Preparation and characterization of a novel bioactive restorative composite based on covalently coupled polyurethane–nanohydroxyapatite fibres

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Abstract

Nanohydroxyapatite (n-HAp) was prepared using a sol–gel method. n-HAp powder was obtained from the gel form by heat treatment followed by grinding using ball milling. A novel polyurethane composite material was prepared by chemically binding the hydroxyapatite to the diisocyanate component in the polyurethane backbone through solvent polymerization. The procedure involved the stepwise addition of monomeric units of the polyurethane and optimizing the reagent concentrations. The resultant composite material was electrospun to form fibre mats. The fibres were less than 1 µm in thickness and contained no beads or irregularities. Chemical structural characterization of both the ceramics and the novel polymers were carried out by Fourier transform infrared and Raman spectroscopy. X-ray diffraction, scanning electron microscopy (SEM), transmission electron microscopy and Brunauer–Emmett–Teller surface area analysis were also employed to observe the crystal lattice and size and surface area of the n-HAp. Further characterization (by energy-dispersive X-ray analysis and SEM) of the spun fibres revealed the presence of elements associated with hydroxyapatite and polyurethane without the presence of any loose particles of hydroxyapatite, indicating the formation of the covalent bond between the ceramics and the polymer backbone.

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Keywords: Polyurethane; Hydroxyapatite; Electrospun fibres; FTIR and Raman spectroscopy; SEM

1. Introduction

It is desirable for a restorative dental material to have bioactive and bonding properties at the interface between the material and the tissue to prevent micro-leakage and ingress of bacteria. Polymeric materials have been used in medical and surgical applications for a number of years [1]. The requirement of specific material differs according to the nature of the application, and there are different techniques in modifying and fabricating different compositions to achieve exact requirements for clinical use [2].

Polyurethane is a versatile class of polymers and is one of the most interesting classes of synthetic elastomers with unique properties that are used in a broad range of applications due to their excellent physical properties and relatively good biocompatibility [3].

A great deal of attention has been given to the synthesis, morphology, chemical and mechanical properties of this family of materials [4]. Polyether-type polyols possess good physical strength, abrasion resistance, water resistance, fatigue life and biocompatible character [5]. Research on polyurethane has focused on its potential application as a facial prosthesis in dentistry, based on its inherent environmental stability, high tear resistance and low modulus without the use of plasticizer, and good ultimate strength and elongation. This material can accept intrinsic colouring...
and is amenable to maxillofacial processing techniques [6–8]. Polyether-based impression materials have been used in restorative dentistry to record intra-oral structure for fabrication of definitive restorations [9]. Urethane-based resins (UDMA) are available for light-activated material formulated specifically for fabrication [10,11].

Nanohydroxyapatite (n-HAp) has been proved to be an osteoconductive material [12] that also binds chemically to enamel and dentine. A significant characteristic of bioactive material is the ability to bond with living tissue through the formation of a hydroxyapatite interfacial layer [13]. From a biological viewpoint, combination of polymer and ceramic material to fabricate a bioactive composite scaffold is a natural strategy. From the material science perspective, a single material type is not able to provide the necessary mechanical and chemical properties required for biomedical applications [14]. Hence, in recent years, considerable attention has been focused towards the development of polymer composites to fulfil the required properties for biomedical applications. The significance of using a composite material is that, through the amount and type of reinforcing material, the mechanical and biological properties can be tailored for each specific application. It has been reported that various polymers, such as polyacrylamide, polyglycolic acid, poly-ε-caprolactone, polyethylene, polyetherketone and polyurethane, have been used with HAp as composite materials [15–22]. The interface adhesion of HAp particles and the polymer matrix is a major factor affecting the properties of the composites [23,24]. Surface modification by surface adsorption and grafting of HAp to polymers provide an effective way to manipulate the surface properties of HAp [15]. When nanoparticles (n-HAp) and polymers form a composite, provided that homogeneous dispersion of the nanoparticles is achieved at the microscopic level, the mechanical properties are expected to be improved and/or new, unexpected features might appear [5]. Liu et al. [23] and Dong et al. [25] studied the reactivity of isocyanate with hydroxyapatite and calcium hydrogen phosphate (CaHPO4, CHP) respectively. They observed that there was a covalent linkage between isocyanate and HAp, and a urethane linkage between hexamethylene diisocyanate (HMDI) and CHP, showing that the hydroxyl (–OH) groups at the surface of n-HAp have reactivity towards organic functional groups.

