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Carbon nanomaterials coatings – Properties and influence on nerve cells response

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ABSTRACT

The goal of this study was to investigate the influence of multi-walled carbon nanotubes (MWCNTs) before and after chemical functionalisation, graphene oxide (GO), and hybrid coating deposited on a titanium (Ti) surface on the nerve cell response in vitro. The physicochemical properties of the surface of the carbon nanomaterial coatings deposited on the Ti substrate using electrophoretic deposition were investigated, followed by biological tests. Scanning and transmission electron microscopy and X-ray photoelectron spectroscopy were used to evaluate the microstructure and chemistry of the carbon nanomaterial coatings. Electrochemical characterisation of the carbon nanomaterial coatings on metal substrates was investigated using cyclic voltammetry and cathodic charge storage capacity. During in vitro analysis, all samples were placed in direct contact with human neuroblastoma SH-SY5Y cells. The viability were analysed after 48 and 72 h of culture. Moreover, the cell morphology in contact with the carbon nanomaterial coatings was observed using fluorescence microscopy. Additionally, the neurite outgrowth and number of pyknotic nuclei were examined using cell microphotographs.

GO exhibited the best biological results among all analysed samples, with a positive effect on cell viability, neural cell morphology, and especially neurite outgrowth, and significantly improved the biological properties of the other hybrid (nanocomposite) coatings. GO coating is not an electrochemically active material, thus its applicability for the production of electrodes for nerve stimulation is limited. However, it may be useful as a scaffold for nerve cell stimulation and regeneration. The advantageous electrochemical activity of MWCNT coatings and a satisfactory cell response greater than the Ti surface alone will pave the way for further research on electrodes for nerve cell stimulation.

1. Introduction

For a few years carbon nanomaterials have been considered in different fields of science, including technical and biological applications [1–6]. Nowadays, the application of these materials in medicine is one of the biggest challenges. Among carbon materials, there are three types of nanomaterials commonly tested for medical applications: carbon nanotubes (CNTs), and graphene and carbon nanofibers. CNTs are the best-investigated carbon nanomaterials used for a wide range of biological applications. These materials are used in drug delivery systems and as potential scaffolds for tissue regeneration, biosensors, and elements for cancer diagnosis and treatment [7–11]. CNTs are of particular interest because of their unusual electrical and mechanical properties and high chemical stability. They are also the subject of growing interest in biological applications, which recently have extended into the area of electrodes for nerve tissue stimulation [6,12,13]. CNTs have been shown to stimulate neurite outgrowth, improve neuronal performance and recording, neuron differentiation, boost neuronal electrical signalling, and function as a substrate for neuronal growth [14,15]. One of the potential applications of these carbon forms are coatings on metal electrodes dedicated for nerve tissue regeneration, especially in the central nervous system. Currently, the majority of neural electrodes are made primarily of metals, such as gold, platinum, titanium (Ti), iridium, and stainless steel. The main disadvantage of these electrodes is

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the poor performance in prolonged stimulation and recording due to poor contact with tissues or scar formation. Therefore, researchers have developed new methods of modifying the surfaces of these electrodes to improve their usability and functionality [14]. The action time of microelectrodes used in brain stimulation largely depends on the electrode materials, which should be characterised by high charge storage capacity and stability [14]. Due to their properties, especially with respect to electric conductivity toward underlying substrates, CNTs can effectively improve neuronal cell behaviour in terms of neurite and axon extension in in vitro investigations and nerve regeneration in animals [16]. Due to its electrical characteristics, graphene is also suitable for electroactive scaffolds capable of emitting electrical signals that stimulate nerve cell regeneration by increasing the branching and regrowth of neurons [17–20].

Among the carbon nanomaterials with good electrical properties, there are some that are also endowed with much lower conductivity than graphene and CNTs, namely graphene oxide (GO). GO is a highly oxidised form of chemically modified graphene [19,21]. The precise GO structure has not yet been fully tested, but it is likely constructed of hydrophobic domains derived from the pure graphite structure and the hydrophilic functional groups generated during oxidation. Due to the coexistence of these groups, GO is characterised by good water solubility, biocompatibility, and affinity for specific biomolecules [22]. These properties provide plenty of opportunities for the development of new biosensors [23–25]. Due to the presence of functional groups, the material is deformed, so its mechanical, electrical, and thermal properties are lower than those of graphene and reduced GO [19]. Interestingly, despite poor electrical conductivity, GO promotes the differentiation of embryonic stem cells into dopamine neurons, unlike CNTs and graphene nanoparticles where no such effects have been observed. Moreover, GO may also stimulate the expression of different integrins responsible for oligodendrocyte survival, differentiation, and myelination. It can also preferentially facilitate neuronal differentiation, even without inductive factors in the culture medium [26–28].

