Effect of a new surface-grafting method for nano-hydroxyapatite on the dispersion and the mechanical enhancement for poly(lactide-*co*-glycolide)

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Abstract. In this paper, a new surface-grafting D, L-lactide (DLLA) for nano-hydroxyapatite (n-HA) with the assist of citric acid was designed. The dispersion of new surface modified n-HA was characterized by Fourier transformation infrared (FTIR) spectroscopy, thermal gravimetric analysis (TGA), X-ray powder diffraction (XRD), transmission electron microscopy (TEM) and dispersion test, and the mechanical enhancement effect for poly(lactide-*co*-glycolide) (PLGA) was evaluated by scanning electron microscopy (SEM), differential scanning calorimeter measurements (DSC) and electro-mechanical universal tester. The results showed that citric acid played a critical role in surface-grafting, which could greatly increase grafting amount and improve dispersion of n-HA, so that it resulted in better interfacial adhesion throughout PLGA matrix, higher crystallinity and better mechanical enhancement for PLGA than the surface-grafting method for n-HA without citric acid, whose bending strength and tensile strength were both over 20% higher than those of pure PLGA when 3 wt% n-HA was added, and it still enhanced 8 and 6% higher than those of pure PLGA even the introduction of 15 wt% n-HA, respectively. The above results suggested that the surface-grafting for n-HA with the aid of citric acid was an ideal novel surface modification method, which could greatly improve the dispersion of n-HA and exhibit excellent mechanical enhancement effect for PLGA, suggesting it has a great potential in the bone fracture internal fixation application in future.

Keywords: polymer composites, nanocomposites, nano-hydroxyapatite, surface modification, mechanical properties

1. Introduction

Poly (lactic acid-*co*-glycolic acid) (PLGA) has been widely studied as bone fracture internal fixation materials [1]. However, the relatively poor mechanical property is insufficient to apply in dense bone, and the lack of bioactivity is unfavorable to bone fracture healing quickly [2, 3]. While nano-hydroxyapatite (n-HA) is similar to the inorganic component of natural bone, which not only has good biocompatibility and osteo-conductivity, but also has good mechanical enhancement effect for polymer as nanoparticles [4, 5]. Therefore, extensive efforts have been devoted to the research of introducing n-HA to

PLGA, which is expected to enhance its mechanical property and improve its bioactivity [6–8].

However, the incorporation of unmodified n-HA would decrease mechanical property of PLGA, due to the agglomeration of the n-HA in the PLGA matrix and the lack of adhesion between the n-HA particles and the PLGA matrix [9], which might limit the application of PLGA as bone fracture internal fixation materials. So surface-modification for n-HA is a very key technique. Consequently, various surface modifications for n-HA have been developed, such as silane coupling agents, zirconyl salt, polyacids, isocyanate, dodecyl alcohol and lac-

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tide (LA) [10–14]. Among the above surface modification for n-HA, grafting ring-opening polymerization of LA onto the surface of n-HA particles is a very effective way to improve the interfacial affinity of n-HA to the PLGA matrix. However, when n-HA is directly treated by LA, the grafting amount is still low because of the poor reactivity of the hydroxyl groups on the surface of n-HA and the bad dispersion of n-HA in hydrophobic solvent. Accordingly, the mechanical enhancement effect for PLGA isn't ideal when higher n-HA content was added into PLGA [15]. However, generally speaking, the higher addition content of n-HA, the better osteological bioactivity of PLGA [16]. Therefore, new surface-grafting methods for n-HA should be explored so as to have both better mechanical enhancement effect and higher n-HA addition content for PLGA. In our previous study, we have investigated some surface-grafting methods for n-HA. For example, we found that the surface modification method of combining stearic acid with surface-grafting L-lactide was an effective modification method for n-HA, where stearic acid could improve the dispersion of n-HA during surface-grafting L-lactide, so the addition of 3 wt% n-HA into PLGA had the best mechanical enhancement for PLGA, while the addition of 15 wt% n-HA decreased markedly its bending strength [17, 18]. Subsequently, we designed another surface-grafting method of combining silane coupling reagent (KH550) with surface-grafting L-lactide (L-LA), where KH550 not only could improve the hydrophobicity of n-HA acting as coupling agent but also could provide amino group to graft-polymerize with L-lactide onto the n-HA surface, so the mechanical enhancement effect was far more excellent than corresponding unmodified n-HA, however, the maximum addition content of n-HA into PLGA was only 10 wt% [19].