Electrospinning is a relatively new process for developing nanofibres. It allows the continuous production of fibres ranging from submicrometres to nanometres [26]. In this process, continuous filaments are drawn from a liquid polymer or melt through a spinneret by high electrostatic forces and later deposited on a conductive collector [27,29].

Due to the high-surface-area to volume ratio of the electrospun fibres and the high porosity on the submicrometre length scale of the obtained non-woven mat, proposed applications for these materials include nanofibre-reinforced composites, nanofibre supports for enzymes and catalysts, and nanofibrous membranes in biomedical applications for drug delivery, wound healing, cardiac grafts, guided bone regeneration and scaffolding for tissue engineering. Electrospun fibres mats are suitable for use as scaffold because of their highly porous three-dimensional (3-D) structure [29–34]. The morphology of electrospun fibres depends on a number of factors, including viscosity, conductivity and surface tension, hydrostatic pressure in the capillary, electric potential at the tip, distance between the tip and the collector, and ambient parameters, including temperature, humidity and air velocity in the electrospraying chamber [26,30,35].

Various polymers, such as polymetaphenylene-isophthalamide, polyetherimide, polyethylene oxide, polyethylene terephthalate, polyaniline, polycaprolactone and poly-ε-lactic acid, have been successfully electrospun into nanofibres [25,28,36–38]. A few studies have reported the culture of chondrocytes and osteoblasts on polycaprolactone [33,34]; in addition, the incorporation of calcium carbonate or HAp has also been reported [29,32,34,39]. It was reported that the presence of non-interacting rigid particles increased the shear viscosity of dilute suspension and decreased the conductivity of the resulting mixture. It was also observed that beaded fibres were obtained; however, this depends mainly on the concentrations of the polymer and particles [32,34]. The resin-based dental polymers 2,2’-bis-(4-(methacryloxypropoxy)-phenyl)- propane and triethylene glycol-dimethacrylate have been investigated with the reinforcing effect of electrospun nylon 6 nanofibres [40]. It was found that the fibres have a crystalline structure and are mechanically strong. The small diameter of nanofibres also provides a high ratio of surface area to volume, which could enhance the intermolecular hydrogen bonding between the filler of nylon 6 nanofibres and the matrix of resin polymers. Electrospraying is the technique used to deposit the particles onto the surface. The basic principle of electrospaying is the generation of a spray of charged, micron-sized droplets. This is done by means of electrostatic atomization. The sprayed droplets are directed towards a heated substrate as a result of an applied potential difference [41].

In this study, a novel bioactive composite material based on covalently linked polyurethane and nanohydroxyapatite particles was synthesized and characterized. The composite was also electrospun to produce nanofibres for morphological characterization.

2. Materials and methods

2.1. Materials and synthesis of hydroxyapatite

n-HAp was synthesized by ammonium hydrogen phosphate 98% ((NH4)2HPO4) and calcium nitrate tetrahydrate 99% (Ca(NO3)2·4H2O) as phosphate and calcium precursors, distilled water and ethanol were used as solvent for precursors respectively. Ammonium hydroxide was used to control the pH value. All materials were purchased from
Sigma Aldrich, UK and were analytically graded. The sol-gel method was utilized to synthesize nanoapatite powder. The ratio of Ca and P precursor was maintained at 1.66. Ca(NO$_3$)$_2$·4H$_2$O (0.083 mol) was mixed in 50 ml of water and the solution was stirred at 85 °C. [(NH$_4$)$_2$HPO$_4$] (0.05 mol) was dissolved in 50 ml of ethanol and ammonium hydroxide was added into the solution to maintain the pH value. The phosphate-containing solution was added dropwise. The equilibrium pH of the mixture was maintained above 10. The mixture was stirred for 4 h as the gel formation occurred. The final gel was aged for 24 h at 40 °C. The sample was heat-treated by heating at a rate of 10 °C min$^{-1}$ up to 400 °C and maintaining this heat for 2 h, before being cooled down to 20 °C at the rate of 10 °C min$^{-1}$. The resulting powder was ground with ball milling and characterized using the methods described in the sections below.

