Vapor-Based Multicomponent Coatings for Antifouling and Biofunctional Synergic Modifications

Meng-Yu Tsai, Yung-Chih Chen, Ting-Ju Lin, Yin-Chu Hsu, Ching-Yu Lin, Ruei-Hung Yuan, Jiashing Yu, Ming-Sheng Teng, Michael Hirtz, Mark Hung-Chih Chen, Chih-Hao Chang, and Hsien-Yeh Chen**

A general concept is introduced featuring an ideal multifunctional surface that can avoid fouling problems while allowing the installed groups to perform with the high efficacy and accuracy necessary for delivering cascading and spontaneous biological activities. The idea is realized by using a direct synthesis of a multicomponent coating containing the two functionalities of 4-methyl-propiolate and 4-*N*-maleimidomethyl that is achieved via chemical vapor deposition copolymerization on various substrates. The novel coating can simultaneously perform specific bio-orthogonal reactions, including the azide-alkyne click reaction and a thiol-maleimide coupling reaction. In the study, azide-terminated polyethylene glycols are first immobilized on the methyl propiolate groups to impart an antifouling property, while bioactivity is enabled by tethering biotinylated thiols or Cys-Arg-Glu-Asp-Val (CREDV) peptides on the maleimide groups. The induced antifouling properties and bioactivities are confirmed by quartz crystal microbalance and cell culture studies. Finally, precisely manipulated endothelial cells, namely, human umbilical vein endothelial cells and bovine arterial endothelial cells, are observed on a complex stent substrate and on confined areas of the poly(methyl methacrylate) substrates.

1. Introduction

The latest developments for the new generation of biomaterial surfaces often eliminate undesired interference from fouling substances, such as small biomolecules, proteins, bacteria, or cell adhesion.[1,2] The fouling (non-specific adsorption) induced by these molecules is usually problematic when uncontrolled, often causing malfunctions and/or reduced operation efficiency during bio-sensing applications, tissue engineering, and cell culture studies.[3] Methods to produce polyethylene glycol (PEG)-ylated biomolecules[4] or surfaces with antifouling modifications[5] are common strategies used to achieve the goals. Surface engineering approaches, however, have also attempted to present active biomolecules, such as small biomolecules and proteins, in a defined manner, mimicking the conditions in natural biological systems. For instance, cells can respond to synthetic cues, such as extracellular matrix (ECM) ligands[6] or chemical signals.[7] These environmental stimuli can induce intercellular and intracellular changes in the cytoskeletal organization, proliferation, cell differentiation, gene expression, and apoptosis,[8] ultimately affecting the rate and quality of new tissue formation.[9] Covalent immobilization strategies that tether these molecules are superior, offering the following advantages: (i) the strong chemical bonds ensure long-term operational efficacy relative to physisorbed molecules;[10] (ii) the surface chemistry can be precisely controlled, while the complicated adsorption/desorption mechanism (process) is usually hard to control.[2] The next-generation surfaces feature designs and modifications that facilitate the installation of multiple chemical functionalities, while these functionalities must concurrently compete with the suppressed background noise arising from fouling materials; specifically, an ideal surface can avoid any fouling problems, allowing the installed groups to perform with the high efficacy and accuracy necessary for delivering cascading and spontaneous biological activities. We introduce an advanced surface based on a

M.-Y. Tsai, Y.-C. Chen, T.-J. Lin, Y.-C. Hsu, C.-Y. Lin, R.-H. Yuan, Prof. J. Yu, Prof. H.-Y. Chen
Department of Chemical Engineering
National Taiwan University
Taipei, 10617, Taiwan
E-mail: hsychen@ntu.edu.tw

M.-S. Teng
Department of Research
Taipei Tzu Chi Hospital
The Buddhist Tzuchi Medical Foundation
Taipei, 23142, Taiwan

Dr. M. Hirtz
Institute of Nanotechnology
Karlsruhe Nano Micro Facility (KNMF)
Karlsruhe Institute of Technology
Hermann-von-Helmholtz-Platz 1
Eggenstein-Leopoldshafen, 76344, Germany

Dr. M. H.-C. Chen, Dr. C.-H. Chang
Department of Orthopedics
National Taiwan University Hospital and
National Taiwan University College of Medicine
Taipei, 10018, Taiwan
E-mail: mike920@gmail.com

DOI: 10.1002/adfm.201303050

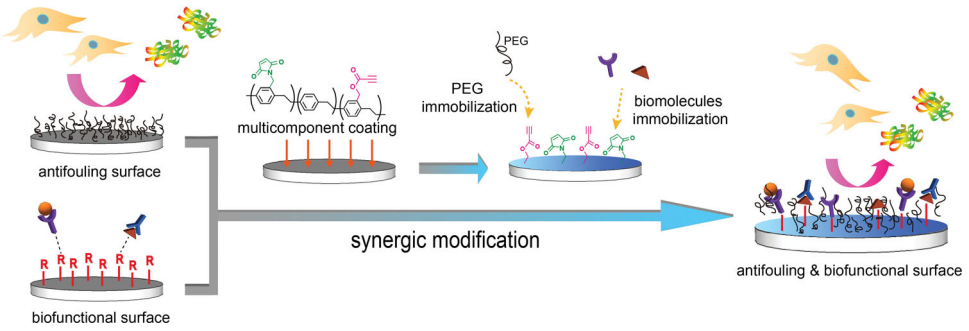

Figure 1. Schematic illustration of using CVD copolymerization to prepare a multicomponent poly-*p*-xylylene coating for co-immobilizing biomolecules. The coating technology can synergically create a surface that resists fouling while addressing a specific biological function.

