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Immobilization of antimicrobial peptide IG-25 onto fluoropolymers via fluorous interactions and click chemistry

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Abstract

We report a practical method for biofunctionalization of fluoropolymers based on non-covalent, fluorous interactions and click chemistry which allows incorporation of biomolecules under physiological solutions. We demonstrate the method by immobilization of an antimicrobial peptide (AMP) on fluorous thin films and fluorosilicone contact lens. The fluorous surfaces were dip-coated with fluorous-tagged oligo(ethylene) chain terminated with a reactive group, such as an alkynyl group. This simple step generates a “clickable” surface. The non-covalent fluorous interaction was strong enough to allow subsequent covalent attachment of IG-25, a truncated version of the most extensively studied human AMP LL-37. The attachment was through copper-catalyzed click reaction between the alkynyl group on the surface and the azido-OEG tag at the N-terminus of IG-25. In comparison to surfaces presenting IG-25 randomly bound via carbodiimide chemistry, the surfaces presenting IG-25 tethering to the surface at the *N*-terminus via click chemistry displayed higher antibacterial activities against an ocular pathogen *Pseudomonas aeruginosa* (strain PA-O1).

Keywords

Fluorinated polymers; antimicrobial peptides; LL-37; click chemistry; contact lens

INTRODUCTION

Owing to their unique properties, including exceptional inertness, stability and oxygen solubilities, fluorinated polymers have been widely used for biomedical research and applications.[1–5] Biofunctionalization on these materials is highly desirable for improving the biocompatibility and cellular responses.[6–9] The conventional approach for modifying fluoropolymers surfaces is mainly based on plasma or ion implantation to generate a mixture of active groups for subsequent covalent modification with biomolecules.[6, 10–12] However, these techniques require specialized equipment and it is difficult to achieve homogenous activation of irregularly-shaped biodevices such as tubing. Recently, a new approach based

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ASSOCIATED CONTENT
Supporting Information.
Synthesis of compound **2,** XPS measurements on contact lenses, estimate of surface density of IG-25 and antibacterial activity on lower density carbodiimide-modify IG-25 is included in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

on non-covalent fluorous interactions was reported.[13] Peptide fluorosurfactant polymer (PFSP) tethering integrin-binding RGD peptides were coated onto ePTFE vascular grafts, leading to great enhancement of endothelial cell (EC) adhesion.[7] While this direct immobilization method is highly efficient, it has to be performed in organic solvents since the fluoropolymers tethering biomolecules are insoluble in water. Therefore, it may not be applicable for proteins and peptides which can be denatured by organic solvents and/or by their carrier (PFSP). Herein, we report a practical approach based on fluorous interactions and click chemistry for biofunctionalization of fluoropolymers, which allows incorporation of biomolecules in physiological solutions. We demonstrate our method by immobilization of an antimicrobial peptide (AMP) on fluorous polymer surfaces. AMPs play an important role in the innate immune system of all organisms. They have broad spectrum activity against bacteria and fungi, while exhibiting low cytotoxicity to host cells and low susceptibility for bacterial resistance.[14–18] With such properties, AMPs have great potential as an antimicrobial coating for preventing bacterial colonization, a major issue for biomedical implants, [19–23] including fluorinated polymers.[24–26] Herein, we demonstrate the first antimicrobial modification of flurouopolymers with antimicrobial peptide IG-25, a truncated version of the most extensively studied human AMP LL-37.[16, 20]

EXPERIMENTAL SECTION

General

All reagents were purchased from Sigma-Aldrich (St. Louis, MO), Thermo-Scientific (Pittsburgh, PA) and Alfa Aesar (Ward Hill, MA) and used without purification.

Reagents

Copper sulfate ($CuSO_4$), ethylenediaminetetreaacetic acid (EDTA), *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide (EDC) and *N*-Hydroxysulfosuccinimide sodiuum salt (sulfo-NHS) were supplied by Aldrich, phospate buffered saline (PBS) and L-Ascorbic acid were purchased from Sigma. **IG-25** and **N_3-EG_{12}-IG-25** (N_3-$(CH_2CH_2O)_{11}CH_2CH_2CO$-IGKEFKRIVQRIKDFLRNLVPRTES-OH)werepurchased from Alpha Diagnostic International (San Antonio, TX). The *Pseudomonas aeruginosa* (PA) strain expressing green-fluorescent protein and a carbenicillin resistance gene(PAO1-GFP) was a generous gift from Dr. Alice Prince (Columbia University, NY).

