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Carbon nanopillars for enhanced stem cell differentiation and dopamine detection

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Introduction
Parkinson's disease is characterized by a deficit of dopamine in the brain, a neurotransmitter involved in the motor function. One of the future ideas for treatment is cell replacement therapy. Our group has previously shown that pyrolysed 3D carbon micropillars induce spontaneous differentiation of human neural stem cells (hNSCs) into dopaminergic neurons and that they can also be employed for detecting dopamine release from mature neurons attached to them [1]. Here, we report 3D carbon nanopillars, fabricated through colloidal lithography, with even more pronounced effect on the electrochemical detection of dopamine.

Fabrication
The 3D carbon nanopillars were obtained using 1 μm polystyrene beads as etching mask and an etching time of 20 min, leading to structures with a height of 1.2 μm and a diameter of 450 nm (before pyrolysis) and a height of 600 nm and a width of 200 nm after pyrolysis.

Stem cell differentiation
Cell line: hVM1-Bd-4xl (human ventral mesencephalic neural stem cell line 1). The cells were seeded and cultured on tissue culture polystyrene (TCP) flat, carbon, micropillars and nanopillars (figures 2 and 3) in similar conditions. Differentiation was tested both in the presence and absence of differentiation factors (DF) on all surfaces.

Electrochemical measurements
The electrochemical behaviour of carbon nanopillars was investigated using cyclic voltammetry [Ru(NH₃)₆Cl₂/Ru(NH₃)₆Cl₃] as standard redox probe (figure 5).

Conclusions
Carbon nanopillars were fabricated using colloidal lithography/pyrolysis and employed as substrate for stem cell differentiation and dopamine detection. Detection of dopamine released from hNSCs differentiated into dopaminergic neurons is improved on the carbon nanopillars.

Literature cited

Figure 1: Schematic process flow for the fabrication of carbon nanopillars (A) and SEM images before (B) and after pyrolysis (C).

Figure 2: Schematic representation of differentiated hNSCs’ attachment on flat or pillared surfaces.

Figure 3: SEM images of stem cells differentiating on carbon nanopillars at different magnifications

Figure 4: Confocal microscopy images of hNSCs after differentiation and immunostaining on TCP (A) and carbon micropillars (B).

Figure 5: Cyclic voltammograms (different scan rates) of ruthenium hexaamine chloride (Ru(III)) on carbon nanopillars

Figure 6: Amperometric detection of released dopamine from differentiated hNSCs (A) and the K⁺-induced depolarization (B).

Figure 7: Comparison of measured charges on different carbon surfaces