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Biological degradation and biostability of nanocomposites based on polysulfone with different concentrations of reduced graphene oxide

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Increasing incorporation of rGO in the polysulfone polymer generates materials with improved chemical and mechanical stability and less prone to biodegradation at the end of the nanocomposite life cycle. The results of ATR-IR and mechanical strength, after exposure to wastewater influent, show that the increasing concentrations of rGO into the polymer matrix reduce changes in the nanocomposite properties. The increasing incorporation of rGO also increases growth inhibition of the wastewater microbial population on the surface of nanocomposites. Highest biofilm inhibition and material stability are observed with nanocomposites containing 3 wt% rGO. These results suggest that reduction in the material biodegradation is linked to the inhibition of biofilm growth on the nanocomposite surface due to the antimicrobial properties of rGO. This study demonstrates, for the first time, that the amount of rGO incorporated in the nanocomposite impact the biodegradability and end of life of polysulfone nanocomposites.

FIGURE FOR ToC_ABSTRACT

Polymer Biodegradation and rGO microbial inactivation
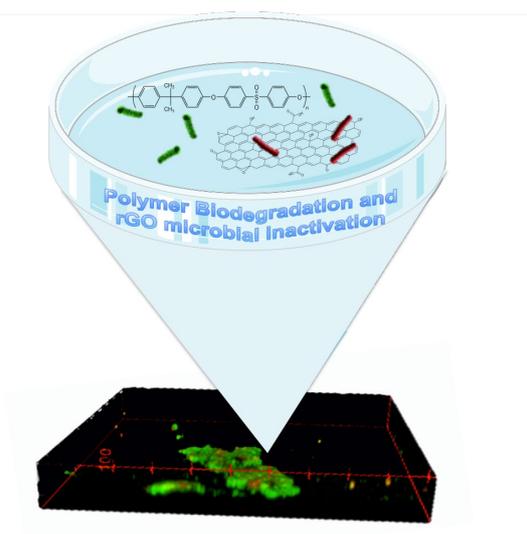
1. Introduction

The rapidly growing role of polymers in diverse industries in the last century requires designing of functional materials with large stability and durability. The incorporation of nanoparticles, such as graphene-based nanomaterials, into polymer matrices have been shown to improve significantly the mechanical, electrical, thermal properties, as well as the chemical stability of nanocomposites.[1-3] In such studies, researchers have focused on understanding the stability of polymers under different chemical conditions, i.e. different temperatures, pH values, and hydrolytic conditions;[4-7] different studies about biodegradable graphene nanocomposites have been reported,[8] however, so far, there is no extensive research about how the incorporation of graphene-based nanomaterials into polymer matrices could influence biological degradation and its effects on stability of rGO nanocomposites.[9]

Chemical and biological degradability of polymers depends very much upon their degree of polymerization, hydrolysis, distribution of functional groups, stereo-regularity, and crystallinity.[4,5] During microbial degradation, microorganisms will produce enzymes that will change the structural and chemical properties of the materials by attacking different chemical bonds of the material. [8,9] Biodegradation frequently requires microorganisms to attach and form biofilms on the surface of the material. Thus, the assessment of the relationship among biofilm formation, biodegradability and polymer structure is of utmost importance to determine the biostability of a nanocomposite.[5,10]

The interactions of microorganisms with nanocomposites will play an important role in determining the nanomaterial's long-term fate and persistence. The first step in biofilm formation is the attachment of planktonic cells to the surface followed by growth and colonization. If the microorganisms survive, they will colonize through proliferation to form biofilms. In contrast, if the surface exhibits antimicrobial properties, cell proliferation can be retarded or even inhibited. If biofilm formation occurs, the attached microorganisms can initiate biodegradation through the release of extracellular enzymes.[9,11]

The polysulfone polymer (PSU) is a thermoplastic with strong thermal, hydrolytic and mechanical properties.[12],[13] These properties make rGO/PSU nanocomposites extremely attractive for diverse applications in biomedical fields and water treatment technologies, such as synthesis of more resistant water filtration membranes. In both applications, biomedical (biomolecules adsorption, bionsensing, dialysis…) and environmental applications (ultrafiltration, membrane bioreactors…) the antifouling properties are critical. Membranes synthesized with PSU for water filtration applications typically will be exposed to microorganisms that could potentially biodegrade the polymer. Similarly, PSUs used to synthesize medical devices [14–18] are exposed to microbes in the human body that could also degrade these polymers. Degradation studies of polysulfone (PSU) have involved mainly chemical and thermal degradation, however, biological degradability of this polymer is still largely unknown.[19],[20] Additionally, not enough attention has been given to the study of durability of thermoplastics according to their preparation techniques.[5] As of today, there are no studies trying to understand the effect on biodegradability when rGO is incorporated in PSU; even though many applications of this polymer will involve direct contact with microbes or biological systems. The present study aims to investigate the biodegradability of PSU and its nanocomposites in the presence of diverse microorganisms.