Alkynyl- and COOH- terminated thin films on fluorous slides and contact lenses

A 1 × 1 cm^2 fluorous slide (Fluorous Technologies Inc., Pittsburgh, PA) or a Fluoroperm 60 contact lens (Paragon Vision Sci, Mesa, AZ) was immersed in a 1 mM solution of the alkynyl-terminated fluorocarbon **1** or COOH-terminated fluorocarbon **2** in methanol for 2 h (Scheme 1). The slides/contact lenses were removed, washed with methanol, and dried in vacuum to completely remove the solvent.

Attaching IG-25 via click chemistry

Solutions of $CuSO_4$ (10 mM), ascorbic acid (154 mM), and the ligand **3** (68 mM) in PBS (10 mM, 140 mM NaCl, pH 7.4) were added to a vial containing the slides/contact lenses modified with the fluorous alkyne **1** (surfaces **A,** scheme 1). After 10 minutes, a solution of **N_3-EG_{12}-IG-25** (20 mg/mL) in PBS was added. After incubation for 4 h under N_2, the films were taken out and immersed in EDTA (10 mM) for 5 minutes followed by washing with methanol and water, and drying in vacuum to completely remove the solvent.

Attaching IG-25 via carbodiimide chemistry

Aqueous solutions of 100 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 60mM *N*-hydroxysuccinimide sodium salt (sulfo-NHS) were added to a vial containing the slides/contact lenses modified with the fluorous carboxylic acid **2** (surfaces **C,** scheme 1). The samples were incubated for 1hour, and rinsed with Millipore water to provide surface **D**. The samples were then incubated in solutions of **IG-25** with a high concentration (200 mg/mL) or a low concentration(20 mg/mL) in PBS for 4 h, and thoroughly washed with PBS and Millipore water.

Antimicrobial activity of IG-25 immobilized on the fluorous glass slides

A single isolated PAO1-GFP colony was inoculated in 5 mL Luria-Bertani Broth (LB) with 300 μg/mL carbenicillin (Sigma-Aldrich) overnight at 37 °C. A 1.0 mL bacterial suspension was used to inoculate 50 mL of fresh LB with 300 μg/mL carbenicillin. The innoculum was incubated with vigorous shaking (250 rpm) at 37 °C for 2 h to achieve mid-log phase growth. After 2 h, a 25 mL sample from the liquid culture was centrifuged at 3000 *rpm* for 10 minutes, and the bacterial cell pellet was resuspended in cold phosphate buffer (PB; 8.2 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4). The optical density (OD) of the suspension was adjusted to 0.28 at 620 nm by adding an appropriate volume of PB. Following a 2 h incubation at 37 °C (without shaking), the substrates were removed. Prior to viewing, a 1 μL of 15 μM propidium iodide (PI) was placed on the substrate and covered by a microscope slide. For the control, the same conditions as above were repeated on fluorous slides without treatment of IG-25. Results from one replicate are expressed as average number of bacteria ± standard deviation performed in 4 different imaged areas (149 × 112 μm^2), and presented in Figure 2d.

Bacterial adhesion measurements on contact lens

Similar to the reportedprocedure, [28] a single isolated *Pseudomonas aeruginosa* (PAO1; ATCC; Manassas, VA) colony was inoculated in 3 mL LB overnight at 37 °C. The bacterial culture was centrifuged at 3000 rpm for 10 minutes, and the bacteria pellet was resuspended in Tryptic Soy Broth (TSB) and the optical density of the suspension was adjusted to 0.1 at 660 nm. Samples were incubated in 1 mL of the bacterial suspension with shaking (150 rpm) at 37°C for 24 h. The lenses were then washed with PBS twice and homogenized using a motorized pestle (VWR Int.) until the contact lenses disintegrated. 10 fold serial dilutions of the lens-bacterial suspension homogenate were made in D/E neutralizing broth (Difco). Ten μL aliquots (in duplicate) for each dilution of the homogenate were plated out on Tryptic Soy Agar (TSA) plates. After incubation overnight at 37°C, viable bacteria were enumerated as colony forming units mm^{-2}. For the controls, the same conditions as the above were applied to contact lenses without any modification (samples designated as “unmodified”), and to contact lenses pre-treated with IG-25 (20 mg/mL) in PBS for 4 h followed by thorough washing with PBS and Millipore water (samples designated as “physisorbed”). Results from one replicate are expressed as the average percent adhesion of the bacteria ± standard deviation (compared to the uncoated control lens) performed in 4 separated imaged areas (149 × 112 μm^2), and presented in Figure 2e.

