Transfer of Vertically Aligned Carbon Nanofibers to Polydimethylsiloxane (PDMS) While Maintaining their Alignment and Impalefection Functionality

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ABSTRACT: Vertically aligned carbon nanofibers (VACNFs) are synthesized on Al 3003 alloy substrates by direct current plasma-enhanced chemical vapor deposition. Chemically synthesized Ni nanoparticles were used as the catalyst for growth. The Si-containing coating (SiN_x) typically created when VACNFs are grown on silicon was produced by adding Si microparticles prior to growth. The fiber arrays were transferred to PDMS by spin coating a layer on the grown substrates, curing the PDMS, and etching away the Al in KOH. The fiber arrays contain many fibers over 15 μm (long enough to protrude from the PDMS film and penetrate cell membranes) and SiN_x coatings as observed by SEM, EDX, and fluorescence microscopy. The free-standing array in PDMS was loaded with pVENUS-C1 plasmid and human brain microcapillary endothelial (HBMEC) cells and was successfully impalefected.

KEYWORDS: carbon nanofibers, nickel, nanoparticles, catalyst, PECVD, transfection, nanomanufacturing

INTRODUCTION

Vertically aligned carbon nanofibers (VACNFs) have shown great utility for chemically and electrically addressing tissues and cells.[1−3] VACNFs are structurally different from carbon nanotubes (CNTs), though the two are often conflated in the literature due to similarities in their morphology and graphitic nature. In carbon nanofibers, graphene layers are arranged in stacked cone or cup structure, whereas in nanotubes, graphene layers are rolled into cylinders that span the whole length of a nanotube. Such structural differences result in diverging physical and chemical properties. Carbon nanofibers are more amenable to chemical and biochemical functionalization as well as possessing much higher electron transfer rates at the graphene edges on the sidewalls than CNTS.[4−10] Vertically aligned carbon nanofibers can be synthesized in a deterministic process that is compatible with most of the microfabrication techniques, with alignment controlled by the plasma environment.[11−17] It has been shown that VACNFs can be grown as an array of free-standing structures.[18] In contrast, vertically aligned carbon nanotubes require a presence of supporting neighbors to maintain alignment perpendicular to a substrate.[19] Second, it has been shown that VACNFs can withstand a variety of postsynthesis processing steps such as silicon dioxide and silicon nitride film deposition, electroplating, photoresist spinning (of various viscosities from S1813 to SU-8) as well as resist removal, and dry/wet etching.[20,21] This made them an attractive structure for integration into microfabricated devices such as electron field emission arrays, scanning probe tips, electrochemical probesn and neuronal multielectrodes arrays, and cell mimic microfluidic devices.[22−32] Third, VACNFs have

been shown to withstand mechanical interfaces with live tissues and cells arrays. For example, hippocampal tissues slices could be pressed onto VACNF multielectrode array and then removed many times without loss of functionality, though it should be noted that a silicon nitride conical side coating was added to enhance the mechanical strength of the freestanding fibers.[33] Even when VACNFs are grown on metal substrates, a thin sacrificial layer of Si was used to add robustness to freestanding nanofibers.[34] In this work, Si microparticles were used for Si source. VACNF arrays have been used in laboratory demonstrations of transfection by cell impalement, resulting in viable cells expressing proteins encoded on DNA physically inserted into their nuclei.[2] The insertion of genetic material into a cell for the purpose of generating desired proteins, by using a physical structure to impale the cell is termed "impalefection."[35] Impalefection is useful for in vitro experiments[23,35−37] associated with exploring gene therapy, but has yet to be used in a therapeutic or diagnostic technology. VACNFs can be grown as free-standing, sparse arrays, similar to a bed of nails making them ideal for impalefection, as opposed to CNTs which can be thought of as a carpet and therefore less useful for impalefection.[7] VACNF arrays are limited in their utility in part by the expensive, inflexible Si substrate that they are typically grown upon. Replacing the Si is a nontrivial task because the substrate must satisfy requirements for chemical compatibility with the catalyst nanoparticles (NPs) and

