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Development of Intrinsically Photoluminescent and Photostable Polylactones

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Abstract

A method of introducing intrinsically photoluminescent properties to biodegradable polymer was introduced, exemplified by the synthesis of intrinsically photoluminescent polylactones that enable non-invasively monitoring and tracking material degradation *in vivo* in real-time and the formation of theranostic nanoparticles for cancer imaging and drug delivery.

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Supporting Information

Detailed experimental section can be found in the Supporting Information. Supporting Information is available online from the Wiley Online Library or from the author.

Keywords

biodegradable polymers; fluorescence imaging; tissue engineering; theranostic drug delivery; *in vivo* degradation

The use of biodegradable polymers, in particular hydrolytically degradable polylactones such as polylactide (PLA), polyglycolide (PGA) and their copolymers (PLGA), has generated a huge scientific and economic impact on a broad range of biomedical applications such as disposable medical devices, tissue engineering, drug delivery, and molecular targeting^[1, 2]. However there have been few innovations in new design of polylactone materials to meet the ever-growing need for functional biomaterials in medical applications, especially where new bioimaging techniques including optical imaging have become important enabling tools for innovation^[3]. Herein, we report a series of newly developed biodegradable polylactone copolymers possessing photostable and *in vivo* detectable intrinsic fluorescence without conjugating or encapsulating traditional photobleaching organic dyes or cytotoxic semiconducting quantum dots. We also demonstrate the applications of these new materials for *in vivo* real-time tracking of scaffold degradation and for theranostic cancer treatment.

Despite the exciting progress, some fundamental understandings on the key elements of tissue engineering are still lacking. For example, although it has been recognized that the scaffold degradation rate should match the rate of new tissue formation, biomaterial designs to control the *in vivo* scaffold degradation rate remain empirical due to the lack of quantitative *in vivo* validation. Currently, *in vivo* scaffold degradation is often estimated based on the outcomes of *in vitro* degradation measurements^[4, 5]. Unfortunately, various factors have been shown to affect biomaterial degradation *in vivo*, which cannot be precisely simulated *in vitro*^[6]. Attempts to quantify polymeric scaffold degradation *in vivo* have been inconsistent due to the difficulty of separating the infiltrated/regenerated tissues from the porous scaffolds^[6]. An *in situ* real-time method to accurately monitor and quantify scaffold degradation and tissue regeneration processes without traumatically explanting samples or sacrificing animals is urgently needed^[2, 7]. To achieve this goal, it is essential that the biodegradable polymers can be used as non-invasive bioimaging probes, in addition to providing a suitable cell growth substrate. Recently, fluorescent dye conjugated polyethylene glycol (PEG)/dextran hydrogels were developed to track the *in vivo* material degradation^[7]. However, such a model system may bring about concerns of photobleaching and cytotoxicity that are often associated with the use of traditional organic dyes. The loss of the conjugated fluorescent dyes on the polymers may complicate the understanding on fluorescence loss and material degradation.

Biodegradable polymers, mostly polylactones, have also been widely used as theranostic nanomedicine platforms for targeted drug delivery and high-throughput imaging^[8]. Generally, imaging agents alone cannot be used as drug delivery carriers and *vice versa*. Therefore, the conjugation/encapsulation of imaging agents onto/into drug delivery carriers is normally required to generate theranostic nanobiomaterials. Polymeric nanoparticles are usually conjugated with additional organic dyes or quantum dots (QDs) for fluorescence

imaging^[9]. However, the poor photobleaching-resistance and low dye-to-particle conjugation ratios of organic dyes as well as the toxicity and poor hydrodynamic stability of QDs prevent their practical uses *in vivo*^[10]. Encapsulating/conjugating imaging agents in/on nanoparticles may result in increased particle sizes, added complexity, and higher risk of adverse biological reactions^[11]. Moreover, targeting ligands have to share conjugation sites with imaging agents, which may result in insufficient conjugation, thus reducing both the targeting and imaging efficiency^[12]. Such challenges might be resolved by using biodegradable fluorescent polymers that exhibit dual-functionality as drug delivery carriers and imaging probes.

