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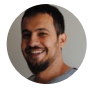

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One-Pot Photo-Induced Sequential CuAAC and Thiol−Ene Click Strategy for Bioactive Macromolecular Synthesis

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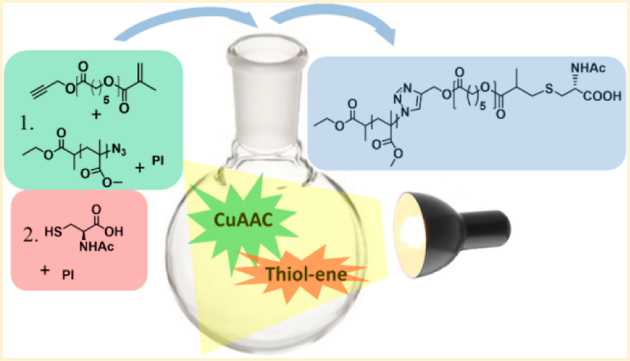
Supporting Information

ABSTRACT: Conceptually new one-pot photoinduced sequential click reactions were implemented to yield novel block copolymers with the ability for cell adhesion. Poly(ε-caprolcatone) possessing clickable functional groups at the chain ends, namely α-alkynyl-ω-alkenyl-poly(ε-caprolactone) (A-PCL-MA), was prepared by ring-opening polymerization of ε-caprolactone using propargyl alcohol in the presence of stannous octoate at 110 °C followed by esterification with methacrylic acid. Azide-functional poly(methyl methacrylate) (PMMA-N_3) was prepared independently by atom transfer radical polymerization (ATRP) followed by an azidation process using sodium azide. Finally, A-PCL-MA was reacted with PMMA-N_3 and *N*-acetyl-L-cysteine (NAC) in a one-pot process through photoinduced sequential click reactions to furnish desired bioactive block copolymer (PMMA-*b*-PCL-NAC). A matrix for cell adhesion was then prepared from the yielded block copolymer PMMA-*b*-PCL-NAC and cell proliferation on the matrix was measured. Cells from the Vero cell line (African green monkey kidney epithelial) were incubated on the matrix, and after 48 h, they showed greater cell proliferation than the commercially available cell culture plates used as comparison.



INTRODUCTION

"Click" chemistry, first coined by Sharpless et al. in 2001[1] has since become one of the preferred weapons of choice for a generation of chemists across many fields of research ranging from the material and polymer sciences[2] to the pharmaceutical and biomedical sectors.[3] It was defined by its founders as any modular chemical transformation with exceptionally high or quantitative yield producing only inoffensive or no side-products and no need for column chromatography among some other requisites.[4] The most famous example of "click" chemistry has to be the copper-catalyzed 1,3- Huisgen dipolar cycloaddition reaction often referred to as the copper-catalyzed azide−alkyne click abbreviated CuAAC as described by Meldal and co-workers in 2001.[5] There are a handful of other transformations that also carry the banner for "click" chemistry such as the so-called thiol−ene and thiol−yne reactions, so well researched by such groups as that of Bowman among others.[6] These are preferred reactions due to their efficacy, modularity, chemoselectivity, and regioselectivity. Another attractive feature of "click" chemistry, especially the CuAAC reaction can be its orthogonal nature whereby it can be carried out in tandem with another process such as RAFT polymerization, with no fear of the two processes interfering or hindering one another.[7] Also this orthogonality has been exploited to combine the use of CuAAC and thiol−ene "click" chemistry in sequential processes which give rise to macromolecules with biomedical application.[8] Atom radical transfer polymerization (ATRP) triggered by the use of a photoinduced reduction of an air-stable copper(II) complex to copper(I) without the use of a reducing agent has been reported by us and several other groups.[9] This concept was then extended and applied successfully thereafter in the copper catalyzed azide−alkyne click reaction.[10] Our group has also divulged this strategy on the synthesis of ABC type miktoarm star copolymers taking advantage once again of the energy provided by light by employing a photoinduced thiol−ene click reaction, in combination with other techniques such as ATRP and a heat induced CuAAC.[11] Recently, the orthogonality of thiol−ene click chemistry was availed of by combining sequentially, thiol-allyl and thiol−acrylate (Michael addition) click chemistry to form graded rainbow holograms.[12] To further explore and take advantage of the orthogonal nature of these types of click reactions, it was the intention here to develop a methodology which similarly availed of a combination of photoinduced copper catalyzed azide−alkyne click chemistry along with a photoinduced thiol−ene click

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reaction to potentially arrive at interesting copolymer macromolecular structures within a one-pot procedure.

