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Synthesis and Properties of a Polymerizable Quaternary Ammonium Salt

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ABSTRACT: Novel polymerizable monomer N,N-dimethyl-2-[(2-methylacryloyl)oxy] ethanaminium 5-carboxy-2,4-bis benzoate (DMAEMA-PMDPM salt) was synthesized by acid-alkali neutralizing reaction and was proved to be successful by Fourier transform infrared spectroscopy. The antibacterial activity and cytotoxicity of monomer were assessed by determination of the minimal inhibitory concentration and minimal bactericidal concentration, time–kill study and methyltetrazolium test assay. Polymerizable efficiency was determined by measurement of degree of conversion. The results indicated that DMAEMA-PMDPM salt which is a kind of liquid polymerizable has some antimicrobial activity and similar cytotoxicity to common dental resin monomers. The new monomer also has a high polymerizable ability. Therefore it might have a great potential to prepare antimicrobial coatings on denture base and soft lining materials, as well as some biomedical applications. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 41002.

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INTRODUCTION

The number of people who suffers from teeth loosing increases as society becomes increasingly elderly. Removable partial denture and complete denture are widely used for the treatment to patients with dentition defect and edentulous. For patients with a highly absorbed alveolar ridge, soft liners could be a good choice for the improvement of denture retention and the relief of the pain during mastication.[1] However, denture base resin, especially soft lining materials often accumulate biofilms in oral cavity and the adhesion of microorganism can induce infection called denture-induced stomatitis.[2] *Candida albicans* are more frequently isolated from the tissue surface of denture base and soft liners as well as the corresponding region of the oral mucosa. Fungi adhesion is the main reason for the occurrence of stomatitis.[3] Therefore, the antimicrobial modification of the common denture base resin materials and soft-liner is necessary currently.

There are several attempts for the preparation of the antimicrobial denture base resin or soft-lining materials currently, such as making surface treatment and adding antibacterial agents. For the methods of surface treatment, there are some studies about using mannan, silica, and polymer to prepare an antimicrobial coating.[4–7] For the methods of mixing antibacterial agents into matrix, silver, fluorine, triclosan, and other bactericide could be used for antimicrobial modification to the denture base and soft lining materials according to some researches.[8–12] However, there are some problems for these modifications such as poor durability of the antimicrobial functions. Moreover, it is still not a successful product for prevention of infection induced by partial and complete removable dentures treatments.

Quaternary ammonium salt (QAS) is well known as a kind of broad-spectrum antimicrobial agent.[13] Polymerizable QAS monomers are developed by combining the antibacterial agent quaternary ammonium and methacryloyl group into one structure. It can copolymerize with other monomers and the antibacterial agent is covalently bonded to the polymer network. The immobilized agent does not leach out from the material but acts as a contact inhibitor against the bacteria which attach to the surface, so the polymerizable QAS applications could

have an excellent biosecurity and a stable antibacterial activity.[14–16] Our research group developed a series of QAS monomers such as DMAE-CB, MAE-HB, MAE-DB, and proved they could endow materials activity of contact inhibitor against the bacteria.[17–19] QAS can be also selected for the preparation of antimicrobial denture base and soft lining materials.

In this study, a novel polymerizable monomer was designed to be liquid and expected to have similar solubility to common dental resins. Therefore, it could be mixed into materials with higher concentration. It also might have lower monomer leachability due to multiple vinyl groups. Moreover, it is expected to form an antibacterial coating by surface grafting technique. This hydrophilic monomer may be used for the development of a novel hydrogel type of soft liners which could improve the adsorptive power with the action of saliva and could be used as a drug delivery for carrying some anti-inflammatory and antiseptic agents. The main purpose of this study was to synthesize this novel QAS monomer via acid–alkali neutralizing reaction and test its properties by some vitro experiments. The monomer was characterized by Fourier transform infrared (FTIR) spectroscopy. The antibacterial activities of the monomer against two oral pathogens as well as its cytotoxicity were assessed by determination of the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC), time–kill study and methyltetrazolium test (MTT) assay. Polymerizable efficiency of N,N-dimethyl-2-[(2-methylacryloyl)oxy] ethanaminium 5-carboxy-2,4-bis benzoate (DMAEMA-PMDPM) salt was determined by the measurement of degree of conversion during the process of poly-DMAEMA-PMDPM salt synthesis. Good antimicrobial activity small cytotoxicity and high polymerization activity of the new monomer was expected.