2.2. Nanohydroxyapatite solution

For electrospraying, n-HAp solution was prepared by mixing the n-HAp with 5% (w/v) dimethylformamide (DMF) solvent.

2.3. Materials and synthesis of polyurethane

All materials were analytically graded and purchased from Sigma Aldrich, UK. Poly(tetramethylene glycol) (PTMG; number average mol. wt. 1400 g mol$^{-1}$) soft segments were freeze-dried for 22 h prior to their employment in the synthesis process. 4,4'-Methylenebisphenylisocyanate (MDI) hard segments and 1,4-butanediol (BDO) chain extender were used as received. $N,N'$-DMF was dried with a 4 Å molecular sieve for 72 h and used as solvent.

2.4. Grafting procedure

A preliminary study was conducted to graft the MDI with n-HAp. The procedure for surface grafting is briefly as follows: 2 g of MDI was dissolved in DMF and put into a three-neck flask. The flask was then stirred under nitrogen flow for 30 min, before 5% of n-HAp was added to the flask. This was then stirred under nitrogen with a flow rate of 200 rpm at 60 °C for 3 h. The resulting surface-modified material was washed with water and methanol three times to remove the residual DMF and allowed to dry in a vacuum pump for 24 h.

Polyurethane was synthesized by the step-growth polymerization method. The ratio of PTMG:MDI:BDO was 1:2.26:1.06 respectively. PTMG was dissolved in DMF and heated at 60 °C, before a solution of MDI and DMF was added dropwise and heated at same temperature for 1 h. BDO solution was then added dropwise. The temperature was increased to 80 °C for 1 h and then to 110 °C for 4 h to allow the solution for complete polymerization.

2.5. Synthesis of PU/n-HAp composite by physical mixing (PM)

The synthesized polyurethane was dissolved in 5% (w/v) DMF solvent. After complete mixing, 10 wt.% n-HAp powder was added to the solution, which was then stirred at room temperature for 24 h.

2.6. Synthesis of PU/n-HAp composite by chemical mixing (CM)

PU/n-HAp composite were synthesized by the step-growth polymerization method without using a catalyst. All glassware was dried in an oven overnight at 150 °C. One mole of PTMG with DMF was placed in a 250 ml three-necked round bottom flask fitted with a magnetic stirrer, a nitrogen inlet and a water condenser. The whole reaction was performed under the continuous flow of nitrogen. The flask was then placed on a hotplate and the reaction mixture was heated at 60 °C for a period of 30 min, before 10 wt.% n-HAp was dissolved in DMF and added to the flask. After 15 min, a solution of MDI (2.26 M) in DMF was added dropwise to the flask through dropping funnel over a period of 30 min. The solution was allowed to react for 30 min to obtain a homogeneous mixture, then BDO (1.06 M) dissolved in DMF was added dropwise to the reaction mixture and the reaction was carried out at same temperature for a further 30 min. The reaction was carried out at 80 °C for 1 h and then at 110 °C for a further 4 h. DMF was added occasionally to the reactor when the viscosity of the reactants was too high prior to polymerization. After 4 h the experiment was finished and the solution was precipitated in ice water followed by reprecipitation in methanol to remove any unreacted DMF and low-molecular-weight polymer. The resulting composite was dried under vacuum at room temperature for 48 h.

For electrospinning, PU/n-HAp composite was diluted in DMF, which has a high dielectric constant; it is also a polyelectrolyte and has been used for enhancing electrospinning polymers in the past [26].
2.7. Electrospinning

Fig. 1 shows the experimental setup for the electrospinning process. The conducting stainless steel nozzle had an internal diameter of 330 μm and was connected to the positive terminal of the high voltage supply (Glassman Europe Ltd), held together by epoxy resin. The composite material was delivered to the nozzle by silicone tubing, which was also attached to the perfusor (Harvard syringe pump, Harvard apparatus Ltd). A flow rate of 15 μl min⁻¹ was deployed and the applied voltage was in the range of 7–9 kV. Fibres were collected 120 mm below the nozzle exit.

