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Effect of polymerizable quaternary ammonium monomer MEIM-x's alkyl chain length and content on bone cement's antibacterial activity and physicochemical properties

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ABSTRACT

Quaternary ammonium monomers with N-alkyl chain length varied from 6 to 18, namely MEIM-x(x = 6–18), were incorporated into acrylic bone cements to prepare antibacterial bone cements. With the increasing of MEIM-x's alkyl chain length, the MEIM-x monomers' minimum inhibitory concentrations (MIC) decreased to 8 μg/mL and 2 μg/mL (MEIM-14, for *E. coli* and *S. aureus,* respectively) at first and then increased. The 50% hemolytic concentrations (HC50) decreased monotonously with the increasing of alkyl chain length. The bone cements contained 2% and 5% of long-chain MEIM-x (x ≥ 10) showed significant antibacterial activity against *E. coli* and *S. aureus*, while the bone cements contained short-chain MEIM-x(x < 10) showed a weaker activity. Overall, adding 2% of MEIM-x had acceptable influences on the bone cements' properties in terms of doughing time, setting time, peak temperature, solubility, fluid uptake, compressive strength, flexural strength, flexural modulus, hemolysis and cytotoxicity, but adding 5% of MEIM-x impaired the bone cements' mechanical properties and hemolysis significantly.

1. Introduction

Polymethylmethacrylate (PMMA) has been widely used as bone cement to fix orthopaedic implants to the bone for decades (Puska et al., 2003). However, there still exist several shortcomings in this kind of bone cement, such as exothermic polymerization reaction, low mechanical properties, shrinkage during the polymerization, toxicity of monomers, dense surface that prevent bone growing into the cement, and so on (Puska et al., 2003, 2016). Several efforts have been done to solve these problems, for example, incorporating hydrophilic oligomer into bone cement to fabricate porous surface for bone ingrowth (Puska et al., 2005), and using fibers to reinforce the cement (Saha and Pal, 1984; Puska et al., 2004a, 2004b).

Infection is one of the most serious complications following joint replacement surgery (Cats-Baril et al., 2013; Akanda et al., 2018). Many efforts have devoted to reducing the infection rate in the past decades as the out-of-control infection usually brings disastrous outcome, yet the infection rate was still reported to be around 1–3% (Schiavone Panni et al., 2016; Sanz-Ruiz et al., 2017). In the surgery, bone cement is a key material used for fixing the prosthesis and filling the space between bone and prosthesis, thus using antibacterial bone cements is considered an effective way to prevent infection.

There have been a lot of researches that introduced traditional antibacterial agents like antibiotics (Slane et al., 2018; Nichol et al., 2017), silver derivatives (Slane et al., 2015; Prokopovich et al., 2015) and other leachable compounds (Persson et al., 2015; Weckbach et al., 2012) into acrylic bone cements. However, problems such as burst release, high risk of developing drug-resistance, and low mechanical performance still have much room for improvement. In recent years, infection-prevented surface prepared by immobilizing quaternary ammonium salts (QASs) onto the surface of biomaterials gradually raised concerns for they could avoid those problems, since the QASs were covalently bonded to the surface (Xu et al., 2017; Calliess et al., 2012; Poverenov and Klein, 2018; Pant et al., 2017). In the bone cement, there are also several quaternary ammonium compounds, such as quaternised chitosan (Shi et al., 2006; Tang et al., 2012; Tan et al., 2012, 2013, 2014), quaternary ammonium dendrimer (Abid et al., 2017, and quaternary amine dimetharylate (QADMA) (Punyani et al., 2007; Deb et al., 2008), have been applied for the antibacterial purpose.

In general, the N-alkyl quaternary ammonium's biological activity

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as well as physicochemical properties were strongly affected by its alkyl chain length (Pavlíková et al., 1995), thereby, it would be desirable to understand how the alkyl chain length will affect the monomer and bone cement's properties. In our previous study (Zhu et al., 2017), a series of N-alkyl imidazolium methacrylate monomers, namely MEIM-x, were synthesized as comonomer for acrylic bone cement, but the discussion was constrained by single monomer concentration (5% in mass) and limited range of carbon chain length (from C10 to C18).

In this study, MEIM-6 and MEIM-8 that had shorter alkyl chain length were studied together with the other MEIM-x that had been reported previously. All the MEIM-x were added into bone cements with two different mass fractions of 2 wt% and 5 wt%. The samples' immobilized antibacterial activities were measured after conducting an extract-culture protocol to eliminate the influence of residual unreacted monomers. The handling and curing characteristics, mechanical properties and biocompatible properties of the prepared bone cements were tested as well to expand the understanding of the relationship between the structure of quaternary polymerizable antibacterial agents and such properties of bone cements.

2. Materials and methods

2.1. Materials

N, N-Dimethyl-p-toluidine (DMPT), methyl methacrylate (MMA), hydroquinone (HQ) were purchased from J&K Scientific Ltd., China. Benzoyl peroxide (BPO) and zirconium dioxide (ZrO_2) were provided by Shanghai Macklin Biochemical Technology Co., Ltd, China. Poly (methyl methacrylate) (Mw ≈ 120,000 by GPC) was a product of Sigma-Aldrich Co., USA. BPO was dried under vacuum at room temperature for 24 h before being used. The other reagents were used directly without further purification.

