The effects of nano-sized carbon fillers on the physico-chemical, mechanical, and biological properties of polyester nanocomposites

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Abstract

Nanocomposites based on poly(ethylene terephthalate-ethylene dilinoleate) (PET-DLA) copolymers of different hard to soft segment ratios (40:60 and 60:40) and three different carbon nanofillers of different aspect ratios (dimensions), as 0D carbon black, 1D multiwalled carbon nanotubes, and 2D graphene, have been prepared *in situ* during two-stage polymerization. Fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR) spectroscopy were used to characterize the chemical structures of the obtained nanocomposites. Scanning electron microscopy (SEM) indicated very good dispersions of all carbon nanofillers in both polymer matrices. Differential scanning calorimetry (DSC) results revealed that the addition of nano-sized fillers eliminated cold crystallization of materials containing 40% hard segments in polymer matrix. We found that the high aspect ratio, 1D nano-filler (multiwalled carbon nanotubes) strongly nucleated crystallization of materials containing 60% of hard segments. This nanofiller also yielded the greatest improvement in the Young's modulus as assessed by tensile tests, both at 24 °C and 37 °C. We did not observe reduced bacterial adhesion to nanocomposites, likely due to increased roughness. Importantly, *in vitro* cytocompatibility tests with L929 murine fibroblasts demonstrated cell viability and growth on all materials except those containing carbon nanotubes. Finally, both high aspect ratio nanofillers markedly improved the barrier properties of obtained nanocomposites. New materials were successfully used for manufacturing of prototype of heart assist device, with pneumatic membrane made of graphene nanocomposite.

Key words: *thermoplastic elastomers; nanocomposites; carbon nanotubes; graphene; carbon black; heart assist device*

Graphical Abstract

1. Introduction

Contemporary engineering materials must be designed to possess multiple, yet specific and desirable features. Biomedical applications are particularly complex and challenging, with the final application of the material driving a number of requirements including mechanical properties, biocompatibility, and/or susceptibility to degradation [1].

Some available polymeric materials for medical applications have a number of limitations related to their chemical structure, leading to strictly defined properties (mechanical, thermal, etc.). In contrast, multiblock copolymers can offer the possibility of tuning a wide range of properties by changing the hard to soft segment ratio—an undeniable advantage [2-3]. For example, poly(ethylene terephthalate) (PET) is an aromatic polyester that exhibits very desirable properties, such as high melting point, very good mechanical properties, and high rigidity [4]. However, modification of PET with bio-based dimeric fatty acid (dilinoleic acid, DLA), yielding PET-DLA multiblock copolyester, can provide enhanced elasticity and improved processing properties, in addition to the very good mechanical properties [5]. These copolymers were recently extensively tested in heart assist devices [6,7], showing good mechanical performance and high bio- and blood compatibility.

Further tuning of properties can be achieved by use of nanofillers and, as a result, polymer nanocomposites have become an important area of research in the field of nanotechnology and biomedicine [8]. The use of various types of carbon nanofillers of different shape (see Table 1), such as carbon black (CB) (spherical objects, aspect ratio $\alpha \sim 1$,

0D), carbon nanotubes (CNT) (tubular systems, aspect ratio $\alpha \gg 100$, 1D), and a layered graphene (aspect ratio $\alpha \sim 100$, 2D), can be used to markedly improve the electrical, optical, mechanical, fire resistance or barrier properties of polymeric materials [9-11]. For example, PET/CNT nanocomposites exhibit improved mechanical, thermal and barrier properties [12,13]. The addition of just 0.4 wt% multiwalled CNT (MWCNT) can improve the Young's modulus and the yield strength of the PET nanocomposites by approx. 25% and 15%, respectively [14]. Similarly, the addition of 1 wt% of functionalized graphene to poly(methyl methacrylate) (PMMA) matrix results in an increase of elastic modulus of about 80% and elongation at break of about 20% [15]. On the other hand, carbon black nanofiller is mainly used to enhance electrical conductivity; the addition of 0.1 vol. % can yield electrically conducting nanocomposites with potential applications as microelectromechanical strain and pressure sensors [16,17].

In this work, we examine the effect of the addition of carbon black nanoparticles, multiwalled carbon nanotubes, and graphene to PET-DLA thermoplastic elastomers with different amounts of hard segments, 40 wt% or 60 wt%, respectively. Our motivation is expanding the material design space available to biomedical engineers working on heart assist devices, such as the POLVAD extracorporeal pump and future implantable devices [6]. These complex devices have various components, from pumping chambers and connectors to membranes and valve leaflets, each with different material requirements. By comparing the effect of carbon nanofillers with different aspect ratios (Table 1), we aim to provide designers and engineers an additional parameter, beyond hard-to-soft segment ratio, that can be used to tune the thermal, mechanical, and barrier properties of the material to the demands of each (sub)component. All nanocomposites were synthesized via *in situ* two-stage polymerization and the results are interpreted in terms of carbon filler aspect ratio, dispersion quality, and in relation to the differences in semi-crystalline morphology of the PET-DLA 40% and PET-DLA 60% matrices. Thus, their potential as next generation materials for heart assist devices will be explored.

