(54) Title: POLYLACTIDE AND APATITE COMPOSITIONS AND METHODS OF MAKING THE SAME

(57) Abstract: A method is provided for synthesizing PLA/apatite composites with improved mechanical strength. In one aspect, a calcium-phosphate/phosphonate hybrid shell is developed to incorporate more reactive hydroxyl groups onto hydroxyapatite (HA) particles. PLA is covalently bonded to HA calcium phosphate hybrid shell, creating a strong interphase between HA and PLA, thus significantly improve the mechanical strength in comparison to that of non-modified HA.

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FOLYLACTIDE AND APATITE COMPOSITIONS
AND METHODS OF MAKING THE SAME

FIELD

[0001]   This application claims the benefit of U.S. Provisional Application Serial No. 61/ 623,483, filed April 12, 2012 which is incorporated herein as if fully rewritten and also claims the benefit of U.S. Provisional Application Serial No. 61/623,490, filed April 12, 2012 which is incorporated as if fully rewritten herein.

[0002]   The present application is directed to compositions including polylactides and calcium phosphate composites and methods for making the same. More particularly, the synthesis methods and resulting composites include polylactides and apatite that have been modified when combined.

BACKGROUND

[0003]   Bioresorabie compositions such as polylactides (PLA) are useful for bone fixation and bone repair and have the advantage of not requiring surgical removal after the bone heals. However, the use of polylactides for bone fixation and bone repair can lead to a variety of undesirable side effects, such as inflammation or allergic reactions.

[0004]   Combining apatite, such as hydroxyapatite (HA), with PLA yields a composition similar to the composition found in bone and teeth *in vivo*. Polylactide / hydroxyapatite (PLA/HA) composites facilitate the osteoinductive properties of an implant plus aides in lessening the side-effect of the PLA composite by neutralizing its acidic bio-degraded by-products. PLA/HA composites have the potential of improving clinical bone healing, but current PLA/HA composites have a significant disadvantage clue to their mechanical weakness. This weakness eliminates the use of PLA/HA composites in load-bearing areas. It is generally suspected that PLA/HA composite weakness is caused by the weak interphase between PLA (hydrophobic) and HA (hydrophiiic) structures.

[0005]   Current PLA/HA composites are prepared by several different methods such as direct blending using nonmodified HA, solution co-precipitation, emulsion, and mechanical mixing. Because of the relatively high hydrophobicity of PLA and hydrophilicity of HA, obvious problems of these methods include weak interfacial adhesion between HA and the PLA

matrix and agglomeration of the HA particles in the matrix. Lack of adhesion between the two phases will result in an early failure at the interface between PLA and HA, usually leading to weak mechanical properties. As an example, the tensile strength of PLA/ HA composites decreased significantly from 54 MPa for pure PLA to 41 MPa even with a HA content of only 18%.

[0006] Increasing interfacial bond strength between PLA and HA is an important factor on a matrix interface to achieve increased mechanical strength. In the last decade, coupling agents such as silanes, isocyanates, and organotitanates have been used to improve the interfacial adhesion between certain ceramic fillers and different polymeric matrices. Although the effects on alumina and silica systems ($SiO?$., bioglass, clay, etc.) were encouraging, the feasibility of using these agents to gain improved interfacial adhesion to HA was not confirmed.

SUMMARY

[0007] It has been unexpectedly found that a polylactide having a general formula of -(OCH(**R**)CO)**n-** , wherein R = H or C1-C10 alkyl and wherein n = 1-4, may be combined with an apatite material having a general formula of $Ca_{10}(PO_4)_6X_2$ where X is OH or F or both, whereby the apatite is modified prior to coupling with the polylactide. In one form, the material may be prepared by combining a hydroxyapatite source having a general formula of $Ca_{10}(PO_4)_6(OH)_2$ with an organic material with phosphonic acid functionality having a general formula of -$PO(OH)_2$ to form an intermediate hydroxyapatite phosphonic acid containing material. The intermediate hydroxyapatite phosphonic acid containing material, which is a reaction product of the hydroxyapatite source and the organic material with phosphonic functionality may then be combined with a lactide material having a general formula of $(OCH\{R\}CO)_r$, wherein n = 1-4, preferably 2 = n, or CH(OH)(R)COOH, wherein R = H or C1-C10 alkyl, to form the polylactide / hydroxyapatite material.

[0008] Further, the polylactide/ modified phosphonic acid apatite material which has been reacted with polylactide may then be combined with additional polylactide to form a composite polylactide/ apatite material. It is believed that such a composite polylactide/ apatite material may have increased tensile strength compared to a poiylactide/apatite material that has not been modified with a phosphonic acid containing material. The amount of additional polylactide to polylactide/ modified phosphonic acid apatite material in the composite may

range from about 1% weight to about 99% weight. In one form, the amount of polylactide/modified phosphonic acid apatite material combined with additional PLA is sufficient to affect an increase in a tensile strength of the composite of at least about 50% when compared to a composite which does not include modified phosphoric acid apatite material as described herein. According to another form, the amount of polylactide/modified phosphonic acid apatite material is sufficient to effect a 100% increase in tensile strength when compared to a composite which does not include modified phosphonic acid apatite material.

[0009]   According to one form, the apatite source is modified with the phosphonic acid containing material to introduce -OH and/ or NH_4 groups on the surface of the apatite source to form the apatite material which is reactive with polylactide. In one form, the intermediate apatite phosphoric acid containing material undergoes surface initiated polymerization with the lactide groups of the polylactide via -OH and/ or NH_2 groups found on a surface of the intermediate apatite phosphoric acid containing material.

[0010]   The method may also include the step of separating unreached phosphonic acid containing material from the intermediate apatite material. Similarly, the method may include the step of separating unreacted lactide containing material from the polylactide/ apatite material.

[0011]   In one form, the phosphonic acid containing organic material includes N-(2~ hydroxyethyl) iminobis(methylphosphonic) acid (HIMPA). In this regard, in one form, the hydroxyapatite source is suspended in an aqueous solution of N-(2-hydroxyethyl) iminobis(methylphosphonic) acid. In another form, the hydroxyapatite is precipitated in the presence of N-(2-hydroxyethyl) iminobis(methylphosphonic) acid. However, other forms of phosphorous containing compounds also may be used.

[0012]   It has been found that there are significant benefits of this method of synthesis, such as significantly increased tensile strength when compared to that of polylactide alone.

[0013]   By one approach, the composition comprises significantly higher polylactide covalently attached to hydroxyapatite than that of current compositions produced by conventional methods.

