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Cell responses to metallic nanostructure arrays with complex geometries

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ABSTRACT

Metallic nanopillar/nanowires are emerging as promising platforms for biological applications, as they allow for the direct characterization and regulation of cell function. Herein we study the response of cells to a versatile nanopillar platform. Nanopillar arrays of various shape, size, and spacing and different nanopillar-substrate interfacial strengths were fabricated and interfaced with fibroblasts and several unique cell-nanopillar interactions were observed using high resolution scanning electron microscopy. Nanopillar penetration, engulfment, tilting, lift off and membrane thinning, were observed by manipulating nanopillar material, size, shape and spacing. These unique cell responses to various nanostructures can be employed for a wide range of applications including the design of highly sensitive nano-electrodes for single-cell probing.

1. Introduction

Metallic nanopillar/nanowire arrays are becoming widely recognized as promising platforms for characterizing and regulating cell functions. For example, nanowire arrays are used as a high-throughput method for direct cytosolic biomolecule delivery, and thus may be used in a wide range of potential applications, such as drug delivery, gene transfection and immunofluorescent staining [1,2]. Furthermore, nanowires have been used for the stimulation and recording of single neuron activities [3,4], localized fluorescent imaging [5], neuron pinning [6], and most recently, exploring mechanisms of cell nanotopography sensing [7]. Nanowires of complex geometries have occasionally been used for specific cellular applications; hollow 100 nm alumina nanowires were able to pierce cells, and form fluidic pipelines efficiently delivering ions directly into the cytosol [8]. Mushroom shaped gold electrodes were used to activate cell phagocytic-engulfment mechanisms, and form strongly sealed membrane–electrode interfaces for detection of subthreshold synaptic and action potentials [9].

Over the recent years, numerous studies have investigated cell–nanowire interactions with a focus on the ability of nanowires to penetrate cells [10–13]. However, with the exception of long nanowires (diameter to height ratios greater than 10), which have been shown to impair cell function [14], in most cases, nanopillars do not penetrate cells. Studies suggest that the overlaying cells form adhesions on the nanopillar surfaces [10,11]. Moreover, when penetration does occur, it is likely mediated by adhesion dependent forces [13]. Herein, we investigate the interactions of 3T3 Swiss Albino fibroblast cells with arrays of metallic nanopillars of various shape, size and spacing. Nickel nanopillars of various geometries were fabricated on a single platform allowing the direct assessment and comparison of single nanopillar–cell interactions. Hollow, x-shaped, c-shaped and mushroom-shaped nanopillars were also fabricated to study the response of cells to nanostructural features of nanopillars. Finally, we assessed the response of cells to

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palladium nanopillars of similar geometries which were weakly bonded to the flat substrate underneath, allowing the inspection of another potential effector of cell-nanopillar interaction.

2. Experimental methods

2.1. Nanopillar fabrication

Nickel and palladium nanopillars were fabricated using electron beam lithography and electroplating techniques [15,16]. Silicon substrates were coated with a ~20 nm adhesive layer of titanium and ~30–100 nm gold using electron beam evaporation. A layer of Poly methyl methacrylate (PMMA) photoresist was then spin-coated on the substrate with various speeds to obtain desired thicknesses. Following PMMA coating, a variety of hole diameters and cross sectional geometries were patterned on the resists by an electron beam with a 100kv acceleration voltage using a Leica EBPG 5000 + electron beam lithography system. Nickel and palladium metals were electroplated into the patterned arrays using Galvanostatic electroplating techniques. Watts-type nickel electroplating solution was made in-house, containing 300 g/l of nickel (II) sulfate hexahydrate (99%, Sigma Aldrich), 30 g/l nickel (II) chloride (98%, Sigma Aldrich), 1.9 g/l of saccharin (98%, Sigma Aldrich) and 30 g/l of boric acid (BX0865, EMD Millipore) [17]. A commercially available palladium tetrammine (II) chloride based electroplating solution (Technology Without Limits, Inc.) was used for fabricating palladium nanopillars. Electroplating duration and current densities were adjusted to obtain desired nanopillar heights. Mushroom shaped nanopillars were fabricated by extending the electroplating duration and allowing the growth of metals above the photoresist thickness. The photoresist films were removed by submerging samples into a bath of acetone and samples were further cleaned with a final rinse of acetone and isopropanol.

