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Study on a series of water-soluble photoinitiators for fabrication of 3D

hydrogels by two-photon polymerization

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Abstract

A series of water-soluble benzylidene cyclanone dyes **T1-T3** were synthesized. Their photo-physical properties were investigated using UV-Vis spectra, fluorescence spectra and two-photon absorption spectra. The maximum two-photon absorption cross-sections (σ_2) of **T1-T3** in deionized water were determined as 567 GM, 808 GM and 231 GM. Using **T1-T3** as photoinitiators (PIs) directly, 2D and 3D nano patterns based on two-photon crosslinking or polymerization of bovine serum albumin (BSA), water-soluble acrylic ester monomer (SR610) and hyaluronic acid derivative (HAGM) were successfully fabricated, respectively. Much lower threshold energies and wider fabrication windows were obtained in the formulations containing these PIs compared with commercial ones. Furthermore, the cytotoxicity study showed a favorable biocompatibility of these PIs, which indicated **T1-T3**

would have application prospects in 3D fabrication of biomaterials.

Keywords: Water-Soluble Photoinitiator; Two-Photon Polymerization; Biomaterials; Tissue Engineering.

Introduction

Tissue engineering is a fresh discipline to study the growth of a fully functional tissue or organ from seed cells and a collagenous scaffold for a medical purpose. It is a combination of cell biology and material science. In human tissues, the natural extracellular matrix (ECM) serves as a 3D substructure, which can be constantly remodeled. Through its intimate interaction with cells, ECM regulates cell differentiation, proliferation, exchanging nutrients and metabolites. ^[1-2] In order to mimick the natural ECM, study on the development of 3D scaffolds with good biocompatibility and proper mechanical strength is of very importance. ^[3-6] Hydrogels are attractive biomaterials for numerous medical applications. Due to their similar mechanical and diffusivity properties to ECM, a number of synthetic and natural hydrogels, such as, PEGDA, Gelatin, Collagen, HA and Lignin, ^[7-11] have been developed to explore their potentials in Tissue engineering.

Two-photon polymerization (2PP) is a powerful tool to construct 3D matrixes with micro- and nanometer precision due to its advantages of large penetration depth and high spatial selectivity. Moreover, with the low photon energy of near infrared light normally used in 2PP, 3D manipulation can be performed in the presence of cells, which guides the formation of biological tissue in all stages of its development. Thus, using 2PP technique to fabricate 3D hydrogels based on natural or artificial

materials under an aqueous environment has attracted much attention in recent years.^[12-15]

Water-soluble photoinitiators (PIs) with two-photon absorption (2PA) capability are the first prerequisite for 2PP based on hydrogels. However, till now the corresponding reports are very limited. Campagnola et al. studied 2PP of acrylamide and two-photon crosslinking (2PC) of protein in aqueous solution using commercially available hydrophilic xanthene dyes, such as Rose Bengal, Eosin and Erythrosine, with amino as coinitiator, and fabricated simple 3D models. [16] However, because the 2PA cross-sections (σ_2) of these dyes were very small (only 10 GM at 800 nm), a high laser intensity (100 mW) and long exposure time (300~400 μ s) were required in their experiments. By using a water-soluble PI (WSPI) with a σ_2 of 120 GM, Liska et al. successfully fabricated 3D scaffolds from PEGDA (PEG diacrylates) with a fast scanning speed of 10 mm/s under laser power of 140 mW.^[17] Irgacure 2959, a commercially available hydrophilic PI, was also used for 2PP of 3D scaffolds due to its good biocompatibility. However, it is only suitable for 2PP at 515 nm wavelength, which may induce the denaturation of proteins.^[18-19] Herein, the exploration of highly active water-soluble PIs with large σ_2 values in near infrared range is a critical requirement.

In our previous work, it was proved that benzylidene cyclanone dyes had large σ_2 values (400~3300 GM) around 800 nm and could be used as

high-efficient photosensitisers or even photoinitiators directly in 2PP based on organo-soluble materials. ^[20-22] Thereafter, through modifying benzylidene cyclopentanone dyes with sodium carboxylate groups, a series water-soluble dyes were synthesized. Among them, BSEA (modified by four sodium carboxylate groups) showed a high efficiency on initiating 2PP of water-soluble acrylate ester (SR610). ^[23,24] Liska et al. also reported similar benzylidene cyclopentanone and cyclohexanone dyes (P2CK and G2CK), which were modified by two sodium carboxylate groups at the terminal alkyl chains, and proved their availability in 2PP or 2PC of biocompatible materials. ^[25-26] These aforementioned works preliminarily revealed the potential of water-soluble benzylidene cyclanone dyes in biomedicine fields.

