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Graphene nanocomposite for biomedical applications: fabrication, antimicrobial and cytotoxic investigations

Catherine M Santos1, Joey Mangadlao2, Farid Ahmed1, Alex Leon1, Rigoberto C Advincula2 and Debora F Rodrigues1

1 Department of Civil and Department of Environmental Engineering, University of Houston, Houston, TX 77204-5003, USA
2 Department of Macromolecular Science and Engineering, Case Western Reserve University, Cleveland, OH 44106, USA

E-mail: rca41@case.edu and dfrigiro@central.uh.edu

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Abstract

Materials possessing excellent bacterial toxicity, while presenting low cytotoxicity to human cells, are strong candidates for biomaterials applications. In this study, we present the fabrication of a nanocomposite containing poly(N-vinylcarbazole) (PVK) and graphene (G) in solutions and thin films. Highly dispersed PVK–G (97-3 w/w%) solutions in various organic and aqueous solvents were prepared by solution mixing and sonication methods. The thermal properties and morphology of the new composite were analyzed using thermal gravimetry analysis (TGA) and atomic force microscopy (AFM), respectively. PVK–G films were immobilized onto indium tin oxide (ITO) substrates via electrodeposition. AFM was used to characterize the resulting topography of the nanocomposite thin films, while cyclic voltammetry and UV–vis were used to monitor their successful electrodeposition. The antimicrobial properties of the electrodeposited PVK–G films and solution-based PVK–G were investigated against Escherichia coli (E. coli) and Bacillus subtilis (B. subtilis). Microbial growth after exposure to the nanocomposite, metabolic assay and live–dead assay of the bacterial solutions exposed to PVK–G presented fewer viable and active bacteria than those exposed to pure PVK or pure graphene solutions. The PVK–G film inhibited about 80% of biofilm surface coverage whereas the PVK- and G-modified surfaces allowed biofilm formation over almost the whole coated surface (i.e. >80%). The biocompatibility of the prepared PVK–G solutions on NIH 3T3 cells was evaluated using the MTS cell proliferation assay. A 24 h exposure of the PVK–G nanocomposite to the NIH 3T3 cells presented ∼80% cell survival.

(Some figures may appear in colour only in the online journal)

1. Introduction

In recent years, there has been a growing interest among scientists in incorporating nanomaterial into polymer matrices to make antibacterial and biocompatible polymer nanocomposites for a wide range of applications, such as thin films for biosensors and biomedical devices [10], fibers for wound dressing [9], membranes for water purification [32], and dispersions with antimicrobial properties [31, 10, 32, 9, 20, 2]. This interest arises as a result of the improved antimicrobial performance and new added properties of the nanomaterial in the nanocomposite form as compared to their individual constituents, such as lower life-cycle cost, design flexibility, and applicability for large-scale fabrication.

Polymer nanocomposites are advanced functional materials composed of nanomaterials as fillers dispersed...
inside the polymer matrix or coated by the polymer. The resulting material combines suitable properties of both its constituents. Carbon-based nanomaterials, specifically graphene (G), graphene oxide (GO), and reduced graphene oxide (rGO), are known to be excellent candidates as filler materials because of their excellent mechanical, thermal, and optical properties [21, 15, 29, 39, 20]. Recently, it has been reported that graphene-based materials also exhibited strong antibacterial toxicity [3, 5, 12, 17, 18, 27].

Even with the outstanding antimicrobial properties of graphene, studies with graphene as an antimicrobial agent have been limited due to its poor solubility and processibility. The incorporation of graphene in a polymer matrix is an effective and alternative way of improving its processibility and dispersibility [38, 25, 37]. Most notably, the use of π-electron-rich polymers as matrices results in a more stable polymer–graphene dispersion due to its ability to form π-stacking with the graphene sheets. Among all the polymers available, poly(N-vinylcarbazole) (PVK) is an excellent candidate, since it has exceptional electronic and mechanical properties, anti-corrosion capability, ease of processibility, and thin film fabrication via electrochemical methods. Compared to other polymers in the market, PVK is commercially available as a preformed polymer, minimizing the need for polymerization optimization. Furthermore, PVK can be readily used as a polymer dispersant [20].

To date, the study of the biomedical applications of graphene containing polymer nanocomposites have been focused on either its antimicrobial property [26] or on its cytotoxicity to mammalian cells [22, 36]. Materials with both low mammalian toxicity as well as increased antimicrobial characteristics are potential candidates for several biomedical applications.

