Supplementary Material (ESI) for Chemical Communications

Tailored Electroactive Nanorods for Biospecific Cell Adhesion and Differentiation

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Experimental Section

***Cell Differentiation Staining**:* Oil Red O stock solution was prepared by weighing out 300 mg of Oil Red O powder and added to 100 ml of 99% isopropanol. The working solution is made by mixing 3 parts of Oil Red O stock solution with 2 parts deionized water and filtered before use. Fresh working solution was made each time and used within two hours. Cell samples were washed in PBS and fixed by 10% formalin for 30 minutes. After discarding the formalin, samples were washed with water, followed by 60% isopropanol for 2-5 minutes, and then Oil Red O working solution for 5 minutes. The sample was then gently rinsed with water to wash away the Oil Red O solution.

Scanning Electron Microscopy of Cells: Cell samples were washed with PBS and fixed by 10% formalin for 30 minutes. After discarding the formalin solution and washing with water, samples were dehydrated stepwise in 30%, 50%, 70%, 90% and 100% ethanol for 30 minutes. After using a critical point drying technique and sputtering 2 nm gold, the samples are ready for SEM imaging.

Microscopy: A phase contrast microscope was used to take cells pictures after staining with Oil Red O. All cell micrographs on flat gold surfaces were taken in transparent mode. The cell micrographs on gold nanorods substrates were taken in reflectance mode, due to the non-transparency of the substrate.

Nanorod Fabrication: Anodic aluminum oxide (AAO) templates (pore size = 50 nm) were purchased from Puyuan Nano, Ltd. (China) 800 nm of Ag (Kurt Lesker, Inc., 99.99%) was thermally evaporated onto one side of the template. The AAO/Ag substrate

was then placed silver-side down on top of a layer of conductive Ag epoxy (Part No. H_2OE, Epoxy Technology) that had been deposited onto a glass slide. The AAO/Ag/Ag epoxy/glass substrate was then cured at 80 °C for 3 hours. This substrate was then used as the working electrode in a three-electrode cell to electrochemically deposit Au into the pores of the AAO template. Electrical contact was made by connecting an alligator clamp to an exposed strip of Ag epoxy. The reference electrode was a Ag/AgCl (3 M NaCl) electrode from Bioanalytical Systems while the counter electrode was a platinum gauze electrode. The electrolyte was a gold electroplating solution purchased from Technic, Inc. (Orotemp 24, diluted by ½ with deionized water). Au nanorods with a length of ~ 500 nm were deposited by applying -1 V vs. Ag/AgCl for 5 minutes. The alumina template was then dissolved with 2 M NaOH (aq) for a period of 30 minutes.

Electrochemical Measurements: All electrochemical experiments were performed using a Bioanalytical Systems CV–100W potentiostat. Electrochemistry on SAMs was performed in 1M $HClO_4$ or Phosphate Buffered Saline (PBS) pH 7.2 using a platinum wire as the counter electrode, Ag/AgCl as reference, and the gold/SAM or gold nanorod/SAM substrate as the working electrode. All cyclic voltammograms were scanned at 50 mV/s.

