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Mild Methodology for the Versatile Chemical Modification of Polylactide Surfaces: Original Combination of Anionic and Click Chemistry for Biomedical Applications

Sarah El Habnoui, Vincent Darcos, Xavier Garric, Jean-Philippe Lavigne, Benjamin Nottelet,* and Jean Coudane

Biodegradable aliphatic polyesters such as polylactide (PLA) are widely used in medical applications. When employed as an implantable material, the control of the surface properties of PLA is of great interest because biochemical reactions occur on the surface or at interfaces. Thus, chemical modifications of the surface of degradable polyesters can be used to tailor the surface properties while preserving the bulk characteristics. This paper proposes a simple and versatile method of preventing polymer degradation and immobilizing simple molecules, macromolecules, and biomolecules on PLA surfaces. The method is based on a one-pot, two-step procedure: anionic activation under selected conditions followed by propargylation to form a “clickable” PLA surface. This surface is extensively characterized by SEC and fluorescence techniques using a fluorescent probe, confirming both the functionalization and the absence of PLA degradation. An example of the surface immobilization of a bioactive compound is then described: a well-defined α -azido-functionalized poly(quaternary ammonium) synthesized via ATRP is covalently bound to the propargylated PLA surface using “click” chemistry. This covalent grafting is confirmed by SEC and XPS analyses. The increase in surface hydrophilicity is demonstrated by water contact-angle measurements. Finally, a primary investigation is conducted to determine the antibacterial activity of modified PLA surfaces against *E. coli* and *S. aureus*.

improve human health. Because of their various compositions, structures and excellent properties, a very-large number of polymers have been evaluated as potential biomaterials for a wide range of biomedical applications.^[1] Special emphasis is placed on biodegradable polymers that are employed as drug carriers, wound dressings, medical devices and scaffolds in tissue engineering;^[2] because of their excellent biocompatibility and adjustable degradability,^[3] these include polyesters such as polylactide (PLA), polyglycolide (PGA) and poly(ϵ -caprolactone) (PCL), which are now in widespread use. As an example, PLA was approved by the US Food and Drug Administration (FDA) in the 1970s, and has since been used in the form of sutures, clips, plates and screws, ultrasound contrast agents, nerve guides and even drug-delivery devices.^[4] In addition to these “classical” uses, PLA is also attractive and under investigation for innovative applications, such as devices bearing magnetic-resonance-imaging contrast agents.^[5] Unfortunately, PLA has a few drawbacks, such as its crystallinity,

1. Introduction

One of today’s most-challenging fields is that of biomaterials, where chemical and medical scientists are working together to

lack of reactive side-chain groups and hydrophobicity. Generally, in order to modulate their physico-chemical and/or biological properties, polyesters are functionalized, and here two main strategies are employed. The first involves the synthesis and (co)polymerization of functionalized lactones,^[6] and the second, the chemical modification of preformed polyesters in solution.^[7] Both yield novel functional polyester chains, lending new properties. However, for implantable materials, it is not always required or even beneficial to change all of a material’s properties by modifying its polymer chains because: i) biochemical reactions occur at the surface of the material or at the interface with body fluids; and ii) as PLA devices are FDA-approved, it is important to ensure that their intrinsic properties, such as their biocompatibility and degradation rate, are unchanged, while at the same time broadening their application possibilities. This seemingly contradictory requirement to both modifying and maintaining their properties explains why such interest focuses on a controlled modification of degradable polyester surfaces.

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Such a surface modification is the aim of current biomedical research as this would improve the biocompatibility and bio-integration, avoid non-specific protein adsorption, control the adsorption of proteins and/or cells on engineered surfaces, or confer antibacterial properties to medical devices.^[8]

Surface-modification methods may be classified as non-permanent (non-covalent attachment of functional groups) or permanent (covalent attachment).^[9] Temporary surface-modification methods include coating,^[10] entrapment^[11] and migratory additives.^[12] Although these methods have been used to modify PLA surfaces, they suffer from the drawbacks inherent to their non-permanent nature: the release of bioactive compounds from such modified surfaces and the subsequent loss of activity potentially makes them unsuitable for most biomedical and consumer applications. For example, in the case of an antibacterial material, the release of leachable biocides that include antibiotics, phenols, iodine, quaternary ammonium or heavy metals such as silver, tin, and mercury in their composition could have serious adverse effects on the durability, useful lifetime, efficiency and toxicity of the treated biomaterial.^[13] As a consequence, it is important for the bioactive compounds to be covalently immobilized on the surface.

Up until now, permanent surface modification has mainly been obtained by plasma treatment, photografting or wet chemistry,^[14] but these techniques have a number of weaknesses. For instance, in plasma treatment, despite the fact that PLA wettability and cell affinity can be improved,^[15] the possibility of surface rearrangement reduces the efficiency of the process,^[16] and can lead to a non-permanent modification.^[17] Furthermore, plasma treatment can also affect PLA degradation.^[18] Photografting has been extensively used to tailor the surface properties of PLA thanks to the advantages it offers: low cost, mild reaction conditions, selectivity of UV light and permanent alteration of surface chemistry.^[19] Also, the penetration of immobilized molecules into the bulk, which may occur when organic solvents are used in the liquid-phase photografting protocol,^[20] can be avoided by using vapour-phase photografting.^[21] However, this technique suffers from its complexity, with new parameters and a new optimization required for each surface functionalization. The third strategy used to covalently modify the PLA surface is wet chemistry. At present, the wet-chemistry modification of PLA is restricted to degrading conditions. An example is surface hydrolysis, by acid/base degradation, which is used to generate carboxylic acids ($-\text{COOH}$) on PLA,^[22] which may then be further activated with phosphorous pentachloride (PCl_5),^[23] thionyl chloride (SOCl_2),^[24] or water-soluble carbodiimides^[25] before subsequent conjugation with amines or alcohols.^[26] Aminolysis is another means used to introduce reactive amino groups onto PLA surfaces.^[27]

In brief, despite the fact that these permanent surface-modification techniques are able to modify PLA surfaces, they lack versatility, since new chemistry has to be developed each time a new functionality/property is sought. Finally, given that PLA is easily degraded, modifications that match the complex requirements imposed by biomedical applications and tissue engineering are quite a challenge. There is therefore a need to develop a simple protocol to functionalize the surface of PLA and enable the immobilization of a wide range of bioactive compounds. Ideally, this method should: i) prevent polymer

