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A versatile and injectable poly(methyl methacrylate) cement functionalized with quaternized chitosan-glycerophosphate/nanosized hydroxyapatite hydrogels

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ABSTRACT

Injectable polymethylmethacrylate (PMMA) bone cement is alluring because it allows a minimally invasive surgical approach, reducing both the cost of treatment and patient discomfort. However, several documented drawbacks have necessitated the design of a more versatile version with adorable properties for future application. In this study, to amend the bulk behavior of PMMA cement, we synthesized a modified version by combining PMMA with quaternized chitosan (N-(2-hydroxy)propyl-3-trimethylammonium chitosan chloride, HTCC)-based hydrogels loaded with nanosized hydroxyapatite (Nano-HA). Then, the physicochemical properties, antimicrobial properties, remineralization capacity and mechanical performance changes under imitated physiological conditions were tested for these cements using a type K thermocouple, scanning electron microscope (SEM), microcomputed tomography (μ-CT), calcium ion test kit and mechanical compression tests. The results demonstrated that the HTCC-GP thermosensitive hydrogel generated interconnected pores, lower the T_{max} value, lengthened the working time, developed appropriate mechanical properties and imparted excellent antibacterial activity to the cement. The Nano-HA particles engendered improved biomineralization ability of the cements without adversely influencing the mechanical performance. Hence, these results indicated that the injectable and multifunctional cement resulting from the p-PMMA/HTCC-GP/Nano-HA combination grips rosy prospect for future applications in bone reconstruction.

1. Introduction

Due to the marked functional and aesthetic character of the craniofacial complex [1], bone defects in this field are among the most enervating forms of defects. Thus, effective bone grafting is vital and imperative for modern expectations of quality of life. In this context, many researchers have devoted to the advancement of manufactured bone regeneration biomaterials, offering mechanical support temporarily for flawed or missing tissue while inducing and guiding the regeneration of fresh, healthy tissue [2]. Therefore, the application of injectable bone substitute (IBS) is progressively alluring in virtue of its marginally invasive surgical approach, reduction for treatment cost and patients' discomfort [3–5].

As a specialized form of IBS widely used in orthopedic surgery since the 1960s [6], polymethylmethacrylate (PMMA) cement is believed to be effective for immediate bone stabilization considering its desirable properties, for instance, ease of use, robustness, plasticity, budget price and FDA approval [7,8]. However, its documented drawbacks [9–12] include risk of infection, unsatisfactory bioactivity, venture of fractures as the result of mismatched mechanical properties between cement and bone, and, during polymerization, strong exothermic reactions, potentially leading to adjacent soft tissue necrosis and pulmonary embolism. Because of the suboptimal properties of PMMA cement, several modification strategies have been employed over the past few decades [13].

Previous studies confirmed that a porous PMMA structure is favorable because it plays a vital part in the proper balance between

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mechanical features and bone ingrowth induction [14–16]. Incorporating osteoconductive materials or drugs into plain PMMA is also considered as a practical approach to upgrate the characterization of PMMA cement [13,17]. For example, loading osteoconductive calcium phosphates (CaPs) into PMMA-based bone cement promotes bone ingrowth while maintaining the mechanical stability of the cement [18]. Nanosized hydroxyapatite (Nano-HA), a specialized form of CaP, is used in this type of strategy owing to its componential and structural comparability to the inorganic matter of human bone, combined with osteophilic, osteoconductive and biocompatible nature [19]. Moreover, because the implantation of synthetic graft materials commonly results in failure due to infection [20], incorporating antibiotic drugs for the local control of infection improves PMMA-based systems [21].

Chitosan (CS), a polysaccharide deacetylated from naturally plentiful chitin which possessing many remarkable features, e.g., excellent biodegradability, bioactivity, biocompatibility and antibacterial properties, is popular for biomedical and pharmaceutical applications [22–25]. Currently, a variety of chitosan based materials have been broadly investigated for drug delivery and tissue engineering, such as chitin/chitosan nanofibers [26], chitosan-starch-sodium benzoate blend films, PLGA-chitosan/PLGA-alginate nanoparticles blends [27], self-assembled chitosan nanofilms or nanoshells [28], carboxymethyl chitosan polymeric micelles [29], chitosan based thermosensitive hydrogel [30], and so forth. Thereinto, considering the immiscibility, we have successfully designed a porous PMMA structure with interconnected tunnels rendered by integrating a CS-based thermosensitive hydrogel phase [17,31]. Due to its low solubility and the protonation of its amino groups under acidic conditions, CS cannot provide adequate antibacterial activity. Therefore, antibacterial agents accompanied by Nano-HA were preloaded into the CS-based hydrogel. All results proposed that the synthesized multifunctional cement grips rosy prospect for future applications in bone reconstruction. However, the system is complicated, and the addition of antibacterial agents such as antibiotics or metal ions may increase the potential risk of drug resistance or cytotoxicity [32]. Thus, a more optimal merger of PMMA/porogen/osteoconductive CaP materials/antibacterial agent should be established and evaluated to simplify the porous construct and circumvent these problems.

