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Application of Polyelectrolyte Multilayers for Photolithographic Patterning of Diverse Mammalian Cell Types in Serum Free Medium

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Abstract

Integration of living cells with novel microdevices requires the development of innovative technologies for manipulating cells. Chemical surface patterning has been proven as an effective method to control the attachment and growth of diverse cell populations. Patterning polyelectrolyte multilayers through the combination of layer-by-layer self-assembly technique and photolithography offers a simple, versatile and silicon compatible approach that overcomes chemical surface patterning limitations, such as short-term stability and low protein adsorption resistance.

In this study, direct photolithographic patterning of PAA/PAAm and PAA/PAH polyelectrolyte multilayers was developed to pattern mammalian neuronal, skeletal and cardiac muscle cells. For all studied cell types, PAA/PAAm multilayers behaved as a negative surface, completely preventing cell attachment. In contrast, PAA/PAH multilayers have shown a cell-selective behavior, promoting the attachment and growth of neuronal cells (embryonic rat hippocampal and NG108-15 cells) to a greater extent, while providing a little attachment for neonatal rat cardiac and skeletal muscle cells (C2C12 cell line). PAA/PAAm multilayer cellular patterns have also shown a remarkable protein adsorption resistance. Protein adsorption protocols commonly used for surface treatment in cell culture did not compromise the cell attachment inhibiting feature of the PAA/PAAm multilayer patterns. The combination of polyelectrolyte multilayer patterns with different adsorbed proteins could expand the applicability of this technology to cell types that require specific proteins either on the surface or in the medium for attachment or differentiation, and could not be patterned using the traditional methods.

Keywords

cell patterning; polyelectrolyte multilayers; layer-by-layer; photolithography; protein adsorption; cell culture

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1. Introduction

Manipulation of mammalian cells has attracted a lot of attention due to its potential application in tissue engineering, biosensors and drug screening devices. Numerous methods, including patterning through surface modifications (1), have been developed to generate proper position and interaction of cells. Various approaches, such as UV lithography (2), laser ablation (3), soft lithography (4,5) and laminar flow patterning in microfluidic channels (5), and materials, such as photoresists (2), polylysine (6), alkanethiolates (4,7), elastomeric PDMS membrane (8), phospholipid bilayers (9), PEO terminated triblock copolymer (10), hyperbranched poly (acrylic) acid films (11), grafted polyethylene oxide (12), polyethylene glycol hydrogels (13), polyelectrolyte multilayers (14), interpenetrating network of polyacrylamide and polyethylene glycol (15), polyglycolic acid (16), functionalized poly-p-xylylenes (17), hyaluronic acid (18) etc., have been successfully used for patterning as well as cell attachment supporting or inhibiting surfaces. One obstacle, which limits the application of these novel technologies in actual devices, is the relatively short lifetime of the created cellular patterns (19). In many cases the patterns are destroyed within a few days after plating, as cells start to grow in the cell resistant areas. Possible causes for this short-term stability of the chemical surface patterns are 1) degradation of the coating material through oxidation or other mechanisms (20) and 2) a slow build up of an adsorbed protein layer, originating from the culture medium (serum) or secreted by the cells themselves, on the top of the surface patterns (21–23).

In earlier experiments, the interpenetrated networks of poly(acrylic acid) (PAA) poly(ethylene glycol) (PEG) and polyelectrolyte multilayers have shown encouraging results in terms of high pattern stability (24–26). In contrast to chemical surface patterns, cell adhesion resistance of polyelectrolyte multilayers is not based on the hydrophobicity of the surface, but on the molecular architecture and physical properties of the film (25). Therefore, they are more resistant to the modifying effect of adsorbed proteins. Moreover, polyelectrolyte multilayers are highly stable and their deposition is a simple process, very similar to biological systems with nanoscale control over thickness, compositions and molecular structure (14).

