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Graphene- and Carbon Nanotubes-Yeast Bionicomposites

L. Valentini, S. Bittolo Bon, S. Signetti and N.M. Pugno

Abstract Nature offers us an enormous amount of ready-to-use templates with various morphologies and functionalities, which can be successfully utilized in fabrication of biosensors, tissue engineering, and microelectronics. The directed combination of such natural templates with graphene or carbon nanotubes results in the development of a novel material which uses the features of both. We produced hybrid materials by giving to microorganisms the nutrient to grow together with graphene nanoplatelets and carbon nanotubes. Such hybrid materials can be considered as bionic because they have the benefits of both biological world which can self-organize and that of non-living materials, which couple functions such as self-healing and electronic transport.

1 Introduction

Biogenic materials are often formed at the nanometer scale through diverse metabolic activities and by passive surface reactions on cell walls or extracellular structures [1, 2]. The exploitation of the processes used by microorganisms to digest

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nutrients for their growth can be a viable method for the formation of a wide range of so-called biogenic materials that have unique mechanical and physical properties that are not produced by abiotic processes [3]. The interaction between living organisms and inorganic materials, including inorganic nanomaterials with higher electrical conductivity, could facilitate extracellular electron transfer as well as the improving of the mechanical properties [4, 5].

Baker's yeast has been used for centuries in wine and bread making and it consists of *Saccharomyces cerevisiae (SaC)*, a unicellular microorganism [6–8]. Yeast is a cellular factory and reproduces asexually through a process called "budding," in which a "mother" cell grows a "daughter" cell that separates to become fully independent. We exploited yeast fermentation to couple graphene on its cells' walls. The technique uses the commercially available and inexpensive bread yeast and can be applied to any living cells without special surface modification of graphene. In turn, this process could lead to the development of hierarchical and interactive structures programmed to self-assemble into specific patterns, such as those on strain sensors, and of self-healing materials capable to sense and repair mechanical damage.

The scientific core is to address the synthesis of hybrid living materials by means of "cold fermentation" processes through alcoholic fermentation and large-scale living microbes' factories. In this context, this research could have future implications of bio-materials in the manufacture of physical smart and multifunctional objects. Encapsulation of the cell with an inorganic shell has already been found to enhance the robustness of the cell and improve its endurance in harsh environments [9]. Some materials such as silica and calcium phosphate have already been used as encapsulating materials to improve the cell stability [10, 11]. Our works [4, 5, 12, 13] present the first attempts in producing bionicomposites and [4, 5] are revised in this book chapter.

2 Graphene-Yeast Bionicomposites

For the preparation of the composite film, 40 mg of graphene nanoplatelets (GNPs, purchased from Cheaptubes®, thickness 8–15 nm) were dispersed for 3 h at room temperature in 40 mL of water using a sonication bath. *SaC* based commercial beer yeast extract was used as the medium for fermentation. The dispersion of GNPs (1 mg/ml) was then added to the yeast solution and stirred at 110 rpm at 30 °C for 1 h. To start the fermentation, sugar (i.e., sucrose, 4 g) was added to the yeast/GNPs solution. The dispersion was heated at 35 °C to allow the fermentation process. The solid films were obtained by evaporating the water in excess, leaving the solutions in a sterilized silicon rubber mold at 30 °C in inert atmosphere for two nights. Field emission scanning microscopy (FESEM) was used to investigate the cross section of the samples obtained by fracture in liquid nitrogen. The current–voltage characteristic was performed by a computer controlled Keithley 4200 Source Measure Unit. The electrical conductivity of the samples was monitored, at

room temperature, by applying a sweeping DC electric voltage across the sample. The tensile properties of films, i.e., Young's modulus and tensile strength, were then measured using a universal tensile testing machine (Lloyd Instr. LR30 K) with a 50 N static load cell. The film samples were cut into strips (30 mm × 12 mm). The gauge length was 20 mm, and the extension rate was set at 2 mm/min.

