPM) has demonstrated the capability of fabricating ultra-precise three-dimensional (3D) microstructures, yet TPP technique has limited applications in 3D-hydrogel microfabrication due to the lack of high-efficiency photoinitiator with good water solubility. In this study, a water-soluble photoinitiator WSPI is prepared via host-guest interaction between 2,6-dimethyl-β-cyclodextrins (2,6-DM-β-CDs) and 2-benzyl-2-(dimethylamino)-4′-morpholinobutyrophenone (PI369). Threshold laser power for TPPM of the "WSPI" pregel solution is measured to 1.9 mW through line microfabrication, which is one-tenth of that reported previously. Furthermore, 3D-hydrogel microstructures are also successfully fabricated with gratifying resolutions, highlighting its potential for high-precision 3D microfabrication.

Keywords Water soluble photoinitiator, Two-photon polymerization, 3D hydrogels, Threshold power

INTRODUCTION

Hydrogels are polymeric materials with 3D network structure. Benefited by their bionic properties, hydrogels have been widely used in biomedical researches such as drug delivery, tissue engineering and so forth [Huang, *et. al.*, 2012]. Hydrogels with high structural precision could support effective simulations of extracellular matrix and great effort had been made to seek approaches for ultra-precise hydrogel fabrication. Among the existing techniques, Two-photon polymerization (TPP) renders diffraction-breaking microfabrication since polymerization is well confined in a small volume near the laser focus, making TPP a good candidate for ultra-precise 3D-hydrogel microfabrication at cellular scale [Kawata, *et. al.*, 2001].

Precedented studies of two-photon polymerization microfabrication (TPPM) of hydrogels mainly focused on the fabrications in organic phase, where cytotoxic organic solvents and surfactants were usually required to accommodate the hydrophobic photoinitiator. Meanwhile, to the best of our knowledge, the so-far reported hydrophilic photoinitiators shared problems of poor water solubility as well as poor polymerization performance. Jan Torgersen et al. fabricated 3D hydrogels in aqueous medium with laser power ranging from 60 mW to 300 mW, and the excessively high power laser could lead to a decrease in resolution and undesired structural damage[Torgersen, *et. al.*, 2012]. Xing et al. synthesized a water-soluble photoinitiator with TPP threshold power of 8.6 mW via introducing a photosensitizer dissolved in N,N-dimethylformamide (DMF) [Xing, *et. al.*, 2014]. In another work, Xing et al. prepared a water-soluble photoinitiator by combining poloxamer (PF127) and 2,7-bis(2-(4-pentaneoxy-phenyl)-vinyl)anthraquinone, the resulted photoinitiator demonstrated a TPP threshold laser power down to 6.29 mW, yet the excessive addition of surfactants also made it less biocompatible [Xing, *et. al.*, 2015]. Thus, water-soluble photoinitiators with high initiating efficiency, *i.e.*, low TPP threshold power, are still in great demand for TPPM of hydrogels.

In this work, a water-soluble photoinitiator (WSPI) was synthesized via host-guest interaction between 2,6-DM-β-CDs and 2-benzyl-2-(dimethylamino)-4′-morpholinobutyrophenone (PI369). Spectral and

morphological characterizations were conducted to investigate the newly synthesized photoinitiator, and it would be shown that the new WSPI is a high-efficiency photoinitiator in aqueous medium. Using the new WSPI, an ultra-low TPPM threshold power down to 1.9 mW with line resolution of 114 nm has been achieved. Meanwhile, 2D patterns and 3D microstructures were also successfully fabricated in aqueous medium with gratifying accuracy, exhibiting the potentials for high-precision microfabrication.

EXPERIMENTAL

Materials

2-benzyl-2-(dimethylamino)- 4′-morpholinobutyro -phenone and (3-aminopropyl) triethoxysilane were purchased from Aladdin company. 2,6-DM-β-CDs was acquired from Shandong Binzhou Zhiyuan Biological Technology Co. Ltd. Tetrahydrofuran was purchased from Jiangtian Chemical Technology Co. Ltd. Acrylamide was obtained from Beijing Chemical Reagent Company. And poly(ethylene glycol) diacrylate (PEGDA, Mn ≈ 600) was acquired from Shanghai Xushuo Biological Technology Co. Ltd.