Polyelectrolyte multilayers can be either cell attachment resistive or promoting depending upon their properties and the cell type (25–30), this makes them promising candidates for patterning diverse cell populations. Although the patterning of several cell types such as NR6 fibroblast (20), neuron (31–33), primary hepatocytes (34), chondrosarcoma cells (35), microvascular endothelial cells (36) and smooth muscle cells (37) has already been demonstrated using polyelectrolyte surfaces, a comparative study with more than two cell types has not been done. Moreover, the combination of polyelectrolyte multilayers with different adsorbed proteins as well as the investigation into the protein resistance limits of the patterns could expand the applicability of this technology to cell types which require specific proteins for attachment or differentiation either on the surface or in the medium, and could not be patterned using the traditional methods.

2. Methods

2.1 Materials

2.1.1 Coatings—Poly(acrylic acid) (PAA) (MW 90,000, 25 wt% solution) was purchased from Polysciences. Poly(acryl amide) (PAAm) (MW 10,000, 50 wt% solution) and Poly(allyl amine hydrochloride) (MW 70,000) were purchased from Sigma Aldrich. Trimethoxysilylpropyldiethylenetriamine (DETA) was obtained from United chemical Technologies Inc.

2.1.2 Cardiac myocyte culture—Calcium and magnesium free Hank's balanced salt solution (HBSS), Trypsin, Trypsin inhibitor, Collagenase and Leibovitz medium (dissolved in

cell culture grade water and filtered through a 0.2 μ m filter) were obtained from Worthington Biochemical Corporation together in their neonatal cardiomyocyte isolation system. Ultraculture (general purpose medium) was obtained from Bio Whittaker Cambrex. Dulbecco's modified eagle medium (DMEM) (containing high glucose 1X, 4.5 g/L D-glucose, L-glutamine, 110/mg/L sodium pyruvate), L- glutamine (200 mM, 100X), Penicillin (10,000 units/mL), Streptomycin (10,000 μ g/mL), B-27, non essential amino acids (MEM NEAA) (100X), HEPES buffer (1M) and Fetal Bovine serum were obtained from Gibco/Invitrogen. Dextrose was obtained from Fisher Scientific. Growth factors L-thyroxine and Epidermal growth factor (EGF) were purchased from Sigma and Hydrocortisone was purchased from BD.

2.1.3 Hippocampal cell culture—Hibernate E medium was purchased from BrainBits (Springfield, IL). Neurobasal E medium, B27, Glutamax and Antibiotic/Antimycotic supplement were obtained from Invitrogen.

2.1.4 NG cells and C2C12 cell culture—DMEM by HyQ (containing 4 mM L-Glutamine, 4500 mg/mL glucose and sodium pyruvate), Fetal Bovine Serum, HAT (100X) and B-27 supplement were obtained from Gibco.

2.1.5 Patch clamp electrophysiology—All chemicals were purchased from Sigma Aldrich. Borosilicate glasses (BF150-86-10) were obtained from Sutter (Novato, CA).

2.2 Multilayer deposition on coverslips

DETA coverslips were prepared as described by Das et al. (38) by cleaning glass coverslips (VWR, 22 $\times$ 22 mm²) with O₂ plasma cleaner (Harrick, Ithaca, NY) for 30 minutes at 100 mTorr. DETA was deposited on clean coverslips by dipping them in 0.1% (v/v) mixture of DETA in freshly distilled toluene. The DETA coverslips were heated to just below the boiling point of toluene for 30 minutes, rinsed with toluene and again heated to just below the boiling point of toluene. The coverslips were then dried in an oven overnight. The DETA coverslips were coated with the polyelectrolyte multilayers on an automatic dipping machine (StratoSequence Slide Stainer). 0.01 M solutions of PAA and PAAM were prepared in deionized (DI) water by taking the molecular weight of the repeat unit of each polymer into consideration; in addition, their pH was adjusted to 3.0 using a 1 M HCl aqueous solution. First, the coverslips were immersed in the PAA solution for fifteen minutes and then rinsed with pH 3.0 water three times in separate beakers for 2 minutes, 1 minute and 1 minute, respectively. The coverslips were then immersed in PAAM solution for fifteen minutes and then rinsed with pH 3.0 water three times as described above. This cycle was repeated 20 times to deposit 20 bilayers of PAA and PAAM. The coverslips were then kept in an oven at 140°C for eight hours for cross linking.