multicomponent poly-*p*-xylylene coating that is prepared using a straightforward synthesis approach based on chemical vapor deposition (CVD) copolymerization processes to install two distinctly addressable reactive moieties: electron-deficient alkynes (methyl propiolate) and unsaturated maleimides. The resulting multicomponent surface enables the performance of specific bioorthogonal reactions (a copper-free 1,3-dipolar cycloaddition and a thiol-maleimide coupling) to create a surface that resists fouling while synergically providing efficient reactive sites for the desired biological activities, as illustrated in **Figure 1**. This coating system provides complimentary selectivity for biological functions to achieve sophisticated surface design and easy access to bioorthogonal conjugations; both coupling reactions are conducted at room temperature with high specificity and rates in the presence of oxygen/water while avoiding expensive and potentially toxic catalysts. In contrast, the previously reported multifunctional surfaces required multiple steps to complete their surface modifications[11] or utilized copper catalysts and acids during their modification.[12] Importantly, our reported multicomponent coating technology is a straightforward and benign modification process that produces a well-defined, synergic biological surface that is biologically inert to repel fouling substances while remaining active toward specific biofunctionalities.

2. Results and Discussion

2.1. CVD Copolymerization to Prepare Multicomponent Coating 3

The new multicomponent coating, poly[(4-*N*-maleimidomethyl-*p*-xylylene)-*co*-(4-methyl-propiolate-*p*-xylylene)-*co*-(*p*-xylylene)] **3**, contains a 4-*N*-maleimidomethyl and a 4-methyl-propiolate moiety; it is synthesized using a one-step process of chemical vapor deposition (CVD) copolymerization using 4-*N*-maleimidomethyl-[2,2]paracyclophane **1** and 4-methyl-propiolate-[2,2]paracyclophane **2**, as indicated in **Figure 2**. A custom-built dual-sourced CVD system was used during the copolymerization process to prepare coating **3**; each source contains independent sublimation and pyrolysis zones, and both sources are connected to the deposition chamber. In this experiment, the sublimed paracyclophanes **1** and **2** (1:1 ratio) were transferred at the same time to the pyrolysis zone and exposed to 580 °C or 510 °C temperatures, respectively in the pyrolysis zone. The subsequent C–C bond cleavage generated the corresponding *p*-quinodimethanes (monomers)[13,14] with different side chain functionalities. During the last step, the monomers copolymerized after they condensed on the cooled substrates (15 °C), forming multicomponent coating **3**. As deposited, **3** contains both methyl propiolate and maleimide side chains, providing the following advantages: i) copper-free accessibility to click reactions with azide-terminated biomolecules, ii) a handle for Michael-type thiol coupling reactions with maleimides, and iii) mild reaction conditions for these conjugation reactions

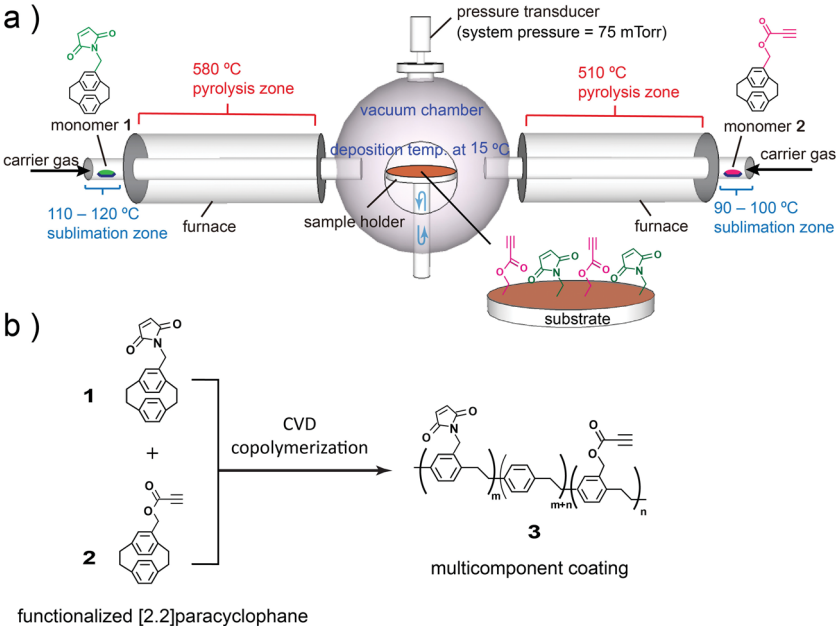

Figure 2. (a) A schematic illustration of a two-sourced CVD copolymerization used to prepare the multicomponent coating. (b) CVD copolymerization of a 1:1 molar ratio of 4-(*N*-maleimidomethyl)-[2,2]paracyclophane (**1**) and 4-methyl-propiolate-[2,2]paracyclophane (**2**) to form poly[(4-*N*-maleimidomethyl-*p*-xylylene)-*co*-(4-methyl-propiolate-*p*-xylylene)-*co*-(*p*-xylylene)] (multicomponent coating **3**) (m:n = 1:1).