<center>**BRIEF DESCRIPTION O F THE FIGURES**</center>

[0014]   Figure 1 illustrates X-ray diffraction patterns of crystallized HA composites prepared according to different methods;

[0015]   Figures 2A-2D illustrate thermogravimetric analysis (TGA) curves of HA composites prepared according to different methods;

[0016]   Figure 3 illustrates pH dependent ζ-potentiai and particles size profiles of HA composites prepared according to different methods;

[0017]   Figure 4 illustrates Diametral Tensile Strength (DTS) of PLA/HA composites prepared according to different methods; and

[0018]   Figure 5 illustrates a schematic representation of PLA/HA composite preparation using initiated polymerization.

<center>**DETAILED DESCRIPTION**</center>

[0019]   Described herein are synthesis methods and compositions comprising PLA/HA composites. The general method provides synthesis of PLA/HA composites by grafting PLA on an HA intermediate, such as via surface-initiated polymerization (SIP) through the non-ionic surface hydroxy! groups. It should be noted that when referring to a composite material, the material includes modified phosphonic acid apatite material along with additional apatite material.

[0020]   In one form, the synthesis methods described herein use a method for preparing a po!ylactide/ apatite material comprising the steps of combining an apatite source with a phosphonic acid containing material to form an intermediate apatite material. In one form, the intermediate apatite material is formed thereby introducing a plurality of -OH and/ or NH_2 groups coupled to the apatite. The intermediate apatite material may then be combined with a lactide containing material to form the polylactide/apatite material which has has a diametral tensile strength that is at least 1.5 times the diametral tensile strength of a polylactide/apatite material prepared without combining a apatite material with the organic material having phosphonic acid functionality.

[0021]   The apatite material may include a variety of apatite containing and/ or providing materials prepared in a variety of manners. In one form, the apatite material has a general formula of $Ca_{10}(PO_4)_6X_2$ where X is OH or F or both OH or F. In this regard, the apatite material may be fluoroapatite and/ or hydroxyapatite. According to one form, the hydroxyapatite material has a general formula of $Ca_{10}(PO_4)_6(OH)_2$. In one form, the hydroxyapatite material includes purified HA which may be prepared as described below.

[0022]   Other forms of calcium phosphate containing materials may also be used in lieu of HA or in combination with HA. For example, such materials include, but are not limited to monocalcium phosphate (MCP, $Ca(H_2PO_4)_2$), dicalcium phosphate (DCP, $CaHPO_4$), tricalcium phosphate (TCP, $Ca_3(PO_4)_2$), amorphous calcium phosphate (ACP, $Ca_3(PO_4)_2$), tetrcalcium phosphate (TTCP), fluoroapatite (FAP, $Ca_{10}(PO_4)_6(OH,F)_2$), octacalcium phosphate (OCP, $Ca_8H_2(PO_4)_6$).

[0023]   The apatite and/ or calcium phosphate containing material may be used in a variety of amounts. For example, the modified apatite and/ or calcium phosphate material may be used in an amount of about 1% weight to about 99% weight based upon the weight of the reaction product of modified apatite and/or calcium phosphate material and lactide. In another form, less than about 60% is used in the overall composite material. It should be noted that a number of the descriptions and examples below describe the use of HA, but other apatite materials and calcium phosphate materials may similarly be used.

[0024]   The organic material with phosphonic acid functionality may include a variety of different materials, such as phosphorous containing molecules, including N-(2-hydroxy ethyl) iminobis(methyl phosphonic) acid, hydroxyethylphophonic acid, any other hydroxy! or amino containing phosphonic acid, phosphoric acid or any kind of phosphorous containing compound. According to one form, the organic material with phosphonic acid functionality has a general formula of -PO3H2. In one form, the source of phosphonic acid includes N-(2-hydroxyethyl) iminobis(methylphosphonic) acid. The amount of phosphonic material will be determined by the amount of phosphate to precipate the apatite. The molar ratio of phosphonic to phosphate may be 1/5 or lower. The phosphonic agent may contain either -OH or -NH2 or both, which can initiate polymerization of the polymers.

[0025]   The lactide used in the method may also include a variety of different sources. For example, the lactide may include a material has a general formula of $-(OCH(CH_3)CO)_n-$, such as PLA (polylactide). Other polymers can he used to composite with HA include polycaprolactone (PCL), polyglycolide (PGA), PLGA (polylactide-co-glycolide), polyhydroxybutyrate or poly(hydroxyvalerate or polycarbonates) or polyphosphazene or polyanhydrides or other polyesters or polyurethane and natural origin degradable polymers such as cellulose or starch or gelatin or chitosan or peptides and their derivatives. The lactide material may be used in a variety of amounts, such as up to about 99 weight % of the overall composite material as described in Figure 5C. In one form, about weight 50% lactide material is used.

[0026]   The method may also further comprise the step of separating unreacted phosphonic acid containing material from the intermediate hydroxyapatite material by washing with an aqueous solution, such as regular water, preferably distilled water.

[0027]   The polylactide/apatite composite material can further comprise the step of combining the polylactide/apatite material with additional polylactide to form a composite polylactide/apatite material. The polylactide/apatite can be separated by precipitation in a solvent such as chloromethane, choroform, prefereably methylene chloride, and precipitated with an excess of methanol, propanol, acetone, preferably ethanol, and then dried in vacuum to remove the residual solvent.

[0028]   In one form, a calcium-phosphate/ phosphonate hybrid shell intermediate is formed thereby creating a greater amount of reactive hydroxyl groups onto the HA moieties. This structure may be formed through a phosphonic based bi-dentate chelating agent that bonds to the HA surfaces. Afterwards, PLA is covalently grafted from the HA through the increased -OH groups by a phosphonic agent. Improved mechanical properties of the PLA/HA composite results from the chemical bonding of the phosphonic group increasing the activity of surface -OH groups on the HA, at the same time surface-initiated polymerization between the HA and PLA particles improves the HA/ PLA interface.

[0029]   Preparation of HA

[0030]   In one form, the HA was synthesized by solution reaction of $Ca(OH)_2$ and H_3PO_4 (or $CaCl_2$ or $Ca(NOs)_2$ or any other calcium containing salt and Na_3PO_4 or any other phosphate

containing salts). in brief, about 300 - 1000 mL, preferably 500 mL of distilled water was boiled in a Teflon-coated pot, equipped with an electric stirring paddle and a reflux condenser with a CO_2-absorbing NaOH trap to protect from atmospheric CO2, under Ar or nitrogen gas for range 10-60 min. One mole of CaO was added to the water, and 300 mL of H_3PO_4 solution (2 mol/L) was slowly (about 0.5 mL/min) added to the $Ca(OH)_2$ slurry to obtain a final Ca/P molar ratio of about 1.67. The reacting mixture was boiled for two days. The precipitated solid was collected by centrifligation and washed with distilled water. The solid was re-dispersed in boiled distilled water and was re-boiled for another two days. These washing and boiling procedures were repeated until the pH of the supernatant was about 6. At pH 6, any traces of anhydrous dicalcium phosphate (DCPA) that might have formed due to possible local more acidic environments were converted to HA. In some cases, the HA precipitate, collected by centrifugation, was used for phosphonic acid coating. In other cases, the HA precipitate was collected by centrifugation, washed with an organic solvent, e.g. methanol, ethanol, preferably acetone, and dried at about 110 °C.