Based on the above results, to further improve the dispersion of n-HA and the mechanical enhancement for PLGA by the addition of higher n-HA content, a new surface-grafting method with the assistance of citric acid was reported in this paper, where molecular structure of citric acid has three carboxyl groups and one hydroxyl group, and the steric hindrance of citric acid is conducive to improve the dispersion of n-HA, so it is expected to improve grafting amount for n-HA by forming a Ca carboxylate bond with citric acid, and D, L-lactide could be ring-opening graft-polymerized onto the n-HA sur-

face by the hydroxyl group of citric acid, based on the literature [20]. Moreover, the new surface-grafted n-HA was introduced into PLGA matrix with different contents of 3, 8, 15 and 25wt% to investigate the mechanical enhancement effect for PLGA, comparing with the surface-grafted n-HA without citric acid. The main aim of the study is to provide an ideal novel surface modification method for n-HA, so that it could improve the dispersion of n-HA and mechanical enhancement effect for PLGA, and it would have a great potential in the bone fracture internal fixation application in future.

2. Materials and methods

2.1. Materials

n-HA was prepared in our laboratory, whose average size of nanoparticles was about 100–120 nm in length and 20–40 nm in width. PLGA, whose copolymer composition (LA:GA) is 95:05 (mol:mol), $M_w = 3.6 \cdot 10^5$ – $3.9 \cdot 10^5$, was also prepared in our laboratory. Citric acid, AR, purchased from chemical reagent factory, Kelong, Chengdu. D, L-lactide (DLLA, Purac company) was recrystallized from ethyl acetate under argon atmosphere. Stannous octanoate was obtained from Sigma Company. All other reagents were of the analytical grade.

2.2. Surface modification of n-HA with the assist of citric acid

10 g citric acid in DMF was added into 10 g n-HA suspended in DMF with stirring. The reaction was maintained at 150°C for 11 h under nitrogen. The powder product was washed by ethanol and vacuum-dried, and 8 g n-HA product powder of surface-modified by citric acid was uniformly dispersed in dimethylbenzene by ultrasonic treatment. Then 8 g DLLA and 0.004 g $\text{Sn}(\text{Oct})_2$ were added under nitrogen. The mixture was maintained at 140°C for 20 h under nitrogen. After it was washed with excessive dichloromethane and vacuum-dried, the obtained product powder was named as g1-n-HA. The n-HA of surface-grafted without citric acid was prepared in the same procedure as the control, which was named as g2-n-HA.

2.3. Characteristics of surface-modified n-HA

The IR analysis of unmodified n-HA and two surface-grafted n-HA powders were performed using Fourier transform infrared spectroscopy (Thermo Nicolet 670, USA) in KBr disks.

The amount of grafted organic materials were determined by TGA (Perkin-Elmer 7 series thermal analysis system) at a heating rate of 10°C/min from room temperature to 700°C under a high-purity nitrogen atmosphere.

X-ray diffraction analysis were carried out by XRD powder diffractometer (Philips, X' Pert Pro, Cu K α). A scan axis of 2 θ was used to obtain diffraction patterns of a scan range from 15 to 70°. The voltage used was 40 kV and the current was 45 mA.

TEM (JME-100CX, Seike Instruments) investigations were taken on a 200 kV acceleration voltage. The specimens were prepared by dripping a drop of 0.1% suspension onto a TEM grid covered with carbon film and evaporated the solvent completely at room temperature.