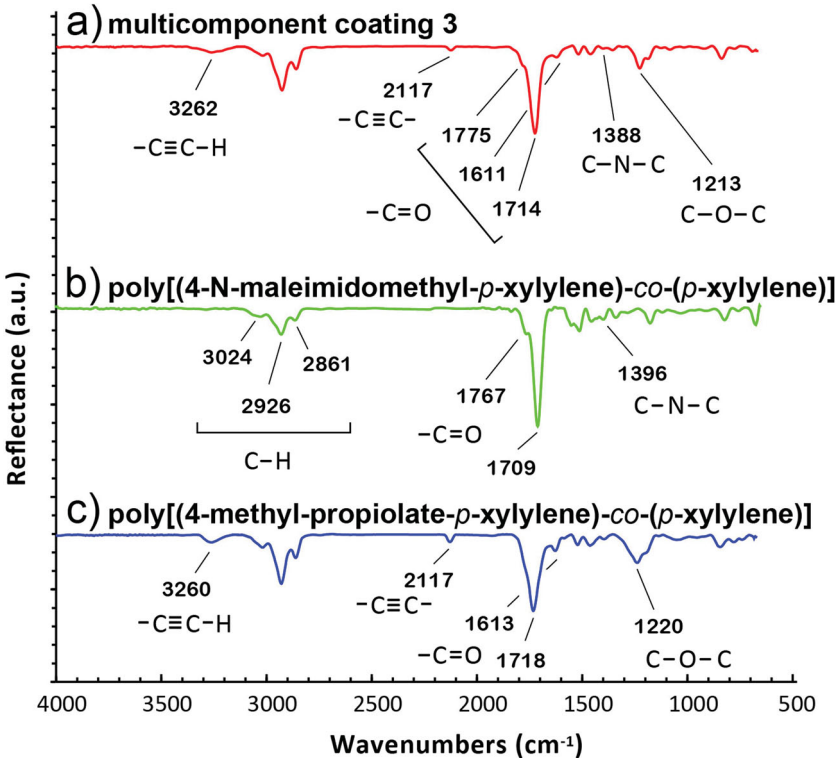

Figure 3. IRRAS spectra comparing the (a) multicomponent coating **3** with mono-substituted (b) poly[(4-*N*-maleimidomethyl-*p*-xylylene)-*co*-(*p*-xylylene)] and (c) poly[(4-methyl-propiolate-*p*-xylylene)-*co*-(*p*-xylylene)]). The characteristic bands of mono-substituted coatings from (b) and (c) were also detected in multicomponent coating **3**, indicating that both methyl propiolate and maleimide group were preserved during the CVD copolymerization process.

that tolerate water and oxygen at room temperature and avoid involving metal catalysts.

2.2. Surface Analysis of Multicomponent Coating 3

Infrared reflection absorption spectroscopy (IRRAS) spectra were recorded for coating **3**, as displayed in **Figure 3**a. The two peaks at 3262 cm^{-1} and 2117 cm^{-1} were attributed to the terminal alkyne groups from methyl propiolate, while the band at 1388 cm^{-1} represented the C–N–C stretching of maleimide. While the characteristic –C=O bands at 1715 cm^{-1}, 1714 cm^{-1}, and 1611 cm^{-1} that were previously observed in related mono-functional coatings (poly[(4-*N*-maleimidomethyl-*p*-xylylene)-*co*-(*p*-xylylene)][15] and poly[(4-methyl-propiolate-*p*-xylylene)-*co*-(*p*-xylylene)])[16] (Figure 3b,c), these signals were also recorded in coating **3**. In addition, the XPS analyses confirmed the chemical composition of coating **3**. As indicated from the survey spectra, coating **3** contained 91.3 atom% carbon, 7.0 atom% oxygen, and 1.7 atom% nitrogen, matching the theoretical values (89.1 atom% carbon, 8.7 atom% oxygen, and 2.2 atom% nitrogen). The high-resolution C_{1s} spectrum of coating **3** (see spectra in the Supporting Information, Figure S1) revealed that the signal at 285.0 eV belonged to the aliphatic and aromatic carbons (C–C, C–H), and the 74.3 atom% intensity was in accordance with the theoretical concentration of 76.0 atom%. The characteristic signals at 285.8 eV (2.8 atom%), 286.8 eV (2.5 atom%), 289.2 eV (1.9 atom%), 286.3 eV (3.0 atom%), and 288.3 eV (4.4 atom%) corresponded to C–C=O, C–O, O–C=O, C–N, N–C=O bonds, respectively, matching the calculated concentrations. The signal at 291.4 eV (2.4 atom%) revealed the $\pi \rightarrow \pi^*$ transitions that are characteristic for mono-substituted[14] and multifunctional[17] poly *p*-xylylenes; the relevant XPS data are listed in **Table 1**. The IRRAS and XPS data confirmed that coating **3** was successfully synthesized via CVD copolymerization using a 1:1 feeding ratio of **1** and **2**; no signs of decomposition or side reactions were observed during the CVD copolymerization process.

2.3. Synergic Conjugation Reaction of Coating 3

To verify that both the methyl propiolate and maleimide groups incorporated into coating **3** were available to perform specific bioorthogonal reactions, we devised a 2-step conjugation procedure to immobilize multiple molecules on separate areas of the same surface. Alexa Fluor-555 azides and fluorescein-labeled cysteines were selected as model substrates for the coupling reactions. The confined reaction areas were created using a microcontact printing (μCP) technique,[18] and selected micropatterns were used as a guide. The conjugation cascade was performed by printing Alexa Fluor 555 azides using μCP with 500 μm × 500 μm array patterns on coating **3** using an azide-alkyne click reaction. Subsequently, fluorescein-conjugated cysteine was printed via μCP with 50 μm × 50 μm array patterns on the same sample surface using a thiol-maleimide coupling reaction. Both reactions were

Table 1. Chemical composition of multicomponent coating **3** determined by XPS. Experimental values are compared with the calculated values.[a)]

Chemical States/ Elements	Binding Energy [eV]	Experimental Concentration [%]	Calculated Concentration [%]
C–C/H	285.0	74.3	76.0
C–C=O	285.8	2.8	2.2
C–O	286.8	2.5	2.2
O–C=O	289.2	1.9	2.2
C–N	286.3	3.0	2.2
N–C=O	288.6	4.4	4.3
$\pi \rightarrow \pi^*$	291.4	2.4	–
O	527.0	7.0	8.7
N	396.0	1.7	2.2

[a)]Experimental values are compared to calculated values based on an equimolar distribution of starting materials.

