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Characterisation of the effects of a titanium micro particle filler on a polyether-*block*-amide host matrix

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Abstract The body of work described in this research article outlines the use of titanium microparticles as fillers in the production of a polyether-*block*-amide (Pebax 5533) based composite for medical applications. Virgin polyether-*block*-amide was compared with titanium filled composites with loadings of 40 and 60% by weight prepared using twin screw extrusion and compression moulding. The materials were characterised using a range of mechanical, thermal, toxicological and surface analysis techniques. Fourier transform infrared spectroscopy indicated that no chemical interaction occurred between the filler particles and the host polymer matrix. Thermal analysis of the composites indicated that as the blend composition varied, so too did the melting behaviour. The inclusion of the titanium microparticles was observed to increase the flow viscosity, tensile strength, hardness and Young's modulus of the composites whilst also resulting in a rougher surface with lower surface energy.

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Introduction

After the discovery of the major commodity and engineering plastics materials in the early to mid part of the twentieth century, the cost of bringing a new polymer material to market began to rise dramatically. As a result, both the polymer industry and academia began to focus on developing polymer blends and composites with novel and valuable properties, in order to enlarge the spectrum of available materials. Various polymeric materials are known for specific or unique characteristics and melt blending of polymers with inorganic particulate fillers during extrusion is a useful method of combining the desired properties of different materials. Processing of polymer composites requires that the compounding equipment quickly melts the polymer(s), and then rapidly and efficiently affect distributive and dispersive mixing of the melt components. Co-rotating twin screw extruders are capable of easily satisfying these elementary steps in blending operations offering a high throughput and a low material loss whilst preparing extrudates that possess excellent homogeneity.

It has been extensively shown that incorporation of fillers significantly changes the various properties of thermoplastic materials [1, 2]. Fillers find use in polymers for a variety of reasons such as cost reduction, improved processing, density control, control of thermal expansion, electrical properties, magnetic properties, flame retardancy and improved mechanical properties. The effect of fillers on the properties of the composites is related to loading levels, interaction with the matrix, degree of mixing and filler size and shape [3, 4]. The use of titanium (Ti) and titanium alloys for the replacement of structural components of the human body has been widely documented [5]. Although titanium has been used since the 1930s, it has only garnered widespread interest in recent years. There

exists a large market for titanium products primarily due to the materials exceptional strength-to-weight ratio, high resistance to elevated temperatures and corrosion resistance in the biological environment combined with a high degree of biocompatibility [6]. The behaviour of composite materials is, in part, controlled by the nature of the interface, which in polymer matrix composites is usually required to be strong [7]. Both thermoset and thermoplastic polymers have been bonded to titanium with varying degrees of success, with thermoplastic materials generally proving more difficult to bond [8]. Pebax polyether-*block*-amides are commercial plasticizer-free thermoplastic elastomers. The 33 series are composed of segments of polyamide 12 (PA12) and polytetramethylene glycol (PTMG) covalently linked via ester groups. The SA subgrade used herein is specifically designed for medical and food uses. Researchers have previously investigated the reinforcement of Pebax thermoplastic elastomer matrices with qramid fibres with results suggesting a good interface [7].

The work described herein investigates the production of composites by melt blending a polyether-*block*-amide (Pebax 5533) with titanium sponge microparticles in varying ratios. The work was carried out in order to modify the properties of the polyether-*block*-amide using an inorganic particulate filler with a high strength-to-weight ratio.

Experimental

Materials

Pebax (polyether-*block*-amide) 5533 polymer was obtained from Arkema Ltd (France). Titanium sponge powder (sized from 75 to 185 μm) was procured from Active metals Ltd (UK).

All chemicals and cell culture reagents were obtained from the Sigma Chemical Co. (Ireland) unless otherwise stated. Tissue culture plastics were supplied by Sarstedt (Ireland) and cell lines were obtained from the European Collection of Animal Cell Cultures (UK). Cytotoxicity detection kit^{PLUS} for lactate dehydrogenase (LDH) testing was procured from Roche Diagnostics Ltd (UK). All materials were used as received with no further purification.

Sample preparation

Hot melt extrusion

The compounding of materials for this work was carried out on a Micro 27 labscale twinscrew extruder (Leistritz Ltd) with a 27 mm screw diameter and a 38/1 length-to-diameter ratio. The required compounding temperature profile was established on the labscale extruder by means of nine temperature controllers placed along the length of the barrel. A tenth temperature controller was used to regulate the temperature at the die. The extrusion conditions used are outlined in Table 1. Feeding of polymer and additive was accomplished via independent K-Tron gravimetric feeders, with the titanium microparticles being added via a downstream feed screw. The batches of polymer extrudates prepared are outlined in Table 2.

Table 1 Extrusion conditions used to examine the effect of variations in processing conditions on PCL/PEO blends

Screw speed (RPM)	Temperature (°C)			
	Feed	Metering 1	Metering 2–8	Die
150	175	185	195	200

Table 2 Batch compositions used to determine the effect of processing conditions on PCL/PEO blends

Batch name	Pebax (% by weight)	Titanium sponge 75–185 μm (% by weight)
Virgin Pebax	100	0
40% composite	60	40
360% composite	40	60

Compression moulding

A Daniels compression press at a pressure of 1,000 psi, fitted with a 5 × 5 cm square mould with a thickness of 0.5 cm was used for the fabrication of plaques of each material under investigation. Following processing trials, a sample weight of 15 g of compounded extrudate, a moulding cycle time of 1 min and a temperature of 200 °C were identified as the most suitable compression moulding settings, as these parameters produced plaques with optimum properties. Teflon sheets were used to avoid polymer adhesion to the mould. Following the moulding operation, the sample was removed from the mould and permitted to cool at room temperature.

