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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.

For comparison, the micropillars we refer to have a height of 11 µm and a diameter of 1.4 µm after pyrolysis.

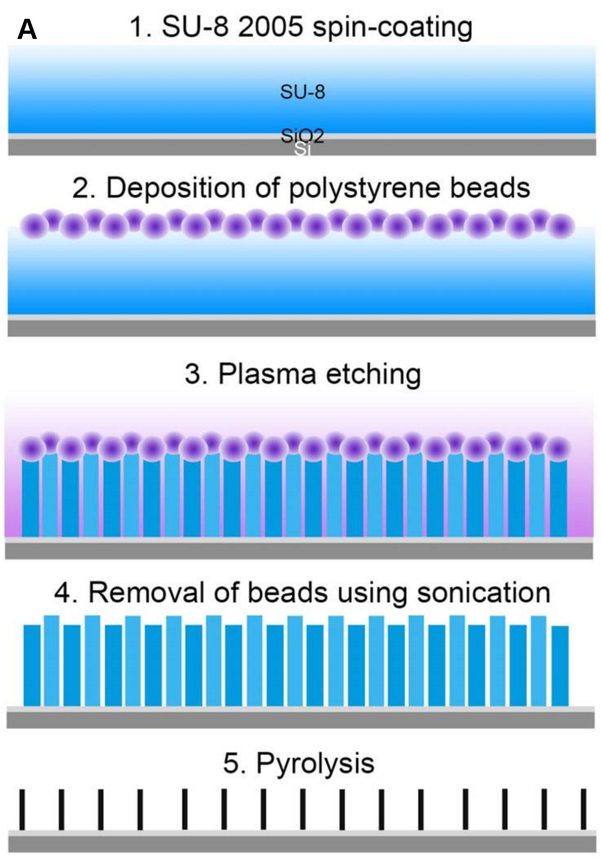

Figure 1: Schematic process flow for the fabrication of carbon nanopillars (A) and SEM images before (B) and after pyrolysis (C).

Stem cell differentiation

Cell line: hVM1-Bcl-x(L) (human ventral mesencephalic neural stem cell line 1).

The cells were seeded and cultured on tissue culture polystyrene (TCPS), 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.

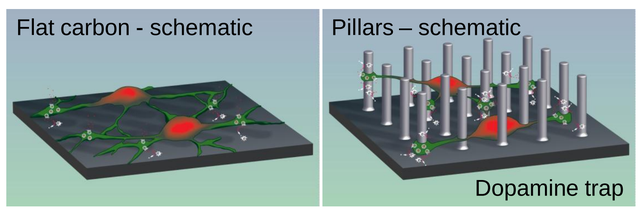

Figure 2: Schematic representation of differentiated hNSCs' attachement on flat or pillared surfaces

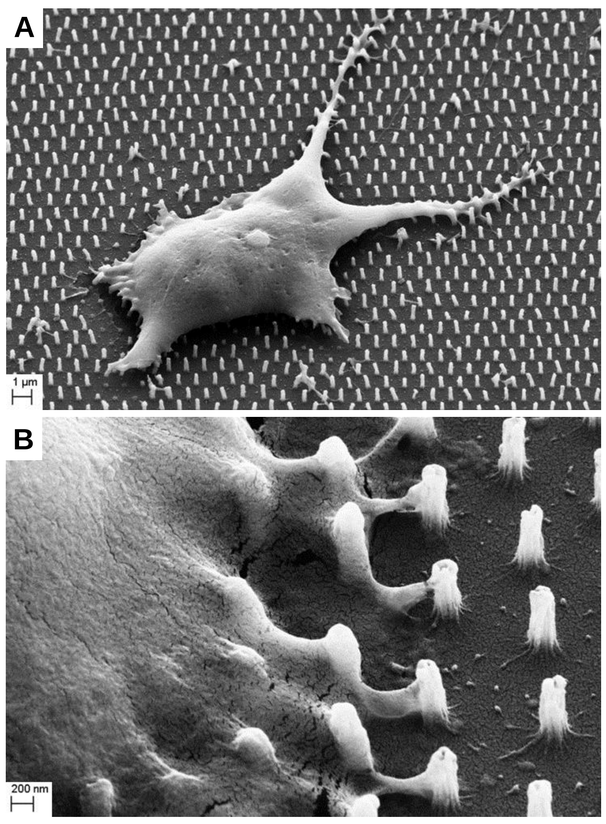

Figure 3: SEM images of stem cells differentiating on carbon nanopillars at different magnifications

Immunostaining was done for nuclei and TH (tyrosine hydroxylase) as indicator for the dopaminergic phenotype (figure 4).