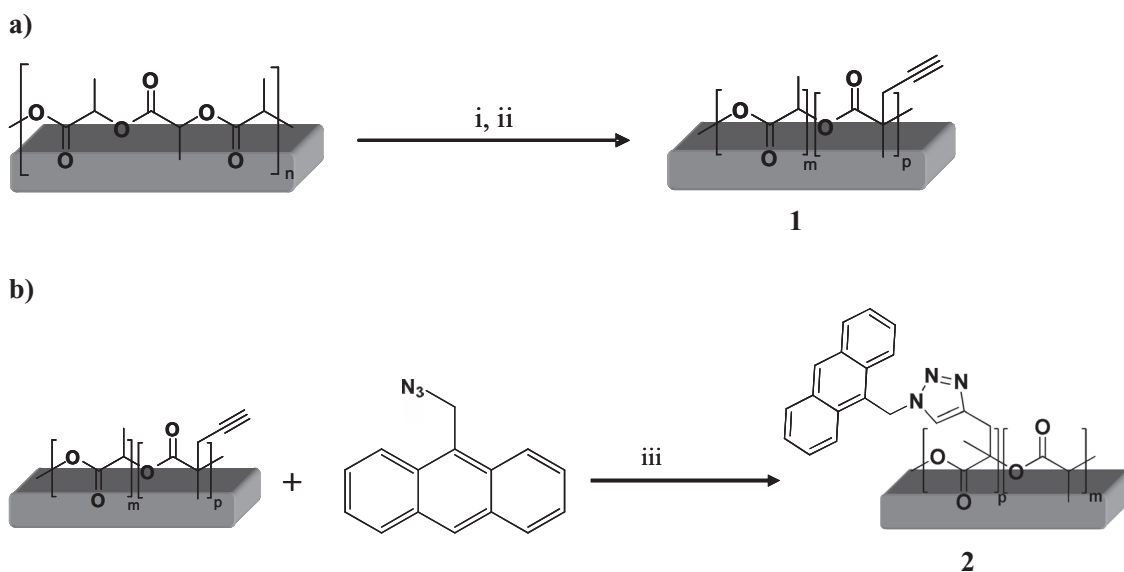
degradation; and ii) yield an activated stable surface that can be derivatized using a simple procedure for the immobilization of small molecules, macromolecules and biomolecules, without altering the structure of these active compounds. These requirements are met by click chemistry, especially the Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition of alkynes and azides (CuAAC), which has demonstrated its superiority as a binding tool in material surface science. Generally speaking, click chemistry is a versatile method, combining mild experimental conditions, tolerance of functional groups, and high yields.^[28] With regard to the modification of surfaces, CuAAC can efficiently couple chemical species to surfaces, thus allowing for the formation of complex molecular architectures on a solid material.^[29] In this paper, we report on the original and straightforward synthesis of a “clickable” PLA surface. An anionic activation, in the presence of lithium di-isopropyl amide (LDA) under selected conditions followed by treatment with propargyl bromide yields an activated and stable clickable PLA surface. Such surfaces were carefully characterized and used to graft a hydrophilic polymer using CuAAC. As proof of the versatility, a quaternized poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) bearing a fluorescent probe (QFPD-MAEMA), synthesized by an ATRP process was also grafted to the PLA surface, yielding a PLA antibacterial surface of interest for implanted biomaterials. Preliminary results concerning antiadherence properties against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) are presented and discussed.

2. Results and Discussion

Our approach to functionalizing the surface of a 3D PLA₉₄ object is based on a three-step strategy combining: 1) synthesis of a propargyl-functionalized surface via anionic activation; 2) synthesis of an azido-functionalized bioactive compound; and 3) Huisgen's 1,3-dipolar cycloaddition (click chemistry) of the bioactive compound to the propargylated surface. This strategy is expected to be versatile and offers a wide range of functionalizations. In this work, as the first example of a bioactive compound, we synthesized a quaternized and fluorescent α -azido-terminated PDMAEMA ($\alpha\text{-N}_3\text{QFPDMAEMA}$) via atom-transfer radical polymerization (ATRP) of 2-(dimethylamino)ethyl methacrylate (DMAEMA) with a fluorescent probe, anthracenylmethyl methacrylate (AMM), followed by quaternization of the amine group.

2.1. Synthesis of the Clickable Surface

The first goal was to make the surface of the PLA₉₄ clickable. Several years ago, we presented a method that can be used to chemically modify aliphatic polyesters. This modification route is based on activation of a polyester chain by proton extraction from the methylene groups in the α -position of the carbonyl group, using lithium *N,N*-di-isopropylamide (LDA) in tetrahydrofuran (THF). The activated polymer chains bearing carbanion sites are then combined with different electrophiles to generate novel copolymers. Despite the fact that the polymer chains underwent limited degradation during the first step



Scheme 1. a) Synthesis of a clickable surface of PLA₉₄: i) LDA/ET₂O/THF, -50 °C to -30 °C, 30 min; ii) propargyl bromide, -30 °C to room temperature (RT), 1 h. b) Fluorescent labelling of the surface by CuAAC: iii) CuSO₄/sodium ascorbate H₂O/EtOH, 40 °C, 48 h.

of the reaction, various functionalized PCLs of rather-high molecular weights were easily obtained in solution ($M_n \geq 20\,000\text{ g mol}^{-1}$).^[30] Preliminary studies on PLA have shown that anionic activation of the surface of a PLA plate can occur under the same conditions, but is accompanied by the degradation of the surface polymer chains.^[31] In this paper, we report on a general method that is mild enough to prevent polymer degradation. This strategy is shown in **Scheme 1**. The surface of a PLA plate was activated by treatment with a non-nucleophilic strong base, LDA, at low temperature in a mixture of chosen solvents.

PLA degradation was avoided by a careful choice of the anionic activation conditions. Various solvent mixtures, reaction times and reaction temperatures were tested. The best results were obtained when the PLA plates were functionalized using LDA in a mixture of THF and diethyl ether (1/2 v/v) for half an hour at -50 °C to -30 °C, followed by the addition of propargyl bromide in two-fold excess versus LDA (Scheme 1a). The electrophile was added over a period of one hour at a temperature rising from -30 °C to room temperature (RT). The plate was then washed with water and diethyl ether to remove any non-covalently grafted species.

The challenge here was to maintain the physico-chemical properties of the core of the plate while modifying the surface. However, since functionalization occurred only at the surface, conventional characterization techniques such as IR or Raman spectroscopy (which are both penetrating techniques), were unable to detect the propargyl group. The presence of propargyl groups on the surface and their reactivity were therefore investigated by an indirect detection method using

a fluorescent probe (Scheme 1b) and 9-azidomethylanthracene was prepared for this purpose in accordance with a described procedure.^[32] This compound was used to detect the presence of propargyl groups on the surface of the PLA and to check their chemical availability for CuAAC. The click reaction of the probe was performed under aqueous click conditions.^[33] The extensively washed plate (2) was analyzed using size-exclusion chromatography (SEC) with refractometric and fluorescence double detection. The SEC traces obtained from the surface to the core showed that only the external layers were fluorescent (**Figure 1**). Therefore, the core was unaffected and the bulk properties were conserved. In addition, SEC using refractometric detection