Thermal analysis

Differential scanning calorimetry

The differential scanning calorimetry (DSC) method was among the techniques used for examination of the extruded pellets. The analysis was performed using a 2010 DSC (TA Instruments). Samples of between 9.0 and 9.8 mg were weighed out using a Sartorius scales having a resolution of 0.00001 g. Samples were then placed in non-perforated

aluminium pans which were crimped before testing, with an empty crimped aluminium pan being used as the reference cell. Calorimetry scans were carried out from −70 to 220 °C for each of the extruded pellets. DSC measurements were carried out at a scanning rate of 10 °C/min. Volatiles were removed from the purging head with nitrogen at a rate of 30 mL/min. Calibration of the instrument was carried out using indium as standard. After each scan was completed the melting points were analysed to determine the heat of fusion and T_m of each batch.

Thermogravimetric analysis

A Perkin Elmer TGA 7 was used for thermogravimetric analysis (TGA) of the materials under investigation. Tests were carried out in triplicate using a sample size of 20 mg. Under a nitrogen atmosphere, the sample was ramped from 30 to 600 °C at a rate of 160 °C/min. The sample was then held isothermally at 600 °C for 3 min.

Rheological analysis

Capillary extrusion rheometery was carried out using a twin bore Rosand RH7 Rheometer. Shear rates of 100–1,000 1/s were used at a test temperature of 200 °C. All tests were carried out in triplicate and conformed with ASTM D 3835-02.

Surface properties analysis

Optical microscopy

Optical microscopy of samples was carried out on a Wild M3Z stereo-zoom microscope using an optical ring illuminator system.

Digital goniometry

Contact angle measurements were obtained in triplicate using the OCA20 digital goniometer using three solvents; water, ethylene glycol and diiodomethane. Liquid droplets were deposited on the polymer surfaces and their contact angles were measured and recorded. The measurements were taken after 10 s so as to let the drop settle on the surface. The measurements were carried out in triplicate and the values obtained were used to calculate surface energy.

White light profilometry

A New View 100 white light profilometer was used to study the surface of the virgin polymer and composite materials using a maximum vertical step height of 100 μm. Objectives were set to 2.5× and 40× (Zoom 0.5× to 2.0×) with a minimum vertical resolution of 0.1 nm. Results obtained from the profilometry analysis were interpreted using Veeco software for R_a (arithmetic mean of the absolute values of the surface departures from the mean plane), R_q (root-mean-squared roughness calculated over the entire measured sample), R_z (average of the 10 greatest peak-to-valley separations on the sample) and R_t (vertical distance between the highest and lowest points as calculated over the entire sample) values.

Mechanical testing

The tensile testing procedure used was based on the ASTM international standard D882-02 test method for tensile properties of thin plastic sheeting. The samples were cut into tensile samples using a dumbbell cutter, which gave a sample test length of 20 mm and a width of 4 mm. Five samples were tested at room temperature from each batch using a Lloyd Lrx Tensile tester, with Nexygen control and analysis software. A crosshead speed of 20 mm/min was used during all tensile tests. A Shore D durometer was used to test hardness of the samples prior to tensile testing. The standard used for this measurement was ISO 868, which is equivalent to ASTM standard D2240. The final value of the hardness was recorded after the indenter had been applied to the test specimen for 15 s.

Fourier transform infrared spectroscopy

Attenuated total reflectance Fourier transform infrared spectroscopy was carried out on compression moulded plaques of each material under investigation using a Nicolet Avator 360 FTIR, with a 32 scan per sample cycle.

Toxicological analysis

Maintenance of cell lines

Human hepatoma HepG2 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) heat-inactivated foetal bovine serum (FBS), 2 mM L-glutamine and 1% non-essential amino acids. The cells were grown at 37 °C/5% CO_2 in a humidified incubator. The cells were screened for mycoplasma contamination by the Hoechst staining method [9] and were cultured with 0.5% Penicillin–Streptomycin (5,000 U/ml). Exponentially growing cells were used throughout.

Treatment of cells with virgin Pebax and Pebax/Ti composites

Prior to cell treatment, discs of each material under investigation, with diameters of 3 cm, were cut out of the

compression moulded samples, weighed and subsequently autoclaved. Within each independent experiment, the polymer/composite specimen discs had similar weights and the average weight of all the specimen discs tested was 2.434 ± 0.207 g.

For all cytotoxicological procedures HepG2 cells were seeded at a density of 3×10^4 cells/cm^2 in 6-well culture dishes and were allowed to attach for 24 h before exposure to the materials. Media was then removed and replaced with DMEM supplemented with 2.5% FBS. A specimen disc was gently placed on top of the cell monolayer, one sample per well, and samples were incubated for 24 h at 37 °C/5% CO_2.

LDH release assay

LDH release was determined as an index of cell viability. LDH is a cytosolic enzyme that is released by cells when they undergo significant membrane damage [10]. The HepG2 cells were incubated in 6-well dishes with virgin Pebax or Pebax-based Ti composites for 24 h. Following treatment, membrane integrity was evaluated by measuring LDH released from cells into the culture medium using a commercial LDH release kit. Experiments were performed as per manufacturer's instructions.

Cell morphology

After a 24 h incubation period, each of the test specimens were carefully lifted off the cells and removed from the wells. Cells were immediately subjected to inspection under an Olympus CK2 inverted light microscope.