2.8. Characterizations

2.8.1. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of the n-HAp, surface-grafted MDI/n-HAp, PU and PU/n-HAp composite were collected using a Thermo-Electron Nicolet 8700 FTIR spectrometer, equipped with a photoacoustic sampling cell (MTech PAS cell). The sample chambers of the PAS cell was purged with dry helium gas (pre-dried over a column of magnesium perchlorate). The spectra were measured at 8 cm⁻¹ resolution, accumulating a total of 256 numbers of scans.

2.8.2. Raman spectroscopy

Raman spectra were collected on a Nicolet Almega XR dispersive Raman spectrometer equipped with a 785 nm laser, accumulating 128 scans and an exposure time of 2 s at 4 cm⁻¹ resolution. The spectra were collected between 4000 and 100 cm⁻¹.

2.8.3. X-ray diffraction (XRD) analysis

PowderXRD patterns were obtained using Simens/Bruker D5000 Diffractometer, with monochromatic Cu Kα radiation (λ = 0.15418 nm). Scans were obtained in the 2θ range = 20–60°, with a step size of 0.02° every 1 s.

2.8.4. Transmission electron microscopy (TEM)

The particles were suited in a JEOL JEM 2010 high-resolution transmission electron microscope operated at 200 KeV. Samples were prepared by ultrasonically dispersing the powders in methanol prior to collection on carbon-coated copper grids (Holey Carbon Film, 300 mesh Cu, Agar Scientific). Morphology and average particle size were estimated from the bright field images.

2.8.5. Scanning electron microscopy (SEM)

The morphology and size of as-synthesized n-HA particles and electrospun fibres were characterized by SEM (JEOL 6300 JSM) by using energy-dispersive X-ray analysis (EDX) and the field emission method. All the electrospun fibres were collected on glass slides and were carbon-coated.

Fig. 2. FTIR spectra of nanohydroxyapatite heat-treated at 800 °C. The peaks at 3571 and 631 cm⁻¹ are from OH groups. The intense peak at 1040 cm⁻¹ and the peaks at 603 and 569 cm⁻¹ are the P-O absorption bands. 1473 cm⁻¹ is of the CO₃ group.
2.8.6. Brunauer–Emmett–Teller (BET) surface area analysis

The specific surface area of the powders was determined by the BET method using a Micromeritics Gemini II 2370 surface area analyser. The five-point multipoint adsorption method was used. All powdered samples (0.2–0.5 g) were degassed with nitrogen using a Flow Prep 060 controller at 200 °C for at least 3 h prior to analyses.

Fig. 3. Raman spectroscopy of nanohydroxyapatite. The peaks at 1077, 1044 and 961 cm\(^{-1}\) are the \(v_3\) and \(v_1\) of P–O respectively. The peak at 603 cm\(^{-1}\) is the \(v_4\) of the O–P–O.

Fig. 4. XRD pattern of nanohydroxyapatite heat-treated at 800 °C.
Fig. 5. TEM (a) and SEM image (b) of nanohydroxyapatite image of n-HAp.

Fig. 6. FTIR (a) and Raman (b) spectra of (i) n-HAp, (ii) MDI and (iii) MDI/n-HAp urethane peaks, isocyanate band, P–C–O peaks.
3. Result and discussion

3.1. Nanohydroxyapatite

FTIR spectra of n-HAp are presented in Fig. 2 and shows the absorption bands that confirm the chemical structural characteristics of hydroxyapatite. Bands at 3571 and 631 cm$^{-1}$ are assigned to stretching mode ($v_s$) and liberation mode ($v_L$), respectively, of the OH group. The band at 1473 cm$^{-1}$ corresponded to the stretching mode, $v_1$, of the CO$_3$ group. Bands at 1040, 946, 603 and 569 cm$^{-1}$ are assigned to vibration of the phosphate group, PO$_4$. The peak at 1040 cm$^{-1}$ is the triply degenerated vibration, $v_3$. 946 cm$^{-1}$ is the non-degenerated symmetric stretching mode, $v_1$, of the P–O bond of the phosphate group. The peaks at 603 and 569 cm$^{-1}$ are assigned to a triply degenerated bending mode, $v_4$, of the O–P–O bond. Fig. 3 shows the Raman spectra, the phosphate peaks $v_3$ at 1077 and 1044 cm$^{-1}$ and the strong $v_1$ stretching peak at 961 cm$^{-1}$, which is dominant in the spectra. $v_4$ and $v_2$ phosphate-bending peaks are at 586 and 432 cm$^{-1}$. All bands were assigned to internal vibrational modes of the phosphate groups [42,43].