The shape of the yeast cells was found to be spheroidal (Fig. 1a). According to previous papers, *SaC* multiplies with a process where a daughter cell is initiated as growth from the mother cell (Fig. 1b, c) [14]. The round protrusion on the cell surface, visible in Fig. 1b, is a bud scar. The bud scar forms on the cell after the process of division has taken place.

The effect on the film morphology when GNPs were added during fermentation is reported in Fig. 1c. The FESEM analysis on the GNP/yeast cell surface indicates the formation of wrinkles which arise from compressive stress when a soft-matter core (i.e., *SaC* yeast cell in our case) is coupled with a thin, high modulus sheet (i.e., GNPs) [15]. The physics and geometry of such system indicates that the wavelength λ of the wrinkles can be estimated as follows:

$$\lambda = 2\pi t \left[\frac{\left(1-\upsilon_S^2\right)}{\left(1-\upsilon^2\right)} \frac{E}{3E_S} \right]^{1/3} \tag{1}$$

where t is the thickness of the GNPs film, E and ν are respectively Young's modulus and Poisson's ratio of the film, while E_s and ν_s refer to the substrate counterparts. Assuming an average thickness $t = 11.6$ nm, $E = 50$ GPa, $\nu = 0.165$, $E_s = 112$ MPa, and $\nu_s = 0.5$ [16] a wrinkling wavelength $\lambda \approx 38$ nm is obtained, in agreement with our experimental value of 30–70 nm (Fig. 1d).

Cell wall can be considered as an elastic encapsulating layer allowing the exchange of substance with the surroundings [16]. *SaC* is a type of yeast widely used in the conversion of sugar because of its ability to produce high amounts of ethanol and glucose as by products [17]. Thus the production of glucose during *SaC* fermentation can be used to couple graphene nanoplatelets onto the yeast cell wall and, when sugar is exhausted, to obtain bionic composite films.

The measured composite, fracture strength σ_f and Young's modulus E, can be related to the properties of the constituent phases via rules of mixture:

$$\sigma_f = f\sigma_{\mathrm{f,\,GNPs}} + (1-f)\sigma_{\mathrm{f,\,yeast}} \tag{2a}$$

$$E = f\,E_{\mathrm{GNPs}} + (1-f)E_{\mathrm{yeast}} \tag{2b}$$

where f is the volumetric fraction of the GNP film and the subscripts refer to each component of the bionic composite. Parallel, a simple system of two fermented cells was modeled via Finite Element Method (FEM). The shape of the cells within the ensemble is approximated as hexagonal prism (Fig. 2a). The dimensions of the cell, mother and daughter, were taken from the work of Ahmad et al. [16] which are

