Construction and Characterization of a Nanowell Electrode Array

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ABSTRACT

Gold nanowell electrode arrays, with a nanowell depth of approximately 600 nm, have been prepared using commercially available anopore membranes as templates. Electrodes were prepared by forming a copper film on anopore, followed by electrodeposition of gold throughout the pore and finally removal of the copper to expose the nanowells. The electrodes were characterized by atomic force microscopy, scanning electron microscopy, and electrochemical methods. Dodecanethiol self-assembled monolayers were found to passivate the electrode.

C. R. Martin coined the term "template synthesis" to describe a method in which the pores of a nanoporous membrane act as templates for the synthesis of nanostructures of a desired material.[1] As the pores within these membranes are nearly cylindrical and of uniform diameter, monodisperse nanocylinders of a desired material can be obtained. This method has been applied to the formation of nanowires,[2] metal nanostructures[3] and hollow metal tubules,[4] conductive[5] and insulating polymers,[6] and semiconductors.[7] (For a review of general template-based methods for the preparation of nanomaterials see ref 1.) The formation of gold metal nanostructures by Martin's group using anopore disks as a template[3] was accomplished by sputtering a thin layer of Ag onto one side of the membrane in order to form an electrode, followed by the galvanostatic deposition of Ag to seal the pores and potentiostatic deposition of Au into the pores on top of the Ag microcylinders. Nitric acid was then used to dissolve away the Ag, leaving Au nanostructures.

In this report we discuss a modification of this method which allows us to form an array of Au nanoelectrodes, 200 nm in diameter and which have a controlled depth, embedded within an anopore template. The nanoelectrode array was characterized by atomic force microscopy (AFM), scanning electron microscopy (SEM), and electrochemical methods. Passivation of the electrodes with alkanethiol molecules in a self-assembled monolayer (SAM) was also investigated.

Anodisc membranes obtained from Whatman were used as the template material for gold nanoelectrode fabrication. The membrane was 47 mm in diameter, 60 μm thick and consisted of 200 nm pores in a hexagonal configuration. Figure 1 shows a schematic of the electrode preparation process. A thin layer of Cu was sputtered on the membrane using a CRC-100 sputtering system. AFM was used to determine the height of the sputtered layer, which was found to be approximately 100 nm. This metal layer did not cover the pores of the anopore, but allowed for electrical contact. The pores were subsequently covered by electroplating Cu onto the sputtered anopore from a 0.1 M $CuSO_4/H_2SO_4$ solution at −0.5 V vs SCE for 1500 s. For this electroplating step, a working electrode was constructed using an ITO coated slide (Delta Technologies) in contact with the Cu-coated anopore to provide electrical contact and a glass slide as the backing.

For deposition of the metal into the template pores a specially constructed cell was used, consisting of a cylindrical Cu base onto which the Cu-coated anopore was placed. This base was screwed into a Teflon cell, with an O-ring to ensure sealing. Au was plated into pores overnight at −0.95 V vs SCE for a commercial gold plating solution, Orotemp 24 gold solution (Technic Inc.). The plating time for the gold to plate throughout the pore was found to be approximately 120 000 seconds. To form the nanopores the initial Cu layer was removed by immersion in a 5 M nitric acid solution, followed by rinsing with a fresh acid solution and distilled water. Dodecanethiol (DT) deposition also occurred under argon in an inert atmosphere drybox. The Au electrodes were coated with monolayers of DT by immersion of the electrode for at least 120 min in a solution of 20 mM DT in ethanol. Upon removal from the deposition solution, the electrode was rinsed with ethanol and isopropyl alcohol.