Fluorescence microscopy

Following exposure to virgin Pebax or Pebax-based Ti composites, standard cytotoxicological evaluations of acute toxicity to the cells were performed using a fluorescent live/dead stain. Briefly, once the test specimen was removed the cells were washed in 1 mL PBS and a solution of fluorescein diacetate (FDA) and ethidium bromide (EtBr) was added to each well. The cells were then incubated at 37 °C for 2–5 min and the samples were analysed at 10× magnifications on a Leica fluorescence microscope with appropriate filter cubes (H3 cube for FDA, N2.1 cube for EtBr). Under these conditions green fluorescence indicates living cells and red fluorescence indicates dead cells.

Statistics

All data points are mean values (±SE) of five independent experiments. Where appropriate, data were analysed by one way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison test. The software employed for statistical analysis was GraphPad Prism, Version 4.

Results and discussion

Extrusion observations and rheological properties

Prior to the compression moulding of the material into test plaques, twin screw extrusion was used to compound the Ti fillers with the Pebax matrix. Extruder torque is a measure of the resistance that the motor experiences as a consequence of the melt viscosity inside the barrel. Verreck et al. [11] discussed the use of the extruder torque values as a method of measuring relative viscosities of polymer melts at set values of processing temperature, feed rate and screw speed. In previous work, our research group [12–15] used a similar method as an indicator for melt viscosity during processing, in the aforementioned contributions, the extruder torque reading was supplemented with measurement of die head pressure. Die head pressure is a measurement obtained by a pressure transducer which records the pressure exerted by the polymer melt at the shaping die. Higher viscosity melts will exert more pressure than melts with lower viscosities. Figure 1 graphically illustrates the torque and die head pressure readings recorded during processing of the virgin Pebax material and composite blends. The information recorded indicates that the melt viscosity of the material in the extruder barrel increases with increasing Ti loading.

The compounding and inclusion of particulate fillers in polymeric materials is a complex process strongly dependent on particle–particle and particle–polymer interactions. Rheological analysis was carried out on each of the extrudates to quantify the effect of titanium microparticles on composite viscosity. Capillary extrusion rheometry is used principally to determine the high rate shear viscosity of molten polymers. Typically, the pressure drops across two cylindrical dies of known dimensions as various volume flow rates are measured (two different die lengths is necessary to correct for entrance pressure drop effects to obtain accurate shear stress values [16]), from which shear viscosity values are calculated following ISO 11443. As shear viscosity is a material property it should be independent of the test geometry used. As expected, results obtained (Fig. 2) are in good agreement with the observations made during the processing of the materials, further indicating that increasing titanium filler content leads to a corresponding increase in material viscosity. In addition, DSC analysis shows (Fig. 3), the melting point of the composites shifts to a slightly higher temperature on addition of the Ti microparticles.

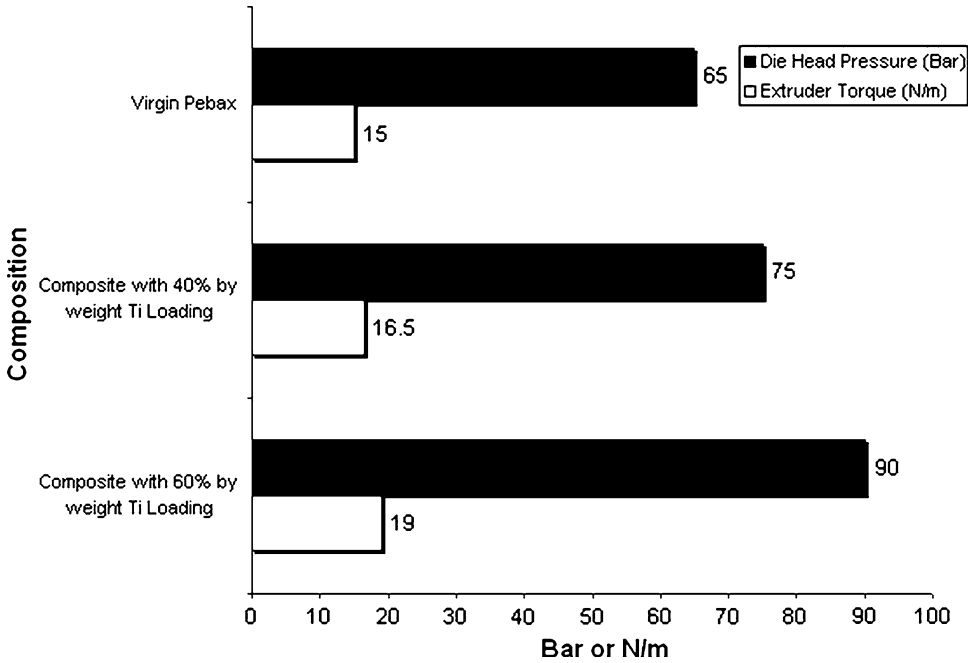


Fig. 1 Peak extruder torque and die head pressure values observed during twin screw compounding of each composition

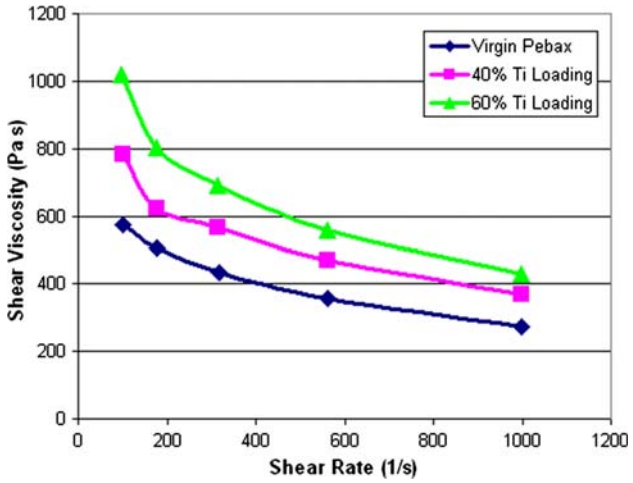


Fig. 2 Capillary rheometry results for each material composition

In order to further confirm that the increases in viscosity were directly related to filler incorporation, a TGA was carried out on each material that had been used for the rheological investigation. Figure 4 depicts an overlay of each of the material compositions and is typical of the TGA results obtained. These results confirm the loading levels of Ti filler in the composites as well as confirming that no other material is present. Thus, filler concentration is solely responsible for the recorded increases in viscosity.

