Materials Science and Engineering C 41 (2014) 65–69

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

# Graphene and carbon nanotube composite enabling a new prospective treatment for trichomoniasis disease

H. Zanin [a,*], A. Margraf-Ferreira [b], N.S. da Silva [b], F.R. Marciano [c], E.J. Corat [a], A.O. Lobo [c]

[a] National Institute for Space Research, Av. dos Astronautas 1758, Sao Jose dos Campos CEP: 12227-010, SP, Brazil
[b] Laboratory of Cell Biology and Tissue, Institute of Research and Development, University of Vale do Paraiba, Av. Shishima Hifumi, 2911, CEP: 12244-000 Sao Jose dos Campos, SP, Brazil
[c] Laboratory of Biomedical Nanotechnology, Institute of Research and Development, University of Vale do Paraiba, Av. Shishima Hifumi, 2911, CEP: 12244-000 Sao Jose dos Campos, SP, Brazil

ARTICLE INFO

*Article history:*
Received 20 September 2013
Received in revised form 23 January 2014
Accepted 7 April 2014
Available online 22 April 2014

*Keywords:*
Graphene oxide
Carbon nanotube oxide
Composite
*Tritrichomonas foetus*
Drug carrier

ABSTRACT

We report the synthesis and application of novel graphene oxide and carbon nanotube oxide (GCN-O) composite. First, pristine multi-walled carbon nanotube was prepared by chemical vapour deposition furnace and then exfoliated and oxidised simultaneously by oxygen plasma etching. The superficial and volumetric compositions of GCN-O were measured by XPS spectroscopy and EDX spectroscopy, respectively. Both XPS and EDX analyses evidence that the GCN-O is composed of up to 20% of oxygen atoms. As a result, GCN-O forms a stable colloidal aqueous solution and shows to have strong interaction with the cell membrane of *Tritrichomonas foetus* protozoa, making easy its application as a drug carrier. Trichomoniasis infection of cattle is a devastating disease for cattle producers, causing some damages to females and fetus, and the abortion is the most serious result of this disease. There is no effective treatment for trichomoniasis infection yet. Therefore, new treatment, especially one with no collateral effects in animals, is required. With this goal in mind, our results suggest that water dispersible composite is a novel nanomaterial, promising for Trichomoniasis infection treatment and as therapeutic delivery agent as well.



## 1. Introduction

Recently graphene is at the centre of a significant research effort with the promise of owning properties as good as or even better than the nanotubes [1]. In the first studies, researchers dealt with the preparation and application of graphene, graphene oxide and reduced graphene oxide [2–6]. The most used method for graphene production is known as Hummer method [7], which consists of chemical intercalation of metal and abrupt thermal expansion of pristine graphite, which promotes their exfoliation. Another attractive possibility for graphene production consists of opening carbon nanotubes, which could be understand as graphene nanosheets rolled up [5]. The multi-walled carbon nanotubes (MWCNTs) can be opened longitudinally by intercalation of metals and exfoliation with acid treatment and abrupt heating. The resulting material consists of: (i) multilayered flat graphitic structures (nanoribbons), (ii) partially open MWCNTs, and (iii) graphene flakes. However, both methods describe above need strong chemical oxidant manipulation, which need special handle care and correct disposal. Into this context, the oxygen plasma etching is a simple, fast, selective and low temperature carbon nanotube exfoliation. The final product of carbon nanotube exfoliation is graphene oxide and carbon nanotube oxide composite. Both graphene and carbon nanotubes are special materials because of their superb physico-chemical properties such as biocompatibility, absence of mutagenic and recombinagenic activity, large specific surface area and potentially low manufacturing cost [8–11]. Carbon nanotubes have been extensively studied in recent years and many technologies were developed using this material [12–14]. There is a great expectation that graphene has similar physico-chemical properties compared to carbon nanotubes [15–17], and should be as good as or better than carbon nanotubes in some applications. However, all those applications should be carefully tested for graphene as well [18–20]. Considering that, we have shown in recent publication the strong *Tritrichomonas foetus* adhesion on to superhydrophylic vertically aligned carbon nanotube (VACNT) surface [21]. That study enabled to apply superhydrophylic VACNT to understand protozoa spreading mechanism and the specific recognition of adhesion proteins. We especially pay attention on this protozoan, because it causes the trichomoniasis, which is a sexually transmitted disease, affecting male and female cattle. Trichomoniasis infection of cattle is a devastating disease for cattle producers [22]. The trichomoniasis can cause some damages to females and fetus, and the abortion is the most serious consequence [23]. When diagnosed in a herd, it causes economic loss and emotional pain [24]. Although there is no treatment for trichomoniasis infection yet [24], there are a couple of reports showing decrease of the protozoa content with estradiol-treatment [25,26]. Studies using ultra-structural cytochemistry and determination of the cellular electrophoretic mobility showed that *T. foetus* possesses a net