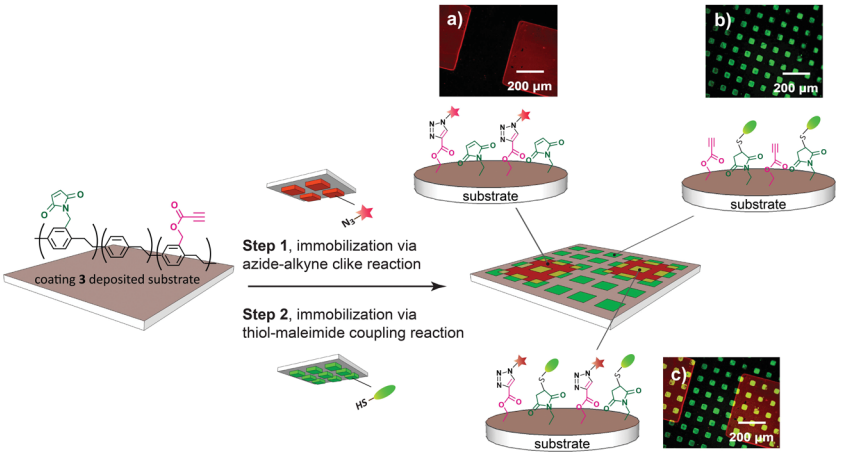

Figure 4. Schematic illustration of the synergic immobilization of multiple biomolecules via bioorthogonal reactions on multicomponent coating **3**. An azide-alkyne click reaction was performed during the first step to immobilize the Alexa Fluor-555 azides, and a thiol-maleimide coupling reaction was used during the second step to immobilize fluorescein-labeled cysteines. The reactions were performed under mild conditions in water/oxygen at room temperature and without a catalyst. The process of μCP was used to confine specific conjugation to selected areas. (a) The red-channeled fluorescent micrograph illustrates the immobilization of the Alexa Fluor-555 azides. (b) The green-channeled fluorescent micrograph reveals the immobilization of the fluorescein-cysteines. (c) Overlaid images of (a,b). The images were captured at the same location in the same sample.

carried out under ambient conditions (pH = 7.4) at room temperature without a catalyst. As displayed in **Figure 4**, the fluorescence micrographs indicated that the fluorescence molecules were addressed on designated areas of coating **3**; the pronounced Alexa Fluor-555 signals (red) were observed in the areas where the azides were printed, while the fluorescein signals (green) appeared on the cysteine (thiol)-printed areas. Moreover, the overlaid image in Figure 4c reveals the parallel immobilization of the two fluorescence molecules in the yellow regions; no cross-reactions occurred. The control experiments used to verify the absence of cross-reactions are reported in the Supporting Information (Figure S2).

2.4. Biological Activity on Fouling-Suppressed Surfaces: QCM Analysis

A surface was created that exhibited reduced background noise from fouling molecules by coupling azide-terminated polyethylene glycol (PEG) molecules via azide-alkyne click reaction on coating 3; utilizing the PEG moiety has remained the gold-standard for suppressing the non-specific binding of fouling molecules and is widely used in surface-engineered biomaterials.[3] The resulting adsorption behavior of streptavidin on this surface was characterized with a quartz crystal microbalance (QCM); this instrument is a well-adopted sensor system suitable for monitoring protein adsorption in real time.[19] In the experiment, QCM crystals were coated with **3** via CVD copolymerization and subsequently coupled with thiol-PEG and azide-PEG via azide-alkyne click reaction and thiol-maleimide coupling reaction, respectively. The frequency changes in the QCM crystal were monitored while a solution of streptavidin was passed over the crystal surfaces. The mass adsorbed onto the surface was quantified from the frequency change according to the Sauerbrey relationship: the adsorbed mass is proportional to the change of frequency.[20] As displayed in **Figure 5**, the streptavidin adsorption curve for the thiol-PEG and azide-PEG modified surface was compared to that of the surface with only coating **3**. A significant amount of fouling was observed on the as-deposited coating **3** surface due to its relatively high hydrophobicity (indicated by its approximately 92 degree water contact angle), and an average frequency of 53.6 Hz that corresponded to a streptavidin surface concentration of approximately 266.1 ng cm^{-2} was observed. In contrast, surfaces that were modified with PEG exhibited extremely low adsorptions of streptavidin and an average frequency of 0.6 Hz, corresponding to a streptavidin surface concentration of approximately 3.0 ng cm^{-2}. To extend the synergic repertoire of coating **3**, biological function was induced on a surface while the fouling activity was reduced. A similar 2-step conjugation procedure was performed by coupling azide-PEG groups to a **3**-coated crystal surface using an azide-alkyne click reaction and subsequently, thiol-terminated biotin molecules were tethered to the surface with a thiol-maleimide coupling reaction. The tightly confined interaction between biotin and streptavidin generated in a high

