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Expanding (Bio)Conjugation Strategies: Metal-Free Thiol-Yne Photo-Click Reaction for Immobilization onto PLLA Surfaces

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Abstract: The study delves into the use of the thiol-yne click reaction to enhance (bio)conjugation methodologies, particularly focusing on immobilizing biomolecules onto PLLA surfaces. The thiol-yne click reaction, known for its efficiency, selectivity, and versatility in forming carbon-sulfur bonds under mild conditions without transition metal catalysts, is explored for conjugating the fluorophore dansyl onto PLLA surfaces. This approach aims to broaden bioconjugation strategies beyond traditional methods like the Michael-type reaction, expanding their applicability to diverse biomolecules. Utilizing a photoinitiator and specific light for photo-immobilization, the thiol-yne click reaction offers spatial and temporal control, with the absence of transition metal catalysts mitigating concerns of cytotoxicity and metal contamination, rendering it suitable for biomedical applications. The objectives of this research encompass demonstrating the feasibility of the thiol-yne click reaction for surface functionalization and enriching bioconjugation strategies for tailoring PLLA surfaces, ultimately advancing biomedical technologies through precise control over surface properties and functionality. For this purpose, PLLA surfaces were activated through hydrolysis and amidation to introduce the activated alkyne moiety (PLLA-Alkyne), followed by photo-induced dansyl immobilization (PLLA-Dns) with Irgacure 651. Various surface characterization techniques, including SEM, WCA, XPS, ATR-FTIR, and fluorescence microscopy and spectroscopy, validated the successful conjugation. This metal-free method preserves the material's bulk properties while enabling thiol-containing molecule immobilization.

Keywords: thiol-yne; coatings; conjugation

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1. Introduction

In recent years, the demand for advanced bioconjugation strategies has led to significant innovations in materials science and bioengineering [1]. Bioconjugation, which involves chemically linking two molecules, including a biomolecule such as a protein, peptide, oligosaccharide, or oligonucleotide, has evolved substantially. Traditional methods often relied on crosslinking agents or enzyme-mediated conjugation [2–5]. However, the advent of click chemistry has revolutionized this field due to its high efficiency, specificity, and operational simplicity [6–8]. Indeed, click chemistry, particularly through photo-induced reactions, has significantly expanded the versatility and applicability of biofunctionalized materials across various emerging fields such as 3D printing, tissue engineering, sensor design, and the biocatalyzed production of chemical building blocks [9].These photoclick reactions offer the advantages of selectivity and high yields combined with light-triggered

reactions [10]. Unlike conventional click reactions, which rely on catalysts or moderate heating to initiate the reaction, photo-mediated reactions provide a level of temporal control, allowing the reaction to be precisely triggered at a specific moment [9,11].

The term "photoclick chemistry" was first coined in 2008 to describe the tetrazole-ene reaction [9,10]. Since then, it has been applied to numerous other photo-mediated reactions, including the thiol-ene and thiol-yne click reactions. Furthermore, compared to other biorthogonal click reactions, such as the renowned Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) reaction [12–14], photoclick reactions integrate into biological systems, widely spreading this tool to biological fields [11]. Among photoclick reactions, the thiol-yne photoclick reaction has garnered considerable attention for its metal-free nature and versatility in bioconjugation processes [15,16]. Thiols are exemplary for bioconjugation due to their high nucleophilicity compared to amino groups in peptides and related biomolecules [17,18]. Thiol-containing amino acids, such as cysteine, and small peptides, like glutathione, are important indicators in disease diagnostics [19,20]. The first report of the addition of sulfides to alkynes mediated by radicals was in the 1930s by Finzi and Kohler [21,22]. Since that time, this reaction has proven to be a very valuable tool for the synthesis of linear polymers, for the subsequent modification of polymers with pendant alkyne groups, or for access to cross-linked networks [23–25]. However, to the best of our knowledge, there are still no examples of the use of this reaction to perform a bioconjugation process on a surface. This method offers several advantages, including the ability to couple two thiols with one alkyne under mild conditions using a chemical radical source, UV irradiation, or even sunlight at ambient temperature [21]. The robustness and adaptability of the radical mechanism make the thiol-yne procedure highly attractive for creating multifunctional materials, offering a robust pathway for enhancing the functionality of polymers for applications such as (bio)coatings [26–28].