The dispersion test was valued by the colloid stability according to the intuitive photographs taken by a conventional camera at different time. The powder was dispersed in dichloromethane (1.0 g/L).

2.4. Preparation of g-n-HA/PLGA composites

The pure PLGA was dissolved in dichloromethane, and pre-calculated two surface-grafted n-HA uniformly dispersed in ethanol and dichloromethane were slowly added into PLGA solution by the addition of 3, 8, 15 and 25 wt%, respectively. Then the mixtures were stirred with ultrasonic treatment for more than 4 h. The precipitates were washed with excessive ethanol and dried under vacuum at 40°C for 48 h.

2.5. Properties of g-n-HA/PLGA composites

Fracture surfaces of PLGA after different addition contents of two surface-grafted were observed by SEM (S-520, Hitachi, Japan) after gold coating, which was used to investigate the dispersion of the g-n-HA nanoparticles in PLGA matrix and the interface microstructure.

The thermal properties of PLGA after different addition contents of two surface-grafted were measured by DSC (Q20, TA instruments, USA) at a heating rate of 10°C/min from room temperature to 190°C with flowing nitrogen gas, holding at 190°C for 5 min to erase thermal history, then cooled to 40°C with 10°C/min cooling rate, finally heated to 190°C with 10°C/min rate again, and the second heat curve was analyzed. The crystallinity (X_c) was calculated according to Equation (1):

$$X_c = \frac{\Delta H_m}{\omega \cdot \Delta H_{m,100\%}} \quad (1)$$

where ΔH_m indicates the melting enthalpy, and ω is the weight fraction of PLGA in the composite. $\Delta H_{m,100\%}$ is the theoretical enthalpy (93.7 J/g) of completely crystalline poly(L-lactic acid) (PLLA) [21].

The mechanical enhancement effect for PLGA after different addition contents of two surface-grafted were measured by an electromechanical universal testing machine (CMT6000, Sans, China). According to GB/T1042-92, the three-point bending strength of rectangular bars of 4 mm×6 mm×60 mm were measured at a crosshead speed of 20 mm/min, and the tensile strength of specimens with dimensions of 50 mm×6 mm×0.5 mm were measured at a speed of 1 mm/min, at 22°C and a relative humidity of 60%. The average value of five measurements was given.

3. Results and discussion

3.1. Characterization of surface modification reactions for n-HA

3.1.1. IR analysis

Figure 1 gives the FTIR spectra of unmodified n-HA and surface-modified n-HA. Some of the main absorption peaks of PO $_4^{3-}$, –OH groups and H $_2$ O were identified for n-HA (shown in Figure 1 curve a). In addition, the split peaks at 1420.0 and 1465.6 cm $^{-1}$ belonging to CO $_3^{2-}$ asymmetric stretch peak are also observed, which was resulted from the minor CO $_3^{2-}$ inclusion during the n-HA synthesis. These results are in accordance with those of n-HA in literature [22]. After surface modification, besides these bands, for g2-n-HA exhibits bands of the carbonyl groups at 1737.5 cm $^{-1}$, and some small peaks observed between 3000 and 2850 cm $^{-1}$ are assigned

Figure 1. IR spectra of (a) unmodified n-HA, (b) g2-n-HA, (c) n-HA of citric acid modified, (d) g1-n-HA

to C–H stretching vibration of the CH_3 - and CH-groups (shown in Figure 1 curve b), suggesting that ester bonds originated from the polymerization of DLLA on the n-HA surface existed. For the n-HA modified by citric acid (shown in Figure 1 curve c), small peaks of C–H stretching vibration emerges and the peak of COO^- at 1597.4 cm^{-1} is observed, implying that calcium carboxylate on the surface of n-HA was formed. While g1-n-HA exhibits a new absorption peak at 1602.1 cm^{-1} belonging to carbonyl groups of PLA, besides for the carbonyl groups at 1735.6 cm^{-1} (shown in Figure 1 curve d). According to the above analysis, we concluded that citric acid and PLA were both successfully grafted onto the surface of n-HA.

