Effect of biodegradation on PLA/graphene-nanoplatelets composites mechanical properties and biocompatibility

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Abstract

Two types of graphene-nanoplatelets (GNP-M and GNP-C) were incorporated in PLA (poly(lactic acid)) by melt blending. Materials were biodegraded during 6 months and characterized by XRD, tensile tests, DMA and biocompatibility assays. For both fillers, low loadings (0.25 wt.%) improved mechanical properties and decreased their decay until 6 months biodegradation. PLA degradation decreased its toughness (AUC) by 10 fold, while for PLA/GNP-M and C after 6 months degradation, toughness was only reduced by 3.3 and 1.7 fold, respectively. Comparing with PLA, PLA/GNP-M and C composites presented similar (HFF-1) fibroblasts adhesion and proliferation at the surface and did not released toxic products (6 months).

Introduction

Graphene is a single layer of sp^2 carbon atoms arranged in a honeycomb structure and possesses extraordinary mechanical strength and an extremely high surface area. [1] A commercial available product, with reduced cost comparing with single layer graphene, graphene nanoplatelets (GNPs), are constituted by few stacked graphene layers, possessing oxygen containing functional groups in the edges. GNPs present high aspect ratio, thus forming a percolated network in composites, with large interfacial interaction between platelets and polymer matrix, mainly in the edges, resulting in effective load transfer and increased strength. [2] Moreover, these materials were shown to be non-toxic when incorporated in low percentages into PLA. [3] The potential of GNPs as polymers fillers, has been observed in our previous study, in which improvements in mechanical properties of PLA thin films were obtained at filler loadings bellow 1 wt.%. Solvent mixing was used for GNPs incorporation, [4] however the use of solvents should be avoided due to the toxicity of residues that may remain in the materials, and for industrial workers [5]. Lahiri *et al.* improved ultrahigh molecular weight polyethylene mechanical properties producing composites by electrostatic deposition of GNPs 1 wt.%. However, composites were toxic to osteoblasts because filler leaching occurred. [2] Thus, melt blending, which assures complete embedding of GNPs in polymer matrix preventing filler leaching, is studied in this work as a green method for production of PLA/GNPs composites.

Materials and Methods

PLA 2003D, was purchased from Natureworks. Graphene-nanoplatelets, grade C750 (GNP-C) and M-5 (GNP-M) were acquired from XG Sciences. PLA/GNP-M and C 0.25 wt.% composites were prepared by melt blending in a Thermo Haake Polylab (180 °C, 15 min, 25 rpm), and moulded in a hot press (190 ºC, 2 minutes) into thin sheets (0.3-0.5 mm). Samples were immersed in 50 mL PBS in sterile conditions and incubated for 6 months (37 °C, 100 rpm). X-Ray diffraction (XRD) analysis, was performed using a Philips X´Pert diffractometer. Tensile properties of the composites (60x15 mm) were measured (Mecmesin Multitest-1d, Mecmesin BF 1000N) at room temperature and strain rate of 10 mm min^{-1}. Dynamical mechanical analysis (DMA) was performed using a DMA 242 E Artemis (Netzsch) in tension assays (6N, 10 minutes) with 10 minutes recovery. Biocompatibility of materials was evaluated using HFF-1 cells cultured at the surface of PLA, PLA/GNP-M and C 0.25 wt.% films (Ø = 5.5 mm) and in direct contact with materials extracts obtained after 6 months incubation in PBS (50 µL in 150 µL DMEM+, after 24h cell grow). In both assays cells were seeded in 96 well plates (7500 cells per well) and 20 µL resazurin solution added at 24, 48, and 72h and incubated for 3h, fluorescence ($\lambda_{ex/em}$=530/590 nm) read and metabolic activity evaluated (Metabolic activity (%) = F_{sample}/F_{PLA} x 100). Suitable controls were performed for both biocompatibility assays.

Results and discussion

XRD

GNP-M and C powders present similar XRD spectra, typical of carbon materials, with an intense peak around 31°, and two broad peaks around 50° and 65°. PLA, before (0M) and after 6 months (6M) biodegradation, presents similar spectra with two broad peaks, the first, around 20°, is more intense than the second, around 35°. PLA/GNP-M 0.25 wt.% 0 and 6M present similar spectra, with PLA and GNP-M peaks being observed, which confirms the filler presence in polymer matrix. For PLA/GNP-C 0.25 wt.% 0M and 6M spectra are also similar, however GNP-C peak is less intense than GNP-M peak.