Among polymers, poly(L-lactic acid) (PLLA), a biodegradable and bioresorbable polymer [29], has become a key material in biomedical applications [30–32]. Its uses range from tissue engineering scaffolds to drug delivery systems [33,34]. PLLA's biocompatibility and mechanical properties make it an ideal substrate for further functionalization through surface modification [32,35–37]. Modifying PLLA surfaces to immobilize bioactive molecules can significantly enhance their interaction with biological environments, thereby improving the material's performance in medical applications [38]. In this context, the thiol-yne photo-click reaction presents a promising strategy for immobilizing bioactive molecules onto polymeric surfaces. This technique can graft various biomolecules onto PLLA surfaces, enhancing their interactions with cells and tissues [39].

In recent years, one of the research lines of our group has focused its attention on the modification of different types of surfaces through click reactions in the absence of metal catalysts. Particularly, we have reported that previously derivatized PLLA surfaces with electron-deficient alkyne moieties can be easily modified with azide-yne and aza-Michael-type click reactions in catalyst-free versions. In fact, different biomolecules, such as tryptophan or amoxicillin, and dyes, such as dansyl, have been effectively conjugated to PLLA surfaces employing these methodologies [40,41]. These types of alkynes with electron-withdrawing substituents have demonstrated to be interesting 1,4-Michael acceptors due to their stability and high reactivity. Although these compounds can react via numerous pathways, they are renowned for their interaction with nucleophiles in conjugate addition reactions [17,40,42]. The practical implementation of the thiol-yne photo-click reaction for polymeric surface modification involves several key steps [24]. Initially, polymer surfaces need to be functionalized with alkyne groups, achievable through various chemical treatments. Subsequently, the thiol-containing biomolecules are introduced, and the system is exposed to UV light to induce the click reaction. This process results in the formation of stable thioether bonds, effectively immobilizing the biomolecules onto the polymer surface. Compared to other metal-free click reactions, a key advantage of thiol-yne reactions is that they enable the bioconjugation of two molecules in a single step. The efficiency and

specificity of this reaction can be further enhanced by optimizing parameters such as light intensity, reaction time, and the concentration of reactants.

In this study, we focus on an activated alkyne featuring an electron-attracting group to promote Michael-type click reactions with thiols without requiring metal catalysts. Propiolic acid serves as the activated alkyne, chosen for its commercial availability and ease of modification. The primary objective is to devise a method for conjugating fluorophore molecules onto polymer surfaces using the alkyne-activated thiol-yne click reaction. Before the conjugation of the fluorophore through the photoclick reaction, it was necessary to carry out different modifications on the poly-L-lactic acid (PLLA) polymeric substrate, to facilitate the covalent attachment of the electron-deficient alkyne moiety. These alkyne-activated substrates have demonstrated a propensity to react with derivatized fluorophore compounds, offering a straightforward means to immobilize diverse molecules through simple modification.

2. Materials and Methods

2.1. Materials

Poly-L-lactide (PLLA) polymer pellets were purchased from Corbion (Amsterdam, The Netherlands). A variety of solvents were used in the synthesis of PLLA films as well as surface functionalization and fluorophore derivatization: acetone (99%, Macron Fine Chemical, Gliwice, Poland), chloroform ($CHCl_3$, >98% Macron Fine Chemicals, Gliwice, Poland), ethanol (EtOH, 99.8%, Macron Fine Chemicals, Gliwice, Poland), methanol (MeOH, 98%, Macron Fine Chemicals, Gliwice, Poland), tetrahydrofuran (THF, 99%, Macron Fine Chemicals, Gliwice, Poland), diethyl ether (Et_2O, 99%, Macron Fine Chemical, Gliwice, Poland), acetic acid (AcOH, Sigma Aldrich, St. Louis, MO, USA), deuterated chloroform ($CDCl_3$, 99.8% Sigma Aldrich, St. Louis, MO, USA), and Milli Q water. Some reagents were employed for the derivatization of dansyl fluorophore: dansyl chloride (99%, Sigma Aldrich, St. Louis, MO, USA), cysteamine hydrochloride (99%, Sigma Aldrich, St. Louis, MO, USA), sodium hydroxide (99%, Panreac, Darmstadt, Germany), sodium hydrogen carbonate ($NaHCO_3$, 99%, Merck, Darmstadt, Germany), sodium carbonate (Na_2CO_3, 98%, Panreac, Darmstadt, Germany), sodium borohydride ($NaBH_4$, 98%, Sigma Aldrich, St. Louise, MO, USA), Irgacure 651 (2,2-dimethoxy-2-phenylacetophenone, Sigma Aldrich, St. Louis, MO, USA), *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC·HCl, 98%, Sigma Aldrich, St. Louis, MO, USA), *N*-hydroxysuccinimide (NHS, 98%, Sigma Aldrich, St. Louis, MO, USA), ethylendiamine (99%, Sigma Aldrich, St. Louis, MO, USA), triethylamine (Et_3N, <99.5%, Sigma Aldrich, St. Louis, MO, USA), propiolic acid (95%, Sigma Aldrich, St. Louis, MO, USA), sodium sulfate anhydrous (Na_2SO_4, 98%, Panreac, Darmstadt, Germany), magnesium sulfate anhydrous ($MgSO_4$, 98%, Panreac, Darmstadt, Germany).