Surface properties

Following the preparation of the test plaques from the virgin Pebax and each of the composite materials, the surface properties of the materials were analysed using a range of techniques. Figure 5 shows the surface of the materials under investigation at a magnification of 10 times. This initial observation revealed pitting on the surface of the composite containing 60% by weight titanium microparticles. White light profilometry was used to quantify this observation by acquiring surface roughness data. The results obtained are presented in Table 3 with a profilometry scan typical of those obtained shown in Fig. 6. The data obtained via the profilometry analysis clearly shows the deterioration of surface smoothness as the titanium material is added with all of the key surface roughness measurements indicating substantial increases as the filler concentration increases.

Surface energies were evaluated using the surface–tension–component theory [17–19]. Thus, the surface energy of a solid, γ_S, incorporates three contributions, Eq. 1:

$$\gamma_S = \gamma_S^{LW} + \left(\gamma_S^+ \gamma_S^-\right)^{1/2}, \tag{1}$$

where γ^{LW} is the Liftshitz/van der Waals component, γ^+ is the Lewis-acid component and γ^- is the Lewis-base component.

γ_S^{LW}, γ_S^+ and γ_S^- are calculated (Eq. 2) by performing liquid–solid contact angle measurements (θ).

For a drop of a liquid at equilibrium with a solid surface:

$$\gamma_L(1+\cos\theta) = 2\left[\left(\gamma_S^{LW}\gamma_L^{LW}\right)^{1/2} + \left(\gamma_S^+\gamma_L^-\right)^{1/2} + \left(\gamma_S^-\gamma_L^+\right)^{1/2}\right], \tag{2}$$

where γ_L is the surface tension (surface energy) of the liquid, with S is the solid and L is the liquid.

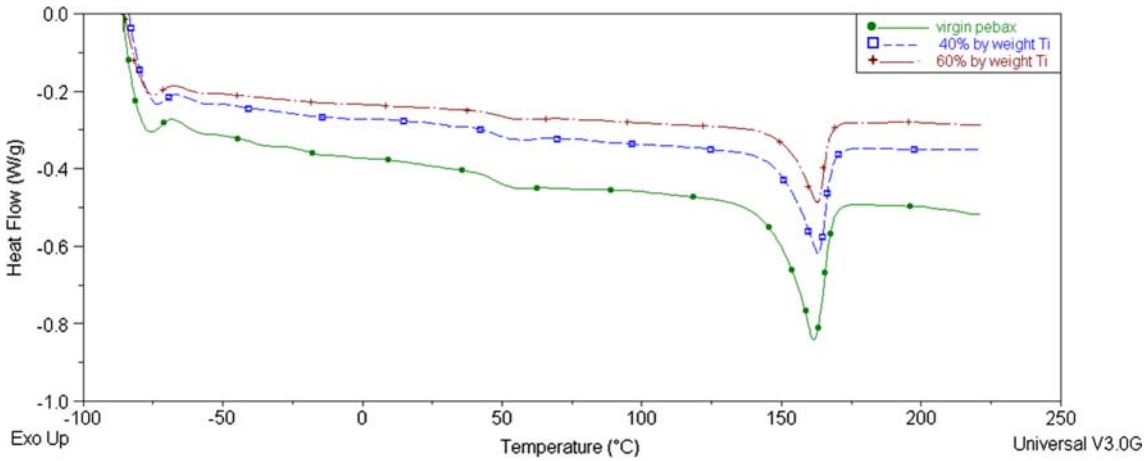
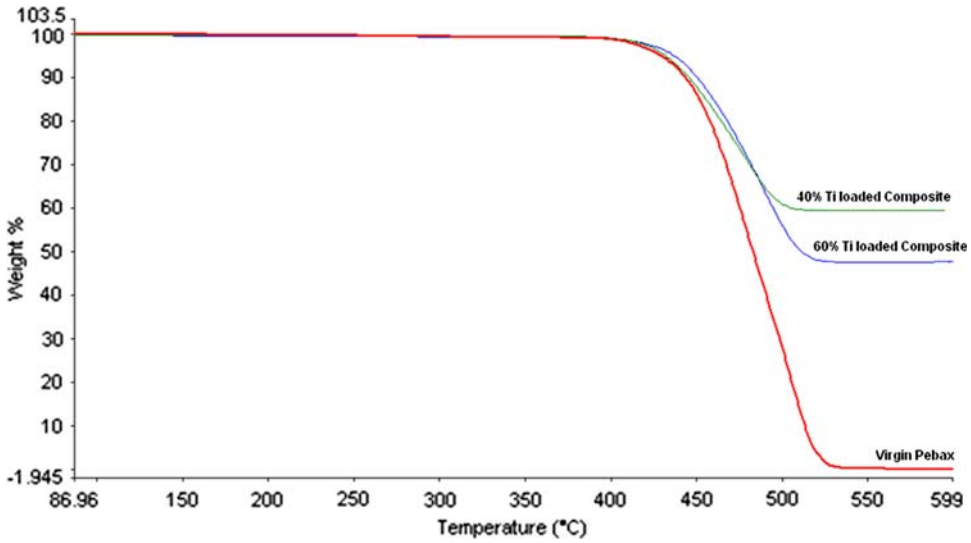