Similarly, PAA/PAH multilayers were deposited by using 0.01 M solutions of PAA and PAH at a pH of 3.5 and 8.5, respectively. The coverslips were immersed in the PAA solution first for fifteen minutes and then rinsed with DI water three times in separate beakers for 2 minutes, 1 minute and 1 minute, respectively. The coverslips were then immersed in the PAH solution for fifteen minutes and then rinsed with DI water three times as described above. This cycle was repeated 20 times to deposit 20 bilayers of PAA and PAH.

2.3 Patterning of the coverslips

The coverslips were patterned using a deep UV (193 nm) excimer laser (LambdaPhysik) at a pulse power of 230 mW and a frequency of 10 Hz for 2 minutes through a quartz photomask (Bandwith Foundry, Eveleigh, Australia). Patterns were visualized by phase contrast microscopy or with an epifluorescent microscope after fluorescent tagging, namely the PAA/PAAM patterned coverslips were dipped in fluorescently tagged PAH at a pH of 8.5.

2.4 X-ray photoelectron spectroscopy

The bare glass, PAA/PAAm, PAA/PAH coatings, Laser ablated PAA/PAAm, PAA/PAH coatings and protein incubated coverslips were examined by X-ray photoelectron spectroscopy (XPS) using a Kratos (Manchester, UK) Axis 165 equipment according to established protocols (38,39). XPS survey scans as well as high resolution C 1s, N 1s, O 1s and Si 2p were obtained using monochromatic Al $K\alpha$ excitation.

2.5 Contact angle measurement

Contact angle measurements were performed according to published protocols (38,39). Briefly, contact angle of a static, sessile drop (5 μ L) of deionized water was measured using a CAM 200 digital goniometer (KSV Instruments, Ltd.). Three measurements were taken and averaged.

2.6 Plating cells on the patterns

2.6.1 Cardiac myocytes—Two-day-old rat pups were euthanized with Halothane. Hearts were dissected and minced in ice cold HBSS. Cardiac myocytes were dissociated by incubation in trypsin (1000 μ g in 10 ml HBSS) for 20 h at 2–8°C followed by collagenase (1500 units in 5 ml L15 medium) treatment for 45 min at 37°C and mechanical trituration. The cell solution was then centrifuged at 50g for 5 minutes at 25°C. The cells were resuspended in DMEM medium supplemented with 10% fetal bovine serum and 1% penicillin streptomycin and preplated in petri dishes and kept in an incubator at 37°C and 5% CO₂ for 45 minutes. The preplating step was carried out to separate fibroblasts from myocytes. The supernatant from the Petri dishes were centrifuged at 50g for 5 minutes at 25°C. The cells were resuspended in the plating medium consisting of: 100 ml Ultraculture medium supplemented with 10 mL B-27, 1 mL L-glutamine, 1 mL Penicillin Streptomycin, 0.375 g dextrose in 800 μ L water, 1 mL non essential amino acids and 1 mL HEPES buffer. Growth factors were added in following concentrations adapted from Mohamed et-al (40) to the medium, L-thyroxine 0.1 μ g/mL, EGF 10 ng/mL and Hydrocortisone 0.5 μ g/mL. Cells were plated at a density of 10⁵ cells/cm² on the coverslips. The medium was changed after 24 hours of plating. Subsequent changing of the medium was carried out every fourth day.