3. RESULTS AND DISCUSSION

As illustrated in Scheme 1, the fluorous polymer surface is modified with perfluorocarbon chains tethering a functional group, such as alkynyl on **A** and carboxylic acid on **C**, through fluorous interactions.[13] As compared to the plasma or ion beam treatment of fluoropolymers, which is less accessible and generates a mixture of limited functional groups (commonly hydroxy, aldehyde, and carboxylic acid), our method is practical, simply involving dipping the substrate into the modifier solution and then washing. It also allows

for attachment of virtually unlimited functional groups with a controlled composition. Biomolecules can then be covalently attached to the modified surfaces presenting the functional groups.[27–29] In particular, biomolecules with an azido handle can be tethered to the alkyne-presenting surfaces via Cu-catalyzed azide-alkyne cycloaddition (CuAAC, a "click" reaction)[30, 31] that is highly specific, regioselective, and can be performed in physiological solutions. The azido handle can be incorporated to the biomolecules through synthesis or metabolically.[32] Furthermore, an oligo(ethylene glycol) (OEG) linker is used to present the functional groups, which is commonly used for minimizing the denaturing and non-specific adsorption of proteins.[28, 33] As shown in Scheme 1, the alkyne- and carboxylic acid-presenting surfaces **A** and **C** were easily prepared by dipping the fluorous substrates into a solution of the fluorous alkyne **1** and carboxylic acid **2**.[13] We have previously shown that the fluorously immobilized films were stable against desorption during the subsequent covalent modification in aquous solutions.[27, 28] The alkynyl surface **A** allows site-specific attachment of the peptide with an OEG-azido tag (**N_3-EG_{12}-IG-25**) via click reaction to provide the surface **B**. Random grafting of the unmodified peptide **IG-25** onto the carboxylic acid surfaces **C** was performed using a procedure similar to a reported one.[34] Thus, the surface **C** was first activated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide sodium salt (sulfo-NHS) to give the surface **D**, followed by treatment with **IG-25** to provide the surface **E**. X-ray photoelectron spectroscopy showed the expected C, O, and F peaks, *e.g.,* those in Figure 1a. After the click reaction of the alkyne surface **A** with **N_3-EG_{12}-IG-25**, a broad N1s signal centered at 399.6 eV appeared (green curve in Figure 1b), indicating the presence of the peptide on the resultant surface **B**. The absence of Cu 2p signal (Figure 1a, inset) indicates that the copper residue in the peptide film was effectively removed by washing with an EDTA solution. Upon activation of the carboxylic acid surface **C**, the presence of NHS groups in **D** was indicated by the appearance of the N1s peak at 402.1 eV (Figure 1b, inset).[35] After reaction with **IG-25**, the N1s peak for NHS disappeared (Figure 1b, indicated by the line). The appearance of a new, broad N1s peak with a lower binding energy (399.8 eV, dark curve in Figure 1b) indicated the presence of the peptide on the surface **E**.

The surface densities of IG-25 were estimated using the C/N ratio obtained from the XPS data (see Supporting Information). Thus, the density of IG-25 tethering with a long OEG linker on film **B** formed via click reaction was approximately 1.4×10^{13} molecules/cm^2. The density of the unmodified peptide**IG-25**on the film **E** formed via carbodiimide chemistry was dependent on the concentration of the peptide reacting with the activated carboxyl surface **D**, being ~1.2×10^{14} molecules/cm^{2}with a high concentration (200 mg/mL) of **IG-25**, and 5.2×10^{13} molecules/cm^2 with a low concentration (20 mg/mL) of **IG-25**.