This study offers a new perspective on the stability of graphene-embedded polymer matrices, a subject that has attracted a lot of attention in recent years. The subject of the biodegradability of the polymer containing nanomaterials have not been investigated so far, thus providing new data for the durability of nanocomposites exposed to complex environments and studies trying to understand the end of life of materials to better determine disposal mechanisms and also the potential persistence of such materials in the environment.

2. Results and Discussion

In the present study, the microbial colonization of the PSU polymer and its nanocomposites, as well as their biodegradability were investigated. Preparation and

characterization of the nanocomposites are detailed in the supporting information. Briefly, nanocomposites with the following rGO concentrations (w/w %) were prepared by extrusion and injection molding: 0, 0.1, 0.5, 1 and 3%. The samples were exposed for nine days in a batch reactor containing a complex microbial community obtained from the Houston Sims Bayou wastewater treatment plant. Wastewater microbial community was used in this study for the biodegradability assays because wastewater is one of the ultimate repositories of waste materials and this environment contains microorganisms able to biodegrade diverse materials and contaminants.[21] If there is any microbial population that could potentially degrade nanocomposites, they would be most likely found in wastewater treatment plants. Biofilm growth and thickness on the material surfaces were investigated with confocal microscopy. For the confocal analysis, biofilms attached to the nanocomposites were stained with the SYTO 9 and Propidium iodine (PI) dyes (to determine live and dead cells, respectively). The confocal images were analyzed using COMSTAT, as previously described.[21–24] Additionally, chemical and physical properties of the nanocomposites were determined by investigating the changes in the FT-IR spectrum, water contact angle, SEM images and mechanical strength before and after exposure to the wastewater microbial community. Controls with filtered-sterilized wastewater were also used to ensure that any modifications in the polymer and nanocomposites were solely due to the presence of microorganisms.

The confocal investigation of the ability of microorganisms to form biofilm in different coupons of PSU with different concentrations of nanomaterials is shown in Figure 1. Results showed that nanocomposites with higher concentrations of nanoparticles reduced the biofilm growth. This was due to the higher concentrations of rGO found on the surface of nanocomposites fabricated with more rGO. Since rGO has been described to have antimicrobial properties,[25–27] the biofilm thickness and the total biomass on the surface decreased significantly (Figure 1 and 2 and Table 1S).

In the nanocomposites with concentrations between 0.1 to 1 wt% of rGO, biofilm growth was clearly detected. In previous studies with carbon based nanomaterials, the authors observed that the microorganisms died in contact with the nanomaterial and formed a layer of dead cells that could serve as a barrier against the nanomaterial.[24] This layer of dead microbes allowed another layer of microbes to grow on top of the film. In the present study, the microorganisms were able to grow on the nanocomposites with low rGO concentrations, but as the polymer started to biodegrade, the surface of the nanocomposite became more and more toxic, which led to the detachment of the biofilm and further inhibition of biofilm growth. We did not observe any layer of dead microorganisms that allowed another layer of microbes to grow on top of the film as previously described (Figure 1 and 3).

