

Nanoscale neuro-integrative coatings for neural implants

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Abstract

Silicon microelectrode arrays (Si MEAs) have great potential in enabling chronic *in vivo* recording of neural activity, but this potential has been hampered by scar tissue formation at the site of implantation. In this study, we report the fabrication and characterization of nanoscale coatings that have the potential of enhancing the biocompatibility of Si electrodes. We use electrostatic layer-by-layer (LbL) assembly to prepare nanoscale bioactive coatings on silicon substrates. We use the response of chick cortical neurons to these coatings to assess potential improvement in biocompatibility *in vitro*. The coatings are built on oxide covered silicon wafers by alternating polycations, polyethyleneimine (PEI) or chitosan (CH), with polyanions, either gelatin or laminin (LN). We use quartz crystal microbalance (QCM) to characterize the coatings. Our analysis confirms that we achieved ~30–110 Å scale coatings via LbL assembly. In contrast to bare oxide covered silicon, coated substrates had significantly enhanced chick cortical neuron adhesion and differentiation, with multilayers of PEI–LN showing the greatest improvement. The multilayers of PEI–LN were stable for at least 7 days in physiological conditions, as determined by an enzyme-linked immunosorbent assay (ELISA). In addition, impedance spectroscopy confirmed that multilayers of PEI and LN did not increase the magnitude of impedance of Si MEAs at the biologically relevant frequency of 1 kHz. Our study demonstrates that electrostatic LbL assembly enables nanoscale bioactive coatings, and that PEI–LN multilayers significantly enhance cortical neuronal attachment and differentiation *in vitro* with no deleterious effects on impedance of the electrodes. Such well-controlled nanoscale coatings have the potential to significantly impact the compatibility and performance of Si MEAs *in vivo*.

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1. Introduction

Neural implants have significant potential to enhance our understanding of normal and pathological states of the brain, and at the same time significantly impact the design and use of prosthetic devices. The potential of neural implants is clear from the prosthetic cochlear implant, a commonly used treatment for deafness that involves implanting electrode arrays into the cochlear region of the brain [1]. Other neural implants in various stages of development include micromachined neural prosthetic devices, which facilitate the functional stimu-

lation of and recording from the central and peripheral nervous system [2–5]. Although these devices have shown great promise in treating neurological disorders such as Parkinson's disease [6], the primary scientific stumbling block remains the inability to sustain long-term functional recordings from these devices following the implantation. The effectiveness of these neural implants decreases with time due to glial scarring and fibrous encapsulation that electrically and mechanically isolates the prosthesis from the nervous system [7].

Several approaches to improve the neuron-implant interface have been explored. These include electrochemical polymerization combined with immobilization of biomolecules [8], covalent immobilization of bioactive peptides [9,10] and microcontact printing [11]. This study describes an alternative approach for surface

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modification for neural implant applications, electrostatic layer-by-layer (LbL) self-assembly. The advantage of this technique is its versatility and fine control over layer thickness, which in turn impacts both the stability and impedance characteristics of the coatings.

The electrostatic LbL technique was introduced by Decher in 1991 [12,13]. The underlying principle for the LbL technique is the attractive electrostatic force between a charged surface and an oppositely charged polyelectrolyte. The LbL technique is a promising approach for the construction of thin films containing macromolecules, such as proteins [14–17], enzymes [18,19] or nucleic acids [20,21], with targeted properties onto a variety of substrates.

In the present study, we used alternating polyelectrolytes, either polyethyleneimine (PEI) or chitosan (CH), and proteins, either laminin or gelatin, to fabricate multilayer films built up by LbL deposition on silicon wafers with a very thin oxide layer. Silicon wafers were chosen as the substrates because silicon is the most widely used material for neuronal electrodes. The constituents of our coatings were chosen for their general ability to promote neuronal attachment and differentiation. For instance, laminin plays a crucial role in the developing and maturing central nervous system, e.g. in cell migration, differentiation and axonal growth [22]. It has been extensively used as a substrate for the studies on the growth of neurons in vitro. Gelatin, a denatured collagen, is also frequently utilized to enhance cell attachment to tissue culture substrates.