Synthesis of WSPI

PI369 (0.1 g) was dissolved in tetrahydrofuran (10 mL) and 2,6-DM-β-CDs (1.0898 g) was dissolved in water (15 mL) at room temperature. Then, the PI369 solution was slowly added to the 2,6-DM-β-CDs solution, after which the beaker containing the mixture was bathed in a thermostatic environment (50 ^{o}C) and was stirred for 2.5 h. The reaction was halted with 8 mL of the solution remained in the vessel. After drying at 30 ^{o}C for 48h, light-yellow powder (1.0596 g, 89 %) was obtained and stored for further analyses.

Instruments and methods

The ^{1}H NMR spectra of 2,6-DM-β-CDs, PI369 and WSPI were performed by a superconducting nuclear magnetic resonance spectrometer (BRUKER AVANCE III HD 400 MHz) setting tetramethylsilane as an internal standard. PI369 was dissolved in CD_3Cl, 2,6-DM-β-CDs and WSPI were dissolved in D_2O, respectively. FTIR spectra of PI369, 2,6-DM-β-CDs, WSPI and mixture of 2,6-DM-β-CDs and PI369 (the molar ratio was 3: 1) were performed with a Thermo Scientific Nicolet iS50 Fourier transform infrared spectrometer. Absorption spectra of PI369 and WSPI were measured with a dual-beam UV-vis spectrophotometer (TU-1901) at room temperature, and fluorescence emission spectra of PI369 and WSPI were measured with a fluorescence spectrometer (Thermo Scientific LUMINA) setting the excitation wavelength and scanning speed to be 320 nm and 1200 nm min^{-1}, respectively. In both measurements, PI369 and WSPI were dissolved in CH_2Cl_2 and H_2O. And the morphologic characterization of the fabricated microstructures was conducted with a scanning electron microscopy (SEM, **FEI** QUANTA FEG 250).

Preparation of the pregel solution

Pregel solution was prepared by co-dissolving the photoiniatior (WSPI, 80 mg), monomer (acrylamide, 80 mg) and crosslinker (PEGDA 600, 120 mg) in water (400 mg). Additionally, 2,6-DM-β-CDs (50 mg) was added and stirred (10 minutes) to improve the stability of WSPI in pregel solution. Afterwards, pregel solution was well sealed to keep away from light.

TPPM of the pregel solution

As shown in Fig. 1, pregel solution was placed at the center of the glass substrate, which was pre-dipped in (3-aminopropyl)triethoxysilane/ethanol solution ((3-aminopropyl)triethoxysilane/ethanol = 4 vol%) for 12 hours to optimize the surface hydrophilicity thereby improving the adhesion performance. A femtosecond laser beam (532 nm, 210 fs, and 80 MHz) was used for initiating the nonlinear two-photon absorption (TPA) of the pregel solution and was tightly focused on the liquid resin using an oil-immersion objective lens (100×) with a numerical aperture of 1.40 (NA = 1.40). With well-defined scanning paths of the piezostage, TPPM of WSPI pregel solution was realized after loading the sample, after which unpolymerized resins were rinsed with deionized water and the sample was collected for further characterizations.

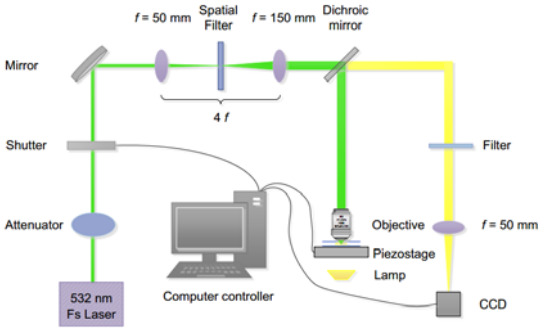

Fig. 1 schematic of experimental setup for TPP microfabrication.

