

# Poly(lactic acid) composites based on graphene oxide particles with antibacterial behavior enhanced by electrical stimulus and biocompatibility

# Paulo Arriagada <sup>(1)</sup>, <sup>1</sup> Humberto Palza, <sup>1</sup> Patricia Palma, <sup>2</sup> Marcos Flores, <sup>3</sup> Pablo Caviedes<sup>4</sup>

<sup>1</sup>Laboratorio de polímeros, Depto. de Ingeniería Química y Biotecnología, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile, Santiago, Chile

<sup>2</sup>Depto. Patología y Medicina Oral, Facultad de Odontología, Universidad de Chile, Sergio Livingstone 943, Santiago, Chile <sup>3</sup>Laboratory of Surfaces and Nanomaterials, Physics Department, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile, Santiago, Chile

<sup>4</sup>Centro de Investigación Clínica y Estudios Farmacológicos, Facultad de Medicina, Universidad de Chile, Santiago, Chile

Received 2 August 2017; revised 7 November 2017; accepted 4 December 2017 Published online 21 December 2017 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.a.36307

**Abstract:** Poly(lactic acid) (PLA) is a biodegradable and biocompatible polyester widely used in biomedical applications. Unfortunately, this biomaterial suffers from some shortcomings related with the absence of both bioactivity and antibacterial capacity. In this work, composites of PLA with either graphene oxide (GO) or thermally reduced graphene oxide (TrGO) were prepared by melt mixing to overcome these limitations. PLA composites with both GO and TrGO inhibited the attachment and proliferation of *Escherichia coli* and *Staphylococcus aureus* bacteria depending on the kind and amount of filler. Noteworthy, it is shown that by applying an electrical stimulus to the percolated PLA/TrGO, the antibacterial behavior can be dramatically increased. MTT analysis showed that while all the PLA/GO composites were more cytocompatible to osteoblast-like cells (SaOS-2) than pure PLA, only low content of TrGO was able to increase this property. These tendencies were related with changes in the surface properties of the resulting polymer composites, such as polarity and roughness. In this way, the addition of GO and TrGO into a PLA matrix allows the development of multifunctional composites for potential applications in bio-medicine. © 2017 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 106A: 1051–1060, 2018.

**Key Words:** graphene oxide, surface characterization, antibacterial activity, biocompatibility, electrical stimulus

**How to cite this article**: Arriagada P, Palza H, Palma P, Flores M, Caviedes P. 2018. Poly(lactic acid) composites based on graphene oxide particles with antibacterial behavior enhanced by electrical stimulus and biocompatibility. J Biomed Mater Res Part A 2018:106A:1051–1060.

## INTRODUCTION

During the past few years, it has been an increase in development of biopolymers as an alternative to petroleumbased materials.<sup>1</sup> Owing to their biocompatibility, these materials can be used in biomedical applications, such as tissue engineering, regenerative medicine, gene therapy, and controlled drug delivery.<sup>1,2</sup> In particular, poly(lactic acid) (PLA), a thermoplastic aliphatic polyester produced from non-fossil renewable natural resources, has emerged as an interesting biopolymer due to its renewability, biodegradability and relatively low cost.<sup>3</sup> Despite the large use of PLA as a biomaterial, it still has some limitations such as poor mechanical behavior and lack of an intrinsic bioactivity. Moreover, similar to any other biomaterial, it is foreseen to be infected due to the absence of antibacterial behavior.<sup>4</sup> Different routes have been reported to overcome these issues where the addition of particles into PLA to produce

composite materials is one of the most used. Different kind of particles had been used such as fibres  $^{\rm 5}$  and micro and nanoparticles.  $^{6,7}$ 

Among the diverse particles used in these composites graphene derivatives (GD) such as graphene nanoplatelets (GNP), exfoliated graphite (EG), graphene oxide (GO), or reduced graphene oxide (rGO)—have emerged as an alternative due to their exciting properties recently.<sup>8</sup> The addition of GD can improve some physical properties of a polymer matrix such as electrical and thermal conductivities, or mechanical behavior, among others.<sup>9,10</sup>

Regarding the antimicrobial behavior of polymer/GD materials, few polymers had been tested, including poly-N-vinylcarbazole (PVK),<sup>11</sup> polyester,<sup>12</sup> chitosan,<sup>13</sup> poly(vinyl alcohol),<sup>14</sup> and PLA. In these cases, GO was mainly used obtaining 100% of antibacterial efficiency at concentrations between  $0.2^{15}$  and  $5^{16}$  wt %. The mechanisms of antibacterial

Additional Supporting Information may be found in the online version of this article.

Correspondence to: P. Arriagada; e-mail. parriagada@ing.uchile.cl

Contract grant sponsor: Fondo Nacional de Desarrollo Científico y Tecnológico; contract grant number: 1150130

behavior include oxidative stress by the formation of reactive oxygen species (ROS) and mechanical disruption of bacterial membranes.<sup>17</sup>

GD particles are outstanding because of their antimicrobial behavior but also because of some studies concluded that their toxicity is relatively low depending on the type, time, production method, and concentration of particles.<sup>18</sup> As for polymer/GD composites, there are few examples about cell viability, for instance, using matrices such as PVK<sup>11</sup> and poly(vinyl alcohol).<sup>14</sup> Regarding PLA, the incorporation of GO was able to improve its biocompatibility toward neuronal,<sup>19</sup> osteogenic cells,<sup>20,21</sup> and foreskin fibroblast.<sup>22,23</sup>

The multifunctional behavior of polymer/GD composites is reflected in the improvements in other parameters related with the physical properties of the polymer matrix such as mechanical and electrical properties. PLA/GD nanocomposites showed an improvement in the Young modulus at low amount of EG.<sup>23-25</sup> On the other hand, electrically conductive PLA composites were found by Kim and Jeong,<sup>24</sup> improving 10 orders of magnitude the conductivity at 7 wt % of EG. Similar results were obtained by Shen et al.<sup>26</sup> and Kashi et al.,<sup>27</sup> at 2 vol % of CrGO and at 15 wt % of GNP, respectively. The electrical behavior is explained by the percolation theory assuming a conductive path formed through the composite. This makes a nonlinear change on the electrical property of the material depending of the additive concentration.<sup>28</sup> Despite the evidence that electrical current through a material can improve several bio-related properties, studies about electrical percolated PLA composites for electrical stimulus have not been reported.