VANCFs, as well as remain intact throughout the VACNF growth process, which typically involves temperatures of ~700 °C and plasma processing. Perhaps the most important role of the Si substrate is providing a silicon source for the SiN_x coating that is formed on the fibers during plasma-enhanced chemical vapor deposition (PECVD) growth. This SiN_x coating imparts fluorescence properties in the visible spectrum, which allows the fibers to be easily imaged by fluorescence microscopy. The coating is also thought to improve the mechanical properties of the fibers as seen by the ability of coated fibers to remain vertically aligned even after spin coating polymer solutions such as photoresist, in addition to possibly reducing the cytotoxicity compared to other uncoated carbon structures.[38−40] Work has also been performed on CNTs, which reveals that CNTs usually deform upon solution coating.[41,42] VACNF arrays on flexible substrates have been attempted previously[29] and, if the substrate is biocompatible, may be particularly useful for biomedical applications.[43] Another attraction to flexible substrates for VACNFs is for impalefection of tissues. The primary goal of this research is to lay the groundwork for an angioplasty balloon with VACNFs, so that upon inflation the blood-brain barrier will be penetrated and genetic material or drugs can be delivered. Current schemes for transferring VACNF arrays to flexible substrates rely on nonbiocompatible materials and processing conditions that are difficult to reproduce.[29] Here, we demonstrate a process that expands the utility of VACNFs toward impalefection schemes by replacing the typical Si substrate with inexpensive Al and replacing the e-beam evaporated catalyst deposition step with air-brushed Ni nanoparticles (NiNPs). Furthermore, we show that the fiber array may be transferred to a free-standing polydimethylsiloxane (PDMS) film (following chemical etching of the Al substrate) and the array will maintain the ability to transfect cells.

■ EXPERIMENTAL SECTION

An overview of the fabrication process from catalyst synthesis to a free-standing PDMS film containing the VACNF array is depicted in Figure 1.

Nickel Nanoparticle Synthesis. 100 nm NiNPs (Figure 2a) were synthesized according to a modified procedure developed by Wang et al.[44] A mixture of, 5.0 mL of 1-octadecene (90%, Sigma Aldrich), 5 mL of oleylamine (80−90%, Acros), and 0.50 g of nickel(II) acetylacetonate hydrate (98%, TCI America) was mixed and allowed to degas under vacuum in a three-necked round-bottomed flash for 120 min at 60 °C. The solution was then backfilled with N_2 and heated at a rate of 10 °C/min to 240 °C, where the temperature was held for 30 min. The Ni NPs were then flocculated by adding ethanol, and the supernatant was discarded following centrifugation at 5000 rpm. The NPs were then redispersed in hexanes. After centrifuging the solution a second time, the supernatant was discarded, and the NPs were redispersed in heptane for airbrushing.

Airbrushing. The heptane solution containing the Ni NPs was airbrushed onto sections of 0.016 in. thick 3003 Al (McMaster Carr) using an Iwata Eclipse HP-CS airbrush operating at 50 psi. Prior to airbrushing, the Al sections were sonicated in acetone and rinsed with methanol to remove any contaminants. The NPs were deposited in short (0.5−2 s) bursts at a 0.3 m distance from the substrate. Heptane was chosen because it gave a more uniform pattern of deposited NPs than other solvents (results not shown).

Silicon Deposition. Si serves an important role in the synthesis of VACNFs for impalefection. During synthesis, Si sputtered by the plasma reacts with ammonia to form a SiN_x coating on the VACNFs. This coating is thought to enhance the mechanical stability of the VACNFs which is important for cell impalement. In order to achieve this same coating when using an Al substrate, small, surfactant-free Si
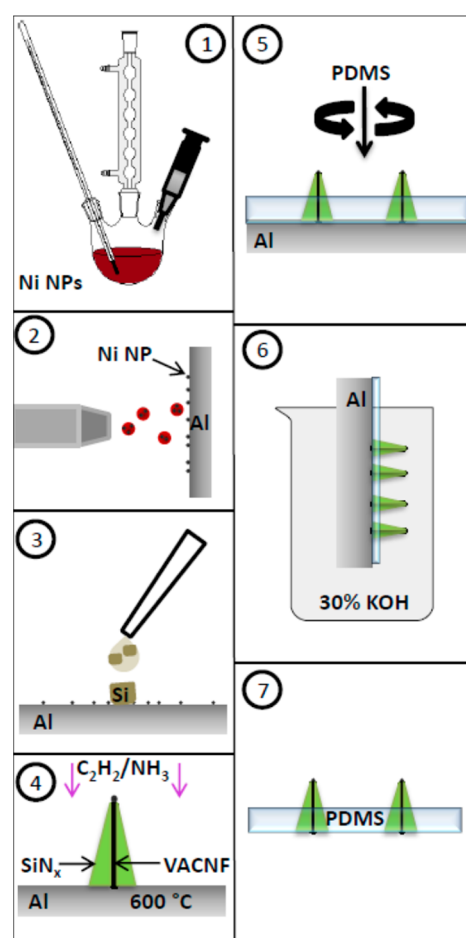