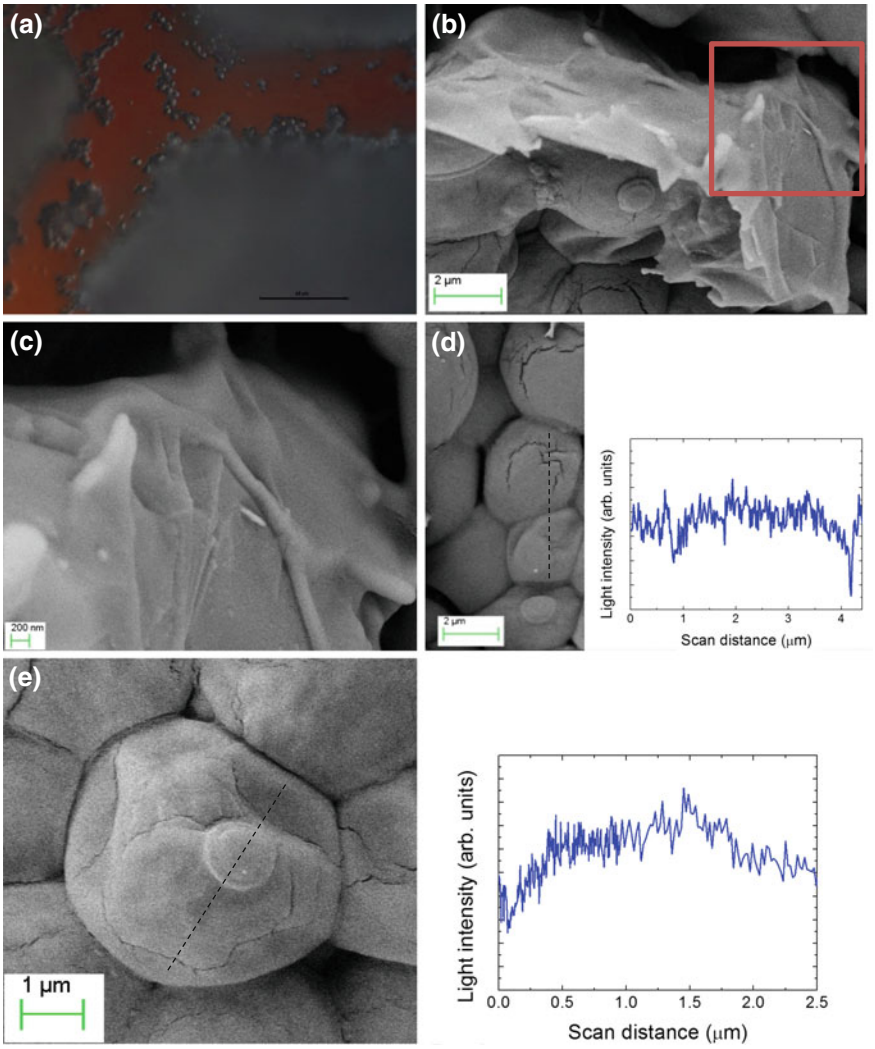

Fig. 1 **a** Optical image of yeast cells (the scale bar indicates 60 μm). **b** FESEM image of the GNPs/yeast cell sample and **c** its magnified view. **d** FESEM image and line scan of the surface of the damaged yeast/GNPs sample. **e** FESEM image and line scan of the surface of the repaired yeast/GNPs sample [5]

comparable to our samples [4]. As regards the yeast constitutive behavior, the curve obtained from the pristine yeast [4] has been adopted as input for simulations, using an elastic material with finite strain capability. The cells are modeled with solid elements. The load is applied as imposed displacements on the lateral face of the cells (Fig. 2a). The interface between the two cells was modeled via a cohesive