Fig. 3 Overlay of DSC thermograms for each material

Fig. 4 Overlay of TGA scans typical of those obtained for the samples under investigation



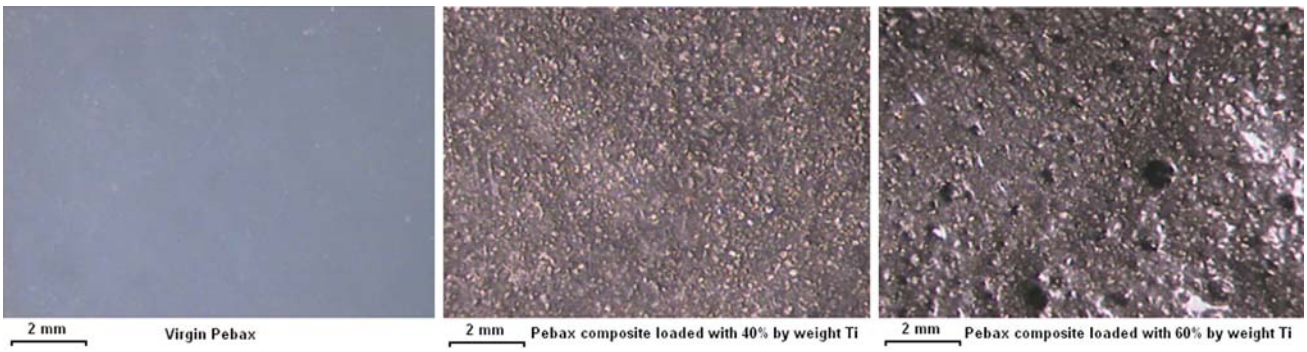


Fig. 5 Optical microscopy images of the surface of each of the materials under investigation

By measuring contact angles with respect to three liquids (water, ethylene glycol and diiodomethane) that are widely reported in the literature in terms of γ_L^{LW}, γ_L^+ and γ_L^- [17–20], three equations with three unknowns are generated and may be solved. Figure 7 illustrates the contact angles observed for water on each of the composite materials and on virgin Pebax. A graph representing the contact angles and the associated surface energies for the materials under investigation herein is presented in Fig. 8.

Table 3 White light profilometry data for each material under investigation

	Area 1	Area 2	Area 3	Image 4	Average
Virgin Pebax sample					
R_a	3.3	2.4	2.6	3.0	2.8
R_q	4.2	3.0	3.3	3.8	3.6
R_z	29.6	22.3	22.7	25.0	24.9
R_t	31.5	24.7	24.1	26.2	26.6
Composite with 40% by weight Ti loading					
R_a	3.8	4.2	4.0	3.9	4.0
R_q	4.9	5.0	5.7	4.9	5.1
R_z	32.7	45.6	32.4	36.8	36.9
R_t	41.0	55.6	45.5	38.7	45.2
Composite with 60% by weight Ti loading					
R_a	7.4	9.4	9.0	9.8	8.9
R_q	9.9	11.9	12.4	11.0	11.3
R_z	64.8	70.0	69.9	72.8	69.4
R_t	65.3	74.8	71.4	75.7	71.8

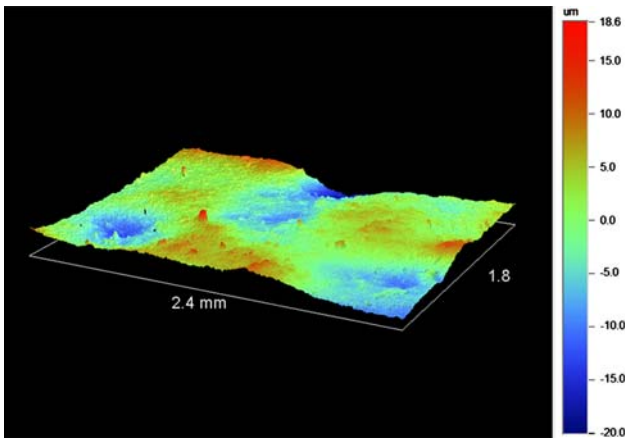


Fig. 6 3D surface representation of Pebax-based composite containing 40% by weight titanium microparticles obtained by white light profilometry

From the results obtained it is clear that the energy of the surface increases with increased loading of titanium particle filler material. However, from observations of the dimensions of solvent droplets, it appeared that penetration of the surface by the liquid (particularly in the case of water) was responsible for the observed reduction in contact angles; hydrogen bonding with oxygen atoms of the ether group coupled with poor adhesion between the Ti particles and Pebax polymer (resulting in void areas and the aforementioned increased surface roughness) may be responsible for the observed phenomenon [19, 21].

Mechanical testing

Tensile and hardness tests were carried out on dumbbell shaped samples cut from the compression moulded plaques of each material under investigation. Selected mechanical properties for the materials are presented in graphical form in Fig. 9. The results obtained from hardness testing are indicative of relative resistance to indentation. However, the Shore Durometer hardness test does not serve well as a predictor of other properties such as strength or resistance to abrasion, or wear, and therefore is used in conjunction with tensile testing in this investigation. Shore hardness is often used as a proxy for flexibility and in combination with the Young's modulus data obtained from tensile testing is very useful. Both the Shore hardness and the Young's modulus of the composite are improved by adding the titanium micro-particles to the polymer matrix as the hard filler material has a much higher stiffness value than the polymer matrix.