Based on the above mentioned, it is clear that the addition of different GD into PLA can produce multifunctional biomaterials with antimicrobial behavior and improved biocompatibility. In this regard, we report a comparative study of the behavior in these properties of PLA nanocomposites having either rGO or GO. Our results not only show that these materials presented improved both physical and bioactive properties, but also for a first time an electrical stimulus biocide behavior was observed in conductive PLA/rGO composites.

# MATERIALS AND METHODS Materials

A commercial grade for 3 D impression *amorphous* poly (lactic acid) (PLA) from Polimersia Global, SL (Spain) with a melt flow rate of 7–9 g/10 min (2.16 kg/210°C) (Norm ASTM D-1238/95) and having a 96:4 L:D ratio content, was used as matrix. G (graphite fine powder extra pure) with a particle size <50 (µm), sulfuric acid (98.08%, H<sub>2</sub>SO<sub>4</sub>), potassium permanganate (99%, KMnO<sub>4</sub>), hydrochloric acid (32%, HCl), and sodium nitrate (99.5%, NaNO<sub>3</sub>) were obtained from Merck (Germany) and used as received. Hydrogen peroxide (5%, H<sub>2</sub>O<sub>2</sub>) was purchased from Kadus S.A.

# Preparation of graphene derivatives

GO was prepared in oxidation process using G as raw material. The graphite oxidation process of Hummers and Offeman was employed.<sup>29</sup> The first step is an oxidation of G with KMnO<sub>4</sub> and NaNO<sub>3</sub> in concentrated sulphuric acid. This oxidation was carried out using 250 mL of concentrated sulfuric acid as dispersion medium per 10 g of G. To the stirred dispersion, 5 g of NaNO<sub>3</sub> was added and after 30 min of stirring, it was cooled down to  $\sim 0^{\circ}$ C using an ice water bath. Then 30 g of KMnO<sub>4</sub> were added during 4 h. When the addition was completed, the resulting dispersion was stirred at room temperature for 1.5 h. The reaction was quenched by pouring the dispersion into 0.5 L of ice water and adding 450 mL of H<sub>2</sub>O<sub>2</sub> (5 wt %) until the excess of KMnO4 was reduced. The GO was filtered off and washed with aqueous HCl and subsequently washed with water. The purified brown GO was dried under vacuum to 110°C for 10 h. Second, the dry GO was thermally reduced to afford TrGO in a nitrogen atmosphere by rapidly heating GO from room temperature up to 600°C during 40 s, using a quartz reactor heated in a vertical tube furnace preheated at 600°C. TrGO was obtained as a black powder with low bulk density. The thermal shock is the prime requirement to achieve reduction of graphene sheets.

#### Synthesis of PLA with graphene derivatives composites

The composites were prepared by using a Brabender Plasticorder (Brabender, Germany) internal mixer at 190°C and a speed of 110 rpm. Filler content ranges from 0 to 5 wt % for GO and 0 to 10 wt % for TrGO. First, a half of polymer was added to the mixer operated at 20 rpm. After 1 min approximately for melting the polymer, the filler was added during 3 min. Finally, the rest of polymer pellets was added and the speed of the mixer was held at 110 rpm for 10 min. Therefore, the total mixing time was around 14 min. After removal from the mixer, the composites were molded in a hot press at 190°C for 2 min, under a pressure of 150 kg/cm<sup>2</sup>, into films of 12x12 cm<sup>2</sup> with approximately 1 mm thickness. After pressing, the films were cooled at room temperature. Samples with different dimensions were cut from these films, depending on the characterization test.

# Characterization

*G*, *GO*, *and TrGO powders*. X-ray diffraction (XRD) analysis was performed on a Siemens D-5000 diffractometer with scintillation detector diffraction system and Bragg–Brentano geometry operating with a Cu K $\alpha$ 1 radiation source filtered with a graphite monochromator (k = 1.5406 Å) at 40 kV and 30 mA in the 2 $\theta$  range of 2–80° at the scan rate of 0.02°/s.

**PLA/graphene derivatives composites.** MFI measurements of PLA and its nanocomposites were done using a Model 2 Digital Melt Flow Indexer (Ray-Ran) at 190°C/2.16 kg according to ASTM D1238.

Fourier transform infrared spectroscopy (FTIR) spectra were obtained through a Thermo Scientific Nicolet 8700 spectrometer, using attenuated total reflectance (ATR) in the 4000 and 400 cm<sup>-1</sup> spectral range averaging 50 scans with a resolution of 4 cm<sup>-1</sup>.

Mechanical properties of composites were examined on a HP model D-500 dynamometer according to ASTM D638-10. Samples of 40  $\times$  16 mm<sup>2</sup> and a thickness of 2 mm were tested at a rate of 50 mm/min at room temperature. Results are the average values out of five measurements.

