Modification of Fluororous Substrates with Oligo(ethylene Glycol) *via* “Click” Chemistry for Long-term Resistance of Cell Adhesion

Rafael Contreras-Caceres,^{a,c,} Catherine M. Santos,^a Siheng Li,^a Amit Kumar,^a Zhiling Zhu,^a
Satya S. Kolar,^b Miguel A. Casado-Rodriguez,^c Yongkai Huang,^a Alison McDermott,^b Juan
Manuel Lopez-Romero,^c and Chengzhi Cai^{a,*}*

^aDepartment of Chemistry, University of Houston, Houston, Texas 77204, USA.

^bCollege of Optometry, University of Houston, Houston, Texas 77204, USA

^cDepartamento de Química Orgánica, Facultad de Ciencias, Universidad de Málaga, Spain,
29071

*E-mail: rcontreras@uma.es (R. Contreras-Caceres) Tel: +34 952137383 Fax:
+34952137383

email addresses: rcontreras@uma.es (R. Contreras-Caceres), cmsantos@central.uh.edu,
(C.M. Santos), sihengli9@gmail.com (S. Li), aks208@yahoo.com (A. Kumar)
jeremial@gmail.com (Z. Zhu), snkolar@uh.edu (S.S. Kolar), miguelcasado204@gmail.com
(M.A. Casado-Rodriguez) hykaiuh@gmail.com (Y. Huang), amcdermott@optometry.uh.edu
(A. McDermott), jmromero@uma.es (J.M. Lopez-Romero), cai@uh.edu (C. Cai).

ABSTRACT

In this work perfluorinated substrates fabricated from SiO₂ glass slides are modified with oligo(ethylene glycol) (OEG) units for long-term resistance of cell adhesion purposes, based on fluorine-fluorine interactions and click chemistry. Specifically, fluorinated substrates, prepared by treatment of glass slides with 1H, 1H, 2H, 2H-perfluorodecyltrimethoxysilane (FAS17), were coated with ethynyl-OEG-C₈F₁₇, followed by covalent attachment of an azido-OEG via copper-catalyzed azide-alkyne cycloaddition (CuAAC) “click” reaction. We demonstrate that the resultant surface avoids fibrinogen adsorption and resists cell adhesion for over 14 days. X-Ray photoemission spectroscopy (XPS) analysis and contact angle goniometry measurements confirm the presence of the OEG molecules on the fluorinated substrates. Bright field optical images show total absence of 3T3 fibroblast cells on the OEG modified fluorinated substrate for 1 and 5 days, and a remarkably decrease of cell adhesion at 14 days.

KEYWORDS: click reaction, fluorinated substrate, fluorine-fluorine interaction, OEG, protein resistance, cell adhesion resistance

1. INTRODUCTION

Controlling the surface chemistry of a material is crucial for the fabrication of devices with capabilities to overcome protein adsorption, being also resistant to cell adhesion. An important challenge for the surface modification of materials is the development of an easy and economic methodology for the fabrication of substrates to be incorporated in biomedical devices as cardiovascular implants, biosensors, microfluidics systems or catheters.¹⁻⁴ During the last years zwitterionics⁵⁻⁶ and oligo- and poly(ethylene) glycol (OEG and PEG, respectively) have been widely used for coating substrate surfaces to reduce adhesion of proteins, bacteria and cells.⁷⁻¹⁰ Due to their unique properties, perfluorinated (fluorinated) compounds and materials have been widely used in biomedical research and applications, such as gas bubbles for ultrasound imaging, contact lenses for enhanced oxygen permeability,