2.6.2 Hippocampal cells—Embryonic rat hippocampal cells were cultured according to established protocols (41). Briefly, the hippocampus was dissected from E18 rat embryos in ice-cold Hibernate E medium. Tissue was minced and mechanically dissociated using a 1 ml pipette. Cells were centrifuged at 300g, 4°C for 2 min and resuspended in the plating medium consisting of Neurobasal E medium supplemented with B27, Glutamax and Antibiotic/Antimycotic. Cells were plated at a density of 100 cells/mm². Cultures were maintained in an incubator at 5% CO₂ and 37°C.

2.6.3 NG108-15 cells—About 1 million frozen NG 108-15 cells were thawed and centrifuged at 300g for 5 minutes in DMEM medium containing 2% HAT and 10% FBS. The cells were resuspended in the same medium and plated in a 75 cm² culture flasks for proliferation. Upon confluency the cells were plated on the PAA/PAH patterned coverslips at a density of 100 cells/mm² in the serum-free differentiating medium consisting of DMEM and 2% B-27.

2.6.4 C2C12 cells—C2C12 cells were cultured and plated according to the same protocol as the NG108-15 cells (42) on PAA/PAAm patterned coverslips. The coverslips were incubated with different proteins before plating the C2C12 cells. The plating density was 300 cells/mm².

2.7 Statistical analysis of the cell resistance of the coatings

The cell resistance and cell adhering properties of the coatings were evaluated statistically by plating all the above cell types on PAA/PAH, PAA/PAAm, and Glass. The cells were counted in each frame at a magnification of 10X and the count was averaged over 10 frames for two coverslips of each type. The counting was done on days 3 and day 6 of the culture.

2.8 Patch clamp electrophysiology

Patch clamp experiments on hippocampal and cardiac cells were performed according to published protocols (38,39). In brief, whole-cell patch clamp recordings were performed in a recording chamber on the stage of a Zeiss Axioscope 2FS Plus upright microscope at room temperature, in the culture medium, where the pH was adjusted to 7.3 with HEPES. Patch pipettes were prepared from borosilicate glass with a Sutter P97 pipette puller and filled with intracellular solution (in mM: K-gluconate 140, EGTA 1, MgCl₂ 2, Na₂ATP 2, Hepes 10; pH = 7.2). The resistance of the electrodes was 6–8 MΩ. Voltage clamp and current clamp experiments were performed with a Multiclamp 700A amplifier (Axon, Union City, CA). Signals were filtered at 3 kHz and digitized at 20 kHz with an Axon Digidata 1322A interface. Data recording and analysis were performed with pClamp 10 software (Axon). Action potentials were evoked with 1s depolarizing current injections from a –70 mV holding potential.

3. Results

3.1 Polyelectrolyte deposition, patterning and visualization

For the initialization of the first layer of the polyelectrolyte, we used trimethoxysilylpropyldiethylenetriamine (DETA) covalently – modified glass coverslips as the substrates. DETA coverslips have a strong inherent positive charge on the surface, which makes the uniform deposition of the multilayers easier in comparison to deposition onto clean glass substrates.

Formation of polyelectrolyte multilayers were verified by contact angle measurements, X-ray photoelectron spectroscopy (XPS) and visual inspection. Static contact angle values for PAA/PAAm and PAA/PAH multilayers were 101.4 ± 5.2 and 67.1 ± 3.3 , respectively. The appearance of large carbon and nitrogen peaks in the XPS spectra verified the formation of thick, uniform polyelectrolyte multilayers.

The ablation time for patterning the polyelectrolyte multilayers was set to remove all measurable traces of the film, which was proven by XPS measurements on the coated and ablated coverslips. The XPS survey spectra obtained on the glass substrate, after the deposition of PAA/PAAm multilayers and after ablation of the film, are shown in Figure 1. The carbon and nitrogen peaks, characteristics of the PAA/PAAm film, were observed on the PAA/PAAm coated glass substrates, but not on the bare glass substrates or the PAA/PAAm coated glass substrates followed by laser ablation. Similarly, large carbon and nitrogen peaks were observed on PAA/PAH modified coverslips, whereas after ablation, there were only traces of carbon present and nitrogen was totally absent.