2. Materials and Experimental Methods

2.1 Materials

PET-DLA multiblock thermoplastic elastomers containing 40 wt% (PET-DLA 40) and 60 wt% (PET-DLA 60) of hard segments (W_h) of poly(ethylene terephthalate) (PET) and soft segments comprising amorphous fatty acid ester sequences based on dilinoleic acid (DLA), as well as nanocomposites containing 0.05, 0.1, 0.25, 0.5, 1 wt% of graphene (Graphene Supermarket, Grade A0-3, USA), 0.05, 0.1, 0.25, 0.5, 1 wt% of carbon black (CB) (Cabot, ELFTEx TP, USA) and 0.05, 0.1, 0.25, 0.5 wt% of MWCNT (CNT) (Nanocyl 7000, Belgium) were synthesized via *in situ* two-stage polymerization, based on previous work [18, 19]. For all nanocomposites containing 0.05 wt% to 0.25 wt% of nanofiller, dispersions were prepared only in ethylene glycol. For nanocomposites containing 0.5 wt% to 1 wt% of nanofiller, dispersions were prepared in both ethylene glycol and dilinoleic acid. Next, two step polymerization was carried out and the obtained nanocomposites were extruded from the

reactor under pressure of nitrogen. We were not able to synthesize nanocomposites containing 1 wt% of MWCNT because the prepared dispersion was so thick and viscous that it was impossible to pour into the reactor during the synthesis. Geometrical characteristics of nanofillers in their pristine form are summarized in Table 1.

Table 1. Geometrical characteristics of used nanofillers and TEM images.

Filler type	Dimensions	Aspect ratio
0D Carbon black (CB)	Length=Width=Thickness ~ 25 nm	1
1D Multiwalled carbon nanotubes (CNT)	Length ~ 15 μm Diameter ~ 15 nm	~ 1000
2D Graphene	Thickness ~ 12 nm Lateral dimensions ~ 4500 nm	~ 375

2.2 Experimental Methods

2.2.1 Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (Spectrometer ATR FT IR Alpha, Bruker) was used to investigate the chemical structure of all obtained materials and to examine the potential of new bond formation between the carbon nanofillers and the PET-DLA matrix. FTIR transmission spectra were recorded between 400 and 4000 cm^{-1} , with 2 cm^{-1} resolution. Samples were vacuum-dried prior to measurement, and 32 scans were averaged across the

spectral range. Polymer and nanocomposites samples were prepared by hot-press compression and discs were cut using a pneumatic punch.

2.2.2 Nuclear magnetic resonance (NMR)

Proton nuclear magnetic resonance (^1H NMR) analysis was performed using a NMR spectrometer (Bruker DPX 400MHz). Nanocomposites samples were prepared by dissolution in a mixture of chloroform and trifluoroacetic acid ($\text{CHCl}_3/\text{CF}_3\text{COOH}$ (2:1)).

2.2.3 High Resolution Scanning electron microscopy (HR-SEM)

High resolution scanning electron microscope (Hitachi SU-70) was used to examine the fracture surface of samples. Injection molded samples were frozen in liquid nitrogen, fractured and metal-sputtered with gold-palladium alloy.

2.2.4 Differential Scanning Calorimetry (DSC)

The thermal properties of the novel nanocomposites were determined using differential scanning calorimetry method (DSC) (Q100, TA Instruments apparatus). Nanocomposite samples, weighing between 10 and 20 mg, were first dried under vacuum at 60 °C for 24h. The DSC measurements were carried out in a triple cycle, "heating-cooling-heating," over a temperature range from -90 to 300 °C. The heating/cooling rate was 10 °C/min. The glass transition temperature was determined from the temperature diagrams by the change of the slope of the obtained curves. The melting and crystallization temperature were determined as the temperature values corresponding to the maximum of endothermic curve and the minimum of exothermic curve, respectively.

2.2.5 Mechanical properties

Tensile tests were performed on Instron 3366 tensile machine with 500 N load cell to determine Young's modulus (at 0.5% strain @ 1 mm/min), yield point, tensile strength and elongation at break for all of obtained nanocomposites containing various carbon nanofillers in 24 °C and 37 °C. Tests were performed at a crosshead speed of 200 mm/min on injected molded, "dog-bone" shaped samples (2 mm thick) according to the PN-EN ISO 527 standard. For tests at 37 °C, an environmental chamber was used; however, due to technical limitation of the chamber, tests had to be stopped at 600% of elongation. As a result, only Young's modulus was calculated, since the machine could not reach tensile strength at break and elongation at break.

2.2.6 Water contact angle

The water contact angle was measured by using a KRUSS DSA100 digital goniometer. Static contact angle measurements were performed on the surface of degreased materials (prepared by using hot press) by placing a 2 μL droplet of deionized water using

the automatic dispenser of the goniometer. Contact angle was calculated using Kruss drop shape analysis software (DSA4).

2.2.7 Surface roughness

Surface roughness analysis was performed with the Keyence VK9700 Laser Scanning Microscope. Nanocomposite samples were prepared by hot pressing films. The surface roughness of individual materials was evaluated on the basis of roughness coefficient R_q , using Keyence Analyzer software:

$$R_q = \sqrt{\frac{1}{n} \sum_{i=1}^n y_i^2} \quad (1)$$

Where: y_i - coarse roughness coordinates.

2.2.8 Bacterial adhesion assay

In order to assess the susceptibility of the materials to bacterial colonization, bacterial adhesion assays were performed using model Gram positive (*Staphylococcus epidermidis*) and Gram negative (*Escherichia coli*) organisms. Only materials containing the highest amount of nanofiller were tested. Briefly, for each organism, discs were inoculated with 40 μ L of 0.5 McFarland inoculum and the bacteria allowed to adhere for 2 hours. After thorough rinsing with phosphate buffered saline (PBS), adherent bacteria were detached via 30 min of vortexing in 1.5 mL of 0.2% saponin in PBS and quantified by plating 100 μ L of serial dilutions and counting colonies after 24 hours. To confirm complete detachment, discs were incubated under brain heart infusion agar for 24 hours. For each material and microorganism, 5 discs were tested and each serial dilution was plated and counted in triplicate.

