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Multifunctional nano-hydroxyapatite and alginate/gelatin based sticky gel composites for potential bone regeneration

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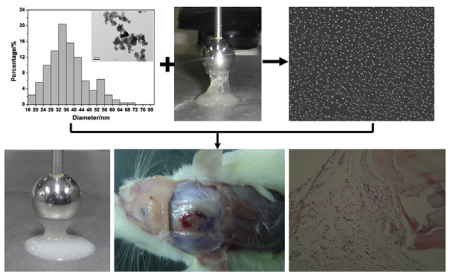
HIGHLIGHTS

- Multifunctional nanohydroxyapatite composite is fabricated.
- The composite consists of nHAP, growth factor, antibiotic and alginate/gelatin gel.
- The composite shows antibacterial effect and good cytocompatibility.
- No adverse effect to the cells tested *in vitro* and *in vivo*.

GRAPHICAL ABSTRACT



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ABSTRACT

To improve the fixations of the implant and implant-bone integration after joint arthroplasty from locally preventing inflammation and promoting the bone regeneration, we design a multifunctional biomaterial consisting of recombinant human bone morphogenetic protein 2 (rhBMP-2) and antibiotic loaded nano-hydroxyapatite with an alginate/gelatin sticky gel. We investigate its role for the prevention of the inflammation and possibility of inducing a new bone growth along with its adhesive ability. The stickiness exists in the composite, which may help to fix itself on the bone fracture surface. The composite sustains the antibacterial effect and promotes the proliferation and differentiation of MG63 cells in *vitro*. *In vivo* experimentation also shows that the composite gel has a role for the reduction of inflammation. It enhances the formation of new bone and blood vessels compared to both the sole rhBMP-2 and non-rhBMP-2/antibiotic loaded composite gels. The multifunctional composite provides a promising material for the prosthetic and bone tissue regeneration.

1. Introduction

The activity limitation, musculoskeletal pain and disability are highly frequent in osteoarthritis. This may negatively affect the quality of life and social participation [1–3]. Joint arthroplasty becomes a popular technology to decrease the pain and improve the function in a cost-effective manner for millions of patients with osteoarthritis [4]. Although bacterial infections following the joint

Abbreviations: nHAP, Nano − hydroxyapitide; VAN, Vanconycin Hydrochloride; rhBMP-2, recombinant human bone morphogenetic protein 2; SA, Sodium alginate; GT, Gelatin; OSA, the oxidized sodium alginate; BHAP, rhBMP-2 loaded nano hydroxyapatite; VHAP, VAN-loaded nano hydroxyapatite; HOG, nHAP/OSA/GT gel; VHOG, VAN-loaded nHAP/OSA/GT gel; BHOG, rhBMP-2 loaded nHAP/OSA/GT gel; VBHOG, VAN- and rhBMP-2 loaded nHAP/OSA/GT gel; SBF, simulated body fluid; SS, silk protein sericin.

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arthroplasty are rare in clinic, these are significant severe complications for the patients [5–8]. The infected implant has to be removed in many of those cases. Usually, the strict antiseptic operative procedures are suggested to prevent the infection including systemic antibiotic prophylaxis combined with the antibiotic-impregnated or antibiotic-loaded cement [9,10]. At present, the systemic antibiotic prophylaxis is thought as a potential treatment procedure in the joint arthroplasty and seems well accepted in clinic [11–13]. However, the usage of antibiotic-impregnated cement remains controversial. The major concerns are the poor drug release kinetic, unreasonable drug-loaded quantity and antibiotic resistance derived from the overuse of topical antibiotics [14]. The research and development of a new antibiotic carrier with more rational drug release kinetics and lower side effect *in vivo* to control and the eradication of infection is still a challenge.

The rapid osteointegration between the implants and the surrounding bones is another important goal for successful long-term survivorship of prostheses especially cement less prostheses to realize biological and earlier stability of implant. Normally, the growth factors including the basic fibroblast growth factor and recombinant human bone morphogenetic protein 2 (rhBMP-2) etc. are combined with the implantable materials to stimulate the proliferation and differentiation of bone-related cells and subsequently to improve the osteointegration of the implants [15–17]. The administration of the factors *in vivo* is very important for a suitable therapy because the rhBMP-2 induces the bone regeneration in a dose-dependent manner [18]. A super-physiological dose may lead to the excessive bone formation and adverse immune responses but inadequate dose is just less effective [19–21]. In order to allow delivery to the treatment site with a therapeutically effective concentration of rhBMP-2 over a prolonged period of time the different biomaterials including hydrogels, capsules and inorganic particles [22–24] are selected as carriers to load and release rhBMP-2. The strategies that can sustain the physiological levels of the factors and to achieve sufficient *in situ* bone formation without any clinical drawbacks are sought for. In our previous study, an antimicrobial composite composed of vancomycin hydrochloride (VAN)-loaded HAP (nHAP) nanoparticles and a mixture of the oxidized sodium alginate (OSA) and gelatin (GT) is prepared [25]. The results *in vitro* show that the composite possesses an adhesive property derived from the gel of OSA and GT. A long-term and continuous antimicrobial performance from the antibiotic-loaded nHAP particles is observed.