For particulate filled composites, strength relies on the effectiveness of stress transfer between the host polymer matrix and the filler material. Particle/matrix interfacial strength and particle loading significantly affect the composite strength. Increased loading of titanium particles results in an increase in the strength of the composite. However, observation of the test specimens after failure indicated poor adhesion between the filler particles and the polymer matrix. Strong interfacial bonding between particles and the polymer matrix is critical for effective stress transfer. In work by Coffey et al. [7] stress transfer between aramid fibres and a Pebax resin was limited by yielding of the host Pebax matrix. However, at higher applied stresses, full debonding of the fibre or fragment ends resulted, and stress transfer was by friction. Thus, to reap the full reinforcing benefit of the titanium incorporation, it would be necessary to treat the surface of the particles or use a coupling agent to increase the affinity of the filler material for the host matrix. FTIR spectra obtained via attenuated total reflectance on the surface of each test plaque showed negligible difference upon incorporation of Ti micro-particles. The spectroscopic analysis indicates little interaction between the Ti particles and the polyether-*block*-amide host matrix.

Toxicological examination

Polymer/composite-induced cytotoxicity was assessed by the LDH release assay. This colorimetric assay measures LDH, an enzyme released by cells as a result of significant membrane damage or cytolysis. Hence, the amount of LDH released is proportional to the number of cells damaged or lysed. After 24 h, treatment with virgin Pebax or Pebax-based Ti composite resulted in a significant ($p < 0.01$)

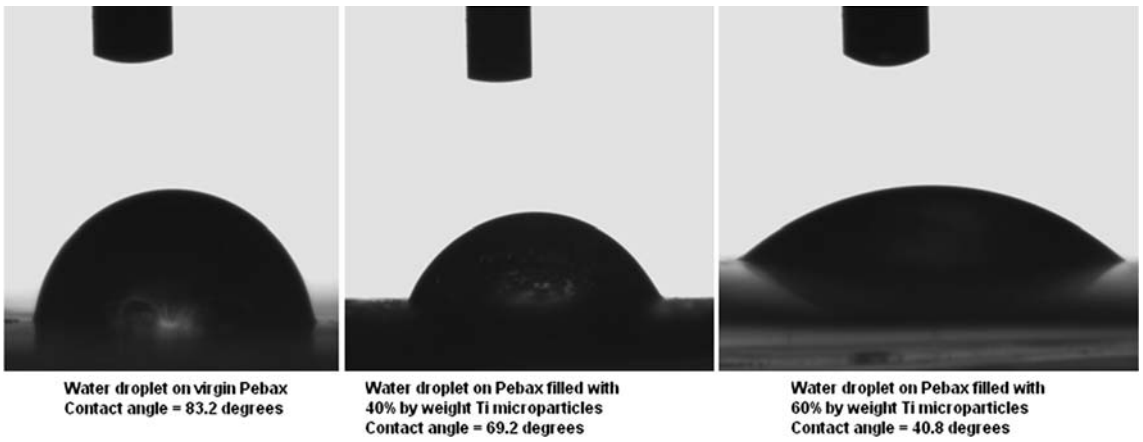


Fig. 7 Contact angle images of water droplets on each of the substrates under examination in this work

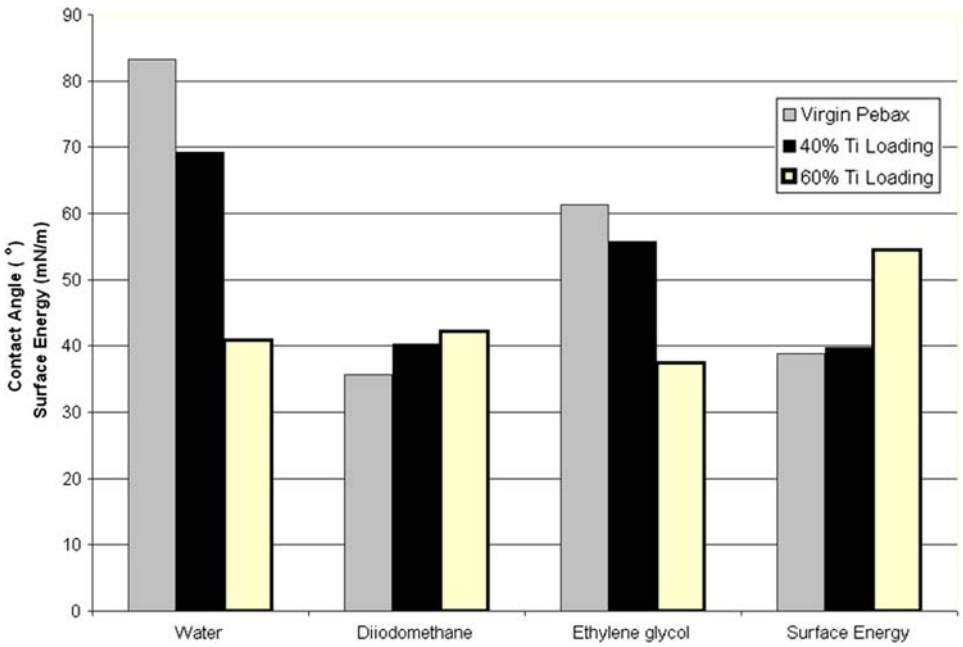


Fig. 8 Contact angles for water, diiodomethane and ethylene glycol on Pebax/Ti composites and surface energies for these materials

increase in LDH release relative to the untreated control (Fig. 10). No differences in LDH release were observed between the virgin Pebax or the Pebax composite loaded with 40% titanium microparticles whilst the amount of LDH released from the cells treated with the Pebax composite loaded with 60% titanium microparticles appeared to be greater.

Changes in HepG2 cell morphology were monitored under an inverted microscope. As shown in Fig. 11, the untreated control cells displayed a normal, healthy shape, whilst direct contact between the cells and the test specimens greatly inhibited the cells' abilities to proliferate as normal. In addition, these effects were predominant in samples treated with the titanium loaded composites, where the majority of HepG2 cells were severely distorted.