2.2.9 *In vitro* cytocompatibility

In vitro adhesion and cytocompatibility studies were performed using mouse fibroblasts, L929. Only materials containing the highest amount of nanofiller were tested. Briefly, L929 cells were maintained in T-25 flasks in Dulbecco's MEM (DMEM) medium with high glucose (4.5g/L) and 10% FBS, 2 mM L-glutamine, 1% antibiotics (penicillin/streptomycin) at 37°C and air atmosphere containing 5% CO₂. For each test material, 4 discs were placed in a 48-well plate and sterilized with ethylene oxide. After degassing, discs were seeded with 30,000 L929 cells in a volume of 300 μ L of medium. An empty well (tissue culture polystyrene, TCPS) served as a control. After 72 hours of culture, medium was exchanged for 200 μ L medium containing 20 μ L of 0.15 g/L resazurin in PBS. After incubation for 5 hours, fluorescence intensity (ex540/em590) was measured using a Biotek Synergy HTX multifunctional platereader. The measured intensity of fluorescence correlates with the viability of the cells. To confirm cell growth, the cells were maintained in culture and the viability assay was repeated after a further 24 hours.

2.2.10 Moisture vapour transmission test

In order to assess barrier properties of the obtained nanocomposites, measurements of moisture vapour transmission were performed with the use of gravimetric moisture analyser (Radwag WPS 110S). Briefly, thin films ($n = 4-5$, 0.2 mm thick and 5.5 cm diameter, prepared by hot-press compression) of each neat copolymer and nanocomposite were used to seal a chamber within the instrument containing 5 g of distilled water. Over the course of 24 hours at 40 °C (the lowest temperature setting of this instrument, but close to body temperature) the mass of the water was monitored; the loss of mass represents moisture vapour transmission through the membrane.

2.3 Statistical analysis

Data is presented as mean $\pm$ standard deviation. Where appropriate, multiple comparison testing was performed via ANOVA followed by Tukey HSD pair-wise or Dunnett's *post hoc* testing using R software (R Studio).

3. Results and discussion

3.1 Structural characterization

3.1.1 FTIR spectroscopy

Two series of polyester nanocomposites were synthesized and their chemical structure (see Fig. 1, 2 and 3 for chemical structure diagrams) was characterized with FTIR spectroscopy as illustrated in Figure S1 (Supplementary Materials). The obtained spectra show bands typical for polyester structure [20] and as expected, because the used nanofillers were not functionalized, PET-DLA nanocomposites exhibited similar absorption bands to those of neat PET-DLA copolymer. The lack of significant changes in band positions indicates that the synthesized nanocomposites and reference material have the same structural properties. The minor changes in peak intensities may be caused by slightly different hard to soft segment ratios of the polymers, since each material was synthesized via *in situ* polymerization, yielding slightly different polymerization degree.

3.1.2 ^1H NMR spectroscopy

^1H NMR spectra of neat PET-DLA containing 40 wt% and 60 wt% of hard segments and prepared nanocomposites containing 0.5 wt% of CNT, 1 wt% of CB, and 1 wt% of graphene are presented in Figure 1 and Figure 2, respectively.

Figure 1. Nuclear magnetic resonance spectroscopy (^1H NMR) spectra of the neat PET-DLA 40 (a) and obtained nanocomposites containing 0.5 wt% of CNT (b), 1 wt% of CB (c) and 1 wt% of graphene (d).

Figure 2. Nuclear magnetic resonance spectroscopy (NMR) spectra of the neat PET-DLA 60 (a) and obtained nanocomposites containing 0.5 wt% of CNT (b), 1 wt% of CB (c) and 1 wt% of graphene (d).

As can be seen from Figure 1 and Figure 2, there is no significant difference in between neat PET-DLA copolymers and the obtained nanocomposites for either composition. All spectra show a peak at 8.17 ppm (g) that can be assigned to aromatic ring from PET sequences in hard segments [21]. The peaks at 4.83, 4.68, 4.58 and 4.42 ppm (e+e'+f+f') belong to $-(CH_2)-(CH_2)-$ from ethylene glycol sequences. Those peaks are attributed to different methylene protons depending on position in polymer structure. The peak at 4.83 ppm is attributed to the methylene groups between PET hard segments (PEP) (Figure 3a), the peaks at 4.68 and 4.58 ppm are attributed to ethylenedioxy groups between PET and DLA (hard and soft segments) units (PED) (Figure 3b), and, finally, the peak at 4.58 ppm is attributed to methylene protons of the ethylenedioxy group between DLA soft segments (DED) (Figure 3c). All possible arrangements of the sequences are shown in Figure 3. The intensities of these peaks depend on the degree of polymerization (DP) and serve as confirmation that obtained polymers are in fact statistical copolymers. The peak at 2.47 ppm is attributed to methylene groups between DLA and carbonyl group in ester bonds. Finally, the three peaks at 1.67, 1.30 and 0.89 ppm are attributed to methylene groups and ending methyl groups in long DLA chains.

Figure 3. Possible structural unit arrangements.

Based on the obtained NMR spectra, we calculated the DP of all obtained materials. Since the peak at 8.17 ppm is assigned to 4 protons in the aromatic ring of PET sequences and the peak at 2.47 ppm is attributed to 4 protons in between segments, we can calculate the DP from Equation (2):

$$DP = \frac{I_{8.17}}{I_{2.47}} \quad (2)$$

where DP is degree of polymerization, $I_{8.17}$ is the intensity of peak at 8.17 ppm and $I_{2.47}$ is the intensity of peak at 2.47 ppm.