The IG-25-modified surfaces were compared for their antibacterial activity against *Pseudomonas aeruginosa* (PA), one of the most common Gram negative pathogens found on medical implants.[36] To facilitate microscopy imaging, we used a strain of PA that was transformed to express green fluorescent protein (PAO1-GFP). By staining with propidium iodide (PI), bacteria with compromised cell membranes were indicated by red fluorescence.[37] After incubation with PAO1-GFP for 2 hours, a much higher density of bacteria fluorescing green adsorbed on both IG-25 modified surfaces **B** and **E** than on the unmodified fluorous substrate (Figure 2a–c). Remarkably, upon adsorption on the surface **B** where the peptide was attached via click chemistry, 91% of the bacteria fluoresced red, *i.e.*, became membrane compromized (Figure 2d). In comparison, disintegration of membranes occurred to only 25%of the bacteria absorbed on the high density but randomly grafted IG-25 surface **E**.[38] Note that42% more bacteria were adsorbed on this surface than on **B** since the peptide density was much higher (1.2×10^{14} vs 1.4×10^{13} molecules/cm^2) thus more efficient in capturing the bacteria via electrostatic interactions. This result is in

agreement with the previous report that the bactericidal activity of LL-37 immobilized on titanium surfaces is greatly enhanced by site-specific immobilization of the peptide via a long PEG linker.[24] The lower activity of the carbodiimide linked surfaces is probably due to the lack of control on the number and location of sites at which the molecule is attached onto the surface since the molecule have multiple amino groups that can react with the carboxy surface. The antimicrobial activity of IG-25 is through the interaction of the amphiphilic molecule with the bacterial cell membrane leading to membrane disruption. The amphiphilic interaction is expected to be greatly reduced if the molecule is covalently bonded to the surface through multiple sites, which not only consumes the amino groups, but also greatly reduces the conformational flexibility. In contrary, the click immobilized IG-25 molecules are attached to the surface at the *N*-terminus through an OEG linker. This flexible linkage favors the molecules to adapt the native conformation to maximize the membrane disruption.

Encouraged by the results obtained on fluorous polymer thin films, we then applied this method to immobilize IG-25 onto Fluoroperm 60 (Paragon Vision Sci, Mesa, AZ), a contact lens made of copolymers of siloxy and fluorinated methacrylate, also known as Paflufocon B. As outlined in Scheme 1, the fluorous alkyne **1** and carboxylic acid **2** were first immobilized on the contact lens via strong fluorous interactions. **N_3-EG_{12}-IG-25** and **IG-25** were then attached via click reaction and amidation, respectively. For the later, OEG linker was not used, since it is inert to the bacteria and is expected to hinder the interaction between IG-25 with the bacteria. XPS results reveal that the peptide attached via click reaction remained on the surface even after incubation in PBS for 24 h (Figure S2), indicating the fluorous interaction is sufficiently strong against desorption. Furthermore, after the CuAAC reaction no traces of Cu 3d signal was found in the XPS (Figure S3). The density of the peptides on the modified lenses was estimated using a reported procedure based on Coumassie staining.[39] Thus, the density of IG-25 attached via amidation was ~20 mg/cm^2, comparable to the reported density (18 mg/cm^2) of AMP grafted on hydroxyethylmethacrylate based contact lens.[25] Similar densities of IG-25 were measured for the surfaces modified via click reactions (~15 mg/cm^2) and the ones by physisorption (~4 mg/cm^2). Antibacterial activity of the IG-25-modified contact lens is indicated by the reduced bacterial adhesion on the surfaces after incubation in PAO1 culture for 24 h. Contact lens pretreated with IG-25 without covalent attachment (samples designated as “physisorbed”) and unmodified contact lens (samples designated as “unmodified”) were used as controls. As shown by Figure 2e, lower bacterial adhesion was observed on the IG-25 modified lenses than the unmodified and physisorbed controls. Remarkably, the lens modified with IG-25 via click chemistry performed the best, reducing the colonization of PAO1 by approximately 98% as compared to the unmodified one.