For electrical conductivity measurements, the material was cut into the shape of a rectangular specimen of length 4 cm, height 1 mm and width 1 cm. Copper wires were used as electrodes, being attached to both ends of the bar. With this set-up, the standard two points method was used. Electrical conductivity of composites was measured with different meghommeters (Megger BM11 with a highest voltage of 1200 V and AEMC 1060 with a highest voltage of 5000 V), depending on the conductivity of the samples. For each electrical value displayed in this investigation, at least four samples were prepared and four measurements for each one were carried out.

A macro lens was used to measure the contact angle of pure water on PLA composites films by the standard sessile drop method at 25°C. Measurements were realized on 5 different regions, and data were obtained with ImageJ software (National Institutes of Health, USA). Equilibrium contact angles considered at 60 s were measured for 5  $\mu$ L droplet volumes.

The surface roughness was estimated from topographic images. The topographic images were obtained by AFM in contact mode. The measurements were performed in an Omicron SPM1 equipment running in vacuum conditions. Several images in different sites on areas of  $5 \times 5 \ \mu\text{m2}$  and  $1 \times 1 \ \mu\text{m2}$  were taken for each sample. The WSxM program<sup>30</sup> was used to analyze the topographic images.

#### **Biocompatibility test**

Cytotoxicity of the PLA/graphene composites was evaluated by 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (USB Corporation, USA) as per standard protocol to assess cell viability (ISO/EN 10993-5 2009). Samples of composites cut in discs with 5  $(\pm 0.5)$ mm of diameter, sterilized by autoclave and UV light during 30 min, were placed at the bottom of each well. Human osteosarcoma cell line (SaOS-2) was seeded in DMEM/F12 supplemented with 10% fetal bovine serum (FBS), gentamicin (40 mg  $L^{-1}$ ) and 1  $\mu$ L ketokonazol (5 mg m<sup>-1</sup> of phosphate buffer solution (PBS))  $mL^{-1}$  culture media, at a density of 5  $\times$   $10^3$  cells per well (150  $\mu L$  of culture media). After 24 h of culture in an incubator at 37°C and humidified atmosphere (5%  $CO_2$  in 95% air), the previous culture media was removed and each well washed with PBS. 100 µL of media with MTT (80 µL of MTT (5 mg  $mL^{-1}$ ) per mL of DMEM/F12 without FBS or phenol red) was added to each well and incubated during 3 h. After incubation, the media containing MTT was removed from the 96-well plate and 200 µL of isopropyl alcohol was added per well to dissolve the formazan crystals. The optical density (OD) of the solution was measured at 540 nm by a microplate reader (Titerket<sup>®</sup> Multiskan<sup>®</sup> MCC/340, MKII) and cell viability calculated. The MTT assay per each bioactive glass was performed with n = 6 and three

independent experiments. The cells cultured with DMEM/ F12 media acted as control. The cell viability was calculated using Eq. (1):

$$Viability (\%) = \frac{OD_{test culture}}{OD_{control culture}} \times 100$$
(1)

# Antibacterial activity test

The antibacterial efficiency PLA/GO and PLA/TrGO nanocomposites was analyzed using the standard protocol of antibacterial activity (ISO 22196:2007(E)) against E. coli ATCC 25922 and S. aureus ATCC 25923. Samples of nanocomposites were cut into 50  $\times$  50 mm<sup>2</sup>, and polyethylene films were cut into 40  $\times$  40 mm<sup>2</sup>. All squares were sterilized by autoclave, and before the test each glass recipient and tool was subjected to UV light for 30 min. After that, 0.4 mL with 2  $\times$  10  $^{6}$  CFU/mL bacterial suspension was seeded on each nanocomposite film sample and covered with polyethylene film so that the bacterial suspension can spread to the edges. For the electrically stimulated composites, each sample was connected to a circuit consisting in six serial batteries (Eveready, 9 V) through two copper electrodes attached in opposites sides of the composite sample, so the electrical current can flow across the entire film. After an incubation of 24 h (37°C, 90% RH), 10 mL of phosphate-buffered saline (PBS) was used to wash the samples and polyethylene films, using gentle shaking for 1 h. Serial dilutions of 10- to  $10^5$ -fold with a gradient method, and 1 mL of each dilution was placed into separate sterile Petri dishes. Immediately, 15 mL of plate count agar was poured into each Petri dish to disperse the bacteria by swirling gently, and incubated at 37°C for 24 h. After incubation, the number of colonies was counted. The antibacterial efficiency (R) was calculated via Eq. (2):

$$R(\%) = \frac{N_0 - N}{N_0} \times 100$$
 (2)

# Statistical analysis

The results are expressed as the mean  $\pm$  SD. Statistical differences were calculated with the two-sample *t* test and were considered significant at  $p \leq 0.05$ . For antibacterial activity and biocompatibility test were used the antibacterial efficiency *R*(%) and the viability (%), respectively, to calculate statistical differences.

# RESULTS

#### Characterization of GD and PLA/GD nanocomposites

X-ray diffraction (XRD) was used to detect the interlayer distance between graphene sheets, as displayed in Figure 1. Graphite (G) presents a sharp diffraction peak at  $2\theta = 26.5^{\circ}$ , corresponding to an interlayer spacing of 3.4 Å.<sup>31,32</sup> The intensity of the G peak is reduced and shifted to  $2\theta = 11.0^{\circ}$  after the oxidation. After thermal reduction of GO to obtain TrGO, there is no evidence of any diffraction peaks.



FIGURE 1. XRD patterns of G, GO, and TrGO.

To analyze the effect of processing on the polymer molecular weight, MFI tests were carried out to some representative samples. As-received PLA presented an MFI value of 7.1 g/10 min, meanwhile after melt mixing (without nanoparticles) and hot-pressing molding, the MFI value of PLA increased to 11.5 g/10 min. Noteworthy, PLA composites with 5 wt % of either GO or TrGO presented an MFI below the resolution of the technique (>30 g/10 min).