In our latest study on biocompatibility of some hydrophilic dyes, it was found that the cytotoxicity of a dye was positively correlated with its amount of cellular uptake. For a similar molecular structure, introducing more hydrophilic groups is beneficial to decrease cellular uptake and enhance biological safety. ^[27-28] In this work, we synthesized a series of hydrophilic dyes using a high-efficient route different from that of BSEA through introducing four sodium carboxylate groups into different benzylidene cyclanone moieties (cyclobutanone, cyclopentanone and cyclohexanone) to further exploit the application of such dyes in tissue engineering. The specific structures of designed PIs (**T1-T3**) are shown in

Scheme 1.

2 Experimental

2.1 Materials

glycidyl Cyclobutanone, cyclopentanone, cyclohexanone and methacrylate were purchased from Arcos Organics; acrylic acid, ethanol, aniline, N,N-dimethylformamide(DMF), phosphorus pentachloride, p-benzenediol, methyl acrylate, light petroleum, acetic ether, dimethyl sulfoxide, Eosin and NaOH were bought from Beijing Chemical Co. Ltd. Chromatographically pure methanol were obtained from Fisher Scientific Company. Other chromatographically pure reagents were from Xilong Chemical Co. Ltd. SR610 was purchased from Sartomer Co. Ltd. Bovine serum albumin (BSA), phosphate buffered solution (PBS), Dulbecco's Modified Eagle's Medium (DMEM), Fetal Bovine Serum (FBS), Penicillin/Streptomycin and MTT reagent were obtained from Beijing Solarbio Science & Technology Co. Ltd. HepG2 was donated by Peking Union Medical College Hospital. Sodium hyaluronate and Poly (ethylene glycol) diacrylate (PEGDA) were from Aladdin, Hyaluronic acid derivative (HAGM) with a degree of substitution 61% was prepared according to the literature. [29] The synthesis and characterization of HAGM were presented in Supporting Information. Y2 and Y3 were synthesized on the basis of protocols reported by our group before. ^[24] The structures of **Y2** and **Y3** are shown in **Figure 1**. All materials were

used directly without further purification.

2.2 Synthesis and characterization

The synthetic routes towards compounds T1-T3 are shown in Scheme 1.

All final compounds were characterized by ¹H-NMR, ¹³C-NMR,

HR-MS and HPLC analysis, conforming a purity of over 95%.

2.2.1. Dimethyl 3, 3'-(phenylazanediyl) dipropanoate (M1).

Aniline (9.31 g, 0.10 mol), methyl acrylate (26.40 g, 0.30 mol), ethylic acid (12.12 g, 0.20 mol) and 0.32 g p-benzenediol were added into a 100 ml round-bottom flask and refluxed for 20 hours at 102°C. After the reaction, excess of the methyl acrylate and ethylic acid were excluded by vacuum distillation, the compounds were purified by Column Chromatograph (silica, light petroleum/CH₃COOCH₂CH₃=8:1) and 25.20 g pure liquid **M1** was obtained. (Yield 95.05%). ¹H-NMR (400MHz, CD₃OD): δ (ppm): 2.53 (t, J = 8 Hz, 3H), 3.49 (s, 4H), 3.62 (s, 6H), 6.64(t, J = 8 Hz, 1H), 6.69~6.71 (m, 2H), 7.15(t, J = 8 Hz, 2H). HR-MS (ESI) theoretical m/z [M+1H]⁺=266.1392, found 266.1378.

