Direct Synthesis of Quaternized Polymer Brushes and Their Application for Guiding Neuronal Growth

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We show that the poly([2-(methacryloyloxy)ethyl]trimethylammonium chloride) (PMETA) brush modified surfaces are excellent substrates for the directed growth of rat hippocampal neurons, which could prove useful for the coating of neural devices. Direct synthesis of quaternized poly(dimethylaminoethyl methacrylate) brushes was achieved by surface-initiated atom transfer radical polymerization (SI-ATRP) of META in protic media at room temperature. PMETA brushes were photolithographically patterned on silicon substrates in combination with patterned regions backfilled with short polyethylene glycol (PEG) SAMs. The resulting hybrid patterned surfaces consisting of both "cytophilic" and "cytophobic" materials were successfully employed to guide a neuronal outgrowth. These surfaces should thus provide an excellent platform for studies of neuronal function, use in neuronal implants, cell-based biosensors, and other applications.

1. Introduction

Careful design of the interface between living tissue and the exposed surfaces of bioimplants is essential for the successful deployment of these devices.[1] Ideally, device surfaces should be compatible with both the growth of eukaryotic cells or tissue (biocompatible) and the suppression of prokaryotic infection (antibacterial), but this combination is difficult to achieve. We examined whether positively charged poly(quaternary ammonium) (PQA) compounds might meet these criteria.

A variety of positively charged PQA compounds have been shown to be antibacterial when applied to surfaces.[2,3] For example, various alkyl halide-quaternized poly(2-(dimethylamino)ethyl methacrylate) polymers are known to have excellent antibacterial activity when deposited on surfaces in the form of polymer brushes.[4−6] In this configuration, one polymer chain end is attached to the selected surface at a sufficiently high density so that the polymer chains are forced to orient themselves away from the substrate. Inspired by the widespread use of positively charged polypeptides (e.g., polylysine) as substrates culturing neurons,[7−10] we examined positively charged polymer brushes for their ability to support neuronal growth. In initial experiments, we synthesized brushes from primary amine monomers. However, these yielded poor survival in cell culture experiments with neurons. In contrast, antibacterial polymer brushes with quaternary ammonium groups grown from META monomers turned out to be suitable substrates to support and guide growth of rat hippocampal neurons.

Polymer brushes with a selection of desired functional groups grown using surface-initiated polymerization can therefore provide a robust, tailored surface modification strategy. Among the many surface-initiated polymerization techniques, surface-initiated atom transfer radical polymerization (SI-ATRP) is most widely used. ATRP is compatible with protic solvents and monomers bearing many types of functional groups. Meanwhile, it also provides controlled polymerization, that is, control over molecular size. Quaternized PDMAEMA brushes have mainly been synthesized by quaternizing preformed PDMAEMA brushes with alkyl halides.[5,6] This method involves multiple synthetic steps and the use of toxic compounds. Direct synthesis of the quaternized brushes is therefore preferable. Osborne et al.[11] reported direct synthesis of PMETA brushes using SI-ATRP in a solution of water and methanol (MeOH/water). However, later, Li et al.[12] studied the ATRP of META in solution polymerization instead of surface polymerization and they showed that transesterification of the monomer with methanol produced significant amounts of methyl methacrylate when the polymerization was done in MeOH/water. Transesterification was avoided by using a solution of isopropanol and water (IPA/water) as the polymerization solvent. We therefore grew PMETA brushes using SI-ATRP and compared brush growth in three different solvents: 3:2 v/v IPA/water mixture, 4:1 v/v MeOH/water mixture (water originally carried in the purchased monomer solution is not counted), and pure water.