1 coating on electrodes, and substrates for microarrays.¹¹⁻¹³ Moreover, bioactive molecules,
2 such as carbohydrates and peptides with an incorporated fluorine tag have been attached onto
3 fluorinated surfaces via strong fluorine interactions.¹⁴⁻¹⁶ For many biological applications,
4 however, the use of fluorinated substrates alone is often not sufficient to prevent biofouling
5 that screens the functional moieties and decrease the biocompatibility of the materials.¹⁵
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7 Therefore, it is highly desirable to develop methods for the modification of fluorinated
8 materials to reduce protein and cell-adhesion, which may also serve as an inert background
9 for attaching biologically active molecules to allow for specific interactions with the targets.
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11 Binary self-assembled monolayers on gold substrates composed by perfluoroalkylthiol and
12 alkanethiol molecules have been recently used for the fabrication of antifouling surfaces.¹⁷
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14 For this particular case, the controlling in the protein adsorption and cell adhesion is
15 restricted to gold substrate, which difficult the implementation in biomedical devices. The
16 cell-resistance capability has also been studied in several perfluoropolymer substrates. For
17 example, polytetrafluoroethylene (PTFE)^{18,19} was used to produce a cell resistance surface up
18 to 14 days, but only after treatment with $\text{NH}_3 + \text{O}_2$ plasma, which increases the production
19 cost at large scale. Another perfluoropolymer with antibiofouling applications is perfluorinated
20 polyether (PFPE).^{20,21} In order to increase the cell resistant capability this surface was
21 functionalized with acrylate-groups obtaining surface with no adhered cells only until 5 days.
22 Fluoropolymer Nafion has also been investigated as solid substrate, being deposited on
23 surfaces as stainless,^{22,23} but cell proliferation at 7 days was found.

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25 Here we present the fabrication of a fluorinated substrate with long-term antibiofouling
26 capacity from commercially available glass slide substrates. Initially, fluorinated surfaces
27 were produced by immersion of SiO_2 slides in a 1H, 1H, 2H, 2H-
28 perfluorodecyltrimethoxysilane (FAS17) solution. This non-toxic and biologically inert surface
29 was then functionalized with an alkynyl-terminated layer (to be used in a CuAAC reaction)

by simply dipping the fluorinated substrate into a solution of compound **1**. The structure of **1** consist in a C₈F₁₇ fluorous tag and a tetraethylene glycol moiety having a terminal alkyne group, also denoted here as C₈F₁₇-EG₄-Alkyne. This layer was strongly bounded to the fluorous substrate *via* non-covalent fluorous interactions, and remained intact during covalent modification with azide **2** under copper-catalyzed alkyne-azide cycloaddition (CuAAC, a “click” reaction) conditions.²⁴ We recently reported a method based on CuAAC reaction for attaching fluorescent molecules, ligands (biotin) and antimicrobial peptides onto fluorous substrates *via* an intermediate fluorous layer terminated with an alkynyl or azido group.^{25,26} For comparison, we also fabricate a fluorinated-EG₄ surface by direct immersion of the fluorinated substrate in a solution with compound **4** (C₈F₁₇-EG₄). Analyses of the surfaces were carried out by XPS, contact angle goniometry (CA) and bright field microscopy. XPS analyses were used to confirm the presence of the incorporated molecules on the surfaces and for protein resistance investigations. Bright field optical images were employed to control 3T3 fibroblast cells proliferation on the OEG modified fluorinated substrate, while CA was used to study the homogeneity of the prepared surfaces. The presented protocol represents an easy and reproducible procedure for the fabrication of protein and cell adhesion resistance surfaces with long-term capabilities and offers the possibility to be implemented in large-scale fabrication of protein and cell adhesion resistance materials.

2. EXPERIMENTAL SECTION

2.1.General.

Air sensitive reactions were performed under a nitrogen atmosphere using Schlenk technique. All reagents were purchased from Sigma-Aldrich or Alfa Aesar, and were used without purification. Flash chromatography was carried out on silica gel (60Å, Sorbent Technologies).