In the case of the nanocomposite with 3 wt% rGO, biofilm growth was almost completely inhibited during the experiment period. Previous studies with other carbon based nanomaterials showed that the biofilm growth inhibition was due to the antimicrobial properties of the nanomaterials.[24,28–30] Studies related to the rGO antimicrobial mechanism concluded that direct contact of nanomaterials with microorganisms can lead to inactivation of cells by membrane damage and cell lysis mechanisms.[31,32] In these studies, rGO was introduced as a suspension or at the surface of the material; therefore, the accessibility of the nanomaterial to microorganisms was higher. In our study, the nanomaterial (specially at lower concentrations) was embedded in the polymer and could only be accessed by microorganisms if the polymer got degraded. The inhibition of biofilm formation on the 3 wt% PSU rGO nanocomposite suggests that this concentration was high enough to produce a nanocomposite with an antimicrobial surface. At this concentration, rGO was more exposed on the surface of the nanocomposite, as displayed by the higher water contact angle results (Figure 2 and Table 1S). These results suggest that the higher the concentration of rGO in the PSU matrix, the lesser was the biofilm growth.
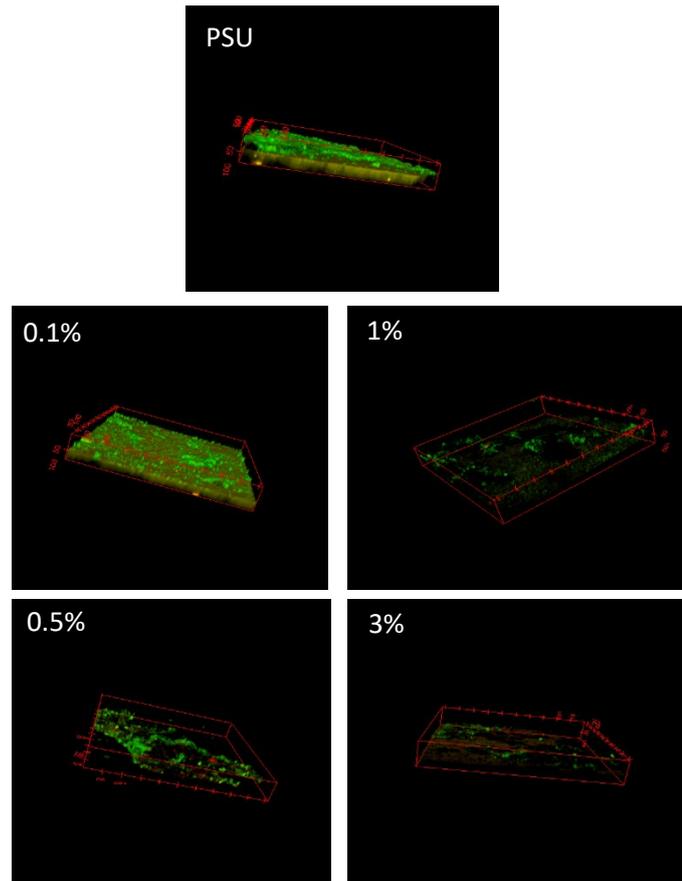
Figure 1. Confocal images of biofilms grown on the surface of nanocomposites after contact for nine days with microorganisms from wastewater.
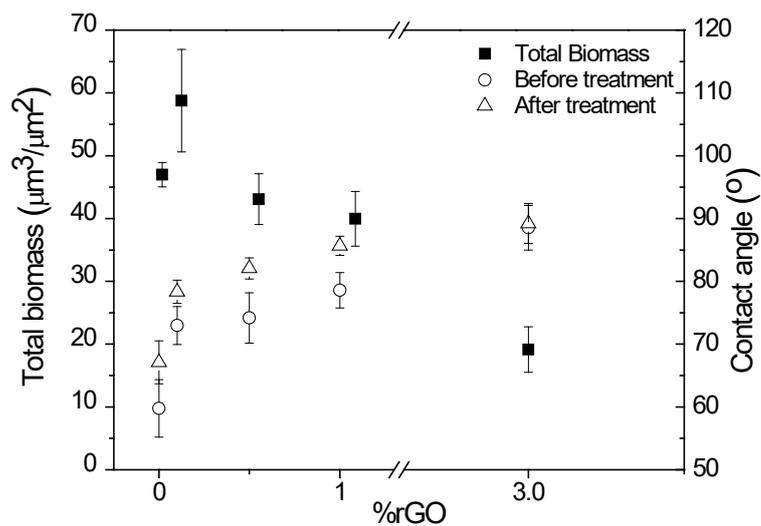

Figure 2. Results of the water contact angle (open dots) and biofilm measurements by confocal microscopy (closed dots), as a function of the % rGO in the nanocomposite. The

contact angle results are presented before and after exposure to the wastewater microbial community.

