Nanotextured Surfaces: New Generation Bioelectronic Interfaces for Nanomedicine

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Abstract:

Bioelectronics is a field of study that contributes to a growing intersection between nanostructures and nanomedicine. One application is using nanostructures for interfacing cells. This can help improve standard signal measurements from cells on a two-dimensional electrode array by using a nanotextured electrode surface instead. Nanotexturing increases the surface area to improve the adhesion of cells to electrodes and provides a more efficient electrical interface. We used a porous alumina membrane as a template to provide uniform nano-scale pores for electrodeposition of gold onto two-dimensional arrays of gold electrodes. We performed extensive characterization of electrodeposition parameters including, current density, deposition rate, nanorod uniformity, and experimental repeatability. Finally, we cultured HL-1 cardiomyocytes on the nanotextured gold arrays and characterized morphology, adhesion, and proliferation rate. These experiments confirmed our ability to culture electrically active cells on nanotextured gold electrodes.

Experimental Procedure:

We fabricated two-dimensional electrode arrays for nanotexturing using standard microfabrication techniques. The lithography was performed on glass substrates using a mask with electrodes and wires leading to bond pads. A 10 nm adhesion layer of chrome was deposited on the glass followed by a 100 nm layer of gold with the Edwards #2 thermal evaporator. Lift-off, agitating the substrate in acetone, patterned the chrome and gold.

The gold electrode array was modified using electro-deposition. Our experimental setup is illustrated in Figure 2. The electrical parameters for the deposition were controlled using a potentiostat. We built this circuit on a proto-board using two operational amplifiers, a resistor, and three electrodes operated using a power supply and a computer-controlled source measurement unit [1]. The potentiostat is used to monitor and control electrochemical reactions utilizing three electrodes. The counter electrode (CE) is used to apply a current, the reference electrode (RE) gives the solution a "chemical ground" and the working electrode (WE) allows for current flow. The counter and reference electrodes go into the solution, but they can be shorted together since they are at the same potential. The working electrode makes electrical contact with the bond pads on the patterned gold substrate. The glass slide patterned with gold was placed in between the two Teflon® cylinders, the top one containing CE, RE, and the ionic solution; this completes the circuit allowing current to flow. The electrodeposition performed with this setup is an electrochemical method for driving gold cations toward an electrode in solution. Potassium aurocyanide ($KAu(CN)_2$) was the gold ion solution placed in the electrochemical cell. Within the solution, the current

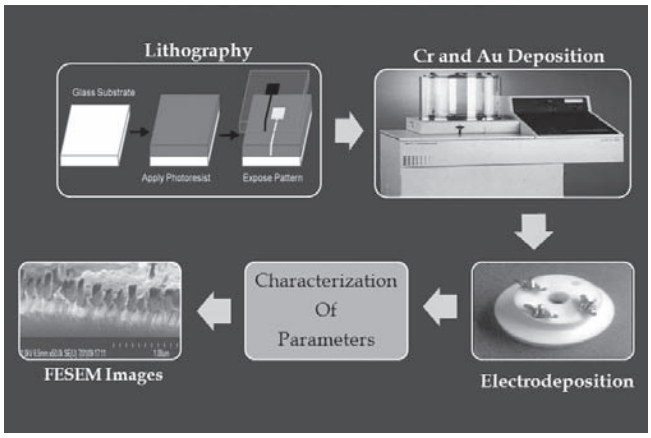
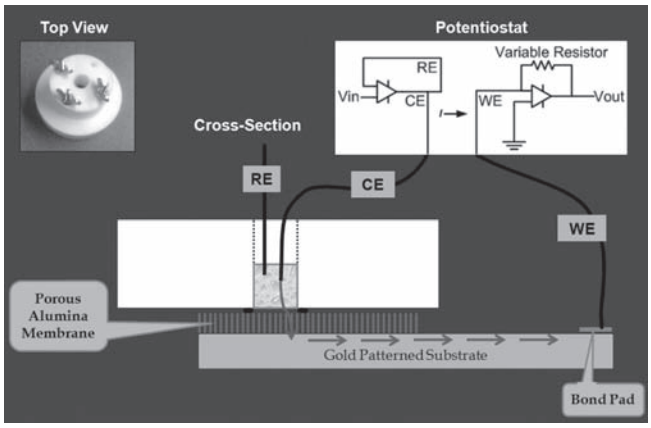


Figure 1, above: Process overview: lithography, deposition, electrodeposition, parameters characterized, and FESEM images.