The ability to guide neuron growth, especially synapse formation, is crucial in the field of neuroelectronic circuits, cell-based biosensors, neurological implants, and pharmaceutical testing.[13] To generate surfaces for neuronal cell patterning, biomolecules that promote neurite growth, such as polylysine or laminin, are microstamped onto a surface.[14,15] Modification of the remaining surface to reduce nonspecific interaction can be challenging due to the instability of physically adsorbed films. Such problems can be resolved by using polymer brushes, which are covalently attached to substrates. Surfaces with patterned polymer brushes can be backfilled with other surface tethered monolayers or polymer chains to form well-defined hybrid surfaces with multiple components.[16]

In this report, photolithography was applied to pattern PMETA brushes and backfilled the patterned surface with a covalently grafted containing poly(ethylene glycol) [PEG] monolayer. Rat hippocampal neurons were grown on the hybrid surfaces and their survival and neurite outgrowth were examined.

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It was found that these neurons grew well and successfully extended neurites on PMETA brush-covered silicon substrates. By using well-defined hybrid surfaces containing both "cytophilic" PMETA brushes and "cytophobic" PEG[17] monolayers, it was possible to guide neuronal growth. Because the patterned hybrid surfaces are very stable and survive further chemical and photolithographic steps, they provide a versatile new tool for applications in cell-surface studies, cell-based biosensing, design of implants, and related uses.

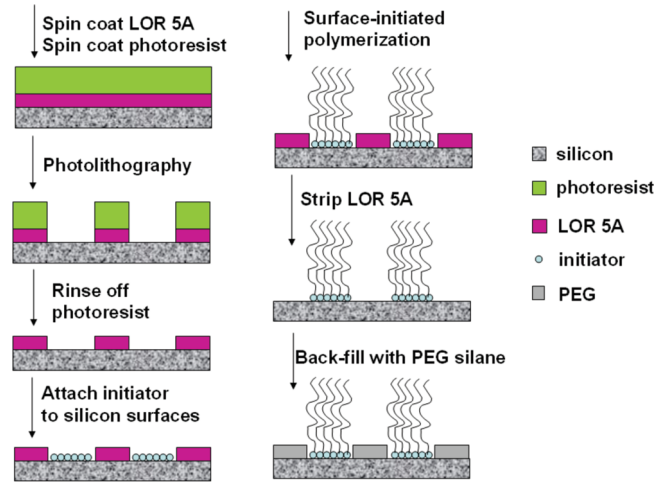
2. Experimental Section

2.1. Materials. Allyl 2-bromo-2-methylpropionate, chlorodimethylhydrosilane, Pt on activated carbon (10 wt %), triethylamine, CuBr, $CuBr_2$, 2,2′-bipyridine, 11-mercapto-1-undecanol, 2-bromo-2-methylpropionyl bromide, anhydrous pyridine, and a 75% w/v aqueous solution of [2-(methacryloyloxy)ethyl]trimethylammonium chloride were purchased from Sigma-Aldrich. 2-[Methoxy(polyethylenoxy)propyl]trichlorosilane (PEGylated silane, $CH_3O(CH_2CH_2O)_{6-9}(CH_2)_3SiCl_3$, 90%) was purchased from Gelest, U.S.A. All solvents used were purchased from Sigma-Aldrich. All the chemicals were used without further purification. Distilled deionized water and high-purity nitrogen gas (99.99%, Airgas) were used throughout. All the gold (~1000 Å) coated silicon wafers (4" diameter) with a titanium adhesion layer were purchased from Platypus Technologies, U.S.A. The wafers were cut into 1 × 2 cm pieces before their usage. Surface initiators for silica substrates and gold substrates were synthesized and immobilized to substrates as described.[18,19] Neurobasal Medium, GlutaMax supplement, pyruvate, antibiotics B27 supplement 0.2% Trypsin, and calcein were obtained from Invitrogen, U.S.A. Hibernate medium was obtained from Brainbits LLC, U.S.A. Poly-D-lysine and paraformaldehyde (37%) with 10% methanol (F-1268) were obtained from Sigma. Fetal bovine serum (FBS) was obtained from Invitrogen, U.S.A. Coverslips (Sargent) were obtained from Warner Instruments, U.S.A. The antagonists D-2-amino-5-phosphonopentanoic acid (D-AP5) and 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo quinoxaline-7-sulfonamide disodium salt (NBQX) were from Tocris, U.S.A. Anti-MAP2 antibody (#M9942) was obtained from Sigma. Secondary Cy3 labeled goat antimouse antibody (#115-165-146) and IgG-free BSA were obtained from Jackson Immunoresearch, U.S.A.