2. Materials and methods

2.1. Preparation of LbL surface coatings on Si substrates

2.1.1. Coating materials

PEI (Aldrich, MW 25,000), CH (degree of deacetylation: 85%, Sigma) and gelatin (type A: from porcine skin, approximately 300 Bloom, Sigma) were used as received. Laminin (laminin-1) was purchased from BD Bioscience and diluted to a concentration of 0.2 mg/ml with phosphate-buffered saline (PBS) solution at pH 7.4. Solutions of PEI (3 mg/ml, pH 7.4), CH (1.5 mg/ml in 0.1 M acetic acid containing 0.14 M NaCl, pH 4) and gelatin (0.5 mg/ml, pH 7.4) were prepared separately using Ultrapure water (Milli Q-plus system, Millipore) with a resistivity of 18.2 M Ω cm.

2.1.2. Nanoscale coating via LbL deposition

The substrates used for film growth were polished silicon wafers with a 10,000-Å-thick oxide layer (University Wafer). The silicon wafers were cut into small squares (1 × 1 cm) and cleaned with acetone, ethanol, deionized water and finally stored in ethanol at room temperature. For the formation of multilayers, a 1 ×

1 cm wafer was dried under nitrogen and a PEI layer was adsorbed for 30 min onto the substrate as a precursor layer to initiate the LbL self-assembly. The targeted multilayer architectures of the designed bioactive coatings were as follows: eight bilayers of PEI–gelatin [(PEI–gelatin)₈], one bilayer of PEI–gelatin followed by seven bilayers of CH–gelatin [(PEI–gelatin)–(CH–gelatin)₇] and eight bilayers of PEI–laminin [(PEI–LN)₈], where the subscript corresponds to the number of bilayers therein (for example, (PEI–gelatin) is considered as one bilayer). The buildup of the multilayer was accomplished by consecutive adsorption of the oppositely charged polycations (PEI or CH) and polyanions (gelatin or laminin) onto the substrate. Between each step, the excess polyelectrolyte was removed by rinsing the sample surface with deionized water. For each layer, an incubation time of 30 min and a rinsing time of 1 min were used.

2.2. Characterization of LbL coatings

2.2.1. Quantification of layer thickness

Quartz crystal microbalance (QCM) is a well-established tool for monitoring the adsorption of small amounts (ng/cm²) of material onto surfaces. As molecules adsorb onto the electrode, the oscillation frequency of the quartz crystal decreases. The adsorbed mass can then be calculated using the Sauerbrey equation, which postulates a linear dependence between the frequency shift and the adsorbed mass [23]. To determine the coating thickness, a MaxTek QCM was used. The multilayer architectures described in the last section were applied to the surface of gold-coated quartz electrode, and the change in resonance frequency was measured. All experiments were carried out at ambient temperature. After each bilayer of coating, the electrode was gently rinsed and dried before performing data collection.

2.2.2. Quantitation of surface-accessible laminin

The amount of accessible laminin present on the surface of the laminin containing multilayers was quantified by enzyme linked immunosorbent assay (ELISA). Unmodified silicon wafers served as controls. Laminin-containing silicon wafers (6 × 8 mm) were placed in a 48-well culture plate and any unbound sites were blocked with bovine serum albumin in PBS for 1 h, followed by incubation with antibodies to mouse laminin (Sigma; diluted 1:1000) for 1 h and anti-rabbit IgG conjugated to alkaline phosphatase (Jackson ImmunoResearch; diluted 1:1000) for 1 h. The reaction was developed using 4-methylumbelliferyl phosphate (MUP; Sigma) in carbonate buffer, pH 9.5, for 1 h. The fluorescent product was then transferred to a 96-well microtiter plate, and the absorbance was read using a 365 nm excitation filter and a 465 nm emission filter on

the microplate reader (Bio-Tek instruments). The relative amount of laminin on the wafers was measured from the densitometric readings against a standard curve obtained for laminin plated onto a 96-well microtiter plate at a concentration ranging from 1 ng to 1 μ g per well.

2.2.3. Coating stability

The stability of the nanoscale (PEI–LN)₈ coating was examined by immersing the laminin-coated wafers in PBS solution and incubating in a CO₂ incubator at 37 °C for 7 days. The wafers were then collected and stored at –80 °C until characterization for coating stability was undertaken. To determine if the number of bilayers had any effect on coating stability, wafers with only two bilayers, (PEI–LN)₂, were prepared in a similar way and used in the stability study as well. The amount of laminin remaining active and accessible on the surface before and after incubation in saline for 7 days was quantified using ELISA as described above.