This study presents the preparation and characterization of highly stable PVK–G dispersions and thin film depositions for biomedical applications. Graphene nanoplatelets were dispersed on a PVK matrix to create PVK–G nanocomposite solutions. Thin film fabrication was performed using electrodeposition of PVK–G dispersions on ITO substrates. The antibacterial properties of different concentrations of PVK–G solutions were investigated against Gram-negative (E. coli) and Gram-positive (B. subtilis) bacteria. The potential biofilm growth inhibition properties were determined on ITO surfaces coated with PVK–G. The biocompatibility of PVK–G nanocomposite on mammalian cells was evaluated against NIH 3T3.

2. Experimental details

2.1. Fabrication of PVK–graphene nanocomposite

2.1.1. Graphene-nanoplatelet (GNP) solutions. Freshly prepared GNP solutions were used prior to each experiment. GNP solutions were prepared by suspending 1 g of GNP (XG Sciences, MI) in 1 ml of water. The solution was shaken for 1 h (150 rpm) followed by sonication for 2 h.

2.1.2. PVK–G nanocomposite solutions. The PVK solution (5 mg ml\(^{-1}\)) was prepared by sonicating PVK in N-cyclohexyl-2-pyrrolidone (CHP). Then, an aliquot of 1 ml was mixed with 4 ml of Milli-Q water, making a 1 mg ml\(^{-1}\) PVK solution. After that, 0.15 ml of the dispersion of GNP in water was dispersed in 4.85 ml PVK solution, followed by a 30 min ultrasonication, obtaining a 97:3 weight ratio of PVK–G dispersion.

2.1.3. Preparation of PVK–G nanocomposite conducting polymer network (CPN) films. The electrodeposition of the PVK–G nanocomposite was done using cyclic voltammetry (CV) in a standard three-electrode cell with platinum wire as the counter electrode, Ag/AgCl wire as the reference electrode, and bare ITO as the working electrode. The films were prepared by sonicating 1 mg of 97:3 PVK–G nanocomposite in 1 ml dichloromethane for 15 min. Then, 38.74 mg of tetrabutylammonium hexafluorophosphate (TBAH) was added to the mixture, resulting in a 0.1 M TBAH solution. The potential was scanned between 0 and 1.3 V for 20 cycles at a scan rate of 50 mV s\(^{-1}\).

2.1.4. Characterization of PVK–G nanocomposite. The PVK–G solutions were characterized by UV–vis absorption measurements, thermal gravimetry analysis (TGA), and atomic force microscopy (AFM).

UV–vis spectra of the PVK–G dispersion and electrodeposited film were recorded using an Agilent 8453 spectrometer. AFM imaging was performed under ambient conditions with a PicoSPM II (Picoplas, Molecular Imaging—Agilent Technologies) with a scan rate of 1.0–1.5 line s\(^{-1}\). Commercially available tapping mode tips (TAP300, Silicon AFM Probes, Ted Pella, Inc.) were used on cantilevers with a resonance frequency in the range of 290–410 kHz. All AFM topographic images (AAC tapping mode) were processed using Gwyddion 2.19 software.

TGA measurements were performed on a TA Instruments 2950 thermogravimetric analyzer. The samples were heated from 20 to 900 °C at a heating rate of 20 °C min\(^{-1}\). The experiments were conducted under N\(_2\) atmosphere at a purge rate of 80–90 ml min\(^{-1}\). The TGA data were analyzed using TA Instruments’ Universal Analysis software.

The electrodeposition of PVK–G CPN films onto ITO were monitored by acquiring the cyclic voltammogram plots (Princeton Applied Research Parstat 2263) at each cycle. The nanocomposite (PVK–G) crosslinked films were characterized using UV–vis measurements.