N-(2-Hydroxy)propyl-3-trimethylammonium chitosan chloride (HTCC), a cationic CS derivative, is already well known for its superior antibacterial properties over a wide pH range [33], which is prepared by the grafting reaction between CS and glycidyl trimethylammonium chloride (GTMAC). The introduced *N*-trimethylated quaternary ammonium salt group notably dilutes the hydrogen bonds between CS chains, meanwhile, enhances water solubility, moisture absorption retention capabilities and antibacterial activity of chitosan [34]. Glycerophosphate (GP), an organic compound naturally found in human body, is served to treat phosphate metabolism imbalance, and certain types of GP, e.g. β-GP, have been proven to act as osteogenic media supplements for human bone marrow stem cells [35]. In this context, an injectable thermosensitive HTCC-GP-based hydrogel was developed [35,36]. Such hydrogel could undergo the sol-gel transition at body temperature (37 °C) in a governable procedure time without any copolymerization agent, organic solvent or externally applied triggers for gelation [37]. Moreover, its excellent injectability, biocompatibility, water solubility and antibacterial ability allow this hydrogel to function as a porogen, antibacterial agent and Nano-HA carrier.

Above all, a novel smart injectable bone substitute was designed as a Nano-HA-incorporated porous PMMA/HTCC-GP construct to combine the advantages of the different components. The objective of this study was to characterize its physicochemical properties, mineralization capacity, antibiotic ability and mechanical properties in vitro for future clinical applications. Because of its easy handling, desirable biomineralization ability and powerful anti-infection capacity, the integration of injectable p-PMMA/HTCC-GP/Nano-HA has potential as a material for prospective clinical bone reconstruction. More importantly, we expected that the smart and optimal system could establish a multifunctional platform to meet different clinical needs in the future by carrying different drugs, like protein and peptide, anti-inflammatory drug, antitumor drug, growth factor, and so forth.

2. Experimental section

2.1. Materials

The commercially available PMMA kit (self-curing PMMA, type II) purchased from Shanghai New Century Dental Materials Co. (Shanghai, China), is a two-component system (powder and liquid). The powder involved pure PMMA, initiator (dibenzoyl peroxide, BPO), reinforce (silicon dioxide) and radiopaque agent (barium sulfate). The liquid component was comprised of methylmethacrylate (MMA) and *N*, *N*-dimethyl-p-toluidine (DMPT) solutions which was used as the accelerator during polymerization reaction.

CS (Mw = 179.17 kD; *N*-deacetylation rate of ≥95%; viscosity = 100–200 mPa.s),$Ca(NO_3)_2{\cdot}4H_2O$, $(NH_4)_2HPO_4$, NH_4OH and $NaHCO_3$ were all obtained from Aladdin Co., Ltd. (Shanghai, China). Glycidyl trimethylammonium chloride (GTMAC) (Mw = 151.63 g/mol) and β-GP (Mw = 216.04 g/mol) were purchased from Adamas Reagent Co., Ltd. (Shanghai, China) and Sigma-Aldrich (Sigma-Aldrich, USA), respectively. All other chemicals were of analytical grade or better.

2.2. Cement preparation

In this study, the HTCC-GP thermosensitive hydrogel was first prepared according to the method reported by Qiuxia Ji et al. [38]. In brief, 0.09 g of HTCC and 0.09 g of CS were blended and dissolved in 4 ml of 0.1 M aqueous lactic acid (LA) solution progressively with sufficient mechanical stirring, then chilled in an ice bath for 15 min. Simultaneously, 0.25 g of β-GP was dissolved in DW at a concentration of 25% (*w/v*) and chilled in ice bath along with the HTCC-CS solution. Afterwards, the β-GP solution was added dropwise to the HTCC-CS solution with stirring over another 20 min. The sol-gel- transition was then proceeded in a thermal cutout at 37 °C for 5 min to get the HTCC-GP hydrogel. For the Nano-HA-loaded hydrogel, 250 mg Nano-HA was added into the homogeneous HTCC-GP solution and mixed evenly by ultrasonication, then converted into hydrogel in thermal cutout for 5 min (Fig. 1).

The feed stock for the synthesis of three types of cements are presented in Table 1. As a control group, solid PMMA cement was fabricated following the manufacturer's instructions by manually blending the powder and liquid parts of the PMMA kit in a mass ratio of 1:1 under ambient conditions. For porous PMMA based cement, the blended PMMA phase was further mixed with the HTCC-based hydrogel at a volume ratio of 3:4 until all welcombined. After that, the hybrid was injected into a Teflon mold to obtain a cylindrical sample with a diameter of 6 mm and height of 12 mm. The diagrammatic drawing for the preparation of p-PMMA-based cements is displayed in Fig. 2. After curing overnight, the products were unmolded, washed with DW, lyophilized and imaged by Nikon D3100 digital camera.

2.3. Polymerization temperature and working time

The temperature variation during exothermic polymerization was detected as soon as the intermixture was stuffed into the Teflon cylindrical mold. A type K thermocouple probe (Victory High Electronic Technology Co., China) connected to a data logger (Victor E86, Victory High Electronic Technology Co., China) was placed on the surface center of the cement to monitor the temperature change every 0.2 s (n = 3) until the temperature began to drop. Meanwhile, the working time, defined as the time span available for safely injection through the access needle and after which the compound gets too stiff to inflood [39], was recorded. Room temperature was 24.9 °C.