2.2. Methods

2.2.1. Synthesis of Dansyl Derivative **2a**

Dansyl chloride (0.50 g, 1.85 mmol) was dissolved in acetone (51 mL) and water (2 mL). Then, previously prepared cysteamine hydrochloride (0.11 g, 0.93 mmol) in aqueous $NaHCO_3$ (0.1 M, 12 mL) solution was added in small portions. In addition, NaOH (0.5 M) was used to keep the solution at a pH of 7.5. Then, the mixture was stirred for 90 min at room temperature. After that, chloroform (100 mL) was added to the solutions, and layers were separated. The organic layer was washed with sodium carbonate solution (4 × 20 mL) and water (1 × 20 mL). Finally, the organic phase was collected, dried over anhydrous $MgSO_4$, and the solvent was evaporated under vacuum to afford *N*,*N*′-(disulfanediylbis(ethane-2,1-diyl))bis(5-(dimethylamino)naphthalene-1-sulfonamide), which was used in the next step without further purification. ^{1}H-NMR (300 MHz, $CDCl_3$) (δ, ppm): 8.54 (d, *J* = 8.5 Hz, 1H, CH_{arom}), 8.24–8.20 (m, 2H, CH_{arom}), 7.56–7.52 (m, 2H, CH_{arom}), 7.17 (d, *J* = 7.6 Hz, 1H, CH_{arom}), 5.24 (t, *J* = 6.3 Hz, 1H, NH), 3.09 (q, *J* = 6.3 Hz, 2H, $NHCH_2$), 2.88 (s, 6H, 2 × CH_3), 2.48 (t, *J* = 6.3 Hz, 2H, S-CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$) (δ, ppm): 152.2 (C_{arom}-N),

134.5 (C_{arom}-S), 132.4 (C_{arom}-H), 130.8 (C_{arom}-C), 130.0 (C_{arom}-H), 129.8 (C_{arom}-H), 129.6 (C_{arom}-H), 123.3 (C_{arom}-H), 118.7 (C_{arom}-C), 115.4 (C_{arom}-H), 45.6 ($N(CH_3)_2$), 41.7 ($NHCH_2$), 37.9($S\text{-}CH_2$).

2.2.2. Synthesis of Dansyl Derivative **2b**

Previously synthesized compound **2a** (0.09 g, 0.16 mmol) was dissolved in THF (6 mL) and water (1 mL) at 0 °C. Then, $NaBH_4$ (0.06 g, 1.63 mmol) was added in small portions. After, the reaction was vigorously stirred for 4 h at room temperature. Following, the solvent was evaporated under vacuum, and the obtained solid was dissolved in water (10 mL). This last aqueous solution was extracted with diethyl ether (3 × 10 mL). Finally, organic layers were collected, dried over anhydrous Na_2SO_4, and solvent evaporated under vacuum to afford 5-(dimethylamino)-N-(2-mercaptoethyl)naphthalene-1-sulfonamide (**2b**) as a brownish oil (0.03 g, 57%). ^{1}H-NMR (300 MHz, $CDCl_3$) (δ, ppm): 8.56 (d, *J* = 8.5 Hz, 1H, CH_{arom}), 8.26–8.23 (m, 2H, CH_{arom}), 7.55–7.52 (m, 2H, CH_{arom}), 7.20 (d, *J* = 7.6 Hz, 1H, CH_{arom}), 5.18 (t, *J* = 6.3 Hz, 1H, NH), 3.10–3.07 (m, 2H, $NHCH_2$), 2.89 (s, 6H, 2×CH_3), 2.51 (dt, *J* = 8.7, 6.4 Hz, 2H, $SHCH_2$), 1.21 (t, *J* = 8.7 Hz, 1H, SH); ^{13}C-NMR (75 MHz, $CDCl_3$) (δ, ppm): 152.1 (C_{arom}-N), 150.1 (C_{arom}-S), 134.6 (C_{arom}-H), 130.7 (C_{arom}-C), 129.9 (C_{arom}-H), 129.5 (C_{arom}-H), 128.6 (C_{arom}-H), 123.2 (C_{arom}-H), 118.5 (C_{arom}-C), 115.3 (C_{arom}-H), 45.9 ($NH\text{-}CH_2$), 45.4 ($N(CH_3)_2$), 24.8 ($SHCH_2$).