2.2. Antimicrobial and cytotoxicity measurements of PVK–graphene nanocomposites

2.2.1. Bacterial cultures. Single isolated colonies of E. coli MG 1655 and B. subtilis 102 were grown in 5 ml of Tryptic Soya Broth (TSB) (Oxoid, England) overnight at 35 °C and 200 rpm. The bacterial culture was centrifuged at 3000 rpm for 10 min. The cells were washed and resuspended in phosphate buffer solution (PBS, 0.01 M, pH = 7.4) (Fisher
2.2.2. Antimicrobial property of nanomaterials on planktonic cells by optical density (OD) measurements. The antimicrobial activity of the nanomaterials (PVK–G, G) on planktonic cells was evaluated by OD measurements. Briefly, bacterial suspensions were exposed for 1 h to different concentrations of nanomaterials. Aliquots of 180 µl of bacterial suspensions (10^7 CFU ml^-1) in PBS and non-inoculated PBS buffer with bacteria (used as blanks) were pipetted in a 96-well flat bottom plate (Costar 3370, Corning, NY) containing triplicates of 20 µl of the following solutions in DI water: (1) G at concentration of 1.0 mg ml^-1; (2) 1.0 mg ml^-1 of PVK; (3) PVK–G nanocomposite at concentrations of 1.0, 0.5, 0.05, and 0.01 mg ml^-1. The amount of graphene for each of the PVK–G nanocomposite dilutions were estimated and are listed in table 1. The control samples contained 20 µl of DI water only with 180 µl of bacterial suspensions. To account for the absorbance of G and PVK–G nanomaterials suspended in the bacterial samples, 20 µl of each concentration of G and PVK–G were added to 180 µl of PBS only and later used as blanks to subtract from the original samples. The plates were then incubated at 37 ºC at 50 rpm for 1 h. After 1 h, 20 µl of the bacteria exposed to the different materials, the negative controls, and the blank samples were transferred into 96-well plates containing 200 µl TSB. The samples were then incubated at 37 ºC at 50 rpm for 3 h and the bacterial growth was monitored using a Synergy MX microtiter plate reader (BioTek) by measuring the OD at 600 nm. The metabolic activity of the bacteria-sample mixture was incubated for 3 h (without shaking) at room temperature. An aliquot of 3 µl was placed in a glass slide, stained, and imaged under a fluorescence microscope. For each mixture of bacteria and nanomaterial, three replicates were made, and six images were taken per each replicate to yield a total of 18 images per solution. The experiment was repeated three times.

To determine the total amount of live and dead cells in the bacteria-samples suspension, the mixture was stained with a live/dead BacLight bacterial viability kit (Invitrogen), as described by the manufacturer in their technical bulletin. The kit employs two nucleic acid dyes: SYTO 9 (green) and propidium iodide (red). The kit stains all cells in green (SYTO9) and dead cells with damaged membranes in red (propidium iodide). The fluorescent images were taken on a BX 51 Olympus Fluorescence Microscope (Leeds Instrument Inc.) equipped with a DP72 digital camera under a 100x objective and a Fluorescein isothiocyanate (FITC) filter. All images were acquired and analyzed using Cell Sens Dimension digital imaging software (Olympus). The same software was used to count the cells for each of the 18 images during each trial. The percentage of inactive cells was expressed as the percentage ratio of the total number of inactive (red) cells to the total number of bacteria (green) attached. Average and standard deviation values were calculated on the percentages based on that cell count.

2.2.5. Biofilm formation measurements on nanocomposite coated surfaces. Biofilm formation was determined on ITO surfaces coated or uncoated with nanocomposites. Unmodified ITO, electrodeposited PVK–G (97-3 wt% PVK–G), electrodeposited PVK, and spin coated G-modified films on ITO surfaces were individually placed in the wells of a 12-well plate (FalconBD, USA). To each well

<table>
<thead>
<tr>
<th>PVK–G (97:3 wt%) concentration (mg ml^-1)</th>
<th>Representative G concentration (mg ml^-1)</th>
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<tr>
<td>1.0</td>
<td>0.03</td>
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<td>0.5</td>
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Scientific, USA) and the bacterial suspension was adjusted to give an optical density (OD) of 0.5 at 600 nm, which corresponds to a cell concentration of 10^7 colony forming units (CFU) ml^-1.

2.2.4. Bacterial viability assay. Fluorescence imaging was carried out to determine the number of total and dead cells exposed to PVK–G, PVK, and G. For this experiment, 180 µl of the bacterial suspension was mixed with 20 µl of sample solution at the most toxic concentration: G (1000 µg ml^-1), PVK–G (1000 µg ml^-1), and control (sterile deionized water). Each nanomaterial–bacteria solution mixture was incubated for 3 h (without shaking) at room temperature. An aliquot of 3 µl was placed in a glass slide, stained, and imaged under a fluorescence microscope. For each mixture of bacteria and nanomaterial, three replicates were made, and six images were taken per each replicate to yield a total of 18 images per solution. The experiment was repeated three times.