3.1.2. TGA

The amount of grafted organic substances is determined by TGA, as shown in Figure 2. According to the TGA curves, the weight losses of unmodified n-HA, n-HA of citric acid modified and two g-n-HA are 4.703, 8.06%, 12.86 and 6.303%, respectively. The surface-grafted amount onto n-HA is calculated as follows by the equation, the grafting amounts [%] = W_1 [%] – W_0 [%], where W_1 is the weight loss of surface-grafted n-HA and W_0 is the weight loss of unmodified n-HA. Therefore, the total grafting amounts onto n-HA of citric acid, g1-n-HA and g2-n-HA are 3.357, 8.157 and 1.600%, respectively, and the grafting amounts of PLA onto g1-n-HA and g2-n-HA are 4.800 (8.157%–3.357%) and 1.600%, respectively. Obviously, it is further proved that n-HA surface was successful grafted quantitatively. Moreover, the grafting amount of g1-n-HA is larger than that of g2-n-HA, which shows that citric acid played an important role in increasing grafting amounts of PLA onto n-HA, and the reason may be attributed to the higher reactivity of the

hydroxyl groups of citric acid and the better dispersion of n-HA due to the steric hindrance of citric acid during the processing of grafting with D, L-lactide, comparing with n-HA directly grafted with D, L-lactide without citric acid. The higher grafting amounts onto n-HA would be benefit to improve the dispersion of n-HA and interface adhesion with PLGA matrix when it was added into PLGA [23].

3.1.3. XRD

Figure 3 gives the XRD patterns of unmodified n-HA and two g-n-HA. The unmodified n-HA exhibits characteristic peaks at 2θ regions of 26, 32–34, 40, and $46\text{--}54^\circ$, which is consistent with the crystalline nature of n-HA in literature [24]. Comparing the XRD patterns of g-n-HA with unmodified n-HA, it can be found that the characteristic diffractions of two g-n-HA nanocrystals are nearly the same as those of unmodified n-HA, which shows that there are no other new apatite crystalline phases, namely, the surface modifications didn't significantly affect the crystalline phases of n-HA. However, it is easy to find that the intensity of characteristic crystalline peaks of 2θ at 26° becomes a little weaker after being modified, and the crystalline peaks of g1-n-HA is weaker than that of g2-n-HA, whose crystallinity of unmodified n-HA, g1-n-HA and g2-n-HA calculated with XRD analysis software Jade are 80.81, 72.00 and 80.53%, respectively. The difference of crystallinity is attributed to the covering of the organic substances grafted on n-HA, and the larger the grafting amount is, the lower the crystallinity of n-HA is, which is in agreement with the results of TGA.

Based on these, the surface-grafting reaction with citric acid for n-HA are assumed as shown in Figure 4. First, Ca on the surface of n-HA reacted with car-

Figure 2. TGA curves of (a) unmodified n-HA, (b) g2-n-HA, (c) n-HA of citric acid modified, (d) g1-n-HA

Figure 3. XRD patterns of (a) unmodified n-HA, (b) g2-n-HA, (c) g1-n-HA

Figure 4. Illustration of the surface grafting with citric acid for n-HA

boxylate of citric acid to form a Ca Carboxylate bond (Step (1)). Then the propagation reaction in the presence of $\text{Sn}(\text{Oct})_2$ included in succession the coordination of both the lactide and the hydroxyl group of calcium citric acid on the surface of HA modified by citric acid with $\text{Sn}(\text{Oct})_2$ followed by cleavage of the acyl-oxygen bond of the D, L-lactide by the Sn-coordinate insertion mechanism and thereby activated hydroxyl group as indicated in (Step (2)) [20].