2.2.2. Dimethyl 3, 3'-((4-formylphenyl)azanediyl) dipropanoate (M2).

 PCl_5 (20.82 g, 0.10 mol) was added slowly to DMF (50 mL) and the solution was cooled in an ice bath. After stirring at 0°C for 30 min, the solution was continuously stirred at room temperature for another 15 min, and then **M1** (12.75 g, 0.05 mol) was slowly added. After stirring at 75°C for 1 h and then cooled at room temperature, the solution was added to

ice water and neutralized with saturated sodium bicarbonate solution. The aqueous layer was extracted three times with CHCl₃ (3×50 mL) and the extraction liquid was washed with a large amount of water. The organic phase was dried over anhydrous MgSO₄ and rotary evaporation, the compounds were purified by Column Chromatograph (silica, light petroleum/CH₃COOCH₂CH₃ =5:1) to give 13.81 g yellow liquid **M2**. (Yield 97.96%). ¹H-NMR (400MHz, CD₃OD): δ (ppm): 2.69 (t, J = 8 Hz, 4H), 3.65 (s, 6H), 3.82 (t, J = 8 Hz, 4H), 6.87~6.89(d, J = 8 Hz, 2H), 7.72~7.74 (d, J = 8 Hz, 2H), 9.74(s, 1H). HR-MS (ESI) theoretical m/z [M+1H]⁺=294.1341, found 294.1328.

2.2.3. Sodium 3,3',3",3"'-(((((2-oxocyclobutane-1,3-diylidene)bis (methanylylidene))bis(4,1-phenylene))bis(azanetriyl))tetrapropanoate
(T1)

M2 (1.05 g, 3.58 mmol) and NaOH (0.29 g, 7.25 mmol) were added to 50 ml H₂O and stirred for a night at room temperature. Then most of the water was excluded by rotary evaporation. NaOH (0.07 g, 1.75 mmol) and cyclobutanone (0.12 g, 1.71 mmol) were added to the above solution and stirred at 80°C for 5 h. After the system cooling down to room temperature, ethanol (50 ml) was added and stirred for 2 h at room temperature. The deep red compound **T1** (0.81 g) was recrystallized from ethanol. (Yield 70.93%). ¹H-NMR (400MHz, D₂O): δ (ppm): 2.43 (t, J=8 Hz, 8H), 3.63 (t, J = 8 Hz, 8H), 3.74 (s, 2H), 6.83~6.85 (d, J=8 Hz, 4H),

7.13 (s, 2H), 7.55~7.57 (d, J=8 Hz, 4H). ¹³C-NMR (100 MHz, D_2O) : δ (ppm): 193.30, 181.35, 150.33, 139.92, 133.35, 130.23, 122.88, 112.96, 48.47, 36.26, 35.14. HR-MS (ESI) theoretical m/z [1/2(M-2Na)+1H]⁺=304.2628, found 304.2608.

2.2.4.Sodium

3,3',3",3"'-((((1E,1'E)-(2-oxocyclopentane-1,3-diylidene)bis(methanylylid ene))bis(4,1-phenylene))bis(azanetriyl))tetrapropanoate (**T2**)

Sodium

3,3',3",3"'-((((1E,1'E)-(2-oxocyclohexane-1,3-diylidene)bis(methanylylid ene))bis(4,1-phenylene))bis(azanetriyl))tetrapropanoate (**T3**)

The deep red compounds **T2** and **T3** were obtained by a similar procedure as described above for **T1**.

T2 (yield 88.67%). ¹**H-NMR (400MHz, D**₂**O**): δ (ppm): 2.43 (t, J=7.4 Hz, 8H), 2.76 (s, 4H), 3.54(t, J=7.4 Hz, 8H), 6.74 (d, J=8.8 Hz, 4H), 7.18 (s, 2H), 7.46 (d, J=8.8 Hz, 4H). ¹³**C-NMR (100 MHz, D**₂**O**) : δ (ppm): 198.74, 181.26, 149.36, 135.93, 134.37, 127.29, 124.17, 112.85, 48.46, 36.01, 27.03. **HR-MS (ESI)** theoretical m/z [M–4Na+5H]⁺ =579.2347, found 579.2337.

T3 (yield 69.33%). ¹**H-NMR** (**400MHz**, **D**₂**O**): δ (ppm): 1.73 (s, 2H), 2.40 (t, J=6 Hz, 8H), 2.85 (s, 4H), 3.59 (t, J=8 Hz, 8H), 6.80~6.82 (d, J=8 Hz, 4H), 7.50~7.52 (d, J=8 Hz, 4H), 7.58 (s, 2H). ¹³**C-NMR (100 MHz, D**₂**O**) : δ (ppm): 193.77, 181.66, 149.17, 139.28, 134.30, 133.05, 123.92, 112.64, 48.43, 36.18, 28.87, 23.22. **HR-MS** (**ESI**) theoretical m/z $1/2[M-4Na+2H]^{2}=295.1149$, found 295.1133.