2.2.3. Preparation, Hydrolysis, and Amidation of PLLA Films

PLLA pellets (1 g) were dissolved in 50 mL of chloroform, followed by reprecipitation in cold distilled methanol and subsequent drying under vacuum at 40 °C. The fabrication of PLLA films followed the established procedure outlined in our previous work [43]. Hydrolysis and amidation conditions remained consistent with those detailed in our earlier studies [40,41].

2.2.4. Immobilization of Dansyl Derivative **2b** onto PLLA Films via Metal-Free Thiol-Yne Click Reaction

The synthesized dansyl derivative **2b** (10 mmol) was dissolved in ethanol, and the photoiniator Irgacure 651 (0.10 mmol) was added. This mixture was homogenized under ultrasound, and then propiolated PLLA (PLLA-Alkyne) surfaces were immersed in the solution for 1 h and exposed to ultraviolet light under an Hg lamp. Afterwards, the obtained dansylate surfaces (PLLA-Dns) were washed with ethanol and water and dried under vacuum at room temperature.

2.2.5. Nuclear Magnetic Resonance (NMR)

For the characterization of the derivatized fluorophore, proton (^{1}H-NMR) and carbon-13 (^{13}C-NMR) nuclear magnetic resonance spectra were obtained using an AV-300 spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C) (Bruker, Rheinstetten, Germany) at room temperature with deuterated chloroform as the solvent. Chemical shifts (δ) are reported in parts per million (ppm) relative to TMS, using the residual signal of the solvent [7.26 ppm (^{1}H) and 77.0 ppm (^{13}C)] as the internal reference. Coupling constants (*J*) are reported in hertz (Hz).

2.2.6. Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR)

PLLA surface functionalizations were analyzed via an FTIR spectrophotometer (NICOLET Nexus 670 FT-IR, Thermo Scientific, Loughborough, UK) equipped with an ATR. 2 × 1 cm PLLA surfaces were measured using 32 scans from 4000 cm^{-1} to 500 cm^{-1} within the wavenumber range and a resolution of 4 cm^{-1}.

2.2.7. Water Contact Angle (WCA)

The alteration in wettability of PLLA surfaces resulting from surface modifications was studied using the static contact angle method (NEURTEK Instruments OCA 15 EC, Eibar,

Spain). Milli-Q water was used as a testing liquid, and measurements were conducted at room temperature using the sessile drop method with 2 μL per drop. Average values were determined from five measurements for each composition.

2.2.8. Scanning Electron Microscope (SEM)

Morphological observations were carried out on functionalized PLLA surfaces employing a HITACHI S-4800 electronic microscope (Hitachi, Singapore). An electron acceleration voltage of 1.5 kV over a distance of 7.1 mm × 5.00 k was applied for different functionalized PLLA surfaces.

2.2.9. X-ray Photoelectron Spectroscopy (XPS)

The elemental analysis of modified PLLA surfaces was performed using X-ray photoelectron spectroscopy (XPS) with a SPECS system (Surface Nano Analysis, Berlin, Germany). This system uses a focus monochromatic radiation source 500 with a dual anode Al/Ag, equipped with a 150 1D-DLD analyzer (Phoibos, Berlin, Germany). The PLLA samples were secured with stainless steel holders and carbon tape during the measurements. Additionally, a carbon reference was employed for the measurements.

2.2.10. Fluorescence Microscopy

The fluorescence of PLLA surfaces, both before and after the thiol-yne click reaction, was analyzed using a Zeiss Axioskop epifluorescence microscope (Zeiss, Jena, Germany).