Figure 1. (1) Synthesis of 100 nm NiNPs. (2) Airbrushing of NiNPs dispersed in heptane onto an Al substrate followed by air drying. (3) Dropcasting of Si microparticles (1−3 μm in size) to provide a Si source for fiber coating. (4) VACNF growth by catalytic activity of the Ni NPs in PECVD from a mixture of acetylene and ammonia. A SiN_x coating forms on the VACNFs as the Si is sputtered from the microparticles. (5) Spin-casting of PDMS on top of the fiber array, followed by curing. (6) Dissolution of the Al substrate in 30% KOH at 80 °C. (7) The VACNF array remains aligned with the tips of the fibers exposed in a free-standing PDMS film.

microparticles ranging from 1 to 3 μm in size (Atlantic Equipment Engineers) were dispersed in acetone (~0.5 g of Si to 10 mL of acetone) for deposition onto the substrate. The dispersion was sonicated for 20 s and 50 μL were immediately dropcast onto each substrate.

VACNF Synthesis. VACNFs were synthesized by direct-current PECVD in a custom built system. The catalyst-coated substrate was heated to 600 °C under a 0.05 Torr vacuum, and then the chamber was backfilled with 4 Torr NH_3. Acetylene was then introduced to the chamber for 10 s prior to striking the plasma. The NH_3/C_2H_2 flow ratio was 85 sccm/45 sccm, and the pressure was maintained at 4 Torr throughout the remainder of the process. The growth took place for 2 h at a current of 0.5 A, and temperature of 600 °C. The chamber was evacuated after the growth, and the substrate was allowed to cool to room temperature under vacuum.

PDMS Deposition. PDMS was mixed with cross-linker (Sylgard 184 kit) according to the standard procedure, in a 10:1 ratio. For deposition onto the Al substrates, a drop of the PDMS mixture was placed on the substrate and spun at 5000 rpm for 3 min, followed by curing on a hot plate at 150 °C for 1 h.

Aluminum Etch. To release the PDMS film and the VACNF array, KOH was used to dissolve the Al substrate. A KOH solution (30% by
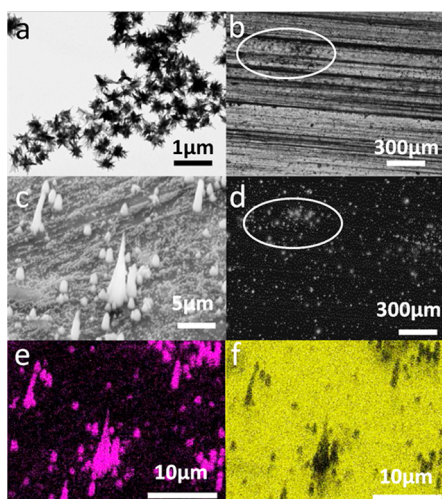
Figure 2. Contrast has been enhanced for easier viewing (a) Bright-field TEM micrograph of chemically synthesized NiNPs; (b) optical micrograph of Al substrate after VACNF growth, circled area contains fibers; (c) secondary electron image of the substrate in b; (d) fluorescence optical micrograph of the same area in b with a 360 nm bandpass excitation filter cube and 410 nm illumination, circled area shows fiber fluorescence; (e) EDX map showing the location of silicon in the same region as panel c; (f) EDX map showing aluminum in the same area as panel c.

mass) was heated to 80 °C, and the substrate was submerged for 20–30 min with continuous stirring, which dissolved it completely. The free-standing PDMS film was removed from the solution and rinsed with deionized water. Changing the etch time and fraction of the substrate immersed can yield a PDMS film with no metal attached or a film with only some areas supported by metal, effectively making windows in the substrate below.

