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Preparation of a nano-hydroxyapatite/chitosan/konjac glucomannan composite as a novel degradable drug delivery system

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A Nano-hydroxyapatite (n-HA)/chitosan (CS)/konjac glucomannan (KGM) composite was prepared by integrating composition and molding. Then, X-ray diffraction (XRD), scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) were used to analyze the physical, chemical and degradable properties of the composite before and after immersion in simulated body fluid (SBF). Moreover, an in vitro test for drug delivery revealed that the amount of released pentoxifylline (1-[5-oxohexyl]-3, 7-dimethylxanthine)(PTX) reached a plateau and equaled 80% of the drug loaded in an implant. The newly developed n-HA/CS/KGM composite may serve as a good degradable biomaterial for an implantable drug delivery system (IDDS) in bone tissue engineering.

Key words: n-HA/CS/KGM composite, In vitro test, Degradability, Drug delivery.

Introduction

Inorganic materials have been widely used as biomaterials. Inparticular n-HA, this material is known for its good biocompatibility and bioactive bone behavior [1]. Recently, more attention has been paid to biodegradable biomaterials. An implantable drug delivery system (IDDS) is an example of a biodegradable biomaterial available for therapeutic use. The major advantages of this system include targeted local delivery of a drug less drug required to teat the diseaseal state, minimization of possible side effects, enhanced efficacy of treatment, and no need for further surgery of remove the implant [2]. konjac glucomannan (KGM) is a neutral polysaccharide isolated from the tubers of Amorphophallus konjac C. Koch, degraded by glycolysis [3]. Besides, chitosan (CS) derived from the natural polymer chitin, is a biodegradable cationic polysaccharide comprised of glucosamine residues. It is known to accelerate wound healing, bone formation and drug delivery [4].

The purpose of this paper is to investigate n-HA/CS/KGM composite as an IDDS: firstly, covering the preparation of this composite; then, make an in vitro study of the physicochemical and degradable behaviors of the n-HA/CS/KGM composite; furthermore, investigating the profile of pentoxifylline (PTX) released in vitro from this composite.

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Experimental Procedures

Firstly, ZnO-bearing n-HA composite powders were prepared in our laboratory and dissolved in ethanol, noted as A solution. Secondly, CS (80% deacetylation with an average molecular weight of 250,000) powders were dissolved into 3 wt% acetic acid solution with stirring for 4 hours to get a transparent solution, noted as B solution. Then, B solution was slowly dropped into A solution with vigorous stirring to a weight ratio of 14 : 1 for n-HA/CS. The speed of dropping was around 4 ml/minute and the rotational speed of the stirrer was adjusted to 1000 rpm. The reaction was carried out at room temperature. After titration, the stirring was maintained for 8 hours, and then the slurry obtained was aged for another 24 hours. The precipitate was filtered and washed with deionized water, and dried in a vacuum oven at 60 °C. Secondly, n-HA/CS composite powders were mixed with KGM (average molecular weight of 400,000) powders according to the weight ratio of 5 : 1. Subsequently, the powders mixed with a special fixing fluid solution (containing Ca^{2+}, $H_2PO_4^-$ and citrate ions) at P/L(powder/liquid) ratio of 1.5 g/ml, and cast in a mould into columnar shape (Φ12.6 mm × 4.0 mm), compressed under 5 Mpa for 30 s, and heated at 60 °C.

The crystallographic structure of composite powders was determined by X-ray diffraction (XRD) using a Philips X' Pert MPD diffractometer using CuKα-radiation. The surface topology of the composite was examined using scanning electron microscopy (SEM, Hitachi S-450). The elemental composition of the films was determined with by energy dispersive spectroscopy

Table 1. Ion concentrations in simulated body fluid (SBF) and human blood plasma

	Ion Concentration/mM							
	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}	SO_4^{2-}
SBF	142.0	5.0	2.5	1.5	147.8	4.2	1.0	0.5
Human plasma	142.0	5.0	2.5	1.5	103.0	27.0	1.0	0.5

The fluid was buffered to pH = 7.40 at 37 °C with tri (hydroxymethyl) aminomethane and hydrochloric acid (Li 1993)

(EDS, Philips 9100-60).