N-(2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethyl)-

2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptafluorononanamide (1), 2-(2-(2-(4-
((methylamino)methyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)ethanol (3), 20-azido-
3,6,9,12,15,18-hexaoxaicosan-1-ol (2) and 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptafluoro-
N-(14-hydroxy-3,6,9,12-tetraoxatetradecyl)nonanamide (4) were prepared according to the
reported procedures.²⁷⁻²⁹

2.2. Preparation of the fluorinated substrate A.

A 1 x 1 cm² glass slide was first cleaned by sonication in acetone for 5 min. After dried with
a N₂ flow the slide was immersed in a piranha solution (70% H₂SO₄/30% H₂O₂) at 70°C for
20 min, and then copiously rinsed with water. Following the procedure previously reported,³⁰
the cleaned glass slides were incubated in a 50 mM solution of FAS17 in chloroform at room
temperature for 12 h. After that, the slide was heated at 100°C under a N₂ atmosphere for 1 h.
Finally, the substrate was rinsed with ethanol and dried with a flow of N₂ obtaining surface
A.

2.3. Preparation of the ethynyl-terminated surface B.

A 1 x 1 cm² fluorinated surface A was immersed in a 1 mM solution of the alkynyl-
terminated fluorocarbon 1 (Scheme 1) in methanol for 6 h under N₂ atmosphere. The slides
were removed and washed several times with methanol, followed by drying in a stream of
argon obtaining surface B.

2.4. General procedure for the surface CuAAC reaction (surface C).

In a reaction vial containing the alkynyl-terminated surface B a solution of CuSO₄ (2.5 mM),
sodium ascorbate (50 mM), and the ligand 3 (25 mM, Scheme 1) in water was added. After

10 min, a solution of the azide **2** (10 mM) in degassed methanol/water (1:1 v/v) was added. After incubation for 4 h under N₂, the treated surface was taken out and immersed in EDTA (10 mM) for 5 min, followed by washing with water and drying under a stream of argon.

2.5. Preparation of surface D.

A 1 x 1 cm² fluorinated glass slide (surface **A**) was immersed in a 1 mM solution of the fluorine-tagged-EG₄ **4** (Scheme 1) in methanol for 6 h under N₂. After this period, the slides were removed and washed with methanol, followed by drying under a stream of N₂.

2.6. X-Ray Photoelectron Spectroscopy (XPS).

XPS was performed with a PHI 5700 X-ray photoelectron spectrometer equipped with a monochromatic Al K α X-ray source ($h\nu=1486.7$ eV) at a takeoff angle of 45° from the surface. The spectrometer was operated both at high and low resolutions with pass energies of 23.5 eV and 187.85 eV, respectively, and an analyzer spot diameter of 1.1 mm. The survey spectra were collected from 0 to 1400 eV, and the high-resolution spectra were obtained for photoelectrons emitted from C1s and N1s. A PHI Multipak software (version 5.0A) was used for all data processing. The high-resolution data were analyzed first by background subtraction using the Shirley routine and a subsequent non-linear fitting to mixed Gaussian-Lorentzian functions. Atomic compositions were derived from the high-resolution scans. Peak areas were obtained after subtraction of the integrated baseline and corrected for sensitivity factors.

2.7. Contact angle goniometry.

Water drops were dispersed onto the substrate surfaces using a micro-Electrapette 25 (Matrix Technologies). Advancing and receding contact angles ($\theta_{a/r}$) were measured using a goniometer (Rame-Hart, model 100). The pipette tip was kept in contact with the drop during

the measurements. At least four drops of probe liquid were measured for each sample, and the mean values were reproducible within $\pm 1^\circ$.

2.8. Evaluation of protein resistance.

The samples were immersed in a solution of fibrinogen from Sigma (1 mg/mL) in PBS buffer (pH 7.4) for 1 h. After that, the samples were thoroughly washed with Millipore water and dried with a flow of N_2 .

