Production and evaluation of biodegradable composites based on PHB–PHV copolymer

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Abstract

In recent years, emphasis in biomaterials engineering has moved from materials that remain stable in the biological environment to materials that can degrade in the human body. Biodegradable materials are designed to degrade gradually and be replaced eventually by newly formed tissue in the body. In this investigation, two particulate bioactive ceramics, i.e., hydroxyapatite (HA) and tricalcium phosphate (TCP), were incorporated into polyhydroxybutyrate–polyhydroxyvalerate (PHB–PHV), which is a biodegradable copolymer, to produce new biomaterials for potential medical applications. All raw materials were commercially available and they were characterised prior to composite production. HA/PHB–PHV and TCP/PHB–PHV composites containing up to 30 vol% of the bioceramics were produced through an established procedure. Compounded and compression moulded materials were evaluated using various techniques including thermogravimetric analysis, scanning electron microscopy, differential scanning calorimetry and dynamic mechanical analysis. The results showed that intended compositions of composites had been achieved and bioceramic particles were well distributed in the polymer. The degradation temperature of PHB–PHV was significantly reduced by the incorporation of bioceramics, while the melting temperature was slightly affected by the addition of bioceramics. The crystallinity of PHB–PHV was also varied with the presence of HA or TCP particles. The storage modulus and loss modulus of the composites increased with the increase in HA or TCP content. Composites containing the highest percentage of bioceramics exhibited the highest stiffness. Preliminary in vitro study indicated enhanced ability of the composites to induce the formation of bone-like apatite on their surfaces.

Keywords: Hydroxyapatite; Tricalcium phosphate; Polyhydroxybutyrate–polyhydroxyvalerate; Structure; Property

1. Introduction

Since the early 1980s, a variety of bioactive composites have been developed for hard tissue replacement [1–3]. Due to the matrix polymers used, most of these composites are non-biodegradable. A shift in emphasis in biomaterials development in recent years has moved attention from materials that will remain completely stable in the biological environment to materials that will, in some way, alter their properties or degrade in response to the cellular environment. Biodegradable materials have the advantage of allowing the new tissue, as it grows naturally, to take over their load-bearing or other functions without having any of the potential chronic problems associated with the presence of bio-stable implants.

Polyhydroxybutyrate (PHB) is a naturally occurring β-hydroxyacid (a linear polyester). Its ability to degrade and resorb in the human body environment makes it a suitable candidate as the matrix for bioactive and biodegradable composite implants that will guide tissue growth and be replaced eventually by newly formed tissue. But its usefulness is limited by its brittleness [4]. However, the addition of polyhydroxyvalerate (PHV) to the PHB polymer chains can improve the ductility and processability of the polymer [5]. Polyhydroxybutyrate-co-hydroxyvalerate (PHB–PHV) with varying molar ratios of HV has been the material of extensive, ongoing research for biomedical applications [6,7]. Being thermoplastic polyesters, PHB and PHB–PHV copolymers can be processed by conventional techniques such as extrusion, injection or compression moulding [4].

Hydroxyapatite (HA) is one of the bioactive calcium phosphates, which exhibits excellent biocompatibility and bioactivity. It has been used for hard tissue replacement and augmentation due to its osteoconductive

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property [8]. Tricalcium phosphate (TCP) is another bioactive calcium phosphate, which has an important role as a resorbable bioceramic. Due in part to its crystalline structure, the dissolution rate of TCP has been shown to be higher than that of HA [9]. In this investigation, particulate HA and TCP were incorporated into a PHB–PHV copolymer separately to produce new bioactive and biodegradable composites for potential medical applications.

2. Materials and methods

2.1. Raw materials and manufacture of composites

Commercially available HA and TCP bioceramics (Taihei Chemicals, Japan) and PHB–PHV copolymer (ICI, UK) were used for making novel composites, with as-received HA and TCP being in the form of fine powders and PHB–PHV being small size granules. The PHB–PHV copolymer contained 12% mol of β-hydroxyvalerate units. All raw materials were used in their as-received state without further treatment and they were characterised using various techniques including particle size analysis, X-ray diffraction (XRD) and scanning electron microscopy (SEM) prior to composite production.