2.2. SI-ATRP of META. Silicon substrates covered with initiator were placed in a dry Schlenk flask. CuBr (28.7 mg, 0.2 mmol), $CuBr_2$ (4.5 mg, 0.02 mmol), and 2,2′-bipyridine (78.4 mg, 0.48 mmol) were added to another dry Schlenk flask tube equipped with a magnetic stir bar. Both flasks were evacuated and backfilled with nitrogen three times. META (2 mL aqueous solution), isopropanol (1.8 mL), and DI water (1.2 mL) were mixed together and bubbled by nitrogen gas for at least 30 min and then the contents were transferred into the Schlenk flask with copper catalysts using a cannula. The mixture was stirred at room temperature under nitrogen for about 10 min and transferred into the other Schlenk flask with silicon substrates using a cannula. Polymerization was carried out at room temperature for a desired period, after which the substrates were taken out of the solution, rinsed thoroughly with DI water and isopropanol, and blown dry by nitrogen gas. The same procedure was used when the polymerization solvent was a methanol/water mixture (1.6 mL methanol and 0.4 mL water) or pure water (1.5 mL). To use grazing-angle reflectance Fourier transform infrared spectroscopy (FTIR) to characterize the synthesized polymer brushes, PMETA brushes were also grown on gold surfaces using the same procedure as described above.

2.3. Characterization of Polymer Brushes by FTIR and Ellipsometry. Gold substrates coated with PMETA brushes were characterized using a VERTEX 80v vacuum FT-IR spectrometer from Bruker Optics equipped with a liquid nitrogen-cooled mercury cadmium telluride (MCT) detector. Before collecting data, the system was left in vacuum for 10 min to minimize signal noise from air. IR spectra were taken with 2 cm^{-1} resolution using 256 scans at 75° incident angle. Spectra from bare gold substrates were used to determine the background signal. OPUS viewer software from Bruker Optics was used to analyze FTIR spectra. Thickness of polymer brushes was measured using a Woollam variable angle spectroscopic ellipsometer. A two-layer model consisting of a 0.5 mm silicon layer and a Cauchy layer, to represent the brush layer, was used to determine both the thickness and refractive index of the brush.

Scheme 1. Generation of Polymer Brushes/PEG Patterned Surfaces Based on Photolithography



2.4. Patterning of Polymer Brushes by Photolithography. PMETA brushes were patterned on silicon surfaces by photolithography as shown in Scheme 1. Lift off resist (LOR) 5A was spin coated on fresh silicon wafers, baked at 180 °C for 10 min, and patterned by standard photolithography.[25] The patterned wafers were cleaned with a Harrick oxygen plasma cleaner (PDC-32G) for 1−2 min. Photoresist was rinsed off with acetone. The silicon wafers covered by patterned LOR 5A were cleaned by plasma cleaner again for 1−2 min. Surface initiator for silicon oxide substrates was attached to the exposed silicon oxide regions on the patterned wafer. SI-ATRP of META was carried out on such surfaces, after which LOR 5A was stripped off by an AZ 300 MIF (tetramethylammonium hydroxide solution) developer, which yielded patterned PMETA brushes. The substrates with patterned brushes were backfilled with PEG-silane by immersing the substrates in 1 mM PEG-silane solution overnight at room temperature under nitrogen protection. After being taken out of solution, the substrates were rinsed with ethanol thoroughly and blown dry. Rat hippocampus neurons were cultured on such PMETA brush/PEG patterned surfaces as described below.