The experimental instrumentation for the electrochemical setup consisted of a EG&G PARC model 263 potentiostat, connected with a GPIB interface (National Instruments) to a Gateway 2000 model P5-60 computer with EG&G PARC

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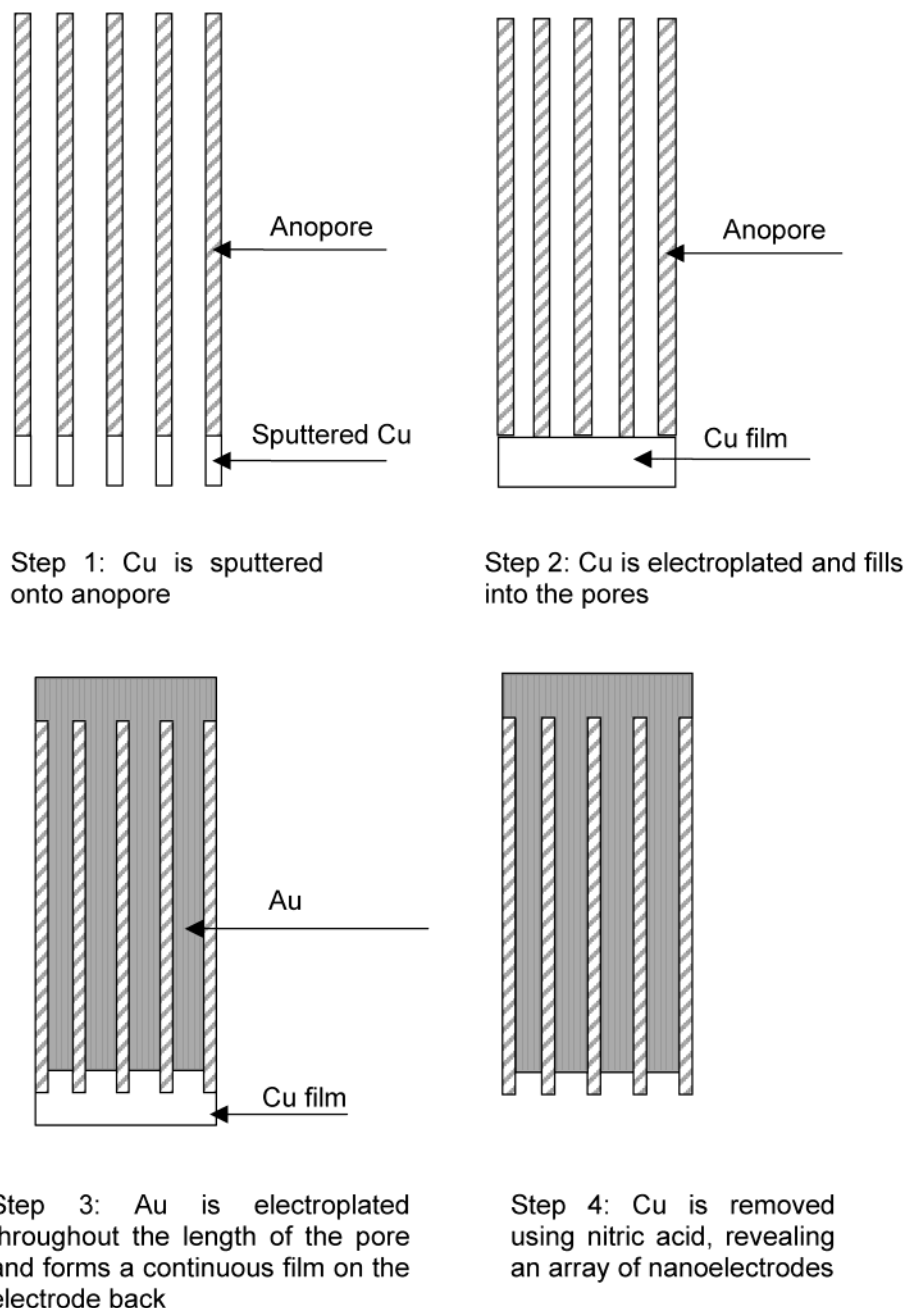
Figure 1. Preparation of a nanoelectrode ensemble. (1) Copper is sputtered onto an alumina membrane. (2) Copper is electroplated, partially filling the pores. (3) Gold is electroplated throughout the pores, until it covers the other side of the membrane. (4) Copper is removed using nitric acid, leaving wells.