2.2.4. Impedance spectroscopy of Si MEA

The effect of the nanoscale multilayers of PEI–LN on the electrical property of Si MEAs was evaluated by impedance spectroscopy. A micromachined single shank acute probe (16 recording sites, catalog number 1cm100_700) obtained from the University of Michigan Center for Neural Communication Technology was coated with multilayers of PEI–LN, and the impedance of the probe sites was measured before the coating, after two bilayers, and after eight bilayers, respectively. The instrument used for this study was built in-house in the Laboratory for Neuroengineering at Georgia Tech [24]. A Tektronix TDS 3014B oscilloscope and a HP function generator were also included. The system was operated under computer control using a MATLAB program. A solution of Hank's balanced saline solution was used as the electrolyte. An AC sinusoid with 50 mV of amplitude was used as the input signal with the DC potential set to 0 V. The value of the impedance was determined around the biologically relevant frequency of 1 kHz.

2.3. Characterization of cortical neuronal interactions with Si substrates

2.3.1. Primary cortical neuron culture

Cortical neurons were obtained from 9-day-old chicken embryos. Cerebral hemispheres were stripped of meninges, cut into small pieces, and enzymatically dissociated with 0.25% trypsin in Ca²⁺/Mg²⁺-free PBS for 20 min at 37 °C. The reaction was stopped by addition of 10% fetal bovine serum. After a brief centrifugation, the cells were resuspended in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal bovine serum, 100 μ g/ml streptomycin, 100 U/ml penicillin G and 292 μ g/ml L-glutamine. Cells were

further dissociated by trituration through a flame-constricted Pasteur pipette. Cells were then plated on a collagen-coated tissue culture dish and incubated for 60 min at 37 °C in 5% CO₂ atmosphere. The non-neuronal cells attach quickly and firmly to the dish while neurons form homotypic neuronal aggregates in the supernatant. Purified neuronal cultures were subsequently prepared by harvesting, rinsing and dissociating these neuronal aggregates by gentle trituration. Cells were seeded on sterile coated and uncoated 1 \times 1 cm wafers, which were placed sterile in individual wells of a 24-well culture dish and cultured for varying lengths of time in 5% CO₂ atmosphere at 37 °C as described in the next section.

2.3.2. Cell adhesion assay

To determine the ability of coatings to influence primary neuronal cell adhesion, cortical neurons were seeded with a density of 75,000 cells/cm² in 24-well plates that contained wafers coated with different LbL coatings, and incubated for 4 h at 37 °C in a humidified air (95%) and CO₂ (5%) atmosphere. Uncoated wafers were used as controls. After 4 h, wafers were gently washed with PBS to remove unattached cells and the number of adherent cells was counted using optical microscope (Zeiss) at 100 \times magnification. Three random 100 \times fields were selected for each substrate for analysis. Experiments were performed in triplicate. Comparisons between sample groups were made using paired Student's *t*-test.

2.3.3. Characterization of neurite outgrowth on coated and uncoated Si substrates

To assess the ability of LbL coatings to support neurite growth, dissociated embryonic (E9) cortical neurons were added to coated and uncoated wafers at a low density of 25,000 cells/cm². After 24 and 48 h of incubation respectively, the percent of neurons with processes greater than one cell diameter were quantified using light microscopy. The number of neurites was counted from three randomly determined regions of each sample and from three replicate cultures. The mean percentage of cells with neurites was calculated by multiplying the ratio of cells with neurites to total cells per three random fields by 100. Comparisons within the different coating groups and between coated and uncoated wafers were made using paired Student's *t*-test.

2.3.4. Characterization of neuronal morphology on coated and uncoated wafers

To study the morphology of neurons on these coatings, α -tubulin immunofluorescence analysis was performed to visualize the neurons. After 5 days of incubation, cortical neuronal cells were fixed for 20 min in Histochoice (Amresco), washed and incubated in PBS containing 4% goat serum to block non-specific binding.