Two series of composites, i.e. HA/PHB–PHV and TCP/PHB–PHV containing up to 30% by volume of HA and TCP, respectively, were produced through an established procedure as outlined in Fig. 1. A HAKKE Rheomix600 internal mixer was used for compounding. The temperature of the mixer chamber was set at 140°C and a pair of cam rotors running at a speed of 25 rpm was utilised. The duration of compounding was controlled to be around 20 min, with high rotor speed being used for materials containing high percentages of bioceramics. The compounded materials were processed into composite powders using a FRITSCH Pulverisette 19 cutting mill. The composite powders were subsequently dried overnight in an oven at 70°C and finally compression moulded into plates (110mm × 110mm × 2mm) using a window type mould and a hot, hydraulic press. Specimens for structural and mechanical evaluations were made from these thin plates.

2.2. Scanning electron microscopy (SEM)

The dispersion and distribution of HA and TCP particles in the PHB–PHV polymer matrix was investigated after compounding and compression moulding. The specimen preparation procedure included sectioning, mounting, grinding, polishing, ultrasonic bath cleaning and drying. Specimens were polished progressively using diamond pastes of 6, 3 and 1μm particle sizes. The polished surfaces were examined under a JEOL JSM5600LV SEM at an accelerating voltage of 20 kV after the specimens had been lightly gold-coated.

2.3. Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) of PHB–PHV and its composites was conducted on a Perkin–Elmer TGA 7 machine at a heating rate of 10°C/min and over a temperature range of 30–700°C. A plot of weight percentage against temperature was obtained for each specimen, showing the weight loss of the PHB–PHV matrix in the composite as temperature was increased, which could be used for calculating the real HA or TCP weight percentage in the composite.

2.4. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) of PHB–PHV and its composites was performed on a Perkin–Elmer DSC 7 thermal analysis system employing three thermal cycles between 30°C and 180°C. A heating/cooling rate of 20°C/min and a sample weight of around 9 mg were maintained for all tests.

2.5. Microhardness testing

The hardness of composites was assessed using a Schimadzu microhardness tester with a pyramid-shaped diamond indenter. For each composition of the composites, a square specimen (10 mm × 10 mm) was cut from the compression moulded plate. The specimens were then embedded in an acrylic resin and ground on various grades of silicon carbide paper. Sufficient flow of tap water flushed away debris during the grinding process. The final preparation of each specimen involved light polishing using diamond pastes with particle sizes from 6 to 3μm. The specimens were then dried by compressed air jets. An indentation load of 300 g was applied to the polished surface for 20s and Vickers’ hardness number (VHN) was subsequently obtained. Each specimen was indented at least fifteen times and each indent was at least two indent diameters away from the other. All indents were made avoiding edges of the specimen.

2.6. Dynamic mechanical analysis (DMA)

Dynamic mechanical properties of the composites were evaluated using a Perkin–Elmer DMA 7 system. A 15mm knife-edge, three-point platform with a 5mm
probe tip was used and DMA tests were performed in a temperature range from 30°C to 130°C at a heating rate of 4°C/min. Nitrogen was used as the purge gas. Assuming the physiological frequency was 1Hz, all measurements were conducted at this frequency. Dimensions of DMA specimens were 22 mm × 5 mm × 1 mm. Specimen surfaces were polished to remove any defects prior to DMA testing.

2.7. In vitro evaluation of composites

An acellular simulated body fluid (SBF) was used for in vitro experiments as it had ion concentrations (in millimoles: Na⁺ 142.0, K⁺ 5.0, Mg²⁺ 1.5, Ca²⁺ 2.5, Cl⁻ 147.8, HCO₃⁻ 4.2, HPO₄²⁻ 1.0, SO₄²⁻ 0.5) that were nearly the same as those of human blood plasma [10]. SBF was prepared by dissolving reagent-grade chemicals of NaCl, NaHCO₃, KCl, K₂HPO₄·3H₂O, MgCl₂·6H₂O, CaCl₂·2H₂O, Na₂SO₄, and (CH₂OH)₃CNH₂ into distilled and de-ionised water and buffered to pH 7.4 at 37°C with HCl.

Polymer and composite specimens were immersed in SBF at 37°C for various periods of time with the specimen surface area to solution volume ratio being 10 mm²/ml. The formation of a biological apatite layer on the composite surface in vitro, which could be detected using SEM, thin-film X-ray diffraction analysis (TF-XRD) and Fourier transform infrared spectroscopy (FTIR), would be indicative of the bioactivity of composites in vivo.