In addition to the biofilm growth investigation, we also examined the effects of this microbial growth on the surfaces of the nanocomposites. For that, we investigated the relationship of the total microbial biomass in relation to contact angle changes (Figure 2). The comparative analyses of the contact angle measurements before and after exposure to the wastewater for nine days are presented in Figure 2 and Table 1S. Results showed that nanocomposites with 3 wt% rGO presented the least changes in the contact angle after exposure to the wastewater microbial community; coincidently, there was less or no biofilm growth on this nanocomposite surface. In contrast, nanocomposites with concentrations between 0.1 and 1 wt%, presented changes in contact angles (i.e. hydrophobicity) after treatment. These changes were attributed to the degradation of the polymer and potential protrusion of rGO on the nanocomposite surface. As the polymer biodegraded after exposure to the wastewater microbial community, the hydrophobicity of these nanocomposites increased and became more like that of the nanocomposite with 3 wt% rGO. The changes on the surface and protrusion of rGO in the nanocomposite surfaces were confirmed by ATR-IR analysis.

Figure 3 shows appreciable changes in the ATR-IR spectra before and after exposure of the nanocomposites to the microorganisms in the wastewater. For comparison, spectra were normalized to the C-O-C stretching peak (1243 cm^{-1}). After exposure, the aromatic and aliphatic C-H stretching peaks in the 2800-3000 cm^{-1} region showed an increased intensity, especially for the neat polymer. Similarly, the peak of the carbonyl group at 1730 cm^{-1} also became more visible in the polysulfone. The ratio between the carbonyl and reference band (C-O-C stretching peak) was 0.5 for all unexposed samples. After exposure to the wastewater, this ratio increased from 0.5 to 0.98 and decreased to 0.16 for the neat polysulfone and PrG(3) nanocomposites, respectively.
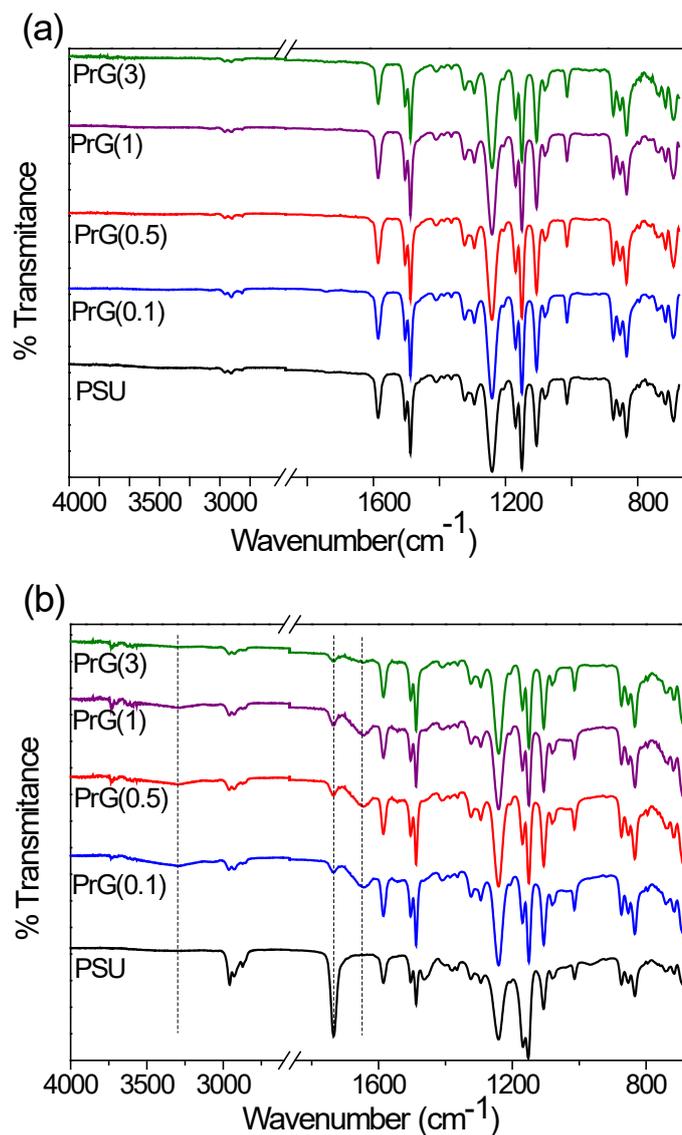
Figure 3. ATR-IR spectra of nanocomposites a) before treatment and b) after nine days in contact with microorganisms in wastewater. Dotted line is a guide for the eye.