The SBF was prepared by dissolving reagent chemicals in the order of NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$, and Na_2SO_4 into deionized water (Table 1) [5].

All specimens were immersed in 6 ml SBF for 4 and 12 weeks. All the test tubes were placed into a water bath with a constant temperature (37 °C). At the end of this experiment, composites were retrieved out of the SBF solution. After rinsing in water and drying at room temperature, the specimens were characterized by XRD, SEM and EDS.

The implants were filled with 3 mg of pentoxifylline (PTX) each. The implants were introduced into 10 ml of phosphatebuffer dissolution medium at pH = 7.35, in round-bottomed flasks which were placed in a water bath (Elpan357) maintained at 37 °C and shaken at 50 strokes/minute. In the course of the release test, samples were withdrawn by a pipette at suitable intervals. pentoxifylline (PTX) concentrations in the collected samples were measured spectrophotometrically (DU-70 Spectrophotometer, Beckman) at a wavelength of 274 nm. The data represented the average of two measurements from independent experiments.

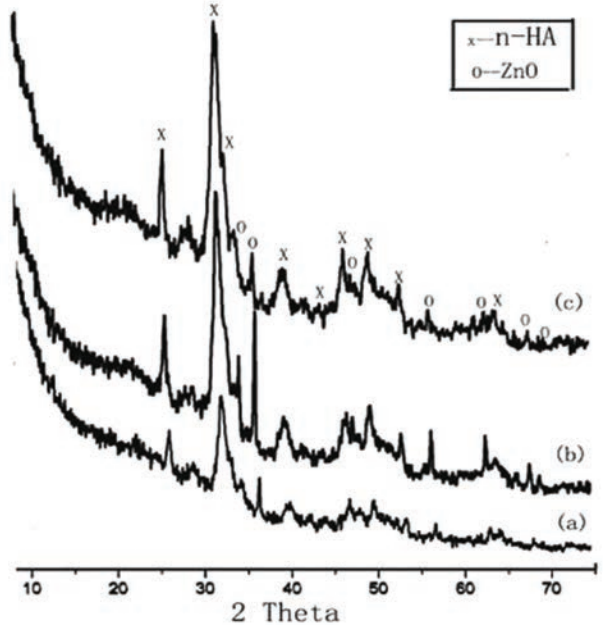

Fig. 1. XRD patterns of composites after up to 12 weeks incubation in SBF at 37 °C: (a) 0 w, (b) 4 w, (c) 12 w.

Results and Discussion

Figure 1 shows the XRD patterns of the composite before and after incubation in SBF. Unsoaked composites (shown in Fig. 1(a)) show a crystalline apatite structure with reflections (002), (211), (112), (202), respectively appearing at 25.9°, 31.9°, 32.4° and 34.1° from the XRD pattern of HA powder (JCPDS # 09-0432). While the reflection peaks was seen at 34.13°, 38.48°, 47.68° and 56.70° at 2-Theta corresponding to ZnO (JCPDS # 36-1451) (100), (002), (102), and (110) planes. This paper employs the method of calcium phosphate cement (CPC) self-setting, which consists of a three phase composition, molding and drug loading simultaneously in one procedure. The ZnO-bearing n-HA composite was incorporated into the CS solution. Hydrogen bonding and coordination bonding are formed between each other. In addition, which KGM introduced into the two phases, new hydrogen bonding formed between CS and KGM, which resulted in relative degrees of the polymer (10° and 20°) which cannot almost be detected in the composite. This is attributed to the hydrogen bonding formed between CS and KGM. The XRD evaluation also confirms that the 12 week incubation of the composite in SBF resulted in increasing peaks around 25.9°, 31-33°, indicating apatite formation during the soaking periods. According to the Scherrer formulation [6], it can be got the values of $D_{(002)}$ were 58.32 nm and 64.12 nm at 4 w and 12 w, respectively. It was found that the soaking time was critical towards obtaining well-crystallized HA particles with uniform morphologies. Occluded impurities are removed and crystal strain is reduced during the soaking time, whereas HA grains redissolve and are re-crystallized into more ordered forms.

