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Nanostructured material formulated acrylic bone cements with enhanced drug release

Shou-Cang Shen [a,*], Wai Kiong Ng [a], Yuan-Cai Dong [a], Junwei Ng [a], Reginald Beng Hee Tan [a,b,**]

[a] Institute of Chemical and Engineering Sciences, A*STAR (Agency for Science, Technology and Research), 1 Pesek Road, Jurong Island, Singapore 627833, Singapore
[b] Department of Chemical and Biomolecular Engineering, The National University of Singapore, 4 Engineering Drive 4, Singapore 117576, Singapore

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ABSTRACT

To improve antibiotic properties, poly(methyl methacrylate) (PMMA)-based bone cements are formulated with antibiotic and nanostructured materials, such as hydroxyapatite (HAP) nanorods, carbon nanotubes (CNT) and mesoporous silica nanoparticles (MSN) as drug carriers. For nonporous HAP nanorods, the release of gentamicin (GTMC) is not obviously improved when the content of HAP is below 10%; while the high content of HAP shows detrimental to mechanical properties although the release of GTMC can be substantially increased. As a comparison, low content of hollow nanostructured CNT and MSN can enhance drug delivery efficiency. The presence of 5.3% of CNT in formulation can facilitate the release of more than 75% of GTMC in 80 days, however, its mechanical strength is seriously impaired. Among nanostructured drug carriers, antibiotic/MSN formulation can effectively improve drug delivery and exhibit well preserved mechanical properties. The hollow nanostructured materials are believed to build up nano-networks for antibiotic to diffuse from the bone cement matrix to surface and achieve sustained drug release. Based on MSN drug carrier in formulated bone cement, a binary delivery system is also investigated to release GTMC together with other antibiotics.

1. Introduction

Due to the excellent tissue compatibility of poly(methylmethacrylate) (PMMA) and the rapid setting with achieved mechanical strength, PMMA bone cement has been widely used clinically to fix total joint prostheses (hip and knee replacements) to bone for decades. It is an integral part of almost all total knee replacements and also used in at least two-thirds of total hip replacements [1]. With the rapid growth of the aged population, there is a dramatic increase in the burden of musculoskeletal disease, over 1,000,000 total hip and knee replacements are performed each year in the US alone. Post-operation infection is a clinical catastrophe, leading to the need for more complex surgery with much higher costs or the increase of morbidity [2]. As with all implant materials, bone cement carries an elevated risk of infection when implanted into the human body because of the possibility microorganism biofilms to form on an inert surface [3–6], usually requiring multiple surgeries for treatment [7,10]. To reduce this risk, an antibiotic (such as gentamicin) has been formulated with bone cement; however, it is known that as the antibiotic is mostly trapped within the substance of the cement, only a small proportion is available for diffusing out to the desired site for antibacterial activity [8]. The therapeutic antimicrobial activity drops rapidly below effective therapeutic levels after surgery within several days [9] and did not show significant advantages in reducing infection rate [10]. Therefore, it is desired to develop functional antibiotic bone cement that can enhance antimicrobial activity and/or prolong the therapeutic levels of antibiotic formulated in bone cement [11–13]. The sustained antimicrobial activity would be of significant impact in improving the results of joint replacement surgeries.

Researchers have explored many approaches to improve the antimicrobial activity of bone cement. It has been reported that the presence of chitosan nanoparticles could effectively prevent viable bacteria from surviving on the surface of the bone cement [14] and the addition of silver nanoparticles to increase its antimicrobial activity [15]. However, those active ingredients cannot be released to protect surrounding tissues and often have adverse cytotoxic effects [16,17]. Many efforts have been made to improve the release of antibiotics from bone cement for applications in total joint replacement, including vacuum-mixing of commercially available antibiotic-impregnated bone cement, but the effects are limited [18–20]. M. Vallet-Regi et al. reported to enhance the release of GTMC from PMMA based bone cement by formulation with HAP [21,22]. Although the drug elution from the PMMA bone cement was enhanced, the high content of fillers could impair mechanical strength. MSN has been formulated with acrylic bone cement and the resulting mechanical behaviors were characterized [23]. Ormsby et al. [24] studied the incorporation of CNT into acrylic based bone cements

