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Highly fluorescent polycaprolactones with tunable light emission wavelengths across visible to NIR spectral window

Shuo Huang,^a Kai Wang,^a Shipan Wang,^b Yue Wang,^b Mingfeng Wang,^{a,}*

^aSchool of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive, Singapore 637459

^bCollege of Chemistry, Jilin University, Changchun, Jilin, China

E-mail: M. Wang (mfwang@ntu.edu.sg)

Abstract

Polycaprolactone (PCL) has been widely used in biomedical applications such as tissue engineering due to its promising biodegradability, biocompatibility and mechanical processibility. However, commercially available PCL without any fluorescence does not meet the increasing demand in biomedical applications, for example, to monitor noninvasively the fate of implanted scaffolds in tissue engineering. To that end, exploration of new fluorescent PCL polymers plays an essential role in tracking their degradation under physiological conditions and distinguishing PCL scaffolds at the interface with biological systems such as cells and tissues. Herein, we report a series of tailor-made fluorescent PCL polymers with various light emission wavelengths ranging from visible to NIR region in solid states. These fluorescent PCL polymers are amenable to be processed into nanofibers using electrospinning, as well as waterborne nanoparticle ink for macroscale stamping, brush writing and microcontact printing. Finally, the application of the NIR fluorescent PCL for *in vitro* imaging and monitoring of scaffold degradation is demonstrated.

Introduction

Polycaprolactone (PCL), as a member of the family of biodegradable polyesters,¹ has attracted much attention from both basic research and pharmaceutical/medical industry.² PCL has been approved by the United States Food and Drug Administration (FDA) for its increasing use in drug-delivery^{3,4} and tissue engineering⁵⁻⁷. The unique properties of PCL compared to other polymers such as poly(lactic-co-glycolic acid) (PLGA) include its slow degradation rate and mechanical properties that enables facile shaping and manufacturing. Particularly, PCL possesses superior rheological and viscoelastic properties over many other biodegradable polymers⁸. Recently, many multifunctional and well-organized PCL three-dimensional scaffolds with various shapes have been developed and applied in tissue engineering, such as bone engineering, cartilage engineering and skin engineering.⁹⁻¹²

The increasing use of PCL scaffolds in any type of tissue engineering makes it essential to study and understand their degradation behavior. Previous methods of tracking degradation of PCL scaffolds have been largely limited to measurement of molecular weight and mass loss as well as change of crystallinity and mechanical properties.^{13, 14} At *in vitro* level, these methods are tedious and time-consuming. At *in vivo* level, these methods often require invasive retrieval of scaffolds from a body.

Recently, Artzi et al.¹⁵ and Kim et al.¹⁶ reported linear correlation between *in vivo* and *in vitro* erosion profiles in fluorescent dye-labeled PEG:dextran hydrogels and collagen hydrogels, respectively, which enables non-invasive tracking of erosion in biodegradable materials based on fluorescence imaging. Nevertheless, this non-invasive fluorescence imaging has never been used for tracking degradation of PCL scaffolds which are widely used in tissue engineering, to the best of our knowledge. It remains challenging to monitor noninvasively the degradation of implanted scaffolds using commercially available PCL which is not traceable *via* fluorescence. To that end, developments of functional PCL

polymers that are highly fluorescent in solid states are important. Furthermore, the fluorescence of PCL scaffolds offers the desired contrast to study the biotic/abiotic interface where the cells or tissues emit fluorescence distinguishable from that of scaffolds.^{17, 18}

Previously reported molecular design, for example, PCL end-capped with small molecular fluorophores, still suffers from the intermolecular aggregation of the fluorophores and consequent fluorescence quenching in solid states.¹⁹⁻²¹ Recently, we reported a facile strategy of enhancing the fluorescence brightness and photostability by covalently linking an organic fluorophore, di(thiophene-2-yl)-diketopyrrolopyrrole (DPP), in the middle of PCL.^{22, 23} When the chain length of PCL is long enough (e.g. $M_n = 38$ kDa), the intermolecular aggregation of the dye moieties in the polymeric matrices can be effectively minimized and the polymer (denoted as PCL-DPP-PCL) shows remarkably bright fluorescence in solid states and significantly enhanced photostability.²³ In addition, the strong covalent bonding between the dye and PCL reduces potential early-stage leaching of dyes compared to physical blends of dyes/polymers. Moreover, these polymers can be processed into highly fluorescent and photostable colloidal nanoparticles which can be internalized by tumor cells and stem cells for cell imaging with minimal cytotoxicity.²² PCL-DPP-PCL has also been electro-spun together with commercial PCL ($M_n = 80$ kDa) into highly fluorescent and photostable nanofibers which could serve as scaffolds for fluorescence-based long-term tracking of cell proliferation and differentiation.¹⁸