SEM examination of the composite surface shows the morphologies before and after incubation in the SBF. It can be seen from Fig. 2(A) and 2(a) that the pre-immersed specimen has a smooth and comparatively dense surface, in which n-HA particles are intimately associated with the organic matrix. With incubation in the SBF, it can be seen that many tiny apatite crystals precipitated on the surface, especially around the pores. Some pores were even filled with apatite particles and more pores appear on the surface (seen in Fig. 2(B) and 2(b)). With a 12-week soak, apatite precipitates pile up on the surface and cover some of these pores. On the other hand, the degradation of CS and KGM made more n-HA particles be exposed and thus induced more apatite

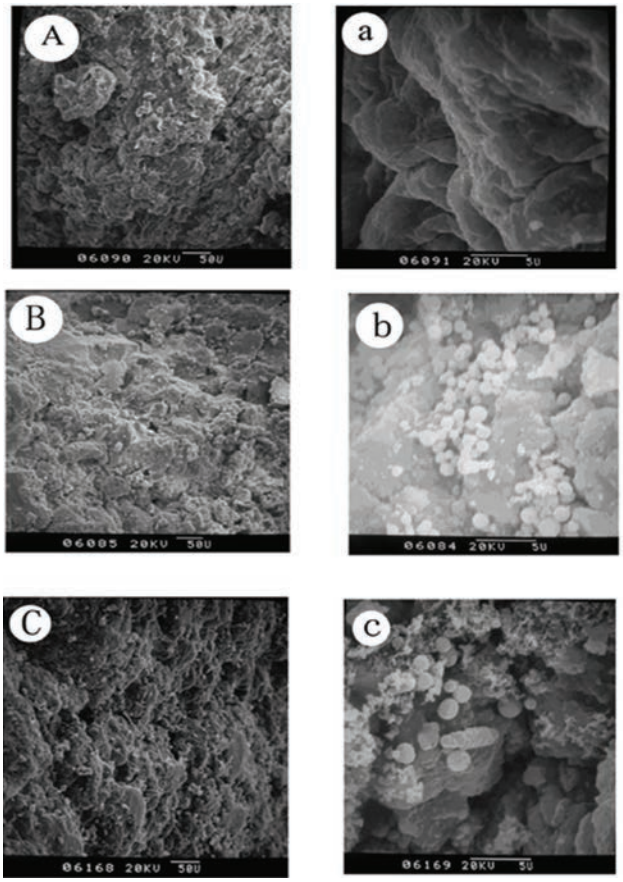

Fig. 2. SEM images of the composite after up to 12 weeks incubation in SBF at 37 °C: 0 w (A) (a) higher magnification, 4 w (B) (b) higher magnification, 12 w (C) (c) higher magnification.