2.9. Cell culture.

Cell adhesion on the different surfaces was assessed by seeding 3T3 fibroblast cells (60,000 cells/mL) in 10% fetal bovine serum (FBS) in Dulbecco's modified Eagle's medium (DMEM) onto the substrates. The media was replaced with fresh 3T3 culture every 3 days. To evaluate the cell adhesion by microscopy, the substrates were rinsed with PBS for 3 times, then fixed by 2% formaldehyde/PBS at room temperature for 30 min. Finally, the substrates were stained with mounting medium DAPI (Vector) and observed with a fluorescent microscope (Eclipse 80i, Nikon) using 20x objective.

3. RESULTS AND DISCUSSION

3.1. Surface characterization.

The immobilization of OEG chains onto the fluorinated surface **A** was begun by treatment of glass slide with FAS17 in order to prepare substrate **A**. Then, an ethynyl-terminated, "clickable" surface, denoted as surface **B** (Scheme 1) was fabricated as we previously described.²⁵ The resultant ethynyl-terminated EG_4 surface **B** was then subjected to CuAAC reaction with EG_6-N_3 **2** in the presence of Cu^+ catalyst, excess of sodium ascorbate to reduce Cu^{2+} (rapidly formed in air) back to the catalytically active Cu^+ , and the ligand **3** to accelerate

the CuAAC reaction to provide surface **C** (Scheme 1).²⁶ Survey spectrum of the resulting click-modified surface showed the presence of the expected elements (C, F, N, and O) for the EG₆ modified surface **C** as shown in Figure 1a. An increase in the nitrogen signal intensity

Scheme 1. Functionalization of fluorinated surface **A** through fluororous immobilization to present EG₄-ethynyl groups on **B**, followed by covalent attachment of EG₆-N₃ **2** via CuAAC reaction to form **C**. Direct attachment of fluororous-tagged EG₄ **4** on the fluorinated substrate forms the EG₄ surface **D**.

was observed due to the formation of the triazole species after the click reaction. Narrow scans of the C 1s and N 1s regions in the XPS of the surface are shown in Figures 1b and 1c, respectively. Successful CuAAC reaction was indicated by the presence of the broad N 1s peak (Figure 1c) at 400.1 eV, which could be deconvoluted into three peaks with the following assignments: 401.3 eV (N–N=N),²⁵ 399.9 eV (N=N)²⁵ and 398.5 eV (O=C–N),²⁵ the ratio being 1.1:2.1:1.0, corresponding to the stoichiometric ratio of 1:2:1 for the CuAAC reaction product from **1** and **2**. Furthermore, unreacted azides were not observed as no peak appeared in the region of 404-405 eV expected for a N₃ group. The narrow scan on the C 1s region (Figure 1b) showed three peaks. The high binding energy peaks at 294.8 eV and 292.5 eV were assigned to CF₃ and CF₂ of **1** and the underlying fluororous substrate, respectively. The remaining C 1s peaks for surface **C** can be deconvoluted into three peaks with the assignment as 289.2 eV (O=C–N), 286.8 eV (C–O) and 285.4 eV (C–N). The copper residue

Figure 1. (a) Survey XPS spectra of the substrate before (black line) and after (red line) click reaction. XPS narrow scans of the C 1s (b) and N 1s(c) regions for the OEG-modified surface **C** with deconvolution and assignment.

was effectively removed by washing with an EDTA solution, as indicated by the absence of the Cu 2p signal (Figure S2). The incorporation of C₈F₁₇EG₄ and ethynyl-C₈F₁₇EG₄ molecules (surface **B** and **D**, respectively) as well as the fluorinated surface **A** and the click-modified surface **C** was also monitored through the atomic F/C concentration ratio in the XPS spectra. Figure 2 represents XPS survey scan for the initial SiO₂ substrate and for surfaces **A**, **B** and **D**. Table 1 shows atomic concentration data for F 1s and C 1s peaks obtained from XPS survey scan for the same aforementioned samples. Figure 2a represents the initial glass slide before FAS17 treatment, and, as expected, only the peaks corresponding to the O 1s and Si 2p were observed. The situation changes when FAS17 was incorporated onto the glass slide. Apart from the decrease in the O 1s peak, the XPS survey scan for the fluorinated surface **A** showed the F 1s and C 1s peaks corresponding to the FAS17 molecule. The XPS F1s narrow scan of fluorinated surface **A** is included in Figure S1. The atomic F/C ratio extracted from XPS was 2.27 and, as expected, the N1s signal was not detected. After incorporation of **1** and **4** to the surfaces, the XPS survey scan showed an increase in the O1s peak produced by the presence of the EG₄ groups, with a decrease in the F/C ratio to 1.87 for