3. Results

The median particle sizes of as-received HA and TCP powders were 24.5 and 11.2 μm, respectively, but the size distributions peaked at around 30 and 10 μm (Fig. 2). Both ceramic powders, especially the HA powder, had significant amounts of small size particles. XRD analysis revealed that the powders were phase-pure HA and α-TCP, respectively (Fig. 3). SEM examination showed that HA particles were spherical whereas TCP particles were irregular in shape and had angular corners (Fig. 4).

After the compounding process, bioceramic particles in the composites were well dispersed. SEM examination revealed a homogeneous distribution of these particles in the PHB–PHV matrix. Subsequent composite processing by compression moulding preserved these characteristics (Fig. 5).

TGA curves were obtained in the temperature range of 30–700°C for PHB–PHV and its composites (Fig. 6). Real weight percentages of bioceramics in composites were determined from these curves and nominal compositions of composites were compared to the results deduced from the TGA analysis (Table 1). It was evident that intended compositions had been
achieved for the composites. TGA analysis also provided thermal degradation temperatures of the composites (Table 2).

A typical DSC thermogram of PHB–PHV is shown in Fig. 7. Similar DSC thermograms were obtained for the composites. DSC analysis was used to study the melting and crystallisation behaviour of composites and crystallinity of the matrix polymer. The mass crystallinity was approximated by using the heat of fusion $\left( H_f^{100\%} \right)$ of totally crystalline PHB, i.e., 146 J/g. This value, found for PHB homopolymer by Barham et al., was considered as a reasonable approximation of the $H_f^{100\%}$ for PHB–PHV copolymers, provided that the copolymers had a low percentage of hydroxyvalerate units [11]. The area of the melting peak endotherm was then directly equated to the heat of melting, $H_f$. The degree of crystallinity, $H^\ast (%)$, of the polymer could thus be estimated by using the following equation:

$$H^\ast (%) = \left( \frac{H_f}{H_f^{100\%}} \right) \times 100,$$

where $H_f$ is the apparent fusion enthalpy of the matrix polymer in the composite specimen and $H_f^{100\%}$ is the fusion enthalpy of the theoretically 100% crystalline polymer. The results of crystallinity studies are summarised in Table 3. It should be noted that crystallinity data listed in Table 3 are not absolute values, but they indicate the trend. As can be seen from Table 3, the crystallinity of matrix polymer decreased with an increase in HA or TCP content in the composites. Melting temperatures of composites, as determined from DSC thermograms, are also listed in Table 3.

An ascending trend of microhardness was observed for HA/PHB–PHV and TCP/PHB–PHV composites with an increase in the volume percentage of particulate bioceramics (Table 4).

DMA has been proven useful to evaluate the viscoelastic properties of polymers. Assuming that the physiological frequency was 1 Hz, all the measurements were therefore made at this frequency. Additionally, a low frequency could give a better insight into the characteristics of the material. For polymeric materials, these equations exist [12]

$$E = \sigma /\varepsilon = E' + iE'',$$  

$$\tan \delta = \frac{E''}{E'},$$

where $E$ is the dynamic modulus, $E'$ is termed storage modulus and $E''$ loss modulus.

The storage modulus was obtained in the temperature range of 30–130°C for both HA/PHB–PHV and TCP/PHB–PHV composites. The obtained results are plotted in Fig. 8. The repeatability of measurements from five specimens of each composition was good. Therefore, only averaged data were plotted without error bars in order to present the results clearly. As expected, the storage modulus increased with the increase in bioceramic content and decreased with the increase in temperature.

The effect of bioceramic particles on the amorphous (viscous) phase of the PHB–PHV copolymer in composites is shown in Fig. 9, in which the loss modulus is plotted against temperature. The general trend is that the loss modulus increased with the increase in bioceramic content and decreased with the increase in temperature.

Results from $\tan \delta$ measurements are shown in Fig. 10. For both HA/PHB–PHV and TCP/PHB–PHV composites, in the temperature range of 30–60°C, the addition of bioceramic particles generally reduced $\tan \delta$.

In the in vitro experiments, a mineral layer was found to form and grow on both HA/PHB–PHV and TCP/PHB–PHV composites after their immersion in SBF (Fig. 11). TF-XRD patterns of immersed specimens showed that mineral crystals formed in vitro were apatite (Fig. 12). Furthermore, FTIR spectra of the
mineral crystals indicated that the apatite formed on the surface of composites in SBF was carbonated apatite (Fig. 13), which was similar in composition and structure to bone apatite [13]. The height of apatite peaks in TF-XRD patterns increased with the increase in immersion time, indicating the growth of apatite in vitro.