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was added 300 µl of TSB media containing bacterial cells at 0.5 OD₅₆₀, then the plate was incubated at 37 °C for 48 h. After incubation, the ITO surfaces were gently rinsed with DI water. The biofilms were fixed using a previously described procedure [24]. Briefly, the ITO surfaces were incubated with 2% glutaraldehyde and subsequently dehydrated with increasing concentrations of ethanol (25%, 50%, 75%, 95% and 100%). The surfaces were dried in vacuum overnight prior to AFM measurements. The surface topography measurements of the biofilm on the ITO were done under ambient conditions with a PicoSPM II (PicoPlus, Molecular Imaging—Agilent Technologies) in the intermittent contact mode. The obtained images were processed using Gwyddion software (2.13).

2.2.6. Cytotoxicity assay on NIH 3T3 fibroblast cells. The cytotoxicity of the PVK–G (1.0 mg ml⁻¹), G (1.0 mg ml⁻¹), and PVK (1.0 mg ml⁻¹) solutions were performed using CellTiter 96 AQueous (Promega) and tested against NIH 3T3 Fibroblast cells. The NIH 3T3 Fibroblasts were a gift from Dr Albee Messing of the University of Wisconsin-Madison. The cells were cultured at 37 °C in a growth media containing: 86% of Dulbecco’s modified Eagle’s medium, 10% fetal bovine serum (FBS), 1% antibiotic (penicillin–streptomycin), 1% l-glutamine, 1% minimum essential medium (MEM) nonessential amino acids solution 10 mM (100 x; GibcoBRL), and 1% 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; (HEPES, 1 M). Fibroblast cells were harvested from culture flasks by 10–12 min incubation with 0.25% trypsin and were resuspended in the growth media. Cell number was determined using a hemocytometer. The assay kits used contained 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) and an electron coupling reagent, phenazine methosulfate (PMS).

Cells were seeded onto a 96-well plate with a seeding density of 2.5 × 10⁴ cells/100 µl. The plate was incubated at 37 °C and 5% CO₂ in humidified air for 24 h. The cell culture medium was then aspirated from the wells and the plates containing cells were gently rinsed with DMEM to remove any non-adherent cells. After this, 100 µl of test sample (PVK–G, PVK, G) was added onto each well containing cells and incubated for 24 h at 37 °C with 5% CO₂. After the incubation, the test sample solutions were aspirated and the wells were rinsed 3 times with DMEM. The adherent cells were evaluated for their viability using MTS cell proliferation assay, as described in the Promega (Promega) technical bulletin [1]. In this method, MTS and PMS detection reagents were mixed, using a ratio of MTS/PMS 20:1, immediately prior to addition to the cell culture media DMEM at a ratio of detection reagents/cell culture medium 1:5. Then, the aspirated wells containing the samples were incubated for 2 h at 37 °C in a 5% CO₂ atmosphere. A sample of culture medium was used as a ‘medium only’ control. The untreated cell suspension was used as a negative control. For the positive control, 4% paraformaldehyde in PBS buffer was added to the cell grown on the plate. The absorbance of the formazan was read using a Synergy MX Microtiter plate reader (BioTek) at a wavelength of 495 nm.

3. Results and discussion

3.1. PVK–G nanocomposite fabrication and characterization

3.1.1. PVK–G nanocomposite fabrication. The preparation of well-dispersed graphene solutions in various solvents is vital for large-scale applications and processes. In this report, stable dispersions of graphene nanoplatelets were created by incorporating graphene in a PVK polymer matrix by solution mixing in different solvents (figure 1(a)). The schematic representation of the PVK–G preparation is shown in scheme 1. A mechanical force, such as sonication, was applied to the graphene nanosheets to control the aggregation of graphene sheets via van der Waals interactions. AFM analysis of the drawn graphene sheets by spin coating on a glass substrate after sonication is shown in figure 1(b). AFM images reveal that the graphene nanoplatelets have an average height of 1.8 nm (figure 1(c)) and lengths that spans 10–20 µm. This result correlates well with previous studies on the morphologies of graphene nanomaterials [19].