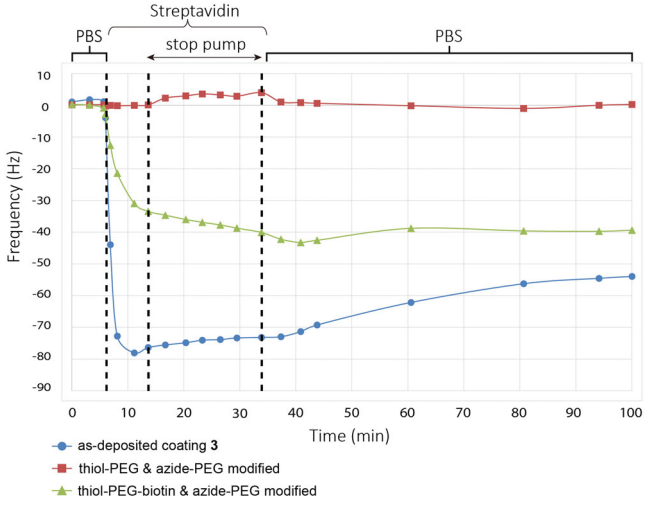

Figure 5. QCM analysis of the antifouling and biofunctional activities on coating **3**. Significant non-specific adsorption of streptavidin was found on coating **3** (an average frequency/adsorption of 53.6 Hz/266.1 ng cm^{-2}). After modifying the PEG groups on coating **3**, the non-specific streptavidin adsorption was dramatically reduced (an average frequency/adsorption of 0.6 Hz/3.0 ng cm^{-2}). Finally, by attaching both PEGs and biotins to coating **3**, the surface utilizes a synergic approach that suppresses the non-specific binding of streptavidin and precisely induces controlled binding through a biotin/streptavidin interaction (an average frequency/adsorption of 39.0 Hz/193.6 ng cm^{-2}). Phosphate buffered saline (PBS) is injected into the flow system.

binding affinity that was detected by QCM, as indicated in Figure 5; the averaged frequency was 39.0 Hz, revealing that approximately 193.6 ng cm^{-2} streptavidin was bound to the synergic surface; for the azide-PEG modified surface, the non-specific binding of streptavidin was significantly reduced. The synergic surface was built based upon this biological inertness; in addition, the installed biotin active sites have rendered the surface specific for binding streptavidin.

2.5. Manipulated Attachment of Endothelial Cells

Low-level, non-specific adsorption by fouling molecules and accurately immobilized biomolecules with specific binding interactions are key factors for developing bioanalytical systems, biosensors, and many devices surgical tools that interact with blood[21] that attain high operational efficiency. We designed a surface to resist fouling and therefore eliminate any undesirable interference from non-specific cell adhesions; concurrently, this surface is equipped with specific molecules to participate in a desired biological process. A stent would benefit from this type of synergically modified surface; as such, we demonstrated the surfaces' properties using this type of device. CVD copolymerization was used to apply coating **3** to a stent substrate for the conceptual demonstration before the following modifications were made: i) functionalizing the entire substrate's surface with azide-PEGs using an azide–alkyne click reaction to render a fouling-resistant surface and ii) immobilizing a cysteine-containing peptide (Cys-Arg-Glu-Asp-Val (CREDV)) that preferentially attracts the binding and proliferation of endothelial cells over other cell types[22] with a thiol-maleimide coupling to impart the biological activity (**Figure 6**a). These two bioorthogonal conjugations were performed under mild conditions, as previously described, and the characteristics of the resulting surface were investigated by examining the in vitro cellular responses when human umbilical vein endothelial cells (HUVECs) were cultured on the surface. The HUVECs adhered to the modified surface, and they were observed after 24 h of incubation. The samples were double-stained with 4′,6-diamidino-2-phenylindole (DAPI, blue) for their nuclei and phalloidin (red) for the distribution of cytoskeletal F-actin in the cells. As indicated in Figure 6b,c, the modified stents were confluent with the cells after 24 h of incubation. The control experiments also indicated that the HUVECs did not adhere to the PEG-modified **3**-coated stent surface (Figure 6d). These results have important implications: i) the HUVEC attachment was promoted only by CREDV peptide ligand and was otherwise prevented by the cell-repellant PEG-modified background, as indicated by the control experiments (Figure 6d); ii) the two equipped functional groups (4-methyl-propiolate and 4-*N*-maleimidomethyl) could synergically perform orthogonal and specific conjugation reactions without cross-reacting, as shown in Figure 4. Cell growth manipulation was also demonstrated on selected areas of poly(methyl methacrylate) (PMMA) substrates in a separate experiment. Similar synergic modifications were performed by clicking azide-PEG to 4-methyl-propiolate groups on a **3**-coated PMMA substrate, and subsequently CREDV peptides were affixed to confined areas using a microcontact printing (μCP)