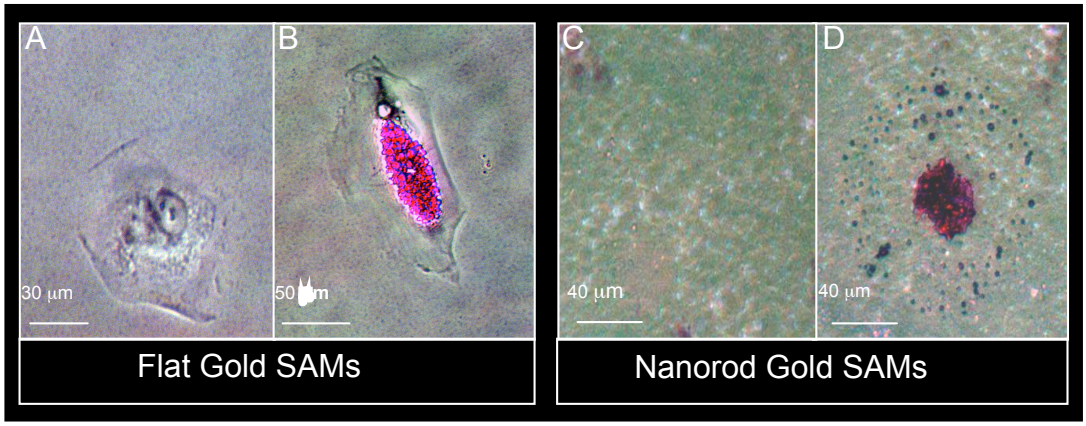


Figure 1. Comparison of phase contrast images of mesenchymal stem cells on flat gold and nanorods surfaces. (A) A undifferentiated stem cell on a flat gold surface. (B) A differentiated stem cell on flat gold surface showing red lipid vacuoles. (C) Undifferentiated stem cells on nanorods are transparent and are not seen by reflectance microscopy. (D) However, differentiated stem cells on nanorods surfaces are visible due to the red lipid vacuoles. All samples were stained by Oil Red O. Micrographs of cells on flat gold were obtained in transparent mode and micrographs of cells on nanorods were taken by reflectance mode microscopy.

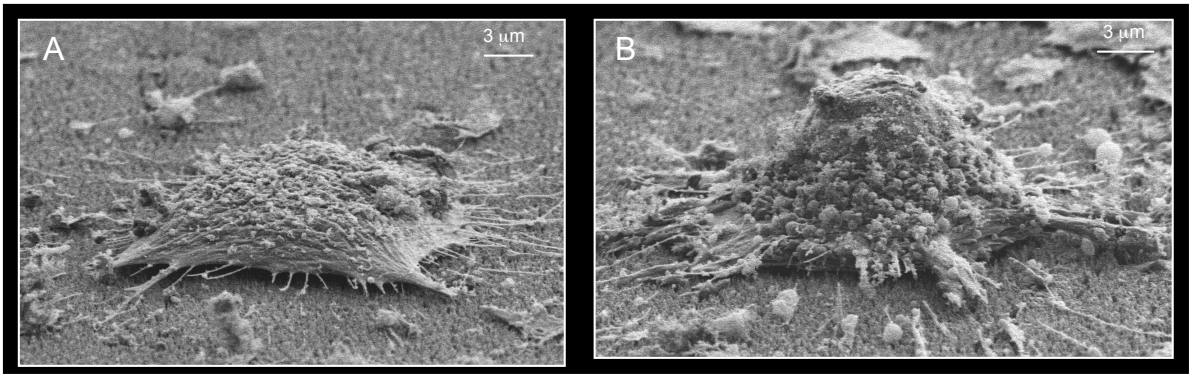
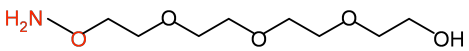
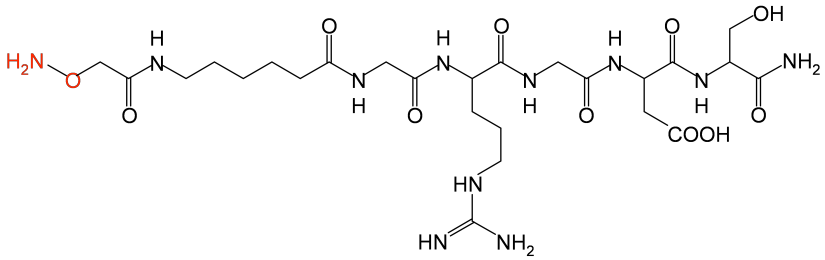


Figure 2. Scanning electron micrographs (SEMs) of fibroblasts adhered on RGD presenting gold nanorod substrates. The cells have many fillopodia appendages on the nanorods where there are much fewer on flat gold surfaces presenting RGD. The surface topology of the nanorods and/or high density of RGD molecules on the nanorods cause the cells to modulate their behavior. All samples were fixed, dehydrated and critical point dried before SEM images were obtained.



Tetra-ethylene(glycol)-oxyamine



RGD-oxyamine

Figure 3. Structures of molecules (RGD-ONH_2 and tetra-ethylene(glycol)-ONH_2). The RGD molecule is a ligand found in the extracellular matrix protein fibronectin and is recognized by cell surface receptors for biospecific adhesion. The tetra-ethylene(glycol)-ONH_2 molecule is used to react with immobilized quinone molecules to generate an inert surface that resists non-specific protein adsorption and cell attachment.