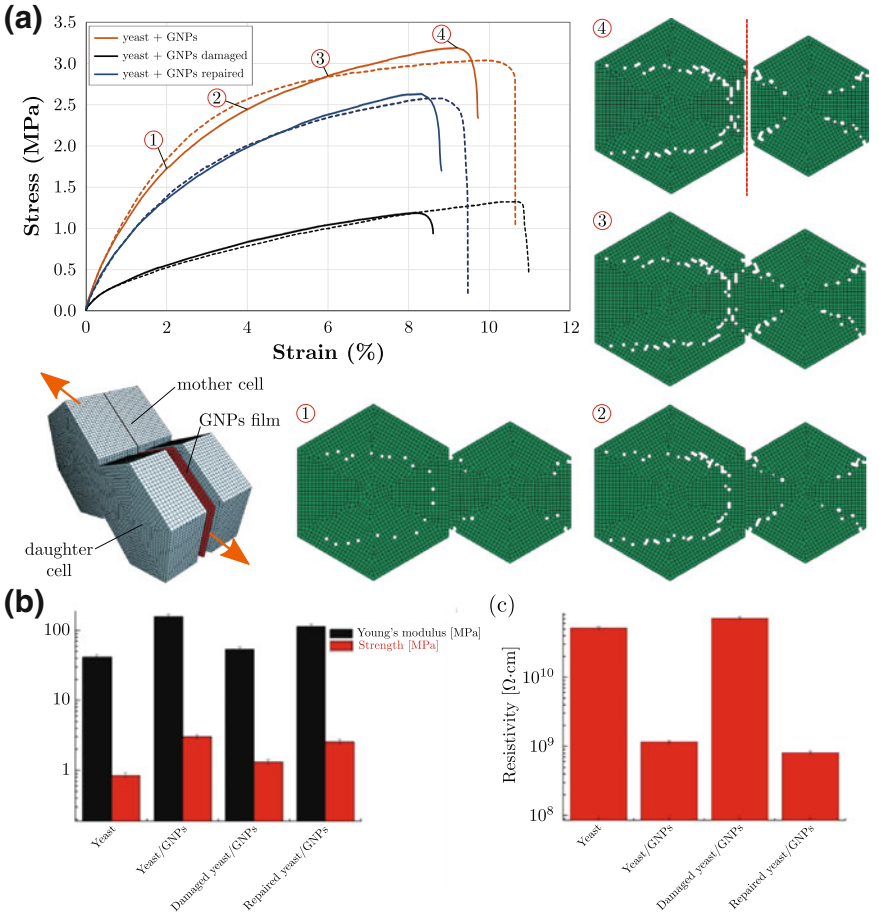

Fig. 2 **a** Simulation of the base "sandwich" composite element made up of a mother cell and a daughter cell with a GNP film in between of 11.5 nm thickness. The simulation-derived curves (continuous) are compared with the corresponding experimental counterpart (*dashed*). The evolution of the fracture pattern in the graphene layers at four different states for increasing tension is depicted: composite failure occurs because of film rupture (state 4) along the interface between the two cells. **b** Mechanical characteristic and **c** electrical resistivity values of neat yeast, yeast/GNPs, damaged and repaired yeast/GNPs samples, respectively [5]

zone model (CZM) based contact for which the force-separation curve was derived in [5] by the authors and associated with an estimated fracture energy of $G_{\text{f-yeast}} = 0.0193$ N/m. GNPs film has been modeled with shell elements assuming an average thickness of 11.6 nm. This corresponds to a total of 34 layers of graphene. Two half cells with a GNPs film in between has been modeled in a sort of "sandwich" structure with periodic boundary conditions at the symmetry planes:

by doing this we simulate an infinite multilayer alternation of yeast cell layer and GNPs film. Constitutive behavior of graphene has been taken from Xu et al. [18], operating a scaling on the failure strength of the curves there reported in order to minimize the deviation of simulation curves with respect to the experimental ones and thus have an indirect estimate of the mechanical properties of GNPs. Perfect bonding was assumed at the interface between the GNPs film and the cell wall, being the constitutive behavior of this interface unknown.

Figure 2a shows the comparison between the curves derived from experiments and simulated tensile tests. The mechanical properties determined from simulation are well comparable with the one estimated with the rule of mixture (Table 1), confirming the mechanical interaction between the GNPs film and yeast cells. A first appearance of diagonal cracks within graphene is followed by their coalescence in the correspondence of the interface between the two cells at which global failure of the composite occurs. This is qualitative comparable with the FESEM experimental images (Fig. 1d, e) and, as obtained for yeast-CNTs composites [4], global failure is governed by interface rather than cell rupture.

Under high-vacuum conditions of FESEM, the formation of cracks on the cell surface indicates a hypo-osmotic shock that is due to the coupling between the GNPs sheets and the cell wall hindering cell dehydration through its membrane (Fig. 1d). We observe that such crack formation can be healed by placing the damaged composite film in aqueous solution of sucrose and GNPs for few minutes; as suggested by FESEM analysis (Fig. 1e), the cracks almost disappear. This self-healing effect is evident on the mechanical and electrical characteristics of the graphene-bionic composite, as reported in Fig. 2b, c. The GNP sheets cover the cell surface and this gives the electrical conductivity and mechanical strength across the cell surface; the decrease of the electrical conductivity and of the mechanical strength as well is attributed to the loss of the graphene connecting network. The nutrient broth reconstructs the GNPs shell and the strain-induced effect on GNP sheets is canceled, partially restoring the mechanical and electrical properties of the undamaged composite.