To address the aforementioned concerns, we create a method to develop biodegradable imaging-detectable polymers, biodegradable photoluminescent polylactones (BPLPLs), via a ring-opening polymerization of lactones initiated by our recently developed biodegradable photoluminescent polymers (BPLPs)^[13, 14]. BPLPs are fully degradable pre-polymers that are synthesized by a polycondensation reaction of biocompatible monomers such as citric acid, α -amino acids, and diols^[13]. BPLPs display high quantum yields (up to 79%), tunable fluorescence emission with varied amino acids (up to 725 nm), and superior photostability compared to organic dyes^[13–15]. Herein, for the first time, we report the synthesis and characterization of thermoplastic BPLPLs, exemplified by BPLP-*co*-Poly(L-lactide) (BPLP-PLLA). The hydroxyl or carboxyl groups of BPLPs initiate ring-opening polymerization of lactone monomers such as lactide, glycolide, and caprolactone to make completely degradable block copolymers (BPLPLs) (Figure 1a). The resulting BPLPL copolymers retain the intrinsic photoluminescence inherited from BPLPs, which enables the feasibility of non-invasive imaging for monitoring the BPLPL scaffold degradation and tracking of cancer targeting BPLPL nanoparticles *in vivo* (Figure 1b and 1c).

The synthesis route of BPLP-PLLA copolymer is illustrated in Figure 1a. The BPLP:lactide molar ratios were varied as 1:20, 1:50, and 1:100. For example, BPLP-Cys-PLLA20 represents a copolymer that was synthesized from BPLP with L-cysteine as the amino acid component and the feeding molar ratio of BPLP-Cys to L-lactide of 1:20. As presented in our previous work^[13], water-soluble BPLPs (WBPLPs) were made when hydrophilic polyethylene glycol (PEG) was chosen as the diol. Therefore, amphiphilic biodegradable photoluminescent WBPLP-PLLAs were also synthesized with WBPLP as the hydrophilic block and PLLA as the hydrophobic block. All polymers synthesized are summarized in Supplementary Table S1. ¹H NMR and FTIR confirm that the chemical structures of BPLP-PLLAs (Figure S1 and S2, Supporting Information) consist of functional groups from both BPLPs and PLLA.

All BPLP-PLLA copolymers emit strong fluorescence, as shown in Figure 2a and 2b. For BPLP-Cys-PLLA, the maximum emission (441 nm) and excitation (377 nm) is slightly different from that of BPLP-Cys pre-polymer, which has maximum emission and excitation wavelengths of 434 and 365 nm, respectively^[13, 14]. At the same concentration (10 mg/ml), the longer PLLA blocks, the relatively weaker fluorescent intensity. BPLP-Ser-PLLA also emits tunable fluorescent light depending on the excitation wavelength as similar to BPLP-Ser^[13, 14]. As shown in Figure 2b, BPLP-Ser-PLLA50 displays fluorescence peak emissions from 350 nm to 700 nm. In addition, BPLP-Cys-PLLA copolymers provide exceptionally

high quantum yields (up to 51.4%, Table S1, Supporting Information), which are in the range of traditional fluorescent materials, including organic dyes, quantum dots, and green fluorescent proteins^[15]. One exceptional advantage of the BPLPs is the excellent photostability over other traditional organic dyes. BPLP-PLLAs inherit this property, which is a key feature for many bioimaging applications^[16], from BPLPs by showing much better photo-bleaching resistance of continuous illumination over photobleaching organic dyes, e.g. Rhodamine B and Fluorescein, especially in the early time period (Figure 2c). The BPLP-PLLA polymers all possess outstanding photostability with >90% signal remaining even after a long (3 hours) illumination. These intriguing fluorescence properties empower our novel degradable BPLPLs for both *in vitro* and *in vivo* detections.