After selective ablation of the PAA/PAAm multilayers through a photomask (patterning), the border between the ablated and non-ablated regions were clearly visible through a standard phase-contrast microscope (Figure 2A). For a more reliable visualization of the electrically charged multilayers, fluorescently- tagged PAH was used to bind with the multilayers and make the films fluorescent (Figure 2B).

According to Yang et al (43) the stability of the polyelectrolyte multilayers was greatly enhanced by crosslinking when exposed to 140°C for 8 hours. In their experiments according to the Fourier Transform Infrared Spectroscopy (FTIR) measurements, the thermal crosslinking resulted in the formation of imide bonds between PAA and PAAm, making the multilayer insoluble in water at a higher pH.

3.2 Cell attachment and growth on polyelectrolyte multilayers and patterns

This study investigated the applicability of polyelectrolyte multilayers for the patterning and manipulation of different mammalian cell types. Cell patterning usually requires two types of surfaces; one promotes cell attachment and growth, while the other prevents cell attachment and growth. In our experiments, two types of polyelectrolyte multilayers, PAA/PAAm and PAA/PAH were used as the patterned substrates, and four cell types, embryonic rat hippocampal cells, neonatal rat cardiac cells, the skeletal muscle C2C12 cell line and the neuroblastoma/glioma NG108-15 neuronal cell line, were studied. In all cases, the cells were cultured in serum-free medium to prevent the cover up of the surface patterns by proteins generally adsorbed from the serum containing medium.

For all studied cell types, the PAA/PAAm multilayer behaved as a negative surface, completely preventing cell attachment and growth. In contrast, the PAA/PAH multilayer showed a cell-selective behavior by promoting the attachment and growth of neuronal cells (embryonic rat hippocampal and NG108-15 cells) to a greater extent and, to some extent, skeletal muscle cells and neonatal rat cardiac cells. The statistical data on the attachment of the various cell types on the different surfaces is provided in Table 1.

Unfortunately, the technical difficulties associated with depositing and patterning more than one type of polyelectrolyte multilayers on the same coverslips prevented us from using this method to enhance the contrast between cell growth enabling and resisting areas. For this a clean glass was used as the alternative surface for cell patterning. Glass was utilized as a positive surface for cardiac myocytes and C2C12 skeletal muscle cells with PAA/PAAm negative background; however, it was used as the negative surface for neurons with PAA/PAH as the positive surface. The patterns of the cells on PAA/PAAm and PAA/PAH multilayers are shown in Figure 3.

However, glass is not an ideal negative surface for promoting physiological development of certain cell types. For example, as reported earlier (42), C2C12 skeletal muscle cells do not form myotubes in serum-free medium without contact signaling that originated from the growth surface. Based on the fact that the attachment of cardiac myocytes is significantly better with fibronectin or serum on the surface, we have taken advantage of the remarkable protein adsorption resistive feature of polyelectrolyte-based cellular patterns and used protein modified patterns to promote cell growth.

3.3 Cell growth on protein – modified polyelectrolyte patterns

In order to assess the protein adsorption resistance of polyelectrolyte based cellular patterns, we incubated the polyelectrolyte patterns in protein containing solutions for different durations of time before cell plating. As noted in Figure 4, cardiac myocytes were complying with the patterns even after 1 h incubation in 0.2 g/L human plasma fibronectin solution. XPS data (Figure 1) show that a thick layer of fibronectin was adsorbed to the ablated portion of the PAA/PAAm coverslips during this time. This amount of protein adsorption on the PAA/PAAm multilayer did not change its cell-attachment and growth resistive properties. In the case of the C2C12 cells, protein absorbed on the glass significantly improved the cell growth and differentiation promoting properties of this surface, enabling the formation of C2C12 myotubes. In the experiments presented in Figure 4, the patterned PAA/PAAm coverslips were

incubated with a 10% serum containing medium (NG proliferation medium) for different time periods. The patterns were observed on the second day of plating. The pictures show that the pattern was formed without the protein incubation, but the formation of myotubes took place only after incubation with the serum containing medium.