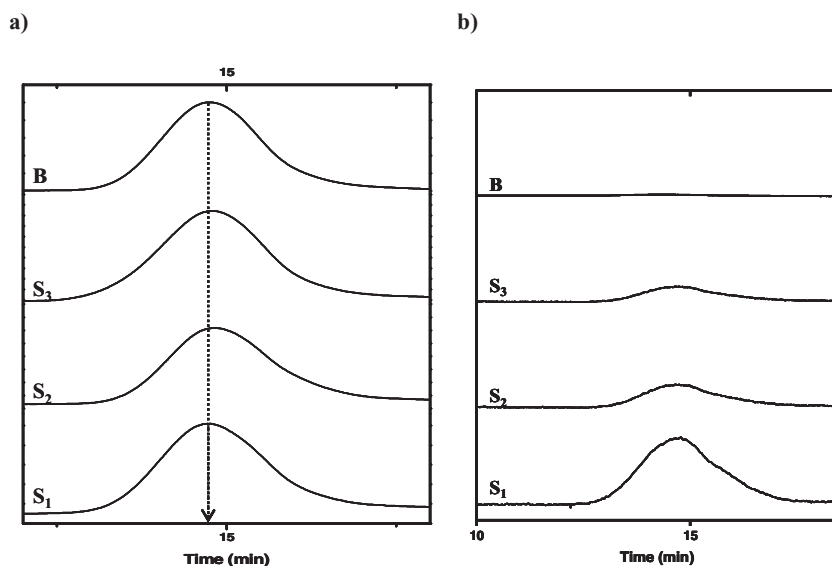


Figure 1. a–b) SEC chromatograms of successive layers from the surface to the core (from S₁ to B) of the fluorescent-labelled surface of PLA₉₄ using refractometric (a) and fluorescence (b) double detection ($\lambda_{\text{ex}} = 370\text{ nm}$, $\lambda_{\text{em}} = 420\text{ nm}$).

showed that the molecular weight and polydispersity of the surface were unchanged compared to that of the core. The external layers and the core had the same molecular weight ($M_n = 100\,000\text{ g mol}^{-1}$) and polydispersity (PDI = 1.9).

Our specific conditions may therefore be considered to yield a stable intermediate, which is the clickable surface of the PLA₉₄ (1), with no degradation and without altering the 3D shape of the plate. To the best of our knowledge, this is the first example of a modified PLA surface obtained in a simple and mild one-step procedure, allowing the specific coupling of azido-modified bioactive molecules by click chemistry.

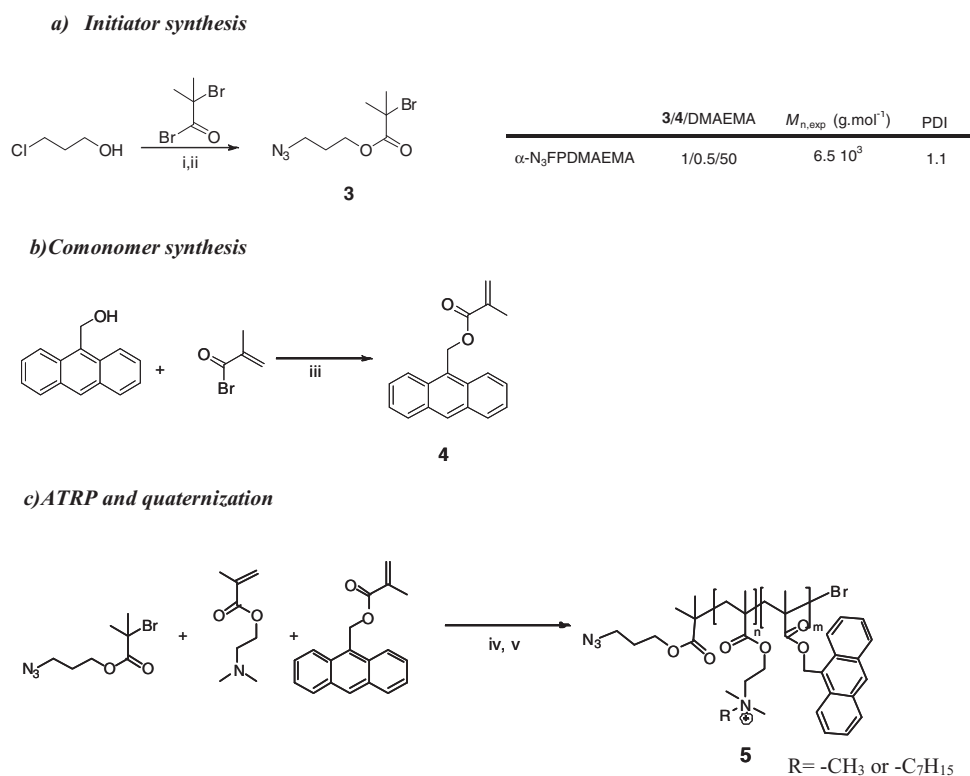
2.2. Synthesis of a Fluorescent, Quaternized and Clickable PDMAEMA (α -N₃QFPDMAEMA)

The grafting of 9-azidomethylanthracene as a fluorescent probe to the clickable PLA surface demonstrated that functionalization by small molecules is possible. Surface modifications with macromolecules are far more challenging. Typically, two routes are used to bind macromolecules to the surface of a polymeric device. The first is the “grafting-from” procedure, involving the polymerization of monomers from surface-anchored initiators. This method ensures the synthesis of polymer chains covalently attached to the surface and can produce a high grafting density. The major drawback of this method is the lack of control over the grafted polymer in terms

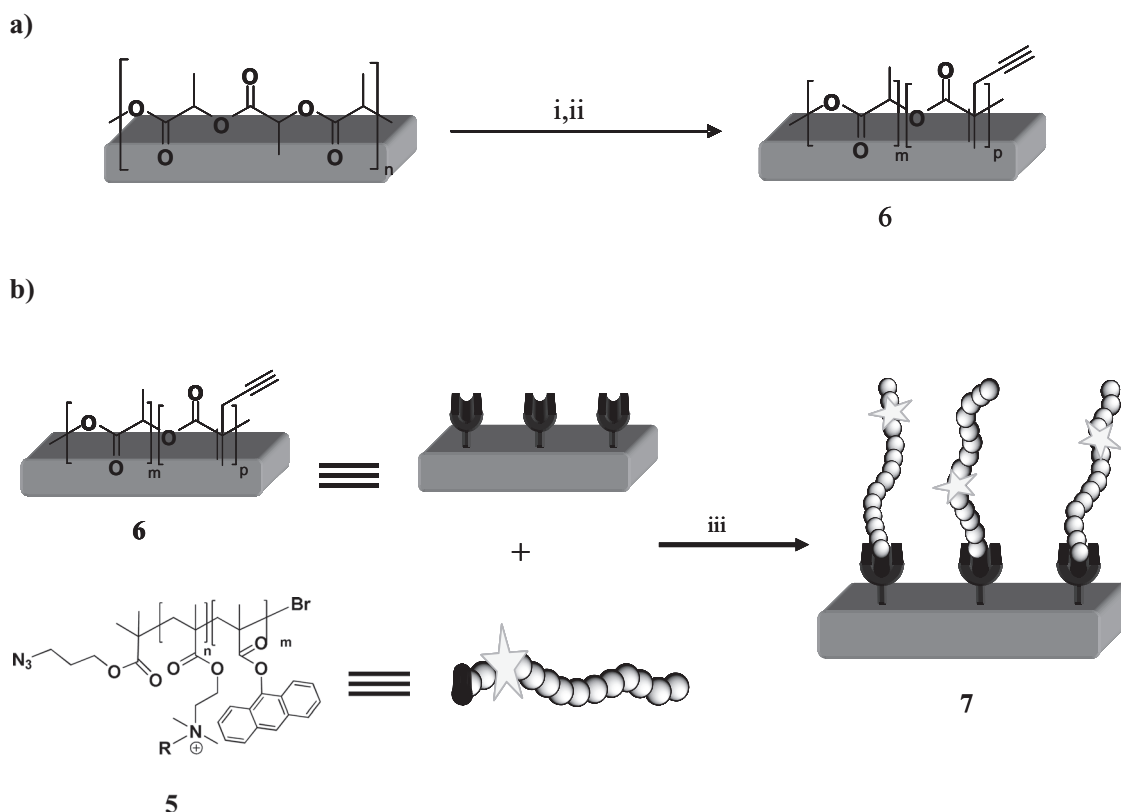
of molecular weight and polydispersity. The second route, called the “grafting-onto” technique, involves the reaction between a functional group of a premade polymer and an active site on the surface. The advantage is that control can be exerted over the molecular weight and polydispersity of the grafted polymer. However, this technique is limited by the low grafting density due to poor accessibility to the reactive surface groups caused by steric hindrance. Thanks to its very-high efficiency, CuAAC is often considered as the tool of choice to overcome this limitation. As an initial demonstration of our methodology, we synthesized a poly(quaternary ammonium) with antibacterial activity and grafted it by CuAAC onto a clickable PLA surface.^[34] PDMAEMA derivatives were therefore synthesized in accordance with well-known chemistry.^[35]