[0031]   The HA may be modified in a number of different manners to form the HA intermediate. For example, the HA may be modified by coating the material and/or the HA may be modified by co-precipitation.

[0032]   In an aspect for preparing the PLA/HA composite, the HA intermediate is prepared by coating with -OH groups using surface modification (PLA-HA-HIMPA-A) (method A which produces a monolayer surface modification as shown in Figure 5). An exemplary method suspends HA particles in an aqueous solution of N-(2-hydroxy ethyl) iminobis(methylphosphonic) acid (HJMPA) (about 2.5%) at a HA/HIMPA mass ratio of about 5:1 or higher. HIMPA was then used as a bidentate chelating agent to link the non-ionic hydroxy! groups to HA (method A).

[0033]   In another form, HA intermediates are prepared by coating -OH groups using *in situ* co-precipitation (PLA-HA-HIMPA-B). In this method (method B which produces core shell structure as shown in Figure 5), HA was precipitated in the presence of HIMPA. In an exemplary method, the phosphate groups on the HA surface were partially substituted by phosphonic acid groups, the $Ca/(PO4^{3-} + PO3H2)$ molar ratio is 1.67 for HA. It should be noted

that in one form, the molar ratio of calcium to phosphorous may be 5/3. Additionally, in one form, the molar ratio of PO_4^{3-}/PO_3H_2 may be 5/1 or higher.

[0034]   In one form, PLA was grafted on the above prepared HA intermediate particles using surface initiated polymerization (SIP) (Hong et al., "Nano-composite of poly(L-lactide) and surface grafted hydroxyapatide: Mechanical properties and biocompatibiliSy," 2005, *Biomaterials* 26:6296-6304). An exemplary grafting method involved suspending HA in 20 ml of toluene containing 10 µL of SnOct?. acting as a catalyst; and separately dissolving 2g of L-lactide in 10 mL of dry toluene or dimethy!formamide (DMF). The HA suspension was heated to about 90 °C, and then dropped into the L-lactide solution under argon protection and with stirring. Argon protects against ring-opening polymerization of lactide, which is sensitive to moisture and impurities. After the reaction continued at about 140 °C for about 48 hours, the reaction mixture was cooled down to room temperature. The PLA-grafted-HA (PLA-g-HA) particles were separated by centrifugation and washed with excess volumes of methylene chloride to remove the free PLA that did not graft on the surface of the HA particles.

[0035]   According to one form, PLA/PLA-g-HA composites were synthesized from PLA-g-HA and additional PLA. An exemplary method involved dispersing non-treated HA or PLA-g-HA with PLA in a ratio of 1/4, with a varied range from 0% to 100% dispersed in methylene chloride and mixed vigorously for about three hours. The composite was precipitated with an excess of ethanoi to remove the residual solvent.

[0036]   The synthesis methods may also include the step of separating unreacted phosphonic acid containing material from the intermediate hydroxy apatite material. Similarly, the method may include the step of separating unreacted lactide containing material from the polylactide/hydroxyapatite material.

EXAMPLES

[0037]   Example 1

[0038]   HA coated with -OH groups prepared by using *in situ* co-precipitation was characterized by powder X-ray diffraction (XRD). XRD was used to determine the crystalline phases and crystallinity of these phases present in the HA/ PLA composites. As found in Figure 1, pure HA displays a typical crystalline HA prepared by a current precipitation method.

The pattern of the HIMFA coated HA prepared by the above described method using surface modification (method A) shows a slight decrease in crystallinity when compared to that of pure HA. In comparison, the HA-HIMPA prepared by using *in situ* co-precipitation (method B) exhibited more discernable peak broadening, signifying significantly lower crystallinity, as a result of partial substitution of the phosphate in HA by the phosphonate.

[0039]   Example 2

[0040]   Thermogravimetric analysis (TGA) was used to estimate the amount of HIMPA coated on HA intermediates. Figure 2A shows the TGA curves of pure HA and HIMPA coated HA. Both the pure HA and HIMPA coated HA was prepared by the above described surface modification (method A) showed a similar 2.5% mass loss when heated to 600 °C, showing that the amount of the HIMPA coating on HA was too low to be observed by TGA. In contrast, the HA-HIMPA prepared by using *in situ* co-precipitation (method B) showed a 5.5% mass ioss (see Figure **2A),** illustrating a **significant** amount of HIMPA coating. Based on the mass loss differences among the pure HA and the HA-HIMPA prepared by surface modification and *in situ* co-precipitation, the HIMPA content **on HA**-HIMPA prepared by *in situ* co-precipitation was estimated to be about 3%. These results support the concept that phosphonic acid can be more effectively coated on HA using *in situ* co-precipitation method (method B), by formation of a hybrid Ca/ $(PO_4^{3-} + R - PO_4^{2-})$ intermediate shell over the core of HA.

[0041]   Example **3**

[0042]   The amount of PLA grafted **on HA** was also evaluated by TGA. Figure **2A** shows the TGA curves for samples of PLA grafted onto HIMPA coated HA samples by method A (PLA-HA-HIMPA-A), using either DMF or toluene as the solvent. The mean values **of** mass loss for both PLA grafted samples were about 4% mass fraction, which are greater than the 2.5% mass loss for the pure HA and HA-HIMPA-A samples. This demonstrated that a small amount of PLA (**1.5%** mass fraction) can be grafted on HA either in DMF or toluene, with toluene being a more effective solvent. Figure 2B shows the TGA curves for the sample series using the *in situ* **co-precipitation** method B. The mean values of mass loss at 600 °C of HA, HA-HIMPA-B, PLA-HA-HIMPA-B-DMF and PLAHA-HIMPA-B-Toluene were 2.5%, 5.5%, 8.5% **and** 12.5%, respectively. Thus, the amounts of PLA coating on the HA particles prepared by method B in DMF and toluene were approximately **3%** and 7%, respectively. These values are 2 and nearly

5 times, respectively, those produce by method A. The data showed that method B together with toluene as the solvent can efficiently produce a large amount of PLA onto the HA particles. Figure 2C shows the first derivatives of the TGA (DTG) curves of the same samples from Figure 2B. The pure HA exhibited a relatively flat curve except for a broad peak around 320 °C.