3.4 Physiology of patterned cells

Visual inspection revealed no obvious morphological difference between the cells grown on the polyelectrolyte patterns and the cells grown on traditional control surfaces (PDL and DETA for neurons, fibronectin for cardiac myocytes). In order to evaluate the physiological properties of the excitable cells, whole-cell patch clamp recordings of spontaneous or evoked action potentials were performed in cardiac and hippocampal cells (Figure 5). Action potential generation is a complex process and distinctive to the type and maturation state of the cells. Most of the recorded hippocampal cells fired repetitive action potentials upon prolonged depolarization, which is characteristic of mature pyramidal cells in culture. Cardiac cells fired spontaneous, short action potentials, which is characteristic of mature postnatal rat cardiac cells (38).

Hippocampal cells were grown on PAA/PAH surfaces as a positive with clean glass as the background negative surface. Cardiac cells were cultured on fibronectin-treated clean glass as the positive and PAA-PAAm as the negative surface. Conventional whole-cell patch clamp recordings were performed on both cell types in current clamp mode. For hippocampal cells repetitive firing were evoked by 1s current injection. Cardiac cells were spontaneously active, thus, no current was injected. Both cell types showed normal characteristic electrophysiological behavior on the patterns

3.5 Long term stability of the pattern

The advantage of using the polyelectrolyte multilayers over other materials for patterning cells is the long term stability of the patterns. In our studies, polyelectrolyte-based cellular patterns were much more stable than the self-assembled monolayer-based patterns reported earlier (42,44). Figure 6 illustrates high fidelity cardiac myocyte patterns after 100 days in culture, which was not achievable with our earlier patterning methods. Also the myocytes were beating for atleast hundred days on the pattern. Moreover, our hippocampal patterns were stable for up to twenty days.

4. Discussion

In this study PAA/PAAm and PAA/PAH polyelectrolyte multilayers were patterned by laser ablation through a photomask in order to create cell attachment resistive and promoting areas on glass coverslips. The patterns were visualized by simple phase contrast microscopy or fluorescence contrast after the fluorescent tagging. PAA/PAAm multilayers prevented the attachment of all studied cell types. PAA/PAH was cell attachment promoting for embryonic rat hippocampal and NG108-15 cells to a greater extent and somewhat for neonatal rat cardiac myocytes and C2C12 skeletal muscle cells. Cellular patterns on the polyelectrolytes were exceptionally stable; cardiac myocytes did not overgrow the patterns and were beating for at least 100 days. Cellular patterns created with PAA/PAAm multilayers as the negative surface showed remarkable protein resistance, they tolerated standard, cell culture surface treatment protein adsorption protocols.

The Layer-by-Layer deposition of the polyelectrolyte multilayers was a simple, reliable process, did not require complex chemical procedures. In comparison with the commonly used covalent surface modification methods, it was simpler, less variable, robust and stable after crosslinking in cell culture conditions. Another advantage of the polyelectrolyte multilayers

was the improved visualization; surface patterns were visible under a normal phase contrast microscope. In specific applications, such as time-lapse imaging or repetitive multi-layer patterning, visualization of the patterns has been a challenging requirement.

Cell attachment inhibiting or promoting features of polyelectrolyte multilayers was determined by the molecular architecture and the physical properties of the layers. Therefore, the tunable and flexible properties of polyelectrolyte multilayers can be used to selectively pattern different cell types. Polyelectrolyte patterns combined with a simple and widely used protein adsorption surface treatment, which does not compromise the cell resistance of the background, could significantly enhance cell selectivity, as well as cell attachment promoting and physiological effects of the foreground. These unique properties could lead to various applications in many cell culture laboratories.