3.1.4. TEM

TEM photographs of unmodified n-HA and two g-n-HA are shown in Figure 5. It can be seen that they are all needles, indicating that the morphology of g-n-HA nanoparticles did not change after being surface-modified. However, unmodified n-HA nanoparticles exhibit aggregation phenomenon in dichloromethane (seen in Figure 5a), while the dispersion of two g-n-HA are obviously improved after being surface-modified, as shown in Figure 5b and

5c. Especially, g1-n-HA has better dispersibility than g2-n-HA, which may be resulted from its higher grafting amount.

3.1.5. Dispersion test

To further investigate the dispersion effect of surface modification for n-HA, the three different samples were dispersed in dichloromethane, and the intuitive photographs taken by a conventional camera are given in Figure 6. As expected, unmodified n-HA particles precipitated in hydrophobic organic solution immediately after ultrasonication. However, after n-HA particles was coated by PLA chains, the surface wettability was greatly improved, so that the two g-n-HA could maintain a stable dispersion in suspension for a longer time. Especially, g1-n-HA exhibits more excellent colloid stability, which can remain stable for one day, while a little precipitate appears in the suspension of g2-n-HA. Obviously, the dispersion of g-n-HA is closely related

Figure 5. TEM photographs of (a) unmodified n-HA, (b) g2-n-HA, (c) g1-n-HA.

Figure 6. Photographs of (a) unmodified n-HA, (b) g2-n-HA, (c) g1-n-HA dispersed in dichloromethane for different time

to the grafting amount on the surface of n-HA. This result shows that the novel method of surface-grafted with the assistance of citric acid for n-HA could change the surface wettability and prevent the aggregation of n-HA particles is more efficient than the method of the directly surface-grafted LA without citric acid for n-HA, and the dispersibility is better than some literature [25], which may be more useful to enhance mechanical properties for PLGA.

3.2. Properties of g-n-HA/PLGA composites

3.2.1. SEM observation

To investigate the dispersion of g-n-HA particles in PLGA matrix and the interfacial adhesion, the SEM

analysis has been performed. The SEM micrographs are given in Figure 7. For g2-n-HA/PLGA composite, there are many cavities between g2-n-HA particles and PLGA matrix, and many agglomerated g2-n-HA particles emerge when the g2-n-HA content is up to 8 wt%, indicating that there was poor dispersion and interfacial adhesion. However, for the g1-n-HA/PLGA composite, there are few cavities and agglomerated particles until the g1-n-HA content increased to 25 wt%. Obviously, it can be concluded that the g1-n-HA dispersed much more uniformly in PLGA matrix than g2-n-HA, so surface-grafted with the assistance of citric acid for n-HA is a novel surface-grafting method to improve

Figure 7. SEM micrographs of different g-n-HA addition content of g2-n-HA at (a) 3 wt%, (b) 8 wt%, (c) 15 wt%, (d) 25 wt% and g1-n-HA (A) 3 wt%, (B) 8 wt%, (C) 15 wt%, (D) 25 wt%. The arrows show the agglomerate g-n-HA particles in PLGA matrix

the dispersion of nanoparticles in PLGA matrix and the interfacial adhesion, which has a close relationship with the mechanical enhancement effect for PLGA [26].

3.2.2. Thermal properties

To further investigate the thermal properties, the DSC curves of PLGA after the different addition of two g-n-HA are shown in Figure 8, and the thermal properties data calculated from DSC curves are listed in Table 1. It can be found that the melting temperature (T_m) seems to have no relationship with the loading of g-n-HA particles, while the crystallinity of PLGA matrix increased with the increasing g-n-HA content. What is more, the crystallinity of PLGA after the addition of g1-n-HA is higher than that of corresponding addition of g2-n-HA, which indicates the g1-n-HA acts as a more effective heterogeneous nucleating agent than g2-n-HA. The reason is that the better dispersion of g1-n-HA in PLGA matrix, the more conducive it is to absorb PLGA matrix onto the surface of g1-n-HA particles,

so that the nucleation and crystallinity are further promoted, which may be another factor to enhance the mechanical property for PLGA [27].