Therefore, the presence of rGO reduced the growth intensity of these bands. These variations on the spectra after treatment showed the degradation of the polymer and the influence of the rGO surrounding the nanocomposite. Figure 3b also shows the appearance of broad bands at about 3400-3200 cm^{-1} and 1644 cm^{-1} in the nanocomposites belonging to hydroxyl and carbonyl amide groups, respectively, due to strong interactions between oxygen groups of rGO and functional groups of the biofilm.[33] The ratio between the carbonyl amide

and reference bands was around 0.30 in PrG (0.1), but decreased to 0.17 in PrG(3), as a consequence of a small biofilm formation.

The biofilm growth results and the changes observed in the PSU clearly show that microorganisms are able to degrade PSU. By degrading the polymer, rGO gets exposed and interacts with the microorganisms inactivating them (Figure 1). Furthermore, the results showed that the degradation process of neat PSU started before nine days and that the inclusion of rGO helped in the preservation of the structure of the nanocomposite for a longer period of time.[19],[34]

The preservation of the nanocomposite structure was confirmed by the mechanical analysis and SEM images of the nanocomposites after nine days of exposure to the wastewater microbial community (Figures 4 and 5). A decrease in the young modulus with respect to the control samples (same nanocomposite but not exposed to wastewater) was observed. For the PSU polymer alone, a 20 % decrease in the young modulus occurred. Stress strain curves are included in the supporting information, Figure 4S. The partial degradation of the polymer at low rGO percentages still caused a significant decay in the mechanical properties of the starting material. At higher rGO loadings, around 1 wt% and above, the decrease in modulus was only 5 %. Figure 5c showed a crack in the PSU surface as a consequence of the partial degradation