From the DP, we were also able to calculate weight percentage of hard segments (%W_h) of obtained materials and compare it with theoretical values using Equation 3:

$$\%W_h = \frac{DP \cdot M_H}{DP \cdot M_H + M_S} \quad (3)$$

where %W_h is weight percentage of hard segments, DP is the degree of polymerization, M_H is the molecular weight of hard segments, and M_S is the molecular weight of soft segments. The results for all obtained materials are presented in Table 2. By knowing the molecular weight of the segments, we were also able to calculate theoretical molar ratio of PEP, PED and DED segments. For PET-DLA 40wt% the theoretical calculated molar ratio of those segments is 0.46/0.44/0.1, respectively and for PET-DLA 60wt% 0.67/0.3/0.03, respectively. Table 2 also shows the values of this molar ratio of PEP, PED and DED segments, as calculated from the NMR spectra for all obtained nanocomposites, confirming that we have synthesized statistical copolymers in all cases. The obtained results fit well with the theoretical values for each copolymer nanocomposite. Importantly, the incorporation of nanofillers did not affect the DP or weight percentage of segments; the small differences between calculated values and theoretical ones can be attributed to slightly different synthesis conditions and/or measurement error.

Table 2. Degree of polymerization and weight percentage of hard segments calculated from NMR spectra for all synthesized materials.

Material	Theoretical DP	Calculated DP	Theoretical %W _h [%]	Calculated %W _h [%]	Theoretical calculated molar ratio PEP/PED/DED	NMR calculated molar ratio PEP/PED/DED
PET-DLA 40%	2.07	2.00	40	39.0	0.46/0.44/0.1	0.45/0.44/0.11
0.05% CB	2.07	1.95	40	39.0	0.46/0.44/0.1	0.45/0.44/0.11
0.1% CB	2.07	1.95	40	39.0	0.46/0.44/0.1	0.45/0.44/0.11
0.25% CB	2.07	1.90	40	38.0	0.46/0.44/0.1	0.43/0.45/0.12
0.5% CB	2.07	1.90	40	38.0	0.46/0.44/0.1	0.43/0.45/0.12
1% CB	2.07	1.90	40	38.0	0.46/0.44/0.1	0.43/0.45/0.12
0.05% Gra	2.07	1.90	40	38.0	0.46/0.44/0.1	0.43/0.45/0.12
0.1% Gra	2.07	1.95	40	39.0	0.46/0.44/0.1	0.45/0.44/0.11
0.25% Gra	2.07	1.90	40	38.0	0.46/0.44/0.1	0.43/0.45/0.12
0.5% Gra	2.07	1.90	40	38.0	0.46/0.44/0.1	0.43/0.45/0.12
1% Gra	2.07	1.90	40	38.0	0.46/0.44/0.1	0.43/0.45/0.12
0.05% CNT	2.07	2.0	40	39.0	0.46/0.44/0.1	0.45/0.44/0.11
0.1% CNT	2.07	1.9	40	38.0	0.46/0.44/0.1	0.43/0.45/0.12
0.25% CNT	2.07	2.0	40	39.5	0.46/0.44/0.1	0.45/0.44/0.11
0.5% CNT	2.07	2.0	40	39.5	0.46/0.44/0.1	0.45/0.44/0.11

Material	Theoretical DP	Calculated DP	Theoretical %W _h [%]	Calculated %W _h [%]	Theoretical calculated molar ratio PEP/PED/DED	NMR calculated molar ratio PEP/PED/DED
PET-DLA 60%	4.65	4.75	60	60.5	0.67/0.3/0.03	0.68/0.29/0.03
0.05% CB	4.65	4.60	60	59.5	0.67/0.3/0.03	0.67/0.30/0.03
0.1% CB	4.65	4.35	60	58.0	0.67/0.3/0.03	0.66/0.30/0.04
0.25% CB	4.65	4.55	60	59.5	0.67/0.3/0.03	0.67/0.30/0.03
0.5% CB	4.65	4.55	60	59.5	0.67/0.3/0.03	0.67/0.30/0.03
1% CB	4.65	4.55	60	59.5	0.67/0.3/0.03	0.67/0.30/0.03
0.05% Gra	4.65	4.17	60	57.0	0.67/0.3/0.03	0.65/0.31/0.04
0.1% Gra	4.65	4.55	60	59.5	0.67/0.3/0.03	0.67/0.30/0.03
0.25% Gra	4.65	4.34	60	58.5	0.67/0.3/0.03	0.66/0.30/0.04
0.5% Gra	4.65	4.75	60	60.5	0.67/0.3/0.03	0.68/0.29/0.03
1% Gra	4.65	4.52	60	59.5	0.67/0.3/0.03	0.67/0.30/0.03
0.05% CNT	4.65	4.35	60	58.5	0.67/0.3/0.03	0.66/0.30/0.04
0.1% CNT	4.65	4.35	60	58.5	0.67/0.3/0.03	0.66/0.30/0.04
0.25% CNT	4.65	4.15	60	57.0	0.67/0.3/0.03	0.65/0.31/0.04
0.5% CNT	4.65	4.75	60	60.5	0.67/0.3/0.03	0.68/0.29/0.03

3.2 Thermal analysis

PET-DLA copolymer exhibit two major transitions (Tables 3 and 4) ascribed to microphase separated structure characteristic for thermoplastic elastomers: low temperature glass transition (T_{g1}) of soft segments and high melting point (T_{m2}) of hard, semicrystalline segments. Additionally, as can be seen from Table 3, neat PET-DLA 40% exhibits a cold crystallization (T_{cc2}). However, none of the obtained nanocomposites exhibit cold crystallization peaks, because the presence of nanofillers favours the formation of crystalline regions during the cooling stage.