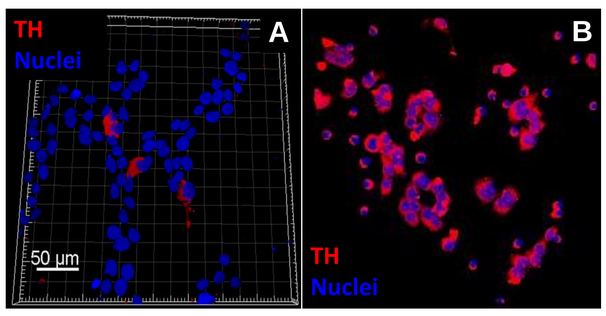

Figure 4: Confocal microscopy images of hNSCs after differentiation and immunostaining on TCPS (A) and carbon micropillars (B)

On all carbon surfaces, ~75% of the cells are TH-positive (regardless of the addition of differentiation factors), while on TCPS only 2.5% (without DF) and 24% (with DF) of the cells are TH-positive.

Electrochemical measurements

The electrochemical behaviour of carbon nanopillars was investigated using cyclic voltammetry $[Ru(NH_3)_6]Cl_2/[Ru(NH_3)_6]Cl_3$ as standard redox probe (figure 5).

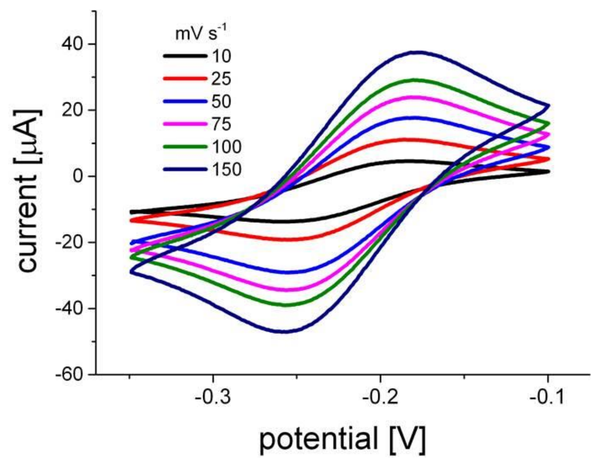

Figure 5: Cyclic voltammograms (different scan rates) of ruthenium hexaamine chloride (II/III) on carbon nanopillars

Dopamine exocytosis from differentiated hNSCs was monitored using amperometry after K^+-induced depolarization (figure 6).

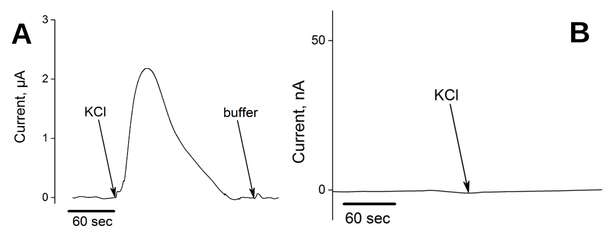

Figure 6: Amperometric detection of released dopamine from differentiated hNSCs (A) and the K^+/buffer effect on the system (B)

The charge measured using amperometry was computed and compared for the hNSCs differentiated on the different carbon surfaces. Nanopillars show the highest measured charges, thus improving detection.

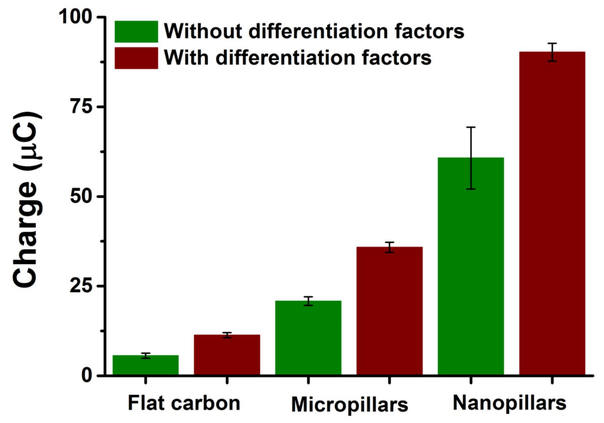

Figure 7: Comparison of measured charges on different carbon surfaces

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

1. L. Amato et. al., Pyrolysed 3D-Carbon Scaffolds Induce Spontaneous Differentiation of Human Neural Stem Cells and Facilitate Real Time Dopamine Detection, *Advanced Functional Materials*, **2014**, Vol. 24, Issue 44, 7042-7052.