Cell viability post-culture with virgin Pebax or Pebax-based Ti composite was assessed using a FDA/EtBr fluorescent dye. Viable cells are stained by membrane-permeant non-fluorescent FDA which is converted by intracellular esterases of living cells to the brightly fluorescent fluorescein, yielding a uniform green fluorescence inside the cells. EtBr is impermeant and is extruded by the intact membrane of live cells; in compromised membranes of dead cells, however, EtBr enters the nucleus and undergoes an increase in red fluorescence. Figure 12 demonstrates a marked increase in cell death as evident by the raise in the number of EtBr-stained (red) cells. Moreover, the quantity of dead cells observed increased substantially following the inclusion of titanium into the polymer. These findings are in line with the previous

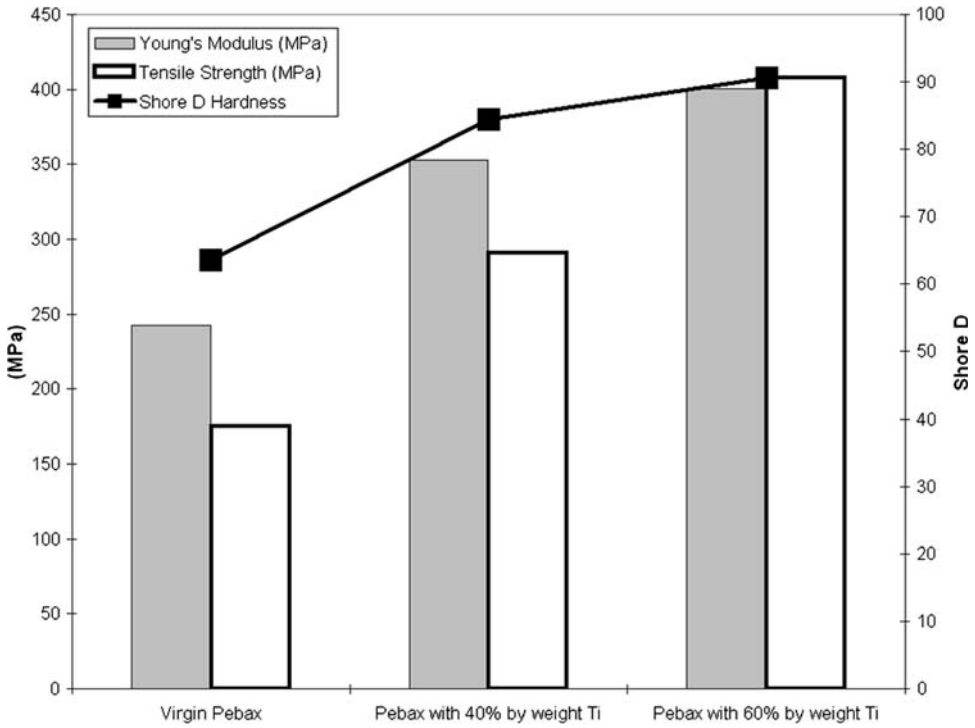


Fig. 9 Selected mechanical properties of virgin and Pebax/Ti composites

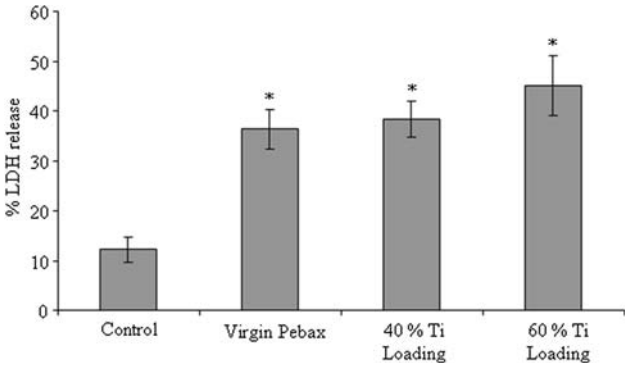


Fig. 10 LDH release from HepG2 cells following 24 h exposure to virgin Pebax, or composites loaded with Ti microparticles. Data represent the mean (±SE) of five independent experiments. * $p < 0.01$, relative to control, ANOVA followed by Dunnett's Multiple Comparison test

results where the composite containing 60% by weight titanium loading was the most cytotoxic composition of all the materials tested.

It is widely reported that titanium metal is spontaneously covered with a surface oxide of up to 10 nm in thickness [22] and it is this thin oxide film that is attributed to the favourable tissue responses to titanium biomedical materials in comparison to many other metals [6]. However, some cytotoxic effects of titanium oxide have been reported and Watanabe et al. [23] found that these effects were dependent on the shape of the material. In addition, Kumazawa et al. [24] showed that toxicity increased with decreasing titanium particle size. Therefore, further work is needed to ascertain if the particle size or the effect of compounding on the surface layer of the Ti materials was responsible for the cytotoxic effects observed in this study.

Conclusion

The work presented describes both the physical effect of the inclusion of Ti microparticles in a polyether-*block*-amide polymer and the effect of the filler materials on composite-induced toxicity. The percentage inclusion of Ti microparticles in the Pebax host matrix was altered in order to investigate the effect of the filler materials on the properties and processability of the matrix. Torque and die head pressure measurements taken during processing, indicate that inclusion of Ti filler increases the viscosity of the material. Thermal analysis of the composites indicated that as the blend composition varied, so too did the melting behaviour. Increasing Ti microparticle loading resulted in composites with increased strength and hardness, with rougher surfaces and lower surface energies.