For all of the PET-DLA 40% nanocomposites, the glass transition temperatures are lower as compared to neat PET-DLA 40% polymer (Table 3), from -19 °C to about -23 °C. Although this change may not be significant, materials containing 60 wt% of PET hard segments (Table 4) showed a very different dependence—their T_{g1} did not changed as compared to the neat PET-DLA 60% copolymer (T_{g1} was approx. -4 °C to -5 °C). The slight shift towards lower temperatures for PET-DLA 40% nanocomposites can be ascribed to a plastifying effect reducing interactions between macrochains and, thus, lowering the glass transition temperature.

The aspect ratio of the nanofiller did not significantly change the melting temperature, T_{m2} of all nanocomposites from PET-DLA 40% series (Table 3), however a slightly increased T_{m2} and melting enthalpy can be observed for materials containing nanofillers with high aspect ratio (graphene and CNTs). In contrast, nanocomposites from the PET-DLA 60%

series (Table 4), exhibit decreased melting temperature, for nano-fillers with low aspect ratio, especially for CB, but also for graphene. Therefore, melting behavior of carbon nanofiller-based nanocomposites can be controlled not only by nanofiller shape, but also by polymer matrix microstructure (PET-DLA 40% copolymer is more amorphous than PET-DLA 60%).

During the cooling stage, all nanocomposites exhibit crystallization that is in the same temperature range, approx. 95-103 °C. No significant differences in T_{c2} can be seen within each series with different content of various nanofillers. Thus, all nanofillers nucleate PET-DLA 40% crystallization, independently of aspect ratio, and therefore strongly affect polymer thermal behavior. In contrast, significant changes can be seen for nanocomposites from the PET-DLA 60% series (Table 4); the crystallization temperature, T_{c2} is increased by 30 °C as compared to the neat polymer and the highest increase in T_{c2} was measured for nanocomposites containing nanofiller with the highest aspect ratio (CNTs). This is an indication of a strong nucleating effect of carbon nanofillers on crystallization of the polymer matrix.

Table 3. Thermal analysis of PET-DLA 40 nanocomposites series

Sample	Soft segments		Hard segments				
	T_{g1} (°C)	ΔC_p (J/g/ °C)	T_{cc2} (°C)	T_{m2} (°C)	ΔH_{m2} (J/g)	T_{c2} (°C)	$W_{c,h}$ (%)
PET-DLA 40	-19.1	0.51	87.2	166.2	11.3	-	8.0
0.05% CB	-22.3	0.39	-	163.2	12.11	95.2	8.5
0.1% CB	-22.5	0.46	-	164.1	12.31	96.1	9.0
0.25% CB	-23.1	0.46	-	164.3	10.84	100	8.0
0.5% CB	-23.4	0.44	-	164.3	11.12	99.3	8.0
1% CB	-23.3	0.43	-	166	11.35	105	8.0
0.05% Gra	-23.1	0.39	-	167.2	21.03	95.3	12.0
0.1% Gra	-23.1	0.38	-	167.4	20.19	95.6	12.5
0.25% Gra	-23.4	0.36	-	167.4	20.02	104.3	13.0
0.5% Gra	-23.3	0.37	-	165.6	18.07	101.2	13.0
1% Gra	-23.1	0.35	-	167.2	17.90	108.4	12.0
0.05% CNT	-23.2	0.42	-	166.2	13.31	104.2	9.5
0.1% CNT	-22.6	0.41	-	165.6	13.20	100.8	9.5
0.25% CNT	-22.5	0.42	-	168.3	12.64	105.3	9.0
0.5% CNT	-22.6	0.41	-	169.2	14.49	104.5	10.5

T_{g1} –glass transition of the soft segments, ΔC_p - change of the heat capacity at glass transition; T_{g2} , T_{m2} , T_{c2} - glass transition, melting, and crystallization temperatures, respectively, of the hard segments; T_{cc2} – cold crystallization; ΔH_{m2} - melting enthalpy of the hard segments; $W_{c,h}$ -mass content of PET crystallites in the polymer.

Table 4. Thermal analysis of PET-DLA 60 nanocomposites series

Sample	Soft segments		Hard segments				
	T_{g1} (°C)	ΔC_p (J/g/ °C)	T_{cc2} (°C)	T_{m2} (°C)	ΔH_{m2} (J/g)	T_{c2} (°C)	$W_{c,h}$ (%)
PET-DLA 60	-5.4	0.35	-	216.3	20.40	120.3	15.0
0.05% CB	-4.2	0.40	-	209.4	17.13	155.3	12.0
0.1% CB	-4.2	0.38	-	209.6	16.03	155.6	12.0
0.25% CB	-5.3	0.38	-	210.0	16.13	157.4	12.0
0.5% CB	-3.7	0.43	-	211.4	17.36	160.1	12.0
1% CB	-5.1	0.38	-	210.7	15.25	158.8	11.0
0.05% Gra	-4.8	0.37	-	208.6	17.29	150.1	12.0
0.1% Gra	-4.7	0.37	-	208.7	17.78	151.7	13.0
0.25% Gra	-4.6	0.24	-	214.3	15.85	167.3	11.0
0.5% Gra	-3.6	0.28	-	213.5	17.08	165.7	12.0
1% Gra	-4.5	0.26	-	213.4	15.11	166.2	11.0
0.05% CNT	-4.2	0.36	-	216.3	22.52	169.7	16.0
0.1% CNT	-4.4	0.34	-	213.4	18.06	160.6	13.0
0.25% CNT	-4.5	0.35	-	214.2	20.09	162.4	15.0
0.5% CNT	-0.9	0.31	-	218.3	22.57	168.4	16.0

T_{g1} –glass transition of the soft segments, ΔC_p - change of the heat capacity at glass transition; T_{g2} , T_{m2} , T_{c2} - glass transition, melting, and crystallization temperatures, respectively, of the hard segments; T_{cc2} – cold crystallization; ΔH_{m2} - melting enthalpy of the hard segments; $W_{c,h}$ -mass content of PET crystallites in the polymer.