Here, we examine whether this strategy of molecular design can be applied to a broad scope of PCL-dye-PCL polymers with different dye structures and thus distinct optical properties. While PCL-DPP-PCL was yellow-fluorescent,²³ three more representative blue-, green-, and red-fluorescent PCL polymers were designed and synthesized. Moreover, a near-infrared (NIR) fluorescent PCL was designed and synthesized to enable deep-tissue imaging, in which biological tissues have relatively low absorption and negligible auto-fluorescence in

the NIR region. These tailor-made PCL polymers not only show strong fluorescence and robust photostability in solid states, but also exhibit good miscibility with PCL compared to physically blending organic dyes with commercial PCL. Moreover, these fluorescent polymers could be processed into nanoparticles stabilized by Pluronic 127 in water, resulting in well dispersible fluorescent ink for patterning at different length scales. Finally, the highly NIR-fluorescent PCL was used to monitor the degradation of PCL scaffolds *via* the evolution of the fluorescence intensity *versus* the mass loss of the scaffolds. As a result, a series of highly fluorescent and photostable PCL-dye-PCL polymers with tunable light absorption and emission wavelengths across visible to NIR spectral window can be achieved for future different end-use applications, for example, fabrication of multi-fluorescent PCL scaffolds with complexed architectures and optical properties for advanced tissue engineering.

Scheme 1. Schematic illustration of preparation (a) of PCL-dye-PCL NPs and the chemical structures (b) of fluorophores with various emission wavelengths.

Results and discussion

Synthesis and characterization of PCL-dye-PCL

Fluorescent polymers PCL-dye-PCL with different emission wavelengths were synthesized by ring-opening polymerization of ϵ -caprolactone from different fluorophore derivatives bearing double –OH groups as the initiators and Sn(II) 2-ethylhexanoate as the catalyst.²³ The details of the synthesis of these functional dye initiators and PCL-dye-PCL polymers are described in Supporting Information. The chemical structures of the synthetic fluorescent polymers shown in **Scheme 1** were characterized by ¹H-NMR spectroscopy (Figure S2-S5). Specifically, the ¹H-NMR spectrum (Figure S2-S5) of each PCL-dye-PCL showed characteristic peaks at 4.1, 2.3, 1.6, 1.4 ppm from the protons of PCL and another peak at 5.2 ppm corresponding to the CH₂ in the benzyl group of the fluorescent dye in the middle of each PCL chain. The number-average molecular weight (M_n) and polydispersity index (PDI) of the obtained polymers were measured with gel permeation chromatography (GPC) (**Table 1**, Figure S1). Most of these fluorescent PCL showed a M_n around 2×10^4 g/mol and a relatively narrow PDI (1.5~2.0). In addition, M_n estimated from the end-group analysis of the ¹H-NMR spectrum was consistent with that measured by GPC (Table 1). The relatively narrow PDI and the good overlapping between the GPC traces recorded with refractive-index (RI) and UV-vis detectors indicate the well-defined structures of these polymers, each of which consists of an organic dye in the middle of the PCL chain.

The thermal properties of different PCL-dye-PCL polymers were examined by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). While all of these polymers showed a similar melting point in the range of 58-60 °C, thermal decomposition temperature varied in the range of 240-310 °C depending on the molecular weight of each polymer (Table 1, Figures S6-S7).

Table 1. Summary of PCL-dye-PCL characterizations.

Polymer	$M_n(*10^3)^a$	M_w/M_n	n^a	n^b	T_d (°C)	T_m (°C)	λ_{ex} (nm) ^c	$\lambda_{max.em}$ (nm) ^c	$\lambda_{max.em,solid}$ (nm)
Blue-PCL	18	1.5	78	80	250	59	389	430,458	434, 458
Green-PCL	23	1.6	100	110	260	60	390	492	489
Yellow-PCL	38	2.0	185	198	306	58	510,550	558, 602	564, 604
Red-PCL	16	1.5	70	69	245	59	491	593	594
NIR-PCL	18	1.7	78	80	265	59	788	942	986

^{a)} Molecule weight was calculated from GPC running in THF as eluent and linear poly(styrene) homopolymers as standards. ^{b)} Molecule weight was calculated from end-group analysis of ¹H-NMR. ^{c)} Measured in THF solutions.

