Functionalization of reduced graphene oxide with hyaluronic acid on CoCr surfaces for biomedical applications

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Electrochemically reduced graphene oxide (ErGO) films on a biomedical grade CoCr alloy have been generated and characterized in order to study the biocompatibility and in vivo behavior for its possible biomedical application. The electrodeposition process was performed by cyclic voltammetry from -2.1 V to -0.5 V at a scanning rate of 10 mV s-1 for five cycles. ErGO/CoCr surface analysis was studied by XPS. Electrochemical techniques such as the measurement of the corrosion potential, Ecorr, and electrochemical impedance spectroscopy (EIS) were applied to assess the corrosion behavior. ErGO films on CoCr were further functionalized with hyaluronic acid (ErGOHA) after 24 hours soaking in a solution containing 3 g/L, a concentration equivalent to the content of the synovial fluid. Biocompatibility and cytotoxicity of CoCr, ErGO/CoCr, and ErGOHA/CoCr studies were performed in mouse macrophages J774A.1 cell cultures by measuring mitochondrial activity (WST-1 assay), plasma membrane damage (LDH assay) and the ratio LDH/WST-1, as relate plasma membrane damage index to the number of metabolically active cells in culture. Pattern distribution and morphology of macrophages on ErGO/CoCr and ErGOHA/CoCr alloys were performed by optical microscopy. The in vivo response of graphene oxide used in the electrochemical reduction was assessed by intraperitoneal injection in wistar rats. Graphene was also used as reference. Graphene (G) and graphene oxide (GO) nanosheets at a concentration of 4 mg per kilogram of weight were suspended into an aqueous solution of 0.9% NaCl in a proportion 1:1 (PBS:G or GO), i.e., 1 mg/ml. After 30 day's post-inoculation, blood extraction was performed in the rats in order to carry out an hematological analysis. XPS revealed sp2 bonding and the presence of C=O and C-O residual groups in the graphene network. ErGO films do not cause significant changes in the good corrosion behavior of CoCr alloys. Macrophages have shown a better biocompatibility on ErGOHA/CoCr than on ErGO/CoCr and CoCr, where they appear widely distributed and in copious number on ErGOHA film. Rats inoculated with graphene and OG showed red cells with smaller size with a high content in haemoglobin.