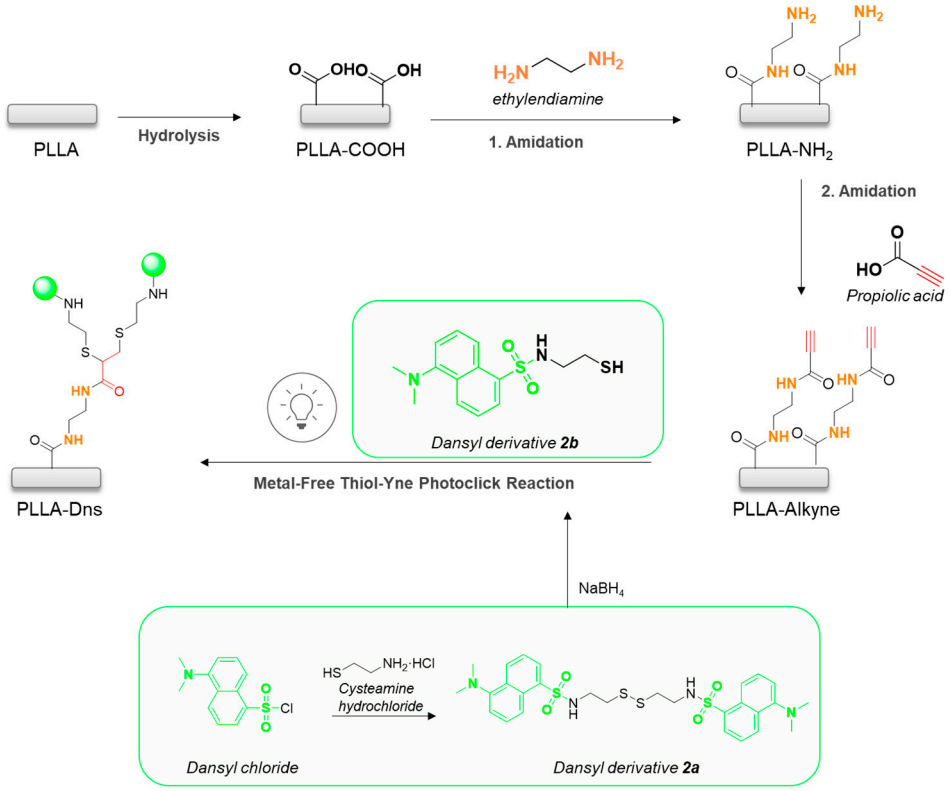
2.2.11. Fluorescence Spectroscopy

The emission spectra of the PLLA films were recorded using an Edinburgh Instruments spectrofluorimeter (Edinburg Instruments, FLSP920 model, Livingston, UK) with UV region excitation in a front-face configuration. The samples were positioned at 40° and 50° angles to the excitation and emission beams, respectively, and tilted at a 30° angle relative to the plane formed by the direction of incidence and detection.

3. Results

To immobilize the desired fluorophore, preactivation of the PLLA surface was essential. The preactivation process was consistent with our previous works [40,41]. Briefly, PLLA was hydrolyzed and then amidated with ethylenediamine, enabling subsequent derivatization with propiolic acid. Both amidation reactions were performed under EDC·HCl/NHS conditions at room temperature. Once the required alkyne group was incorporated onto the surface, the synthesized fluorophore derivative was immobilized onto the PLLA surfaces using thiol-yne click chemistry. This method excludes the need for a catalyst and employs a photoinitiator, providing a straightforward and efficient approach to surface modification (Scheme 1).

The appearance of new bond formation was followed by ATR-FTIR analysis (Figure 1A). In fact, after hydrolysis and the first amidation step, prominent bands at 3300–3250 cm^{-1} and 2900 cm^{-1} appeared in the PLLA-COOH and PLLA-NH_2 spectra, attributed to the stretching vibrations of N-H, O-H, and C_{sp3}-H, respectively, which corroborated the successful preactivation process. Upon further grafting with propiolic acid, the presence of distinctive stretching bands at 3350 cm^{-1} and 2090 cm^{-1} confirmed the incorporation of the terminal alkyne moiety onto PLLA surfaces. Furthermore, the presence of a band at 1650 cm^{-1}, indicative of C=O stretching and consistent with the typical appearance of C=O in amides, provided strong evidence of the success of the second amidation reaction. Finally, following conjugation with dansyl derivative **2b** via a thiol-yne photoclick reaction (PLLA-Dns), the C_{sp}-C_{sp} stretching signal disappeared, confirming the effective immobilization of the dansyl derivative onto PLLA.