Cell Transfection. A 5 mm ×5 mm section of the Al substrate with VACNFs embedded in the PDMS film was back-etched for 3 h in 1 M KOH at room temperature, yielding a substrate with some portion of thin Al and free-standing PDMS film. The conventional cell pellet on concavity slide method (pellet of cells, prewet DNA functionalized chip, press chip against cell pellet) was used to integrate the VACNFs with the cells, as previously described.[2] HBMEC cells and the plasmid pVENUS-C1 were used. The PDMS side of the chip was etched with a water plasma for about 15 s to promote wetting and cell adhesion prior to wetting with DNA solution. This initial experiment yielded low transfection rates. Only a small number of cells on the DNA-spotted region of the 5 mm × 5 mm chip were successfully transfected. However, none of the impalefected cells adhered to the substrate as previously described using Si substrates.[2] The transgene-expressing cells appeared pierced, but were elevated above the substrate. Thus, a larger number of cells may have been transfected, but not retained on the device during overnight imaging because of poor adhesion. Figure 4 shows a transfection, and an internalized nanofiber (visible with TRITC green/red filter).

■ RESULTS AND DISCUSSION

VACNFs were grown by direct-current PECVD on Al foil using solution-processed NiNPs as the catalyst material (Figure 1).[45] (See Supporting Information for review only) 100 nm NiNPs were synthesized by previously reported methods,[44] then suspended in heptane and airbrushed onto sections of Al 3003 alloy foil. Al was chosen because it is inexpensive and can be easily dissolved in KOH. The substrates were heated to 600 °C under vacuum and subjected to an acetylene and ammonia plasma at 4 Torr for 2 h. Initial growths without a Si source resulted in small fibers that were overetched by ammonia

during synthesis (results not shown). Depositing Si micro-particles by dropcasting prior to VACNF growth results in the formation of SiN_x coatings on the fibers that are comparable to growth on Si substrates, where the Si is sputtered and reacts with ammonia ions (Figure 1). Energy-dispersive X-ray spectroscopy (EDX) mapping indicates that Si is present on the VACNFs but not on the Al substrate between the fibers, except for the Si microparticles added prior to growth.[45] Given the combination of green luminescence and EDX signal for Si (Figure 2), it is likely that a SiN_x species forms preferentially on the sidewalls of the fibers. Dangling Si, C, and N bonds can account for the green emission.[46]

Transferring VACNFs to a flexible membrane while leaving enough of the fibers protruding for cell impalement requires control over the film thickness. Spin-casting is universally used in the semiconductor industry to produce polymer films of photoresist on flat substrates with well-defined thickness and uniformity. Spin-casting was used here to produce a PDMS film thin enough to allow for many VACNFs to protrude, while remaining thick enough to have the mechanical robustness to be free-standing. PDMS was chosen because it is biocompatible, flexible, and chemically inert. The Al substrates have a roughness (standard deviation of height measurements) of about 3 μm, as measured by profilometry (results not shown), which determines the lower limit for PDMS film thickness in order to obtain a continuous film. After growth, the VACNFs varied in height between ∼4–20 μm because of agglomeration of the catalyst NPs. Fibers grown from smaller, non-agglomerated catalyst particles run out of catalyst more quickly due to sputtering, which subsequently leads to etching back of the fiber over the long growth time.[47] Therefore, we chose to produce PDMS films 7–10 μm thick, in order to sufficiently exceed the surface roughness of the substrate. This thickness is close to that of LDPE plastic wrap commonly used for food storage (12.7 μm). The films were deposited by spin coating a standard Sylgard 184 PDMS mixture according to methods reported for PDMS on Si.[48] Capillary action of the PDMS solution on the sidewalls of the VACNFs during spin coating causes the film to be thicker near the fiber base but still leaves much of the fibers exposed (Figure 3a).

Release of the film from the Al substrate is performed by KOH etching at elevated temperature (80 °C). PDMS is largely resistant to KOH etching and no noticeable damage or swelling occurred during release. The free-standing arrays (Figure 3) contain a sparse population of fibers with the mechanical robustness and length necessary to impale and transfect cells.