2.5. Rat Hippocampus Neuron Culture. Rat hippocampal neurons were obtained from newborn rats (P0−P5). Hippocampi were dissected in cold Hibernate E-Low Fluorescence medium (HibE, Brainbits LLC) and transferred to a 15 mL tube containing cold (4 °C) HibE. The solution was then exchanged for a freshly prepared 1:1 (v/v) mixture of room temperature 0.25% (w/v) Trypsin in Hank's balanced salts solution (Invitrogen) and warm (37 °C) HibE. Hippocampi were digested for 15 min at 37 °C. Trypsin was quenched with three changes of Neurobasal medium supplemented with 10% FBS. The hippocampi were then triturated 10 times with a 5 mL serological pipet and undissociated tissue allowed to settle. The supernatant, which contained dissociated neurons, was then transferred to a new 15 mL tube for counting and dilution. Prior to plating, the patterned PMETA brush/PEG surfaces were sterilized in ethanol and transferred to Neurobasal medium supplemented with 10% FBS. Neurons were plated in Neurobasal Medium supplemented with 1% GlutaMax, 1 mM pyruvate, 1% penicillin/streptomycin, 2% B27 supplement, and 10% FBS After 12 h, the medium was changed to Neurobasal medium with the same supplements but no serum. Cells were maintained in a water-jacketed incubator maintained at 37 °C and 5% CO_2.

2.6. Microscopy of Living Hippocampal Neurons Labeled with Calcein. To visualize living cells on the substrates, calcein-AM ester (1 mg/mL in DMSO) was diluted 1:2000 in HibE medium or

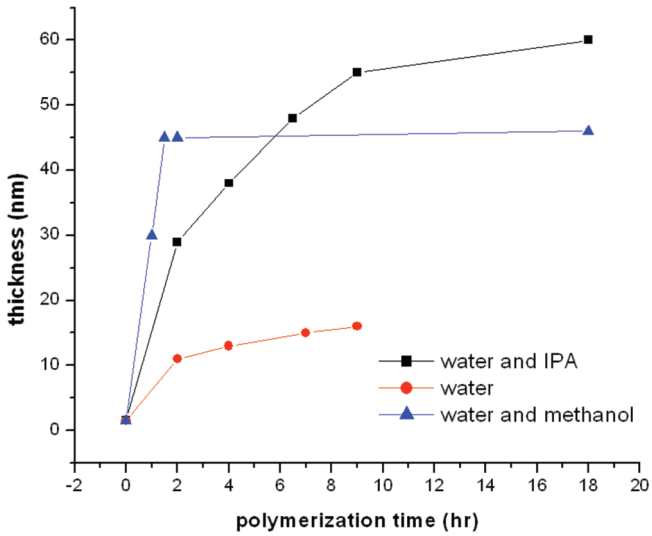


Figure 1. Thickness of PMETA brushes vs polymerization time in different solvents. Lines have been added to guide the eye.

Ringers solution for 10 min. The solution was then exchanged for new HibE medium or Ringers solution interchangeably. Ringers solution contained in millimolar concentration: NaCl, 119; KCl, 2.5; $CaCl_2$, 2; $MgCl_2$, 2; HEPES (pH 7.4), 25; glucose, 30. In both cases, the cell medium was supplemented with 50 μM AP-5 and 1 μM NBQX to block NMDA and AMPA/Kainate receptors, respectively.

2.7. Immunocytochemistry of Hippocampal Neurons. Cells were fixed with 3.7% formaldehyde and 1−1.5% methanol in phosphate buffered saline (PBS) solution for 15 min. Free aldehydes were quenched with 50 mM NH_4Cl in PBS for 5 min. The cells were then permeabilized in the presence of 0.1% Tween-20 in PBS (PBST) for 5 min and blocked with 0.1% Tween-20 in PBS supplemented with 3% IgG free bovine serum albumin (Jackson Immunoresearch; PBSTB) for 30 min. Primary antibodies were added for 1−3 h in PBSTB and rinsed 3 × 10 min in PBSTB. Secondary antibodies (Cy3 labeled antimouse, 1:500) were added for 1 h in PBSTB and the cells rinsed 3 × 10 min in PBS. Patterned substrates with cultured cells atop were stored in PBS before imaging.