The effect of sonication on the dispersibility of graphene was substantial; however it was still not enough to create a homogeneous dispersion of graphene. To address this issue, we incorporated PVK, a precursor polymer containing aromatic groups. The use of such polymer as a dispersant is usually more effective for G in organic and aqueous solvents than small molecules or monomers, since there are more π−π interactions between the two materials.

Various amounts of graphene were tested for dispersion (2 wt%, 3 wt%, 4 wt%, 5 wt%) in the PVK matrix with different solvents (CHP, DMF, THF, DCM, and water). It was observed that the amount of graphene in the nanocomposite greatly affects the stability of the solution. More stable exfoliations were observed at lower graphene loadings and the greatest change in stability time was acquired for the PVK–G nanocomposite containing 3 wt% graphene (results not shown). The stability of the nanocomposite dispersion was tested using both organic and aqueous solvents for 48 h. Figure 1(a) shows the digital images of the exfoliated graphene in PVK matrix using the most commonly used organics (CHP, DMF, THF, DCM) and aqueous solvents (i.e. water).

3.1.2. PVK–G nanocomposite characterization. The presence of PVK and graphene in the nanocomposite was verified using UV–vis spectroscopy. Similar UV–vis spectra were observed for all the PVK–G samples prepared with different graphene loadings (figure 2(a), table 1). As expected, higher intensity peaks were observed for the more concentrated nanocomposite solution (1 mg ml⁻¹). The signature peak of graphene was observed between 240 and 250 nm and is attributed to itsπ−π* transition [23], while the peaks at 295, 331 and 344 nm correspond to the π−π* and n−π* transitions of the PVK [30].

TGA analysis was acquired to determine the thermal stability of the bulk PVK–G nanocomposite (figure 3). As a control, the TGA curves for PVK and pure graphene were also acquired. Similar to previous results, the thermal properties
of pure graphene remained highly stable up to 600 °C. For
the pure PVK, the TGA thermogram exhibited a two-step
degradation process, which starts with a slight steady loss at
around ~200 °C due to degradation of the PVK chains and
ends with the sudden drop in weight % at ~410 °C attributed
to the polymer backbone degradation [28]. Compared to PVK,
the TGA data for the PVK–G (97-3 w/w%) nanocomposite
showed an enhanced thermal stability and lower weight loss,
primarily because of the incorporation of thermally stable
graphene. It is also worth noting that even after heating to
600 °C ~3% of the graphene in the nanocomposite material
was still present. This result indicates that the prepared
PVK–G nanocomposite contains ~3 wt% of graphene.

The facile and robust preparation of thin films of
graphene is useful for a wide range of applications in the
semiconductor industries and in the field of biotechnology.
As noted earlier, the incorporation of PVK serves not only
as an effective polymer dispersant for the nanomaterial but
also facilitates the immobilization of graphene sheets onto
surfaces by the cyclic voltammetry electrochemical method.
Figure 2. UV–vis spectra of (a) PVK–G (97-3 w/w%) nanocomposite solution and (b) the electrodeposited PVK–G film on ITO surface.

Figure 3. Thermal properties of PVK–G nanocomposite, pure graphene, and PVK.

Figure 4(a) depicts the CV plot of PVK–G (97-3 w/w%) nanocomposite films, with potential scans from 0 to 1.3 V. In the first cycle, the appearance of the anodic peak at ~1.1 V corresponds to the oxidation and crosslinking of carbazole units. This is in agreement with the previously reported literature [8, 11]. Furthermore, the electrodeposition of the nanocomposite is also substantiated by the increase of the current as the number of cycles increases [25].

The successful immobilization of PVK–G on the ITO substrate was verified by UV–vis spectroscopy. The results showed that the peaks at 342 and 352 nm that were present prior to the electrodeposition were replaced by a new broad peak in the UV–vis region of 425–475 nm (figure 2(b)), which signifies that the electrochemical crosslinking of the carbazole moieties [34] of PVK created a highly robust and insoluble PVK–G film onto the surface. These results were consistent with previous studies of electro-crosslinked PVK with the CPN approach [25, 11].