3.3 Mechanical properties

The mechanical properties of neat PET-DLA copolymers containing 40 and 60 wt% of hard segments, as well as the obtained nanocomposites are presented in Figures 4 and 5, respectively.

Figure 4. Mechanical properties of nanocomposites based on PET-DLA 40 wt% polymer matrix. a) Young's modulus at 24 °C; b) Young's modulus at 37 °C; c) tensile strength at 24 °C; d) elongation at break at 24 °C.

All PET-DLA 40 wt% nanocomposites exhibit increased values of Young's modulus, compared to neat PET-DLA 40 wt%, especially at 37 °C (Figure 4b). For the PET-DLA 40 wt% matrix, the addition of CNT nanofiller resulted in the largest increase in Young's modulus at 24 °C and 37 °C, up to 30% (for material containing 0.5 wt% of CNT). All the other nanocomposites showed an increase in Young's modulus values at 24 °C and 37 °C up to approx. 15%, in comparison with neat polymer. Due to the technical limitations of our environmental chamber, it was impossible to reach tensile strength and elongation at break values (up to 600%) at 37 °C, therefore we are not able to compare those values. However, at 24 °C, the tensile strength values of all PET-DLA 40 wt% nanocomposites were comparable, approx. 15 MPa (Figure 4c) and elongation at break values were lower than neat PET-DLA 40 wt%, but no trend was observed (Figure 4d). The change in tensile strength is very marginal, indicating the poor interaction of the nanofillers with the PET-DLA polymer matrix. However, this is to be expected, since we used unmodified carbon nanofillers and, as FTIR and ^1H NMR spectroscopy indicated, there is no significant chemical interaction between nanofiller and polymer matrix.

Figure 5. Mechanical properties of nanocomposites based on PET-DLA 60 wt% polymer matrix. a) Young's modulus at 24 °C; b) Young's modulus at 37 °C; c) tensile strength at 24 °C; d) elongation at break in 24 °C.

For the case of PET-DLA 60 wt% matrix, the addition of CNT as nanofillers also yielded the largest increase in Young's modulus, up to approx. 30%; however, the measured values were not linearly dependent on concentration at 24 °C. For materials containing CB and graphene nanofillers, Young's modulus values were comparable, or slightly lower, to neat copolymer matrix at 24 °C and at comparable level at 37 °C, with the exception of 1.0% graphene, which is 15% higher. Interestingly, tensile strength for all of obtained nanocomposites is higher than for neat material (Figure 5c), except of materials containing 0.1% CNT.

Since our materials are multiblock copolymers, containing hard and soft segments, their useful application temperature range is limited by the glass transition temperature (T_g) of soft segments and the T_g of hard segments [22]. For PET-DLA 40 wt% the T_g is approx. -18 °C and PET-DLA 60 wt% has T_g of approx. -5 °C [7]. Thus, below the T_g of the DLA soft segments, the materials are in a glassy state with no elastomeric properties, however with high tensile strength. Above the T_g of the soft segments, on the other hand, the tensile strength of the materials is much lower. The upper temperature limit is related to the softening of hard segments and it depends on hard segments length, domain size, and phase

separation. Importantly, our results at 37 °C (human body temperature) indicate that the mechanical properties are stable, so we assume that the materials will maintain their mechanical properties if used as a biomaterial within the human body.

3.4 High resolution scanning electron microscopy (HR-SEM)

High resolution scanning electron microscopy (HR-SEM) was used to examine the dispersion of carbon nanofillers within the polymer matrices and to investigate the morphology of obtained nanocomposites. Neat PET-DLA 40 wt% and PET-DLA 60 wt% polymers are shown in Figure 6.

Figure 6. HRSEM micrographs of neat a) PET-DLA 40 wt% and b) PET-DLA 60 wt%.

In Figure 7 and 8, we present HR-SEM micrographs of PET-DLA 40 wt% and PET-DLA 60 wt% nanocomposites, respectively. Overall, the SEM images indicate that we obtained very good dispersion of each carbon nanofillers in both the PET-DLA 40 wt% matrix and the PET-DLA 60 wt% matrix. The micrographs clearly show the difference between amorphous carbon black (Figures 7 and 8, c,d) and the crystalline particles of both carbon nanotubes (Figures 7 and 8, a,b) and graphene (Figures 7 and 8, e,f).

Figure 7. HR-SEM micrographs of PET-DLA 40 wt% containing a,b) PET-DLA 40 wt% + 0.5% CNT, c,d) PET-DLA 40 wt% + 1% CB and e,f) PET-DLA 40 wt% + 1% graphene (right panel shows sample surface at higher magnification).

Figure 8. HR-SEM micrographs of PET-DLA 60 wt% containing a,b) PET-DLA 60 wt% + 0.5% CNT, c,d) PET-DLA 60 wt% + 1% CB, and e,f) PET-DLA 60 wt% + 1% graphene (right panel shows sample surface at higher magnification).

3.5 Water contact angle

The wetting properties of polymeric materials are very important for applications in biomedical devices or implants. For example, in general hydrophilic surfaces exhibit reduced protein adsorption [23], potentially improving biocompatibility, but tend to foster bacterial cell attachment [24]. To study the surface wetting character of our materials, water contact angle measurements of neat polymers and nanocomposites containing maximum amount of each nanofiller were performed and the results are presented in Table 5.

Table 5. Water contact angle and surface roughness of nanocomposites with highest content of carbon nano-fillers.