Immunostaining of neuronal cells was performed for 1 h at room temperature with mouse monoclonal anti- α -tubulin (Sigma) diluted 1:200. Then the cells were rinsed in 0.5% triton in PBS and incubated with a goat anti-mouse IgG1 antibody conjugated with Alexa Fluor 488 (Molecular Probes) diluted 1:100 in 0.5% triton in PBS for an hour at room temperature. After being washed, the wafers were mounted on glass microscope slides with Fluoromount-G (Southern Biotechnology Associates, Inc.) and the immunostained cells were examined with a Zeiss LSM/NLO 510 confocal/multi-photon microscope.

3. Results

3.1. Nanoscale coatings produced by LbL assembly

3.1.1. Layer thickness

The buildup of the protein multilayers was monitored by QCM, a technique that has been well developed for characterizing LbL assemblies. In Fig. 1, frequency changes upon film formation were plotted as a function of the number of bilayers. In all three cases, the frequency decreased as the number of bilayers increased, indicating the mass increase on the QCM electrode as a result of the LbL assembly. All three coatings are very thin, with (PEI-LN)₈ being the thickest at 110 Å.

3.1.2. Surface-accessible laminin and coating stability

To quantify the amount of laminin that was present and accessible on the surface of the coatings, ELISA was used. ELISA was performed on wafers coated with two bilayers of PEI-LN as well as eight bilayers of PEI-LN using laminin antibodies. The results (Fig. 2) showed that there was little difference in the laminin concentration presented on the surface between the two-bilayer

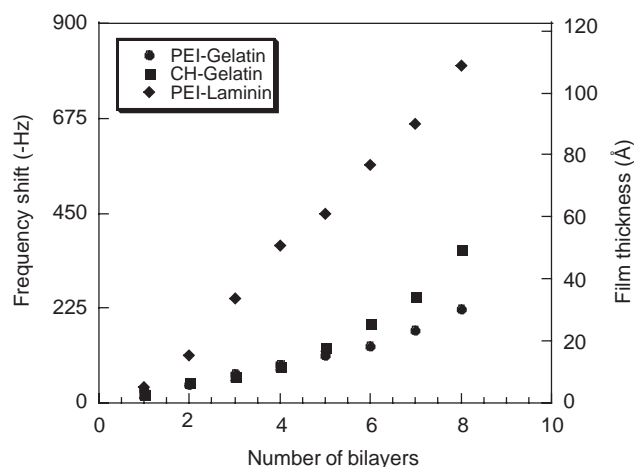


Fig. 1. QCM characterization of coatings: frequency shift and film thickness of each assembly bilayer for (●) (PEI-gelatin)₈, (■) (PEI-gelatin)-(CH-gelatin)₇, and (◆) (PEI-LN)₈ on QCM electrodes.

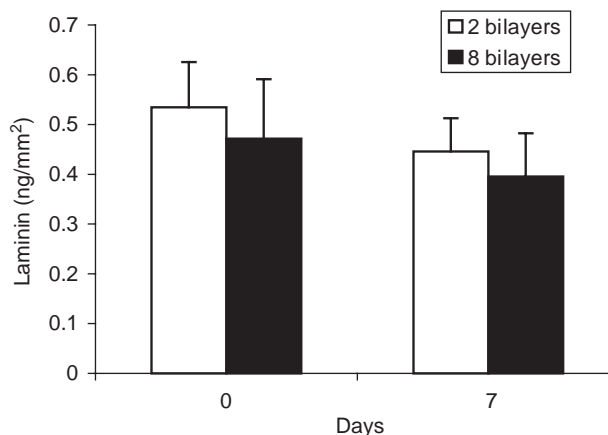


Fig. 2. Silicon wafers coated with two bilayers or eight bilayers (solid bars) of PEI-LN were incubated in PBS at 37 °C for 7 days to examine the stability of the bound laminin. The amount of laminin remaining was quantified using laminin antibodies by ELISA. There was no significant decline in the amount of laminin remaining in either the two-bilayer coating or the eight-bilayer coating over a 7-day period. Data are shown as means \pm SEM ($n = 3$).

coating (0.53 ± 0.09 ng/mm²) and the eight-bilayer coating (0.47 ± 0.12 ng/mm²). To examine the stability of the laminin multilayers coating on the Si/SiO₂ wafers, over a 7-day period the substrates were incubated at 37 °C in PBS and tested for surface-accessible laminin using ELISA. The LN ELISA showed no significant change in the surface laminin concentration over this time period for both the two-bilayer coating and the eight-bilayer coating (Fig. 2).