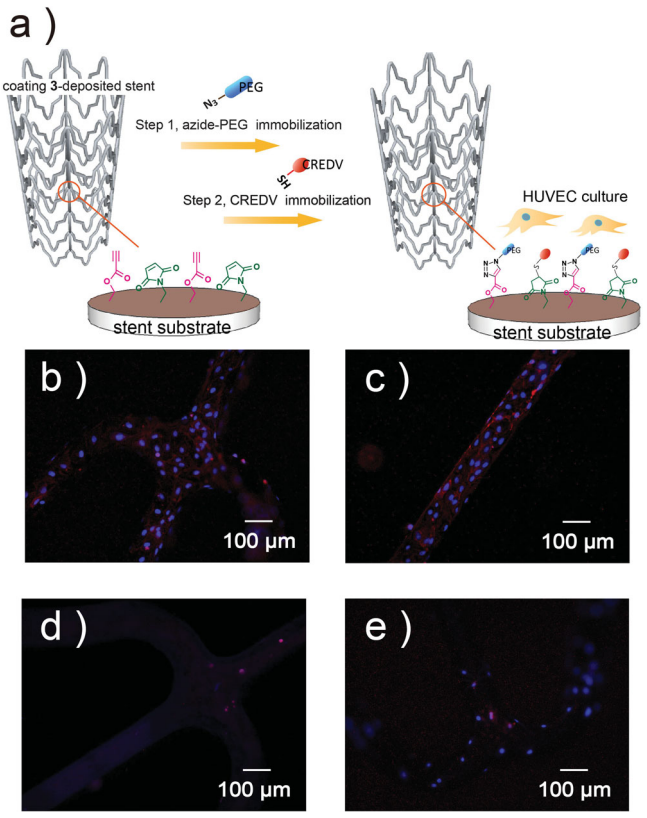

Figure 6. (a) The PEG/CREDV-modified synergic surface of a coating **3**-deposited stent sample. The modified surface was used for a cell culture study of HUVAECs. Azide-PEG was first tethered to the coated stent substrate using an azide-alkyne click reaction; second, the CREDV peptide was immobilized with a thiol-maleimide coupling. The fluorescence micrographs display the HUVAECs that were incubated for 24 h on (b) a conjunction area, and (c) a string area of the modified stent sample. (d) The control experiments have revealed that HUVAECs avoided adhering to only the PEG-immobilized stent. (e) The control experiments indicated that noticeable HUVAECs grew on the surface with coating **3** due to non-specific attachment. Staining procedures were performed on HUVAEC's actin cytoskeleton using rhodamine-phalloidin (red), and the nucleus was stained using DAPI (blue).

process and a thiol-maleimide conjugation reaction. Bovine arterial endothelial cells (BAECs) were subsequently allowed to adhere to the modified surfaces and observed after 10 and 24 h of incubation time. As illustrated in **Figure 7**, confluent distributions of BAECs were found only on the areas where CREDV was immobilized; in contrast, the PEG-exposed areas exhibited no BAECs adhesion or growth. Additional control experiments confirmed these results by investigating the attachment of the BAECs to separate samples of either PEG- or CREDV-modified substrates, and the data are provided in the Supporting Information (Figure S3). These results suggest that the surfaces synergically modified with coating **3** exhibit a biologically inert background that eliminates non-specific cell attachments while accurately and effectively inducing bioactive functions (i.e., conjugate with CREDV peptide to attract HUVEC or BAEC attachments) that provide compatibility and the growth-conducive interactions needed for substrates/devices.

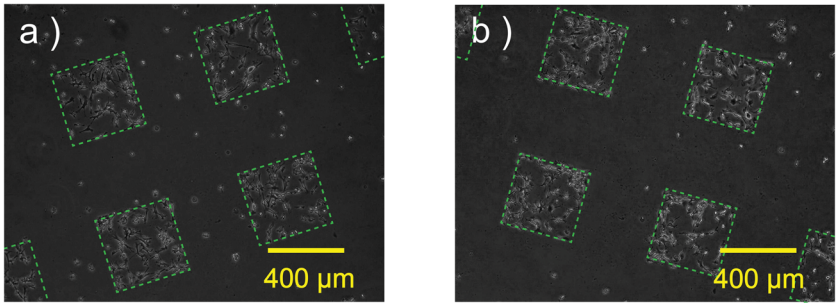

Figure 7. BAECs were cultured on a PEG/CREDV-modified synergic surface of a PMMA substrate. Azide-PEGs were first clicked homogeneously on the modified substrate using an azide-alkyne reaction; second, CREDV molecules were immobilized on confined areas of the same sample from the first step, and the covalent linkage was reinforced with a thiol-maleimide reaction. The phase contrast images display the BAECs that were incubated after (a) 10 and (b) 24 h on the synergic surface. The green dotted lines mark the perimeter of the CREDV modified areas, and they are drawn to guide the eye.

3. Conclusion

The multicomponent coating features a novel combination of alkynyl and maleimide groups and is synthesized by CVD copolymerization into a one-step coating system available for a wide range of substrate materials and complicated devices, such as a stent. The functionalities are designed to control the immobilization of biomolecules both regio- and stereoselectively under mild conditions similar to biological environments. Moreover, synergically presented biological activities, such as a bioinert surface with both a resistance toward protein fouling and specified cell attachments, are precisely manipulated for cell culture studies. The performance of the coating technology is not limited to the demonstrations in this study and is expected to extend across various applications in biomaterial designs, sophisticated biosensors devices, tissue engineering, and regeneration medicine fields.

4. Experimental Section

Materials: The following materials were obtained commercially and used as received unless otherwise noted: fluorescein-conjugated cysteine (95%, Kelowna International Scientific Inc., Taiwan), Alexa Fluor-555 conjugate azides (95%, Yao-Hong biotechnology Inc., Taiwan), methoxypolyethylene glycol azide M.W. 5000 (Sigma-Aldrich), thiol-PEG-biotin (Nanocs Inc.), CREDV (95%, Yao-Hong biotechnology Inc., Taiwan), polystyrene (Taifonacrylic Co., Taiwan), silicon wafer (Goldeninent Inc., Taiwan). The gold substrates were fabricated using thermal evaporation on a 4-in silicon wafer; a titanium layer of 300 Å thickness was formed, followed by a 700 Å thick gold layer (Kao Duen Technology Co., Taiwan). A self-expanding stent (RX Acculink Carotid Stent System, Abbott) that had a 7 mm I.D. on one end, a 5 mm I.D. on the other end, and a length of 40 mm, was cleaned with ethanol before CVD deposition.

CVD Copolymerization: Poly[(4-*N*-maleimidomethyl-p-xylylene)-*co*-(4-methyl-propiolate-*p*-xylylene)-*co*-(p-xylylene)] **3** was prepared from 4-*N*-maleimidomethyl-[2,2]paracyclophane **1** and 4-methyl-propiolate-[2,2]paracyclophane **2** via CVD copolymerization using a dual-sourced CVD installation; the 1:1 feeding ratio (molar) of **1** and **2** was controlled during the CVD copolymerization process. The pyrolysis temperatures were 580 °C for **1** and 510 °C for **2**. The polymerization of **1** and **2** to form copolymer **3** was spontaneously induced on substrates placed on top of a rotating and cooled (15 °C) sample holder in the deposition chamber. The system pressure was maintained at 75 mTorr during the entire CVD copolymerization process.