A fluorescent-azide-containing PDMAEMA (α -N₃FPDMAEMA) (5) precursor was prepared following a similar procedure to that reported by Agut et al.^[36] Well-defined α -N₃FPDMAEMA was synthesized by ATRP at 60 °C in toluene in the presence of CuBr/*N,N,N',N'',N'''*-pentamethyldiethylenetriamine (PMDETA) as a catalytic system, using a specific azide-functionalized initiator, (3-azidopropyl)bromoisobutyrate (3), and a fluorescent probe (4) (Scheme 2).

SEC showed a monomodal trace with polydispersity below 1.2. The molecular weights (M_n) determined by SEC were relative to polystyrene standards, so more-accurate values were provided by ¹H-NMR spectroscopy. Accordingly, $M_{n,NMR}$ of 5 was determined to be 6500 g mol⁻¹, in good agreement with



Scheme 2. a) Synthesis of α -azido initiator: i) NaN₃, tetrabutylammonium hydrogen sulfate (TBAHS)/water, 80 °C, 24 h then RT, 12 h; ii) TEA/DCM 0 °C, 1 h then RT, 17 h. b) Synthesis of fluorescent comonomer: iii) TEA, DCM, 0 °C, 1 h then RT, 17 h. c) Copolymerization with DMAEMA by ATRP and quaternization: iv) CuBr, PMDETA, toluene, 60 °C, 20 min; v) CH₃I or C₇H₁₅I, THF, RT, 17 h. The table insert provides an overview of the proportions of 3/4/DMAEMA, M_n determined by ¹H-NMR spectroscopy and the PDI determined by SEC.



Scheme 3. a) Synthesis of a clickable surface of PLA₉₄: i) LDA/ET₂O/THF, -50 °C to -30 °C, 30 min; ii) propargyl bromide, -30 °C to RT, 1 h. b) Covalent immobilization of α -N₃QFPDMAEMA by CuAAC: iii) CuSO₄/sodium ascorbate H₂O/EtOH, 40 °C, 48 h.

the theoretical value of 7000 g mol⁻¹. In addition to the molecular weight, the SEC chromatograms, using a photodiode-array (PDA) detector, confirmed the incorporation of the fluorescent methacrylate dye during the ATRP process. The presence of azide groups was evidenced by the characteristic azide absorbance peak at ≈ 2100 cm⁻¹ in the FTIR spectrum (S1–S4, Supporting Information). Finally, quaternized α -N₃QFPDMAEMA (α -N₃QFPDMAEMA) was obtained by a conventional technique, using methyl iodide or heptyl iodide as an alkylating agent, in THF for 18 h at room temperature.

2.3. Click Reaction of α -N₃QFPDMAEMA on Propargylated PLA₉₄ Surface

The next critical step was the “click-chemistry” reaction of α -N₃QFPDMAEMA on the propargyl-functionalized PLA surface. Although already described for a number of other surface types, we sought mild conditions for the heterogeneous click reaction of a macromolecule onto the surface of the propargylated PLA. CuAAC was performed under aqueous conditions, following the same procedure as that described for the labelling of the surface 2. The reaction was carried out for 48 h at 40 °C (Scheme 3).

The plate was then extensively washed with water and ethanol to remove all physically adsorbed molecules. SEC analysis of the surface, using refractometric and fluorescence double

detection, showed this approach to be effective. No degradation was observed by refractometric detection and the peak corresponding to the PLA exhibited fluorescence properties due to the covalent binding of the fluorescent PDMAEMA on the plate (S5, Supporting Information). The same experiment, carried out without the propargylic functionalization step, was performed as a blank. Specifically, after reaction of the PLA plate with LDA, all of the carbanionic sites were quenched with water and immersed in the “click” reaction medium with α -N₃QFPDMAEMA under the same conditions as described above. This control reaction was carried out to ensure that no fluorescence was detected at the PLA retention time, and that washings were able to remove all of the α -N₃QFPDMAEMA that was potentially adsorbed on the surface. SEC analysis of the surface with fluorescence detection showed that no peaks were present (S6, Supporting Information). It may therefore be stated that the fluorescence signal observed for the surface of 7 (see Scheme 3) was solely due to the covalent binding of the QFPDMAEMA to the surface.

2.3.1. X-Ray-Photoelectron-Spectroscopy (XPS) Analysis

XPS was used to characterize the surfaces further. Given its high sensitivity and non-destructive nature, XPS was chosen to analyse the surface composition after the grafting of the QFPDMAEMA. Table 1 gives the XPS binding energies, and the full-widths-at-half-maximum (fwhms) for the C 1s, O 1s, and N

Table 1. Comparison of assignments (eV) of XPS peaks, fwhm for PLA, and PLA-graft-QFPDMAEMA surface and composit N₃QFPDMAEMA.

PLA ₉₄ ^{a)}	C 1s					N 1s		O 1s		
	C-H/C-C	C-N	C-N ⁺	C-O	O-C=O	N-C	N ⁺ -C	O-C	O=C	
BE (eV)	284.77	–	–	286.79	288.86	–	–	532.23	533.67	
fwhm	1.44	–	–	1.33	1.22	–	–	1.65	1.65	
composition (at.%)	23.45	–	–	19.85	19.27	–	–	19.13	18.29	
overall composition (at.%)	62.57					–		37.42		
PLA-g-PDMAEMA ^{a)}	BE (eV)	284.76	285.66	286.02	286.82	288.88	399.55	402.28	532.08	533.52
fwhm	1.70	1.50	1.49	1.50	1.50	1.50	1.62	1.62	1.73	1.73
composition (at.%)	27.55	1.11	1.46	17.70	17.45	17.45	0.48	0.42	17.88	15.93
overall composition (at.%)	65.27					0.9		33.81		
PDMAEMA ^{b)}	composition (at.%)	26.03	13.23	17.07	8.68	8.68	4.69	4.27	8.68	8.68
overall composition (at.%)	73.69					8.68		17.36		

^{a)} Experimental results obtained from XPS analysis; ^{b)} Theoretical atomic composition of the grafted chain based on the chemical structure.