2.2. Cell culture

3T3 Swiss Albino fibroblasts were maintained in Dulbecco's Modified Eagle Medium (DMEM; Lonza, USA) supplemented with Fetal Bovine Serum (FBS; Lonza, USA) and penstrep (Invitrogen, NY, USA) at 37 °C, 5% CO_2 and 95% humidity. Cell culture medium was replaced every 2–3 days. Samples were sterilized using UV

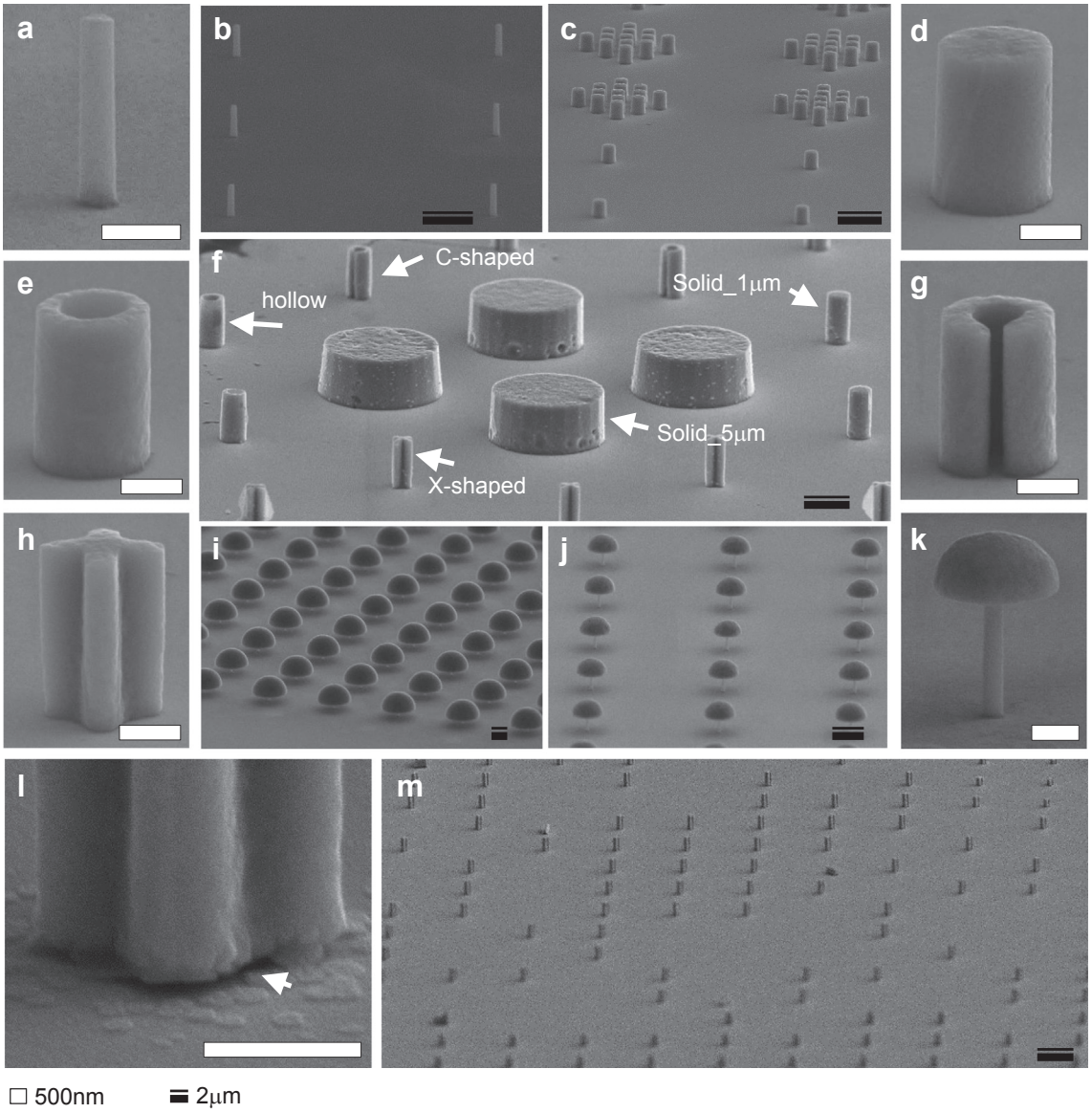

Fig. 1. SEM micrographs of nanopillars of various size, shape and materials. (a) a representative image of ~220 nm diameter, high aspect ratio nickel nanopillar, (b) array of 220 nm nickel nanopillars with 10 μm spacing, (c) arrays of clustered and individual 600 nm nanopillars on a single substrate, (d) a representative image of 1 μm solid nanopillar, (e) representative image of 1 μm outer diameter hollow nanopillar, (f) arrays of solid, hollow, c-shaped and x-shaped nanopillars with 1 μm outer diameters and 10 μm spacing, and solid 5 μm disk shapes, (g) representative image of c-shaped nanopillar with 1 μm outer diameter, (h) representative image of x-shaped nanopillar with 1 μm outer diameter, (i), (j) arrays of mushroom-shaped pillars with various mushroom cap sizes and 10 μm spacing (k) representative image of a mushroom shaped nanopillar with a ~220 nm stem, (l) image of palladium cross-shaped nanopillar showing a small gap at the interface of the nanopillar with the gold substrate underneath (arrow), (m) arrays of palladium nanopillars, several pillars were detached from the substrate during fabrication, indicating weak interfacial bonds between nanopillars and the gold substrate. All white scale bars represent 500 nm and black scale bars represent 2 μm.