Poly(ε-caprolactone) (PCL) was chosen as the scaffold to click to as it is recognized as a biocompatible and biodegradable biomaterial. It is also versatile in its preparation, allowing for easy introduction of clickable moieties. Hvilsted and collaborators have described a very convenient method for obtaining a terminally bifunctional "alkyne-alkene" PCL in two steps that avails of a ROP of ε-caprolactone (CL) catalyzed by stannous octoate with various alcohols being effective as initiator.[13] Olefin functionality can then be introduced subsequently using a Mitsonobu transformation. For the purpose of synthesizing a bifunctional PCL for CuAAC and thiol−ene click chemistry, Hvilsted et al. described this using propargyl alcohol as the initiating alcohol in the ROP with 4-pentenoic acid or methacrylic acid being used in the Mitsunobu reaction, thereafter, to provide the bifunctionality.

Poly(methyl methacrylate) (PMMA) has also been employed in biomedical applications such as in bone cements; however, it is recognized as a poorly biocompatible material.[14] For the purposes of our study, we would use PMMA poly(methyl methacrylate) as a matrix to which biocompatible entities would be clicked to in a facile one-pot procedure. *N*-Acetyl-L-cysteine (NAC) was chosen due to its primary thiol amino acid side chain which could be exploited in a thiol−ene click reaction to attach the amino acid to the block copolymer base. The free carboxyl moiety of the amino acid could thereafter be availed of in the future to initiate peptide growth, facilitate peptide attachment or linking of other biomolecular entities via the available C-terminus.

It was desired to combine these components in a one-pot and photoinduced procedure taking advantage of the selectivity and orthogonality of the copper catalyzed 1,3-Huisgen dipolar cycloaddition and thiol−ene click reactions. It was apparent that these transformations would need to be employed in a sequential fashion beginning with the CuAAC reaction due to the reactivity of alkyne functionality to also carry out thiol−yne click chemistry.[15] The strategy envisioned consisted of first clicking PMMA-N_3 to an alkyne terminus residing on PCL through CuAAC chemisty followed by, in the same reaction vessel, the clicking of NAC to an alkene terminus of PCL using thiol−ene click chemistry.

And with regard to biotechnological investigations, cell cultivation on a matrix is one of the most important experimental procedures and is widely used to follow cell-material interactions as well as cell based bioanalyses and *in vitro* toxicity of drug candidates. The biologically active clicked block copolymer potentially obtained from this novel approach could be applied to obtain a cell culture platform and the Vero cell line (from African green monkey kidney epithelial) as a model could be cultured with the aim of successful proliferation on this copolymer.

EXPERIMENTAL SECTION

Materials. Propargyl alcohol (99%, Aldrich), stannous octoate (95%, Aldrich), diethyl azodicarboxylate (DEAD, Aldrich), methacrylic acid (98%, Aldrich), ethyl 2-bromopropionate (99%, Aldrich), copper(I) bromide (98%, Acros), triphenylphosphine (98.5%, Fluka), sodium azide (99%, Aldrich), copper(II) chloride (98%, Aldrich), 2-benzyl-2-dimethylamino-4′-morpholinobutyrophenone (DBMP, Ciba Specialty Chemicals), and 2,4,6-trimethylbenzoyl)-diphenylphosphine oxide (TMDPO, Ciba Specialty Chemicals) were used as received. Methyl methacrylate (MMA, 99%, Aldrich) was filtered over basic Al_2O_3 to remove inhibitor. Toluene was distilled over $CaCl_2$ before use. THF was distilled over sodium and benzophenone (Aldrich) before use. *N*,*N*,*N*′,*N*″,*N*″-Pentamethyldiethylenetriamine (PMDETA, 99%, Aldrich) was distilled before use.