<table>
<thead>
<tr>
<th>Wavenumber (cm$^{-1}$)</th>
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<tr>
<td>3310</td>
<td>Strong vs. (N–H) bonded N–H</td>
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<tr>
<td>2930</td>
<td>Strong vs. (CH$_3$)</td>
</tr>
<tr>
<td>2860</td>
<td>Strong vs. (CH$_3$)</td>
</tr>
<tr>
<td>1730</td>
<td>Very strong free C=O</td>
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<tr>
<td>1702</td>
<td>Very strong bonded C=O</td>
</tr>
<tr>
<td>1600</td>
<td>Strong vs. (C=C)</td>
</tr>
<tr>
<td>1530</td>
<td>Very strong δ (N–H) + ν (C–N)</td>
</tr>
<tr>
<td>1410</td>
<td>Strong vs. (C–C) in benzene ring</td>
</tr>
<tr>
<td>1310</td>
<td>Strong δ (N–H) + ν(C–N), β(C–H)</td>
</tr>
<tr>
<td>1230</td>
<td>Strong δ (N–H) + ν(C–N)</td>
</tr>
<tr>
<td>1110</td>
<td>Very strong vs. (CH$_2$–O–CH$_3$) of aliphatic ether</td>
</tr>
<tr>
<td>1020</td>
<td>Weak β (C–H) in benzene ring</td>
</tr>
<tr>
<td>818</td>
<td>Weak γ (C–H) in benzene ring</td>
</tr>
</tbody>
</table>
Fig. 4 shows the XRD pattern of heat-treated n-HAp at 800 °C. It was observed that the major phase is HAp and that there is no other crystalline phase. The peaks at the diffraction angles of almost 26, 32, 33 and 40 are attributed to (002), (211), (300) and (310) [43]. It is suggested that the heat treatment plays an important role in the formation of HA. As the temperature was 800 °C, n-HAp lines become distinct and narrower, suggesting an increase in the crystalline degree. It was reported that the heat treatment above 800 °C shows some additional crystalline phases, such as β-TCP (tricalcium phosphate) and CaO (calcium oxide) [44].

TEM and SEM images (Fig. 5a and b) shows the morphological patterns of the particles, which exhibit the nanostructure of hydroxyapatite particles. The figure shows that the particle sizes are in the range 40–150 nm. The structural characteristics of HAp are affected by the synthetic precursors, pH values, reaction temperature and post-treatment processes, including ageing and heat treatment [45]. The n-HAp is of biological interest because of its similarity in chemical composition and size to the mineral in bones and teeth. Thus, it may create a bioactive bond between the material and the tooth structure, such as enamel and dentine, and provides better mechanical properties due to its high-surface-area to volume ratio, superior chemical homogeneity and microstructural uniformity [46]. The BET results showed that the average surface area of synthesized n-HAp powder was 30 m²/g. This value is due to heat treatment of n-HAp.

3.2. FTIR of PU and PU/n-HAp composite

Fig. 6a and b shows the comparative Raman and FTIR spectra of (i) n-HAp, (ii) MDI and (iii) MDI/n-HAp, respectively. The FTIR spectrum (Fig. 6a) indicated that certain functional groups on the surface of nanoapatite have reactivity towards isocyanate. An N–H stretching peak was observed at 3330 cm⁻¹, which clearly shows the formation of urethane linkage. There is an increase in intensity of the peak, and CH₂ peaks were observed at 3010 and 2930 cm⁻¹. A marked reduction in the isocyanate band at 2400–2100 cm⁻¹ was observed and clearly shows the reaction of isocyanate. Other urethane peaks were observed at 1710, 1575, 1447 and 1236 cm⁻¹. A noticeable change in peak was observed at 1100 cm⁻¹, which is due to asymmetric stretching vibration of P–O–C. Similar behaviour was observed in the Raman spectrum (Fig. 6b). The isocyanate peak (2260 cm⁻¹) was completely removed after reaction and the characteristic urethane–hydrogen bond peak was observed at 1680 cm⁻¹. Like FTIR, urethane peaks (1445, 1350, 1300 cm⁻¹) were observed and obvious change was observed at 960 cm⁻¹, which shows the linkage of the phosphate band.