2.8. Confocal Microscopy. Images of labeled neurons were obtained with a Leica TCS SP2 confocal microscope equipped for spectral detection. A 40× 0.8 NA long working distance water immersion lens was used to acquire Z-series of labeled neurons. All images were acquired at room temperature. To visualize polymer brushes and cell morphology, reflection images with a highly attenuated laser were acquired and enhanced using a shadow function in ImageJ.

2.9. Analysis of Neurites. Images were inspected using ImageJ[20] (v 1.41o) Z-projections of confocal stacks were obtained by summing intensity values and the summed stacks used for subsequent analysis. Neurite lengths were measured interactively with the NeuronJ plugin.[21] Statistical analysis was conducted using Igor (v. 6.05, Wavemetrics, Lake Oswego, OR).

3. Results and Discussion

3.1. Synthesis of PMETA Brushes. Direct synthesis of PMETA brushes was first reported by Osborne et al.[11] They showed that PMETA brushes can be grown in a controlled manner on gold surfaces using SI-ATRP of META in methanol/water mixture at room temperature. However, it was also shown by Li et al.[12] that transesterification of META occurred during polymerization, which produced significant amounts of methyl methacrylate in the final polymer. Unexpected transesterification of tertiary amine methacrylates during methanolic ATRP has been reported before.[22] Li et al.[12] also showed that when methanol was replaced by IPA, such transesterification was avoided because the secondary alcohol was less prone to transesterification. In the present work, direct synthesis of PMETA brushes on silicon surfaces was carried out in IPA/water mixture to avoid transesterification (Scheme 2). The brush growth was also compared in three different solvents: IPA/water mixture, methanol/water mixture, and pure water (Figure 1). The goal of these studies was brush growth, but despite the limited study of reaction conditions, it is worth commenting on the differences in the rate and extent of brush formation.

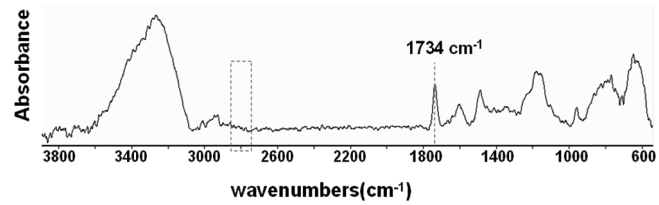


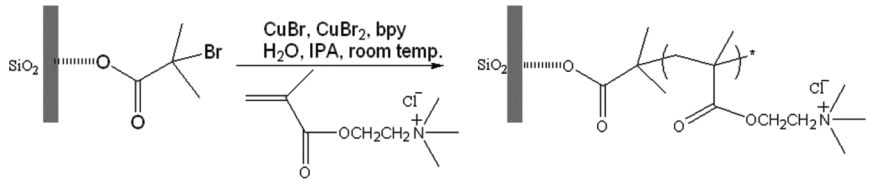
Figure 2. Grazing angle reflectance FTIR spectra of PMETA brushes synthesized directly from META. Absence of absorption bands at ~2800 cm^{-1} shows that amine groups in directly synthesized PMETA brushes are quaternized.

The growth of PMETA brushes in water stopped after a short time of polymerization, and resulted in a small final thickness (~15 nm) of the brushes. In an IPA/water mixture, PMETA brushes grew in a controllable manner for a longer time (~10 h), and the largest final brush thickness (~60 nm) was achieved. However, PMETA brushes grew faster in the early stage in methanol/water mixture than in an IPA/water mixture. Such polymerization reactivity is quite different from the behavior in solution reported by Li et al.[12] In solution, polymerization of META in an IPA/water mixture was faster than in a methanol/water mixture. Such differences imply that polymerization kinetics in solution and on a surface can be quite different. The brush thickness observed in a methanol/water mixture is inconsistent with what Osborne et al.[11] reported. The difference could be due to differences in either initiator efficiency or initiator density on gold surfaces and silica surfaces.

An FTIR spectrum of PMETA brushes is shown in Figure 2. The absence of the absorption bands at ~2800 cm^{-1}, which are attributed to symmetric stretching vibrations of -CH_3 of the tertiary amine groups, indicated that the amine groups were quaternized in PMETA brushes synthesized directly from META. The absorption peak at 1734 cm^{-1} is a characteristic peak corresponding to the C═O stretching vibration.