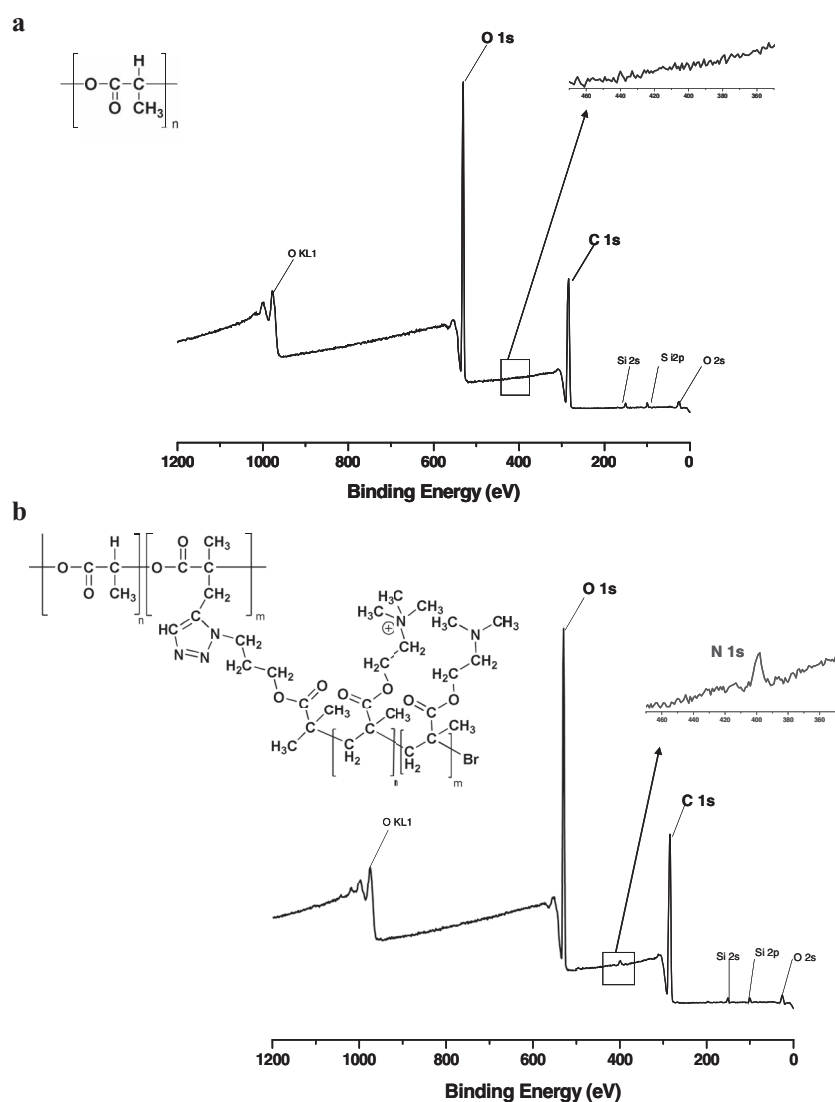


Figure 2. a–b) XPS survey scan spectra of PLA (a) and PLA-graft-QFPDMAEMA (b) surfaces.

1s spectra, together with assignments to the chemical bonds based on the observed chemical shifts.

Figure 2–b shows XPS survey-scan spectra for neat PLA and PLA-graft-QFPDMAEMA, respectively. Figure 2a shows two peaks located at binding energies of 283 and 531 eV, corresponding to C 1s and O 1s, respectively. It may be calculated from the XPS survey scans of the neat PLA that the C/O ratio was 1.7 (i.e., close to the theoretical value of 1.5, which is based on the chemical structure of PLA). The small difference is due to the so-called adventitious carbon induced by the adsorption of impurity hydrocarbons, and is used for binding-energy calibration by setting its energy to 284.8 eV, to correct for sample charging.^[37] XPS survey scans of the PLA-graft-QFPDMAEMA showed one additional element: N 1s at 398 eV. Since PLA does not contain nitrogen, it was used as an elemental marker for the QFPDMAEMA immobilization. Therefore, the presence of nitrogen confirmed successful QFPDMAEMA binding. It is important to mention here that the use of copper and its partial removal from materials is often a limitation of “click” chemistry, when targeting biomedical applications. Our experiments showed that extensive washing removed all copper, as none was detected in the 933 eV region by the XPS analyses of the PLA surfaces.

QFPDMAEMA immobilization on the surface was further investigated by high-resolution XPS in the C 1s, O 1s and N 1s regions (Figure 3). The high-resolution scan of the neat PLA was deconvoluted into three C 1s peak components of approximately the same composition, corresponding to the three types