The origin of the cell resistance of the PAA/PAAm multilayers was based on the physical properties of the layers not the chemical properties. The high degree of swelling (about 3.5 times) of PAA/PAAm in PBS, with the same ionic strength as the cell culture medium, makes the coatings soft and water like that they do not provide a rigid support for cell attachment (14). PAA/PAH coatings deposited at the pH of 3.5/7.5, respectively, have been reported to be cell adhesive as they swell only to 130% of their original thickness in the buffered conditions (25).

Laser ablation through a photomask has proved to be a simple and effective way to create polyelectrolyte surface patterns; this method was high-throughput and compatible with standard silicon manufacturing process. Technical difficulties prevented the 'backfill' of the ablated areas with a second/different polyelectrolyte multilayer, instead, protein modification of the background clean glass was utilized, as it is widely used in most cell culture practices.

5. Conclusions

Photolithographic patterning of polyelectrolyte multilayers is a simple, versatile and robust method to pattern cells with exceptional long-term stability and protein adsorption resistance. High fidelity beating patterns of neonatal rat cardiac myocytes were observed after 100 days on polyelectrolyte patterns in our serum-free medium. Pre-made polyelectrolyte patterns combined with commonly used protein adsorption, surface modification methods could extend the applications of the patterned cultures.

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A) Clean glass

B) PAA/PAAm

C) Ablated PAA/PAAm

D) Ablated PAA/PAAm + Fibronectin

E) PAA/ PAH

F) Ablated PAA/PAH

Figure 1.

XPS survey scans of A) Clean glass, B) PAA/PAAm coating, C) Ablated PAA/PAAm D) Ablated PAA/PAAm after fibronectin incubation, E) PAA/PAH, F) Ablated PAA/PAH

Figure 2. Visualization of patterned polyelectrolyte multilayers by A) phase contrast microscopy B) fluorescently tagged PAH. Scale bar depicts 100 μm .

Figure 3.

Cell attachment and growth on patterned polyelectrolyte multilayers PAA/PAAm was negative for all cell types. PAA/PAH was positive for neurons and negative for cardiac myocytes and skeletal muscle cells. Glass was negative for neurons, but allowed attachment and growth of cardiac myocytes and skeletal muscle cells. Scale bar depicts 100 μm .

Figure 4.

Effect of protein incubation on the patterns and cell morphology A) Cardiac myocytes on glass vs. PAA/PAAm surface patterns incubated with fibronectin B) C2C12 cells did not form myotubes without incubation with serum containing medium C)–F) C2C12 cells plated on glass vs. PAA/PAAm surface patterns incubated with 10% serum containing medium for 1 – 8 hours. Scale bar depicts 100 μm.

Figure 5. Electrophysiological characterization of hippocampal (A) and cardiac cells (B) grown on polyelectrolyte patterns for 2 weeks.

Figure 6. Long term stability of the polyelectrolyte-based patterns. Neonatal cardiac myocytes on day 3 (A) and day 100 (B). Hippocampal cells on day 3 (C) and day 20 (D). Scale bar depicts 100 μm .

Table 1

A) Statistical analysis, Day 3 of the culture				
Surface	Cardiac	C2C12	Hippocampus	NG
PAA PAAm	1.9 ± 1.3	0 ± 0	0 ± 0	0 ± 0
PAA PAH	10.9 ± 5.4	10.6 ± 2.3	25 ± 6.8	27.5 ± 7.3
Glass	82.7 ± 15.6	24 ± 4.7	3.8 ± 2.2	1.4 ± 1

B) Statistical analysis, Day 6 of the culture				
Surface	Cardiac	C2C12	Hippocampus	NG
PAA PAAm	1.2 ± 1.1	0 ± 0	0 ± 0	0 ± 0
PAA PAH	27 ± 11.6	3.6 ± 2.4	19.1 ± 5.3	19.1 ± 5.7
Glass	89 ± 16.8	31.4 ± 6.1	1.5 ± 1.3	1 ± 1.9