In this work, rhBMP-2 is loaded into the hydroxyapatite nanoparticles system along with antibiotic and sodium alginate/gelatin to construct a multifunctional composite. The composite is designed to induce the bone regeneration together with preventing inflammation and reducing the mobilization on the bone fracture surface. The *in vitro* antimicrobial property and cytocompatibility of the composite and the *in vivo* bone repairing capacity in the rat cranium are investigated. The results indicate that this nanocomposite matrix may play an important role for implant − bone integration by promoting bone tissue regeneration.

2. Experimental section

2.1. Materials

Vancomycin hydrochloride (VAN, Eli Lilly Japan K.K), silk sericin proteins (SS, Molecule weight 8 kDa, Huzhou Aotesi Biotechnology Co., Ltd.), sodium alginate (SA, Mai Chao Import & Export Trading Co., Ltd.), gelatin (GT, Mai Chao Import & Export Trading Co., Ltd.), fetal bovine serum and DMEM powders (Gibco BRL Co. Ltd., USA), DiO (3,3′-dioctadecyloxacarbocyanine perchlorate), enhanced BCA protein assay kit and MTT cell proliferation kit and ALP assay kit (Beyotime Institute of Biotechnology, Country), all other chemicals of analytical grade (Hangzhou Mike Chemical Instrument Co., Ltd., China). *Staphylococcus aureus* (*S. aureus*) and osteosarcoma MG-63 cells (Shanghai Institute for Biological Sciences, Chinese Academy of Sciences.) were purchased/obtained for this experimentation.

2.2. Synthesis and characterization of drug-loaded nano-hydroxyapatite (nHAP)

Nano-hydroxyapatite (nHAP) particles were fabricated according to the method described previously with minor modification [25]. Briefly, 50 mL $CaCl_2$ solution (0.05 M) was added into 150 mL silk sericin (SS) solution (0.83 wt%) at 50 °C with agitation for 30 min. 50 mL Na_2HPO_4 solution (0.03 M) was then added dropwise into above SS-$CaCl_2$ solution. The pH value of the reaction system was kept at 10 using 1 M NaOH aqueous solution. The obtained precipitates were collected after 2 h of reaction time by centrifugation and rinsed with ddH_2O and absolute ethyl alcohol for 3 times alternatively. Finally, precipitates were lyophilized and sintered at 650 °C for 3 h to obtain the nHAP particles. For the preparation of VAN-loaded nHAP particles, 100 mg nHAP particles were dispersed in 2 mL SBF (pH 7.4) containing 5 mg VAN, which were then kept in a vacuum at ambient temperature for 24 h. The precipitates (named as VHAP) were collected by centrifugation, and the residual VAN in the supernatant was determined by measuring its concentration by UV–vis spectrophotometer (Hitachi U-2900). The amount of VAN loaded on the nHAP particles was then calculated using the weight differential method.

The recombinant human bone morphogenetic protein 2 (rhBMP-2) was expressed in prokaryotic expression system, purified by Ni ion affinity chromatography column, and then renaturated using urea gradient dialysis according to the method reported previously [26]. To select an optimum load condition of rhBMP-2 on nHAP, 100 mg nHAP particles were dispersed in 2 mL SBF solution containing 100 μg, 200 μg, 300 μg and 400 μg rhBMP-2 at 37 °C for 6 h, 12 h, 24 h and 36 h, respectively. The rhBMP-2 loaded nHAP particles (named as BHAP) were collected by centrifugation and the residual rhBMP-2 in the supernatant was determined by BCA protein assay kit and spectrophotometric microplate reader (BIO-RAD 680, USA). The total amount of rhBMP-2 loaded on the nHAP particles was calculated using the weight differential method. The release of rhBMP-2 from BHAP was performed at 37 °C in an incubator shaker after 100 mg BHAP particles were dispersed in 5 mL SBF for 1 d, 3 d, 5 d, and 7 d, respectively. 0.5 mL SBF was sampled at every time point to detect the release of rhBMP-2. Meanwhile, 0.5 mL fresh SBF pre-warmed at 37 °C was added into the release system to maintain its constant volume.

The chemical composition of obtained particles were analyzed with XRD (ARL X'TRA, Thermo Electron) using a monochromatic CuKα radiation ($\lambda = 1.54056$ nm) in a range of $2\theta = 10–70°$ with a speed of 5°/min and voltage of 40 kV. The morphology of nHAP was investigated using a TEM (JEM-1230, JEOL) at 80 KV after they were dispersed in the absolute ethyl alcohol and the size distribution of nHAP particles was obtained after statistical analysis to 100 nHAPs particles in TEM figure.

2.3. Preparation of the composites and their characterization in vitro

The oxidized sodium alginate (OSA) was prepared by the method described previously with minor modification [27]. Firstly, 2.0 g $NaIO_4$ was added into 200 mL SA solution (2 wt%) with agitation at 25 °C for 4 h in the dark environment. 2 mL glycol was