FTIR-ATR analysis was carried out to PLA and its nanocomposites, where significant differences were found in the intensities of the peaks of the pure matrix and composites. As could be seen in Supporting Information, Figure S1, the peaks at 2995 and 2944 cm<sup>-1</sup> were assigned to -CHstretching, the peak at 1746 cm<sup>-1</sup> was assigned to C=0 carbonyl, the peaks at 1452, 1381, and 1360 cm<sup>-1</sup> were assigned to asymmetric and symmetric -CH- deformation in CH<sub>3</sub> bending, the peaks at 1262 and 1179 cm<sup>-1</sup> and at 1128, 1079, and 1042 cm<sup>-1</sup> were assigned to -C-O- stretching in -CH-O- and -O-C=O-, respectively, and the peak at 866 cm<sup>-1</sup> was assigned to -C-C- stretching.<sup>33-35</sup> By adding both type of fillers into the PLA, the peaks at 956, 1042–1262, and 1746 cm<sup>-1</sup> decreased its bands area, while the peak at 922 presented an increase and a decrease in the band area for GO and TrGO, respectively, as could be seen in Supporting Information, Figure S2.

The sessile drop method was used to determine the water contact angles on the surfaces of the resulting composites as displayed in Figure 2. The contact angle of PLA/GO composites decreased  $>10^{\circ}$  comparing with pristine PLA, meanwhile the water contact angle of PLA/TrGO composites increased around 13° comparing with PLA.

The topography of the surface of the different composites was further evaluated. In particular, AFM topographic images, with some representative images shown in Figure 3, were used to study surface roughness as displayed in Figure 4. The root mean square (RMS) roughness of PLA measured at sections of 1 and 5  $\mu$ m<sup>2</sup> was affected by the incorporation of both GO and TrGO carbon nanostructure, with the decreasing of its value.

# Antibacterial activity

Antibacterial efficiencies against *E. coli* and *S. aureus* of PLA and its composites with GD are shown in Table I. Noteworthy, the antimicrobial behavior of the composites depended on the kind and the amount of particle, with GO presenting the best results. For instance, 5 wt % of GO nanofillers sacrificed 100% of both bacteria. For TrGO nanofillers, only composites with 10 wt % showed some antibacterial behavior for both *E. coli* and *S. aureus* bacteria, increasing its value.

#### **Electrical properties**

Electrical properties of the PLA/GD composites are showed in Figure 5. As is observed, the PLA/GO composites did not show electrical percolation, meanwhile the PLA/TrGO



FIGURE 2. Water drop profiles and contact angles of (a) PLA, (b) PLA/5% TrGO, (c) PLA/10%TrGO, (d) PLA/3% GO, and (e) PLA/5% GO composites (values indicated are the corresponding contact angle; n = 3).



FIGURE 3. Three-dimensional representation of AFM images of (a) PLA, (b) PLA/5%TrGO, (c) PLA/10%TrGO, (d) PLA/3%GO, and (e)PLA/5%GO.

composites showed an electrical percolation Around 5 wt %. Composite with 10 wt % of TrGO showed an electrical conductivity of  $10^{-2}$  [S/m].

# **Biocompatibility**

Viability of SaOS-2 cells after 24 h of exposure to the different nanocomposites is shown in Figure 6. Cell viability in PLA is lower than control, and by adding low amount of TrGO (<1 wt %) and GO in the whole range studied, the samples increase the cell viability. For high amount of TrGO biocompatibility did not show statistical difference respect to PLA.

## **Mechanical properties**

Figures 7 and 8 shows the changes of Young's modulus and strain at break, respectively, of PLA/GO and PLA/TrGO



**FIGURE 4.** RMS roughness of PLA composites in different studied areas (n = 3).

composites as a function of graphene content. The mechanical behavior depended of the filler used with GO particles producing higher values of Young moduli than TrGO.

Dashed lines in Figure 7 show the Young modulus predicted by the Halpin-tsai model varying the aspect ratio,  $\alpha$ , to the required value fittings the experimental data. For PLA/TrGO composites  $\alpha$  gets the value of 60 meanwhile in PLA/GO this value increased to 500.

# DISCUSSION

# Characterization of GD and PLA/GD nanocomposites

As displayed in Figure 1, the intensity of the G peak is reduced and shifted to  $2\theta = 11.0^{\circ}$  after the oxidation, meaning that the presence of functional groups disrupts the layer structure of pure graphite.<sup>32</sup> After thermal reduction, there is no evidence of peaks associated with GO, confirming the disruption of the interlayer order.<sup>31</sup> Both GD, meaning TrGO and GO, were used as fillers in PLA nanocomposites.

MFI values suggest a thermal degradation of PLA matrix during melt processing. Moreover, incorporation of GD into PLA increased its MFI value, which is attributed to a pronounced degradation of the polymer matrix<sup>22,36</sup> by hydrolysis of its ester bond.<sup>33,34</sup> This process is accelerated by the presence of GD, due to the defects caused by the fillers



**FIGURE 5.** Effect of the graphene derivatives on the electrical conductivity of PLA composites (n = 5).

producing different interfaces in the polymer matrix, generating penetration channels for the infiltration of water molecules.<sup>22,34</sup> Additionally, for GO, oxygen-containing groups induce the scission of the PLA molecular chains.<sup>34</sup>