Polyurethane was characterized by FTIR (see Fig. 7a). The assignments of the FTIR spectra are tabulated in Table 1. Fig. 7b and c shows the FTIR spectra of grafted n-HAp and PU composite by PM and CM, respectively. In CM, the polymer on the surface of n-HAp was identified by the presence of a urethane linkage (3313 cm⁻¹), car-
bonyl vibration (1700 cm$^{-1}$) and C–O–C vibration (1110–950 cm$^{-1}$). The peak at 3313 cm$^{-1}$ belongs to the grafted peak of HN–CO (linkage of OH (n-HAp) and NCO (PU)), showing that a covalent bond was formed between n-HAp and PU, whereas it is interesting to see that the PM spectrum (Fig. 7b) shows a separate –OH peak at 3570 cm$^{-1}$ (characteristic stretching peak of hydroxyl group of hydroxyapatite). An N–H peak was observed, but it was from the polyurethane linkage. Hence, PM only provide the n-HAp as a filler particle but did not show any linkage and coating of the polymer on the surface. The composite without coating will show weight loss and abrasion on load application, the presence of hydroxyapatite on the surface will weaken the material due to release of particles, and the insoluble particles will be released from the polymer and move into the adjacent area. It is reported that the dissolution of the high-surface-area particulate will effectively minimize the fall in pH associated with the healing process as hydroxide ions are released from the hydroxyapatite [51].

Due to the relatively complex structure of n-HAp, it is suggested that the urethane linkage is bonded with apatite structure: It is expected that there is sharing of bond between –C, –O and apatite. –O and –C have charges before reaction (N=C=O), but after bonding (HN–C–O) they show the dipole moment. The peaks at 2939, 2855 and 2796 cm$^{-1}$ are assigned to the $\nu_a$, $\nu_b$ and CH$_2$ stretch vibration respectively. It is established that the isocyanate peak usually appears at 2220 cm$^{-1}$, which was not observed in this study, so it is suggested that there is no unreacted isocyanate. The peak at 1732 cm$^{-1}$ is the free C=O, but the more intense peak at 1700 cm$^{-1}$ is assigned to the bonded $\nu_a$ C=O. The 1598 cm$^{-1}$ peak belongs to the vibrational C=C benzene ring, and 1539 and 1224 cm$^{-1}$ are the $\delta$ (N–H) + vibrational (C–N). 1311 cm$^{-1}$ is the strong $\delta$ (N–H) + $\nu$ (C–N), $\beta$ (C–H) [49,50].