* Corresponding author. Tel./fax: +55 1232086558.
*E-mail address:* hudsonzanin@gmail.com (H. Zanin).

http://dx.doi.org/10.1016/j.msec.2014.04.020
0928-4931/

negative surface charge [27], which plays a fundamental role on their ability to adhere to inert and biological substrata.

In this work, we study a novel method to prepare graphene by oxygen plasma etching of MWCNTs. We propose a novel composite of graphene oxide and carbon nanotube oxide (GCN-O), exfoliating nanotubes. Further, we evaluate this new material using scanning and transmission electron microscopy; Raman spectroscopy, energy-dispersive X-ray spectroscopy and X-ray photoemission spectroscopy; zeta potential; surface tension tests and semi-quantitative adhesion to parasitic protozoan *T. foetus*.

## 2. Experimental

### 2.1. Synthesis of MWCNT and GCN-O

The MWCNTs were prepared using a mixture of camphor (85 wt.%) and ferrocene in a thermal chemical vapour deposition (CVD) furnace, as reported elsewhere [28]. The mixture was vapourised at 220 °C in a pre-chamber and then, the vapour was carried by an argon gas flow at atmospheric pressure, to the chamber of the CVD furnace set at 850 °C. The time elapsed during the process used to produce the MWCNTs was only a few minutes. The sample was etched by chloridric acid at 100 °C for 5 h for residual catalytic iron particle removal. And then, it was extensively water washed and finally dried. The incorporation of oxygen-containing groups was carried out in a pulsed-direct current plasma reactor with an oxygen flow rate of 1 sccm (standard cubic centimetres per minute), at a pressure of 185 mTorr, −700 V and with pulse frequency of 20 kHz [29]. We have developed an apparatus that shakes and deagglomerate the carbon nanotube powder during oxygen plasma etching. This apparatus is a hollow cathode with a helix on the base for powder shaking and grinding. The plasma forms inside this hollow cathode, allowing the plasma to access three-dimensionally the nanotube powder in motion for up to 1 h.

### 2.2. GCN-O and parasitic protozoa T. foetus in TYM medium

The K strains of *T. foetus*, isolated from the urogenital tract of a bull were maintained in TYM Diamond's medium (medium of trypticase, yeast extract, maltose, and serum used to detect the presence of *Trichomonas vaginalis*), supplemented with Fetal bovine serum (10%, Gibco/BRL) at 37 °C [30]. A colloidal solution of $10^4$ *T. foetus* K was cultivated during 24 h (logarithmic growth phase) and used in each experiment with GCN-O. A stock solution (1 mg/ml) of GCN-O composite was prepared in phosphate-buffered saline solution (PBS). A suspension of *T. foetus* ($10^4$ protozoa/ml) was aliquoted into individual wells of a microdilution plate (24 wells) and 10 μg/ml or 100 μg/ml of GCN-O composite was added and the mixture was incubated at 37 °C for 1 h. The adhesion was evaluated by an inverted optical microscope (DMIL — Leica) and the SEM microscopy. Control groups (protozoa without GCN-$O_2$ and GCN-$O_2$ only) were performed as well.