The thermal properties of BPLP-PLLAs were characterized by Differential Scanning Calorimetry (DSC) and Thermal Gravimetric Analysis (TGA). Figure S3a (Supporting Information) presents the DSC thermograms of BPLP-Cys-PLLA copolymers with various PLLA chain lengths. The glass transition temperature (T_g) increases gradually with the increasing of PLLA block lengths: 0°C, 12.2°C, and 40.1°C for BPLP-Cys-PLLA20, BPLP-Cys-PLLA50, and BPLP-Cys-PLLA100, respectively. The same trend could also apply to BPLP-Ser-PLLA and WBPLP-PLLA (Figure S4, Supporting Information). Figure S3b (Supporting Information) also shows a representative DSC thermogram of BPLP-Cys-PLLA100 with a clear crystallization peak at 106.1°C and a melting peak at 115.3°C, which is lower than the typical melting point of PLLA (around 170°C)^[17], probably due to the incorporation of softer BPLP chains. Figure S5 (Supporting Information) characterizes the thermal decomposition temperatures of BPLP-Cys-PLLA copolymers. BPLP-Cys-PLLA20 exhibits a slight weight loss at 120°C, possibly due to the loss of water from carboxyl group. However, no weight loss was observed before 280°C for BPLP-Cys-PLLA100, in common with commercial PLLA^[17]. Unlike BPLPs, all BPLPLs cannot be thermally crosslinked upon heating, suggesting they are thermoplastic materials. With similar thermal properties to PLLA, our BPLP-PLLAs can be processed into various forms, including films, scaffolds, particles, micelles and fibers for a broad range of biomedical applications. BPLP-Ser-PLLA particle sizes and drug delivery profiles can also be easily tuned by varying the molecular design of polymers (Figure S6, Supporting Information). Previously mentioned amphiphilic WBPLP-PLLAs were also prepared into micelles with controllable critical micelle concentration (Figure S7, Supporting Information). Both nanoparticles and micelles exhibit strong fluorescence in aqueous solutions (Figure S8, Supporting Information). Importantly, BPLP-Ser-PLLA50 nanoparticles that were taken up by 3T3 fibroblasts *in vitro* can be imaged by fluorescent microscopy, verifying the cell labeling capability of BPLPLs that is similar to quantum dots (Figure 2d). BPLP-PLLA nanoparticles were also tested as drug delivery carriers using 5-fluorouracil as a model drug and the release profile could be tailored simply by varying the molecular weight of PLLA chain (Figure S9, Supporting Information). In addition, BPLP-Ser-PLLA50 polymers were electrospun into uniform ultra-fine fibers that exhibited strong photoluminescence (Figure S10, Supporting Information). Electrospun fibers have been widely used in tissue engineering as the nano-fibrous structures were considered mimicking the structures of extracellular matrix such as collagen and elastin. Thus, BPLPLs demonstrate excellent processability, which is exemplified by the fabrication of films, particles, fibers and scaffolds suggesting that BPLPLs may serve as

candidate materials for a number of biomedical applications such as tissue engineering, drug delivery, and bioimaging.

To assess the cytotoxicity of BPLPLs, BPLP-Cys-PLLA nanoparticles were cultured with mouse 3T3 fibroblasts. As shown in Figure S11 (Supporting Information) BPLP-Cys-PLLA nanoparticles exhibit lower cytotoxicity than the pristine BPLP-Cys nanoparticles at high concentrations and similar toxicity *in vitro* to the widely used PLLA nanoparticles. To assess the *in vivo* host response of BPLP-PLLA, BPLP-Ser-PLLA20 films were implanted subcutaneously in 1-year-old male Sprague Dawley rats. PLLA (Mw=20kDa) films and crosslinked BPLP-Ser films were chosen as controls. Histological analysis shows the presence of inflammatory cells at both 1 and 10 weeks of implantation (Figure S12, Supporting Information). After 1 week of implantation, the acute inflammatory response was mild for all samples. There is no significant difference ($p>0.05$) observed among PLLA, CBPLP films and BPLP-Ser-PLLA20 in terms of cell density surrounding the implants, while the cell numbers for all samples decreased significantly ($p<0.01$) at week 10 (Figure S12b). Noticeably, the fibrotic capsule thicknesses for CBPLP films and BPLP-Ser-PLLA20 are significantly thinner than that for PLLA ($p<0.01$) at both week 1 and week 10 (Figure S12c), likely due to the soft, elastic and hydrophilic nature of CBPLP and BPLP-Ser-PLLA20 compared to PLLA. The fibrotic capsule layer thickness for each sample also decreased and stabilized at week 10. One week after the implantation, CD11b+ inflammatory cell were identified around the implanted materials (Figure S12a). This immunohistochemical analysis (Figure S12d) reveals that almost the same amount of CD11b + inflammatory cells around CBPLP and BPLP-Ser-PLLA20 implants as around PLLA ($p>0.05$). The above animal studies indicate that BPLP-PLLAs have comparable or even better *in vivo* tissue responses as compared to the commercial PLLA.