Optical properties

The optical properties of PCL-dye-PCL polymers are presented in Figure 1. The UV-VIS-NIR absorption spectra in THF (Figure 1) show the maximum absorption peaks at 389, 390, 491 and 788 nm for Blue-, Green-, Red- and NIR-PCL, respectively. While the emission spectrum of Blue-PCL shows a vibronic double peak at 430 and 458 nm, respectively, the other fluorescent PCL polymers (including Green-, Red- and NIR-PCL) all show a broad featureless emission band centered at 492, 593 and 942 nm, respectively, largely due to the strong intramolecular charge transfer.

Similar to Yellow-PCL reported in our previous publications,^{22, 23} all of the other fluorescent PCL polymers exhibited strong fluorescence in solid states including bulk powders and solid films. Moreover, there was no significant change between the emission spectra of these polymers in films and those in dilute solutions such as in THF (Figure 1), which implies that the PCL chains grafted from the organic dyes effectively suppress the aggregation-caused FL quenching caused by intermolecular π - π stacking such as *J*-aggregation. The slight red-shift of the emission peak in NIR-PCL in solid states vs. in dilute THF solution implies stronger π - π interaction of BBT units compared to other organic dyes in the solid states. Particularly, the NIR-PCL shows a relative high FL quantum yield ($\Phi = 0.01$)

in solid states compared to commercial NIR dyes such as IR 1048 ($\Phi = 0.004$ in dichloromethane), which enables the NIR-PCL as a promising fluorescent contrast agent for *in vivo* bioimaging.

Figure 1. Absorption and emission spectra of Blue-PCL (a), Green-PCL (b), Red-PCL (c) and NIR-PCL (d) in THF and in films. Excitation wavelengths are 390, 390, 490 and 790 nm, respectively. The inset digital photographs show fluorescence images of these polymers both in solution and in solid powders under UV light (365 nm) (a-c), or 808 nm laser (d) irradiation.

Colloidal PCL-dye-PCL nanoparticles as fluorescent ink for patterning

To render the hydrophobic PCL-dye-PCL polymers described above dispersible into water, they were encapsulated into colloidal nanoparticles (NPs) *via* a nanoprecipitation^{23, 24} process in the presence of Pluronic 127 as the stabilizer. The morphology and the size of the

prepared NPs were characterized by scanning electron microscope (SEM) and dynamic light scattering (DLS) (Figure. S8). The average diameter measured from the SEM images of the spherical NPs, which was 63 ± 11 nm for Blue-PCL, 54 ± 10 nm for Green-PCL and 57 ± 14 nm for Red-PCL, was similar for different colored PCL but generally smaller than that of the NPs measured by DLS in hydrated states. Moreover, these NPs composed of PCL-dye-PCL in the cores are highly fluorescent and can be used as water-borne ink for patterning such as macro-scale stamping (Figure. 2a), brush writing (Figure. 2b-c) and micro-contact printing (Figure. 2d-f) at different length scales. These fluorescent patterns maintained their features and brightness after being stored for at least one year under ambient conditions.

Figure 2. (a-c) Fluorescence images of PCL-dye-PCL patterns under UV light 365 nm. The patterns were prepared by stamping (a) and brush writing (b and c) the colloidal NPs as water-borne ink on filter paper. (d-f) Confocal laser scanning microscope images of patterns

prepared by micro-contact printing of colloidal NPs made of Blue- (d), Green- (e) and Red-PCL (f) on glass substrates.

Fluorescent nanofibers of PCL-dye-PCL and photostability

To investigate the effect of the chemical structure of the organic dye on the fluorescence properties of PCL-dye-PCL in solid states, we further fabricated fluorescent nanofibers by electrospinning PCL-dye-PCL (10% w/w) mixed with commercial PCL in trifluoroethanol. (Experimental details are described in Supporting Information). All of the nanofibers (Figure S9) appeared more uniform in diameter (ca. 2 μ m) than the physically blended mixture of commercial PCL and the dye initiator under the fluorescence microscope. In addition, the nanofibers exhibited good photostability, maintaining their high fluorescence intensity after 100 s of light irradiation (50 W Mercury light source) under a fluorescence microscope. The relatively lower photostability of blue-PCL nanofibers compared to the green-PCL and the red-PCL nanofibers could be due to the shorter PCL chains in the former and/or the higher susceptibility of the blue fluorophore (DPTF) against photobleaching.