2.3 Methods

UV-vis spectra were recorded on a JascoV-530 spectrophotometer. Steady-state fluorescence was performed at room temperature using Hitachi F-4500 spectrometer. Fluorescence quantum yields (Φ_f) in water were measured using rhodamine B dissolved in methanol as a standard $(\Phi_f = 0.7)$.^[30] The singlet oxygen quantum yield (Φ_A) was determined by a photochemical bleaching method using 1,3-Diphenylbenzofuran (DPBF) as ¹O₂ acceptor and methylene blue (MB) as the reference with a yield of 0.75 in CH₃OH.^[31] ¹H-NMR and ¹³C-NMR spectra were obtained on a Bruker DPX 400 spectrometer. The HR-MS analyses were carried out on a Bruker apex IV FT mass spectrometer. 2PP fabricated microstructures characterized by scanning were electron microscopy (SEM, HitachiS-4300FEGd).

2PA cross-section (σ_2) values of compounds were determined using two-photon excited fluorescence (2PEF) method with a femtosecond laser.

In 2PEF measurement, the light source is a Tsunami mode-locked Ti: sapphire system (750~840 nm, 80 MHz, <130 fs). Up-converted fluorescence spectra were recorded by a fiber spectrometer (Ocean Optics USB2000 CCD). The concentrations of samples were 5.0×10^{-5} M,

 1.0×10^{-4} M and 1.0×10^{-4} M for **T1-T3**, respectively. Rhodamine B in methanol solution (10^{-6} M) was used as reference. The 2PA cross-section of sample was calculated by equations reported previously.^[32]

In 2PP experiments, **T1-T3** and Eosin were used as PIs, monomers were SR610, BSA and HAGM. All formulas are showed in **table 1**. The mixed photo-curable resins were poured onto glass substrate. The laser (Tsunami Ti: sapphire, 820 nm, 82 MHz, 80 fs) was tightly focused via an oil-immersion objective lens (100×, NA=1.45, Olympus) into the sample which was fixed on a xyz-step motorized stage controlled by a computer. The fabrication process was monitored by a CCD on live, after the fabrication, the unpolymerized resin was washed out by deionized water. 2PP linear structures and 2PC patterns were watched by Nikon TI-U inverted microscope and recorded by Low Temperature refrigeration high touch bar camera (PIXIS: 100B). The obtained 3D microstructures were characterized by SEM (HITACHI S-4300F).

The resins **R2-R4** (listed in Table 1) were used in one-photon polymerization experiments. The mixed solution was poured into a glass cell made in our laboratory, the thickness of the sample was 0.5 mm. A 532 nm laser was used as light source. Its intensity of irradiation was 60 mW/cm². The conversion rate of double bonds during the photocuring of the formulation was recorded by real-time Fourier transform infrared spectroscopy (Nicolet Magna 5700 real-time FTIR). The polymerization

conversion efficiency was calculated by the change of the double bond absorption peak area at 6082-6230 cm⁻¹ in near-IR region.

The evaluation of biocompatibility of **T1-T3** was carried out by MTT assay using hepatoma cells HepG2, while Y2 and Y3 were used as contrasts. The cells were incubated in the culture media of DMEM containing 10% FBS and 1% Penicillin/Streptomycin. HepG2 cells were seeded onto a 96 well plate at a density of 1×10^4 cells per well in 100 µl culture media and incubated for 24 h for cells to attach in an incubator in a humid atmosphere with 5% carbon dioxide at 37°C. After that, 100 µl culture media containing different concentrations of PIs (T1-T3, Y2 and Y3) were added into the 96 well plates to achieve the final PIs' concentrations of 0.6, 0.9, 1.3, 2.0 and 3.0 mM in wells, respectively. The culture media and the culture media plus HepG2 were used as blank group and control group, respectively. After another 24 h incubation, 20 μ l MTT reagent (dissolved in PBS, 5 g/L) was added into the plates and well-distributed, then the 96 well plates were put back into the incubator for additional 4 h incubation. Afterwards, the culture media was removed and all wells were washed three times with PBS, 150 µl dimethyl sulfoxide was added into the wells to dissolve the formazan crystals. The plates were incubated on a shaker in the dark at room temperature for 20 min, and the absorbance in each well was measured at 570 nm on a Multi-mode microplate detecting instrument (Perkin Elmer).