Degradation is a critical property of biomaterials, since it affects cytotoxicity, cell penetration/proliferation, drug release rate, mechanical performance, and many other properties^[18]. As expected, the *in vitro* weight loss of BPLP-PLLAs mainly depends on the length of hydrophobic PLLA blocks (Figure 3a). BPLP-Cys-PLLA20 completely degraded after 12 weeks of incubation in PBS, while the degradation rate of BPLP-Cys-PLLA100 was similar to that of PLLA homopolymer with a similar molecular weight^[19]. One of the most significant advantages of BPLP-PLLAs is that their degradation can be measured by fluorescent signal decay in addition to the traditional weight loss measurements. We first confirmed that the normalized fluorescence signal loss matched the weight loss of BPLP-PLLAs *in vitro* (correlation coefficient of two covariance = 0.997) (Figure 3b). This phenomenon supports our hypothesis that fluorescence decay can be used as a measurement of material degradation. Thus, it is possible that the *in vivo* degradation of BPLP-PLLAs can be monitored non-invasively by tracking the fluorescence decay of polymers under an *in vivo* fluorescent imaging system without extracting the implants or sacrificing animals (Figure 3d). As shown in Figure 3c, the weight of BPLP-Ser-PLLA20 decreased much faster in PBS *in vitro* than in the living body at all time points, especially for the first 5 weeks (correlation coefficient = 0.946), likely due to the relatively large volume of liquid in *in vitro* degradation compared to the relatively small volume of subcutaneous fluid in animals. Interestingly, by comparing *in vivo* fluorescence decay and *in vivo* material degradation, we

find that, the rate of fluorescence loss is slightly higher than the rate of actual weight loss in first two weeks, and a significantly higher fluorescence signal loss was observed at week 3. Although the cause has yet to be determined, it is possible that the acute inflammatory cells and protein-rich interstitial fluids block some fluorescence^[20]. Nonetheless, the fluorescence decay still shows a better correlation to the *in vivo* weight loss compared to *in vitro* weight loss. When acute inflammation (loose connective tissues) is transformed into mild chronic inflammation (thin fibrotic capsules) after 4 weeks, it is expected that fluorescence decay should better represent the actual material degradation^[5, 21]. Indeed, we observe that the fluorescence decay after 4 weeks matched (correlation coefficient = 0.987) the *in vivo* weight loss of the BPLP-Ser-PLLA20 samples. Histological images also confirm that after 10 weeks *in vivo* implantation, BPLP-Ser-PLLA20 films degraded into small pieces and the infiltrated tissues occupied the spaces of the degraded materials (Figure S13, Supporting Information). Overall, these results indicate that our BPLP-PLLAs provide intrinsic fluorescence, which enables accurate, non-invasive monitoring of the implant degradation *in vivo*. It is noteworthy that BPLPs and PLA are all aliphatic polyesters, thus the degradation mechanism of both is the hydrolysis of ester bonds. During a bulk degradation process, the ester bonds cleave randomly, resulting in that fluorescence loss rate matches the whole polymer degradation rate. Conferring *in vivo* detectable fluorescent properties to biodegradable polymers may advance the field of tissue engineering as the enabling imaging tool may facilitate obtaining the true understanding on material degradation and tissue material interactions *in situ* and in real time.

With the unique intrinsic fluorescence, BPLP-PLLAs could be used as a label-free *in vivo* imaging tool for cancer detection and treatment. To demonstrate the feasibility, targeting ligand folates were conjugated on BPLP-Ser-PLLA50 nanoparticles^[22], which are stable in physiological conditions (Figure S14, Supporting Information). After i.v. injection through the tail vein, these nanoparticles quickly accumulate at the dorsal orthotopic breast tumors (MCF7 cell injected subcutaneously) of a nude mouse within 4–6 hours as detected by *in vivo* fluorescent imaging system (Figure 2e). *Ex vivo* fluorescent imaging (Figure S15, Supporting Information) confirms that substantial higher fluorescent intensities are found in tumor tissue than in liver. These results support that BPLP-PLLAs nanoparticle preferentially accumulated at tumor sites. Since drugs can be easily encapsulated with BPLP-PLLA nanoparticles as well (Figure S9), the feasibility of applying BPLPLs into theranostic cancer management is obvious. Furthermore, the high targeting efficiency is probably a result of high density of targeting folates on the nanoparticle surfaces, since folates do not need to share conjugation sites with imaging molecules as in most existing nanoparticle systems^[23]. Thus, our BPLP-PLLA nanoparticles could potentially serve as ideal candidates for *in vivo* cancer fluorescence imaging and drug delivery, without the need of conjugation with any organic dyes or quantum dots.