Hydrolytic degradation could be studied by FTIR spectra, as is shown in Supporting Information, Figure S1. Compared with PLA, the PLA/GD composites exhibits visible alteration in the FTIR spectra, related to the changes in area of the peak bands. As seen in Supporting Information, Figure S2, a decrease in the area of the peaks between 1042 and 1262 cm<sup>-1</sup> of a 15% and a 60% for G0 and TrGO, respectively, was detected, confirming that hydrolysis of the ester bond is the predominant degradation mechanism.<sup>33,34</sup> Decrease in the peak at 1746 cm<sup>-1</sup> also confirms the disruption of the ester chemical bond.<sup>37</sup> Furthermore, GD change the crystallinity of the PLA matrix, as is confirmed by the decrease in the peak at 956 cm<sup>-1</sup>, related to the amorphous region of the polymer, and a slight increase in the peak at 922 cm<sup>-1</sup>, related to the crystalline region.<sup>34,35</sup>

For the surface properties have a huge effect on the biological properties of a sample, such as protein adsorption and cell adhesion,<sup>38</sup> the sessile drop method was used, as displayed in Figure 2. The contact angle of PLA/GO composites decreased  $>10^{\circ}$  comparing with pristine PLA, showing that addition of GO in the PLA matrix increases its

<b>TABLE I. Antibacterial Efficiency</b>	<i>R</i> (%) and	CFU/mL of	f PLA/Graphene	Derivatives	Composites
--	------------------	-----------	----------------	-------------	------------

Material	E. coli		S. aureus	
	R (%)	CFU/mL	R (%)	CFU/mL
PLA <sup>a</sup>	_	8.82×10 <sup>7</sup>	-	8.09×10 <sup>7</sup>
PLA/3% GO	3.1 ± 3	8.55×10 <sup>7</sup>	1.8 ± 3	7.95×10 <sup>7</sup>
PLA/5% GO	100 ± 0	3.13×10 <sup>1</sup>	100 ± 0	3.88×10 <sup>1</sup>
PLA/5% TRGO	13.8 ± 8	7.60×10 <sup>7</sup>	0 ± 6	8.62×10 <sup>7</sup>
PLA/10% TRGO	44.9 ± 11	4.34×10 <sup>7</sup>	35.5 ± 3	5.22×10 <sup>7</sup>
PLA/10% TRGO e-	$100 \pm 0$	9.63×10 <sup>1</sup>	$100 \pm 0$	3.93×10 <sup>4</sup>

<sup>a</sup>PLA was used as control.

e- denotes that the composite was subjected to an electrical stimulus.



**FIGURE 6.** Cytocompatibility of PLA nanocomposites. \* Statistically significant difference between experiment groups with control. + Statistically significant difference between experiment groups with PLA (n = 6; p < 0.05).

hydrophilicity. In contrast, the water contact angle of PLA/ TrGO composites increased around  $13^{\circ}$  comparing with PLA, meaning an increase of hydrophobicity. Therefore, the presence of GD affects the surface properties, particularly its wettability, as was found by Pinto et al.<sup>20</sup> and Pei et al.<sup>38</sup> These results further confirmed the high polarity of GO associated with its functional groups as compared with TrGO with poor polarity because of the high content of carbon atoms.

The topography of the surface was further evaluated as it has an effect on cellular adhesion.<sup>38</sup> In particular, AFM topographic images, with some representative images shown in Figure 3, were used to study surface roughness as displayed in Figure 4. The root mean square (RMS)



**FIGURE 7**. Young's modulus of PLA/GO and PLA/TrGO composites as a function of graphene content (n = 5).



**FIGURE 8.** Strain at break of PLA/GO and PLA/TrGO composites as a function of graphene content (n = 5).

roughness of PLA measured at sections of 1 and 5  $\mu$ m<sup>2</sup> was affected by the incorporation of both GO and TrGO carbon nanostructure, decreasing its value. However, an increase in roughness at 3% of GO content was observed likely associated with the presence of GO particles in the surface of the composites.<sup>20</sup> The presence of carbon nanostructures further decreased the RMS dispersion meaning an increase in the surface homogeneity. An opposite tendency has been reported, founded an increment in roughness with the addition of GD.<sup>20,38</sup> However, the method of mixing PLA with GD fillers should be considered as the solvent mixing used in previous reports improve the presence of GO and TrGO agglomerates in the surface.<sup>20</sup>

# Effect of composite properties in antibacterial activity

As expected, pure PLA did not present any antimicrobial effect although the presence of GO and TrGO added a biocide behavior, as shown in Table I. In addition, there is not a significant statistical difference between both tested bacteria, so the differences in the outer bacterial membrane are not a relevant issue in this analysis. It is noteworthy that the antimicrobial behavior of the composites depended on the kind and the amount of particle, with GO presenting the best results. For instance, 5 wt % of GO nanofillers sacrificed 100% of both bacteria. This behavior agrees with studies by An et al.<sup>15</sup> and Huang et al.<sup>16</sup> The increased hydrophilicity of the composites (Fig. 2) creates an inhibition surface for bacteria proliferation, which is potentiated by the amphiphilic behavior of the surface.<sup>20</sup> These results further confirmed the high polarity of GO associated with its functional groups as compared with TrGO. Roughness of the composites should be further considered in the antimicrobial behavior as bacterial adhesion is sensitive to this parameter, and can explain the absence of antibacterial behavior in composites with 3% of GO.  $^{\rm 39}$ 

For TrGO nanofillers, only composites with 10 wt % showed some antibacterial behavior. In this case, the surface



FIGURE 9. Schematic illustration of graphene effect in electron chain of bacteria membrane.

polarity of the composite cannot explain this behavior due to its less hydrophilic characteristic, so the antimicrobial behavior should relate with both the reduced roughness and the electrical behavior. Regarding the surface roughness, it is reported that a reduced surface roughness (as can be seen in Fig. 3 for PLA/TrGO composite) decreases bacterial adhesion, resulting in minor colonization.<sup>39</sup> Regarding the electrical, as displayed in Figure 5, PLA with 10 wt % of TrGO presented an electrical percolated state. This electrical conductivity can render antimicrobial behavior to the composite as recently reported, associated with the production process of energy in bacterial cells requiring electron transport chain to carry out respiration process,<sup>40</sup> so bacteria membrane exhibits a negative net charge.<sup>41</sup> As proposed by Li et al.,<sup>42</sup> presence of graphene brings the facile transfer of electrons from bacteria membranes to graphene acceptors. This process produces an interruption of the electron chain transfer in the membrane, killing bacteria.40 In this form, electrical conductive nanocomposites with 10 wt % of TrGO show around 40% of antibacterial behavior, in contrast with not conductive nanocomposites with 5 wt % of TrGO without any effect. This process is illustrated in Figure 9, where graphene sheets embedded in the polymer attracts electrons from bacterial membrane.