To determine if there was any interpenetration between the layers, ELISA was also carried out on laminin multilayers ending with PEI instead of LN. In these experiments, we were able to detect small amounts (>0.1 ng/mm²) of accessible LN on these surfaces.

3.1.3. Impedance spectroscopy

To determine the effect of PEI-LN multilayers on the electrical properties of neural implants, the electrical impedance of electrode sites on the Michigan single shank acute probes with and without coatings was carried out. The magnitude of impedance for the probe sites measured at three different time points—before the coating, after two bilayers of PEI-LN, and after eight bilayers of PEI-LN was plotted in Fig. 3. As we can see, such nanoscale coatings did not change the magnitude of impedance for the probe sites significantly at the frequency of 1 kHz.

3.2. Neuronal cultures on the nanoscale coatings

3.2.1. Cell adhesion

Cortical cell adhesion was quantified on all surfaces and the results are shown in Fig. 4. All three coated substrates promoted cell attachment as compared to the

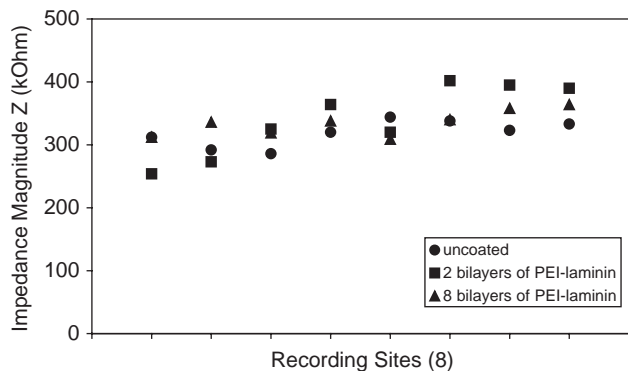


Fig. 3. Graph depicting the magnitude of impedance for the recording sites on the Michigan single shank acute probe before coating (●), after two bilayers of PEI-LN coating (■) and after eight bilayers of PEI-LN coating (▲). There were 16 recording sites on the probe; eight sites were randomly selected for the measurement. The frequency applied was 1 kHz, which is in the mid-range of the biologic relevant frequency of neural activity.

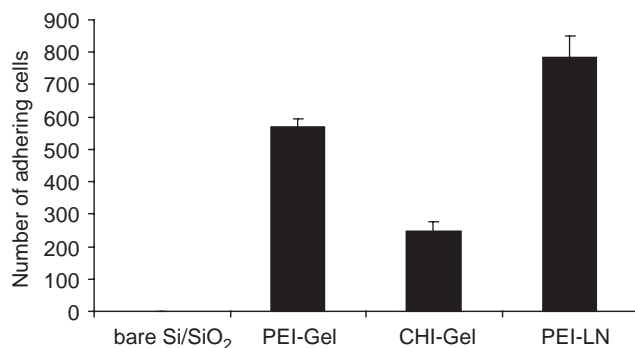


Fig. 4. Neuronal cell adhesion on (PEI-gelatin)₈, (PEI-gelatin)-(CH-gelatin)₇ and (PEI-LN)₈ coated Si/SiO₂ wafers 4 h after plating of cells. The number of cells was represented as the sum from three random 100 × fields. The control bar denotes adhesion on uncoated Si/SiO₂ wafers (no cells adhered to bare Si/SiO₂).

pristine sample where very few neurons adhered 4 h after plating. Among the three types of coating, (PEI-LN)₈ had the most number of cortical neurons attached. Although both ended with gelatin, coatings (PEI-gelatin)₈ and (PEI-gelatin)-(CH-gelatin)₇ showed different effects on cell attachment. When PEI was used as the counter ion for layering, the number of cells attached was significantly higher than in the case where CH was used as the counter ion.