In conclusion, we have successfully synthesized and characterized biodegradable photoluminescent polylactone (BPLPL), representatively BPLP-PLLA copolymers. These fully degradable polymers exhibit similar thermal properties and biocompatibility to the commercial polylactones. Most importantly, BPLPLs enable non-invasive fluorescence imaging for tracking *in vivo* degradation of polymers. BPLPLs could be fabricated into

different forms such as nanoparticles for potential theranostic applications. We demonstrated that incorporating BPLP units into biodegradable polymer design could be an effective way to integrate intrinsically photoluminescent properties to biodegradable polymers. Given the already significant impact generated by using polylactone materials, conferring intriguing *in vivo* detectable photobleaching-resistant photoluminescence to polylactone represents an innovation that should advance the understanding, design, and use of biodegradable polymers in a broad range of biomedical and biological applications where fluorescence imaging and sensing have gained increasing importance.

Experimental Section

Experimental details are describes in the Supporting Information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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References

1. Hench LL, Polak JM. *Science*. 2002; 295:1014. [PubMed: 11834817] Langer R, Tirrell DA. *Nature*. 2004; 428:487. [PubMed: 15057821] Lutolf MP, Hubbell JA. *Nat. Biotech.* 2005; 23:47. Wang Y, Ameer GA, Sheppard BJ, Langer R. *Nat. Biotech.* 2002; 20:602. Nair LS, Laurencin CT. *Prog. Polym. Sci.* 2007; 32:762. Tian H, Tang Z, Zhuang X, Chen X, Jing X. *Prog. Polym. Sci.* 2012; 37:237.
2. Vert M. *Biomacromolecules*. 2005; 6:538. [PubMed: 15762610]
3. Ntziachristos V. *Annu. Rev. Biomed. Eng.* 2006; 8:1. [PubMed: 16834550]
4. Yang J, Webb AR, Ameer GA. *Adv. Mater.* 2004; 16:511. Gong Y, Zhou Q, Gao C, Shen J. *Acta Biomater.* 2007; 3:531. [PubMed: 17350355]
5. Dey J, Xu H, Shen J, Thevenot P, Gondi SR, Nguyen KT, Sumerlin BS, Tang L, Yang J. *Biomaterials*. 2008; 29:4637. [PubMed: 18801566]
6. Lu L, Peter SJ, Lyman MD, Lai H-L, Leite SM, Tamada JA, Uyama S, Vacanti JP, Robert L, Mikos AG. *Biomaterials*. 2000; 21:1837. [PubMed: 10919687]
7. Artzi N, Oliva N, Puron C, Shitreet S, Artzi S, bon Ramos A, Groothuis A, Sahagian G, Edelman ER. *Nat. Mater.* 2011
8. Jokerst JV, Gambhir SS. *Acc. Chem. Res.* 2011; 44:1050. [PubMed: 21919457] Wadajkar AS, Kadapure T, Zhang Y, Cui W, Nguyen KT, Yang J. *Adv. Healthc. Mater.* 2012; 1:450. [PubMed: 23061030]
9. Gao X, Cui Y, Levenson RM, Chung LWK, Nie S. *Nat. Biotech.* 2004; 22:969. Jokerst JV, Gambhir SS. *Accounts of Chemical Research*. 2011; 44:1050. [PubMed: 21919457]
10. Smith AM, Duan H, Mohs AM, Nie S. *Advanced Drug Delivery Reviews*. 2008; 60:1226. [PubMed: 18495291] Soo Choi H, Liu W, Misra P, Tanaka E, Zimmer JP, Ito Ipe B, Bawendi MG, Frangioni JV. *Nat Biotech.* 2007; 25:1165.
11. Pridgen EM, Langer R, Farokhzad OC. *Nanomedicine*. 2007; 2:669. [PubMed: 17976029]
12. Kobayashi H, Longmire MR, Ogawa M, Choyke PL. *Chem. Soc. Rev.* 2011; 40:4626. [PubMed: 21607237] Koo H, Huh MS, Sun I-C, Yuk SH, Choi K, Kim K, Kwon IC. *Acc. Chem. Res.* 2011; 44:1018. [PubMed: 21851104]