Characterization. ^{1}H NMR of the intermediates and final polymers taken in $CDCl_3$ with $Si(CH_3)_4$ as an internal standard were recorded at room temperature at 500 MHz on a Agilent VNMRS 500 spectrometer. FT-IR spectra were recorded on Perkin−Elmer FT-IR spectrum one spectrometer with an ATR Accessory (ZnSe, PikeMiracle Accessory) and cadmium telluride (MCT) detector. Resolution was 4 cm $^{-1}$ and 24 scans with 0.2 cm/s scan speed. Molecular weights and polydispersities of polymers and the block copolymer were measured by gel permeation chromatography (GPC) employing an Agilent 1100 instrument equipped with a differential refractometer by using THF as the eluent at a flow rate of 0.3 mL min $^{-1}$ at 30 °C. Molecular weights were determined using polystyrene standards.

Preparation of Bromo-Functional Poly(methyl methacrylate) by ATRP. MMA (4 mL, 37.55 mmol), toluene (2 mL), ethyl 2-bromopropionate (0.098 mL, 0.751 mmol), PMDETA (0.16 mL, 0.751 mmol), and CuBr (0.107 g, 0.751 mmol) were added to a flame-dried tube under N_2 in that order. The reaction was purged of oxygen by fluxing through N_2 for 10 min. It was heated directly to 75 °C with a preheated oil bath and it was stirred at that temperature for 10 min before being removed from the oil bath and being allowed to cool. It was diluted with THF and filtered over a small column of Al_2O_3. Solvent was reduced in *vacuo* before the product was precipitated in excess, cold MeOH. The precipitate was filtered and dried in *vacuo* to yield a white solid (0.790 g, conversion = 21%). M_{nGPC} = 8087 g/mol. PDI = 1.45. ^{1}H NMR ($CDCl_3$, δ, ppm, TMS): 0.86 ($-CH_2C(CH_3)-CH_2-$), 0.93 ($CH_3CH_2OC(O)CH(CH_3)-$) 1.03 ($-CH_2C(CH_3)-CH_2-$), 1.09 ($CH_3CH_2C(O)O-$), 1.82−2.07 ($-CH_2-$), 2.05 ($-C(CO_2CH_3)(CH_3)Br$), 2.31 ($-CH_2C(CH_3)(CO_2CH_3)Br$), 3.61 ($-CH_3OC(O)-$), 4.10 ($CH_3CH_2OC(O)-$). IR (υ_{max}): 3491 broad, 2999, 2937, 2331, 2064, 1956, 1724, 1668 cm^{-1}.

Azidation of PMMA. Above obtained PMMA (0.790 g, 0.098 mmol) and sodium azide (0.031 g, 0.476 mmol) were added to a 100 mL round-bottom flask under N_2 before DMF (10 mL) was added on top, and the reaction was heated to 60 °C and was stirred for 22 h. It was then allowed to cool before being precipitated in cold, excess MeOH. The azido-functional PMMA (PMMA-N_3) product was finally filtered and dried in a vacuum oven (0.460 g, yield =59%). M_{nGPC} = 8378 g/mol. PDI = 1.43. IR (υ_{max}): 3616, 3441, 2995, 2943, 2321, 2137 (azide), 1725, 1674 cm^{-1}.

Preparation of α-Hydroxyl−ω-Alkyne−Poly(ε-caprolactone). The preparation of this polymer was carried out according to an altered, previously described procedure.[16] To a 100 mL round-bottom flask was added ε-caprolactone (5 mL, 45.12 mmol), toluene (5 mL), propargyl alcohol (0.14 mL, 2.26 mmol), and stannous octoate (0.15 mL, 0.45 mmol). The reaction was purged of oxygen by fluxing through N_2 for 30 min. It was then heated directly to 110 °C with a preheated silicon oil bath and stirred for 23 h. The reaction was then allowed to cool before it was precipitated in cold hexane. It was then filtered and dried in a vacuum oven for 2 days. Product was isolated as a white solid (4.537 g, conversion = 86%). M_{nGPC} = 4705 g/mol. PDI = 1.39. ^{1}H NMR ($CDCl_3$, δ, ppm, TMS): 1.38 (2H × m, $-CH_2CH_2CH_2-$), 1.57−1.65 (4H × m, $-CH_2CH_2CH_2O-$, $-OCH(=O)CH_2CH_2CH_2-$), 2.31 (2H × m, $-OCH(=O)-CH_2CH_2-$), 2.5 (1H, $-C\equiv CH$), 3.65 (2H, $-CH_2CH_2OH$), 3.95−4.05 (2H × m, $-CH_2CH_2O-$), 4.68 (2H × s, $-OCH(=O)CH_2C\equiv CH$). IR (υ_{max}): 3524 (br), 3277, 3928, 2316, 2095, 1720 cm^{-1}.