Table 1 GNPs properties determined from experiments through rules of mixture or from numerical simulations. For both estimates of GNPs properties we assumed an average film thickness $t_{GNP} = 11.5$ nm

Material	Experiments				Simulations			
	σ_f	E	$\sigma_{f\text{-}GNP}$	E_{GNP}	σ_f	E	$\sigma_{f\text{-}GNP}$	E_{GNP}
	(MPa)	(MPa)	(MPa)	(GPa)	(MPa)	(MPa)	(MPa)	(GPa)
Yeast+GNPs	3.1	159	909	48.8	3.2	135	882	50.1
Yeast+GNPs damaged	1.3	54	196	5.1	1.2	50	126	5.3
Yeast+GNP repaired	2.6	115	719	30.5	2.6	107	670	31.7

3 CNTs-Yeast Bionicomposites

An analogous procedure was used to produce CNTs-based yeast bionicomposites (Fig. 3a) [4]. Water dispersion of CNTs (1 mg/ml) were prepared by tip sonication. This dispersion of CNTs was then added to the yeast solution and stirred at 110 rpm at 30 °C for 1 h. Then the yeast/CNTs solution was put into a sterilized circular aluminum mold, and the liquid medium was left to evaporate at 30 °C during the night. In another sterilized flask the same procedure was adopted by adding sugar to promote the fermentation of the yeast/CNTs solution. After fermentation, the liquid medium was dried in a sterilized mold and a composite film was obtained. This procedure results in a complete encapsulation of CNTs by the yeast cells (Fig. 3c, d).

The strength of the composite can be estimated according to Eq. 2a in which the strength of the GNPs is substituted by the pull-out strength of CNTs, derived from the pull-out energy G at the CNT-yeast interface. The fiber volume fraction was estimated to be $f \approx 0.021$ from FESEM images (encircled region in Fig. 3b). The strength of the pristine yeast and for CNTs-yeast fermented composite were respectively 0.0297 MPa and 0.240 MPa obtaining a value of CNTs pull-out strength $\sigma_{f,CNTpo} \approx 10.3$ MPa. From this calculation it is clear how the strength of the yeast is much lower than the pull-out tension, indicating that the strength of the composite is governed by the adhesion energy between the CNTs and the matrix.

Analogously to the previous case, a FEM model of a system of two cells was developed. The interface between the two cells implements a cohesive zone model based contact and its adhesion energy, $G_{f,comp}$ assumed of pure Mode I fracture, is a combination by a rule of mixture of the interface fracture energy of the pristine

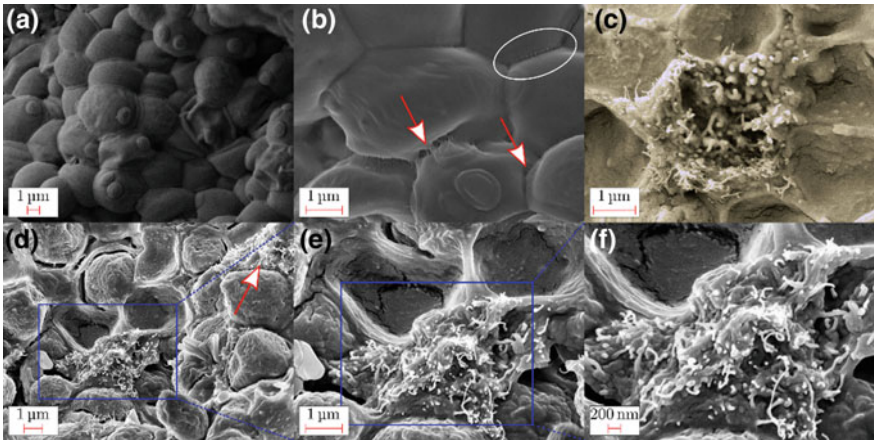

Fig. 3 **a** FESEM image of Saccharomyces cerevisiae. **b** FESEM image showing CNTs bridging yeast cells. The arrows indicate the CNTs bridging the yeast cells. **c** FESEM image of the cross section of the fermented yeast/CNTs film after prolonged exposure to FESEM where CNTs protruding from a broken yeast cell are visible. **d, f** FESEM images of the yeast/CNTs film without fermentation taken at different magnifications [4]

