Fabrication and characterization of conductive anodic aluminum oxide substrates

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ABSTRACT

Biomaterials that allow the utilization of electrical, chemical and topographic cues for improved neuron–material interaction and neural regeneration hold great promise for nerve tissue engineering applications. The nature of anodic aluminum oxide (AAO) membranes intrinsically provides delicate control over topographic and chemical cues for enhanced cell interaction; however their use in nerve regeneration is still very limited. Herein, we report the fabrication and characterization of conductive AAO (CAAO) surfaces for the ultimate goal of integrating electrical cues for improved nerve tissue behavior on the nanoporous substrate material. Parafilm was used as a protecting polymer film, for the first time, in order to obtain large area (50 cm²) free-standing AAO membranes. Carbon (C) was then deposited on the AAO surface via sputtering. Morphological characterization of the CAAO surfaces revealed that the pores remain open after the deposition process. The presence of C on the material surface and inside the nanopores was confirmed by XPS and EDX studies. Furthermore, I–V curves of the surface were used to extract surface resistance values and conductive AFM demonstrated that current signals can only be achieved where conductive C layer is present. Finally, novel nanoporous C films with controllable pore diameters and one dimensional (1-D) C nanostructures were obtained by the dissolution of the template AAO substrate.

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1. Introduction

Development of effective biomaterials for neural tissue engineering requires the optimization of topographic, chemical and electrical cues that influence the cell–material interactions [1–4]. Significant amount of research has been conducted to utilize combinations of these cues on a rainbow of different substrates for increased cell adhesion, proliferation and alignment as well as enhanced neurite outgrowth. For instance, using biomaterials with nanoscale topography that resembles the hierarchical structure of the extracellular matrix promotes select protein adsorption/bioactivity and cell interaction [3–6]. Patterned surfaces with these features create opportunities to align the neurons which have potential to be used as neuron guidance conduits for successful neuroregeneration. Regarding chemical cues, a variety of different strategies were employed for promoting nerve regeneration. These involve the modification of substrate surface with ECM proteins or neuroactive peptides as well as doping the substrate with biochemicals such as drugs and growth factors [6,7]. Finally, studies over the past few decades have demonstrated that electrical stimulation can accelerate neural tissue regeneration. Here, bulk or nanofiber-based conducting polymeric scaffolds [5,7,8] and carbon nanotubes [9,10] are emerging as novel conductive platforms that can improve the modulation of neuronal responses.

Nanoporous anodic aluminum oxide (AAO) membranes are a unique class of biomaterials that can be synthesized by anodization of high purity aluminum [11,12]. Their intrinsic properties allow one to tune several parameters for obtaining improved tissue regeneration, and hence these biomaterials are widely used especially for bone tissue engineering applications [13,14]. For instance, the native porous structure provides a nanoscale topography upon which improved osteoblast adhesion and matrix formation was attained when compared with non-porous counterpart that does not promote osseointegration [15]. Furthermore, the pores of this

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biocompatible material can be filled with chemicals or bioactive materials to promote osseointegration [11,15]. Despite the ability to manipulate such topographic and chemical cues, the research for utilizing AAO membranes for neural tissue engineering is still very limited and the related studies mainly focus on the role of topography in cell–material interaction [16–18]. Hence, exploiting the full potential of AAO as a promising substrate for neuroregeneration by utilizing the topographic, chemical and electrical cues has a great potential for neural tissue engineering applications, however, it currently still remains to be a challenge.

In this study, we focus on the fabrication and characterization of conductive AAO (CAAO) substrates to eventually control nerve tissue behavior via utilizing electrical cues. We first introduce a new approach for producing large area free-standing AAO membranes by the use of paraffin as an AAO protecting layer. We then show that CAAO surfaces with open nanopores can be prepared by carbon (C) sputtering through morphological, chemical and electrical characterization of the material. Finally, we report the formation of novel nanoporous C films and one dimensional (1-D) C nanostructures after the removal of the AAO templates.

