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Graphene derivative scaffolds facilitate in vitro cell survival and maturation of dopaminergic SN4741 cells

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The emerging carbon nanomaterial Graphene (G), in the form of scaffold structure, has an efficient bioconjugation with common biomolecules and activates cell differentiation of neuronal stem cells, providing a promising approach for neural regeneration. We propose the use of G as a scaffold to re-address the dopaminergic (DA) neurons and the residual axons from dead or apoptotic DA neurons in Parkinson´s disease (PD). G could act as a physical support to promote the axonal sprout as a "deceleration" support for the DA cells derived from neural stem cells or DA direct cell conversion, allowing the propagation of nerve impulses. We cultured a clonal substantia nigra (SN) DA neuronal progenitor cell line (SN4741) in presence of G as scaffold. This cell line derived from mouse embryos was cultured in Dulbecco's modified Eagle's medium/10% FCS to about 80% confluence. Cells were incubated in three chemically different G derivatives and two different presentation matrixes as powder and films: 1) G oxide (GO); 2) partially reduced GO (PRGO) which is hydrophobic; and 3) fully reduced GO (FRGO). Cell viability was determined using the MTT assay after adding the following G concentrations: 1mg/ml; 0.1mg/ml; 0.05mg/ml; 0.02mg/ml and 0.01mg/ml, in each type of GO. To study cellular morphology and assessment of cell engraftment into GO films (GO film, PRGO film, FRGO film), we analyzed the immunostaining of the anti-rabbit neuron-specific DNA-binding protein (NeuN) antibody, the anti-rat Beta-3-tubulin antibody in combination with the mitochondrial marker mouse anti-ATP synthase antibody, and the anti-rabbit DCX as immature neuronal marker. Hoechst label was used as nuclei marker. Reactive oxidative species (ROS) were measured by flow cytometry to study the influence of G on the cell redox-state. With this purpose, cells were loaded with dihydroethidium. The mitochondrial membrane potential after JC-1 incubation was studied by flow cytometry. Our results show an increase of survival and metabolism (30-40%) at low concentrations of PRGO and FRGO (0.05-0.01 mg/ml) respect to the higher concentration (1 mg/ml), while no changes were seen in the GO group. LDH concentration was measured in the supernatant using a COBAS analyzer showing a neuroprotective action at low concentrations. Furthermore, either PRGO film or FRGO film show an increase in the effective anchorage capacity to nest into the G matrix and in the maturation of the SN 4741 cells. We conclude that the use of G scaffolds in the research of neurological diseases like PD could offer a powerful platform for neural stem cells, direct cell conversion techniques and neural tissue engineering.

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