RESULTS AND DISCUSSIONS

Characterizations

^{1}H NMR spectra of 2,6-DM-β-CDs (I), PI369 (II) and WSPI (III) are shown in Fig. 2(a). As depicted by spectrum III, proton peaks that are supposed to be attributed to the benzene protons of PI369 appear at 6.8-8.4 ppm. And the two peaks, which are identified to be attributed to the heterocycle of PI369, show up at 3.9 and 2.3 ppm. Besides, the peaks at approx. 0.7, 2 and 3-4 ppm, which are believed to be contributed by the alkyl chain of PI369, demonstrate lower intensities. The above ^{1}H NMR study implies that the heterocycle of PI369 (guest) has been incorporated into 2,6-DM-β-CDs (host) via host-guest interaction.

To prove that WSPI was not the plain physical mixture of 2,6-DM-β-CDs and PI369, we next investigated the FTIR spectra of PI369 (I), 2,6-DM-β-CDs (II), a physical mixture of PI369 and 2,6-DM-β-CDs (III) and WSPI (IV), as shown in Fig. 2(b). It can be seen that spectrum III could be regarded as the superposition of I and II: characteristic peaks marking the carboxyl groups and benzene ring of PI369 as well as the hydroxyl groups of 2,6-DM-β-CDs could simultaneously be found in III. However, in spectrum IV, characteristic peaks representing the benzene ring of PI369 still appear at 1596 cm^{-1}, 755 cm^{-1} and 700 cm^{-1} but with lower intensities. Meanwhile, the carboxyl band of PI369 exhibits a blue shift of 50 cm^{-1} while the peak shape becomes broader with peak intensity being significantly decreased. Moreover, the characteristic peak of 2,6-DM-β-CDs, which is expected to be observed at 1642 cm^{-1}, almost declines to zero. These could be ascribed to the formation of O-H...O and C-H...O hydrogen bonds between the host and guest and the FTIR results further suggest the occurrence of the host-guest interaction and that WSPI is not just the physical mixture of 2,6-DM-β-CDs and PI369.

UV-vis absorption and photoluminescence properties of WSPI were then tested. As shown in Fig.2(c-d), the absorption peaks of WSPI (red line) appear at approx. 229 nm and 318 nm, which are blue-shifted from the 232 nm and 322 nm peaks of PI369 (black line). Furthermore, the fluorescence peak of WSPI demonstrates a red shift of 5 nm from that of PI369 (400 nm) and appears at 405 nm. The deviations further support our conclusion that the guest had been successfully included into the host, and the change of electronic configurations is reflected on the shifts of the absorption and emission peaks.