3. Results and discussions

3.1 Photo-physical properties

The normalized absorption spectra and the florescence emission spectra of **T1-T3** in deionized water are shown in **Figure 2.** Their comprehensive photo-physical characteristics are listed in **Table 2.** It is shown that all of the three compounds present a strong intramolecular charge transfer (ICT) absorption band within 400~650 nm. This absorption band is attributed to symmetrical charge transfer from the electron-donating amino groups on both ends to the central electron-withdrawing carbonyl group. Compared with **T1**, the absorption peaks of **T2** and **T3** exhibit a blue-shift of 9 nm and 44 nm, respectively, while their emission peaks also have similar shifts to the short wavelength region, which should be due to the increase of the ring size. From **T1** (cyclobutanone) to **T3** (cyclohexanone), the planarity of the cross-conjugated system decreases, which diminishes the delocalization of the π electrons.

The Stokes shift of **T3** is much larger than that of **T1** and **T2**, which means that the geometry change between the ground state and the first excited state of **T3** is much greater compared with the other two compounds. A decrease in fluorescence quantum yield (Φ_f) can be observed with an increase of the size of the middle ring from **T1** to **T3**, which should be due to the better flexibility of the larger ring, thus promoting non-radiative relaxation of electronic excited states.

The Φ_{Λ} value of a compound is positively correlated with its photocytotoxicity after being taken by cells or tissues when it is exposed to light. All of **T1-T3** present a low Φ_{Λ} data within 0.01~0.02, which ensures a certain security when they are applied in biological fields.

3.2 2PA cross-sections

Using the 2PEF method, the two-photon-excited spectra of compounds **T1-T3** in deionized water within 750~840 nm were measured using Rhodamine B as a reference. The results are showed in **Figure 3**. It can be seen that the 2PA peaks of three compounds within the measured range all appear in 820 nm. The maximum σ_2 values of **T1-T3** are 567 GM, 808 GM and 231 GM, respectively, which are much higher than the corresponding data of commercial hydrophilic initiators. It is known that the 2PA intensity of a compound is strongly dependent on its intramolecular charger transfer (ICT), which is critically relative with its co-planarity. This result further confirms that the cross-conjugated system is distorted by the flexible six-membered ring of cyclohexanone.

3.3 One-photon polymerization

The monomer's double bond conversion (DBC) is an efficient indicator to character the initiation capacity of a PI. A higher conversion of monomers means better initiation efficiency of PIs. The conversion efficiency of three resins **R2-R4** with SR610 as monomer containing **T1-T3** as PI, respectively, was characterized by plotting the DBC during

the polymerization against the time (**Figure 4**). It can be easily found that in the same formula of resins, **T2** exhibits the best initiation efficiency.

3.4 Two-photon polymerization

A series of resins were made up using different formula with SR610, BSA and HAGM as monomers, **T1-T3** as initiators, respectively, to investigate their potentials in 2PP. Blank monomers and monomers plus Eosin were both used as contrasts. Using line scanning method, the 2PP threshold energy (\mathbf{E}_{th}) and the laser-induced breakdown threshold energy (\mathbf{E}_{br}) of these resins under illumination of an 820 nm fs laser could be observed by CCD on live. Here, \mathbf{E}_{th} is defined as the lowest laser power at focus point that can fabricate a solid line and \mathbf{E}_{br} is defined as lowest laser power at focus point that can induce intense damage to materials under the same scan speed. The power between \mathbf{E}_{th} and \mathbf{E}_{br} is defined as the fabrication window (\mathbf{FW}). ^[33] The obtained \mathbf{FW} (\mathbf{E}_{th} and \mathbf{E}_{br}) data for PIs in all formulas are listed in **Table 1**.

It is pursued for a formula to have a broad **FW** and lower \mathbf{E}_{th} in application, which can ensure a high quality and efficiency in mass production. In **Table 1**, it is shown that **T1-T3** all exhibit lower 2PP \mathbf{E}_{th} than that of Eosin when a same monomer is used. Furthermore, it is obvious that **T2** performs an overwhelmingly excellent fabrication capacity in SR610. The \mathbf{E}_{th} of resin **R3** (containing **T2** as PI) is much lower than those of other SR610 resins, while the corresponding

differences among BSA or HAGM resins are limited. It should be due to the lack of enough reactive groups in BSA or HAGM monomers so that they are not sensitive to the amounts of free radicals or other active species generated by PIs.