13. Yang J, Zhang Y, Gautam S, Liu L, Dey J, Chen W, Mason RP, Serrano CA, Schug KA, Tang L. Proc. Natl. Acad. Sci. USA. 2009; 106:10086. [PubMed: 19506254]
14. Zhang Y, Tran RT, Qattan IS, Tsai Y-T, Tang L, Liu C, Yang J. Biomaterials. 2013; 34:4048. [PubMed: 23465824]
15. Zhang Y, Yang J, Mater J. Chem. B. 2013; 1:132.
16. Li K, Liu B, Mater J. Chem. 2012; 22:1257.
17. Abe H, Takahashi N, Kim KJ, Mochizuki M, Doi Y. Biomacromolecules. 2004; 5:1606. [PubMed: 15244485]
18. Nair LS, Laurencin CT. Progress in Polymer Science. 2007; 32:762.
19. Leenslag JW, Pennings AJ, Bos RRM, Rozema FR, Boering G. Biomaterials. 1987; 8:311. [PubMed: 3663810]
20. Remes A, Williams DF. Biomaterials. 1992; 13:731. [PubMed: 1391394]
21. Wang Z, Wang S, Marois Y, Guidoin R, Zhang Z. Biomaterials. 2005; 26:7387. [PubMed: 16019065]
22. Lu Y, Low PS. Adv. Drug Deliv. Rev. 2002; 54:675. [PubMed: 12204598]
23. Park J-H, von Maltzahn G, Zhang L, Derfus AM, Simberg D, Harris TJ, Ruoslahti E, Bhatia SN, Sailor MJ. Small. 2009; 5:694. [PubMed: 19263431] Kohler N, Fryxell GE, Zhang M. J. Am. Chem. Soc. 2004; 126:7206. [PubMed: 15186157]

Figure 1. Schematic illustration of biodegradable photoluminescent polylactide (BPLPL-Cys-PLLA) synthesis and its applications in scaffold imaging and nanoparticle tracking *in vivo*.

Figure 2.

Photoluminescent and imaging characterizations of BPLP-PLLAs. **a)** Fluorescent excitation and emission spectra of BPLP-Cys-PLLA with various polylactide chain lengths (insert, optical imaging of BPLP-Cys-PLLA50 solution under UV light). **b)** Fluorescent emission spectra of BPLP-Ser-PLLA50 shift depending on the excitation wavelengths (320 nm to 500 nm). **c)** Photo-stability of various BPLP-PLAs polymer solutions compares to Rhodamine B and Fluorescein solutions that were subjected to 3 hours of continuous illumination. **d)** Fluorescence microscopic image of 3T3 mouse fibroblasts with BPLP-Ser-PLLA50 nanoparticles uptake (insert, TEM image of BPLP-Ser-PLLA50 nanoparticles, scale bar = 100 nm). **e)** Representative *in vivo* white light and fluorescence images of a nude mouse with a human MCF7 breast tumor xenograft growing on its back after tail intravenous injection of BPLP-Ser-PLLA50 nanoparticles.

Figure 3.

Degradation profiles of BPLP-PLLAs. **a)** *In vitro* weight loss in PBS of BPLP-Cys-PLLA with different polylactide chain lengths at 37°C. **b)** Comparison of *in vitro* fluorescence loss and *in vitro* weight loss of BPLP-Ser-PLLA20. **c)** Comparison of *in vivo* fluorescence loss, *in vitro* weight loss, and *in vivo* weight loss of BPLP-Ser-PLLA20. **d)** Top, white light images of a representative nude mice with a subcutaneously implanted BPLP-Ser-PLLA20 film at different time points; bottom, *in vivo* fluorescent images of the same implant at each time point.