The isocyanate group has high reactivity. They often occur in prepolymerized forms. The reaction of isocyanate group with OH group can form a urethane linkage, which is characterized by an absorption band at 1539 cm$^{-1}$. The carbonyl absorption band at 1695–1615 cm$^{-1}$ is one of the secondary amide absorption bands. If P–O–CO–NH existed, the carbonyl absorption band would be shifting to a higher wave number of 1716 cm$^{-1}$ [25]. The presence of the peak at 1715 cm$^{-1}$ provides the evidence of P–O–CO–NH bond formed between MDI and PO$_4$. Non-stoichiometric structure of HAp contains HPO$_4^{2-}$ on the surface of apatite. It was claimed that there was no linkage between HPO$_4^{2-}$ and isocyanate [15], however, another study group [25] proved the linkage between calcium hydrogenphosphate (CHP) and isocyanate (asymmetric stretching vibration of P–O–C). This study also found that, by the grafting procedure and the synthesis of PU and PU/n-HAp composites, there is a change in the peak intensity and emergence of new shoulder peaks in the range of 1108–850 cm$^{-1}$, which are attributed to the linkage OP–HC–O (linkage of PO$_4^{3-}$ and vibrational (CH$_2$–O–CH$_2$). The shoulder peaks at 1000–940 cm$^{-1}$ are evidence of P–O–C formation. The covalent bonding of n-HAp with PU has the potential to improve the interface of the composite matrix, thereby leading to significant improvement of the bioactive, bonding and mechanical properties. The –OH group present on the surface of n-HAp seems to be a reactive group, which can be used to make a covalent linkage with polymer, due to n-HAp and the large surface area, and there are relatively large amounts of atoms on the surface and large fraction of surface OH groups [15]. The FTIR spectrum indicates that certain functional (hydroxyl) groups on the n-HAp have reactivity towards isocyanate groups and also form a urethane linkage. It has been reported that the reactive groups on the surface of nanoapatite shows three types of reactive species which can react with isocyanate groups. They are surface-absorbed water, HPO$_4$ and OH$^-$. In this study it was observed that after grafting the change of the peak intensity at 850 cm$^{-1}$ emerged as a new peak from P–O–C. It was previously reported that only hydroxyl groups of nanoapatite have any reactivity towards isocyanate [15], but this study shows that phosphate groups also have a tendency to show reactivity with isocyanate.
3.3. Raman spectroscopy of PU and PU/n-HAp composite

Raman spectroscopy has a minimal required sampling volume and large frequency ranges for spectral observation. In this study Raman spectroscopy (Fig. 8a–c) was used to acquire the intensities of polyurethane and PU/n-HAp PM and CM composite. The peaks at 2920, 2869 and 2801 cm\(^{-1}\) are assigned to CH\(_2\), as same in FTIR spectra. The region in between 1800 and 1570 cm\(^{-1}\) is assigned to carbonyl symmetric stretch vibration, which consists of three characteristic bands: 1700 cm\(^{-1}\), assigned to the ordered hydrogen bond in hard phase. In this study, a shoulder was observed in this region; 1712 cm\(^{-1}\) is the amide I (C=O, carbonyl) peak and corresponds to the disordered hydrogen bond; and 1730 cm\(^{-1}\) is assigned to the free carbonyl group. There is an aromatic breathing mode symmetric stretch vibration (C=C) at 1619 cm\(^{-1}\). The peak at 1528 cm\(^{-1}\) is assigned to \(\nu\) (aromatic, C=C), urethane amide II: \(\nu\)(C–N) + \(\delta\)(N–H). This band is similar to the isocyanate asymmetric and symmetric stretching vibrations. The literature suggests that this band arises from para-(4,4\(^{\prime}\)-isomer)-disubstituted phenylene ring vibration in MDI. However, the other isocyanate peak at 2200 cm\(^{-1}\) is not visible, so it is suggested that this peak belongs to urethane amide II. The peaks of 1443 and 1305 cm\(^{-1}\) are assigned to \(\delta\)(CH\(_2\)) bending vibration and \(\delta\)(CH) respectively. It is also reported that the 1305 cm\(^{-1}\) peak is assigned to the urethane III band, which is predominantly due to the in-phase combination of N–H in-plane bending and C–N stretching vibrations. A band corresponding to C–O–C stretch is observed at 1118 cm\(^{-1}\) and a weak peak at 1080 cm\(^{-1}\) is attributed to C(O)–O–C stretch of the hard segment [53–56]. Fig. 8b shows the spectrum of the PM composite and an intense characteristic phosphate peak can be seen at 950 cm\(^{-1}\), which indicates the presence of n-HAp particles on the surface of polymer. The observed spectra for PU/n-HAp CM (Fig. 8c) composites showed some additional shoulder peaks in the region of aromatic (C=C), urethane amide II (1541 cm\(^{-1}\)), \(\delta\)(CH\(_2\)) (1448 cm\(^{-1}\)), urethane III (1302 cm\(^{-1}\)) and C–O–C (1120 cm\(^{-1}\)). These additional shoulder peaks show that the linkage between polyurethane and n-HAp have some effect on the structure of material. It is reported that in Raman spectroscopy, fluorescence spectra due to organic materials often dominate the much weaker Raman signals. Therefore, Raman spectroscopic studies have been limited.
to tissues that contain only a few per cent of organic materials. Hence, the peaks of hydroxyapatite did not have any significant effect on the intensity of the polyurethane peaks, but the additional peaks support the hypothesis that there exists a linkage between the polyurethane and n-hydroxyapatite.