2. Experimental details

2.1. Preparation of the free-standing AAO membranes

High purity Al foils (99.999%, Puratronic, 1 mm thickness, Alfa Aesar) were sanded with 600 grit sand paper, rinsed with deionized water (18 MΩ, Sartorius) and annealed at 450 °C for 4 h. After cooling to room temperature, an electrochemical polishing step at 15 V was applied to the foils using a Pb cathode for 90 min at 75 °C. The electroplating solution consisted of 95 wt% H3PO4 (BDH Prolabo), 5 wt% H2SO4 (Fluka) and 20 g/ml CrO3 (Fluka). These foils were then subjected to single or two-step anodization processes depending on the desired final nanopore size. Nanopores with ∼250 nm diameter were obtained using a single step anodization where 160 V anodization potential was applied to the Al foils in a 0.4 M H3PO4 electrolyte at 0 °C against a stainless steel cathode. The nanopores were then widened in an aqueous chromic acid solution at room temperature for 22 min.

In order to obtain monodisperse nanopores with ∼100 nm pore diameter, the well-known two-step anodization method [19,20] was followed. Here, the first anodization step was carried out at 50 V in a 5 wt% aqueous oxalic acid electrolyte solution for 18 h at 5 °C. A thick non-uniform alumina (Al2O3) film that forms on both sides of the Al foil was removed using an aqueous solution composed of 0.4 M H3PO4 and 0.2 M CrO3 (Fluka) at 75 °C. A second anodization step was then applied at the same conditions of the first one for 5 min and the substrate was then immersed into a 5 vol% H3PO4 solution to widen the nanopores to yield AAO films with 100 nm ordered uniform nanopores were attained. Note that through this report, AAO structures are named as films if they are bound to the underlying Al, and membranes when they are liberated.

AAO films form on both sides of the Al foil and obtaining free-standing AAO membranes requires the removal of the Al foil that is sandwiched between these films (Fig. 1). This is typically achieved by applying a protecting polymer layer on the surface of one of the AAO films. The unprotected AAO film and the Al foil were then removed in appropriate solutions and free-standing AAO membranes were achieved after the protecting polymer layer was dissolved in an organic solvent (Fig. 1). In our case, paraffin (Bemis) was used, for the first time, as the protecting layer and the unprotected AAO film was dissolved in 1 M NaOH (BDH Prolabo) to expose the metallic Al surface. Al was then oxidized in 0.1 M CuCl2·2H2O (Alfa Aesar) + 6.1 M HCl (Merck) solution, and AAO membranes were obtained by dissolving the paraffin in n-hexane (Sigma–Aldrich). Nail polish (Plomar), Poly (methyl methacrylate) (PMMA, Aldrich, 350,000 MW) and lacquer (Polisan) were also used as alternative protecting layers; however, best results were obtained with paraffin as discussed in the following sections.

2.2. Preparation and characterization of CAAO surfaces

The released AAO membranes have two distinct surfaces, the barrier side and the solution side [21]. Nanopores with ∼100 or 250 nm pore diameters are located on the solution side of the membranes. In order to create CAAO surfaces, the solution sides of the membranes were coated with 20-nm-thick C layer via sputtering (GATAN Precision Etching & Coating System) unless mentioned otherwise. The C thickness value was also verified by independent ellipsometer studies which confirmed the thickness reading from the sputtering instrument with less than 10% deviation (data not shown). These CAAO surfaces were then characterized by using Scanning Electron Microscope (SEM) with an Energy Dispersive X-ray (EDX) detector (ESEM, Quanta 200 FEG), Atomic Force Microscope (AFM, EZ-AFM, tapping mode, PPP cantilever, Nanomagnetics), X-Ray photoelectron spectroscopy (XPS, K-Alpha, Thermo Scientific). The electrical characterization of the CAAO surfaces was made by measuring the surface resistance of the structures via a DC Voltage/Current Source (Yokogawa GS 210 sourcemeter). Here, CAAO surfaces were first fabricated by
sputtering AAO membranes with C or Au (as a control) layers. A mask with 100 μm width was then placed on the CAAO surface and a second 50-nm-thick Au layer was sputtered (Fig. 2).

The mask was removed and five different potentials were applied across the top Au pads and the corresponding current values were recorded. The slope of the resultant I–V curve was then used to obtain the surface resistance value corresponding to each coating condition. Further confirmation of surface conductivity was characterized by conductive AFM (hp-AFM, Nanomagnetics). In this setup, part of an AAO substrate was masked and then the whole AAO surface was exposed to C sputtering. After the mask was removed a voltage bias (3.5 V) was applied between a conductive AFM tip (Pt/Ir cantilever) and the substrate. The current between the conductive tip and the partially C coated substrate surface was then monitored to distinguish the C coated areas.