model 270 Research Electrochemistry Software, or a 263A potentiostat, controlled by Electrochemistry Powersuite software (PerkinElmer). Ethanol (AAPER), dodecanethiol (Aldrich), sulfuric acid (Fisher Scientific), nitric acid (Fisher Scientific), ferricyanide (Fisher Scientific), and copper sulfate (Mallinckrodt AR) were reagent grade and were used without further purification. Aqueous solutions were prepared with deionized water. Scanning electron microscopy (SEM) was done using a Hitachi S-2500 scanning electron microscope. Atomic force microscopy was carried out using a MultiMode SPM with a JV scanner controlled by a NanoScope IIIA system controller, from Digital Instruments. Both contact and tapping mode tips were purchased from the same manufacturer.

The goal of this work was to develop a method for the fabrication of a gold nanowell electrode that is reproducible and allows for control of the well depth. Initially silver was used as the metal for removal and well formation, but this was not very successful due to the solubility of the sputtered silver in both commercial and homemade silver plating solutions, which led to frequent failures with the plating step. When plating was successful, the silver film was found not to adhere well to the anopore. The substitution of copper was found to be a good alternative. Although the sputtered copper does dissolve with time in the copper plating solution, this can be overcome by beginning plating very quickly after the electrode is placed in solution. The electroplated copper films are brightly colored and adhere well to the anopore.
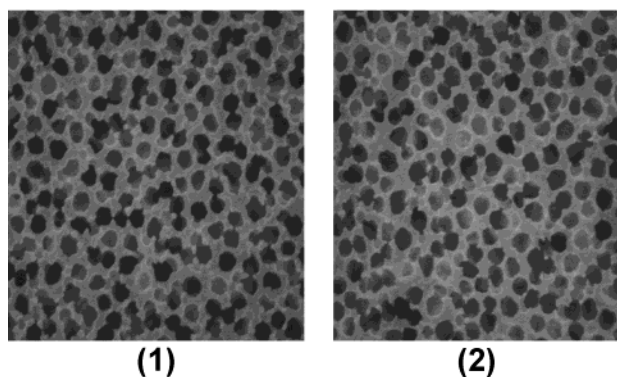
Figure 2. (1) SEM image of the anopore membrane with wells, fabricated through the procedure illustrated in Figure 1. (2) SEM image of a clean anopore membrane, as purchased, only sputtered with gold to allow the SEM image. Both are taken with a magnification of 20 000, and they are 2.35 μm wide. The diameter of the wells ranges from 150 to 200 nm. The two images do not differ significantly.

Figure 3. AFM showing a section analysis of Cu negative.

To determine how effectively 5 M nitric acid removes the copper from the electrodes, they were characterized using SEM. Images of a clean anopore and of the electrode surface after removal of Cu were taken. These images demonstrated that the initial electroplating step deposits enough Cu into the pores to form wells upon removal of Cu. The Cu removal leaves a homogeneous array of wells over a surface that is as clean and regular as the original anopore membrane (see Figure 2). However, it was not possible to determine the depths of the wells using SEM. To determine if the Au nanoelectrodes formed were of uniform height, the electrode was cleaved to reveal a cross section and the anopore was removed using a 1 M NaOH solution. The remaining gold rods were imaged by SEM at an angle of 45° and found to be uniform in height and diameter on the micrometer scale. All the pores contained a nanorod, with no visible gaps.