The distribution of the PVK–G (97-3 w/w%) nanocomposite on the surface was investigated using AFM (figure 4(b)). The film had an averaged roughness of 24.7 nm and exhibited globular domains covering the surface. The presence of such features is commonly observed for PVK containing thin films. Estimation of film thickness was conducted by comparative AFM analyses of areas with and without the thin films using the AFM scratching method [7, 4]. AFM results revealed that the PVK–G surface produced an estimated thickness of 150 nm after 20 cycles of electrodeposition with a scan rate of 50 mV s⁻¹ (figure 4(c)).

3.2. Antimicrobial properties of PVK–G

3.2.1. Antimicrobial activity of different PVK–G concentrations. Antibacterial tests of different PVK–G (97-3 w/w%) solution concentrations were tested against E. coli and B. subtilis. As shown in figure 5, higher cell inactivation of E. coli and B. subtilis was observed for solutions with higher concentrations of PVK–G (1000 and 500 µg ml⁻¹). This result suggests that the antibacterial performance of PVK–G is dependent on the concentration of the nanomaterial in the matrix. In addition, the results correlate well with previously reported antibacterial toxicity dependences on concentration for SWNT and graphene oxide [18, 20, 2]. Furthermore in figure 5, bacterial toxicity for both bacteria was observed for the PVK–G nanocomposites and pure graphene while bacterial inactivation was not observed for the PVK control. This result indicates that the presence of the PVK did not hinder the known antibacterial property of graphene, as was observed in previous studies, but rather enhances its bactericidal properties. We hypothesize that the improved antibacterial activity of the nanocomposite is possibly due to a better dispersion of graphene in the presence of PVK. It was noted from previous studies that dispersibility of graphene-based nanomaterials plays a huge factor in its antibacterial toxicity. Although results were not verified in this study, it is also possible that the incorporation of a polymer might have contributed to the enhanced antimicrobial property due to the synergistic effect caused by either electronic interactions or morphological interaction between the polymer and the nanomaterial.

3.2.2. Viability measurements and metabolic assay tests of PVK–G solutions. Viability measurements using
Figure 4. Electrodeposited PVK–G films on ITO: (a) CV plot of electrodeposited PVK–G. (b) AFM image of PVK–G film (c) AFM image of scratched PVK–G used for thickness estimation.

Figure 5. Toxicity effect of PVK–G (97-3 w/w%, concentration: 1.0, 0.5, 0.05, 0.01 mg ml$^{-1}$), PVK (concentration: 1.0 mg ml$^{-1}$) and graphene (concentration: 1.0 mg ml$^{-1}$) solutions on E. coli and B. subtilis. To perform the fluorescence-based assay were conducted to determine the antibacterial efficacy of the G containing nanocomposite against E.coli and B. subtilis. For this analysis, only the most toxic concentration of PVK–G (1000 µg ml$^{-1}$) was tested for viability and the results were compared with those observed for the PVK and pure graphene as controls. Figure 6 indicates that bacterial inactivation after 1 h exposure to the samples is found to be the highest for PVK–G. Pure graphene also showed bacterial inactivity, while no toxicity was observed for the PVK solution.

In parallel, cell metabolic assay was conducted to determine the effect of nanomaterial toxicity on the metabolic activity of bacterial cells after exposure to the nanomaterials. This assay has been extensively used to detect cell metabolic activity by measuring the reduction of a nonfluorescent resazurin to a red-fluorescent resorufin by viable bacteria [33, 35]. Previous investigators have shown that the bacterial toxicity of nanomaterials is related to the disruption of the cell’s intracellular metabolic pathway by oxidative stress, which leads to cell death [18, 14, 31, 20]. Figure 6 depicts the effect of the nanomaterial on the % of metabolically active bacteria after 1 h exposure. Clearly, bacteria exposed to the PVK–G solution presented fewer viable bacteria than those exposed to PVK. These results corroborate well with the results observed for the live–dead assay and the OD measurements. Furthermore, these results suggest that the
Figure 6. Correlation between % metabolically active and % viable bacteria after 1 h exposure of (a) B. subtilis and (b) E. coli to PVK–graphene (97.3 w/w%; concentration: 1.0 mg ml\(^{-1}\)), PVK (concentration: 1.0 mg ml\(^{-1}\)), and graphene (concentration: 1.0 mg ml\(^{-1}\)) solution. The (+) control is the bacterial solution that is not exposed to the nanomaterial.