Scheme 1. PLLA surface preactivation and dansyl flurophore **2b** photo-immobilization via thiol-yne click chemistry.

The success of each functionalization step was corroborated by means of XPS in order to determine the chemical composition of the surfaces (Figure 1B). After hydrolysis, two major contributions were observed at 284 eV and 535 eV, corresponding to C1s (69.9%) and O1s (29.8%), respectively. Following amidation with ethylenediamine, a new peak at 400 eV attributed to N1s (1.8%) emerged in the spectrum of PLLA-NH_2, confirming the successful amidation of hydrolyzed PLLA. Moreover, high-resolution carbon spectra corroborated the introduction of nitrogen, as evidenced by the enhanced peak of the C-O/C-N contribution at 285.8 eV. Subsequent to the second amidation process employing propiolic acid (PLLA-Alkyne), similar carbon, oxygen, and nitrogen content values were obtained compared to PLLA-NH_2, namely 62.0%, 26.0%, and 3.2%, respectively. However, notable changes were detected in the nitrogen high-resolution spectra of PLLA-NH_2 and PLLA-Alkyne (Figure 1C). Specifically, contributions observed at 398.9 eV, 399.7 eV, and 400.6 eV corresponding to NH_2, N-C=O, and C-NH functionalities on PLLA-NH_2 surfaces disappeared, while NH-C=O contributions increased on PLLA-Alkyne surfaces. These findings confirmed the introduction of the activated terminal alkyne group crucial for facilitating the attack of the corresponding nucleophilic partner, in this case the thiol moiety of the fluorophore derivative. Upon further functionalization with the dansyl derivative, a new peak around 169 eV corresponding to the S2p (0.8%) element was observed. These results reaffirmed the immobilization of the dansyl derivative **2b** onto the PLLA surface through the metal-free thiol-yne photoclick reaction.

For this thiol-yne click reaction, an Irgacure 651 photoinitiator was used to photoimmobilize the dansyl derivative **2b**. PLLA-alkyne samples carrying the dansyl derivative were exposed to irradiation for 1 h across a broad spectrum, ranging from 260 to 400 nm. Notably, the UV maximum absorption peak of the photoinitiator lies between 310 and 350 nm. Through confocal microscopy (Figure 2), the fluorescence emission from the PLLA-Dns surface was observed. Upon light excitation of both pristine PLLA and PLLA-Dns, only PLLA-Dns exhibited a green emission, corresponding to the dansyl region. These

findings certainly confirmed the completion of the immobilization of dansyl derivative **2b** via the thiol-yne click reaction.