It was attempted to determine well depth using tapping mode AFM, but the diameter and depth of the wells prevented the AFM tip from reaching the well floor. Attempts to construct electrodes with shallower wells by using shorter Cu plating times of less than 1000 s failed, as the Cu films formed at these plating times were not completely continuous and allowed leakage of the Au plating solution. Therefore, an alternative way of evaluating the depth of the wells was adopted. The anopore was initially sputtered with gold, instead of copper, obtaining a metallic layer of ~100 nm. Copper was then plated on this membrane for the amount of time used for the regular electrodes. The membrane was then attached to an adhesive tape and immersed in NaOH 1 M in order to dissolve the alumina. This process left a series of copper rods of a height equivalent to the depth of the wells. A small region was etched with concentrated nitric acid to create a step that was measured afterward using AFM. Figure 3 reports the image of the step and the average transverse section. The average height of the wells was estimated to be 680 $\pm$ 313 nm. A SEM image was also acquired of the cross section of a copper "negative" plated for 2000 seconds. This showed rods of approximately 1 μm that have rough surfaces.
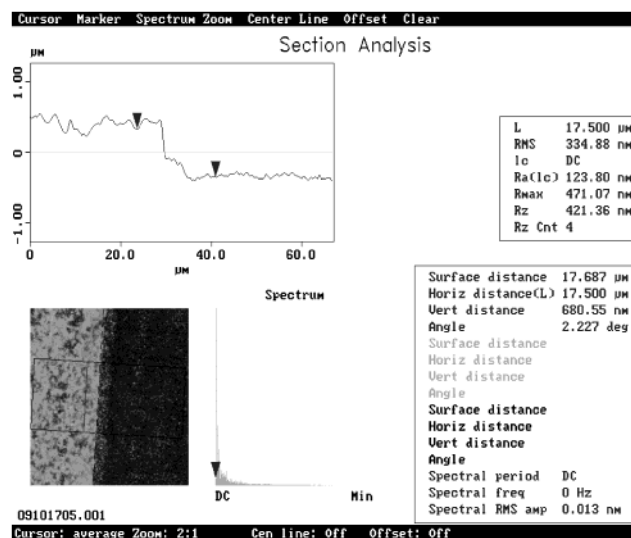
For electrochemical experiments, the anopore was attached to a piece of indium tin oxide (ITO) with electroplating tape (3M), which was cut to expose a known area of the nanowell electrode to the solution and to mask the ITO from the solution. Blank experiments were run to ensure that the taped ITO did not react in the solutions under investigation. To ensure good electrical contact between the gold and ITO, either Au was sputtered onto the back of the electrode to thicken the gold layer or the anopore was attached to the ITO with silver epoxy (EPO_TEK). Occasionally the electrodes formed with epoxy showed contamination due to cracking of the nanowell electrode allowing solution to reach the epoxy. However, electrodes formed by both methods showed identical behavior. This confirms that solution cannot penetrate to the back of the electrode in an undamaged metal-filled anodisc, because otherwise all electrodes formed by the epoxy method would show contamination.

Faradaic electrochemical experiments were carried out in order to further characterize the electrodes. Electrochemical area calculations were considered important as AFM and SEM experiments do not give us information about the seal between each individual gold nanoelectrode and the surrounding anopore. This has been observed to be a problem for nanoelectrode arrays prepared with other templates.[2] It is also possible that there are some cracks in the electrode matrix where the sides of the gold electrodes may be unattached from the anopore and can come in contact with solution.

Electrodes were initially characterized by cyclic voltammetry in a ferricyanide/sodium sulfate solution. In a 0.01 M /$K_3Fe(CN)_6$/0.05 M Na_2SO_4 solution, the ferricyanide redox peaks occur with an $E_{1/2}$ of 0.138 V vs SCE. However, the shape of the CV differs from that of a planar gold electrode in the same solution (see Figure 4), due to higher background current in the array. Peak currents were proportional to the square root of sweep rate, in accordance with the Randles−Sevcik equation which applies to a diffusion controlled reaction at a planar electrode.[8] The Randles−Sevcik equation was used to estimate the electrode area by calculating the
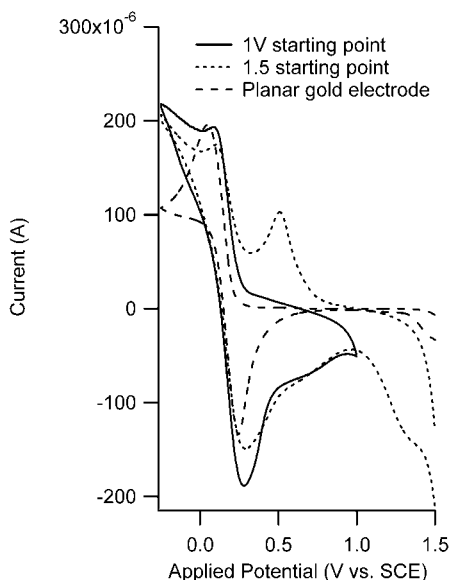
Figure 4. Cyclic voltammetry at 20 mV/s, in a Na$_2$SO$_4$/K$_3$Fe(CN)$_6$ solution showing the shape of the redox curve compared to that of the planar electrode and the presence of an extra peak for the nanoelectrode when the upper limit is set at 1.5 V.