3.2. PMETA Brush-Modified Surfaces for Neuron Culture. It is well-known that neuronal cells grow well on surfaces covered with positively charged molecules.[23,24] Polylysine and

Scheme 2. Synthesis of Cationic Polymer Brushes by SI-ATRP of [2-(Methacryloyoxy)ethyl]trimethylammonium Chloride (META)



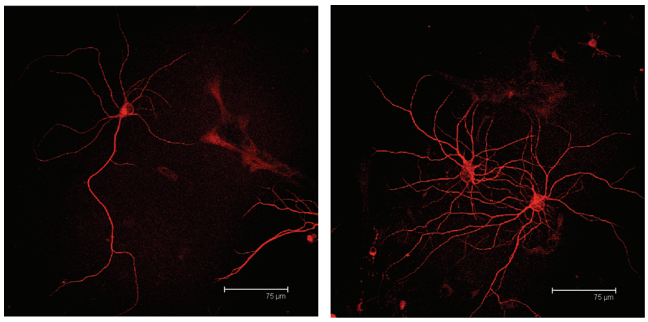


Figure 3. Confocal fluorescence image of rat hippocampal neurons cultured on PMETA brushes modified silicon substrates for ~20 days. Neurons were labeled by primary antibody raised against MAP2 and Cy3 labeled secondary antibody sequentially.

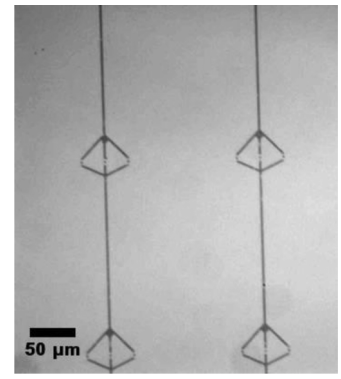


Figure 4. Optical image of a patterned PMETA brush/PEG surface. The black lines are PMETA brushes and the rest of the surface is covered with a PEG monolayer.

laminin are the most commonly used compounds to promote neuron growth.[14,15] Surfaces covered by such molecules are, however, not stable over time and are thus usually freshly prepared before neuron culture. Rühe et al.[7] have shown that polymer brush modified surfaces are more stable than polylysine covered surfaces during undirected culturing of cerebellar cells. Polymer brushes provide robust surface modification with desired functional groups for different applications. Quaternized PDMAEMA brushes have been shown to possess antibacterial activity.[5] Thus, quaternized PMETA brushes are able to provide stable modified surfaces free of antibacterial contamination over time, suitable for neuron culture substrates. Rat hippocampal neurons were cultured on PMETA brush modified silicon substrates and labeled for the neuronal marker microtubule associated protein 2 (MAP2), Figure 3). MAP2 is localized to the cell bodies and dendrites, which are clearly evident in the immunofluorescence image (Figure 3).

3.3. Creating Patterned Hybrid PMETA/PEG Surfaces. The ability to control and direct neurite outgrowth is critical for neurological implants and neuroelectronic circuits. It can also be very useful for design of cell-based biosensors and fundamental studies of neuronal signaling. These studies require precise fabrication of hybrid patterned surfaces containing molecules that direct cell adhesion to precise locations and promote process outgrowth in desired directions. As mentioned before, chemical modification and lithographic processes can be performed on patterned polymer brush surfaces because of their high stability. The flexibility and robustness of the process can be very useful in generating multicomponent hybrid patterned surfaces for various applications and are among the most promising features of the approach described here. To direct neuronal growth, we created a hybrid surface consisting of "cytophilic" PMETA brushes and "cytophobic" PEG monolayer.