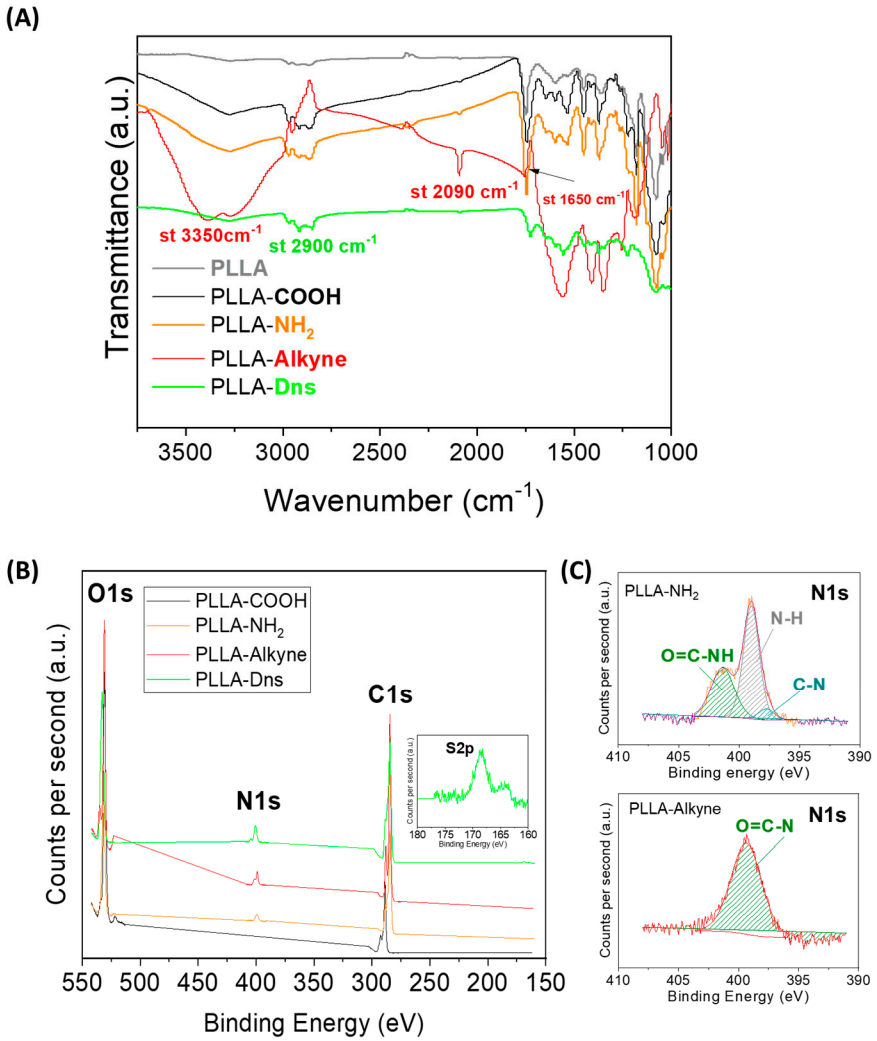


Figure 1. (**A**) ATR-FTIR spectra of pristine and functionalized PLLA surfaces. (**B**) Total XPS spectra of PLLA-COOH, PLLA-NH_2, PLLA-Alkyne, and PLLA-Dns surfaces. (**C**) High-resolution N XPS spectra of above PLLA-NH_2 and bottom PLLA-Alkyne.

Similarly, steady-state fluorescence spectroscopy was also employed for the corroboration of the fluorophore immobilization (Figure 2B). Pristine PLLA and PLLA-Dns were excited at the same wavelengths, but only the PLLA-Dns sample exhibited a maximum peak at 405 nm, which could be related to dansyl derivative **2b** fluorescence emission. These findings, along with the confocal fluorescence emission, confirmed the successful photoimmobilization of dansyl derivative **2b** using the thiol-yne click reaction.

Regarding surface morphology, SEM images in Figure 3A revealed that PLLA surfaces experienced fissures and cracking after hydrolysis and amidation reactions, resulting in heterogeneous surfaces. Following the photo-immobilization of the dansyl derivative **2b**, the surface exhibited minimal non-uniform protrusions. These specific surface variations further indicated the successful surface functionalization and effective immobilization of the dansyl derivative.

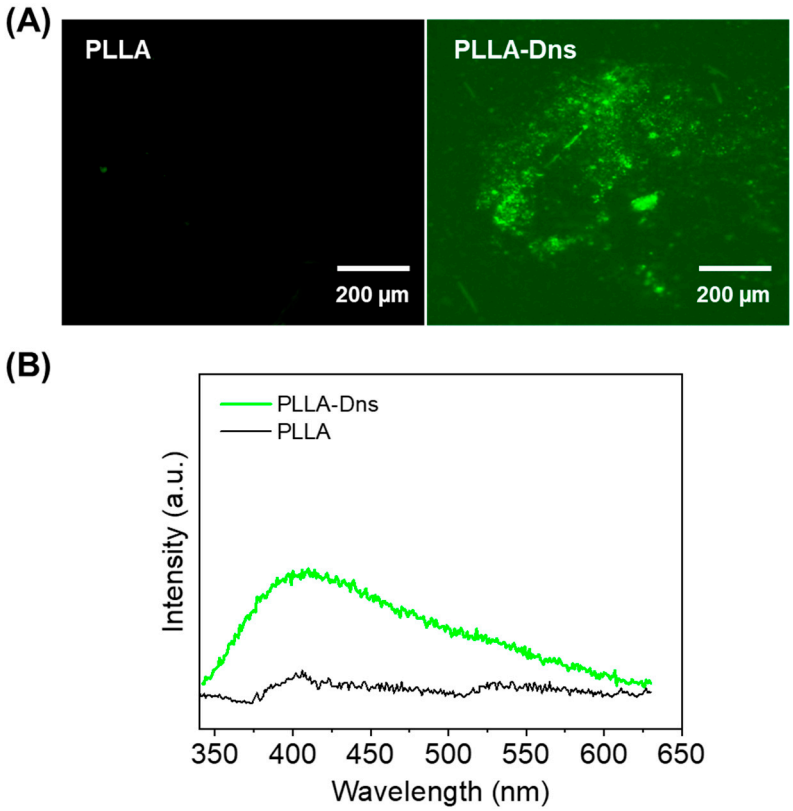


Figure 2. (**A**) Confocal microscopy of PLLA and PLLA-Dns surfaces. (**B**) Steady-state fluorescence spectroscopy of PLLA and PLLA-Dns surfaces.