4. CONCLUSIONS

In conclusion, we have presented a versatile and practical method for biofunctionalization of fluoropolymer substrates based on the combination of fluorous interactions and click chemistry. Specifically, we demonstrated the immobilization of antimicrobial peptide IG-25 on fluorous substrate slides and on implantable fluoropolymers (fluorosilicone contact lens), leading to a very high antimicrobial efficiency, as compared to the conventional method via carbodiimide chemistry. Our method can be applied to the modification of other fluorous-based biomaterials to tether biomolecules to enhance the desired biological responses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Krafft MP, Reiss JG. J Polym Sci, Part A: Polym Chem. 2007; 45:1185–1198.
2. Xie T. Nature. 2010; 464:267–270. [PubMed: 20220846]
3. Nicolson PC, Vogt J. Biomaterials. 2001; 22:3273–3283. [PubMed: 11700799]
4. Dylan, JB. Advances in Fluorine-Containing Polymers. Vol. 1106. American Chemical Society; 2012. The State of Fluoropolymers; p. 1-7.
5. Lindstrom AB, Strynar MJ, Libelo EL. Environ Sci Technol. 2011; 45:7954–7961. [PubMed: 21866930]
6. Wang H, Kwok DTK, Wang W, Wu Z, Tong L, Zhang Y, Chu PK. Biomaterials. 2010; 31:413–419. [PubMed: 19811820]
7. Larsen CC, Kligman F, Kottke-Marchant K, Marchant RE. Biomaterials. 2006; 27:4846–4855. [PubMed: 16762410]
8. Moby V, Boura C, Kerdjoudj H, Voegel JC, Marchal L, Dumas D, Schaaf P, Stoltz JF, Menu P. Biomacromolecules. 2007; 8:2156–2160. [PubMed: 17585807]
9. Lejars M, Margaillan A, Bressy C. Chem Rev. 2012:4347–4390. [PubMed: 22578131]
10. Takahashi N, Suzuki Y, Ujiie H, Iwaki M, Hori T, Yamada T. Surf Coat Technol. 2007; 201:8150–8154.
11. Chen-Yang YW, Liao JD, Kau JY, Huang J, Chang WT, Chen CW. Macromolecules. 2000; 33:5638–5643.
12. Dufour T, Hubert J, Viville P, Duluard CY, Desbief S, Lazzaroni R, Reniers F. Plasma Processes Polym. 2012; 9:820–829.
13. Ko KS, Jaipuri FA, Pohl NL. J Am Chem Soc. 2005; 127:13162–13163. [PubMed: 16173741]
14. Hancock REW, Sahl HG. Nat Biotechnol. 2006; 24:1551–1557. [PubMed: 17160061]
15. Peschel A, Sahl HG. Nat Rev Microbiol. 2006; 4:529–536. [PubMed: 16778838]
16. McDermott AM. Ophthalmic Res. 2009; 41:60–75. [PubMed: 19122467]
17. Durr UHN, Sudheendra US, Ramamoorthy A. Biochim Biophys Acta. 2006; 1758:1408–1425. [PubMed: 16716248]
18. Burton MF, Steel PG. Nat Prod Rep. 2009; 26:1572–1584. [PubMed: 19936387]
19. Gao G, Cheng JTJ, Kindrachuk J, Handcock REW, Straus SK, Kizhakkedathu JN. Chem and Biol. 2012; 19:199–209. [PubMed: 22365603]
20. Gabriel M, Nazmi K, Veerman EC, Amerongen AVN, Zentner A. Bioconjugate Chem. 2006; 17:548–550.
21. Meyers SR, Grinstaff MW. Chem Rev. 2012; 112:1615–1632. [PubMed: 22007787]
22. Gao G, Yu K, Kindrachuk J, Brooks DE, Hancock REW, Kizhakkedathu JN. Biomacromolecules. 2011; 12:3715–3727. [PubMed: 21902171]
23. Narbat MK, Kindrachuk J, Duan K, Jenssen H, Hancock EW, Wang R. Biomacromolecules. 2010; 31:9519–9526.
24. Sweeney DF, Jalbert I, Covey M, Sankaridurg PR, Vajdic C, Holden BA, Sharma S, Ramachandran, Willcox LM, Rao GN. Cornea. 2003; 22:435–442. [PubMed: 12827049]
25. Willcox MDP, Harmis NY, Holden BA. Aust J Optom. 2002; 85:172–175.
26. Willcox MDP, Hume EBH, Aliwarga Y, Kumar N, Cole N. J Appl Microbiol. 2008; 105:1817–1825. [PubMed: 19016975]
27. Santos CM, Kumar A, Zhang W, Cai C. Chem Commun. 2009; 20:2854–2856.
28. Li Y, Santos CM, Kumar A, Zhao M, Lopez AI, Qin G, Mc Dermott AM, Cai C. Chem Eur J. 2011; 17:2656–2665. [PubMed: 21264959]