yeast interface (Fig. 4a) and of the adhesion energy of CNTs determined above with the pull-out model (details can be found in Ref. [4]). Under tensile load, failure occurs when for the current interface energy G is $G/G_{\mathrm{f,comp}} > 1$. Figure 4 shows the superposition of the FEM simulations curves on experimental ones. Figure 4a refers to mother–daughter cells in fermented yeast, while in Fig. 4b the cases of equal

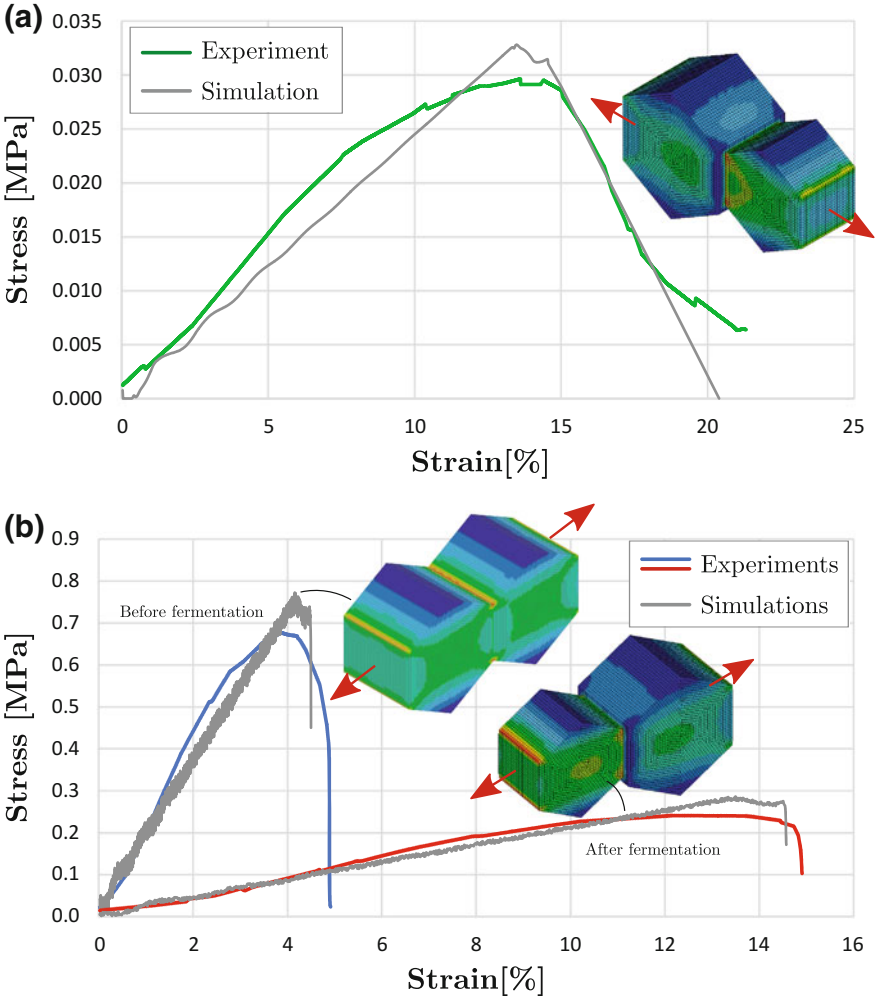

Fig. 4 Stress-strain curves obtained from tensile tests on **a** fermented yeast sample (*green curve*) and **b** yeast/CNT composites prepared before fermentation (*blue curve*) and after fermentation (*red curve*), respectively. Note that for the fermented system two different cell type in terms of dimension and mechanical properties were adopted in order to take into account the mother-daughter cells systems. The comparisons with the result of FEM simulation of traction of a two-cell system are also reported [4]

cells with CNTs before fermentation and mother–daughter cells with CNTs after fermentation are reported. A decrease in the CNTs volume fraction after fermentation is estimated. This result is interpreted with a higher contact area between cells due to the increase of the cells volume after fermentation explaining the lower failure stress and the higher failure strain observed for the composite film after fermentation. Further investigations on this are required.