3.2.2. Neurite outgrowth

The ability of the coatings to induce differentiation of neurons was determined by following neurite outgrowth on the surfaces 24 and 48 h post cell seeding. As seen in Fig. 5, cortical neurons were able to differentiate and generate neurites on all the coatings, and the percentage of cells bearing neurites increased as the culture time increased. Although the number of cells adhered on the (PEI-gelatin)-(CH-gelatin)₇ coating was less than that on the (PEI-gelatin)₈ even after 2 days of culture, the

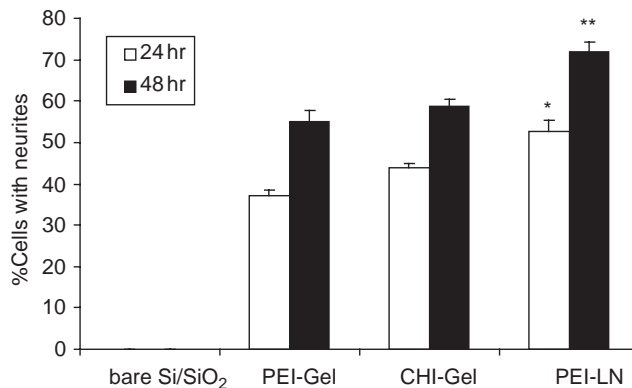


Fig. 5. Cortical neuron differentiation quantified as percentage of cells with processes greater than one cell diameter on nanoscale LbL coatings, (PEI-gelatin)₈, (PEI-gelatin)-(CH-gelatin)₇ and (PEI-LN)₈ after 24 and 48 h culture. Uncoated wafers served as control. * $p < 0.05$ vs. both gelatin containing coatings and control after 24 h; ** $p < 0.05$ vs. both gelatin containing coatings and control after 48 h by the paired Student's *t*-test.

percentage of cells with neurites was comparable between these two. As Figs. 4 and 5 indicate, the level of neurite growth was significantly higher ($p < 0.05$) on the laminin multilayers.

The morphology of neurites varied greatly as a function of the coating. By day 5 in culture, a network of connections was formed for neurons growing on the laminin multilayer coated substrates, as revealed by immunostaining with anti- α -tubulin antibodies (Fig. 6C). The process extended from the cell body was confirmed to be neuritic outgrowth by anti-160-kDa neurofilament staining (data not shown). In contrast, on the gelatin multilayers coated samples (Fig. 6A and 6B), most of the neurons remained separated with much shorter neurites, and smaller networks formed by a few neurons were present occasionally. It is noteworthy that on the laminin coating, we were able to identify growth cones that were nicely formed and spread (indicated by the arrows in Fig. 6E), which further confirmed the role of laminin in stimulating growth cone formation and in promoting neurite outgrowth.

4. Discussion

LbL assembly produces nanoscale films on solid substrates by alternate adsorption of oppositely charged macromolecules. Since water-soluble proteins can be induced to be charged species similar to polycations or polyanions, functional assemblies can be prepared via the LbL technique. In our experiments, gelatin and laminin were used for making the functional, neuro-adhesive multilayers. The pH of the protein solutions was set apart from the isoelectric point so that proteins were sufficiently charged under the experimental conditions. The isoelectric point of gelatin is 4.68 [25].

