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Nanostructured biointerfaces created from carbon nanotube patterned porous silicon films

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ABSTRACT

Interfacing mammalian cells with single-walled carbon nanotubes (SWCNTs) has been considered a potential route for various bioengineering applications. Here, the interaction between human neuroblastoma cell line, SK-N-SH, with catalyst-free SWCNT-decorated porous silicon (pSi) substrates is investigated. SWCNT-decorated surfaces were fabricated by chemically attaching carboxy-functional SWCNTs to pSi functionalized via an amino silane. SWCNT attachment was confirmed by atomic force microscopy and Raman spectroscopy. Patterning of the amino silane on pSi by photolithography permitted the creation of patterned SWCNT-decorated pSi substrates. The number of cells attaching to the SWCNT decorated surface was observed to correlate with SWCNT density, implying that vertically aligned SWCNTs allowed the capture of cells. By incorporating a low fouling PEG silanization surface modification into the procedure, cell patterning was achieved on the fabricated SWCNT patterns. These SWCNT-decorated pSi substrates could potentially find application in lab-on-chip devices, particularly as platforms for the electrical stimulation of neuronal cells.

1. Introduction

Carbon nanotubes (CNTs) have generated a great deal of interest in the field of nanotechnology due to their unique physical, electrical, and mechanical properties [1]. Much of the interest has focused upon the integration of CNTs into devices for improved performance, particularly in the areas of field emission [2–4], electronics [5,6], and enhanced functional materials [7,8]. The utilization of CNTs in the majority of these fields requires the control of their placement, orientation and coverage on a surface [9]. Therefore, it is important to develop methods for the precise placement of CNTs on a surface. Creating such surface environments has been pursued intensely by means of techniques such as lithographically designed catalyst placement for chemical vapor deposition (CVD) CNT growth [10–12], soft lithography manipulation of CVD grown CNTs [13], inkjet printing of CNT solutions [14], and CNT adsorption or chemical attachment onto lithographically designed surface patterns [15].

It is well established that mammalian cells are able to detect and process topographical cues on a surface down to the nanoscale [16]. CNT-decorated surfaces are prime examples of controlled nanostructured topographies which can be presented to cells in order to achieve cell guidance and cell stimulation. The ability of such surfaces to effectively interface with cells may be useful for tissue regeneration in general, bone bioengineering, gene transfection and neural stimulation in particular [17–20]. Several recent studies have focused on cell interactions with CNTs bound to planar surfaces [21,22]. These studies have provided insights into cell morphology, proliferation and migration in direct response to surfaces decorated by CNTs. For example, Giannona et al. [23] showed that spreading, adhesion and proliferation of osteosarcoma cells were enhanced and cell bodies were markedly elongated on vertically aligned multi-walled CNTs on a silicon substrate in comparison to conventional tissue culture plastic. Recent reports have shown that patterned silicon nanotubes and nanowires can record and stimulate neuronal activity in cultures of rat cortical neurons [24]. This work allows electrical currents to be measured within many cells and permits control of neurons over several days with single-cell resolution. However, the patterned nanowires investigated did not lead to an improvement in resolution over other methods of cellular interrogation, such as patch-clamp recording [25], due to the large diameter of the nanowires (150 nm). An alternative approach to achieve high-resolution single-cell interrogation could be to use a patterned array of vertically aligned CNTs.

Carbon nanotube specific cytotoxicity is not an issue for surface-bound CNTs because CNTs are not taken up into the cell interior, although they have been reported to pierce cell membranes [20,26,27]. However, the effect of catalyst particles from CNTs grown by CVD potentially compromises cell viability, since commonly used transition

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metal catalysts such as nickel and cobalt have been found to induce cytotoxicity [28]. For this reason, recent research has focused on fabricating catalyst-free surface bound CNTs. The simplest approach to achieve this is the chemical attachment of functionalized CNTs to a chemically modified surface [9,29]. Indeed, smart architectures of vertically aligned single-walled CNTs (SWCNTs) have been prepared for application in field emission [30], photovoltaics [31], and biological and chemical sensing [32,33].

Porous silicon (pSi) is an ideal biomaterial due to its biodegradability, large internal surface area and biocompatibility, and has found application in biosensor design, tissue engineering and drug delivery [34–38]. We have recently reported both the chemical attachment of functionalized SWCNTs to amino silane modified pSi [39] and the formation of silane patterns on pSi [40]. Here, we present the chemical attachment and patterning of SWCNTs on pSi for the first time (Fig. 1). The ability of the SWCNTs to capture human neuroblastoma cells is investigated and cell patterning is achieved.

2. Materials and methods

2.1. pSi fabrication

pSi fabrication was completed in a custom-built Teflon etching cell following methods described in detail previously [41]. Silicon wafers (100 orientation, boron doped, 3–6 Ω cm resistivity, Virginia Semiconductor, USA) were etched in a 1:1 (v/v) solution of 48% aqueous hydrofluoric acid (HF, Merck, Germany):ethanol (100% undenatured, Chem-Supply, Australia) for 90 s at an applied current of 66 mA over a surface area of 1.767 cm^2. After etching, the HF was removed and the wafer was rinsed sequentially with methanol, acetone, and dichloromethane before being dried in a stream of nitrogen gas.