[0043]   In contrast, HA-HIMPA-B shows a large peak around 450 °C, due to the loss of HIMPA that was incorporated within the HA. PLA grafted HA from both solvents showed the same mass loss profile around 450 °C In addition, PLA HA-HIMPA-B-toluene shows a significant peak around 260 °C, which can be attributed to the loss of PLA. The sharpness of this peak provides evidence of a large amount of PLA on HA. The above results indicate that phosphonic acid (HIMPA) can be used as an efficient coupling agent to coat HA particles, especially by using *in situ* co-precipitation of HA in the presence of HIMPA (method B). PLA can also be grafted on HIMPA coated HA through surface initiated polymerization. Due to the greater amount and different kind of -OH groups on the HIMPA-HA than that of uncoated HA, more PLA can be grafted on HIMPA-coated HA by using method A or method B than on HA alone. Furthermore, the amount of grafted PLA by method B is greater than that of method A, suggesting that HIMPA coating produced by method B is a more efficient approach to graft more PLA onto HA particles.

[0044]   Example 4

[0045]   The pH dependent ζ-potential and particles size profiles provided further information regarding grafting of PLA onto the HA. The weighted average value *(n = 3)* of median particle size and ζ-potential of the non-treated HA and PLA grafted HA (HA-PLA) are shown in Figures 3A and 3B, respectively. At pH 13, HA presented a negative ζ-potential (Figure 3A). These negative net surface charges prevented the agglomeration of HA, resulting in an average particle size of approximately 4 μm. Titration from pH 13 to pH 4 led to a gradual change of potential from -25.1 mV to -0.6 mV (Figure 3A). Due to the decreasing ^-potential, HA particles tend to conglomerate, resulting in an increase in the average size of HA from 4 μm (pH 13) to 12 μm (pH 4). The sudden decrease in the size of HA below pH 4 is caused by the significant dissolution of HA in the highly acidic solution. Unlike HA, the HA-PLA showed distinctly different pH dependent ζ-potential and particle size profiles (Figure 3B). At pH 13, HA-PLA and HA present similar negative ζ-potential. However, upon titration of acid,

ζ-potential of HA-PLA shows a positive peak (-5.43 mV) at around pH 8, corresponding to the least net surface charge. Additionally, acid titration led to increases in negative charges and reached a peak at pH 5, which then decreased with further decreases in pH. In addition, the mean size of HA-PLA remained nearly constant between pH 11 and pH 5, this is consistent with the concept that PLA coating significantly altered the surface charge and agglomeration properties of HA. The association of -COO- with H+ at below pH 5 reduced the net surface charge of FIA-PLA, causing particle agglomeration and increasing the particle size from 4 µm at pH 5 to 8 µm at pH 2. The PLA coating protected the HA from rapid dissolution in strong-acidic environments.

[0046]   Example 5

[0047]   Figure 4 shows the load-strain curves and corresponding DTS values for the PLA/HA composites. PLA/HA composite from non-treated HA was (17.4 ± 1.0) MPa (mean ± standard deviation; $n = 3$), which is significantly lower than that (30.3 MPa, unpublished data) of the PLA alone samples prepared from the same polymer. The decrease in strength, which is in agreement with the literature results, can be attributed to the weak interfacial adhesion between the PLA matrix and the non-treated HA.

[0048]   In contrast, the DTS of the two composites prepared from interfacially improved HA (PLA/HA-PLA-A and PLA/ HA-PLA-B) were (37.3 ± 1.4) and (38.3 ± 2.3) MPa, respectively. These values are more than twice (p < 0.05) that of the composite from non-treated HA (17.4 MPa) and 23% higher than that of the PLA itself. The increased DTS values are also higher than the DTS values of composites with a similar HA ratio. These results demonstrate that combination of SIP with the phosphonic acid agent can significantly improve the tensile strength of the PLA/HA composite. Because HIMPA can lead to a strong interfacial binding between HA and non-ionic -OH groups of HIMPA, the PLA initially grafted on the coated -HA can be considered as covaiently bond to HA, and the mechanical properties of PLA/HA can be significantly improved.

[0049]   Disclosed herein are methods of improving the interfacial interactions between PLA and HA by grafting PLA directly from the surface of HA via surface initiated polymerization (SIP). This approach consists of several conceptual steps as depicted schematically in Figure 5. The lactide monomer was initiated by the non-ionic -OH groups on the surface of HA in the

presence of **Sn(Oct)2**as the catalyst (Figure 5b). Because literature data indicated that the innate hydroxy] groups (-OH) on the surface of HA may not be sufficiently reactive and the amount of PLA that can be grafted was limited, modification of the FIA surface to increase the amount and the type of -OH groups with greater reactivity was necessary to improve PLA grafting.

[0050]   In one form, HIMPA, a molecule with two phosphonic acid groups on one end and an -OH group on the other end, was used as a **bidentate** chelating agent to link the non-ionic hydroxy! groups to HA (Figure **5a**). It is suspected that HIMPA may lead to a stronger binding with FIA, and a higher amount of HIMPA can be coated on HA than that of mono-phosphonic acid molecule. Of the two approaches used in the present study to attach non-ionic -OH groups on HA particles, method B, an *in situ* co-precipitation method, **in which** HA **was** precipitated in the presence of HIMPA, led to a significantly higher HIMPA incorporation than that by method A. The resulted HIMPA coated HA from method B showed a lower crystallinity than that from method A and standard HA (Figure **1),** suggesting that some phosphonate ions were embedded within the HA crystal lattice, possibly forming a hybrid shell of $Ca/(PO_4^{2-} + R-PCV^-)$.

[0051]   Initiated by the surface non-ionic hydroxy! groups from the HIMPA coating, PLA could be successfully grafted from FIA by using surface initiated polymerization in the presence of SnOct2. Because ring-opening polymerization of lactide (LA) is sensitive to moisture or impurities in the solvent or **reactants,** in one form, all the solvents including toluene and DMF must be dried over sodium or calcium hydride, respectively. Due to the reactivity of the surface -OH from HIMPA coated HA, **the** amount of grafted PLA either from method A or method B was greater than that of non-modified HA. In particular, the greater amount of HIMPA coating formed on HA by method **B** led to a higher amount **of** grafted PLA *(7%* mass fraction), which was higher than that previously reported in **the** literature **(5%).** The combination of phosphonic acid coupling agent and surface initiated polymerization facilitated the PLA to link with **the** HIMPA coated HA surface through **covalent** bonding, and a strong **interfacial** adhesion can thus be established.

[0052]   Due to the improved interface and the entanglement of the PLA on the surface of HA and the PLA matrix, the mechanical properties of PLA/ HA composites prepared from **PLA-grafted** FIA was significantly improved in comparison to that of the composite prepared

using non-grafted HA (17 MPa). This suggests that the interfacial improvement plays a role in the mechanical properties of the composite. The design of biocomposites is more complex than that of conventional monolithic materials because of the iarge number of design variables that must be considered. It is believed that the mechanical properties of PLA/HA composite are affected by the inherent characteristic of PLA such as chemical configuration, crystaliinity, relative molecular mass and polydispersity index, and characteristics of the HA filler such as morphology (particulate or whisker), size distribution, crystaliinity (amorphous or crystalline), preparation method (sintered or solution precipitated). Additional considerations include mass fraction of HA, composite preparation methods (solvent casting, hot pressing, compression molding, melt extrusion, biomimetic process, etc.), interfacial treatment as well as specimens fabrication techniques and conditions (heat pressing, casting, sintering, machining, together with molding temperature, pressure, and processing time).