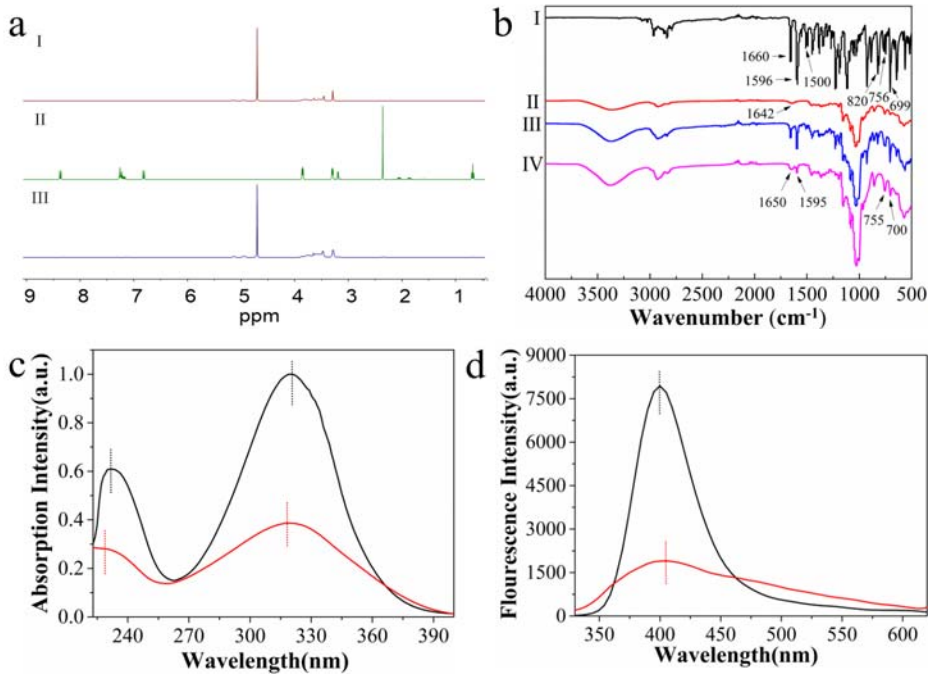

Fig. 2 (a) ^{1}H NMR spectra (400 MHz) of 2,6-DM-β-CDs (I), PI369 (II) and WSPI (III); (b) FTIR spectra of PI369 (I), 2,6-DM-β-CDs (II), a physical mixture of PI369 and 2,6-DM-β-CDs (III) and WSPI (IV); (c) Normalized UV-vis of PI369 (black line) and WSPI (red line). WSPI and PI369 are dissolved in H_2O and CH_2Cl_2, respectively. (d) Fluorescence of PI369 (black line) and WSPI (red line). The excitation wavelength for fluorescence measurement is 320 nm. WSPI and PI369 are dissolved in H_2O and CH_2Cl_2, respectively.

Solubility of WSPI

The resulted WSPI demonstrates optimized water solubility and could be well dissolved in deuterated water. Solubility of WSPI in water was determined to be 48 g / 100 mL at room temperature, which was about 28-times better than the commercially available water-soluble photoiniator Irgacure 2959 (1.7 g / 100 mL)[Liska, *et. al.*, 2014].

Two-photon polymerization microfabrication of pregel solution

To characterize the initiation efficiency of WSPI, linear writing tests were conducted to determine the lowest laser power required to fabricate polymer lines at a constant linear-scanning speed of 10 μm s^{-1}. As shown in the in Fig.3(a), as the laser power shrink down from 4.1 mW to 1.9 mW, linewidth of the fabricated polymer lines gradually narrow down (Fig. 1c) and reach the minimum of approx.114 nm (Fig. 1b), which indicates that 1.9 mW is the critical value that could provide sufficient energy to form mechanically robust microstructures on the glass

substrate. The ultralow threshold energy of 1.9 mW is about one-tenth of the previously reported value of 20 mW in Jhaveri's work [Jhaveri, *et. al.*, 2009].