29. Wang X, Ishii Y, Ruslinda AR, Hasegawa M, Kawarada H. ACS Appl Mater Interfaces. 2012; 4:3526–3534. [PubMed: 22697908]
30. Meldal M, Tornoe CW. Chem Rev. 2008; 108:2952–3015. [PubMed: 18698735]
31. Hvilsted S. Polym Int. 2012; 61:485–494.
32. Sletten EM, Bertozzi CR. Angew Chem Int Ed. 2009; 48:6974–6998.
33. Prime KL, Whitesides GM. J Am Chem Soc. 1993; 115:10714–10721.
34. Steven MD, Hotchkiss JH. J Appl Polym Sci. 2008; 110:2665–2670.
35. Bocking T, Wong ELS, James M, Watson JA, Brown CL, Chilcott TC, Barrow KD, Coster HGL. Thin Solid Films. 2006; 515:1857–1863.
36. Luo J, Porteous N, Sun Y. ACS Appl Mat Interfaces. 2011; 3:2845–2903.
37. Banning N, Toze S, Mee BJ. J Appl Microbiol. 2002; 93:69–76. [PubMed: 12067376]
38. For the other surface E having a low peptide density (5.2×10^{13}) grafted via amidation with a low concentration of IG-25, only 15% of the adsorbed bacteria were membrane-compromised (see Figure S4 in Supporting Information).
39. Cole N, Ralston G. Int J Biochem. 1994; 26:971–976. [PubMed: 8088417]

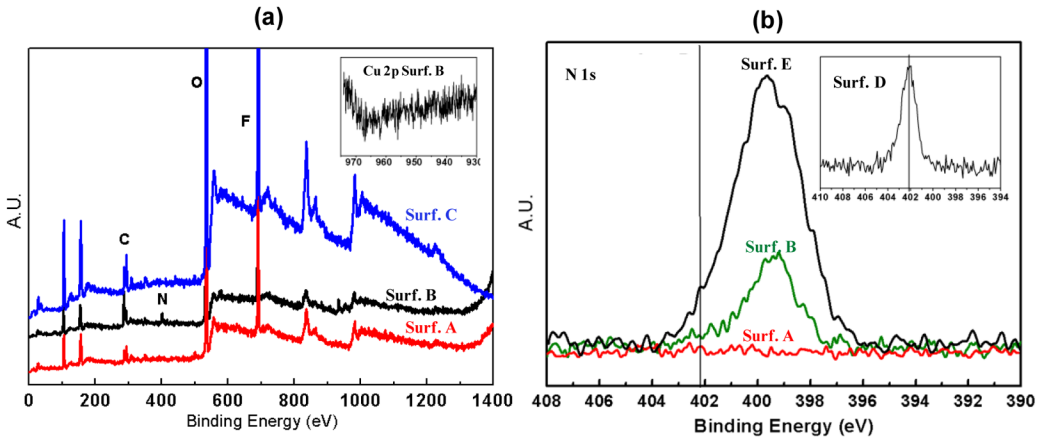


Figure 1.

XPS data for the modified fluorous surfaces. (a) Survey spectrum of the alkynyl-modified surface **A** (red), clicked IG-25 surface **B** (black) and the COOH-modified surface **C** (blue). The inset shows the narrow scan for Cu 2p of surface **B** after washing with EDTA. (b) Narrow scan for N 1s of the ethynyl-terminated surface **A**(red), the IG-25-modified surface **B** via click reaction (green) and the IG-25-modified surface **E** via amidation using a high concentration (200 mg/mL) of **IG-25** with the activated surface **D** (black). The inset shows the N 1s region of the EDC/NHS activated surface **D** before reaction with the peptide. The lines mark the peak positions of theN1s signal from NHS groups.

