EFFECTS OF NANOCRYSTALLINE GLASS-LIKE CARBON THIN FILMS IN THE SN4741 DOPAMINERGIC CELL PARKINSON MODEL

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The interest in carbon nanomaterials has grown within the last decade in view of a wide variety of applications. In this work we test the biocompatibility of a particular nanometer-thin nanocrystalline glass-like carbon films (NGLC), a disordered structure of graphene flakes joined by carbon matrix [1].
We used a cell line (SN4741) from substantia nigra dopaminergic cells derived from transgenic mouse embryo cells [2]. Some cells were cultured on top of NGLC films (5, 20 and 80 nm) and other with NGLC microflakes (approx. 5-10 mm2) in increasing concentrations: 1, 5, 10, 20 and 50 μg/ml, during 24 h, 3 days and 7 days. Cells growing in normal conditions were defined under culture with DMEM supplemented with 10% FCS. Microflakes were resuspended in DMEM at the stock concentration (2 g/l). We use 96 well plates (Corning) using 2500 cells per well. For MTT analysis a positive control with a 10% Triton X-100 and a negative control. As apoptosis/necrosis assay we used LIVE/DEAD® Viability/Cytotoxicity Assay Kit (Invitrogen). In a separate experiment, cells were cultured on top of the NGLC films for 7 days. Primary antibodies: anti-synaptophysin (SYP, clone SY38, Chemicon) and goat anti-GIRK2 (G-protein-regulated inward-rectifier potassium channel 2 protein) (Abcom) for immunofluorescence. WB was performed with a polyclonal anti-rabbit proliferating cell nuclear antigen (PCNA).
We demonstrate the biocompatibility with different concentration of NGLC varying the degree of survival from a low concentration (1 g/ml) in the first 24 h to high concentrations (20-50 μg/ml) after 7 days, corroborated by the PCNA analysis. Cells cultured on top of the film showed after 7 days axonal-like alignment and edge orientation as well as net-like images. Neuronal functionality was demonstrated through the analysis of coexistence between SYP and GIRK2.
This nanomaterial could offer a powerful platform for biomedical applications such as neural tissue engineering.

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