Preparation of α-Methacrylate−ω-Alkyne−Poly(ε-caprolactone). A previously described Mitsunobu procedure was employed to end functionalize the α-hydroxyl−ω-alkyne−poly(ε-caprolactone).[17] The PCL (2.26g, 0.69 mmol) and triphenylphosphine (0.650 g, 2.48 mmol) was stirred under nitrogen at room temperature in 12 mL of distilled THF in a 100 mL round-botttom flask before methacrylic acid was added slowly. It was stirred for 30 min before the temperature was lowered to 0 °C with an ice bath. It was stirred for 30 min at 0 °C before DEAD (0.36 mL, 2.27 mmol) was added drop by drop. It was stirred for 30 min at 0 °C before the ice bath was removed and it was

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allowed to warm up, stirring overnight. It was stirred thereafter for 24 h before being precipitated in cold methanol (120 mL) and dried in a vacuum oven for 48 h to yield a white solid (2.057 g, yield =81%, conversion of hydroxyl to methacrylate = 100% (^{1}H NMR). M_{nGPC} = 4530 g/mol. PDI = 1.36. ^{1}H NMR ($CDCl_3$, δ, ppm, TMS): 1.38 (2H × m, $-CH_2CH_2CH_2-$), 1.57−1.65 (4H × m, $-CH_2CH_2CH_2O-$, $-OCH(=O)CH_2CH_2CH_2-$), 1.94 (3H × s, $CH_3C(CH_2)C=O$), 2.31 (2H × m, $-OCH(=O)CH_2CH_2-$), 2.5 (1H, $-C\equiv CH$), 3.95−4.05 (2H × m, $-CH_2CH_2O-$), 4.68 (2H, $-OCH(=O)CH_2C\equiv CH$), 5.55 (1H × s, $-C=C(H)H$), 6.09 (1H × s, $-C=C(H)H$). IR (υ_{max}): 3437, 3257, 3941, 1721 cm^{-1}.

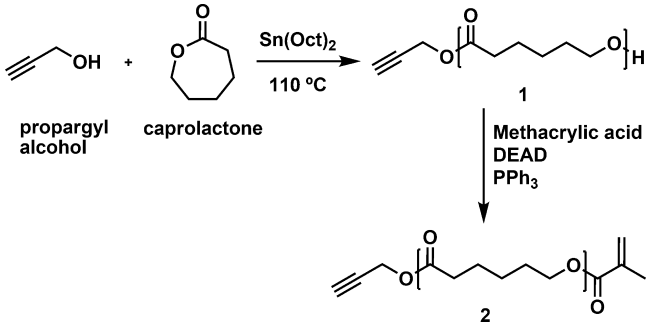
Sequential Photoinitiated CuAAC/Thiol−Ene Click Reactions. PMMA-N_3 (0.28 g, 0.018 mmol), A-PCL-MA (0.08 g, 0.019 mmol), $CuCl_2$ (0.012 g, 0.089 mmol), and TMDPO (0.028 g, 0.08 mmol) were added to a flame-dried glass tube fitted with a rubber septum under nitrogen followed by 1:1 THF/DMSO (8 mL). To this mixture was added PMDETA (0.017 mL, 0.08 mmol) before it was purged of oxygen by fluxing through nitrogen gas for 20 min, at which point the tube was irradiated at λ > 350 nm. After 2 h of irradiation, NAC (0.031 g, 0.19 mmol) and DBMP (0.07 g, 0.19 mmol) were added to the tube, and after that, it was again purged of oxygen by fluxing through nitrogen gas for 20 min. It was once again irradiated at the same wavelength for 16 h. The reaction mixture was diluted with THF and filtered over neutral Al_2O_3 before being reduced in *vacuo*. It was then precipitated in cold methanol (80 mL) before being gravity filtered and dried in a vacuum oven for 24 h to yield a white solid (0.359 g, 92%). M_{nGPC} = 11,141g/mol. PDI = 1.31. ^{1}H NMR ($CDCl_3$, δ, ppm, TMS): 0.86 (3H × d, $-CCH_3$, PMMA), 1.03 (3H × s, $-CCH_3$,PMMA) 1.38 (2H × m, $-CH_2CH_2CH_2-$, PCL), 1.13 (3H × d, J = 6.9 Hz, CH_3CHCH_2-S-), 1.57−1.65 (4H × m, $-CH_2CH_2CH_2O-$, $-OCH(=O)CH_2CH_2CH_2-$, PCL), 1.82−2.07 ($-CH_2-$, PMMA) 1.94 (3H × s, $CH_3C(CH_2)C=O$, PCL), 2.31 (2H × m, $-OCH(=O)CH_2CH_2-$, PCL), 2.71 (2H × s, $-SCH_2CH$-(NHAc)COOH−), 2.76 (1H × s, $-CH_3CHCH_2S-$), 2.83 (2H × s, $-CHCH_2SCH_2-$), 3.61 (CH_3-O-, PMMA), 3.95−4.05 (2H × *m*, $-CH_2CH_2O-$, PCL), 5.3 (2H × s, $-CH_2-C_2HN_3$), 7.45−7.55 (1H × m, C_2HN_3), 7.70−7.80 (1H × m, $-NH-$). IR (υ_{max}): 3447 (br), 3000, 1724 cm^{-1}.