Surface Characterization: The infrared reflection absorption spectroscopy (IRRAS) spectra were recorded using a Thermo Nicolet NEXUS 470 FT-IR spectrometer equipped with a liquid nitrogen-cooled MCT detector; the spectra were corrected for any residual baseline drift. The samples were mounted in a nitrogen-purged chamber. The CVD coatings were affixed to gold-coated silicon substrates (Au/Si) for the measurements, and a blank Au/Si substrate was used as a reference during the experiments. X-ray photoelectron spectroscopy (XPS) data were recorded with a Theta Probe X-ray photoelectron spectrometer (Thermal Scientific, UK) using a monochromatized AlKα X-ray source. The XPS spectra were recorded with an X-ray power of 150 kW. The pass energies were 200.0 eV and 20.0 eV for the survey spectra and the high-resolution C_{1s} elemental spectra, respectively. The XPS atomic analysis was reported based on the atomic concentrations (%) and was compared to the theoretical values calculated on the basis of structure **3** (m:n=1:1).

Immobilization of Biomolecules: The immobilization of the fluorescein-conjugated cysteine was performed via microcontact printing (μCP) using a poly(dimethylsiloxane) (PDMS) stamp consisting of square arrays with 50 μm sides and a 100 μm center-center spacing; the stamp was inked with fluorescein-conjugated cysteine in deionized water (5 mM) and used to print on top of coating **3** for 2 h. The resulting sample was washed three times with phosphate-buffered saline (PBS pH = 7.4, contains Tween 20, Sigma), one time with PBS (pH = 7.4, Sigma) and finally rinsed with deionized water. Similarly, the fluorescein-labeled azide was immobilized by inking a PDMS stamp (square arrays with 500 μm sides and a 1000 μm center-center spacing) with fluorescein-labeled azide solution (5 mM) in deionized water, and printing over coating **3** for 2 h. The PDMS stamps were treated with 10 W oxygen plasma for 2 min before the inking process to render surface hydrophilic. μCP was performed at 25 °C and 55% humidity. The resulting samples were analyzed, and the fluorescent micrographs were recorded with a fluorescence microscope (Nikon TE2000-U).

Quartz Crystal Microbalance: A QCM instrument (ANT Technologies Corp., Taiwan), equipped with a flow injection analysis (FIA) device and continuous frequency variation recording device quantitatively monitored the protein binding in real time. The sensing element of this instrument was an AT-cut piezoelectric quartz disc with a 9 MHz resonant frequency and a 0.1 cm^2 total sensing area. Co-immobilization of the azide-PEG (200 mg/mL) and biotin-thiol (150 mg/mL) was performed on **3**-coated quartz discs under previously described reaction conditions. PBS (pH = 7.4) was used as a carrier solution for the QCM system and was continuously delivered into the flow channel until a stable frequency response was obtained. Subsequently, a streptavidin solution (312.5 μg/mL in PBS, pH=7.4) was injected into the flow system, and the time-dependent change in frequency was continuously monitored while detecting the streptavidin adsorption. The 36.5 μg/mL flow rate was maintained using a peristaltic pump connected to the FIA device.

Cell Culture: A **3**-deposited stent substrate was exposed to 200 mg/mL azide-PEG for 2 h and washed thoroughly with deionized water to remove any unreacted azide-PEG. For the second step, the same stent sample was exposed to CREDV (4 mg/mL) for 2 h. The resulting stent sample was finally rinsed with deionized water and PBS (pH=7.4) before use in the cell culture study. Human umbilical vein endothelial cells (HUVECs) were obtained commercially from ScienCell Research Laboratories (catalog #8000), cultured in M199 (Sigma-Aldrich) containing 10% fetal bovine serum (FBS) for 24 h and observed without washing. After microscopic analysis, the samples were washed with PBS (pH=7.4) and fixed with 3.7% formaldehyde (Acros) solution for 10 min. For staining, 4',6-diamidino-2-phenylindole (DAPI,

0.29×10^{-3} M, Sigma-Aldrich) and Alexa Fluor 543 phalloidin (6.3 μM, Molecular Probes) solutions were used to detect the adhesion and morphology of the cells on the substrates. The samples were incubated with DAPI and phalloidin solutions simultaneously at room temperature for 30 min and washed with PBS to remove any extra stain solution. The stained samples were analyzed with fluorescence microscopy (Nikon TE2000-U). Coating **3**-modified PMMA substrates were used to confine CREDV (4 mg/mL) molecules and used for a cell culture study with bovine arterial endothelial cells (BAECs). The coated PMMA substrate was reacted with 200 mg/mL azide-PEG for 2 h and thoroughly washed with deionized water to remove any unreacted azide-PEG. Next, a PDMS stamp patterned with squares that had 400 μm sides and a 800 μm center-center spacing was used. The stamp was inked with CREDV (4 mg/mL) and printed for 2 h on the samples. The resulting PMMA samples were finally rinsed with deionized water and PBS (pH = 7.4, Sigma) before they were used for BAECs cell culture studies. The BAECs were obtained from Academia Sinica, Institute of Biomedical Science and were cultured in low-glucose DMEM containing 10% fetal bovine serum (FBS). The modified surfaces were fixed at the prepared wells and briefly maintained in Dulbecco's modified Eagle's medium (DMEM) (Hyclone) without FBS (Biolegend) until the cells were ready for seeding. The cells were re-suspended in DMEM without FBS and added to the modified surfaces. The cells were allowed to adhere to the substrates for 2 h at 37 °C in DMEM without FBS and subsequently observed with phase contrast microscopy. After the initial 2 h, the medium was exchanged to FBS-containing DMEM, and the substrates were incubated for another 48 h before being observed without washing.