As shown in Scheme 1, polymer brushes were patterned using a photolithographic procedure, which is modified from a previously reported approach.[25] Due to the good solubility of standard photoresists in organic solvents, immobilizing surface initiator onto surfaces with patterned photoresists does not yield a well-defined patterned initiator layer. As a result, most reported procedures for polymer brush patterning to date have been based on microcontact printing.[14,24] Anderson et al.[25] showed that LOR 5A, a lift-off resist, is compatible with the chemical conditions required for molecular self-assembly. Here, we used a modification of the procedure reported by Anderson et al. to pattern PMETA brushes. Briefly, conventional photolithography was done on a silicon wafer precoated with a layer of LOR 5A. The pattern generated on the photoresist layer was transferred into the LOR 5A layer. Because LOR 5A is stable in organic solvents, it prevents modification of the underlying silica regions. Surface-initiated polymerization took place in the exposed regions. After polymerization, LOR 5A was removed using a base developer. This process provides a standard method to pattern polymer brushes on both silicon and gold surfaces. The unmodified silica regions were backfilled with a PEG layer. To avoid contamination or possible structural changes during the patterning process, a PEG monolayer was deposited onto the patterned surface at the end of the whole process. An optical image of the patterned hybrid surface is shown in Figure 4. Large 10 × 10 μm squares were designed for neuron cell bodies to settle. Thin lines (2.5 μm wide) were designed for axon and dendrite processes. Such patterned hybrid surfaces were used to guide neurite outgrowth.

3.4. Neurite Outgrowth on Patterned PMETA Brush/PEG Surfaces. Although much effort has been devoted to the creation of in vitro neuronal networks with geometrically defined features, most published procedures continue to rely on physically adsorbed polylysine or laminin, which is not stable over time. The polymer brushes are covalently attached to the substrate and are very stable in further chemical or photolithography processes. Hippocampal neurons became localized to the brush patterns and, growth of neurites along the pattern line was clearly evident (Figure 5A,B).

Although the PEG between patterned polymer brush surfaces was designed to minimize cell attachment and neurite outgrowth, we found that some cells did attach to this surface and extend neurites. To assess the utility of the brush surface, we compared the lengths of neurite labeled with Cy3/anti-Map-2 antibody on the patterned brush to that on the intervening PEG areas of patterned chips. Cumulative histograms (Figure 5C) of neurite length indicate that neurites on the PMETA pattern lines (Figure 5C, blue line) were markedly longer than those extending on the cytophobic PEG surface (Figure 5C, green line; brush median length 39 μm, mean 60 μm; PEG median length = 20 μm, mean = 24 μm; $H = 48$, $p < 0.0001$, Kruskal−Wallis test). For comparison, also, the neurite length distribution of cells grown on a conventional polylysine surface (Figure 5C black line) is shown (median length = 57 μm, mean = 81 μm). On unpatterned PMETA surfaces neurite length was intermediate (Figure 5C, red line). This suggests that the constrained geometry of the pattern promotes neurite outgrowth, perhaps by limiting the metabolic cost of outgrowth in the nonpreferred (pegylated) directions.

Overall, neurons grew not quite as well on PMETA as on polylysine, their growth on these novel antibacterial surfaces is still quite good. Nevertheless, there is room for further

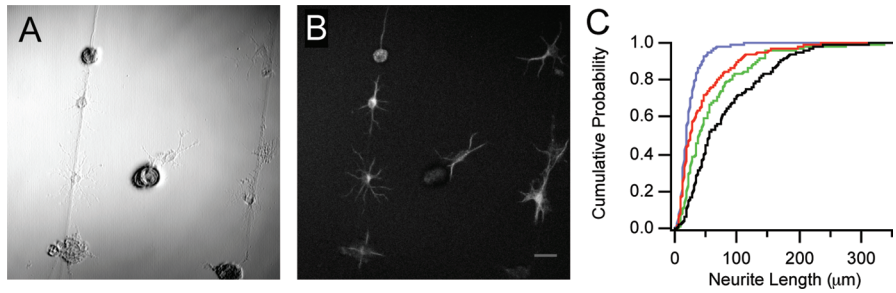


Figure 5. Reflectance (A) and fluorescence (B) images of hippocampal neurons acquired by confocal microscopy. The neurons were cultured on a PMETA brush/PEG hybrid patterned surface for ~10 days and labeled with Cy3/anti-Map-2 antibody. (C) Cumulative histograms of length of neurite labeled with Cy3/anti-Map-2 antibody on patterned lines (green), on PEG-surface between PMETA patterns (blue), on unpatterned PMETA surface (red), and unpatterned poly lysine (black). The scale bar is 50 μm.