Carbon nanomaterials can interact with cells in different ways, depending on their structure, purity, chemistry, and form of contact with cells. Regarding the application of biomaterials, it seems that the most appropriate would be both CNTs and other forms of nanocarbons in the form of coatings. The most popular technique used for carbon nanomaterial-based coatings on metal substrates is electrophoretic deposition (EPD) [29,30]. The advantages of this method include repeatability, simplicity, low price, homogeneity of deposits, and control of thickness [29]. Carbon nanomaterial coatings on metallic surfaces carry a large potential for modern medical applications, e.g., as sensors, electrodes, and stimulators of the nervous system and scaffolds for nerve tissue regeneration [31–35]. Carbon nanomaterials may be endowed with a different morphology, structure, chemistry, and electrical properties. Therefore, their use as coatings provides the modified surfaces with different properties and consequently a different interaction with the cells. Both the presence of the specific chemical groups on carbon nanomaterials and variations in topography and microstructure of the coatings based on these nanomaterials could affect the cellular response. Nanomaterials are considered biomimetic materials because they can mimic nature, unlike micrometric materials [36]. For example, CNTs are morphologically similar to neural fibres. Therefore, they can be used to stimulate nerve growth [37].

The objective of this study was to evaluate the physicochemical and morphological properties of CNTs and GO coatings deposited on the metal substrates using the EPD method and their interaction with nerve cells (SH-SY5Y). Human neuroblastoma SH-SY5Y cells are widely used as a neuronal-like cell model particularly in in vitro neuroprotection/neurotoxicity research [38–40]. However, they are also commonly acceptable as a model for neuroregeneration [20,41–43]. Moreover, most carbon nanomaterials are electrically conductive, which is why the nervous system serves as an ideal breakthrough model as its functions are based on electrical responses [20,44,45]. Electrically active human neuroblastoma SH-SY5Y cells were therefore chosen for our in vitro analysis [45–47].

2. Materials and methods

Pristine (MWCNTs) and functionalised (MWCNT-Ks) CNTs, both multi-walled, and one type of GO were used in this study. All the tested materials were purchased from NanoAmor. The pristine MWCNTs exhibit a diameter > 50 nm and are 10–20 μm long. The functionalised MWCNT-Ks exhibit a diameter of 10–20 nm and are 0.5–2 μm long. According to the manufacturer, the functional group (—OH) content of MWCNT-Ks is 2.91–3.21 wt%. The presence of hydroxyl groups on the surface of the MWCNT-Ks provide a negative surface charge, thus during EPD, the CNTs are deposited on the anode [30,48,49]. On average, the GO plate is 0.5–3 μm long and 0.55–1.2 nm thick, and is endowed with a negative surface charge.

In this study, the pristine and functionalised MWCNTs and GO were deposited on pure Ti Grade 2 substrates of 11 mm in diameter using the EPD method. Before the EPD procedure, the Ti plates were degreased with acetone and ethanol and etched in 5% hydrofluoric acid (HF) for 1 min. For EPD, both GO and MWCNT-Ks (35 mg) were introduced into a mixture of ethanol:acetone:water in a 62:21:17% ratio (12 mL) and sonicated for 5 min using a tip sonicator (model: CP 130 PB, Palmer Instruments) as previously described [50]. Only the MWCNTs were deposited on the Ti plates using the EPD technique from isopropanol. The selection of solvents and preparation of a stable dispersed carbon nanomaterial suspension in a solvent are key steps to ensure the success of EPD. One of the parameters determining the particles' stability in a solvent medium is their high zeta potential (ζ), while sustaining the low ionic conductivity of the suspension [51]. The ζ values of MWCNT-Ks and GO in the solution were −20 and −30 mV, respectively. This measurement was taken using a combination of electrophoresis and the laser Doppler velocimetry technique (Malvern Zetasizer Nano ZS).

The EPD process was carried out from 5 to 15 s at a voltage of 30 V, depending on the type of coating. The distance between two electrodes, where one of them was the substrate for the carbon nanomaterial, was 0.5 cm. Four kinds of coatings were obtained using EPD, and three were prepared in a form of pure GO, MWCNT, and MWCNT-K coatings, and the fourth was prepared as a hybrid coating containing two types of different nanomaterials. The solutions for the hybrid coatings were prepared by mixing two types of solutions containing GO and MWCNT-K in a 1:1 ratio. The samples investigated in this study are summarised below:

- GOTi – GO coating deposited on a Ti substrate;
- MWTi – MWCNT coating deposited on a Ti substrate;
- MWKTi – functionalised MWCNT-K coating deposited on a Ti substrate; and
- GO:MWKTi – hybrid coating made of functionalised MWCNT-K and GO in a 1:1 ratio deposited on a Ti substrate.

Since titanium is a well-recognised biocompatible material commonly applied in implantology [52,53] and used as a substrate for the deposition of carbon nanomaterial coatings, it was used as a reference. In our investigation, the reference samples were Ti plates without a carbon nanomaterial coating after etching in 5% HF.

2.1. Physicochemical properties of the carbon nanomaterial coatings

The elemental composition of the samples was determined using the μ-proton-induced X-ray emission method. In this method, the characteristic X–ray lines, corresponding to the elements present in the sample, are induced by proton beam scanning of its surface. The beam energy was 2 MeV, while the beam current intensity was approximately 200 pA, focused to a spot of approximately 20 μm in diameter. To collect more reliable data, i.e., averaged over larger surfaces, the