2.3. Liberation and characterization of nanoporous C films and 1-D C nanostructures

C coated AAO membranes were dissolved in 1 M aqueous NaOH solution for 4 h. Template synthesized nanoporous C films and 1-D C nanostructures were imaged by SEM after this solution was filtered and extensively washed with water and isopropyl alcohol. Here, a 5-nm-thick Au–Pd layer was sputtered prior to imaging for improved image quality. Further confirmation of nanoporous C films and 1-D C nanostructures were carried out by Transmission Electron Microscopy (TEM, FEI Technai Biotwin). TEM images were obtained after the filtered nanostructures were transferred to a minute volume of ethanol and this concentrated dispersion was dropped on a 300-mesh TEM grid.

3. Results and discussion

The general rationale for obtaining free-standing AAO membrane is to protect one of the two AAO faces that form on both sides of the Al foil (Fig. 1). Popular protecting layers are polymer-based materials including lacquer [22], nail polish [23] or PMMA [24]. Prior to developing our own methodology, we have tried these three agents as protecting layers. Despite being successful to a certain extent, several problems involving surface adherence or the final removal of the protecting layer were faced. It should be noted that the oxide or metal removal steps, occurring in low pH CuCl₂ solutions, are extremely vigorous and proceed at elevated temperatures. These conditions result in surface adherence
problems for the protecting layer in addition to the breaking of the AAO membrane to small pieces. On the other hand, the difficulty observed in the final polymer removal step should be due to extensive crosslinking of the polymer layers [25] as the temperature of the solutions can rise well above 110 °C. The application of parafilm as protecting layer proved to be useful during the first NaOH-based unprotected AAO film removal as well as the second acidic Al dissolution step. After the observation of the metal removal, the AAO-parafilm assembly was washed and parafilm was dissolved in hexane to reproducibly yield ~50 cm²-area free-standing AAO membranes (Fig. 3a).

The conventional approach for coating C on AAO is via chemical vapor deposition (CVD) [26] where carbon nano tubes (CNTs) [27,28] or CNT membranes [29] can be successfully produced at elevated temperatures. Here, we have used physical vapor deposition (PVD)-based sputtering as an alternative, practical and versatile tool to create CAAO surfaces. They can be visually distinguished from the AAO membranes after ~20 nm C coating and as the material turns into a gray color compared to the white AAO (Fig. 3b). The electron micrograph of a CAAO substrate is illustrated in Fig. 3c which reveals that the pore diameters are relatively large (235.28 ± 14.72 nm) as expected from an AAO membrane prepared in H3PO4 electrolyte.

AFM was also used to demonstrate the topography of the CAAO surface, and in particular, the state of the pores (Fig. 4). The dark circular areas indicate the positions of the pores. A line across this image was chosen to demonstrate the pore to pore distance of ~300 nm which is parallel to the SEM data. More importantly, the depth profile across this line illustrates that the pores are open and pore depth is ~90 nm. This value is much smaller than the real pore depth (20 μm) and stems from the limited ability of the AFM tips inside the nanochannels, but nevertheless, it is apparent from Fig. 4b that the periodic pores are not blocked due to C deposition.

The chemical characterizations of CAAO surface as well as the nanopores are shown in Fig. 5. High resolution C 1s XPS spectrum of the CAAO surface (Fig. 5a) illustrates the presence of a broad peak where the maximum intensity corresponds to the binding energy of 285 eV, and dictates the dominant presence of amorphous C on the CAAO surface [29]. In order to confirm the presence of C inside the nanopores, a C coated AAO membrane was broken into half and the membrane cross-section was analyzed by SEM and EDX. Two different areas, one, close to the conductive surface, and the other, further down from this surface were investigated.

The EDX data (Fig. 5b and c) displays the abundant presence of C near the conductive surface and comparably low C content on areas that are placed further deep into the membrane in addition to the components of Al2O3-based material. This was expected from a C deposition by a regular sputterer for
electron microscopy use as there is no control of working pressure nor substrate temperature that are essential in obtaining conformal coating [30]. It is also worth mentioning that, despite the improved characteristics for sputtering, PVD methods generally suffer from conformal coating of substrates with deep features [30, 31].

The electrical properties of the CAAO surfaces were investigated by calculating their surface resistance values and by imaging a partially C coated AAO substrate with a conductive AFM. In order to obtain surface resistance values, different DC voltages were applied on masked substrates (see Section 2) and the resultant I–V curves were used to extract the resistance data. As a comparison, uncoated and Au coated AAO substrates were also investigated (Table 1). As expected, the naked AAO substrates were insulators, and Au coated AAO surfaces had lower resistivity values compared with C coated ones. In both cases, surface resistance value increased as the coating got thicker, and similar trend was reported by other researchers in the literature [32]. It should be noted that, the traditional use of four-point probes for surface resistance calculation failed in our study due to the fragile nature of the AAO membrane.

