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Nanocrystalline glass-like carbon thin films may be an useful tool in nerve cells regeneration

The interest in carbon nanomaterials with high transparency and electrical conductivity has grown within the last decade in view of a wide variety of applications, including biocompatible sensors, diagnostic devices and bioelectronic implants. The aim of this work is to test the biocompatibility of 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 nanoflakes (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, Glucose (0,6%), penicillin-streptomycin (50U/ml) and L-glutamine (2mM) at 5%CO2 humidified atmosphere. Nanoflakes were resuspended in DMEM at the stock concentration (2 g/l). The experiments were conducted in 96 well plates (Corning) using 2500 cells per well. For MTT analysis, the manufacturer recommendations were followed (Roche, MTT kit assay): a positive control with a 10% Triton X-100 treatments (15 minutes) and a negative control without neither Triton X-100 nor NGLC. 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) following protocol for immunofluorescence. WB for proteins detection performed with a polyclonal anti-rabbit proliferating cell nuclear antigen (PCNA). Results demonstrated the biocompatibility with different concentration of NGLC varying the degree of survival from a low concentration (1 μg/ml) in the first 24 h to high concentrations (20-50 μg/ml) after 7 days as it is 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 to a certain extent through the analysis of coexistence between SYP and GIRK2. In conclusion, this nanomaterial could offer a powerful platform for biomedical applications such as neural tissue engineering. Supported by UMA, Campus Excelencia Internacional Andalucia Tech, Spain. We thank Prof. Arenas, Karolinska Institutet, Stockholm, Sweden, for SN4741 cell line. R.G.V. gratefully acknowledges Spanish Ministry of Science and Innovation for funding through a Ramon y Cajal fellowship.

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