Cell Culture Studies. Vero cell line (from African green monkey kidney epithelial) was supplied from ATCC. Cell culture supplies including fetal calf serum (FCS Gold) and penicillin/streptomycin (P/S, 100×) were purchased from Lonza (Basel, Switzerland). Vero cells were grown in DMEM (Dulbecco's modified Eagle medium) containing 10% FCS and 1.0% P/S. All cells were cultivated in medium and incubated with samples and reagents at 37 °C in a humidified environment with 5.0% CO_2. Cell adhesion on the poly(methyl methacrylate)][polycaprolactone]cysteine functionalized block copolymer **4** was observed via fluorescence microscopes and proliferation studies using the MTT Assay. Poly(methyl methacrylate)][polycaprolactone]cysteine-functionalized block copolymer **4** was compared to the 96-well cell culture plates to observe their properties as cell culture materials. Thus, 1.0 mg of the copolymer was suspended in 100 μL of THF and 1900 μL of PBS, and then, 50 μL of the suspension was added to each well in 96 well tissue plates (Sarstedt). Plates were dried for 24 h at room temperature. Afterward, the copolymer-coated plates were sterilized under UV radiation for 15 min and used for the cell adhesion cell experiments. In proliferation assay, the cells cultivated on the 96 well tissue plates without copolymer were applied as a negative control. Cells were incubated for 3. 24, 48, and 72 h (time for adherence). At the end of each cultivation time, cells were treated with 110 μL/well 10% MTT solution (5.0 mg/mL phosphate buffer saline (PBS)) in medium for 4 h. Then 100 μL of SDS solution (1.0 g of SDS in 10 mL of 0.01 M HCl) were added per well and after 24 h of incubation, UV−vis absorption was measured at 570 nm with 630 nm as reference wavelength using a microplate reader (Bio-Tek Instruments, Inc., Winooski, VT). Additionally, DAPI cell nuclei control staining was performed for the cultivated cells. The cell staining medium was removed and cells were washed with PBS, and wells were treated with DAPI solution (1.0 mg/mL) for 15 min at 37 °C and then washed again twice with PBS. Fluorescence of samples was monitored using an Olympus BX53F fluorescence microscope equipped with a CCD camera (Olympus DP72). To monitor fluorescence after DAPI staining, a U-MWU excitation filter, BP330−385 (exciter filter) and BA420 (barrier filter) were used.[18]