3.2.3. Mechanical properties

The initial mechanical property is usually a critical technical index for bone fracture internal fixation materials. The enhancement dependency of bending strength and tensile strength for PLGA after different addition content of two g-n-HA are shown in Figure 9. Obviously, it can be seen that the maximum enhancement of bending strength and tensile strength is at 3 wt% surface-grafted, and it leads to a reduction in the bending strength and tensile strength with the further increasing g-n-HA content. Moreover, g1-n-HA has more improvement in mechanical properties than g2-n-HA, whose bending strength and tensile strength were both over 20% higher than those of pure PLGA when 3 wt% g1-n-HA was added. Especially, even 15 wt% g1-n-HA was added, whose bending and tensile strength are still 8 and 6% higher than those of pure PLGA, respectively. However, in our previous study, the maximum addition content of n-HA into PLGA was only 10 wt%, and the addition of 15 wt% n-HA decreased markedly its bending strength [18, 19]. Obviously, the mechanical enhancement for PLGA is by far the most excellent as comparing to some related literatures [28]. The main reason may also be ascribed to the higher grafting amount for g1-n-HA, which might improve the dispersion of g1-n-HA nanoparticles in PLGA matrix and the interfacial adhesion as well as the increased crystallinity for PLGA.

Figure 8. DSC thermograms of samples of (a) PLGA, (b) 3 wt%, (c) 8 wt%, (d) 5 wt%, (e) 25 wt% of g2-n-HA, (f) 3 wt%, (g) 8 wt%, (h) 15 wt%, (i) 25 wt% of g1-n-HA

4. Conclusions

In this study, a new method of surface-grafting D, L-lactide (DLLA) with the assistance of citric acid

Table 1. Thermal properties of PLGA after different addition content of two g-n-HA determined by DSC

Sample	Addition g-n-HA content [wt%]	T_m [°C]	ΔH_m [J·g ⁻¹]	X_c [%]
PLGA	0	166.78	2.423	2.586
g2-n-HA/PLGA	3	164.24	5.221	5.744
	8	161.98	10.45	12.12
	15	161.68	13.99	17.57
	25	161.93	18.14	25.81
g1-n-HA/PLGA	3	161.86	9.623	10.59
	8	161.71	16.05	18.62
	15	161.13	18.56	23.30
	25	160.92	22.96	32.67

Figure 9. Mechanical properties of different g-n-HA content (a) bending strength, (b) tensile strength (red curve is g1-n-HA, and black curve is g2-n-HA)

for n-HA was designed, comparing with the surface-grafting without citric acid, and the two surface-grafted n-HA were respectively introduced into PLGA matrix with different contents. The results showed that the two surface-grafting methods for n-HA were both successful. However, citric acid played a critical role in surface-grafting, which had much higher grafting amount and better dispersion than surface-grafting without citric acid, so that the g-n-HA/PLGA composites exhibited more excellent mechanical properties owing to the better dispersion and interfacial adhesion throughout PLGA matrix, and the higher crystallinity for PLGA, whose bending strength and tensile strength were both over 20% higher than those of pure PLGA when 3 wt% n-HA was added. Especially, even 15 wt% n-HA was added, whose bending and tensile strength were still 8 and 6% higher than those of PLGA, respectively. The above results suggested that the new surface-grafting with the aid of citric acid for n-HA was an ideal novel surface modification method, which could greatly improve the dispersion and achieve a better mechanical strength for g-n-HA/PLGA composite with higher n-HA content, suggesting that it has a great potential in the bone fracture internal fixation application in future. For the degradability and biological properties of the g-n-HA/PLGA composite, the work is in progress, and the results would be reported in another paper in the future.

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