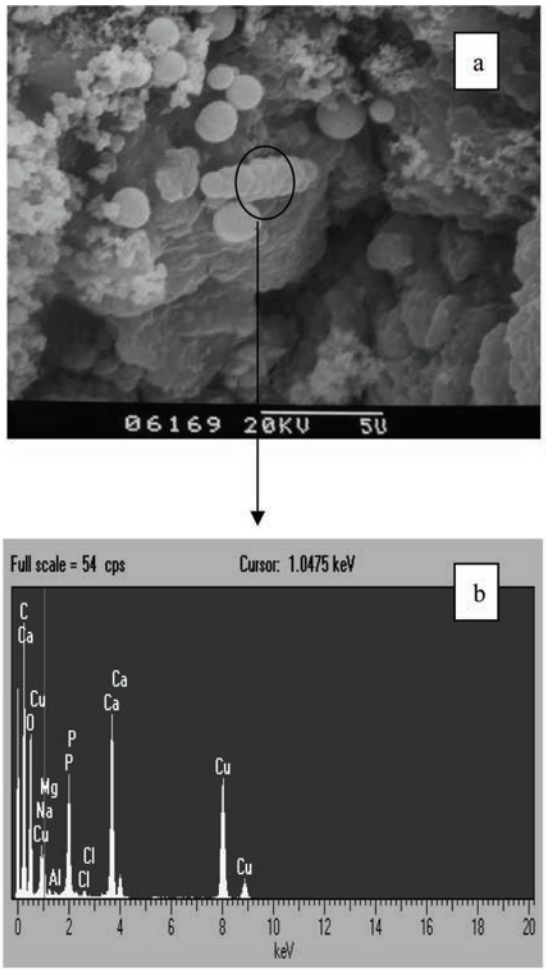

Fig. 3. (a) SEM of implant soaked in SBF for 12 w; (b) EDS of the circled area.

crystals to be deposited. Therefore, the intensity of diffraction peaks for n-HA was strengthened. Comparing Fig. 2(C) with Fig. 2(c), it can be seen that the crystallized apatite particles are in a more ordered form than for others, which is the same as the XRD conclusion.

Table 2 lists the Ca/P ratio changes of composite surface composities before and after incubation in SBF as determined by EDS. These analyses reveal that the Ca/P ratio of the composite surface is higher than the theoretical value for HA, i.e. 1.71 instead of 1.67. With soaking time for weeks on the specimen surfaces, the crystals, from Ca/P deposition, formed and became more compact, and the whole mass becomes squeezed together into a more dense form (shown in Fig. 2). Further, as a new layer forms and buries the old layer, the layers of surface become compact to form a much denser type of specimen, which has little pore space. Meanwhile, in the process of recrystallization, the growth of new crystals (presented in Fig. 3) at the expense of old ones, reduces the the percentage of porosities with time.

Table 2. EDS measurement of the Ca/P ratio of the composite after incubation in SBF.

Week	Ca	P	Ca/P ratio
0	44.68	26.13	1.71
4 w	46.38	28.81	1.61
12 w	50.25	31.61	1.59

The composite promotes the deposition of the apatite crystallites from the SBF, and suggests that some secondary nucleation could occur on the surface of the composite. The dissolution-precipitation mechanism is prominent on the surface of the composite in this process. Also the deposition of Ca and P as well as the change of the Ca/P ratio are similar to the process of formation of new apatite crystallites. The partial dissolution of n-HA is intimately involved in causing the release of Ca^{2+}, HPO_4^{2-} and PO_4^{3-} and increasing the supersaturation of the micro-environment. In addition, new apatite crystallite forms, such as dicalcium phosphate dihydrate (DCPD), octacalcium phosphate (OCP) and Mg-substituted HA [7, 8], can form directly in the physiological environment, using the calcium and phosphate ions released. From the partially dissolved n-HA and from the SBF which contain other electrolytes, notably Mg^{2+}, causing So the Ca/P ratio of the composite to vary between respectively 1.71 to 1.59 during the incubation time in the SBF.

According to the classical nucleation theory, heterogeneous

nucleation takes place always preferentially in a supersaturated solution due to the lower free energy of nuclei. Taking into account the composition of SBF, some of the possible reactions in the solution are [9, 10]:

$$10Ca^{2+} + 6PO_4^{3-} + 2OH^- \longrightarrow Ca_{10}(PO_4)_6(OH)_2 \quad (HAp) \quad (1)$$

$$3Ca^{2+} + 2PO_4^{3-} \longrightarrow Ca_3(PO_4)_2 \quad (2)$$

$$Ca^{2+} + HPO_4^{2-} \longrightarrow CaHPO_4 \cdot 2H_2O \quad (DCPD) \quad (3)$$

$$9Ca^{2+} + 6PO_4^{3-} + H_2O \longrightarrow Ca_9(HPO_4)(PO_4)_5(OH) \quad (CDHA) \quad (4)$$

$$10Ca^{2+} + (6-X)HPO_4^{2-} + (X+Y)CO_3^{2-} \longrightarrow Ca_{10}(PO_4)_{6-X}(CO_3)_{X+Y}(OH)_{2-y} \quad (CHA) \quad (5)$$