[0053]   In order to specifically demonstrate the effect of interfacial improvement proposed in this application, the composites for this study were prepared from the same PLA under the same experimental condition, e.g., filler ratio, composite technology, temperature, molding pressure, etc., with the interfacial optimization of the HA particles (non-treated HA or PLA grafted HA) being the only difference.

[0054]   The foregoing descriptions are not intended to represent the only forms of the compositions and methods according to the present application. The percentages provided herein are by weight unless stated otherwise. Changes in form and in proportion of components, as well as the substitution of equivalents, are contemplated as circumstances may suggest or render expedient. Similarly, while compositions and methods have been described herein in conjunction with specific embodiments, many alternatives, modifications, and variations will be apparent to those skilled in the art in light of the foregoing description.

CLAIMS

What is claimed is:

1.   A method for preparing a polylactide/apatite material comprising the steps of:
combining an apatite material selected from the group consisting of a phosphate having a general formula of $Ca_{10}(PO_4)_6X_2$, where X = -OH or F, monocalcium phosphate, dicaicium phosphate, tricalcium phosphate, amorphous calcium phosphate, tetrcalcium phosphate, fluoroapatite, octacalcium phosphate with an organic material having phosphonic acid functionality to form an intermediate apatite and phosphonic acid containing material; and

combining polymers selected from the group consisting of a mterial having a general formula of $-(OCHRCO)_n-$ where R = C1-C10 alkyl or $-(OCH_2CO)_n-$ where n=l-4, or copolymers thereof, polycaprolactone and mixtures thereof with the intermediate apatite and phosphonic acid containing material to form the polylactide/apatite material.

2.   The method of claim 1 further comprising combining the polylactide/ apatite material with additional polylactide to form a composite polylactide/apatite material.

3.   The method of claim 1 wherein the combining the apatite material with the organic material having phosphonic acid functionality introduces -OH groups on the surface of the apatite material to form the intermediate apatite and phosphonic acid containing material.

4.   The method of claim 1 wherein the combining the lactide containing material with the intermediate apatite and phosphonic acid containing material includes surface initiated polymerization of lactide onto the intermediate apatite and phosphonic acid containing material via -OH or $-NH_2$ groups on a surface of the intermediate apatite and phosphonic acid containing material.

5.   The method of claim 1 further comprising separating unreacted organic material having phosphonic acid functionality or other phosphorous containing groups from the intermediate apatite and phosphonic acid containing material.

6.   The method of claim 1 further comprising separating unreacted lactide material from the polylactide/apatite material.

7.   The method of claim 1 wherein the combining the apatite material with the organic material having phosphonic acid functionality includes combining N-(2-hydroxyethyl) iminobis(methylphosphonic} acid or hydroxyethvlphophonic acid or any other hvdroxyl or amino containing phosphonic acid or phosphoric acid or any kind of phosphorous containing compound with the apatite material.

8.   The method of claim 7 wherein the apatite material is suspended in an aqueous solution of N-(2-hydroxyethyi) iminobis(methylphosphonic) acid or hydroxyethylphophonic acid or any other hydroxy! or amino containing phosphonic acid or phosphoric acid or any kind of phosphorous containing compound.

9.   The method of claim 7 wherein apatite is precipitated in the presence of N-(2-hydroxy ethyl) iminobis(methylphosphonic) acid or hydroxyethylphophonic acid or any other hvdroxyl or amino containing phosphonic acid or phosphoric acid or any kind of phosphorous containing compound.

10.   A po!ylactide/ apatite material prepared by a process comprising the steps of:
        combining an apatite material selected from the group consisting of a phosphate having a general formula of $Ca_{10}(PO_4)_6X_2$, where X = -OH or F, monocalcium phosphate, dicalcium phosphate, tricalcium phosphate, amorphous calcium phosphate, tetrcalcium phosphate, fluoroapatite, octacalcium phosphate with an organic material having phosphonic acid functionality to form an intermediate apatite and phosphonic acid containing material; and
        combining a lactide material having a general formula of $-(OCHRCO)_n-$ where R = C1–C1Oalky! or $-(OCH_2CO)_n-$ where n=l-4, or copolymers thereof, polycaprolactone and mixtures

thereof with the intermediate apatite and phosphoric acid containing material to form the polylactide/apatite material.

11.   The polylactide/apatite material of claim 10 wherein the polylactide/apatite material has a diametral tensile strength that is at least 1.5 times the diametral tensile strength of a polylactide/apatite material prepared without combining a apatite material with the organic material having phosphonic acid functionality.

12.   The polylactide/apatite material of claim 10 wherein the method further comprises the step of combining the polylactide/apatite material with additional polylactide to form a composite polylactide/apatite material.

13.   The polylactide/apatite material of claim 10 wherein combining the apatite material with the organic material having phosphonic acid functionality introduces -OH or -NH2 groups on the surface of the apatite material to form the intermediate apatite and phosphonic acid containing material.

14.   The polylactide/apatite material of claim 10 wherein combining the lactide containing material with the intermediate apatite and material includes surface initiated polymerization of lactide onto the intermediate apatite material via -OH or -NIT2 groups found on a surface of the intermediate apatite and phosphonic acid containing material.

15.   The polylactide/apatite material of claim 10 wherein the method further comprises the step of separating unreacted organic material having phosphonic acid functionality from the intermediate apatite and phosphonic acid containing material.

16.   The polylactide/apatite material of claim 10 wherein the method further comprises the step of separating unreacted lactide material from the polylactide/apatite material.

17.   The polylactide/ apatite material of claim 10 wherein combining the apatite material with the organic material having phosphonic acid functionality includes combining N-(2-hydroxy ethyl) iminobis(methylphosphonic) acid or hydroxy ethylphophonic acid or any-other hydroxyl or amino containing phosphonic acid or phosphoric acid or any kind of phosphorous containing compound with the apatite material.

18.   The polylactide/ apatite material of claim 17 wherein the apatite material is suspended in an aqueous solution of N-(2~hydroxyethyl) iminobis(methyiphosphonic) acid or hydroxyethylphophonic acid or any other hydro xyl or amino containing phosphonic acid or phosphoric acid or any kind of phosphorous containing compound.

19.   The polylactide/ apatite material of claim 17 wherein hydroxyapatite is precipitated in the presence of N-(2~hydroxyethyi) iminobis(methy!phosphonic) acid or hydroxyethylphophonic acid or any other hydro xyl or amino containing phosphonic acid or phosphoric acid or any kind of phosphorous containing compound.

20.   A poly lactide / apatite composition comprising:
an apatite and phosphonic acid containing substrate having lactide coupled to at least a portion of the apatite and phosphonic acid containing substrate through -OH or -NH groups on the substrate having lactide functionality.