Figure 2, below: Electrodeposition experimental setup includes: potentiostat circuit, three electrodes, membrane on patterned gold substrate, and potassium aurocyanide.



flow described is the movement of gold cations toward the patterned gold electrodes forming the deposited gold.

A porous alumina membrane was used as a template for the nanorod growth. The membrane is 40 μm thick and has pores with a 200 nanometer diameter. When placed above the electrode array as shown in the Figure 2, the flow of cations was restricted to the pore interior. Current flows through the pores of membrane then to the electrodes, resulting in the precipitation of gold within the pores. This results in the deposition of the 200 nm diameter gold rods with thickness depending on experimental parameters. This creates free-standing gold rods once the membrane is dissolved with sodium hydroxide.

Results and Conclusion:

One of our goals for this research was to correlate many experimental parameters with the resulting nanorod growth characteristics. We were able to use a MATLAB program for controlling the potentiostat to systematically vary the following experimental parameters: voltage, time, sampling rate, gain, and experimental run number using our potentiostat control function. All of the experiments were monitored in real-time using MATLAB plots of voltage vs. time, current vs. time, and total charge vs. time.

A wide array of experiments were performed, and we used an Excel spreadsheet to record the parameters described above, the substrate patterning and membrane use. Our experimental runs included deposition onto a gold surface with and without the membrane and gold electrodes with and without a membrane. Initial experiments allowed us to verify the complete experimental setup and determine the appropriate values for experimental parameters. We performed a total of 32 experiments, each with a different set of experimental conditions.

We used a Dektak II profilometer and a field emission scanning electron microscope (FESEM) to verify nanorod thickness and appearance. The profilometer allowed us to measure the film thicknesses. The FESEM allowed us to obtain high magnification images of our nanorods. A single row of nanorods is shown in Figure 3; the top image has a wide view of the sample at 35,000X and the bottom image focuses on the nanorods at an increased magnification of 50,000X. These images verify that the nanorods have dimensions consistent with the pore geometry in the membranes. Since we had to break the substrate to obtain a sample small enough for the FESEM, the nanorods were damaged in the process.

Ultimately we were able to culture HL-1 cardiomyocytes on the gold nanorod arrays. HL-1 cells are an electrically active cardiac cell line derived from rats. A transmitted light image was taken of the cells on the gold electrodes as seen in Figure 4. We were able to demonstrate the growth and viability of the cells on the nanorod electrodes surface. In addition we verified excellent cell adhesion, normal morphology and standard proliferation rate. Further testing will be required for a comparative study of the adhesion characteristics.

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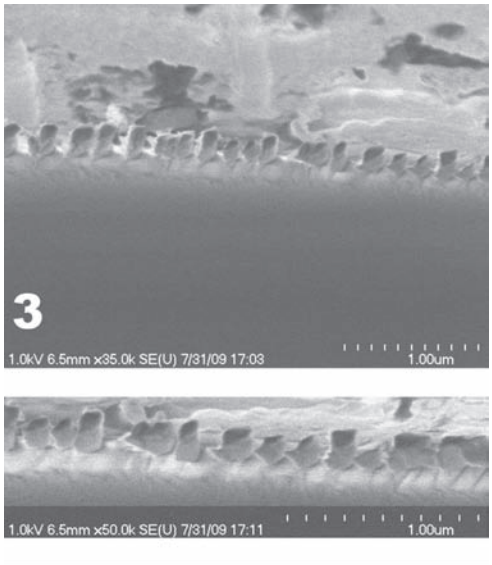

Figure 3: FESEM images; single line of gold nanorods at 35,000X and 50,000X magnification.

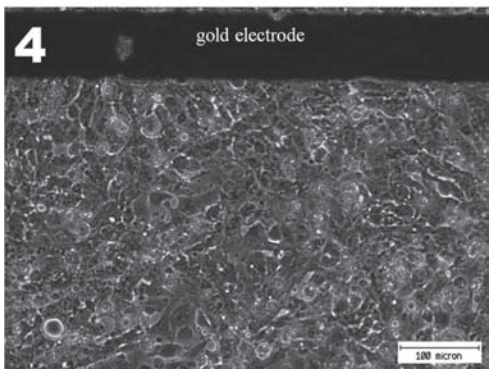

Figure 4: Phase contrast image of cultured HL-1 cardiomyocytes on Au nanorods.