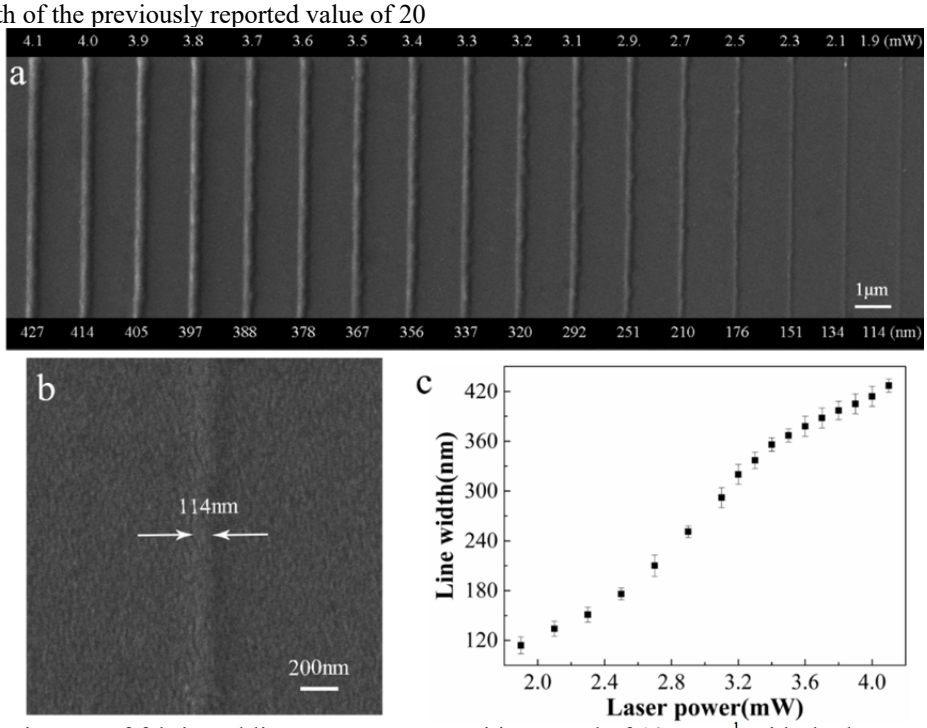

Fig. 3 (a) SEM images of fabricated lines at a constant writing speed of 10 μm s^{-1} with the laser power ranging from 1.9 mW to 4.1 mW. (b) Magnified SEM image of the line of 114 nm at the power of 1.9 mW. (c) Laser power-line width relationship of (a).

2D patterns and 3D microstructures were also fabricated to examine the potential of WSPI for 2D/3D microfabrications. As demonstrated in Fig.4 (a-c), geometric figures of line, 45^{o} angle, equilateral triangle, square and circle were designed and successfully fabricated on glass substrate with powers ranging from 3 mW to 5 mW. Morphology of the microstructures in Fig.4 (a) reveals low structural contrasts since the employed laser power is inadequate to produce sufficient radical ions for TPP. And the morphology becomes better with increased power (Fig.4 (b-c)) because of sufficient radical ions to support thorough polymerization. 3D hydrogel microstructures were also successfully acquired with gratifying spatial resolution utilizing the efficient WSPI pregel solution (Fig.4 (d-e)), indicating its great potential for complex 3D hydrogel microfabrication.

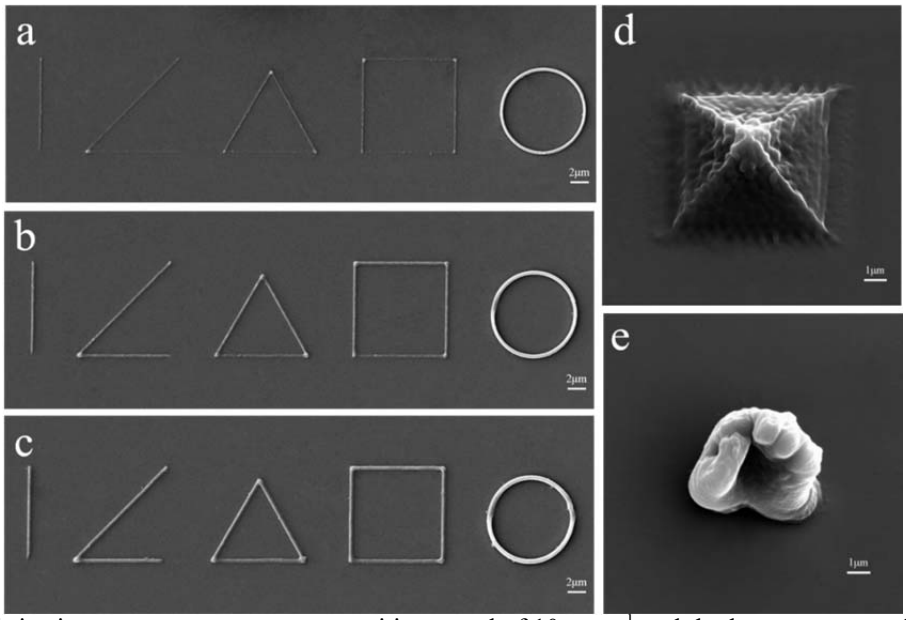

Fig. 4 (a-c) Fabrication geometry at a constant writing speed of 10 μm s^{-1} and the laser power are 3 mW, 4 mW and 5 mW, respectively. (d) The pyramid structure was fabricated with laser power of 3 mW and a scanning

speed of 30 μm s^{-1}. (e) Prepare the hand structure of OK with laser power of 3 mW and a scanning speed of 20 μm s^{-1}.