Figure 7. Biofilm studies on surface coated PVK–G. AFM topography (top) and friction (bottom) images of (a) PVK–G, (b) PVK, and (c) ITO surfaces after 24 h incubation in E. coli (10\(^7\) CFU ml\(^{-1}\)). Scale bar: 20 µm.

The antimicrobial activity of the G containing solutions (PVK–G, G) is possibly related to inducing oxidative stress and thus interfering with the metabolic activity of the bacteria.

3.2.3. Evaluation of biofilm inhibition on PVK–G films. Antimicrobial coating materials are essential for the control of biofilm formation [6]. To compare the antimicrobial property of PVK–G, PVK, and G films on surfaces, thin films were produced on ITO surfaces by spin coating of graphene and electrodeposition of PVK–G and PVK. These coated surfaces were incubated with bacterial solutions for 48 h. As shown in figure 7, both the PVK and the bare ITO surface allowed biofilm formation. Under the same conditions, the surfaces of the electrodeposited PVK–G and the spin coated graphene showed little bacterial coverage. The biofilm growth inhibition on the surface of PVK–G films can be attributed to the antimicrobial activity of graphene nanoplatelets, where inactivation of the bacteria is caused by the direct contact with the film. It is also possible that the presence of the nanomaterial on the surface could be sensed by the bacteria; therefore they would avoid the surface.

3.3. Biocompatibility tests of PVK–G on NIH 3T3 fibroblasts

The application of antimicrobial material for biomedical applications requires effective concentrations of nanomaterials
needed to rapidly kill bacteria, but with low cytotoxicity to human cells. The fact that some carbon-based nanomaterials have been shown to be toxic to human cells [16, 13] makes it imperative to test the biocompatibility of this new nanocomposite. So far, no previous study has addressed the human cytotoxicity of polymers, such as PVK, impregnated with nanomaterials.

To address this issue, we measured the cytotoxicity of a solution containing a PVK–G concentration with the highest antibacterial property. As controls, we also prepared solutions containing PVK and pure graphene. The test used to determine the nanocomposite cytotoxicity was the MTS cell proliferation assay with NIH 3T3 fibroblast cells. Figure 8 shows that cell survival was ∼80% after 24 h exposure time to the PVK–G nanocomposite solution, while the pure G solution resulted in ∼60% cell viability. The PVK control on the other hand, showed ∼90% cell viability. Therefore, the polymer nanocomposite is less toxic to the NIH 3T3 cells than the pure G.

4. Conclusion

In conclusion, we have presented the facile and simple fabrication of a PVK–G nanocomposite solution and thin film with antimicrobial and biocompatible properties that can potentially be used for diverse biomedical applications. Highly stable exfoliations of PVK–G (97-3 w/w% solution) were obtained by mechanical mixing and sonication. The prepared solutions were found to be well dispersed in various solvents, such as CHP, NMP, water, and THF. Electrodeposition of the PVK–G dispersion was facilitated by the electro-crosslinking of the carbazole unit of the PVK on the ITO substrate, which created a robust conducting polymer nanocomposite network thin film. Biomedical applications of the prepared PVK–G solutions and thin films were evaluated by investigating their antimicrobial properties against B. subtilis and E. coli, as well as their biocompatibility to NIH 3T3 fibroblast cells. Antimicrobial tests via OD measurements of PVK–G presented higher bacterial toxicity than its individual constituents (PVK and G). In addition, the antimicrobial property of the nanocomposite is concentration dependent since higher concentrations (1.0 and 0.5 mg ml⁻¹) of PVK–G depicted greater bacterial toxicity. The live–dead assay and the metabolic activity results demonstrated that a lower percentage of viable bacteria were present after exposure to the graphene containing solutions (PVK–G and G) as compared to PVK. AFM comparisons of biofilm formation on modified ITO surfaces with PVK–G, PVK, G-modified, and unmodified ITO surfaces revealed that PVK–G presents the best biofilm inhibition capability. The biocompatibility of the exfoliated PVK–G solution by the MTS cell proliferation assay with NIH 3T3 fibroblast cells showed ∼80% cell survival, which suggest that this nanocomposite is highly biocompatible.

These results support the potential use of the PVK–G for a wide variety of biomedical applications where bactericidal properties coupled with low cytotoxicity to mammalian cells are critical. Further investigation on the long-term stability of the PVK–G nanocomposites and on its effect to other types of host cell are necessary to determine their application for biomedical devices and implants.

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