### 2.3. Characterisation of GCN-O composite

The composite was characterised by several techniques such as: high resolution scanning electron microscopy (SEM), transmission electron microscopy (TEM), Raman spectroscopy, X-ray photoemission spectroscopy (XPS), and energy-dispersive X-ray (EDX) spectroscopy, and zeta potential analysed by dynamic light scattering (DLS). The agglomeration status was analysed by inverted optical microscope (DMIL — Leica). Morphological analyses were performed with SEM-FEG (JEOL 6330) operated at 20 kV and coupled with an EDX for chemical analysis, operating with a Si (Li) detector with an energy resolution of 126 eV. Raman spectra were recorded using a Renishaw microprobe, employing an argon laser for excitation ($\lambda$ = 514.5 nm) with a laser power of approximately 6 mW. The XPS spectra were taken using VSW H100 spectrometer with residual pressure less than $10^{-10}$ Torr. All measurements were conducted at room temperature. The GCN-O zeta potential was measured by dynamic light scattering (DLS) (Zetasizer, Malvern — UK). All samples were diluted at 150 μg $ml^{-1}$ in KCl 1 mM solution and were deagglomerate with two different ways: i) conventional sonication during 30 min and ii) using a ultra power sound irradiation (UI) at 200 W during 30 min. Then samples were placed to a polystyrene zeta cuvette. Measures were performed 2 h after re-suspension. The EasyDyne tensiometer (Kruss, K20 model) was used to measure the surface tension of the GCN-O, *T. foetus* and its conjugate using the Du Noüy Ring method [31] for an average of 10 data sets.

## 3. Results and discussion

Fig. 1(a–g) shows the typical morphology of GCN-O. Fig. 1(a) is the top view of GCN-O, which is magnified at higher resolutions (Fig. 1(b–g)) to better visualisation of the effect of the oxygen plasma etching at MWCNT. From all those SEM micrographs, we could see that oxygen plasma exfoliated preferentially the carbon nanotube tips, opening its walls and disclosing its fundamental structure: graphene [32-34]. It is well known that carbon nanotubes are composed of graphene nanosheets rolled into cylinders. In addition, graphene nanosheet rolling up into a tube is a standard picture to explain the carbon nanotube formation. In that tubular structure, the nanotube tips are the most defective and are more suitable to interact direct with plasma, causing the pitch of corrosion and then starting exfoliation from there. Fig. 1(h) shows the TEM micrograph of GCN-O, which the exfoliation started from the tip and continued. We noted that depending on plasma conditions we could etch the whole nanotubes or simply attach oxygen groups to them without exfoliation. At higher plasma pressures (higher than 180 mTorr) or for longer processing times, the erosion was completed. With diluted plasma, exfoliation does not show up but wettability increased significantly because of oxygen groups' attachment [40]. The GCN-O composite was achieved at quite specific conditions. Further, all those micrographs point out GCN-O as porous structure, which the real surface area and specific density were measured by BET and helium pycnometry to be ~38.5 $m^2/g$ and ~2.3 $g/cm^3$, respectively.

Fig. 2(a & b) shows first- and second-order Raman scattering spectra of MWCNT and GCN-O. The deconvolutions were performed using Lorentzian shapes for the D, G and G′ bands, and Gaussian shape for bands around 1250, 1480 and 1611 $cm^{-1}$ (D′ shoulder) [33,35]. The D band is usually assigned to the disorder and defect of the carbon crystallites [36]. The G band is assigned to one of the two $E_{2g}$ modes corresponding to stretching vibrations in the basal plane ($sp^2$ domains) of single crystal graphene [37]. The high intensity G′ band reveals that these materials present high crystallinity [38]. In the GCN-O first order Raman, for set aside deconvolution fitting, two Gaussian peaks centred at around 1250 and 1480 $cm^{-1}$ were added necessarily. Probably the shoulder has its origin in double resonance, because its Raman shift (~1200 $cm^{-1}$) is a point on graphene phonon dispersion curves [39]. The origin of the 1480 $cm^{-1}$ band is probably correlated with the polar group grafting on to GCN-O surfaces [40].

We identified and semi-quantified chemical elements and chemical bond in as-grown MWCNT and GCN-O samples using two different techniques. Fig. 3(a–d) presents the results that we have taken from as-grown MWCNT and GCN-O by XPS and EDX measurements for an average of 5 data sets. Fig. 3(a & b) shows the (a) C1s and (b) O1s fitted photoemission spectra recorded and deconvoluted for GCN-O. The spectra were deconvoluted assuming a Lorentzian–Gaussian sum of functions (20% Lorentzian maximum contribution) by Fityk software [41]. Fig. 3(a) presents the C1s spectrumdecomposed into five Gaussian components, referring to the bonds: $sp^2$ carbon (~284.5 eV), C–O (~285.8 eV), C═O and –COO– (288.7 eV), and the last one at 291.2 eV assigned to the shake-up peak (π–π* transitions) [42,43]. Fig. 3(b) shows deconvolution of the O1s spectrum using three bands. The first is in the range from 531.0 to 532.4 eV, attributed to hydroxyl