Figure 5. Cyclic voltammetry at 10 mV/s, in a H$_2$SO$_4$ solution showing gold oxide formation and stripping.

ferricyanide diffusion coefficient at a planar gold electrode of known area. Variation was found between electrodes, but in general the experimental area was in the range of 1.3 to 2.2 times greater than the measured geometric area. We know from SEM data that 44% of the anopore surface consists of pore and therefore the gold nanorods should make up this percentage of the total geometric area. From SEM images of the copper negative used to form the nanowells it can be seen that the surfaces of the electrodes are quite rough, and this could account for the experimentally measured area being larger that the geometric area. However, from SEM and AFM imaging of the anopore membrane we know that the pore distribution is quite regular and therefore it would be expected that the ratio of experimentally measured area to geometric area would not vary very much between electrodes. This suggests that there may be a small crack density that varies from electrode to electrode.

Increasing the anodic limit for cyclic voltammetry to 1.25 V in the ferricyanide/sodium sulfate solution leads to the emergence of a peak on the cathodic sweep at 0.52 V vs SCE, which is not present when the limit of the CV is less than 1 V. On the subsequent anodic sweep a peak occurs at 0.6 V. The peak current for the cathodic peak increases by a factor of 3 on increasing the upper limit from 1.25 to 1.5 V vs SCE, and a peak is observed at 1.25 V on the anodic sweep. This behavior is similar to gold oxide formation and stripping seen in acidic solutions. These peaks do not occur for a planar gold electrode in the same solution: after bubbling oxygen through the solution, a small peak emerged at 0.58 V, but the peak height was insignificant compared to that observed with the nanowell electrode. However, it is possible that there is oxygen present on the nanoelectrode due to the anopore. This could happen in two ways. Oxygen may be selectively adsorbed onto the anopore surface, making it available for oxide formation. Alternatively, as
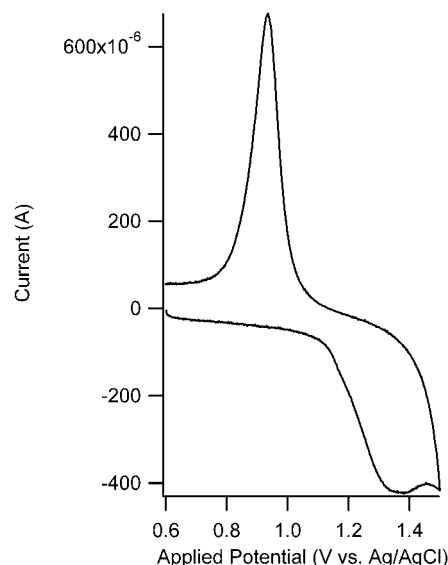
the anopore is formed of aluminum oxide, the anopore itself may be the source.