In order to confirm the conductive characteristic of the CAAO surface, part of an AAO substrate was selectively blocked from C sputtering via the use of a mask. After the mask was removed, a voltage bias (3.5 V) was applied between a conductive AFM tip (Pt/Ir cantilever) and the substrate. A large area topographic view of this selectively blocked CAAO surface as well as its current profile is shown in Fig. 6. The tiny darker-colored dots in Fig. 6a correspond to the locations of nanopores that are ∼250 nm in diameter, and they do not appear clearly in this large area AFM scan. However, when the AFM is switched to the conductive mode, a sharp distinction between the C coated vs. non-coated area becomes apparent (Fig. 6b). There is no current flow between the tip and the masked, non-conductive AAO substrate where as current values up to 0.05 nA can be obtained while the tip scans the conductive C-sputtered region. The imperfections in the masking should be responsible for the local current signals observed in the masked AAO area.

Complete dissolution of a CAAO substrate yields two distinct group of structures, namely, nanoporous C films and 1-D C nanostructures as demonstrated in Fig. 7. The diameters of the pores in the nanoporous C film (Fig. 7b) as well as the diameter of 1-D structures (Fig. 7b and c) are on the order of ∼240–270 nm, which reflects the starting template pore size attained by using H3PO4 electrolyte. Utilizing the versatile nature of template synthesis [33], it is also possible to fabricate porous C films with more uniform and smaller diameter nanopores. Here, AAO templates with monodisperse nanopores having ∼100 nm pore diameters were first synthesized via the use of two-step anodization [19] in oxalic acid electrolyte (Fig. 7d). After C coating and dissolution of template, nanoporous C films with ordered and smaller nanopores (∼50 nm diameter) were attained (Fig. 7e, curled film resting on the filter material). To the best of our knowledge, the template-based formations of such structures are reported for the first time in the literature and potential applications involving filtration [34] and sensing [35] can be foreseen by the control over initial template pore size.

Careful investigation of the 1D nanostructures and comparison between the previously published electron micrographs of template synthesized CNTs [27–29] dictates that these particles are not tubular. Note that, after C coating, the pores of the CAAO material were open. One reason behind this observation can be the collapse of the C-coating inside the nanopores during the template dissolution process. It is also suspected that the non-uniform conformal C coating by sputtering has a role in the observation of such structures as opposed to the theoretical formation of CNTs upon dissolution. Note that, by fine tuning the deposition conditions involving substrate temperature and pressure, CNT fabrication by sputtering is plausible. Systematic control over such conditions for CNT production via sputtering and detailed characterization of the 1-D C nanostructures is beyond the scope of this work, and is currently under investigation for a separate report.

Table 1
The surface resistance values of C or Au coated AAO substrates.

<table>
<thead>
<tr>
<th>AA0 coating material</th>
<th>Thickness (nm)</th>
<th>Resistance (MΩ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon (C)</td>
<td>0</td>
<td>∞</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>195.24 ± 2.97</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>624.54 ± 10.13</td>
</tr>
<tr>
<td>Gold (Au)</td>
<td>0</td>
<td>∞</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>22.77 ± 1.10</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>28.65 ± 2.05</td>
</tr>
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</table>
4. Conclusion

We have fabricated conductive AAO surfaces to be used as a biomaterial in nerve tissue engineering. Large area free-standing AAO membranes were first fabricated by a novel procedure that utilizes parafilm as a protecting film. These substrates were then coated with C via sputtering. The morphology of the open nanopores after C coating was characterized by SEM and AFM studies. Presence of C on the CAAO surface and within the pores that are close to the sputtered face was characterized by XPS and EDX analysis. The conductivity of the CAAO surfaces was confirmed by DC voltage–current reading as well as conductive AFM setups by using masked CAAO substrates. Unique nanoporous C films with controllable pore dimensions and 1-D C nanostructures were obtained by the virtue of template synthesis upon the complete dissolution of sputtered AAO templates. The potential of AAO and CAAO substrates for neural adherence and regeneration is currently being investigated and promising results including cell growth.
stimulation was observed, and these will be the topic of an upcoming report.

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References