# **Electricidal effect**

Some studies<sup>43–45</sup> found that a prolonged exposure to low-intensity electrical current reduced the presence of *Staphylococcus* and *Pseudomonas* in material surfaces, a phenomenon that was called "electricidal effect" by Schmidt-Malan et al.<sup>45</sup> Electrostatic forces between bacteria and surfaces are generally repulsive, as most of biomaterials are negatively charged as are bacteria.<sup>44</sup> It has been proposed that these repulsive forces can be enhanced by the application of electrical current provoking the surface detachment of bacterial biofilms.<sup>44</sup> Additionally, it could produce oxidative stress<sup>46</sup> or changes in pH and temperature.<sup>45</sup> Despite these results, application of electrically conductive polymer composites to antimicrobial application has been barely carried out. To our knowledge, there is no report about polymer/graphene nanocomposites for electrical antimicrobial behavior.

When the electrical percolated composite was subjected to an electrical stimulus (PLA/10% TrGO e-), the antimicrobial behavior dramatically increased, reaching values as high as 100% of antimicrobial activity. We hypothesized that the charge transfer process is potentiated by the electrical current, as is explained by van der Borden et al.<sup>44</sup> Therefore, it is possible to control the bacterial attachment to the surface by applying an electrical current.<sup>47</sup> The antibacterial behavior is based on the generation of an electrostatic effect that causes bacterial cell death.<sup>42,44,48</sup> As presented early, in this case there are also not statistical differences between both tested bacteria, and again the differences in outer bacterial membrane are not considered.

# Effect of composite properties in biocompatibility

As is shown in Figure 6, cell viability in PLA is lower than control because of the hydrophobic nature of PLA (Fig. 2), reducing the adhesion of the extracellular matrix (ECM) proteins, avoiding cells stick on the surface.<sup>20</sup> Noteworthy, by adding low amount of TrGO (<1 wt %) and GO in the whole range studied, the samples increase the cell viability. For composites with low amount of TrGO, the increment in cell viability could be due to the increased surface roughness,<sup>38</sup> as reported by Pinto et al.<sup>20</sup> However, this positive effect was vanished at high TrGO content due to the negative effect associated with the increment of the surface hydrophobicity (Fig. 2) hindering cell adhesion.<sup>16,20,38</sup> PLA/GO nanocomposites otherwise have more hydrophilic surfaces (Fig. 2), enhancing ECM protein absorption and consequently cell adhesion, as reported previously.<sup>19,21</sup>

# **Mechanical properties**

Mechanical behavior is an important aim in designing biomaterials, particularly biocomposites. As was mentioned, GD produces a reinforcing effect in polymer matrices.<sup>24</sup> As can be seen in Figures 7 and 8, the mechanical behavior depended of the filler used, where both nanoparticles produced a decrease in strain at break (Fig. 8). However, GO particles produced higher values of Young moduli than TrGO (Fig. 7). This tendency can be associated with a better dispersion of GO sheets in the PLA matrix.<sup>24,25,49</sup> Moreover. a good adhesion between GO and PLA because of hydrogen bonds should further be considered in the analysis.<sup>50</sup> However, our results of FTIR analysis (Supporting Information, Fig. S1) do not indicate the formation of chemical bonding between GO and PLA. In this case, the reinforcement of the polymer matrix could be due to the increase in crystallinity, as is indicated by the increase of the peak at 922  $\text{cm}^{-1}$ (Supporting Information, Fig. S2).<sup>35</sup>

To analyze our results, the experimental Young modulus were compared with theoretical values predicted by the Halpin-tsai model, assuming random distribution of both GO and TrGO fillers in the PLA matrix.<sup>51</sup> This model is

expressed by the values and equations stated in Supplementary Information. Varying the aspect ratio,  $\alpha$ , to the required value fittings the experimental data (Fig. 7), it was shown that for PLA/TrGO composites,  $\alpha$  gets the value of 60 meanwhile in PLA/GO this value increased to 500, meaning completely separated GO sheets. In this way, change in the filler aspect ratio can explain the findings observed in Figure 7.

# CONCLUSION

Incorporation of graphene derivatives into a PLA matrix can add some functionalities to the polymer matrix that are relevant for future bioapplications. In particular, changes in the wettability of the surface of the composites were obtained with a surface more either hydrophilic for PLA/GO composites or hydrophobic for PLA/TrGO composites, as compared with pristine PLA. The addition of GO inhibited the proliferation of bacteria at a concentration of 5 wt % of GO, while improving 40% the cell adhesion as compared with PLA, showing no cytotoxic effects. Additionally, by applying an electrical stimulus the antibacterial behavior in PLA/TrGO composites was dramatically enhanced. Cell adhesion in PLA/TrGO composites did not show a significant difference with PLA. Both GO and TrGO present an improvement of Young's modulus, with the former being a better reinforcement for the PLA matrix. In this way, this investigation shows good results of PLA/graphene derivatives composites for its potential use in biomedical applications.

# CONFLICT OF INTEREST

The authors declare no competing financial interest.