2.2. pSi functionalization

After etching, the pSi surfaces are Si–H terminated. In order to attach SWCNTs, the pSi was first oxidized and then reacted with 3-aminopropyltriethoxysilane (APTES, 99%, Sigma, Australia). Ozone oxidation was carried out by exposing freshly etched pSi to ozone using a Fischer ozone generator with the current set at 1.2 A and an ozone flow rate of 3.2 g h^{-1} for 20–60 min (20 min for entire surface attachment, 60 min for patterned attachment). The oxidized pSi wafer was then immersed into a solution of 0.5% (v/v) APTES in dry toluene for 5 min. The wafer was washed sequentially via immersion in chloroform, acetone (both Ajax-Finechem, Australia), and MilliQ water, then dried under a stream of nitrogen gas. The APTES modification produces an amine-functionalized surface, which can be used for carbodiimide mediated attachment of carboxy-functional SWCNTs.

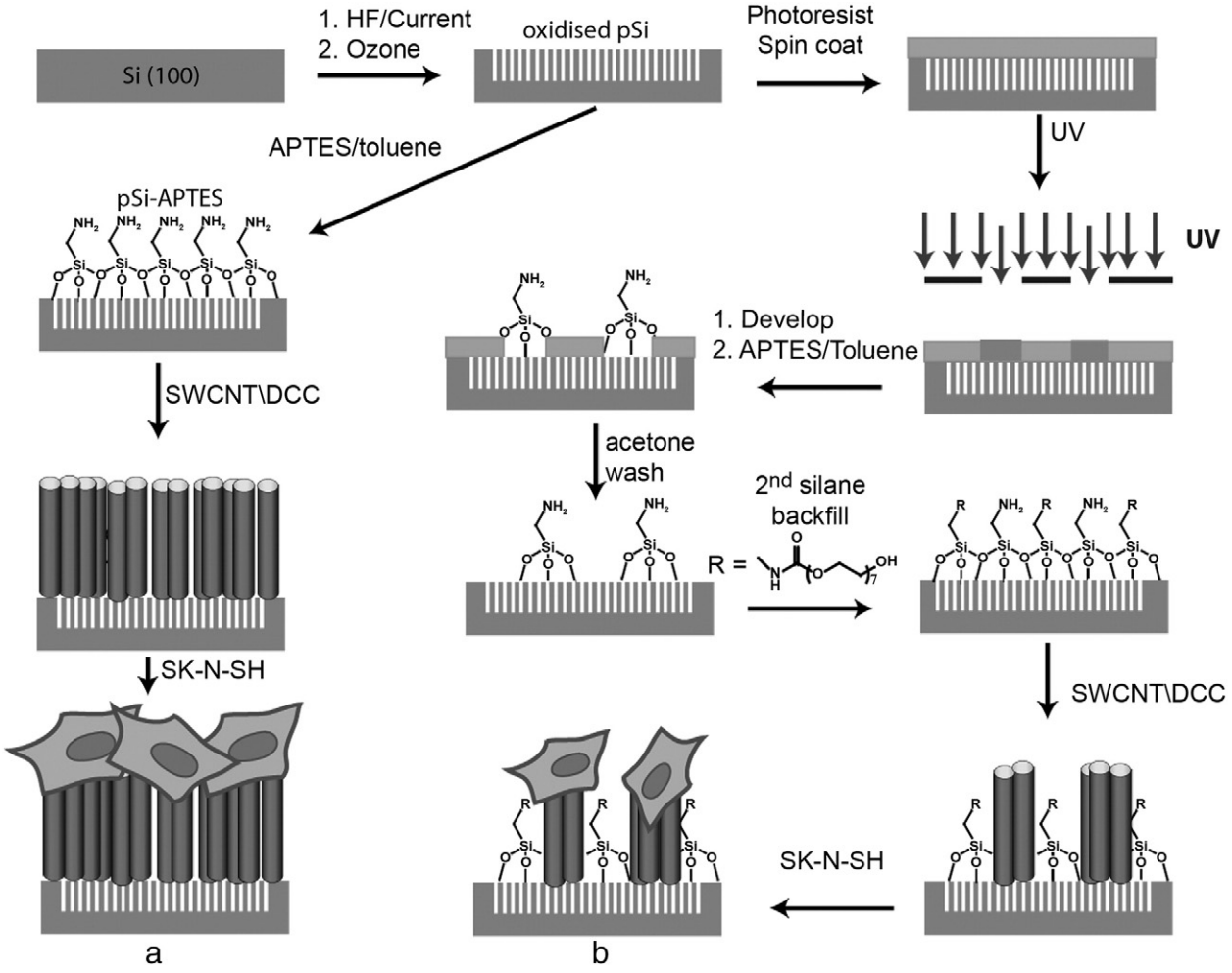

Fig. 1. Schematic detailing the preparation of (a) SWCNT-decorated pSi substrate surfaces and (b) patterned pSi-SWCNT substrate surfaces and the resulting attachment of neuroblastoma cells (SK-N-SH). Both preparation schemes involve the chemical attachment of SWCNTs to an amino silane (APTES) on pSi via dicyclohexyl carbodiimide (DCC) assisted coupling.