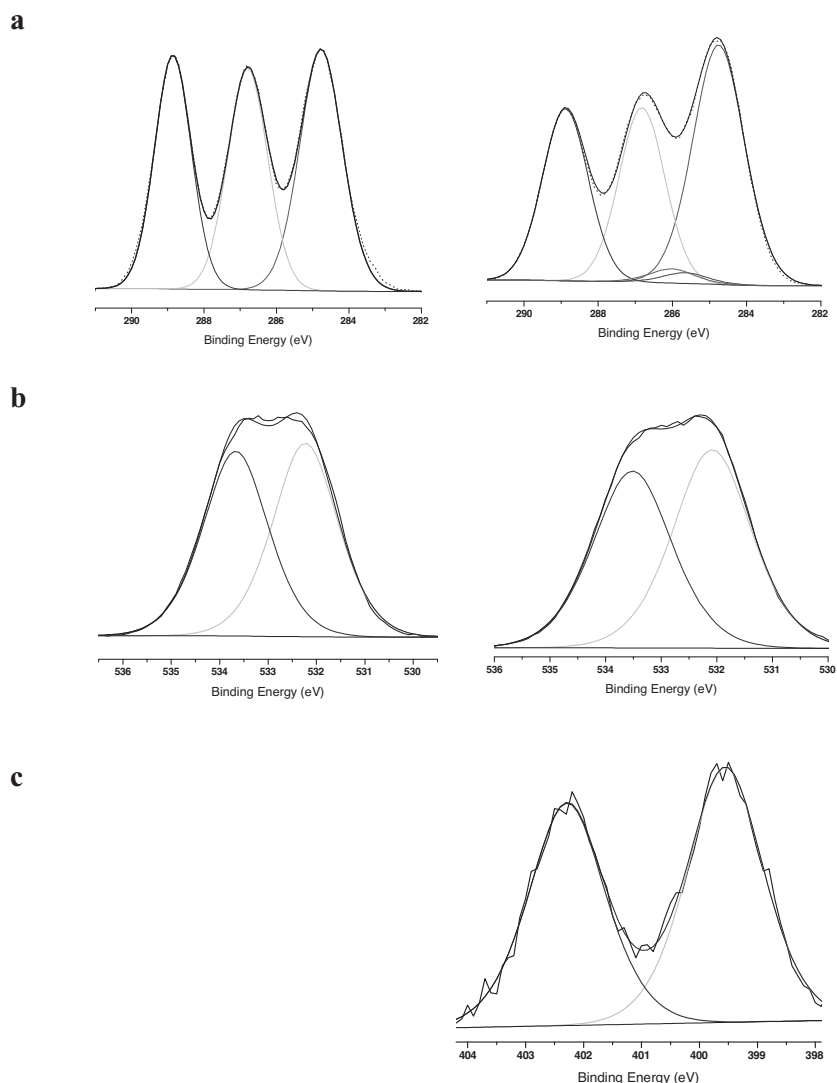


Figure 3. a–c) XPS high-resolution spectra of the C 1s (a), O 1s (b), and N 1s (c) regions of the PLA (left) and PLA-graft-QFPDMAEMA (right) surfaces.

of carbon atoms present in PLA. The peaks at 284.77, 286.79 and 288.86 eV were assigned to C–H/C–C, C–O and O=C=O bonds, respectively. The O 1s peaks at 532.23 and 533.67 eV correspond to the binding energies of the C–O and O=C=O groups in PLA, respectively. These results were expected and are consistent with previous reports.^[38]

The high-resolution scan in the N 1s region shows two peaks of approximately equal intensity. The low binding energy at 399.55 eV is characteristic of the C–N species in QFPDMAEMA, and the peak at 402.28 eV was attributed to C–N⁺.^[39] The presence of neutral nitrogen on the QFPDMAEMA chains indicates partial quaternization. The absence of a peak corresponding to free azido groups at BE = 405 eV^[40] is additional evidence that the QFPDMAEMA was covalently attached on the surface. No peaks corresponding to the nitrogen atoms of the triazole ring (doubly bonded, 400.3 eV and singly bonded, 398.2 eV)^[41] were detected as a result of the low grafting density of the PDMAEMA on the PLA surface, and of the 1/50 ratio between

the triazole chain-ends and the DMAEMA units. The C 1s core-level spectrum shows five peak components at BE \approx 284.76, 285.66, 286.02, 286.82 and 288.88 eV, which were attributed to C–H/C–C, C–N, C–N⁺, C–O and O=C–O, respectively, in accordance with the literature.^[42] The two additional peaks corresponding to the binding energies of C–N and C–N⁺ confirmed the QFPDMAEMA grafting onto the surface of the PLA. Finally, the high-resolution scan of O 1s showed two peaks at BE \approx 532.08 and 533.52 eV. These were assigned to O–C and O=C–O.

These results confirmed that the PLA surface had been chemically modified by the covalent immobilization of QFPDMAEMA, without any degradation of this fragile material. Two surface properties were then investigated, namely wettability and antiadherence against bacteria.

2.3.2. Static Contact-Angle Measurements

The surface wettability was measured using a static angle technique (S7, Supporting Information). As expected, the PLA plate was hydrophobic, as demonstrated by an 84° contact angle. After immobilization of the FPDMAEMA quaternized with methyl iodide (C1 QFPDMAEMA $M_{n,NMR} = 6500 \text{ g mol}^{-1}$), the contact angle decreased to 55° because of the presence of hydrophilic quaternary ammonium (QA).

2.3.3. In Vitro Antibacterial Activity

The ability of the two modified surfaces to prevent bacterial adherence was tested with *E. coli* and *S. aureus*. The data are reported in Table 2. The two surfaces modified by FPDMAEMA quaternized with either a methyl (C1 QFPDMAEMA) or a heptyl (C7 QFPDMAEMA) group reduced the adherence of *E. coli* and *S. aureus* compared with neat PLA. For the C1-QFPDMAEMA-grafted surfaces, the colony-forming-unit (CFU) ratio was 2.7×10^{-4} for *E. coli* and 3.3×10^{-5} for *S. aureus*, as compared with genuine PLA. This anti-adherence activity was

Table 2. Comparison of antiadherence activity against *E. coli* and *S. aureus* of PLA, PLA-graft-QFPDMAEMA plate with –CH₃ as alkylating agent and PLA-graft-QFPDMAEMA plate with –C₇H₁₅ as alkylating agent.

	CFU <i>E. coli</i>	CFU <i>S. aureus</i>
PLA	3×10^8	6×10^8
PLA-graft-QFPDMAEMA (C ₁) ^{a)}	8×10^4	2×10^4
PLA-graft-QFPDMAEMA (C ₇) ^{b)}	8×10^1	4×10^2

^{a)}QFPDMAEMA with –CH₃ as alkylating agent; ^{b)}QFPDMAEMA with –C₇H₁₅ as alkylating agent.

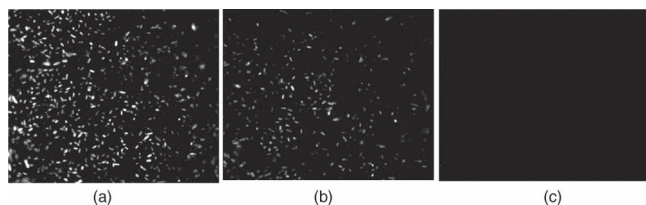


Figure 4. a–c) Visualization of adherence of *E. coli gfp+* on: neat PLA (a) and PLA-graft-QFPDMAEMA plate with $-\text{CH}_3$ as an alkylating agent (b) and $-\text{C}_7\text{H}_{15}$ as alkylating agent (c).

improved with the C7-QFPDMAEMA-grafted surfaces, which gave ratios of 2.7×10^{-7} and 6.7×10^{-7} , respectively.

Another experiment using a genetically modified strain of *E. coli* expressing the *gfp* gene (*E. coli gfp+*) was performed to visualize the bacterial adherence on the modified surfaces and on neat PLA by fluorescence microscopy. The microscopy images are shown in **Figure 4**. Fewer adherent bacteria were present on the C1-QFPDMAEMA-modified surface than on the PLA unmodified surface, and this phenomenon was even more marked with C7 QFPDMAEMA, where no fluorescence was detected, showing that no bacteria were retained on this surface.

These preliminary results for the antibacterial activity of the modified PLA surfaces are encouraging, and biological investigations, such as biofilm assays and cell cultures, will follow. Furthermore, this antibacterial activity demonstrates that the strategy proposed herein enables PLA surfaces to be functionalized via the covalent grafting of (macro)molecules while preserving the bioactivity of these (macro)molecules and the bulk properties of the material.