Supporting Information

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

Acknowledgements

H.-Y. Chen gratefully acknowledges the National Science Council of Taiwan (NSC 101-2628-E-002-034-MY3, NSC 100-2221-E-002-086-MY2, and NSC-DAAD 102-2911-I-002-507) for their financial support. This work is further supported by NSC 101-2622-B-002-007-CC3 and NTUH.102-S2144 for Dr. C.-H. Chang. We thank Prof. Hao-Ming Hsiao of the Department of Mechanical Engineering, National Taiwan University, for providing stent substrates.

Received: September 1, 2013
Revised: October 28, 2013
Published online: January 2, 2014

[1] N. Nath, J. Hyun, H. Ma, A. Chilkoti, *Surf. Sci.* **2004**, *570*, 98.
[2] D. G. Castner, B. D. Ratner, *Surf. Sci.* **2002**, *500*, 28.
[3] I. Banerjee, R. C. Pangule, R. S. Kane, *Adv. Mater.* **2011**, *23*, 690.
[4] R. Müller, A. Eidt, K.-A. Hiller, V. Katzur, M. Subat, H. Schweikl, S. Imazato, S. Ruhl, G. Schmalz, *Biomaterials* **2009**, *30*, 4921.
[5] S. Krishnan, C. J. Weinman, C. K. Ober, *J. Mater. Chem.* **2008**, *18*, 3405.
[6] a) M. M. Stevens, J. H. George, Science **2005**, *310*, 1135; b) D. Lehnert, B. Wehrle-Haller, C. David, U. Weiland, C. Ballestrem, B. A. Imhof, M. Bastmeyer, *J. Cell. Sci.* **2004**, *117*, 41; c) J. H. Huang, S. V. Grater, F. Corbellinl, S. Rinck, E. Bock, R. Kemkemer, H. Kessler, J. D. Ding, J. P. Spatz, *Nano Lett.* **2009**, *9*, 1111.
[7] a) R. L. Juliano, *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 283; b) J. P. Spatz, B. Geiger, *Cell Mech.* **2007**, *83*, 89.
[8] a) B. Geiger, J. P. Spatz, A. D. Bershadsky, *Nat. Rev. Mol. Cell Biol.* **2009**, *10*, 21; b) N. J. Sniadecki, A. Anguelouch, M. T. Yang, C. M. Lamb, Z. Liu, S. B. Kirschner, Y. Liu, D. H. Reich, C. S. Chen, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 14553; c) M. J. Dalby, M. O. Riehle, D. S. Sutherland, H. Agheli, A. S. G. Curtis, *Eur. J.Cell Biol.* **2004**, *83*, 159.
[9] B. D. Boyan, T. W. Hummert, D. D. Dean, Z. Schwartz, *Biomaterials* **1996**, *17*, 137.
[10] H. Shin, S. Jo, A. G. Mikos, *Biomaterials* **2003**, *24*, 4353.
[11] a) K. C. Wood, H. F. Chuang, R. D. Batten, D. M. Lynn, P. T. Hammond, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 10207; b) N. Herzer, C. Haensch, S. Hoeppener, U. S. Schubert, *Langmuir* **2010**, *26*, 8358; c) C. A. DeForest, B. D. Polizzotti, K. S. Anseth, *Nat. Mater.* **2009**, *8*, 659.
[12] a) X. Deng, J. Lahann, *Macromol. Rapid Commun.* **2012**, *33*, 1459; b) X. Deng, T. W. Eyster, Y. Elkasabi, J. Lahann, *Macromol. Rapid Commun.* **2012**, *33*, 640.
[13] a) W. F. Gorham, *J. Polym. Sci., Part A-1: Polym. Chem.* **1966**, *4*, 3027; b) P. Kramer, A. K. Sharma, E. E. Hennecke, H. Yasuda, *J. Polym. Sci. Polym. Chem. Ed.* **1984**, *22*, 475.
[14] J. Lahann, R. Langer, *Macromolecules* **2002**, *35*, 4380.
[15] M.-Y. Tsai, C.-Y. Lin, C.-H. Huang, J.-A. Gu, S.-T. Huang, J. Yu, H.-Y. Chen, *Chem. Commun.* **2012**, *48*, 10969.
[16] X. Deng, C. Friedmann, J. Lahann, *Angew. Chem. Int. Ed.* **2011**, *50*, 6522.
[17] a) Y. Elkasabi, H. Y. Chen, J. Lahann, *Adv. Mater.* **2006**, *18*, 1521; b) H.-Y. Chen, T.-J. Lin, M.-Y. Tsai, C.-T. Su, R.-H. Yuan, C.-C. Hsieh, Y.-J. Yang, C.-C. Hsu, H.-M. Hsiao, Y.-C. Hsu, *Chem. Commun.* **2013**, *49*, 4531.
[18] S. Alom Ruiz, C. S. Chen, *Soft Matter* **2007**, *3*, 168.
[19] K. A. Marx, *Biomacromolecules* **2003**, *4*, 1099.
[20] G. Sauerbrey, Z. Physik **1959**, *155*, 206.
[21] a) S. Hoffman Allan, in *Biomaterials: Interfacial Phenomena and Applications*, Vol. *199*, ACS, Washington **1982**; b) Z. Liu, N. Khan, H. Hu, B. Yu, J. Liu, M. Chen, F. Zhou, *Macromol. Rapid Commun.* **2008**, *29*, 1937; c) T. B. McPherson, H. S. Shim, K. Park, *J. Biomed. Mater. Res.* **1997**, *38*, 289.
[22] B. D. Plouffe, D. N. Njoka, J. Harris, J. Liao, N. K. Horick, M. Radisic, S. K. Murthy, *Langmuir* **2007**, *23*, 5050.