#### REFERENCES

- Nair LS, Laurencin CT. Biodegradable polymers as biomaterials. Prog Polym Sci 2007;32:762–798.
- Singh A. Biopolymers in drug delivery: A review. Pharmacol Online 2011;674:666–674.
- Drumright RE, Gruber PR, Henton DE. Polylactic acid technology. Adv Mater 2000;12:1841–1846.
- Bin AM, Jokar M. Silver/poly (lactic acid) nanocomposites: Preparation, characterization, and antibacterial activity. Int J Nanomed 2010;573–579.
- Pickering KL, Efendy MGA, Le TM. A review of recent developments in natural fibre composites and their mechanical performance. Compos Part A Appl Sci Manuf 2016;83:98–112.
- Raquez J-M, Habibi Y, Murariu M, Dubois P. Polylactide (PLA)based nanocomposites. Prog Polym Sci 2013;38:1504–1542.
- Basu A, Nazarkovsky M, Ghadi R, Khan W, Domb AJ. Poly(lactic acid)-based nanocomposites. Polym Adv Technol 2017;28:919– 930.
- 8. Geim AK, Novoselov KS. The rise of graphene. Nat Mater 2007; 6: 183–191.
- Khanam PN, Ponnamma D, AL-Madeed MA. Electrical properties of graphene polymer nanocomposites. Polym Compos Mater 2015;36–38.
- Garzón C, Palza H. Electrical behavior of polypropylene composites melt mixed with carbon-based particles: Effect of the kind of particle and annealing process. Compos Sci Technol 2014;99:117– 123.
- Mejías Carpio IE, Santos CM, Wei X, Rodrigues DF. Toxicity of a polymer-graphene oxide composite against bacterial planktonic cells, biofilms, and mammalian cells. Nanoscale 2012;4: 4746.

- Bora C, Bharali P, Baglari S, Dolui SK, Konwar BK. Strong and conductive reduced graphene oxide/polyester resin composite films with improved mechanical strength, thermal stability and its antibacterial activity. Compos Sci Technol. Elsevier Ltd 2013; 87:1–7.
- Lim HN, Huang NM, Loo CH. Facile preparation of graphene-based chitosan films: Enhanced thermal, mechanical and antibacterial properties. J Non Cryst Solids. Elsevier BV 2012;358:525–530.
- Jankovic A, Mitric M, Matic I, Juranic ZD, Rhee KY, Jin S, Ljiljana Z, Hui D. The effect of graphene loading on mechanical, thermal and biological properties of poly (vinyl alcohol)/graphene nanocomposites. J Ind Eng Chem 2016;34:250–257.
- An X, Ma H, Liu B, Wang J. Graphene oxide reinforced polylactic acid/polyurethane antibacterial composites. J Nanomater 2013; 2013:1–6.
- Huang Y, Wang T, Zhao X, Wang X, Zhou L, Yang Y, Liao F, Ju Y. Poly(lactic acid)/graphene oxide-ZnO nanocomposite films with good mechanical, dynamic mechanical, anti-UV and antibacterial properties. J Chem Technol Biotechnol 2015;90:1677–1684.
- Tegou E, Magana M, Katsogridaki AE, Ioannidis A, Raptis V, Jordan S, Chatzipanagiotou S, Chatzandroulis S, Ornelas C, Tegos GP. Terms of endearment: Bacteria meet graphene nanosurfaces. Biomaterials. Elsevier Ltd 2016;89:38–55.
- Syama S, Mohanan PV. Safety and biocompatibility of graphene: A new generation nanomaterial for biomedical application. Int J Biol Macromol 2016;86:546–555.
- Yoon OJ, Jung CY, Sohn IY, Kim HJ, Hong B, Jhon MS, Lee NE. Nanocomposite nanofibers of poly(d, I-lactic-co-glycolic acid) and graphene oxide nanosheets. Compos Part A Appl Sci Manuf. Elsevier Ltd 2011;42:1978–1984.
- Pinto AM, Moreira S, Gonçalves IC, Gama FM, Mendes AM, Magalhães FD. Biocompatibility of poly(lactic acid) with incorporated graphene-based materials. Colloids Surf B Biointerfaces 2013;104:229–238.
- Luo Y, Shen H, Fang Y, Cao Y, Huang J, Zhang M, Dai J, Shi X, Zhang Z. Enhanced proliferation and osteogenic differentiation of mesenchymal stem cells on graphene oxide-incorporated electrospun poly(lactic-co-glycolic acid) nanofibrous mats. ACS Appl Mater Interfaces 2015;7:6331–6339.
- Pinto AM, Gonçalves C, Gonçalves IC, Magalhães FD. Effect of biodegradation on thermo-mechanical properties and biocompatibility of poly(lactic acid)/graphene nanoplatelets composites. Eur Polym J Elsevier Ltd 2016;85:431–444.
- Gonçalves C, Pinto AM, Machado AV, Moreira J, Gonçalves IC, Magalhães FD. Biocompatible reinforcement of poly(lactic acid) with graphene nanoplatelets. Polym Compos 2016.
- Kim IH, Jeong YG. Polylactide/exfoliated graphite nanocomposites with enhanced thermal stability, mechanical modulus, and electrical conductivity. J Polym Sci Part B Polym Phys 2010;48: 850–858.
- Pinto AM, Cabral J, Tanaka DAP, Mendes AM, Magalhães FD. Effect of incorporation of graphene oxide and graphene nanoplatelets on mechanical and gas permeability properties of poly(lactic acid) films. Polym Int 2013;62:33–40.
- Shen Y, Jing T, Ren W, Zhang J, Jiang ZG, Yu ZZ, Dasari A. Chemical and thermal reduction of graphene oxide and its electrically conductive polylactic acid nanocomposites. Compos Sci Technol Elsevier Ltd 2012;72:1430–1435.
- Kashi S, Gupta RK, Baum T, Kao N, Bhattacharya SN. Morphology, electromagnetic properties and electromagnetic interference shielding performance of poly lactide/graphene nanoplatelet nanocomposites. Mater Des 2016;95:119–126.
- Linares A, Canalda JC, Cagiao ME, Garcia-Gutierrez MC, Nogales A, Martin-Gullon I, Vera J, Ezquerra TA, Broad-band electrical conductivity of high density polyethylene nanocomposites with carbon nanoadditives: Multiwall carbon nanotubes and carbon nanofibers. Macromolecules 2008;41:7090–7097.
- Hummers WS, Offeman RE. Preparation of graphitic oxide. J Am Chem Soc 1958;80:1339–1339.
- Horcas I, Fernández R, Gómez-Rodríguez JM, Colchero J, Gómez-Herrero J, Baro AM. WSXM: A software for scanning probe microscopy and a tool for nanotechnology. Rev Sci Instrum 2007; 78:013705.