21.   The polylactide/ apatite composition of claim 20 further comprising a plurality of poly!actide/apatite molecules joined to one another via lactide containing compounds thereby forming a polylactide /apatite composite.
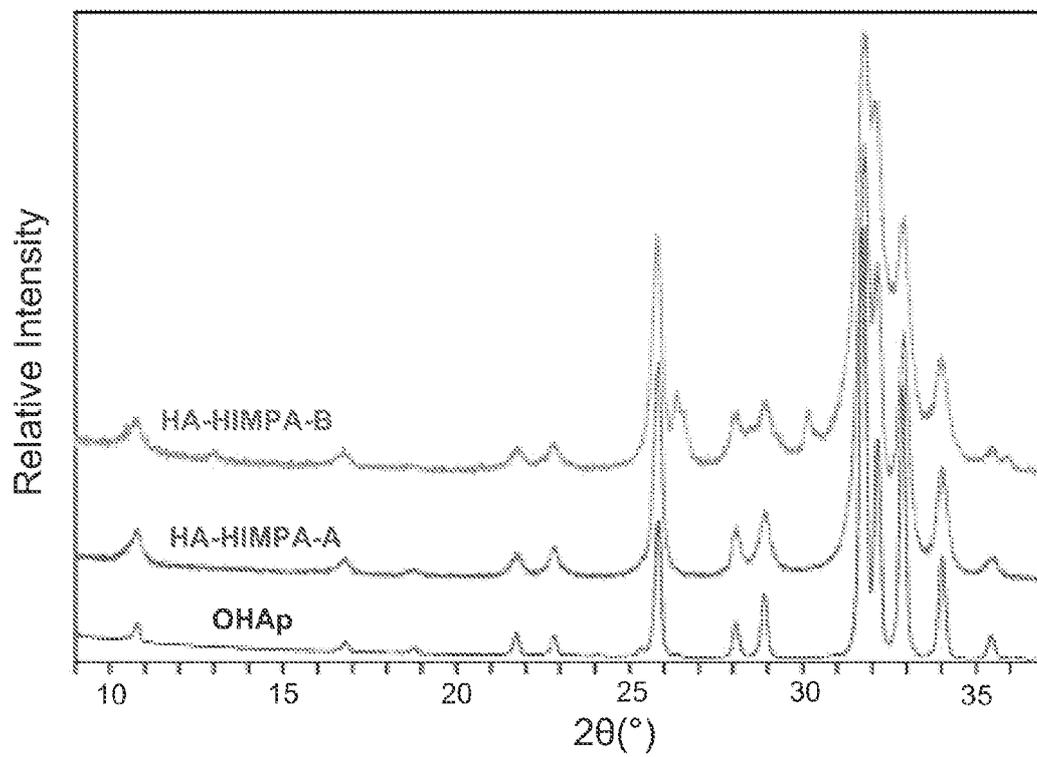
22.   The polylactide/ apatite composition of claim 21 wherein the polylactide/ apatite composite has a diametral tensile strength that is at least 1.5 times the diametral tensile strength of a polylactide/ apatite material that does not contain phosphonic acid functionality.

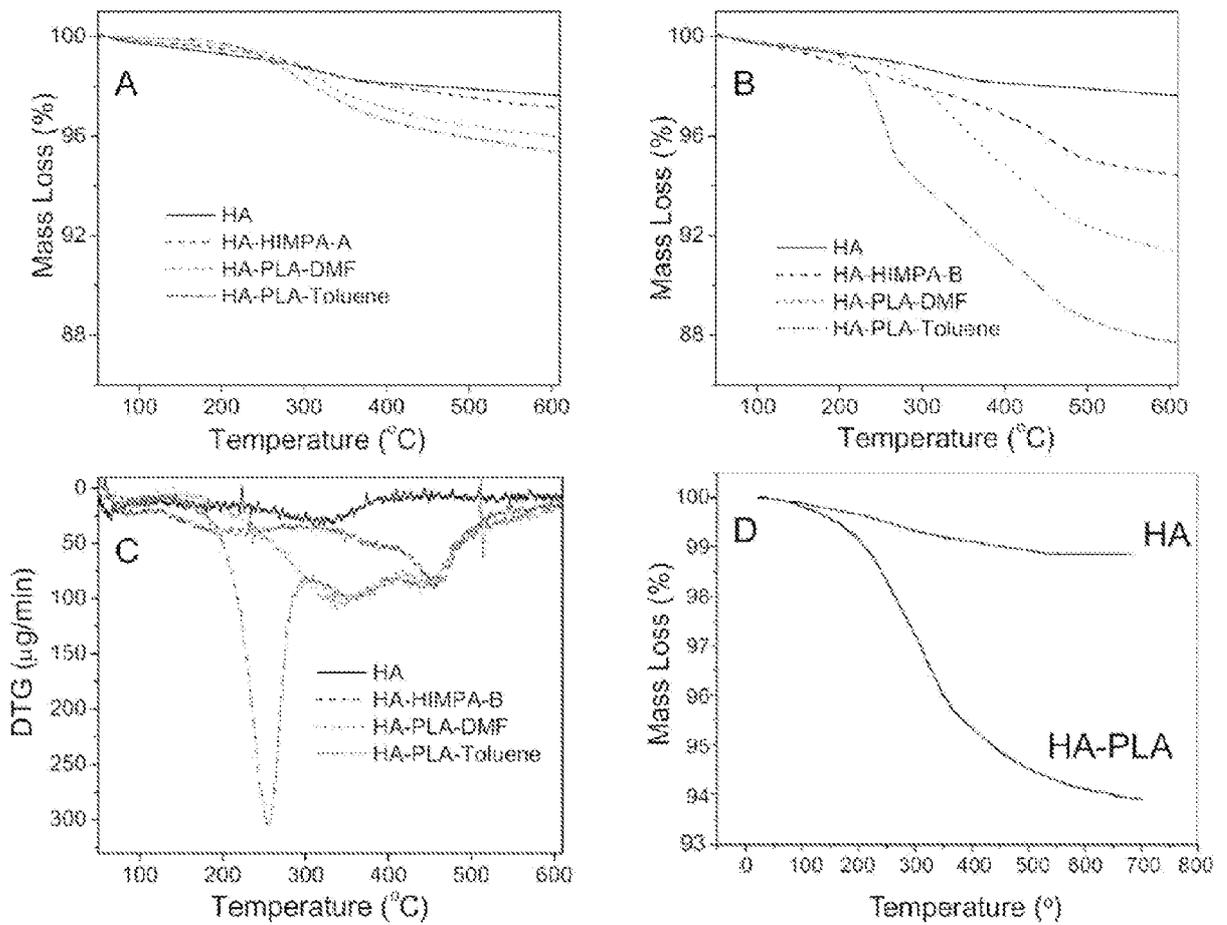23.   A lactide/ calcium phosphate containing composition prepared by a process comprising the steps of:

combining a calcium phosphate containing material selected from the group consisting of a phosphate having a general formula of $Ca_{10}(PO_4)_6X_2$, where $X = $ -OH or F, monocalcium phosphate, dlcakium phosphate, tricalcium phosphate, amorphous calcium phosphate, tetrcalcium phosphate, fluoroapatite, octacalcium phosphate with an organic material having phosphonic acid functionality to form an intermediate calcium phosphate and phosphonic acid containing material; and

combining a biodegradable polymeric material selected from the group consisting of polyhydroxybutyrate, poly(hydroxyvaierate), polyphosphazene, polyester, poiyurethane and cellulose, starch, gelatin, chitosan, peptide and derivatives thereof with the intermediate calcium phosphate and phosphonic acid containing material to form the polylactide/ calcium phosphate material.