For **R1-R4**, both of 2D and 3D nanopatterns can be fabricated by 2PP. A representative line array obtained using **R2** (**T1** as PI) by changing the incident energies with a fixed line scan speed (10μ m/s) and a carbon nano-tube from **R3** (**T2** as PI) with 0.72mW power and 110 µm/s scan speed are shown in **Figure 5a** and **5b**, respectively. Due to the poor rigidity of protein BSA, only 2D nanopatterns were obtained using **R6-R9** by 2PC. **Figure 5c** displays the designed model (left) and real pattern (right) of the 2D logo of our laboratory made using **R8**.

All 3D structures fabricated using **R11-R14** based on HAGM partially collapsed. A typical result is shown in **Figure 6a**. It should be due to their poor mechanical strength. As mentioned above, the reactive groups in both BSA and HAGM are limited, which may result in low degree of crosslinking or polymerization. Moreover, the water contents of **R5-R9** and **R11-R14** are rather high, which will decrease the rigidity of the final structures. Through adding some PEGDA into **R11-R14**, a new formula (10 wt% HAGM, 5 wt% PEGDA, 0.16 wt% PI and 84.84 wt% H₂O) was studied. A well-developed 3D carbon nano-tube was obtained, as shown in **Figure 6b**. This indicates that HAGM may have potentials in cells

adhesion and encapsulation through improving its mechanical property, the real performance will be further investigated.

3.5 Cytotoxicity Studies

Biocompatibility is a vitally important factor for the application of water-soluble PIs. Figure 7 displays the cell viability after 24 h cultivation in substrates containing these PIs with a concentration of 3 mM, which approximates to the concentration of **T1-T3** used in the 2PP. It is shown that Y2 and Y3 have severe cytotoxicity and over 95% of HepG2 cells are dead, while nearly 60% of HepG2 cells keep alive incubated with T1-T3 for 24 h. The differences among Y2, Y3 and T2 are mainly the number of sodium carboxylate groups. Obviously, introducing more sodium carboxylate groups is beneficial to achieve a better biological safety for these compounds, which should be due to the improved water solubility and lowered cellular uptake of compounds by modifying more water-soluble groups as we reported. ^[27-28] With the increased size of the middle ring from T1 to T3, there is a gradual enhancement of the cell viability, which could be owing to their different molecular structures or different cellular uptakes. Overall, **T3** enjoys the best biocompatibility at this concentration.

4. Conclusion

Three water-soluble benzylidene cyclanone dyes **T1**, **T2** and **T3** were designed and synthesized by introducing four sodium carboxylate groups

into different benzylidene cyclanone moieties (cyclobutanone, cyclopentanone and cyclohexanone, respectively) through simple and high-efficient Michael addition reaction and Aldol reaction. Due to their different middle ring, these compounds exhibited different photo-physical and photo-chemical properties. Compared with commercial water-soluble PI Eosin, all of these compounds had larger σ_2 values and showed better potentials to initiate 2PP or 2PC of water-soluble acrylate (SR610), bovine serum albumin (BSA) and hyaluronic acid derivative (HAGM). Much lower threshold energies and wider fabrication windows were obtained in the formulations containing these PIs, especially for T2. The cytotoxicity of **T1-T3** was investigated by MTT assay using HepG2 cells. Nearly 60% of cells kept alive after 24 h incubation with these PIs when the concentration of them reached the same level used in 2PP or 2PC, indicating **T1-T3** would have potential application prospects in 2D and 3D nanostructures fabrication based on biomaterials under aqueous environments.

Conflict of interest

The authors declare no competing financial interest.

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Figure captions

Figure 1. Structures of Y2 and Y3

Figure 2. Normalized one-photon absorption spectra (left) and florescence emission spectra (right) excited at 510 nm of PIs in deionized water at concentration of about 10^{-6} M.

Figure 3. Two-photon absorption cross-sections of compounds T1-T3 in deionized water.