Figure 2. XPS survey scan for (a) the initial SiO₂ surface, (b) fluorinated glass slide after FAS17 incorporation (surface **A**), (c) ethyl-EG₄-C₈F₁₇ surface **B** and (d) direct EG₄-C₈F₁₇ surface **D**.

surface **B** and 1.95 for surface **D**. After click reaction of azide **2**, due to the presence of triazol groups, the XPS survey scan showed the N1s peak (see Figure 1a), and the incorporation of EG₆ groups reduced the F/C ratio to 1.32.

The packing density of OEG units can be calculated by angle resolved XPS analysis for organic films deposited on flat silicon wafer substrates.^{30,31} However, our initial substrate was an amorphous glass slide and we did not obtain appropriate results by angle resolved XPS (ARXPS) to properly determine the packing density for the different incorporation steps. We consider that the lack of homogeneity of the glass slide surfaces compared with that for H-Si(111) surface is crucial to monitor the packing density by ARXPS analysis. However, water contact angle is an easy and rapid tool to compare the density and homogeneity of monolayers that are prepared under different conditions. Therefore we used this parameter as a way to monitor the packing density and homogeneity of the surface substrates. The advancing and receding water contact angles ($\theta_{a/r}$) on the fluorinated surface **A** were 109/107°. The low hysteresis ($\Delta\theta = 2^\circ$) indicates smooth and homogeneous surface. After direct deposition of the fluorine-tagged EG₄ to form surface **D** the $\theta_{a/r}$ values decreased to 76/70°. This high hysteresis value ($\Delta\theta = 6^\circ$) indicates a not homogeneous surface. This water contact angle value is high if it is compared with that for EG units on H-Si(111) surfaces.^(ref) The reason for such difference will be discussed in the next section. On the contrary, the CuAAC-modified OEG surface **C** exhibited a significantly more hydrophilic surface with $\theta_{a/r}$ values of 50/48°. This water contact angle data is very close to the advancing and receding water contact angle for ethylene glycols thin films on H-Si(111) prepared by hydrosilylation reaction,⁷ which suggests a homogeneous layer incorporated on the fluorinated surface.

3.2. Protein resistance.

The protein resistance of the fluorinated surface **A**, and the directly deposited EG₄ surface **D** and the CuAAC-modified surfaces **C**, respectively, was tested by immersing a 1 x 1 cm² sample in a solution of fibrinogen (1 mg/mL) in PBS for 1 h,³² followed by evaluation by XPS of the changes in the N 1s signal intensity. An increase in the N 1s signal is attributed to