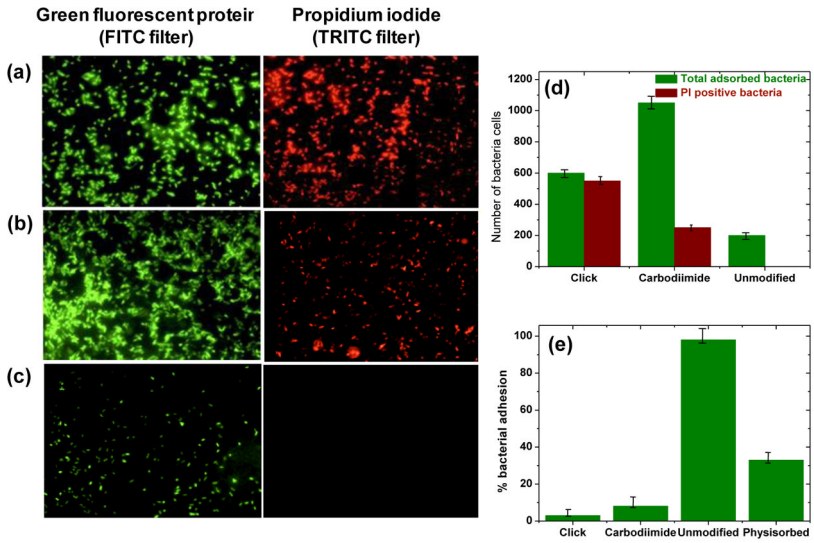
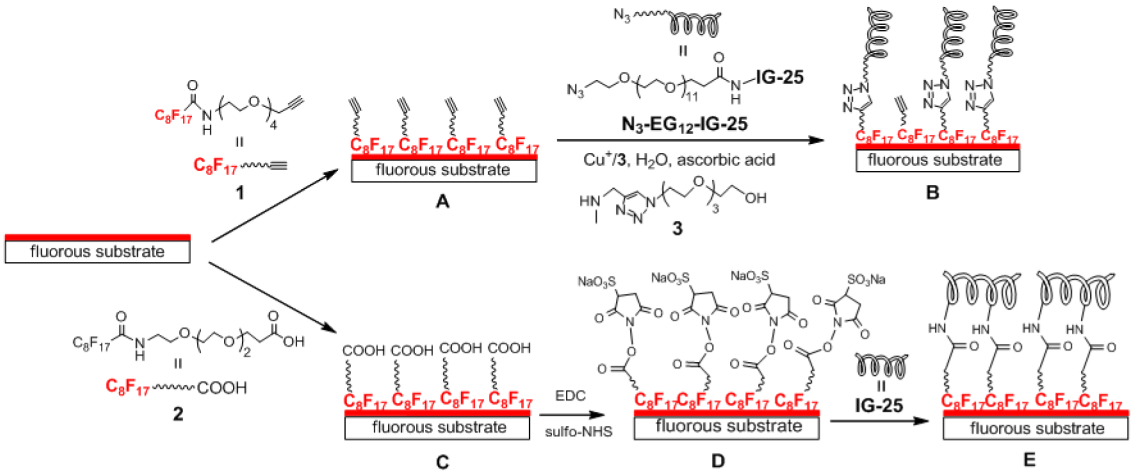


Figure 2.

(a–c): Fluorescence images of the GFP-transformed *Pseudomonas aeruginosa* (Area: 149 × 112 μm^2) on (a) IG-25 functionalized surface **B** via click chemistry, (b) IG-25 modified surface **E** via reaction of the activated surface **D** with a high concentration (200 mg/mL) of IG-25 and (c) unmodified fluorous substrate. Images were obtained with a 60× water immersion objective using a FITC filter for green fluorescence from GFP in all bacteria presented on the left column and a TRITC filter for red fluorescence from PI in membrane-compromised bacteria presented on the right column. (d) Plot of the total number of bacteria (green) and among them the number of PI-positive bacteria (red) on the field of view (149 × 112 μm^2), which were adsorbed on the surfaces **B** and **E** where IG-25 was attached onto surface **A** via click reaction with **N_3-EG_{12}-IG-25** (20 mg/mL) and onto surface **D** via amidation with **IG-25** (200mg/mL), respectively, with the unmodified fluorous slide serving as the control. (e) Relative % adhesion of PAO1 on the filed of view (149 × 112 μm^2) after 24 h incubation on contact lenses modified via click chemistry, carbodimide chemistry or physisorption, with the unmodified contact lens serving as the control.



Scheme 1.

Biofunctionalization of fluorous substrates through fluorous immobilization to present functional groups, such as alkynyl on **A** or COOH on **C**, both with an OEG linker, followed by covalent attachment of biomolecules, e.g., N3-EG12-IG-25 to **A** via click reaction forming **B** or IG-25 to **C** via amidation forming **E**.