Current–voltage characteristic of the yeast-CNTs composite was then measured (Fig. 5). We observed that the increase in conductivity is more prominent with the CNTs. Furthermore, the addition of sucrose to the neat yeast resulted in a better conductivity being this effect more pronounced for the fermented yeast/CNTs system. The source of this behavior is the bridging of conductive CNTs within the yeast during the fermentation that improves the percolation pattern for electron transfer. On the contrary the formation and confinement of isolated CNTs bundles between the cell walls (Fig. 3d, f) result in an interruption of the percolation pattern leading to a lower conductivity for the non-fermented yeast/CNTs sample.

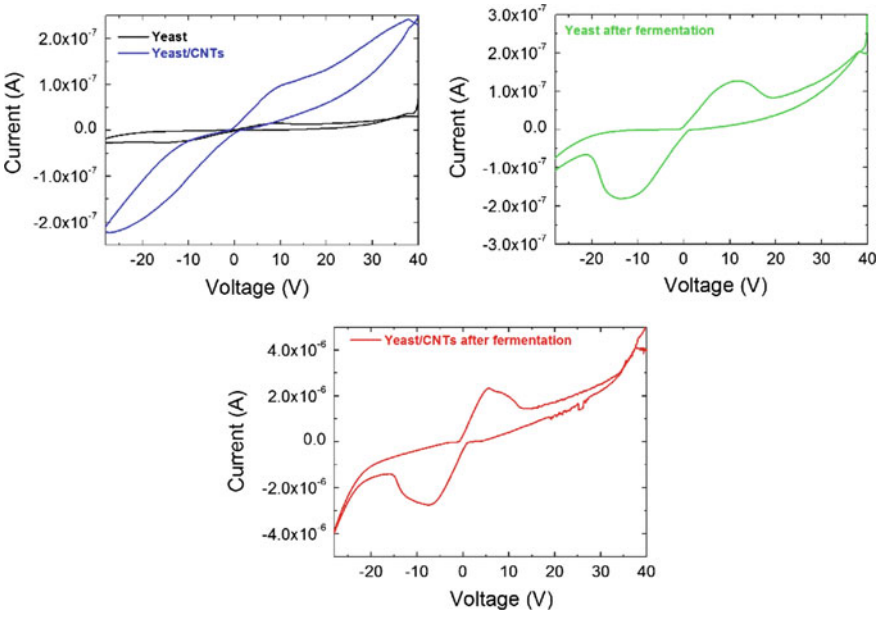

Fig. 5 I-V characteristics of the prepared samples [4]

4 Conclusions

In this study, we demonstrated that the fermentation of commercially available and inexpensive bread yeast with graphene sheets leads to the development of interactive bionic structures programmed to self-assemble into living templates. Encapsulation of the cell with graphene nanoplatelets has been found to enhance the yeast mechanical strength as well as the electrical conductivity. The main result of this study is the development of nanostructured shells that do not disturb the cell proliferation which acts as an electrical conductor and as mechanical reinforcement. At the same time they show self-healing capacity, being able to re-assemble onto the cell surface when the encapsulation is broken by external harsh environment. Similar results were also obtained in CNTs-based fermented yeast composites, in which the conductivity and mechanical properties are enhanced by a nanotube bridging mechanism. In this context, these works [5, 6] aim at the development of future applications of such bionic materials, e.g., in the manufacture of physical smart objects with multifunctional properties (bioelectronics), in the fields of self-healing and functional bionic composites.

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