MEIM-x (x = 6, 8, 10, 12, 14, 16 and 18, structures were shown in Fig. 1) was synthesized according to the method reported previously (Zhu et al., 2017). All of monomers were column-purified with ethyl acetate and isopropanol (3:1, V:V) and methanol as eluent.

Defibrous rabbit blood was brought from Nanjing Maojie Microbiological Technology Co., Ltd., China, which was collected from healthy New Zealand White rabbits. *Escherichia coli* (ATCC 25922) *and Staphylococcus aureus* (ATCC 6538) were brought from Guangdong Culture Collection Center, China. Rat bone marrow derived stroma cells (rBMSC) were provided by National Engineering Research Center for Tissue Restoration and Reconstruction, South China University of Technology, which were collected from rats aged 2 weeks.

2.2. Evaluation of MEIM-x's antibacterial and hemolytic activity

Minimum inhibitory concentrations (MICs) of MEIM-x were tested in accordance with CLSI microbroth dilution. Briefly, at first, bacteria were cultured in nutrient broth (NB) for 24 h and the suspension was adjusted to 1×10^6 CFU/mL. Every MEIM-x was dissolved by sterilized NB to obtain a stock solution of 8192 μg/mL. 100 μL of the solution was then diluted by 2-fold serial dilutions using NB in 96-well plate. The obtained diluted solutions were mixed with equal volume of bacteria suspension (1×10^6 CFU/mL) to gain a series of bacteria suspension

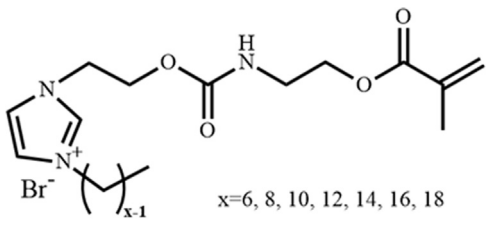

Fig. 1. Chemical structure of MEIM-x.

where MEIM-x's concentration were from 4096, 2048, 1024–2 and 1 μg/mL and bacteria's concentration were 5×10^5 CFU/mL. After 24 h culture in ambient air at 37 °C, each plate well was checked for their visual turbidity. The MIC of each MEIM-x was determined as the lowest concentration that could keep the culture medium clear. The assays were run in triplicate (n = 3) for both two strains.

50% hemolytic concentration (HC50) is the compound's concentration at which 50% of the red blood cells are lysed. At first, diluted rabbit blood was prepared by mixing 8 mL defibrous rabbit blood with 10 mL saline (0.8% NaCl solution), and 8192 μg/mL MEIM-x solutions were prepared by dissolving MEIM-x in saline. The monomer solutions were 2-fold serially diluted by the saline to produce 1 mL dilutions, and then they were supplemented by 20 μL diluted rabbit blood. For negative and positive control, 20 μL of the diluted rabbit blood were added into 1 mL of saline and deionized water, respectively. All of the solutions were then placed in an incubator at 37 °C for 1 h. Subsequently, the solutions were centrifuged at 2800 rpm for 5 min. The supernatant's absorbance at 545 nm was measured by a UV–vis spectrophotometer (Shanghai Youke Instrument Co.,Ltd, China) with deionized water as reference. The hemolysis rate was determined as follows:

$$HR\ (\%) = (A_t - A_N)/(A_P - A_N) \times 100\% \quad (1)$$

where A refers to the absorbance, subscription t means the test solution, subscription N means negative control, subscription P means the positive control.

HR (%) was plotted versus MEIM-x's concentration, and the concentration that caused 50% hemolysis (HC50) was estimated by linear interpolation. The assays were run in triplicate (n = 3).

2.3. Preparation of bone cements with MEIM-x

The PMMA beads were grinded and sieved to obtain beads of 100 mesh size or less. The formulation of each bone cement was shown in Table 1, where the mass ratio of powders and liquid components were 2:1. MEIM-x were dissolved into the liquid component of the bone cement (MEIM-6 could not dissolve completely, the liquid components contained MEIM-6 were shaken extensively before mixing the two components). Two components of bone cement were manually stirred in a plastic bowl at 23 ± 1 °C until dough shape being formed. Afterwards, the dough was immediately filled into different molds to form samples with specific dimensions required by different measurements. After 1 h of curing time, cured samples were removed from the molds for further processing.

2.4. Measurement of doughing time and polymerization exotherm

The powders and liquid components of each bone cement were added into a polypropylene bowl at 23 ± 1 °C. The mixture was stirred till the finger gloved with an unpowdered non-water-rinsed surgical latex glove (SafeTouch 1144 C,A.R. Medicom Inc. Healthcare (Shanghai) Ltd., China) could be first cleanly separated from the freshly-exposed surface of the mixture. The doughing time (t_{dough}) was defined as the time from when two components were contacted to each other to when the mixture no longer adhered to gloves. Subsequently, the dough was filled into a PTFE mold in accordance to ISO-5833

Table 1
Formulations of experimental bone cements containing MEIM-x (the total mass is 100 g).

Formulation	Powders (g)			Liquid (g)		
	PMMA	ZrO_2	BPO	MMA/MEIM-x	DMPT	HQ
Plain cement	55.37	10	1.33	32.58/0	0.67	0.05
MEIM-x-2%	55.37	10	1.33	30.58/2	0.67	0.05
MEIM-x-5%	55.37	10	1.33	27.58/5	0.67	0.05