Material	Contact angle [°]	Surface roughness R_q [μm]
PET-DLA 40	90 +/- 2	0.095
PET-DLA 40 + 0.5% CNT	87 +/- 3	0.137
PET-DLA 40 + 1% CB	91 +/- 2	0.218
PET-DLA 40 + 1% graphene	90 +/- 1	0.293
PET-DLA 60	87 +/- 3	0.817
PET-DLA 60 + 0.5% CNT	85 +/- 2	0.826
PET-DLA 60 + 1% CB	87 +/- 2	0.762
PET-DLA 60 + 1% graphene	86 +/- 4	0.506

Both neat PET-DLA polymers exhibited water contact angle values of approximately 90°. Modification of either matrix with carbon nanofillers did not have a strong effect on the water contact angle. In Table 5, we also present the surface roughness measurements (root mean square roughness, R_q) as determined using laser scanning microscopy. According to the Wenzel model, surface roughness enhances the effect of surface chemistry on contact angle [25], according to the equation (4):

$$\cos\theta_z = r \cdot \cos\theta_y \quad (4)$$

where: θ_z is the measured contact angle, θ_y is the Young contact angle, of ideal, flat surface, and r is the roughness ratio between the actual surface area and the projected, flat area.

And so, because $r > 1$, $|\cos\theta_z| > |\cos\theta_y|$ and thus, with increasing roughness hydrophobic materials become more hydrophobic, while hydrophilic materials become more hydrophilic. However, as shown in Table 5, both neat copolymer matrices, as well as all nanocomposites exhibited water contact angles near 90°, so $\cos\theta_z \approx 0$. Therefore, $\cos\theta_y$ must also be close to 0. This explains the lack of significant changes in the contact angle despite the differences in roughness of the surface.

3.6 Bacterial adhesion

Figure 9 presents the results of bacterial adhesion studies on PET-DLA 40 and PET-DLA 60 nanocomposites containing the highest amounts of each carbon nanofiller. For the PET-DLA 40 materials, only the inclusion of graphene nanofiller resulted in statistically significant increase in Gram-negative *E. coli* adhesion, as compared to neat PET-DLA 40, as well as PET-DLA 40 containing CNT nanofiller ($p=0.014$ and $p=0.003$, respectively). For

Gram-positive *S. epidermidis*, adhesion was approximately 10 times greater than *E. coli*, but a similar trend was observed, with highest adhesion on copolymer containing graphene nanofiller ($p=0.001$ vs. PET-DLA 40, $p=0.013$ vs PET-DLA 40+CNT), however inclusion of carbon black also resulted in a statistically significant increase in adhesion as compared to neat copolymer ($p=0.008$).

Figure 9. Bacterial adhesion of PET-DLA 40 (a,b) and PET-DLA 60 (c,d) nanocomposites containing the highest amounts of each carbon nanofiller.

For the PET-DLA 60 materials, no significant differences were observed in Gram-negative *E. coli* adhesion. For Gram-positive *S. epidermidis*, adhesion was again approximately 10 times greater than *E. coli*, as was observed for the PET-DLA 40 nanocomposites. Likewise, the observed trend *S. epidermidis*, adhesion was similar to that of *E. coli*, with the graphene nanocomposite being the lowest, however, it only significantly differed in adhesion from the CNT nanocomposites ($p=0.033$).

Bacterial adhesion to surfaces is a complex process, depending on characteristics of the microorganism, the surface, as well as environmental conditions [26,27]. In light of this, we interpret our results with caution. The most substantial observed difference, that between the two model organisms, is likely a function of differing surface free energy (SFE) of the

organisms, due to their differing cell wall composition; *E. coli* has a greater (SFE) than *S. epidermidis* and exhibits markedly reduced adhesion to hydrophobic surfaces [27]. However, the differences between the nanocomposites may be the result of differences in surface roughness, which has a positive, though non-linear effect on adhesion, particularly when the roughness is on the same scale as the size of the organism [26]. For the nanocomposites presented here, the differences in roughness of the PET-DLA 40 nanocomposites are in the range where a large effect would be predicted and the trend in adhesion follows the roughness measurements (Table 5). Meanwhile for the PET-DLA 60 nanocomposites, the surface roughness of all of the materials exceeds the radius of a microorganism and as a result the differences in roughness do not substantially influence adhesion.

3.7 *In vitro* cytocompatibility

Figure 10 shows graphs of L929 mouse fibroblast viability after 72 and 96 hours on PET-DLA 40 and PET-DLA 60 nanocomposites containing the highest amounts of each carbon nanofiller. The results are presented as percent of fibroblast viability on tissue culture polystyrene (TCPS). For all tested materials, the results indicate markedly less cell viability as compared to near-confluent cells on TCPS. However, it is important to note that these materials do not possess any charged function groups or biofunctionalization, which could facilitate cell adhesion and thus growth of this adherent cell line. For PET-DLA 40 and PET-DLA 60, as well as their nanocomposites containing carbon black and graphene as nanofillers, the reduced viability is likely due to relatively poor cell adhesion, as additional 24 hours of culture results in an increase in viability, indicating growth and sustained viability. Importantly, we do not observe any significant decrease in viability for these two nanocomposites, as compared to the neat polymers. On the other hand, for materials containing CNT nanofillers, viability at both time points was minimal, indicating both poor adhesion and no growth—poor cytocompatibility. Statistical analysis with ANOVA followed by Dunnet's *post hoc* analysis indicates that this reduced viability on material with CNT nanofillers is statistically significant, as compared to corresponding neat copolymer ($p=0.015$ for PET-DLA 40 and $p=0.007$ for PET-DLA 60).

Figure 10. L929 fibroblast proliferation on nanocomposites surfaces a) PET-DLA 40, b) PET-DLA 60, normalized to TCPS (tissue culture polystyrene) (mean $\pm$ SD, n=4 discs).