* Corresponding author.
** Correspondence to: R.B.H. Tan, Institute of Chemical and Engineering Sciences, A*STAR (Agency for Science, Technology and Research), 1 Pesek Road, Jurong Island, Singapore 627833, Singapore.
E-mail addresses: shen_shoucang@ices.a-star.edu.sg (S.-C. Shen), reginald_tan@ices.a-star.edu.sg (R.B.H. Tan).

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and reinforced mechanical properties were found. However, the effect of these nanoparticles on delivery of antibiotics has rarely been studied. The sustained release of antibiotics from bone cement is essential to prevent the formation of biofilm on implanted bone cement and prosthesis parts. Thus, it is necessary to develop antibiotic bone cements with enhanced and prolonged antimicrobial activity to protect the surrounding tissues, as the operation sites are prone to infection for up to 6 to 8 weeks after surgery and usually need at least 6 weeks of parenteral antibiotic administration [25].

In this study, nanostructured materials, such as CNT, HAP nanorods and MSN, are formulated into PMMA based bone cement to enhance the release of antibiotics. The presence of hollow nanostructured particles in bone cement builds up diffusion networks and improves the loaded antibiotics to diffuse from bone cement to surface. Moreover, novel bone cement enabled effective delivery of gentamicin in combination with other antibiotics, such as vancomycin.

2. Experimental

2.1. Materials

Mesoporous silica nanoparticles (MSN) were prepared using fluorocarbon-surfactant-mediated synthesized as reported by Han et al. [26]. Typically, 0.5 g of Pluronic P123 and 1.4 g of FC-4 were dissolved in 80 ml of HCl solution (0.02 M), followed by the introduction of 2.0 g of TEOS under stirring. The solution was continuously stirred at 30 °C for 24 h and then transferred into a polypropylene bottle and kept at 100 °C for 24 h. The resultant solid was recovered by centrifuging and washed with deionized water twice, subsequently it was dried at 55 °C for 12 h. To remove the template molecules, the material was heated from room temperature to 550 °C at a heating rate of 2 °C/min and followed by calcination in air for 6 h.

Nanorods of hydroxyapatite (HAP) were prepared by wet gel steaming method [27]. Typically, 15 ml of 0.5 M $Ca(NO_3)_2 \cdot 4H_2O$ (Alfa Aesar) solution was added to 15 ml of 0.5 M $(NH_4)2HPO_4$ (Alfa Aesar) solution to achieve the desired Ca^{2+}/PO_4^{3-} ratio of 1.0 for precipitation of di-calcium phosphate dihydrate ($CaPO_4 \cdot 2H_2O$, Ca: P = 1:1) at 25 °C. The pH of the mixture was adjusted to 11.0 by using aqueous ammonia solution (NH_4OH, 25 wt.%, Merck). The precipitate solid was recovered by filtration and then transferred to a 25 ml beaker, which was placed in an autoclave with a polytetrafluoroethylene (PTFE) liner. 5 ml of the ammonia solution was poured into the bottom of the PTFE cup and physically separated from the solid sample. The autoclave was then placed into an oven at 180 °C for 20 h. After the steam-assisted treatment, the resulting white solid was dispersed in water using ultrasound and recovered by centrifugation. The washing procedure was repeated twice. The obtained solid material was dried in an oven at 55 °C, and finally ground to a fine powder.

The multiwall carbon nanotubes (CNT) were provided by Shenzhen Nanotech Port Co., Ltd.

Three kinds of commercial bone cements were used: CMW Smartset-HV and CMW Smart GHV (DePuy International Ltd. UK) and Simplex P (Stryker Co, UK). The compositions of the bone cements, as indicated in the products' brochure, are shown in Table 1.