RESULTS AND DISCUSSION

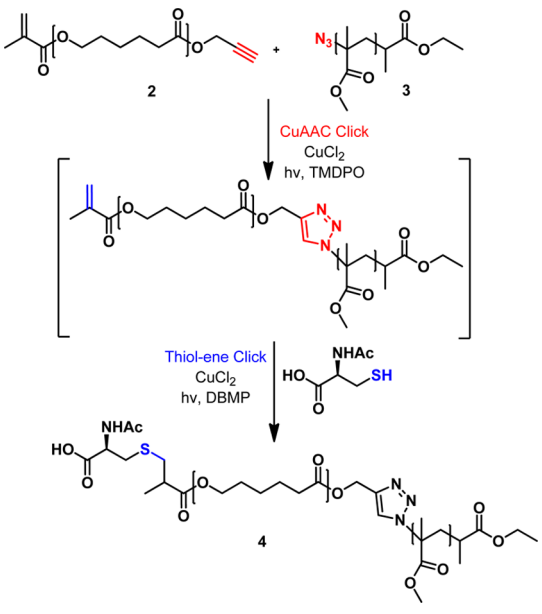
PCL possessing both acetylene and methacrylate functionalities at the chain ends (A-PCL-MA) was prepared according to the literature by first carrying out a ring-opening polymerization of CL initiated by propargyl alcohol with stannous octoate as catalyst.[13c,16] After obtaining the desired polymer, the alkene functionality was installed at the hydroxyl terminus of the polycaprolactone via a Mitsunobu coupling employing methacrylic acid as observed in Scheme 1.

Scheme 1. Preparation of Alkyne−Alkene Terminally Bifunctional PCL 2



Once the bifunctional PCL, **2**, was obtained a novel one-pot photoinduced CuAAC thiol−ene click procedure was attempted as demonstrated in Scheme 2. PMMA-N_3, **3**, prepared according to literature precedent,[19] was irradiated under UV−vis at >350 nm with A-PCL-MA in the presence of $CuCl_2$ and TMDPO with PMDETA to solubilize the copper species.

Scheme 2. Sequential One-Pot Photoinduced CuAAC and Thiol−Ene Click Reactions Yielding Bio-Active Block Co-Polymer 4



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TMDPO was used as the photoinitiator molecule due to its efficacy in formation of radicals giving rise to the reduction of Cu(II) to Cu(I), necessary in the photoinduced CuAAC reaction as described previously by our group and collaborators.[20] This process has been shown to be quick, with usually times of between 20 min to 1 h employed in a successfully carried out, photoinduced CuAAC reaction and in this instance 2 h was employed for the sake of surety. To carry out the thiol−ene click step of the reaction scheme, the reaction was opened to the atmosphere to add in the NAC, being the thiol bearing moiety, along with additional photoinitiator before being sealed and purged of oxygen once again. For the thiol−ene click step a different photoinitiator was added to the reaction in the form of DBMP. It was chosen for its solubility in the employed solvent system along with its abundance at hand and the mixture of different photoinitiators imposed no hindrance to the reaction system.

Once irradiation was stopped the product was filtered over neutral Al_2O_3 to remove copper and the copolymer was obtained after precipitation in cold methanol with subsequent drying. An analysis of the polymer using GPC (Figure 1) could

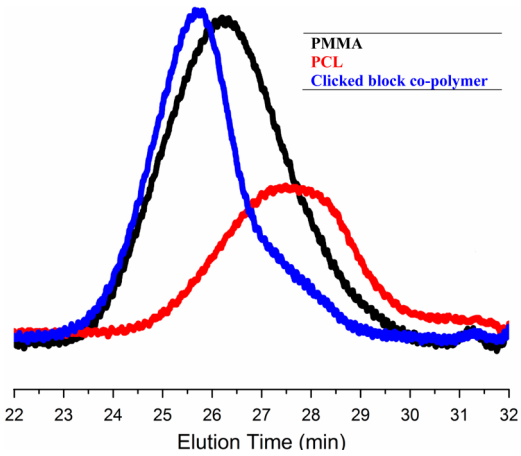

Figure 1. GPC trace of NAC bound block copolymer **4** in blue with PMMA in black and PCL in red shown for comparison.

be used to confirm the successful clicking of PMMA to PCL. As can be observed from Figure 1, the clicked block copolymer, **4**, had a shorter elution time compared with that of the precursors PMMA and PCL.

The GPC analysis outlined the successful coupling of the click components through the consequetive click reactios (Table 1).

The ^{1}H NMR of the resulting NAC bound block copolymer, **4**, was used to confirm the presence of all the expected peaks corresponding to both PMMA and PCL segments (Figure 2). Absence of characteristic and obvious peaks corresponding to

Table 1. Molecular Weight Characteristics[a] of the Precursors and Final Click Product

polymer	M_n (g mol^{-1})	M_w (g mol^{-1})	PDI
PMMA-N_3	8087	11 729	1.45
A-PCL-OH	4705	6541	1.39
A-PCL-MA	4530	6158	1.36
PMMA-*b*-PCL-NAC	11 141	14 625	1.31

[a]Determined by GPC using PS standards.