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FIGURE 1
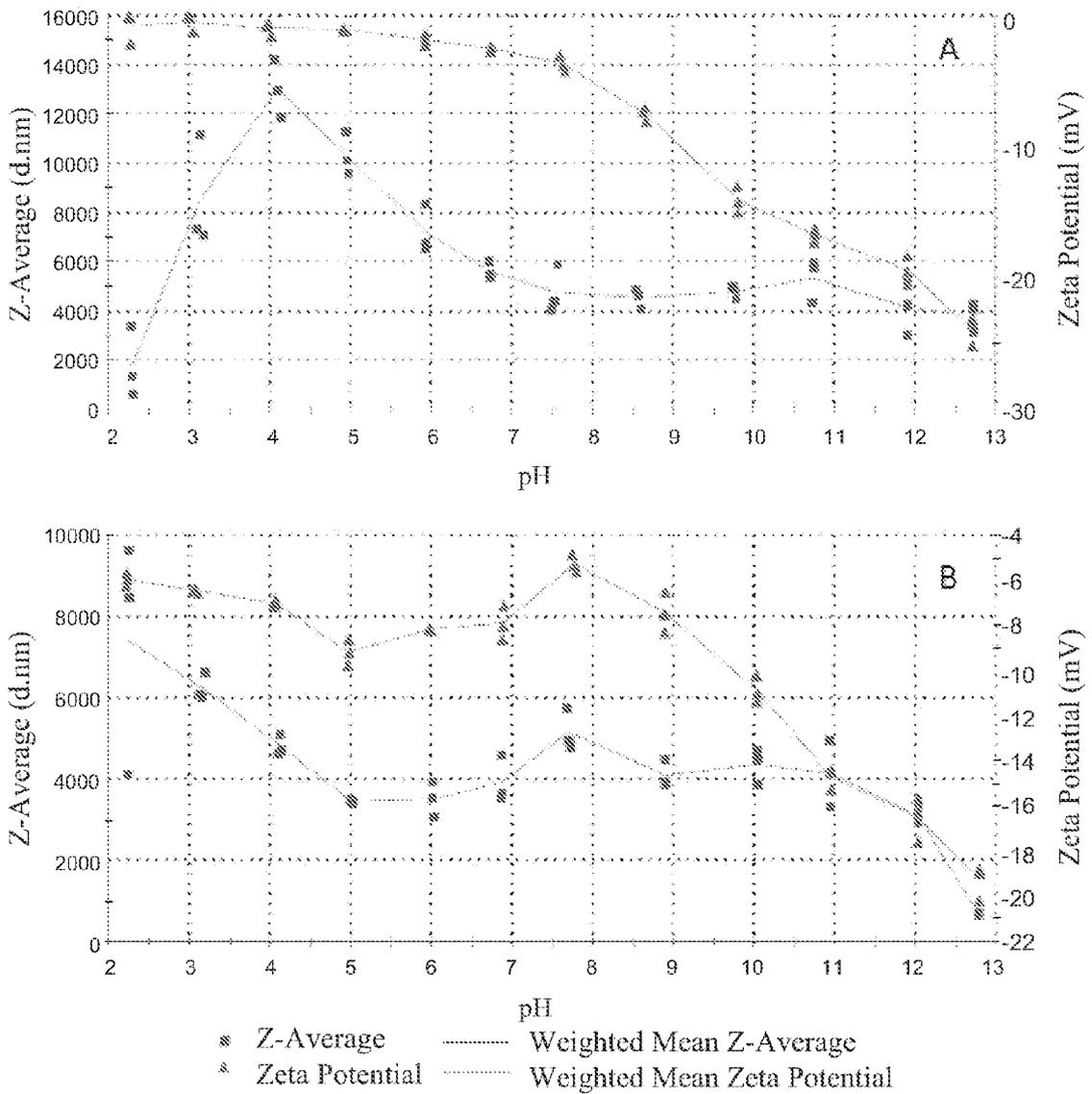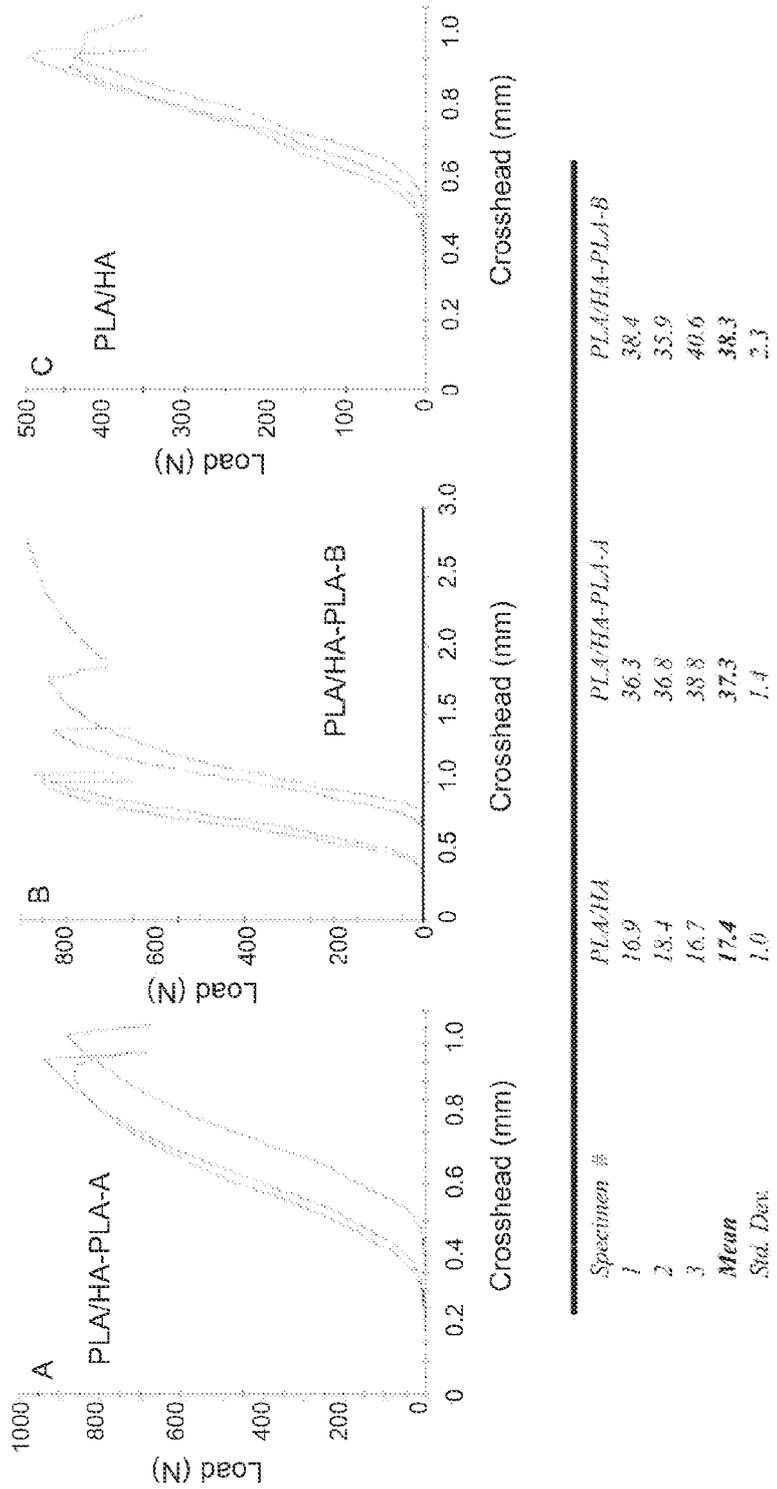
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FIGURES 2A-2D