CONCLUSION

To summarize with, we have successfully prepared a water-soluble photoinitiator (WSPI) through host-guest chemical interaction. The threshold laser power of the WSPI pregel solution for realizing photopolymerized features is pushed down to 1.9 mW with a spatial resolution of 114 nm, which is much lower than the previous record and could provide promising accuracy for TPPM. The synthesized photoinitiator, WSPI , exhibits good water solubility and extraordinary photoinitiating property, offering a viable solution for TPPM of 3D hydrogel in aqueous medium.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (61405100) and the Natural Science Foundation of Shandong Province (ZR2012EMQ006). Dr. Yaoyu Cao thanks the financial supports from the National Science Funds of China (Grant No. 61605061, 61522504), the Natural Science Foundation of Guangdong Province (Grant No. 2016A030313088) and Guangdong Provincial Innovation and Entrepreneurship Project (Grant 2016ZT06D081).

REFERENCES

Coenjarts C A, Ober C K. Two-Photon Three-Dimensional Microfabrication of Poly(Dimethylsiloxane) Elastomers [J]. Chemistry of Materials, 2004, 16(26), 5556-5558.

Huang G, Wang L, Wang S Q, et al. Engineering three-dimensional cell mechanical microenvironment with hydrogels[J]. Biofabrication, 2012, 4(4), 042001.

Jhaveri S J, Mcmullen J D, Sijbesma R, et al. Direct Three-Dimensional Microfabrication of Hydrogels via Two-Photon Lithography in Aqueous Solution [J]. Chemistry of Materials, 2009, 21(10), 2003-2006.

Kawata S, Sun H B, Tanaka T, et al. Finer features for functional microdevices[J]. Nature, 2001, 412(6848), 697-698.

Liska R, Herzog D. New photocleavable structures. II. α-Cleavable photoinitiators based on pyridines[J]. Journal of Polymer Science Part A Polymer Chemistry, 2004, 42(3), 752-764.

Peppas N. Hydrogels in biology and medicine: from fundamentals to bionanotechnology[J]. Adv. Mater. 2006, 18(11), 1345-1360.

Tomatsu I, Peng K, Kros A. Photoresponsive hydrogels for biomedical applications[J]. Advanced drug delivery reviews, 2011, 63(14-15), 1257-1266.

Torgersen J, Ovsianikov A, Mironov V, et al. Photo-sensitive hydrogels for three-dimensional laser microfabrication in the presence of whole organisms[J]. Journal of Biomedical Optics, 2012, 17(10), 105008.

Torgersen J, Qin X H, Li Z, et al. Hydrogels for Two-Photon Polymerization: A Toolbox for Mimicking the Extracellular Matrix[J]. Advanced Functional Materials, 2013, 23(36), 4542-4554.

Two-photon polymerization microfabrication of hydrogels: an advanced 3D printing technology for tissue engineering and drug delivery[J]. Chem. Soc. Rev. 2015, 44(15), 5031-5039.

Xing J, Liu L, Song X, et al. 3D Hydrogels with High Resolution Fabricated by Two-photon Polymerization with Sensitive Water Soluble Initiator[J]. Journal of Materials Chemistry B, 2015, 3(43), 8486-8491.

Xing J, Liu J, Zhang T, et al. A water soluble initiator prepared through host-guest chemical interaction for microfabrication of 3D hydrogels via two-photon polymerization[J]. Journal of Materials Chemistry B, 2014, 2(27), 4318-4323.

Xiong Z , Zheng M L , Dong X Z , et al. Asymmetric microstructure of hydrogel: two-photon microfabrication and stimuli-responsive behavior[J]. Soft Matter, 2011, 7(21), 10353-10359.