Additionally, there is a trend towards higher viability of L929 mouse fibroblast on materials containing 60 wt% of hard segments. This effect is probably related to greater adhesion of cells, which may be the result of a combination of increased roughness and stiffness of this matrix—stiffness of the polymer substrate can promote fibroblast adhesion [29,30].

3.8 Barrier properties

In a pneumatic pulsatile heart assist device, such as POLVAD [6], a polymer membrane plays a key role in transmitting the pumping force (gas) to the blood (liquid) in the pump chamber. For this component, barrier properties are important, yet can be challenging due to the mechanical properties dictating a thin elastic membrane. The addition of a nanofiller can improve the barrier properties by either reducing the solubility of the permeating molecule (typically for low nanofillers content, <0.05 vol%) or by reducing the diffusivity of the permeating molecule due to the physical presence of nanofillers [10]. In the latter case, the aspect ratio and orientation play a key role, with increased aspect ratio and alignment perpendicular to the path yielding greater reduction in permeability and thus improved barrier properties. Here, in order to assess barrier properties of the obtained nanocomposites, we performed gravimetric measurements of moisture vapour transmission through 0.5 mm thick films over the course of 24 hours at 40 °C (Table 6). As can be seen from Table 6, both of the high aspect ratio nanofillers (CNT and graphene) markedly reduce the moisture vapour transmission rate, indicating improved barrier properties. Despite the lower aspect ratio, the 2D geometry of graphene is likely responsible for the trend towards greater improvement as compared to CNT. Meanwhile, the difference between the neat copolymers may be explained by the higher (nearly double) crystallinity ($W_{c,h}$) of the PET-DLA 60 copolymer.

Table 6. The results of water vapour transmission resulting in final barrier properties (the higher percentage and negative value, the better barrier properties).

Material	Water mass loss [g]	Moisture vapour transmission rate [mg/(cm² hr)]	% difference [%]*
PET-DLA 40	0.097	0.170	-
PET-DLA 40 + 0.5% CNT	0.050	0.088	-48.5
PET-DLA 40 + 1% CB	0.083	0.145	-14.4
PET-DLA 40 + 1% graphene	0.047	0.082	-51.5
PET-DLA 60	0.026	0.046	-
PET-DLA 60 + 0.5% CNT	0.021	0.037	-19.2
PET-DLA 60 + 1% CB	0.030	0.053	+15.4
PET-DLA 60 + 1% graphene	0.016	0.028	-38.5

*- in reference to the unfilled PET-DLA material; negative value represents reduced moisture vapour transmission rate indicating improved barrier function

The combination of mechanical performance with superior barrier properties obtained by addition of 1% graphene to PET-DLA 40 matrix has been utilized in the pneumatic membrane (Figure 11a) of a heart assist device prototype (Figure 11b) made entirely of PET-DLA copolymers with different hardness and mechanical properties, but with sufficient transparency, in collaboration with the Professor Zbigniew Religa Foundation of Cardiac Surgery Development, Zabrze, Poland.

Figure 11. Compression thermoformed pneumatic membrane from PET-DLA 40 (a, right) and PET-DLA 40 containing 1% graphene (a, left); prototype of heart assist device made of PET-DLA copolymers (b).

4. Conclusions

In the present work, we successfully synthesized segmented PET-DLA nanocomposites containing carbon nanofillers with different aspect ratios (dimensions): carbon black (0D), multiwalled carbon nanotubes (1D), and graphene (2D). The unmodified carbon nanofillers did not affect the chemical structure of obtained nanocomposites. HR-SEM imaging indicates very good dispersion in polymeric matrices. DSC data revealed that high aspect ratio nanofiller (1D multiwalled carbon nanotubes) strongly nucleates PET-DLA 60% crystallization and, therefore, strongly affects the thermal behavior of the nanocomposite. Mechanical properties of PET-DLA 40 wt% nanocomposites showed an increase in Young's modulus compared to neat materials, but no significant improvement in tensile strength and elongation at break were observed. Probably, the nanofiller-matrix interaction is too small to effectively carry the load from the polymer matrix to nanofillers. In the case of PET-DLA 60 wt% nanocomposites, the improvement of the Young's modulus was observed only for CNT-containing nanocomposites, which is related to the high value of the aspect ratio, which influences the effective transfer of load between the nanofiller and the polymer matrix. Tensile strength of all nanocomposites was higher than for neat materials. In terms of bacterial adhesion, only for PET-DLA 40 nanocomposites do we observe an effect of nanofillers: a trend toward greater adhesion for all but CNT, likely due to increased roughness, of the same order as the size of a bacterial cell. Meanwhile, *in vitro*

cytocompatibility tests with L929 murine fibroblasts demonstrated cell viability and growth on all materials, except for nanocomposites containing CNT. Finally, the two high aspect ratio nanofillers yielded nanocomposites with improved barrier properties. Thus, we conclude that nanofillers type and content provide additional tunable parameters, in addition to hard-to-soft segment ratio, for optimizing materials for applications in biomedical devices. In fact, the family of PET-DLA copolymers has proven suitable for manufacturing a prototype heart assist device (in collaboration with the Professor Zbigniew Religa Foundation of Cardiac Surgery Development, Zabrze, Poland), fully composed of PET-DLA copolymers with different hardness/mechanical characteristics (while maintaining transparency) and with the pneumatic membrane thermoformed from graphene-containing nanocomposite. Further studies are on going, however these prototypes provide proof-of-concept of the possible translation of these materials to medical products.

Acknowledgements

This work received financial support from the National Centre for Research and Development (PBS1/A5/2/2012). The authors thank Dr. Karol Fijałkowski (Faculty of Biotechnology and Animal Husbandry, ZUT) for access to the microplate reader and Monika Michaliszyn and Aleksandra Weisłek for help with microbiological experiments.

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