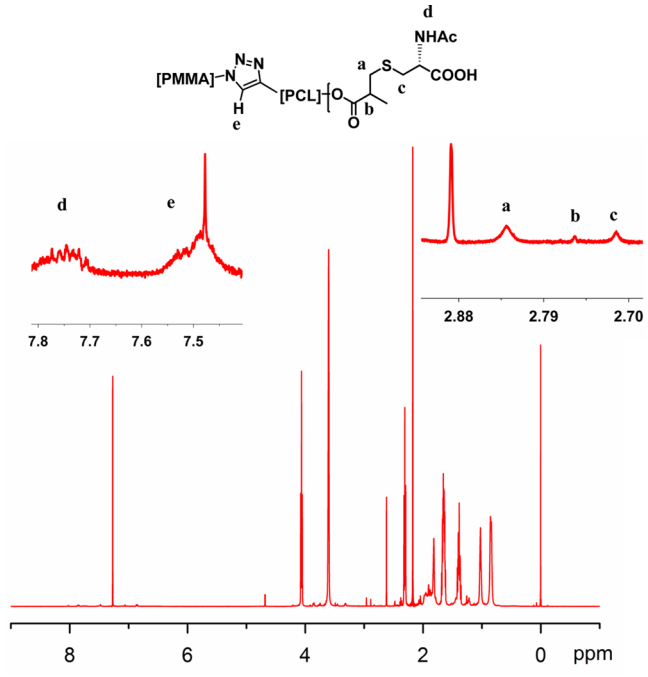

Figure 2. ^{1}H NMR spectra of cysteine-bound block copolymer **4**.

the propargyl CH_2 at 4.68 ppm and propargyl terminal CH at 2.47 ppm of the PCL starting material immediately suggested a successful CuAAC click reaction while the appearance of triazole −CH− peak between 7.44 and 7.56 ppm was confirmed. The absence of the alkene peaks at 5.55 and 6.09 ppm also suggests a successful thiol−ene click reaction has taken place on the PCL methacrylate functionality and examining the ^{1}H NMR spectrum closer the amino acid -NH- could be observed between 7.70 and 7.80 ppm. At 2.71 ppm the amino acid R group $-CH_2-$ can be appreciated, at 2.76 ppm the newly formed tertiary −CH− derived from the reduction of the methacrylate can be observed while at 2.83 ppm the methacrylate derived $-CH_2-$ peak is apparent. At 1.13 ppm, a peak can be observed corresponding to the methyl group of the reacted methyl acrylate species. It is noted that the *N*-acetyl group of cysteine cannot be appreciated due to it being overcrowded by PCL peaks.

The FT-IR spectra of the clicked block copolymer (Figure 3) confirmed that no azide remained as a peak at 2135 cm^{-1} was no longer visible. Although a new triazole C−H stretch could not be appreciated where expected, around 3000 cm^{-1} due to overlying peaks belonging to PMMA, a new N=N triazole stretch could be observed at 1598 cm^{-1}.

PMMA-*b*-PCL-NAC was covered on the surface of 96 well plates. Vero cells were used for the studying of cell adhesion properties on this novel matrix. Proliferation behaviors of Vero cells were searched using MTT method. Figure 4 shows that similar proliferation features were obtained with the commercial cell culture plates. It can be stated that this novel material could be successfully applied for cell cultivation on different surfaces. This is especially important to construct cell based biosensor platforms which could be used in different shapes and dimensions.[21]

Additionally, DAPI staining was used to image cell adhesion on both copolymer and well plate surfaces (Figure 5). No significant differences between two surfaces were observed. This means that this novel synthetic approach could be a