Deep-tissue imaging of NIR fluorescent PCL scaffolds

To examine the application of NIR-PCL in fluorescence-based biological imaging and the penetration depth, we acquired NIR fluorescence signal from a model of #-like scaffold prepared by drop-casting NIR-PCL (50%, w/w) blended with commercial PCL ($M_n = 70000$ g/mol). The scaffold was embedded inside two pieces of pork tissues (Figure 3a). The fluorescence images (Figure 3b) of the NIR-PCL scaffold show strong fluorescence signal at various thicknesses of the tissues, indicating deep penetration of tissues up to 10 mm. The shape of the NIR-PCL scaffold appeared blurry with increase of the tissue thickness, mainly caused by light scattering from tissues. Nevertheless, the fluorescence signal was strong enough for *in vivo* imaging applications. In addition, the NIR-PCL retained its photostability

after NIR laser ($2\text{W}/\text{cm}^2$, 808 nm) irradiation for 3 hours, in contrast to the significant photodegradation of indocyanine (denoted as ICG, a commercially available and widely used NIR imaging probe) under the same irradiation condition (Figure S12). Similar to PCL-DPP-PCL,²² this NIR-PCL polymer in the form of colloidal nanoparticles also showed no obvious cytotoxicity (Figure 13). These results indicate robust photostability and good biocompatibility of the NIR-PCL polymer, which is important for long-term fluorescence imaging and tracking.

Figure 3. (a) Digital photographs of the #-like NIR-PCL scaffold on the top of a piece of pork tissue (top view, left) and sandwiched between two pieces of pork tissues (side view, right). (b) The fluorescence images of the NIR-PCL scaffold embedded inside two pieces of pork tissues with various thicknesses as indicated under each image.

Quantifying *in vitro* degradation of NIR-PCL scaffolds

To investigate the application of NIR-PCL for monitoring the degradation of the scaffolds non-invasively, we monitored the rate of the scaffold degradation by measuring changes of

NIR fluorescence signals from the scaffold using a NIR camera. Specifically, a prepared star-like tetrapod scaffold made of NIR-PCL was soaked in aqueous solution of sodium hydroxide (NaOH) at various concentrations. For comparison, we also measured the mass loss of the same scaffold during the degradation process. Figure S10 shows the degradation rate of the NIR-PCL depending on the concentration of NaOH, in which the mass of the scaffold remained less than 10% after 96 h in NaOH (5 M). Figure 4a shows that the decrease of the NIR fluorescence intensity is consistent with the mass loss of the scaffold after degradation over one week, which means the degradation result monitored by NIR fluorescence is reliable for future *in vivo* applications. The evolution of the NIR-PCL scaffold after being soaked in NaOH *aq.* over different periods could be monitored clearly by the NIR fluorescence images (Figure 4b) obtained by a shortwave infrared (SWIR) Indium Gallium Arsenide (InGaAs) camera. These results suggest the promising potential of these NIR-PCL in monitoring the degradation of PCL *in vivo* with a non-invasive manner.

Figure 4. Quantification of NIR-PCL scaffold degradation *in vitro* in NaOH aqueous media. (a) Degradation measurement of NIR-PCL by fluorescence intensity and remaining mass, respectively, within 8 days. (b) NIR digital photos of a NIR-PCL scaffold after being soaked in NaOH *aq.* over different periods. Scale bar = 1 cm.

Conclusion

In summary, we have presented a series of well-defined fluorescent PCL polymers with tunable emission wavelengths across blue to NIR region. The water-borne ink consisting of the PCL-dye-PCL colloidal nanoparticles with bright fluorescence and remarkable photostability can be used in macro-scale stamping, brush writing and micro-contact patterning. These unique properties may offer promising applications of these fluorescent PCL-dye-PCL, for example, for bioimaging and tissue engineering. Moreover, PCL-DPTBBT-PCL with relatively strong NIR fluorescence enables deep-tissue imaging and long-term tracking the degradation of PCL scaffolds in a non-invasive way.

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Notes and references

^a School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive, Singapore 637459. E-mail: M. Wang (mfwang@ntu.edu.sg)

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A series of tailor-made fluorescent PCL polymers with tunable light emission wavelengths from visible to NIR are amenable to be processed into waterborne nanoparticle ink for multiple-length-scale patterning and scaffolds for deep-tissue imaging.