Figure 4. DBC versus time of resins R2 (containing T1 as PI), R3 (T2) and R4 (T3).

Figure 5. (a) **2D** micropattern fabricated in **R2** by changing the incident energy with a fixed line scan speed of 10 μ m/s; (b) 3D carbon nano-tube fabricated in **R3** with 0.72mW power and 110 μ m/s scan speed; (c) 2D logo of our laboratory made using **R8**, the designed model (left) and real pattern (right).

Figure 6. 3D carbon nano-tube structures fabricated using (a) R14 and (b) R14 plus PEGDA with 0.85 mW power and 110 μ m/s scan.

Figure 7. Cell viability after 24 h cultivation in substrates containing different PIs with a concentration of 3 mM.

Scheme 1. Structures and synthetic routes for T1, T2 and T3.

Table 1. The formulas and fabrication window of all resins.

Table 2. One- and two-photon photo-physical properties of PIs indeionized water.

Resin	SR610(wt%)	BSA(wt%)	HAGM(wt%)	$H_2O(wt\%)$	Eosin(wt%)	T1(wt%)	T2(wt%)	T3(wt%)	E_{th} (mW)	$E_{br}(mW)$
R1	79.87	0	0	19.97	0.16	0	0	0	0. 51	5.24
R2	79.87	0	0	19.97	0	0.16	0	0	0.37	5.93
R3	79.87	0	0	19.97	0	0	0.16	0)	0.08	6.94
R4	79.87	0	0	19.97	0	0	0	0.16	0.43	5.65
R5	80	0	0	20	0	0	0	0	—	7.79
R6	0	15	0	84.84	0.16	0	0	0	3.31	5.12
R7	0	15	0	84.84	0	0.16	0	0	2.14	5.78
R8	0	15	0	84.84	0	0	0.16	0	1.87	6.22
R9	0	15	0	84.84	0	0	0	0.16	1.97	5.57
R10	0	15	0	85	0	0	0	0	_	7.43
R11	0	0	10	89.84	0.16	0	0	0	1.07	5.54
R12	0	0	10	89.84	0	0.16	0	0	0.84	7.08
R13	0	0	10	89.84	0	0	0.16	0	0.59	7.37
R14	0	0	10	89.84	0	0	0	0.16	0.38	8.05
R15	0	0	10	90	0	0	0	0	-	10.16

Table 1. The formulas and fabrication window of all resins.

"-" means no polymerization was found during the experiment.

Comp	λ ^{abs} (nm)	$\epsilon_{max} \ (10^4 \text{ M}^{-1} \ \text{cm}^{-1})$	λ ^{fl} (nm)	⊕ _f (10 ⁻²)	Φ _Δ (10 ⁻²)	Δν (cm⁻¹)	σ ₂ (GM)
T1	522	2.87	661	1.28	1.72	4028	567
T2	513	5.50	644	1.10	1.40	3965	808
Т3	478	4.94	636	0.52	1.18	5197	231

Table 2. One- and two-photon photo-physical properties of PIs indeionized water.

 λ_{max}^{abs} is the maximum absorption wavelength in diluted solutions; ε_{max} is the molar absorption coefficient at λ_{max}^{abs} in diluted solutions; λ_{max}^{fl} is the maximum fluorescence emission wavelength at the excitation of 510 nm; Δv is stoke's shift; Φ_f is the fluorescence quantum yield in water; Φ_{Δ} is the singlet oxygen quantum yield in methanol; σ_2 is the maximum 2PA cross-section within the wavelength range of 750~840 nm measured in diluted solutions; 1 GM = 10⁻⁵⁰ cm⁴s/photon.









CERTIN MARKE











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Highlights

- Three water-soluble benzylidene cyclanone dyes **T1-T3** with high initiation efficiency and relatively large two-photon absorption cross-section in near infrared region were synthesized.
- Using **T1-T3** as photoinitiators directly, 2D and 3D nano patterns based on two-photon crosslinking or polymerization of water-soluble acrylate (SR610), bovine serum albumin (BSA) and hyaluronic acid derivative (HAGM), respectively, were successfully fabricated. Much lower threshold energies and wider fabrication windows were obtained in the formulations containing these PIs compared with commercial ones.
- Cytotoxicity test showed a favorable biocompatibility of these PIs, indicating their application prospects in 3D fabrication of biomaterials.

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