adsorbed protein. XPS is not as sensitive method as others to calculate the amount of adsorbed protein, but is normally accepted to evaluate the protein resistance.^{32,33} As expected, after incubation, the results showed the presence of a substantial amount of fibrinogen on the fluorinated surface **A**, as is shown in Figure 3a. Moreover, direct modification of the fluorinated substrate with fluorous-tagged EG₄ (**4**, Scheme 1) to generate surface **D** did not result in an efficient protein-resistant surface, as indicated by the large increase of the N 1s signal represented in Figure 3b which is associated to the adsorbed protein. Both results indicate that a fluorinated substrate and a surface with fluorous-tagged EG₄ chains alone is not enough to achieve efficient protein resistance. This is probably due to the short length of the EG chains, in accordance with the protein resistance monolayers for EG units incorporated on H-Si(111) surface.^{7,33} For a quantitative comparison, a freshly prepared SiO₂ surface was subjected to the above conditions. Assuming a full monolayer adsorption of fibrinogen on SiO₂, the ratio (N_{EG}/N_{SiO_2}) of the integrated areas of the N 1s peak at ~401 eV from the adsorbed protein on surface **D** and SiO₂, respectively, correspond to the degree of protein adsorption on the EG surface (Figure 3d). This method results in 42% monolayer adsorption on surface **D**, which is a slightly higher compared with the previously results obtained for OEG units incorporated on H-Si(111) surface by photo-induced hydrosilylation reaction.^{32,34} We suppose that this circumstance can be due to two principal reasons; *i*) a lower packing density for C₈F₁₇-EG₄ groups on surface **D** compared with that performed by photo-induced hydrosilylation under high vacuum conditions, (refs) and *ii*) the length of the EG₄ units is not enough to screen the underlying fluorous surface. However, for the OEG modified surface **C**, analysis showed a 3% monolayer adsorption of protein (Figure 3d). It is important to note that before fibrinogen incubation surface **C** contains nitrogen, due to the triazole groups (Figure 2a). For this reason we present in Figure 3c the N 1s narrow scan

1 before and after immersion in protein solution. As is shown no apparent change in the N
2 intensity was observed on the CuAAC-modified
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7 **Figure 3.** N 1s scans for: (a) fluorinated surface **A** (b) directly functionalized surface **D** and
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9 (c) CuAAC-modified surface **B** before (black line) and after (red line) incubation in
10 fibrinogen solution (d) SiO₂ (redline), surface **D** (black line) and surface **C** (blue line) after
11 immersion in protein solution
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19 surface **C**. This data is consistent with the previously published for OEG groups on H-Si(111)
20 substrates, where almost a completely resisted to the adsorption of fibrinogen was observed
21 from EG₉ films. The presented XPS analysis is also consistent with water contact angle data,
22 thus longer OEG chains incorporated by CuAAC reaction screen the underlying fluoruous
23 substrate and enhance the adsorption of water to the film, which is necessary for efficient
24 protein-resistance.^{7,8}
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36 **3.3 Cell adhesion assays.**

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38 Cell adhesion on the fluorinated substrate **A**, direct deposited fluoruous-EG₄ surface **D** and
39 CuAAC-modified OEG surface **C** was assessed by seeding 3T3 fibroblast cells (60,000
40 cells/mL) in 10 % fetal bovine serum (FBS) in Dulbecco's modified Eagle's medium
41 (DMEM) onto the surfaces. As mentioned in experimental, the media was replaced with fresh
42 3T3 culture every 3 days. Representative optical microscopy images and the measured
43 density of the cellular attachment at 1 day, 5 days and 14 days are shown in Figures 4 and 5,
44 respectively. The cell density was calculated by counting the number of cells in a specific
45 area provided by the bright field optical microscope images and lead to counts/mm². As
46 expected, the fluorinated surface **A** showed significant cell adhesion at the three measured
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times (Figure 4a and Figure 5). Direct deposited fluororous-EG₄ substrates (surface **D**) effectively resisted the cell adhesion at 1 and 5 days, but a substantial increase of cell density was observed after 14 days (Figure 4b and Figure 5). The moderate cell-resistant ability of surface **D** is also attributed to the relative short length of the EG₄ chains deposited on the fluororous substrate, which could not prevent deposition of extracellular matrix proteins, such as fibrinogen (Figure 3c), leading to cell adhesion. On contrary, effective cell adhesion resistance over 14 days was achieved on the CuAAC-modified OEG substrate **C** as shown in Figure 4c and Figure 5. For a quantitative comparison, cell counting from the fluorescence images was also carried out, showing that the density of adhered cells for the CuAAC-modified surface **C** at 14 days was approximately 25 times lower than that for the untreated surface **A**, and about 20 times lower if it is compared with the direct modified surface **D**.