The compounding process outlined herein may provide a potential alternative route to the manufacture of wear resistant biomaterials. By incorporating bioactive and/or biodegradable polymers as the host matrix and improving titanium adhesion, the fabrication of biomaterials with enhanced osseogenetic properties without the use of expensive coating technologies may be possible. However,

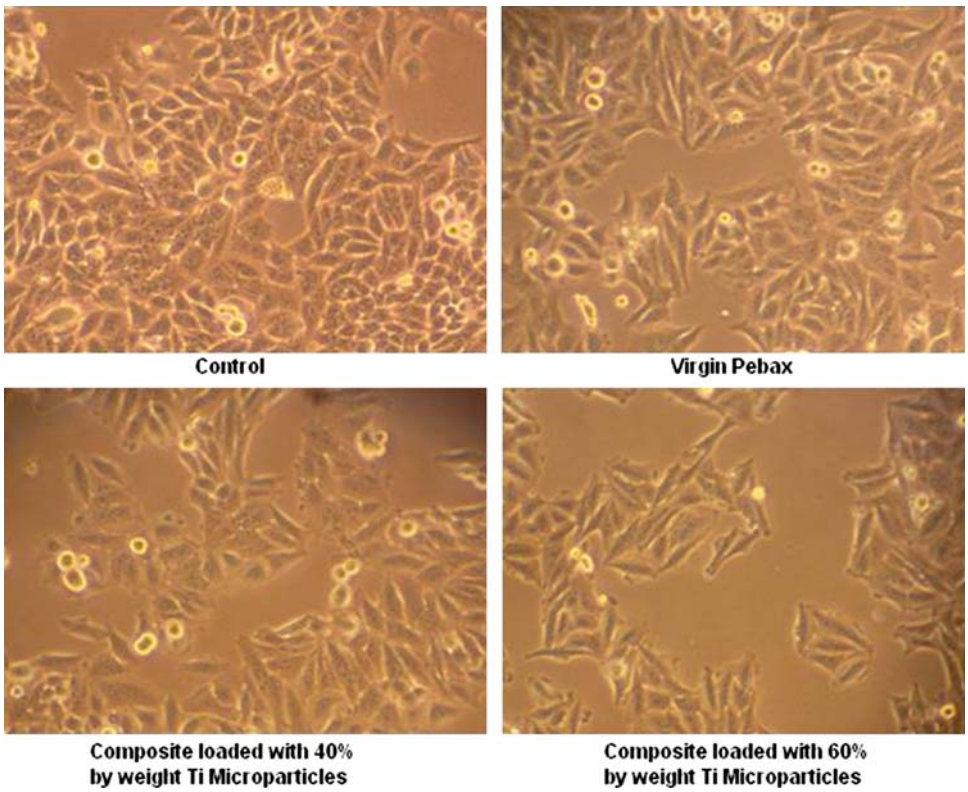


Fig. 11 Morphology of HepG2 cells after 24 h exposure to virgin Pebax and Ti loaded composites (magnification, ×10)

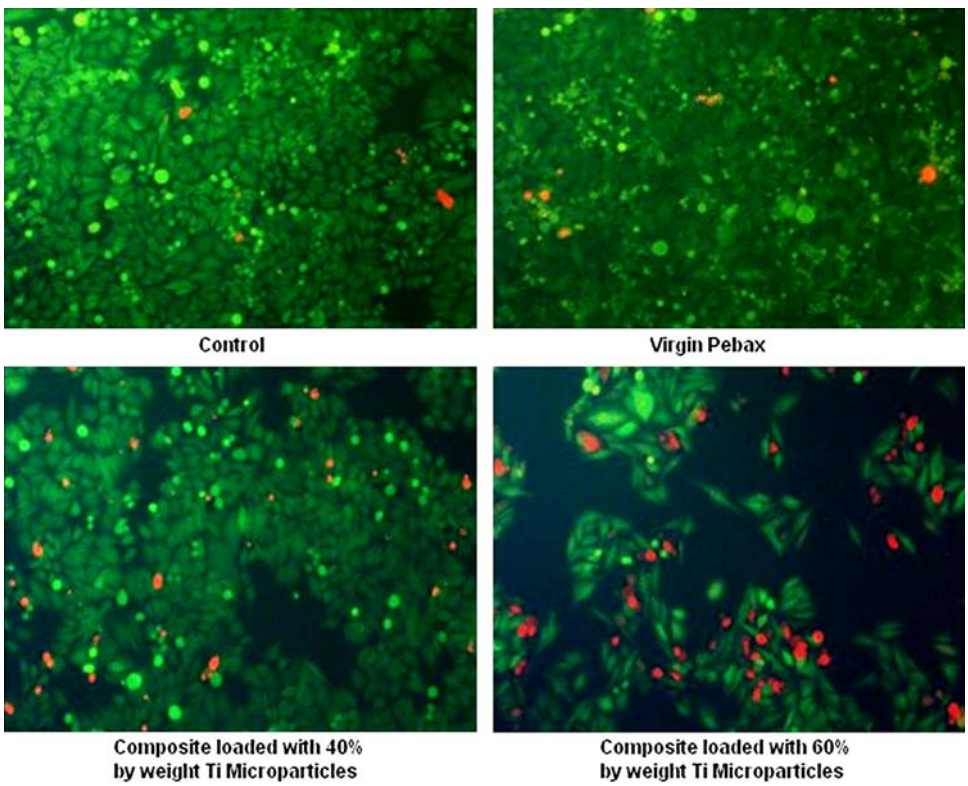


Fig. 12 Fluorescent images of FDA/EtBr-stained HepG2 cells with live cells fluorescing green and dead cells fluorescing red

the observed toxicological effects of the Ti microparticles inclusion indicate that he material described herein would require further refinement before being deployed in a biomedical environment.

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