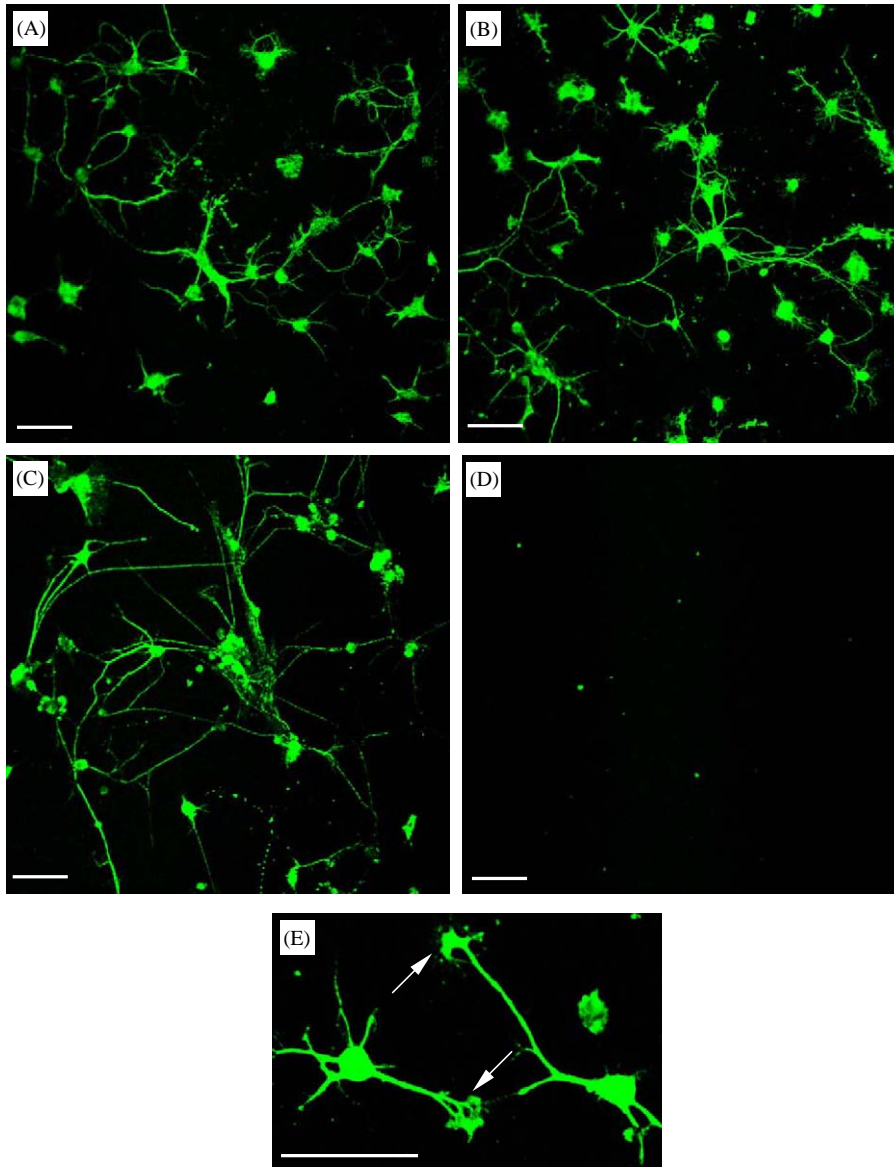


Fig. 6. Anti- α -tubulin immunofluorescence of chick cortical neurons grown for 5 days on silicon wafers coated with: (A) (PEI-gelatin)₈; (B) (PEI-gelatin)-(CH-gelatin)₇ and (C) (PEI-LN)₈. Uncoated wafer was used as control (D). Picture (E) was taken at a higher magnification from samples coated with (PEI-LN)₈, where a growth cone was observed. Scale bar equals 50 μ m on all the images.

Laminin is a major structural component of basement membranes and is a glycoprotein composed of three peptide chains (α , β , γ) with a multi-domain structure [26]. The isoelectric points for α , β and γ peptide chains are 6.26, 4.89 and 5.08, respectively [27]. Therefore, at a pH of 7.4, both gelatin and laminin are negatively charged.

In order to achieve successful assembly of protein multilayers, an alternation with polyion layer is important [23]. Hence, PEI or CH was used as “electrostatic polyion glue” for the protein assembly.

The multilayers were constructed on the surface of Si/SiO₂ wafers, which are negatively charged because of the oxide. The Si/SiO₂ surface is representative of many Si multi-electrode array systems including the Michigan

microelectrode system, which is used for chronic unit recording in the cerebral cortex [5]. LbL coatings always started with PEI, a highly positively charged polycation that readily attaches to oxidized surfaces. All three targeted multilayer architectures, (PEI-gelatin)₈, (PEI-gelatin)-(CH-gelatin)₇ and (PEI-LN)₈, were obtained as clear and transparent coatings on the Si/SiO₂ wafers, and the color was uniform across the substrate upon visual inspection.

LbL assembly is known to have the capability of producing nanoscale films with a nanometer-ordered thickness [28]. In this study, we demonstrated that coatings less than 11 nm could be prepared via this method. Important factors for controlling the assembly thickness include ionic strength of the solution, mole-

cular weight of the material and pH. When tracking the assembly process with QCM, it is observed that the protein adsorption step correlated approximately with the molecular weights, i.e. the frequency shift caused by laminin adsorption was much larger than that of gelatin considering the 800 kDa molecular weight of laminin to around 100 kDa of gelatin. Such nanoscale coatings are excellent candidates for surface modification of the neural implants such as microelectrodes.