2.1.1. PBS buffer

Buffered saline solution pH 7.4 was prepared as described in the British Pharmacopoeia (BP A79): anhydrous di-sodium hydrogen phosphate 2.38 g; anhydrous potassium hydrogen phosphate 0.19 g and sodium chloride 8 g to 1000 ml with deionized water (Milli-Q).

2.1.2. o-Phthaldialdehyde reagent

o-Phthaldialdehyde reagent was prepared according to a procedure reported by Zhang et al. [28]. It was formulated by adding 2.5 g of o-phthaldialdehyde, 62.5 ml methanol and 3 ml of 2-mercaptoethanol to 560 ml of 0.04 M sodium borate in a deionized water solution. The reagent was stored in the darkness and settled for at least 24 h prior to use.

Table 1
Composition of commercial bone cements.

	Simplex P (w/w%)	Smartset HV (w/w%)	Smartset GHV (w/w%)
Powder			
Methylmethacrylate methacrylate copolymer	15.00	84.00	80.46
Methyl methacrylate–stylene copolymer	73.72	–	–
Zirconium dioxide		15.00	14.37
Barium sulfate	10.00		
Benzoyl peroxide	1.28	1.00	0.96
Gentamicin	–	–	4.22
Liquid			
Methylmethacrylate	97.49	97.50	97.50
N,N-dimethyl-*p*-toluidine	2.50	2.50	2.50
Hydroquinone	0.0075		

% by weight (w/w) of powder component and liquid component.

2.2. Preparation of antibiotic-loaded bone cements

GTMC was loaded onto the nanostructured materials (MSN, HAP or CNT) by wet impregnation. Typically, 0.20 g of GTMC was dissolved in 3 ml deionized water. 0.30 g of MSN powder was impregnated with GTMC solution under stirring and aged for 24 h. The mixture was dried under vacuum at room temperature under vacuum for 48 h. The dried GTMC loaded nanoparticles were ground to fine powder and a certain amount of GTMC loaded nanoparticles were mixed with commercial bone cement solid powder by manual grinding. When HAP and CNT are used as drug carriers, the same impregnation method was used and the drug-to-carrier ratio may be adjusted to avoid too high drug loading in the final formulation. Samples of A-1–A-9 prepared by using GTMC–MSN with different drug loadings are listed in Table 2.

The samples of antibiotic loaded bone cement were prepared by mixing the powder with the liquid monomer in a ratio of 2 g/ml in a beaker in a laminar flow hood, in accordance with the manufacturer's instruction. Monomer liquid was added to the polymer–GTMC–MSN mixture in a bowl and was stirred using a spatula until the powder was fully wetted. The soft mixture was inserted into the mold with dimensions of 6 mm in diameter and 12 mm in height. The filled mold was pressed between two glass plates to harden overnight at room temperature. The hardened bone cement cylinders were pulled out of the mold and stored under sterile conditions at room temperature for the in-vitro drug release test and compression test. In addition, rectangular beams with dimension of 25 × 10 × 2 mm were prepared for a bending test and an antibacterial property test.

2.3. In vitro drug release study

The drug release study was conducted by soaking two cylinder samples of each composition in 5 ml PBS buffer (pH 7.2). The sample

Table 2
Composition of nanomaterial formulated antibiotic bone cements.

	Simplex-P powder (g)	MMA (ml)	Filler %	Drug%
A-1	1.90	1.0	MSN 0	3.40
A-2	1.90	1.0	MSN 2.04	1.36
A-3	1.80	1.0	MSN 4.08	2.72
A-4	1.70	1.0	MSN 6.12	4.08
A-5	1.60	1.0	MSN 8.15	5.44
A-6	1.50	1.0	MSN 10.19	6.79
A-7	2.0	1.0	HAP 11.56	3.59
A8	1.0	1.0	HAP 32.33	4.85
A9	2.0	1.0	CNT 5.36	3.21