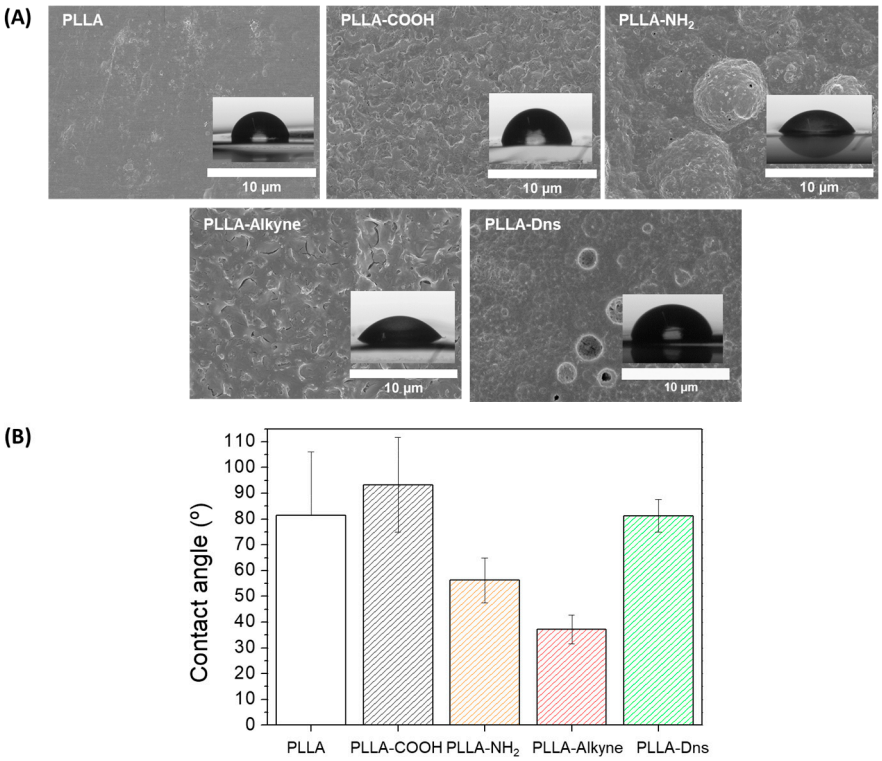


Figure 3. (**A**) SEM images of pristine and functionalized PLLA surfaces (10 µm) (**B**) Water contact angle values of pristine and functionalized PLLA surfaces.

Moreover, the wettability properties of the surface were analyzed after each functionalization step by means of water contact angle (Figure 3B). Hydrolysis and both amidation reactions significantly increased the surface's hydrophilicity, reducing the contact angle value from 111 ± 4.7° to 38.7 ± 4.6°. However, after the photoimmobilization of the dansyl derivative **2b**, the water contact angle (WCA) value increased to 81.3 ± 6.3°. This increase corroborated the presence of aromatic groups and sulfur elements on the polymer surface. These findings, consistent with those obtained in other studies, align with the presence of aromatic functional groups in dansyl, which make the surface less prone to forming hydrogen bonds with water [40]. This change in WCA value reaffirmed the successful immobilization of the dansyl derivative **2b** through the thiol-yne click reaction.

4. Conclusions

This study developed a surface treatment strategy for the conjugation of fluorophore compounds onto polymer surfaces using a metal-free thiol-yne photoclick reaction. The success of surface pre-functionalization and the covalent attachment of the fluorophore to it were confirmed through several characterization techniques, including ATR-FTIR, XPS, water contact angle measurements, SEM, and fluorescence analysis. This work provides a new route for conjugating biological compounds via click reactions, effectively overcoming the drawbacks of cytotoxic metals. By combining simple amidation reactions with advanced metal-free Michael-type click reactions, it is possible to create bioactivated materials suitable for a wide range of biomedical applications, such as implant coatings, biosensors, and tissue engineering. Future investigations will focus on broadening the research scope and assessing the degradability and cell cytotoxicity of the proposed coating. This study not only expands the potential of metal-free click chemistry in developing novel conjugation strategies but also highlights the promising future trends in thiol-yne click chemistry in creating advanced biomedical materials.

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