After the KOH etch, a UV-ozone plasma treatment was performed to increase the hydrophilicity of the substrate and consequently increase cell adhesion. 100 ng of pVENUS-C1 plasmid was suspended in one μL of nuclease-free water and dried onto the surface of a small region of the VACNF-PDMS film. The side of the film with the exposed VACNFs was then pressed into a pellet of human capillary endothelial cells (HBMEC) and incubated overnight using methods described elsewhere.[2] The sparse density of long, robust fibers in the DNA-spotted region of the array resulted in a small number of transfections, evident by the expression of the Venus fluorescent protein in the localized spotted region (Figure 4). The low transfection rate could stem from the low-density of emergent fibers from the PDMS, allowing cells to roll off the nanofiber tip and down to the substrate. Future efforts to provide higher density of emergent nanofibers are anticipated to increase transfection rates by improving the penetration and
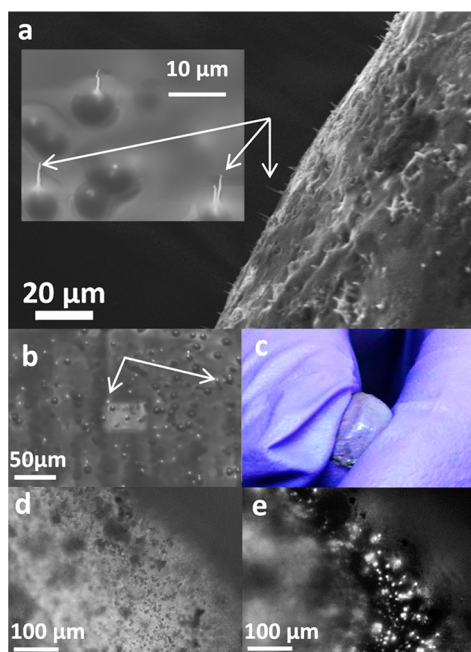
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Figure 3. SEM micrographs of (a) free-standing PDMS film taken at a fold in the film showing protruding VACNFs, inset shows fibers on a planar area of the PDMS film, with a great deal of charging. Arrows denote fibers; (b) 30° tilted image of PDMS-coated VACNF array prior to KOH etch, arrows point to fibers protruding from PDMS film; (c) free-standing PDMS film with VACNF array stretched over a gloved finger; (d) transmission optical micrograph of free-standing PDMS film with an embedded VACNF array; (e) fluorescence optical micrograph of the same area of the film as in (d) under a 360 nm bandpass excitation filter cube with 410 nm illumination.
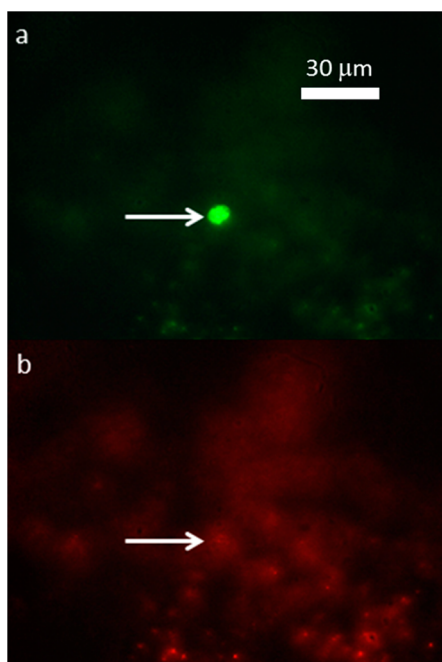

Figure 4. Fluorescence microscopy images of (a) a transfected cell expressing the Venus fluorescent protein using a YFP filter set that transmits fluorescence from both the Venus fluorescent protein and the VACNFs and (b) the same location with a TRITC filter, such that the Venus fluorescent protein is not excited, but the VACNFs emit red.

retention of individual cells. This successful transfection shows that our simple and inexpensive approach to conduct impalefection using flexible substrates is effective, which can be further optimized by controlling the density of VACNFs protruding through the PDMS film.

CONCLUSIONS

We have demonstrated the ability to grow VACNFs on Al substrates by PECVD using chemically synthesized Ni NP catalysts. EDX and optical fluorescence measurements indicate that the SiN$_x$-containing coating observed in fibers grown on silicon can be reproduced for fibers grown on Al when Si microparticles are added to serve as a source of Si. The fibers can successfully be incorporated into PDMS films, while maintaining their alignment and impalefection functionality. These results demonstrate a simple and reliable method, by which we have brought the impalefection platform to flexible substrates. This development is a critical first step toward the creation of an angioplasty balloon with blood−brain barrier piercing capabilities for gene and drug delivery.

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Notes

The authors declare no competing financial interest.
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