Figure 4. Representative bright field images of the (a) surface **A** (b) EG₄-modified fluororous surfaces **D** and (c) CuAAC-modified OEG surfaces **C**, after 1, 5 and 14 days of incubation in 3T3 fibroblast cell culture.

Figure 5. Measured cell densities on the surfaces in log scale with the error bars.

4. CONCLUSIONS

In conclusion, we have presented a simple and versatile method of creating a highly cell-resistant surface on fluororous substrates. The fluorinated substrates were fabricated by immersion of glass slides in a solution of FAS17. Then, they were modified with C₈F₁₇-EG₄-ethynyl groups by fluorine-fluorine interactions. Azido-EG₆ was incorporated by easy and reproducible CuAAC click reaction. XPS and water contact angle analysis confirmed the presence of the incorporated moieties. The protein resistance was evaluated showing a

1 reduced adsorption of fibrinogen to ~3% monolayer. Bright field optical microscopy
2 demonstrated total absence of 3T3 fibroblast cell on the CuAAC-modified substrate at 1 day
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4 and 5 days of analysis and an important decrease at 14 days compared with fluorinated
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6 substrates and directly deposited C₈F₁₇-EG₄ surfaces, obtaining a density of adhered cells 25
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8 times lower. Our presented method offers the possibility to be extended for potential
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10 applications in biomedical devices as well as to be used for the functionalization of different
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12 fluorous substrates.
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16 ASSOCIATED CONTENT

17 Supporting Information (SI)

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22 High resolution C 1s scan for FAS17 glass slide and Cu 2p scan for surface C after CuACC
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24 reaction are included in SI. This information is available free of charge via the Internet at
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26 <http://pubs.acs.org/>.
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32 AUTHOR INFORMATION

33 Corresponding Author

34
35
36
37 E-mail: rcontreras@uma.es
38
39

40
41
42 E-mail: cai@edu.edu
43
44
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46 Author Contributions

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53 approval to the final version of the manuscript.
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Scheme 1. Functionalization of fluorinated surface **A** through fluororous immobilization to present EG₄-ethynyl groups on **B**, followed by covalent attachment of EG₆-N₃ **2** via CuAAC reaction to form **C**. Direct attachment of fluororous-tagged EG₄ **4** on the fluorinated substrate forms the EG₄ surface **D**.

FIGURES

Figure 1. (a) Survey XPS spectra of the substrate before (black line) and after (red line) click reaction. XPS narrow scans of the C 1s (b) and N 1s(c) regions for the OEG-modified surface C with deconvolution and assignment.

Figure 2. XPS survey scan for (a) the initial SiO_2 surface, (b) fluorinated glass slide after FAS17 incorporation (surface **A**), (c) ethyl- $\text{EG}_4\text{-C}_8\text{F}_{17}$ surface **B** and (d) direct $\text{EG}_4\text{-C}_8\text{F}_{17}$ surface **D**.

Figure 3. N 1s scans for: (a) fluorinated surface **A** (b) directly functionalized surface **D** and (c) CuAAC-modified surface **B** before (black line) and after (red line) incubation in fibrinogen solution (d) SiO₂ (redline), surface **D** (black line) and surface **C** (blue line) after immersion in protein solution

Figure 4. Representative bright field images of the (a) surface **A** (b) EG₄-modified fluororous surfaces **D** and (c) CuAAC-modified OEG surfaces **C**, after 1, 5 and 14 days of incubation in 3T3 fibroblast cell culture.

Figure 5. Measured cell densities on the surfaces in log scale with the error bars.

Table 1. Atomic concentration table extracted from the XPS survey scans for the OEG modified substrates

Sample/Atom	C1s	F1s	F/C
Surface A	28.6	69.4	2.27
Surface B	34.42	64.64	1.87
Surface D	33.56	65.73	1.95
Surface C	41.74	55.45	1.32