4. Discussion

It has been shown that a good dispersion and a homogeneous distribution of bioceramic particles in the PHB–PHV matrix were achieved after the compounding process for both HA/PHB–PHV and TCP/PHB–PHV composites, which indicates that the technique and equipment chosen for compounding are suitable for these composites. A homogenous distribution of bioceramic particles in composites for implant applications is essential as, when a biological apatite layer develops between these particles and the surrounding tissue, the particles will provide uniformly distributed anchors for the prosthesis while at the same time the matrix polymer will degrade. The homogenous distribution of bioceramic particles in the composites is also important for obtaining composites having high mechanical performance.

It appeared that there was no chemical bond between bioceramic particles and the polymer matrix in the composites produced via the current manufacturing process. Mechanical bond existed at the bioceramic-polymer interface, which had resulted from the shrinkage of PHB–PHV around individual bioceramic particles during thermal processing. In this respect, particles of irregular shapes are preferred to spherical particles in order to have strong mechanical interlock between bioceramic particles and the polymer.

TGA results indicated that the differences between real mass percentages of HA or TCP in the composites and theoretical values (Rule of Mixtures calculations)
were negligible and hence intended compositions for the composites had been achieved (Table 1). The thermal degradation temperature of PHB–PHV polymer was generally decreased by the incorporation of bioceramic particles (Table 2).

DSC analysis showed that, contrary to the belief that the introduction of minute particles into the polymer melt would enhance the nucleation of crystallites in the polymer upon cooling and hence improve the crystallinity of the polymer after cooling, the incorporation of HA or TCP particles into PHB–PHV led to the decrease in crystallinity of PHB–PHV matrix polymer. Furthermore, an increase in the HA or TCP content resulted in the decrease in crystallinity of PHB–PHV (Table 3), which suggests that high percentages of bioceramic particles in the composites hindered the growth of crystallites in the polymer. The consequence of lowered crystallinity of the polymer is that the in vivo degradation rate of HA/PHB–PHV and TCP/PHB–PHV composites maybe higher than that of unfilled PHB–PHV, as a lower degree of crystallinity was found to lead to a higher degradation rate of degradable polymers [14]. DSC analysis also showed that the melting temperature of PHB–PHV was slightly affected by the incorporation of bioceramic particles.

Microhardness of HA/PHB–PHV and TCP/PHB–PHV composites increased with an increase in the volume percentage of particulate bioceramics (Table 4). As microhardness may be used as an indicator of Young’s modulus of a bioactive composite [15], the hardness results suggest that with an increase in HA or TCP content the resultant composites became harder.

**Table 1**
Theoretical and real weight percentages of bioceramics in HA/PHB–PHV and TCP/PHB–PHV composites

<table>
<thead>
<tr>
<th>Nominal bioceramic content (vol%)</th>
<th>Bioceramic mass (wt%)</th>
</tr>
</thead>
</table>
| Theoretical value^
value        | TGA analysis          |
| Compounded                       | Moulded               |

<table>
<thead>
<tr>
<th>HA/PHB–PHV:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1.14</td>
</tr>
<tr>
<td>10</td>
<td>21.93</td>
<td>21.48</td>
</tr>
<tr>
<td>20</td>
<td>38.73</td>
<td>37.50</td>
</tr>
<tr>
<td>30</td>
<td>52.00</td>
<td>50.39</td>
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<table>
<thead>
<tr>
<th>TCP/PHB–PHV:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>20.10</td>
<td>20.49</td>
</tr>
<tr>
<td>20</td>
<td>36.14</td>
<td>37.43</td>
</tr>
<tr>
<td>30</td>
<td>49.25</td>
<td>50.23</td>
</tr>
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^
Calculations based on Rule of Mixtures.

![Fig. 6. TGA curves of compression moulded composites. (a) HA/PHB–PHV and (b) TCP/PHB–PHV.](image)

![Fig. 7. DSC thermogram of compression moulded PHB–PHV.](image)

![Table 2](image)

<table>
<thead>
<tr>
<th>Nominal bioceramic content (vol%)</th>
<th>Degradation temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HA/PHB–PHV</td>
</tr>
<tr>
<td>0</td>
<td>279.6</td>
</tr>
<tr>
<td>10</td>
<td>280.2</td>
</tr>
<tr>
<td>20</td>
<td>266.9</td>
</tr>
<tr>
<td>30</td>
<td>252.2</td>
</tr>
</tbody>
</table>
TCP content, Young's modulus of the composites increased and hence the composites became stiffer.