Tensile tests

Incorporation of GNP-C and M in PLA increased its Young´s modulus by 14 %. Also, tensile strength is increased by 20% with GNP-C incorporation and by 6% with GNP-M. Improvements in toughness of 20% are only observed for GNP-C. After 6 months biodegradation no significant changes are observed in Young´s modulus for all materials tested. Decreases in tensile strength, elongation at break, and toughness are respectively, for PLA of 2.6, 2.5, and 10 fold, for GNP/PLA-M of 1.6, 1.8 and 3.3 fold, and for GNP-C of 1.4, 1.4 and 1.7 fold. Thus, the presence of the fillers prevents decreases of PLA mechanical properties with biodegradation, namely tensile strength, elongation at break and toughness. Also, GNP-C incorporation seems to have a more beneficial effect than GNP-M, especially in toughness.

DMA

Figure 1 shows that for PLA, dL_f (final, at 6N) after 10 cycles before degradation was of 14.2 µm, being of 13.7 and 13.2 µm for PLA/GNP-M and C 0.25 wt.%, respectively. After 6 months degradation, PLA sample ruptured after 4 cycles (1.A) reaching a dL_f of 56.3 µm, PLA/GNP-M and C 0.25 wt.% did not ruptured (1.B,C) and presented a slight increase in dL_f, which were of 16.8 and 16.7 µm, respectively.

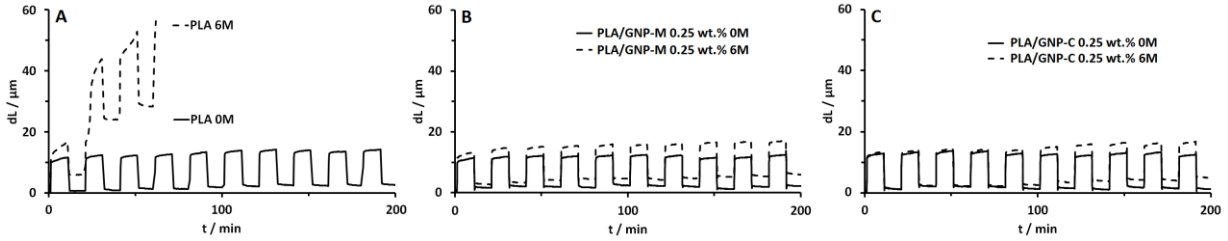

Figure 1. Multi cycle DMA of PLA and composites before and after 6 months degradation.

These results are in agreement with those obtained in tensile tests, with a significant decay in PLA mechanical properties after 6 months biodegradation and small effects observed for PLA/GNP-M and C 0.25 wt.%. Thus, fillers are reinforcing the polymer matrix and retarding decrease of its mechanical properties. Materials degradation was confirmed by GPC-SEC and SEM (results not shown).

Cell adhesion and proliferation assays

HFF-1 cell metabolic activity at PLA surface was 75% at 24 and 48h, and 94% at 72h, comparing with cells at tissue culture treated surface of 96 well plates. PLA/GNP-M and C 0.25 wt.%, metabolic activity never decreased below 90%, for both composites in comparison with PLA. Thus, fillers incorporation has no impact in cell adhesion and proliferation at materials surface.

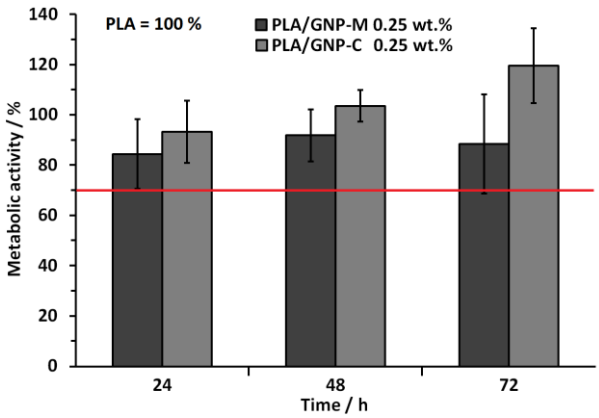

Figure 2. Cytotoxicity of PLA/GNP-M and C 0.25 wt.% 6 months degradation products, comparing with PLA in the same conditions. Assays were performed in triplicate, with 6 replicates for each condition tested. Error bars represent standard deviation. Red line represents cytotoxicity limit of 70%.

Degradation products cytotoxicity

A control performed with PBS (37 ºC, 100 rpm, 6 months) presented similar cell metabolic activity (24, 48, 72h) to PLA 6M degradation products, which shows that they are not toxic. Figure 2 shows that degradation products of PLA/GNP-M and C 0.25 wt.% 6M are not toxic (24, 48, 72h), comparing with PLA 6M, according to ISO 10993-5:2009(E), which considers toxic a material that decreases cell viability below 70% of negative control for cell viability. Also, cell morphology is normal and similar for all conditions tested (images not shown).

Conclusions

GNP-M and GNP-C incorporation in PLA matrix at low loadings (0.25 wt.%) improved mechanical properties and decreased their decay until 6 months biodegradation. These nano-fillers can be used to tune PLA mechanical performance during biodegradation. PLA/GNP-M and C composites allow similar HFF-1 cell adhesion and proliferation at the surface and do not release toxic products.

References

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