The Faradaic response of the nanoelectrode array was also investigated in acidic media (See Figure 5). In 0.5 M H$_2$SO$_4$ solution, the electrode shows the onset of gold oxidation at 1.1 V vs SCE with gold oxide stripping peak at 0.9 V. This behavior is identical to that observed for a planar gold macroelectrode in the same solution. The oxide stripping peak area was used to estimate the gold surface area of the electrode by assuming a value of 450 μC/cm^2 for gold oxide stripping at +0.9 V vs SCE.[9] The electrode area found by this method did not correlate well to the geometric electrode area, being on average 10 to 20 times greater than the geometric area. This also disagrees with the area obtained by cyclic voltammetry in ferricyanide as discussed above. One possible explanation is that the nanoelectrodes have small leakages at the sides and the size of the ferricyanide ion prevents it from entering these cracks, while the sulfuric acid may do so. Another explanation could be that along the side of the nanorods the gold is reacting with oxygen from the alumina and, upon stripping, gold oxide is being removed from the sides as well as the top surface. This is in agreement with the presence of the gold oxide stripping peak in the ferricyanide solution.

For most of the electrodes prepared, the anopore was positioned with pores normal to the sputtering target. Therefore some copper is expected to penetrate into the nanopores. During the plating step no effort was made to seal the back of the sputtered anopore electrode, and therefore copper can plate from both sides. This may be a factor in the shape of nanowells. For comparison, electrodes were prepared by sputtering at a 45° angle to see if this would affect the electrode behavior. However, no significant difference was observed as a result of this change. The area calculated by both methods showed the same discrepancy.

It has been shown that on Au surfaces dodecanethiol forms a SAM that effectively passivates the Au surface to
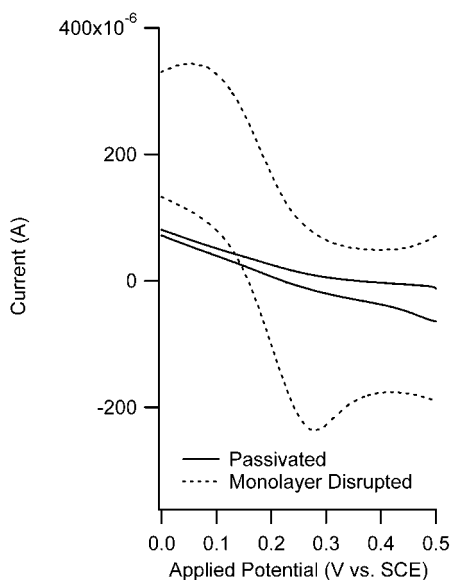
Figure 6. Cyclic voltammetry at 20 mV/s, in a $Na_2SO_4/K_3Fe(CN)_6$ solution of the passivated gold nanoelectrode ensemble shows a complete suppression of the cathodic and anodic peaks. After cycling repeatedly between 1.5 V and −0.5 V, the dodecanethiol SAM was disrupted and the free gold surfaces were exposed to the solution. The cyclic voltammetry after this process shows the expected cathodic and anodic peaks for the ferricyanide reaction.

electrochemical processes in solution.[10] Up to 99% of the surface can be passivated to cyclic voltammetry of ferro-cyanide with a small residual current being attributed to pinhole defects in the SAM. Passivation was carried out initially using a piece of the anopore epoxied to a piece of ITO. A drop of the SAM in ethanol was placed over the entire area of the anopore and left for at least 2 h under argon in a glovebox atmosphere. Then electroplating tape was used to mask the ITO and leave a small area of the nanoelectrode open to the solution. The presence of the monolayer was visible in that there was an increased reflectivity/bluish cast from the electrode. Cyclic voltammetry was carried out

initially between 0.4 and −0.1 V vs SCE and the ferri/ ferrocyanide redox peak was seen to be suppressed. As the exact area of the exposed electrode was not known, it was necessary to strip away the SAM to have a comparison. This was done by repeatedly cycling the electrode from 1.5 V to −0.5 V vs SCE. This was successful at disrupting the SAM, as can be seen in Figure 6.

In conclusion, a nanowell electrode was prepared using an anopore membrane as the template with copper electro-plating as the means to form the wells, which were found to have a depth of 680 nm on average. Cyclic voltammetry was used to characterize the electrodes, which display a charac-teristic shape and show a peak due to oxygen stripping attributed to the presence of the anopore membrane. The nanowell electrode can be successfully passivated by a dodecanethiol SAM.

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