3. Conclusions

A novel method has been developed to chemically modify the surface of biodegradable polyesters. Using carefully selected anionic reaction conditions, we have demonstrated that wet chemistry can be used to obtain propargyl-functionalized surfaces without altering the core. It is expected that this bio-orthogonal functionalization enables the covalent immobilization of simple molecules, macromolecules and biomolecules by a click methodology, while preserving the other functionalities and/or activity. As an initial example, we have reported the grafting of an $\alpha\text{-N}_3$ -functionalized poly(quaternary ammonium) using click chemistry. We then used SEC and XPS analyses to show that: i) only the external layer was modified, without any degradation of the PLA core; ii) poly(quaternary ammonium) immobilization without adsorption was achieved by click chemistry in heterogeneous medium; and iii) no copper, used as a catalyst, was detected, which is of importance to ensure biocompatibility. This surface modification firstly improved the hydrophilicity of the modified PLA, with its water contact angle decreasing by approximately 29° . The biological activity of this modified surface was also determined in terms of its potential antibacterial properties against *E. coli* and *S. aureus*. A marked antiadherent effect was shown, with up to 10^7 fewer bacteria on the treated surfaces

as compared to the bare PLA. This study is ongoing with investigations focusing on other bacterial strains, the formation of biofilms, and an evaluation of the surface biocompatibility using cell cultures. Other biomedical applications for PLA will also be investigated using this strategy for the synthesis and surface immobilization of other azido-modified bioactive compounds.

4. Experimental Section

Materials: PLA₉₄ was synthesized by the ring-opening copolymerization of L-lactide (88%) and D-lactide (12%) (PURAC, Lyon, France), in bulk, using tin 2-ethylhexanoate as a catalyst. ($M_n = 100\,000\text{ g mol}^{-1}$; PDI = 1.9). The PLA₉₄ plate was shaped using a Carver hydraulic heated press (4120-289). The plates were heated at 130°C and PLA powder was pressed for 5 min before cooling under pressure ($1.5 \times 10^7\text{ Pa}$). PLA₉₄ plates with a thickness of $500\ \mu\text{m}$ were obtained. DMAEMA (98%, Aldrich, St Quentin Fallavier, France) was passed through a column of basic alumina to remove the stabilizing agents. Toluene (Aldrich) and dichloromethane (DCM) (Aldrich) were dried by refluxing over CaH_2 and were then distilled before use. Triethylamine (TEA) (Aldrich) was distilled over KOH. THF (Aldrich) was dried by refluxing over a benzophenone/sodium mixture until a deep-blue color was obtained and was then distilled. All of the other solvents and chemicals were purchased from Aldrich and were used as received.

NMR Spectroscopy: The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra were recorded using a Bruker spectrometer (AMX300) operating at 300 MHz and 75 MHz, respectively. Deuterated chloroform or deuterated dimethyl sulfoxide (DMSO) were used as solvents. The chemical shifts are expressed in ppm with respect to tetramethylsilane (TMS).

Attenuated Total Reflection (ATR)-FTIR Spectroscopy: The FTIR spectra were recorded using a Perkin-Elmer Spectrum 100 FTIR spectrometer using the attenuated-total-reflectance (ATR) method.

Size-Exclusion Chromatography: SEC was performed at room temperature using a Waters system equipped with a guard column, a 600 mm PLgel 5 mm Mixed C column (Polymer Laboratories), a Waters 410 refractometric detector and a Waters 470 scanning fluorescence detector. Calibration was established with polystyrene standards from Polymer Laboratories. THF and THF/TEA (95/5; v/v) were used as eluents for the PDMAEMA analyses, with a flow rate of 1 mL min^{-1} .

XPS: XPS was performed using an ESCALAB 250 photoelectron spectrometer with Al K_{α} radiation (1486.6 eV) and an overall instrument resolution of 1.1 eV. All of the spectra were collected at an electron take-off angle of 90° to the sample surface. Surfaces of $400\ \mu\text{m}^2$ were analyzed. The binding energies were corrected by referencing the binding energy of the C–C component of C 1s to 284.8 eV.

Contact-Angle Measurements: The water contact angles were measured using the sessile-drop method. The system was composed of a sample stage to hold the substrate, a syringe to apply a droplet of ultra-pure water, a light source on the back and a camera to capture the profile of the drop on the PLA surfaces. Image J software (NIH, USA) was used to measure the contact angle. A water-drop volume of $50\ \mu\text{L}$ was placed on the film surface and allowed to stabilize for 30 s.

Synthesis of the Clickable Surface of PLA₉₄ (1): Typically, a PLA₉₄ plate ($m = 125\text{ mg}$, thickness = $500\ \mu\text{m}$, surface = 1.76 cm^2) was immersed in a stirred solution of 2.0 M lithium di-isopropyl amide (1.5 mL, 3 mmol) in an anhydrous THF (60 mL)/diethyl ether (120 mL) mixture at -50°C under an inert argon atmosphere. This activation step was carried out for 30 min before the addition of propargyl bromide (700 μL , 6 mmol) at -30°C . The mixture was stirred for 1 h at -30°C and then raised to room temperature. The PLA plate was quenched and washed with water, diethyl ether and methanol. Any residual solvent was removed under vacuum.

Synthesis of 9-Azidomethylanthracene: 9-Azidomethylanthracene was synthesized from commercially available 9-chloromethylanthracene according to a previous report.^[32] (99% yield): $^1\text{H NMR}$ (300 MHz, CDCl_3 ,

δ): 8.49 (s, 1H, H₁₀ anthracene), 8.29–8.26 (m, 2H, H₁H₈ anthracene), 8.05–8.02 (m, 2H, H₄H₅ anthracene), 7.60–7.24 (m, 4H, H₂H₃H₆H₇ anthracene), 5.32 (s, 2H, CH₂); FTIR (ATR): ν = 2093 cm⁻¹ (N₃).

Synthesis of PLA₉₄ Fluorescently Labelled Surface (2): A clickable plate of PLA (1) was immersed in a solution of 9-azidomethylanthracene (100 mg, 0.4 mmol) in an ethanol (3 mL)/water (1 mL) mixture. The Cu^I catalyst was then generated in situ by the addition of copper sulfate (300 μ L of a 1 M aqueous solution) and sodium ascorbate (600 μ L of a 1 M aqueous solution). The reaction was performed at 40 °C for 2 days. The PLA plate was then extensively washed with water and ethanol, rinsed with methanol and finally dried under vacuum prior to analysis. The plate was immersed for 30 s in three successive THF baths and each solution (S1, S2, and S3) was analyzed by SEC. Afterwards, the plate was entirely dissolved in THF in order to analyse the bulk (B).

Synthesis of 3-Azidopropanol: 3-Chloropropanol (5.1 mL, 5.77 g, 61 mmol), sodium azide (7.94 g, 122 mmol), tetrabutylammonium hydrogen sulfate (TBAHS) (40 mg), and water (5 mL) were placed in a round-bottom flask. The reaction mixture was stirred first at 80 °C for 24 h, and then at room temperature for 12 h. The mixture was extracted with diethyl ether (3 \times 100 mL). The organic phase was dried over MgSO₄ and the solvent was removed under vacuum. 4.8 g of 3-azidopropanol were obtained after distillation (78% yield). ¹H NMR (300 MHz, CDCl₃, δ): 3.69 (t, 2H, CH₂O), 3.40 (t, 2H, CH₂N₃), 1.78 (m, 2H, CH₂CH₂CH₂).