3.4. Electrospinning

Fig. 9a and b shows the electrospaying of n-HAp powder particles. The powders are distributed on the surface. The parameters that influence the results are the applied voltage, solvent type, polymer solution flow rate and polymer concentration [57]. A thick coating of the nanoparticles was not achieved in this study due to the short time period. This study focuses mainly on the electrospinning process for the production of nanofibres and assesses the morphological behaviour. SEM images of electrospun mats of polyurethane, polyurethane/n-HAp (PM) and polyurethane/n-HAp (CM) are shown in Figs. 10 and 11. Unlike conventional polymer fibres, the morphological appearance of the PU/n-HAp (CM) composite fibres shows a mostly uniform size. The deviation in size is in the regions of nanometres and submicrometres. The reduction in diameter from the millimetre scale to the nanoscale is due to instability, where the jet is stretched by whipping and bending. Electrospinning is driven by electrostatic forces and requires only a small amount of polymer solution [58]. However, the polyurethane fibres were shown to have a beaded structure, whereas the composite was smooth and no beaded nanofibres were observed in the SEM images. It was reported that the electrospun beaded nanofibres of the polymers are related to the instability of the jet of polymer solution [58,26]. The smoothness and splaying of nanofibres was attributed to the increasing DMF volume fraction and decreasing surface.

<table>
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<th>Element</th>
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<tr>
<td>O</td>
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<td>P</td>
<td>3.20</td>
<td>1.44</td>
</tr>
<tr>
<td>Ca</td>
<td>5.20</td>
<td>1.81</td>
</tr>
</tbody>
</table>
| Total   | 100.00

![Fig. 12. SEM images (a, b) and EDX (c) spectra and map of electrospun fibres of PU/n-HAp CM composite.](image-url)
tension and viscosity, while conductivity and dielectric constant increased. It is also reported that the solvent composition and various solution properties conducted an important role in determining the fibre formation [26]. The size of nanofibres depends on the incorporation of n-HAp that could cause the increased viscosity of the solution. The increase in viscosity of the solution was due to the increased molecular entanglement or linkage between polyurethane and nanoparticles. In order to confirm the presence of n-HAp in fibres, EDX was performed with SEM. The elements calcium and phosphate were not present in polyurethane fibres, whereas they were present in the n-HAp in the PU/n-HAp composite (Table 2 and Fig. 12). Hence it was confirmed that the n-HAp particles existed in the composite fibres. It was further observed that these particles could be observed on the surface of the PM fibres, but not on the CM fibres. As the CM fibres were shown to have covalent bonds between the polyurethane and n-HAp by the FTIR spectra, the SEM images confirmed that these bonds are strong and the n-HAp particles were fully embedded in the polyurethane matrix. Hence, unlike the PM fibres, the n-HAp particles in the CM fibres would not be easily lost by mechanical abrasion. The significance of presence of n-HAp within the polymer is its bioactivity. It is established that the presence of Ca\(^{2+}\) stimulates osteoblastic proliferation and depresses osteoclast-mediated bone resorption through negative feedback loops [59], while a high concentration of P induces osteoblast apoptosis [60]. A high concentration of Ca\(^{2+}\) promotes osteoblastic differentiation, leading to bone mineralization. This response contributes to the maintenance of bone homeostasis and differential reactivity [39]. HAp is also known to enhance cell adhesion through an inherently high capacity of adsorbing proteins. Through the electrospinning process, nanofibres produced by the CM technique can be produced to form two- or three-dimensional structures for mechanical devices in specific medical and dental applications.

4. Conclusion

This study shows that covalently linked PU/n-HAp composite and the nanofibres of this composite were successfully collected by the electrospinning process. The morphological appearance of the CM nanofibres was smooth and uniform in size, with no visible n-HAp particles on the surface. These high-surface-area nanofibres could potentially be used in biomedical and dental applications because of their bioactivity and because their mechanical properties can be adjusted to suit.

References