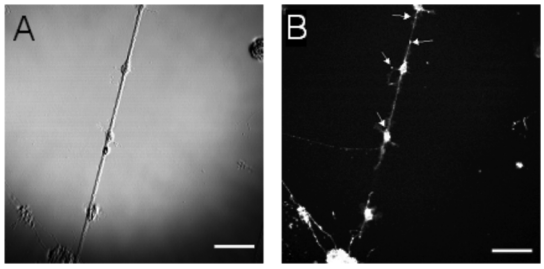


Figure 6. Reflectance (A) and fluorescence (B) images of hippocampal neurons acquired by confocal microscopy. The neurons were cultured on a PMETA brush/PEG hybrid patterned surface for ~10 days and labeled with anti-synaptobrevin antibody. The scale bar is 50 μm.

optimization in the future. The successful growth of neurons on the PMETA surfaces naturally raises the question of how the cells adhere to the brushes. The mechanism of cellular attachment is not yet known. One unusual feature of neurons in culture is that they express proteins that are post-translationally modified with high levels of polyanions (e.g., polysialic acid and chondroitin sulfate). Electrostatic interactions between the positively charged brushes and negatively charged post-translational modifications may thus play a role. It is also possible that the quaternary amines present on the brushes may interact with ion channel pores on the neuronal surfaces and in this way act as noncanonical trophic factors. Similar soluble quaternary compounds are, for example, employed to convert ion channels into a photactivatable form for functional studies. A third possibility is that the brushes and cells may bind through an intermediary layer. During the initial plating of the neurons, the brushes are incubated in serum-containing medium; unfortunately, without the trophic factor support provided by the serum proteins, hippocampal neuron survival upon plating on any surface is low. During this period, serum proteins may adsorb to the brushes and provide suitable landing spots for neurons. It is worth noting in this regard that both laminin, a cell attachment protein often used to promote axon growth, and thrombospondin, a synapse-promoting molecule, are present in serum.

To examine whether neurons that grew on PMETA were healthy enough to support axon outgrowth and synapse formation, immunofluorescence labeling of neurons for the synaptic vesicle protein synaptobrevin was performed (Figure 6). These experiments showed that there is directed growth of axons along the PMETA brush patterns and puncta (arrows), indicating synaptic varicosities are clearly evident. Future applications may extend to spotting proteins to promote synapse formation at the predesignated location in the neuronal network. Neuronal networks with precise control of synapses should be extremely useful for hybrid neuronal-electronic circuits and investigating neuronal signaling.

Conclusions

PMETA brushes were directly synthesized by SI-ATRP of META in protic media. Brush growth in IPA/water mixture enabled growth of PMETA brushes in a controllable manner for ~8 h, resulting in the largest final brush thickness (~ 60 nm) among three solvents. PMETA brush modified surfaces were shown to be effective neuron culture substrates. Rat hippocampal neurons showed long-term viability, extended processes, and formed synapses on PMETA brushes coated silicon substrates. With previously reported antibacterial activity and the ability to provide good neuronal cell viability demonstrated here, quaternized PMETA polymer brushes are excellent candidates for biointerface materials in bioimplanting, biosensing, and the like.

Hybrid surfaces with both "cytophilic" PMETA brushes and "cytophobic" PEG monolayers were patterned by a procedure based on photolithography. Such hybrid surfaces showed excellent spatial control of rat hippocampal neuron outgrowth by restricting cell growth to the PEG-free areas. We see, however, the significance of the PMETA brush approach in its additional capabilities. The PMETA brush is antibacterial, while it does allow neuronal growth. In addition, the PMETA brush enables surface functionalization using a much more precise and high resolution patterning process than possible using microcontact printing or other methods.

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