A somewhat lower Ca/P ratio of the deposited apatite crystallites can be observed in the SBF compared with the in vivo situation [11], which is due to the effect of proteins on the formation of HA in vivo [12]. Nevertheless, this observation confirms that the introduction of n-HA provides the composite with bioactive properties.

A local delivery application is one way of targeting a drug to a desired site. In bone tissue, sustained drug release can be achieved using hydroxyapatite implants loaded with a given drug. Conventionally, the method of drug loading in IDDS is that implants immerse into the drug solution [13] or sealed with wax [14]. This study employed the method of the CPC self-setting three phases composition£¨shaping and drug loading simultaneously in one procedure. ZnO-bearing n-HA complex was incorporated into the CS solution. Due to the hydrogen bonding and coordination bonding, n-HA/CS composite was formed. As the soaking time was increased, a preferred growth along the (002) axis of the n-HA was observed in XRD patterns, resulting in an increasingly rodlike morphology and a larger average particle size. It was found that the soaking time was critical towards obtaining well-crystallized HA particles with uniform morphologies.

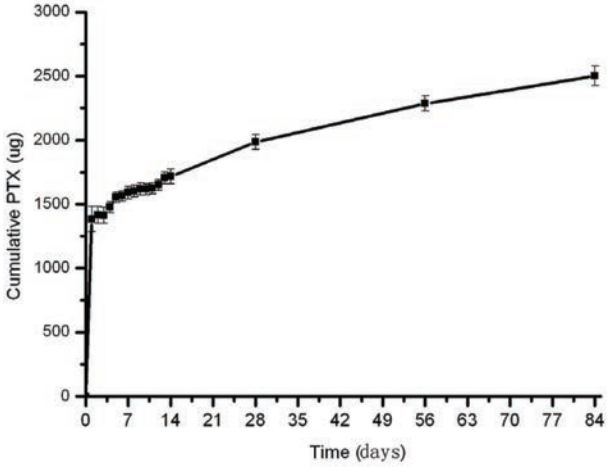

Fig. 4. The cumulative PTX delivery from the n-HA/CS/KGM composite implant with time.

In the past, porous hydroxyapatite implants have been evaluated for IDDS [15, 16]. The amount of drug released reached 80% in less than 40 h [17]. However, to prolong the therapeutic level of drug at the site, a longer release time is required which is important. While in this n-HA/CS/KGM system, the curve which described the amount of PTX released with time had a typically sigmoid-like pattern with a lag time of approximately 3 h, after 60 h, the amount of PTX released reached a plateau and almost equaled 80% of the total amount of drug loaded in an implant as shown in Fig. 4.

In this n-HA/CS/KGM system, the role of the carrier is fulfilled by the n-HA and CS-KGM polymer. With the polymer degradation, the pores on the surface increase with time, which induces an improved rate of drug release. But the surface of implants also changes due to the precipitation-dissolution mechanism. The size and structure of pores become smaller and denser, which induces a lower rate of drug release. After 60 days, the rate is appreciablely decreased.

Conclusions

A n-HA/CS/KGM composite was successfully prepared by integrating composition and molding. And n-HA particles were homogeninally dispersed in the organic matrix. With incubation time in the SBF, some new pores appeared and many apatite particles were deposited on the composite surface. An in vitro test for drug delivery revealed that the amount of PTX released reached a plateau of up to 80% after 60 days. Therefore, the composites developed have a great potential for use as degradable biomaterials for IDDS.

Acknowledgements

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