DMA has been used to characterise polymer blends and filled polymers [16,17]. Since PHB–PHV is a viscoelastic material, it shows both the capability to store mechanical energy without dissipation of it (measured as storage modulus, $E'$) and the ability to dissipate energy but not to store it (measured as loss modulus, $E''$). The dissipation of energy manifests itself as internal friction or damping in a polymeric material. In a polymer matrix composite, the energy dissipation may also come from the filler-matrix interface where friction between the two phases can occur. The internal friction can be quantified by $\tan \delta$ which is the ratio of the energy dissipated per cycle to the energy stored during the cycle (i.e., $E''/E'$). A high damping capability is accompanied by a decrease in dimensional stability. It

<table>
<thead>
<tr>
<th>Nominal bioceramic content (vol%)</th>
<th>Melting temperature (°C)</th>
<th>Crystallinity of PHB–PHV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>137.4</td>
<td>137.4</td>
</tr>
<tr>
<td>10</td>
<td>144.1</td>
<td>137.4</td>
</tr>
<tr>
<td>20</td>
<td>144.7</td>
<td>136.7</td>
</tr>
<tr>
<td>30</td>
<td>142.7</td>
<td>136.7</td>
</tr>
</tbody>
</table>

Table 3
DSC results for HA/PHB–PHV and TCP/PHB–PHV composites

<table>
<thead>
<tr>
<th>Nominal bioceramic content (vol%)</th>
<th>Microhardness (VHN)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HA/PHB–PHV</td>
</tr>
<tr>
<td>0</td>
<td>8.56 ± 0.14</td>
</tr>
<tr>
<td>10</td>
<td>10.12 ± 0.46</td>
</tr>
<tr>
<td>20</td>
<td>13.19 ± 0.50</td>
</tr>
<tr>
<td>30</td>
<td>15.73 ± 0.44</td>
</tr>
</tbody>
</table>

Table 4
Microhardness of HA/PHB–PHV and TCP/PHB–PHV composites

![Fig. 8. Storage modulus versus temperature for composites. (a) HA/PHB–PHV and (b) TCP/PHB–PHV.](image)

![Fig. 9. Loss modulus versus temperature for composites. (a) HA/PHB–PHV, (b) TCP/PHB–PHV.](image)
appeared that, in the temperature range of 30–60°C, the addition of bioceramics had limited the mobility of the amorphous phase in the matrix polymer and hence reduced the damping of the composites, the degree of which depended upon bioceramics volume percentage (Fig. 10). This can be expected, because an increase in the bioceramic content will decrease the polymer volume and thus the overall damping of the composite. In the dissipation of energy during DMA stress cycling, a relatively large amount of the energy is assumed to be dissipated at the bioceramic-polymer interface due to the existence of interfacial mechanical bond.

Like microhardness, the dynamic modulus \((E' + iE'')\) of HA/PHB–PHV or TCP/PHB–PHV increased with an increase in bioceramic content. As expected, it decreased with an increase in temperature. A similar effect was observed for other ceramic particle filled polymers [18]. The incorporation of particulate bioceramics into PHB–PHV has resulted in composites with higher modulus values (both Young’s modulus and dynamic modulus) as compared to PHB–PHV copolymer itself.

In vitro experiments showed that a biologically active apatite (i.e., bone-like apatite) layer formed on the surface of composites, indicating bioactivity of the
composites. Furthermore, different composites (HA/PHB–PHV or TCP/PHB–PHV) or the same composite of different compositions (10, 20 or 30 vol% of the bioceramic) had different degrees of ability to induce the formation of bone-like apatite on their surfaces [19]. Therefore, by varying the type of bioceramics or the amount of the bioceramic in the composite, a novel material can be tailor-made to meet the specific requirements for mechanical as well as biological performance. The HA/PHB–PHV and TCP/PHB–PHV composites can thus be used in various clinical situations.

5. Conclusions

HA/PHB–PHV and TCP/PHB–PHV composites containing up to 30% by volume of bioceramic particles have been manufactured. HA and TCP particles are well dispersed and homogeneously distributed in the PHB–PHV matrix using the established processing technology. It is demonstrated that the modulus and microhardness of the composites increase with an increase in the volume percentage of bioceramic particles. In vitro study shows enhanced ability of both series of composites to induce the formation of bone-like apatite, indicating bioactivity of the composites. Due to biodegradability of their constituents, HA/PHB–PHV and TCP/PHB–PHV composites have the potential for various medical applications.

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References