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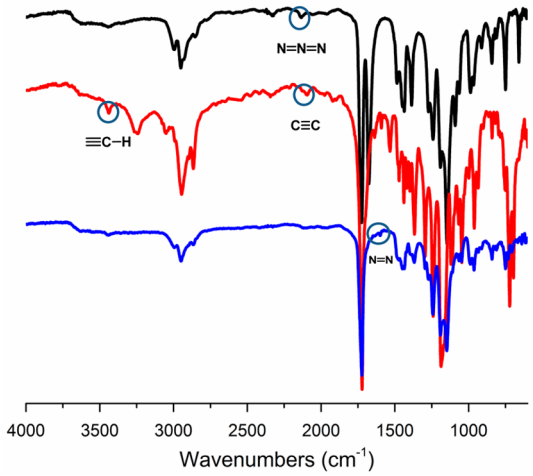

Figure 3. FT-IR spectra of the clicked block copolymer **4** (blue), the A-PCL-MA (**2**) (red) and PMMA-N_3 (**3**) (black).

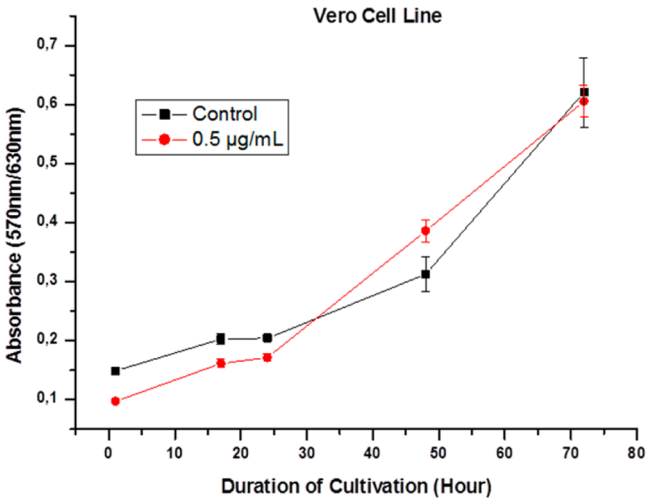

Figure 4. Time dependent proliferation behavior of Vero cell line on the copolymer **4** (red line) and on the commercial cell culture plates (black line).

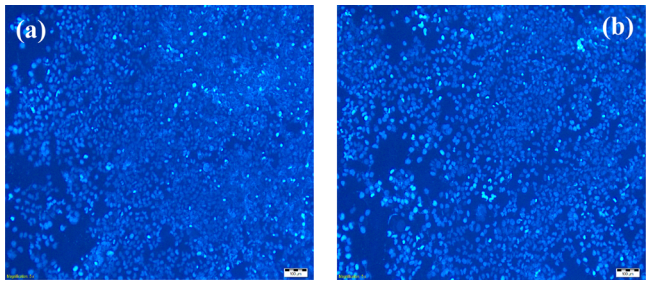

Figure 5. Fluorescence microscope images of DAPI stained Vero cells cultivated on the commercial well plates (a) and copolymer **4** coated plates (b) after 24 h (5× magnification).

promising alternative to fabricate efficient and practical materials for cell culture studies. Also, this matrix could be easily modified for the design of targeted surfaces for cancer cells which could be selectively adhered and proliferated due to the presence of some of the overexpressed molecules.[15]

CONCLUSIONS

In conclusion, in this work a strategy was laid out whereby photoinitiated CuAAC and thiol–ene click chemistry could be availed of in a one-pot procedure and was successfully applied. This one-pot photoinduced CuAAC/thiol–ene click sequential reaction system was employed to bring about the biologically active block copolymer PMMA-*b*-PCL-NAC. The newly formed blocked copolymer was applied as a matrix in cell proliferation studies showing favorable results. These results make it clear that the orthogonal nature of the photoinitiated CuAAC and thiol–ene click reactions can be taken advantage of in a one pot system with the potential for widespread application.

ASSOCIATED CONTENT

Supporting Information

Overlay of GPC traces and ^{1}H NMR spectra. This information is provided free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

PMMA, poly(methyl methacrylate); PCL, polycaprolactone; GPC, gel permeation chromatography; CuAAC, copper-catalyzed azide–alkyne click; RAFT, raft reversible addition–fragmentation transfer; ATRP, atom transfer radical polymerization; PDI, polydispersity index; THF, tetrahydrofuran; DMSO, dimethyl sulfoxide; DEAD, diethyl azodicarboxylate; PMDETA, pentamethyl diethylenetriamine; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DAPI, 4′,6-diamidino-2-phenylindole; PBS, phosphate-buffered saline

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