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pH Titration Graph

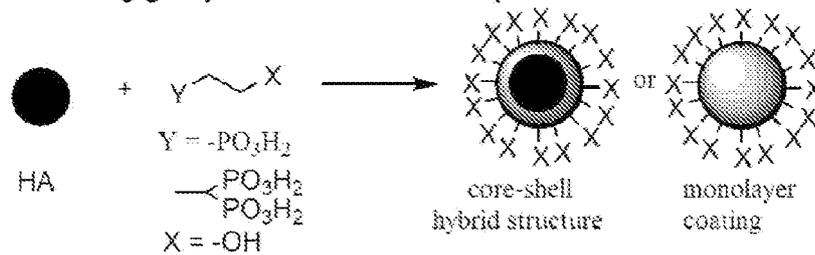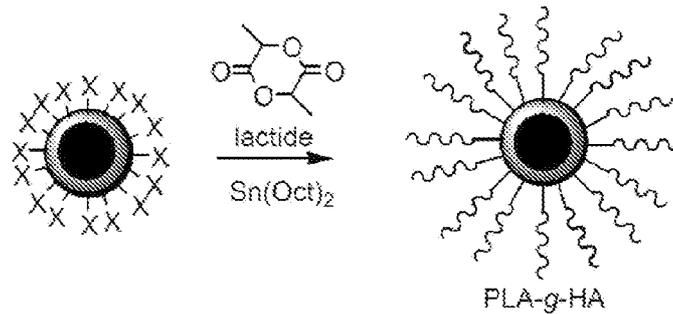FIGURES 3A-3B

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Specimen #	PLA/HA	PLA/HA-PLA-A	PLA/HA-PLA-B
1	16.9	36.3	38.4
2	18.4	36.8	35.9
3	16.7	38.8	40.6
Mean	17.4	37.3	38.3
Std. Dev.	1.0	1.4	2.3

FIGURES 4A–4C

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(a) Coat initiating groups onto surface of HA particle:

Y = -PO$_3$H$_2$

—$\langle$ PO$_3$H$_2$
        PO$_3$H$_2$

X = -OH

HA

core-shell
hybrid structure

or

monolayer
coating

(b) Graft PLA onto HA particle through surface initiated polymerization:

lactide

Sn(Oct)$_2$

PLA-*g*-HA

(c) Prepare PLA/HA composite:

PLA

PLA-*g*-HA

PLA/PLA-*g*-HA composite

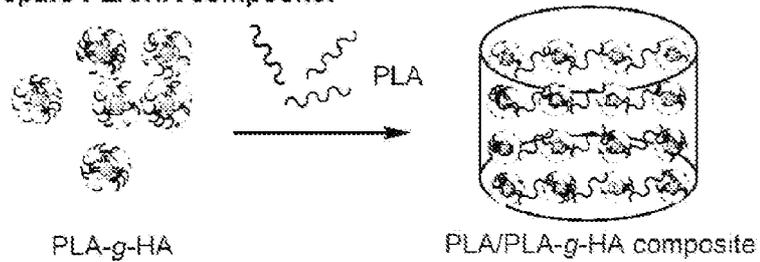*FIGURE 5*

International application No.

PCT/US 13/29839

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C08G 69/1 4 (201 3.01)
USPC - 528/31 8

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C08G 69/14 (2013.01)
USPC - 528/318

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8) - C07F 2/00, 5/00; A61L 27/00, 27/32, 27/46, 27/54; A61 P35/00; A61K31/6
USPC - 424/423, 426, 486; 514/108; 562/13

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Minesoft Patbase, Google Patent, Google Scholar; keywords: Apatite, hydroxyapatite or (octacalcium w2 phosphate) or (tricalcium w2 phosphate) or (carbonated w2 hydroxyapatite) or (fluorinated w2 hydroxyapatite) or brushite or (magnesium w2 hydroxyapatite) or (dicalcium w2 phosphate) or (amorphous calcium phosphate), (posphon* or diphosphon* or bipo

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2010/0160467 A1 (Lee et al.) 24 June 2010 (24.06.2010) para [0002], [0012], [0013], [0014], [0018]	I , 3 , 5-8, 10, 13, 15-18, 23 ------------------------------
Y		2 , 4 , 9 , 11, 12, 14, 19-22
Y	US 2004/0023048 A1 (Schwartz'048 et al.) 05 February 2004 (05.02.2004) para [0022], [0023], [0024], [0070]	2 , 4 , 12, 14, 20
Y	US 2008/0226547 A1 (Larsen et. al.) 18 September 2008 (18.09.2008) para [0033]	9 , 19
Y	US 2004/0253290 A1 (Kim et al.) 16 December 2004 (16.12.2004) para [0016]	2 1
Y	US 2006/0194008 A1 (Schwartz'008 et al.) 3 1 August 2006 (31.08.2006) para [0022]	I I , 22

☐ Further documents are listed in the continuation of Box C.   ☐

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier application or patent but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later, than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 April 2013 (22.04.2013)	**2 0 MAY 2013**

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450	Lee W. Young
Facsimile No. 571-273-3201	PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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