- Song P, Cao Z, Cai Y, Zhao L, Fang Z, Fu S. Fabrication of exfoliated graphene-based polypropylene nanocomposites with enhanced mechanical and thermal properties. Polymer (Guildf) 2011;52:4001–4010.
- 32. Guo Y, Bao C, Song L, Yuan B, Hu Y. In situ polymerization of graphene, graphite oxide, and functionalized graphite oxide into epoxy resin and comparison study of on-the-flame behavior. Ind Eng Chem Res 2011;50:7772–7783.
- Agarwal M, Koelling KW, Chalmers JJ. Characterization of the degradation of polylactic acid polymer in a solid substrate environment. Biotechnol Prog 1998;14:517–526.
- Duan J, Xie Y, ning Yang J, hui Huang T, Zhang N, Wang Y, ZJ. hong Graphene oxide induced hydrolytic degradation behavior changes of poly(L-lactide) in different mediums. Polym Test Elsevier Ltd 2016;56:220–228.
- Huang HD, Xu JZ, Fan Y, Xu L, Li ZM. Poly(I-lactic acid) crystallization in a confined space containing graphene oxide nanosheets. J Phys Chem B 2013;117:10641–10651.
- Scaffaro R, Botta L, Maio A, Gallo G. PLA graphene nanoplatelets nanocomposites: Physical properties and release kinetics of an antimicrobial agent. Compos Part B Eng Elsevier Ltd 2017;109: 138–146.
- Maria M, Sylwia K. Comparison of photochemical and thermal degradation of poly (lactic acid) doped by nanosilver. J Chem Chem Eng 2015;9:23–30.
- Pei S, Ai F, Qu S. Fabrication and biocompatibility of reduced graphene oxide/poly(vinylidene fluoride) composite membranes. RSC Adv 2015;5:99841–99847.
- Hegab HM, Elmekawy A, Zou L, Mulcahy D, Saint CP, Ginic-Markovic M. The controversial antibacterial activity of graphenebased materials. Carbon N Y Elsevier Ltd 2016;105:362–376.
- Harris HW, El-Naggar MY, Bretschger O, Ward MJ, Romine MF, Obraztsova AY, Nealson KH. Electrokinesis is a microbial behavior that requires extracellular electron transport. Proc Natl Acad Sci USA 2010;107:326–331.

- Timofeeva L, Kleshcheva N. Antimicrobial polymers: Mechanism of action, factors of activity, and applications. Appl Microbiol Biotechnol 2011;89:475–492.
- Li J, Wang G, Zhu H, Zhang M, Zheng X, Di Z, Liu X, Wang X. Antibacterial activity of large-area monolayer graphene film manipulated by charge transfer. Sci Rep 2015;4:4359.
- Obermeier A, Matl FD, Friess W, Stemberger A. Growth inhibition of *Staphylococcus aureus* induced by low-frequency electric and electromagnetic fields. Bioelectromagnetics 2009;30:270–279.
- Van Der Borden AJ, Van Der Mei HC, Busscher HJ. Electric block current induced detachment from surgical stainless steel and decreased viability of Staphylococcus epidermidis. Biomaterials 2005;26:6731–6735.
- Schmidt-Malan SM, Karau MJ, Cede J, Greenwood-Quaintance KE, Brinkman CL, Mandrekar JN, Patel R. Antibiofilm activity of low-amperage continuous and intermittent direct electrical current. Antimicrob Agents Chemother 2015;59:4610–4615.
- Boles BR, Singh PK. Endogenous oxidative stress produces diversity and adaptability in biofilm communities. Proc Natl Acad Sci USA 2008;105:12503–12508.
- Poortinga AT, Bos R, Busscher HJ. Charge transfer during staphylococcal adhesion to TiNOX® coatings with different specific resistivity. Biophys Chem 2001;91:273–279.
- Blenkinsopp SA, Khoury AE, Costerton JW. Electrical enhancement of biocide efficacy against Pseudomonas aeruginosa biofilms. Appl Environ Microbiol 1992;58:3770–3773.
- Cao Y, Feng J, Wu P. Preparation of organically dispersible graphene nanosheet powders through a lyophilization method and their poly(lactic acid) composites. Carbon N Y 2010;48:3834–3839.
- Liang J, Huang Y, Zhang L, Wang Y, Ma Y, Guo T, Chen Y. Molecular-level dispersion of graphene into poly(vinyl alcohol) and effective reinforcement of their nanocomposites. Adv Funct Mater 2009;19:2297–2302.
- Halpin JC, Kardos JL. The Halpin-Tsai equations: A review. Polym Eng Sci 1976;16:344–352.