For a micromachined neural interface system, it is important to keep the interface impedance low because the neuronal signals generally have very small amplitude; therefore, large impedance can adversely affect the charge transfer capability of the interface. Our impedance spectroscopy study confirms that modifying silicon microelectrodes with the LbL assembly had little influence on the electrical impedance of these devices, because the coatings were ultrathin.

Another concern is the long-term stability of the coatings, because neural implants are intended as prosthetic devices for patients with sensory and/or motor deficits and for fundamental studies in neuroscience that involve chronic or long-term recording. Using the ELISA assay, we have demonstrated that the protein multilayers obtained by the electrostatic LbL technique were stable under the *in vitro* simulated physiological conditions for at least 7 days.

Compatibility of these coatings with neuronal cells was tested *in vitro*. Primary cell culture was chosen over cell lines because it is a better representation of the *in vivo* situation. Purified culture of neurons was obtained following the method of Hanson et al. [29] that provided a purity of 97%. Neuronal cell adhesion and neurite outgrowth assays verified that these coatings are indeed neuro-integrative. As expected, all coatings improved neuron attachment and supported neurite growth. Among them, coatings that contained laminin showed the most improvement both in terms of cell attachment and neurite extension. This is consistent with previous studies showing that laminin promotes the neuronal cell adhesion which is often mediated by integrins [30]. For coatings built up with gelatin, our studies showed that using PEI as the counter ion seems to provide better neuron adhesion than when CH was used. This could be explained by examining the structures of PEI and CH. PEI is a linear polymer while CH is a polysaccharide with bulky $\beta(1-4)$ linked D-glucosamine repeat units. As indicated from the ELISA study, the interface between layers is not sharp and a partial interpenetration between neighboring layers takes place [28]. For the protein multilayers, even in the case when the counter ion was left as the outermost layer, ELISA assay was still able to detect the underlying protein. We hypothesize that due to the penetration of the underlying layer, there could be fewer gelatin molecules available for the

cells to access when CH was the counter ion as opposed to the linear PEI.

Although all the coatings supported neuron differentiation, there were significant differences in cell morphology. Individual cells or clusters of few cells were observed on the gelatin multilayers, while on the laminin multilayers, very few cell aggregates formed. Neuronal cells are more flattened and spread on the laminin layers, with either bipolar or multipolar shapes. Neurites on the laminin layers are generally longer and thicker than those on the gelatin layers. As culture time increased, neurons on the laminin layers seem to migrate to form small neuronal aggregates and organize into networks by producing long processes. On gelatin substrates, they remain mostly as individual or clustered with shorter processes and smaller networks.

We suggest it might be advantageous to coat the entire implant with neuro-integrative coatings instead of only coating the recording/stimulation sites [8]. Whole implant coatings potentially can attract more neurons to the surface of the implant, leading to a better integration of the entire implant in the host tissue. Furthermore, modification of the surface with the whole laminin molecule instead of simply grafting laminin-based peptides, such as IKVAV [10], can potentially be more effective for neuronal cells. The whole molecule is more efficient in promoting neurite adhesion and growth compared to peptides [31].

5. Conclusion

In this study, the formation of a series of bioactive films based on alternate adsorption of PEI and gelatin, CH and gelatin, or PEI and laminin on Si/SiO₂ substrates through the electrostatic LbL technique was investigated. All the films were in the nanometer scale (≤ 110 Å), and did not alter the electrical impedance of neural implants. The coatings are stable under simulated physical condition for at least 7 days. Experiments with primary cortical neuronal cultures demonstrate that these nanoscale coatings significantly improved neuron cell adhesion and differentiation. In comparison to conventional coating methods, the LbL technique is simple, inexpensive and amendable to coating with proteins. Additionally, LbL coatings lend themselves well to incorporation of bioactive drugs, growth factors, or nucleic acids into the layers for local delivery. Ongoing efforts are focused on incorporating neurotrophic factors and anti-inflammatory agents into the coatings for their sustained release after implantation *in vivo*. We suggest that neuro-adhesive properties demonstrated in this study, when combined with sustained release of bioactive agents, will greatly enhance the chronic recording stability of Si MEAs *in vivo*.

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