of the polymer that was produced by the bacteria in the wastewater. Figure 5d did not show any difference with sample PrG(3) before treatment. These results confirmed that the microorganisms in wastewater were able to biodegrade the polymer and the presence of rGO generated a protective effect against biodegradation of the nanocomposite.
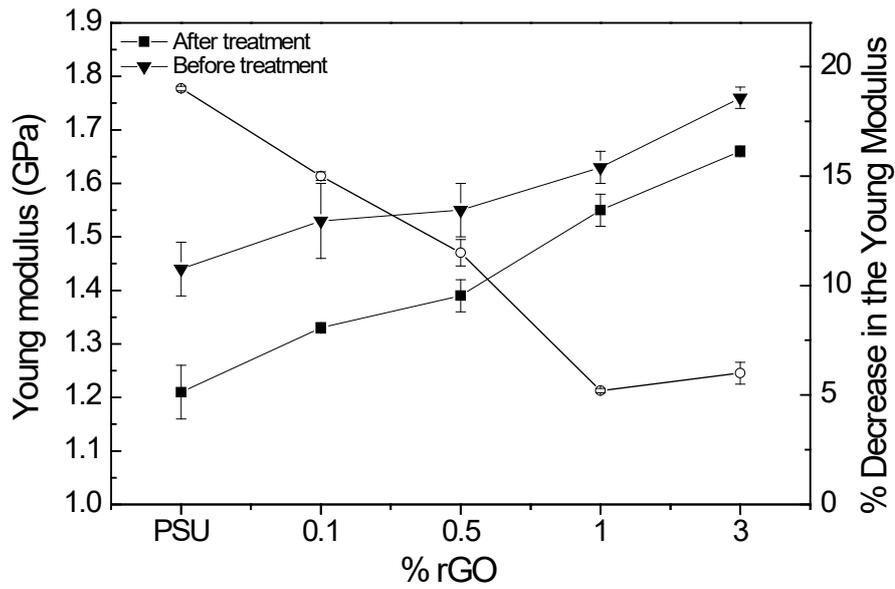
Figure 4. Young modulus of all nanocomposites (dots, left axis) and percent decrease in Young modulus after exposure to wastewater with respect to non-exposed material (open dots, right axis). Lines are used as a guide for the eyes.
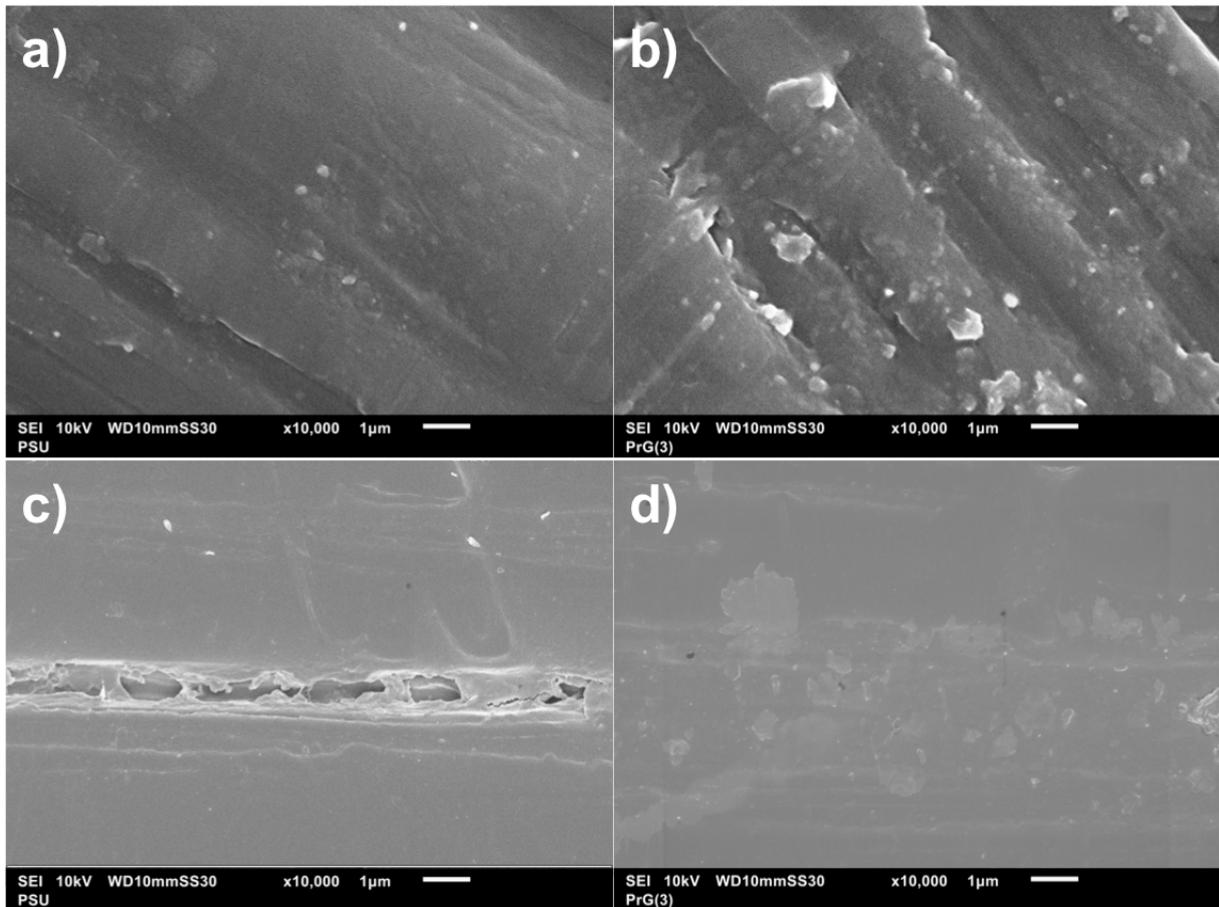

Figure 5. SEM images before treatment of, a) PSU and b) PrG(3) and after treatment of c) PSU and d) PrG(3).

3. Conclusions

In summary, increasing amounts of rGO embedded in a polymer nanocomposite directly decreases both biofilm formation and biodegradability of the nanocomposite, thereby maintaining the structural and chemical integrity of the nanocomposite. More specifically, the addition of rGO to the PSU matrix allowed the inhibition of biofilm formation, and also increased the resistance of the nanocomposite to biodegradation by microorganisms present in wastewater. This suggests that nanocomposites of rGO can potentially be more persistent in the environment, but at the same time, the incorporation of rGO can produce a more robust and resistant material for applications in the environmental and biomedical fields requiring biostability over long periods of time.

Supporting Information

Electronic Supplementary Information (ESI) is available from the Wiley Online Library: rGO characterization include XRD and XPS (Figure 1S and 2S, respectively). Figure 3S include nanocomposite characterization. Table 1S summarize contact angle measurements and confocal microscopy. In Figure 4S, strain-stress curves are presented.

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