Synthesis of (3-Azidopropyl)bromoisobutyrate (APBIB) (α -Functionalized ATRP Initiator) (3): 3-Azidopropanol (2 g, 19.8 mmol), triethylamine (4.1 mL, 29.7 mmol) and dry CH₂Cl₂ (50 mL) were placed in a round-bottom flask. Bromoisobutryl bromide (3.67 mL, 29.7 mmol) was then added slowly at 0 °C, and the mixture was stirred at room temperature for 17 h. The solution was filtered to remove the triethylammonium bromide and the solvent was removed under vacuum. The viscous residue was dissolved in DCM and washed with a saturated sodium hydrogenocarbonate (NaHCO₃) solution. The product was dried over MgSO₄ and the volatiles were removed under vacuum. The crude product was purified by column chromatography on silica using a heptane/ethyl acetate (9:1) mixture as the eluent. (90% yield). ¹H NMR (300 MHz, CDCl₃, δ): 4.24 (t, 2H, CH₂OC=O), 3.41 (t, 2H, CH₂N₃), 1.92 (m, 2H, CH₂CH₂CH₂), 1.91 (s, 6H, Br(CH₃)₂CC=O).

Synthesis of Anthracenylmethyl Methacrylate (4) (AMM) (Fluorescent Probe): A solution of 9-(methanol)anthracene (3 g, 14.4 mmol, 1 eq.), triethylamine (3.04 mL, 21.6 mmol, 1.5 eq.) and hydroquinone (0.622 g, 5.6 mmol, 0.4 eq.) in dry DCM (75 mL) was cooled at 0 °C. Then, methacryloylchloride (2.18 mL, 21.6 mmol, 1.5 eq.) was added dropwise under an inert atmosphere. After stirring at room temperature for 17 h the crude product was washed three times with 1 M hydrochloric acid (3 \times 100 mL), 1 M sodium hydrogenocarbonate (3 \times 100 mL) and deionized water (100 mL). The product was dried over MgSO₄ and filtered, and the solvent was removed under vacuum. The product was purified by column chromatography on silica gel using chloroform as the eluent. (m = 3.2 mg, 82% yield). ¹H NMR (300 MHz, CDCl₃, δ): 8.49 (s, 1H, H₁₀ anthracene), 8.37–8.34 (m, 2H, H₁H₈ anthracene), 8.03–8.00 (m, 2H, H₄H₅ anthracene), 7.58–7.45 (m, 4H, H₂H₃H₆H₇ anthracene), 6.21 (s, 2H, CH₂O), 6.03 (s, 1H, CHC(CH₃)(CO)), 5.48 (s, 1H, CHC(CH₃)(CO)), 1.90–1.89 (m, 3H, CH₃); IR (ATR): ν = 1711 cm⁻¹ (CO).

Synthesis of α -Azido FPDMAEMA: Polymerization was carried out using the standard Schlenk technique under an argon atmosphere. CuBr (118 mg, 0.82 mmol, 1 eq.), the initiator (3) (206 mg, 0.82 mmol, 1 eq.) and the fluorescent probe (4) (115 mg, 0.41 mmol, 0.5 equiv) were placed in an oven-dried Schlenk tube. The tube was fitted with a rubber septum. Degassed toluene (7 mL) and DMAEMA (7 mL, 41.5 mmol, 50 equiv) were transferred to the tube via a degassed syringe. The solution was further degassed by three freeze-thaw-pump cycles. The mixture was stirred rapidly under argon and PMDETA was added. The resulting mixture was placed in a thermostatically controlled oil bath at 60 °C for 20 min. The reaction was stopped by immersion in liquid nitrogen, and the catalyst was removed by passing through a column of activated basic alumina prior to molecular-weight analysis. The conversion was measured using ¹H-NMR spectroscopy. ¹H NMR (300 MHz, CDCl₃, δ): 3.98 (CH₂OC=O), 3.33 (CH₂N₃), 2.49 (CH₂N), 2.21 (N(CH₃)₂),

1.6–2.0 (CH₂C(CH₃)C=O), 0.65–1.11 (CH₂C(CH₃)C=O); $M_{n,SEC}$ = 13 600 g mol⁻¹; M_w/M_n (PDI) = 1.1; $M_{n,NMR}$ = 6500 g mol⁻¹.

Quaternization of α -Azido-FPDMAEMA (5): α -Azido FPDMAEMA (500 mg) was dissolved in THF (100 mL) in a round-bottom flask. The mixture was stirred and an excess (2 eq.) of quaternizing agent (heptyl iodide or methyl iodide) was added. The solution was stirred at room temperature overnight. The crude copolymer was then purified by dialysis against deionized water using a dialysis tube (cut-off = 3 500 Daltons). The final aqueous solution was freeze-dried to yield the quaternized α -azido FPDMAEMA.

Synthesis of PLA-graft-QFPDMAEMA Surface (6): A clickable plate of PLA (1) was immersed in a solution of α -azido QFPDMAEMA (5) (100 mg, 0.6 mmol) in a water (1 mL)/ethanol (3 mL) mixture. A Cu^I catalyst was then generated in situ using copper sulfate (300 μ L of a 1 M aqueous solution) and sodium ascorbate (600 μ L of a 1 M solution). The reaction was performed at 40 °C for 2 days. The PLA plate was extensively washed by dialysis against water until no fluorescence was detected in the dialysis medium. The plate was then rinsed with ethanol and methanol, and was finally dried under vacuum prior to analysis. The surface was analyzed by water-contact-angle measurements and XPS. SEC analyses were performed using refractometric and fluorescence double detection after dissolution of the surface in THF (30 s in 1 mL, 5 mg).

Antiadherence Activity: i) Two strains of bacteria were used, *E. coli* NECS19923, genetically modified to express the *gfp* gene (*E. coli gfp+*) and *S. aureus* NSA4201. Before in vitro antibacterial tests, the bacterial strains were grown aerobically overnight on Müller–Hinton medium at 37 °C under stirring.

ii) Evaluation of the bacterial adherence on the modified plates was performed following a procedure described previously.^[42] The PLA plates were immersed in a bacterial solution (DO₆₀₀ = 0.05) for 1 h; then, non-adherent bacteria on the sample surface were removed by washing several times with sterilized water before incubation of the PLA objects in a neutral medium. After 24 h of incubation at 37 °C, the plates were submitted to a vortex and ultrasound in physiological saline buffer to remove any adherent bacteria from the surface. Detached viable bacteria were counted by series dilutions, whereas adherent viable bacteria were quantified by pressing the samples about fifteen times directly onto the surface of a Müller–Hinton-type agar plate. Finally, the total bacterial adherence was calculated by adding the count of the CFUs, after overnight incubation of the agar plates at 37 °C, to all cultivated bacteria.

iii) For the visualization of the bacteria on the surfaces, the samples were immersed in a diluted culture solution of *E. coli gfp+* (DO₆₀₀ = 0.05). After 1 h, the PLA objects were washed with sterilized water and observed using a fluorescence microscope.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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