EXPERIMENTAL

Synthesis of DMAEMA-PMDPM Salt

2-(Dimethylamino) ethyl methacrylate (DMAEMA, Sigma-Aldrich Chemical Co.) was added to 4,6-bis{[(5-methyl-4-oxo-hex-5-en-l-yl)oxy]carbony}benzene-1.3-dicarboxylic acid (PMDPM, synthesized by Dental Materials Department, School of Stomatology, Fourth Military Medical University) dropwise under magnetic stirrer at room temperature and the final mol ratio was 1 : 1. Reaction temperature was observed by thermometer. When the temperature of mixture no longer rose and dropped to the room temperature, stopped stirring and reaction was ended. Colorless and transparent liquid was obtained finally after removing of deposit. The pH value of product was recorded by pH meter (420A, ORION, USA) and the density was measured by electronic balance (AR2130, Ohaus, China) using weighing method. FTIR spectra of the starting materials and products were collected using KBr powder pellet method in the 4000–400 cm^{-1} region with a wavenumber expanded uncertainty of 0.5 cm^{-1} (EQUINOX55, Bruker Co., Germany).

Antimicrobial Test

MIC&MBC Test. MIC&MBC of DMAEMA-PMDPM salt monomer was determined in this test and methacryloxylethylcetyl dimethyl ammonium chloride (DMAE-CB) was the positive control group. *Streptococcus mutans* (*S. mutans*, ATCC 25175) and *Candida albicans* (*C. albicans*, ATCC 90028) were used in this test. *S. mutans* was maintained in brain heart infusion (BHI) Broth (Hopebio, Qing Dao, China) and were cultured anaerobically at 37°C (5% CO_2 by volume). *C. albicans* was maintained in sabouraud's glucose broth medium (Hopebio, Qing Dao, China) and were cultured aerobically at 37°C. For DMAEMA-PMDPM salt (1 mL), pure monomer was as the initial concentration and start diluting. DMAE-CB monomer was diluted in cultural medium (1 mL) with concentration of 5 mg mL^{-1} for the series dilutions. Serial two-fold dilutions were made into 1 mL volumes of culture solution. Overnight cultures of each bacteria was adjusted to 1×10^7 colony-forming units (CFU) mL^{-1} with BHI broth or sabouraud's glucose broth medium, and 100 μL microbial suspensions were added to each tube containing 1 mL of a series of antibacterial monomer dilution broths. Culture solutions with 100 μL of microbial suspensions served as the negative control. Tubes of *C. albicans* were read for turbidity aerobic after 24 h culture, and tubes of *S. mutans* were read for turbidity after 24 h anaerobic culture; both of them were referenced by the negative and positive control tubes. MIC was defined as the endpoint where no turbidity could be detected with respect to the controls. Then an aliquot of 100 μL from each test tube without turbidity was inoculated on their appropriate agar plates, BHI agar plates (Hopebio, Qing Dao, China) or sabouraud dextrose agar (SDA) plates (Hopebio, Qing Dao, China) and after 24 h of incubation, plates containing no bacterial colonies and their corresponding concentrations were recorded. The MBC value was defined as the lowest concentration of antibacterial monomers that produced no bacteria on the plate.[20] The tests were performed in triplicates.

Time–Kill Study. *Candida albicans* (ATCC 90028) was used to test the killing kinetics of DMAEMA-PMDPM salt monomer suspensions. Concentrations of monomer solutions were adjusted to one, two, and four times of the MBC value (MBC value was previously determined). About 200 μL sabouraud's glucose broth suspensions with *C. albicans* which colony density was 1×10^7 CFU mL^{-1} were added to 1.8 mL monomer solutions. Then suspensions were incubated aerobically at 37°C with gentle agitation in a shaking water bath. After 10 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, and 16 h of incubation, 100 μL of the suspensions were serially diluted and inoculated on the SDA plates. The number of viable bacterial colonies was counted after 24 h incubation at 37°C. All experimental procedures were performed in triplicate. Time–kill assay results were analyzed by determining the numbers of viable cell counts (log^{10} CFU mL^{-1}) obtained at the different contact times and an average time–kill curve was constructed.[21]

Cytotoxic Test of Monomer

Cell Culture. In this study human gingival fibroblasts (HGF) cells were used. Primary HGF cells were obtained from the attached gingival of healthy premolar which were extracted for the reason of orthodontic treatment. The HGF cells were cultured by the tissue explant technique. Tissues were minced into small pieces by a surgical knife, placed on the culture dishes and grown in Dulbecco's modified eagle's medium (DMEM, Gibco) supplemented with 10% foetal bovine serum (FBS, Gibco), 100 U mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin.

They were grown at 37°C in a humidified atmosphere of 5% CO_2 and were passaged after reaching confluence of 80%.[22,23]

Cytotoxicity Test. The third passage HGF cells were used in MTT assay. The concentrations of DMAEMA-PMDPM salt monomer for test were 1.264 mg mL^{-1}, 632 μg mL^{-1}, 126.4 μg mL^{-1}, 63.2 μg mL^{-1}, 12.64 μg mL^{-1}, 6.32 μg mL^{-1}, and 1.264 μg mL^{-1}. 2,2-bis[4-(3-methacryloyloxy-2-hydroxypropoxy)phenyl]propane (Bis-GMA, Sigma-Aldrich Chemical Co.) was tested as control group with the same concentrations. The two monomers were firstly prepared in dimethyl sulfoxide (DMSO, Gibco) and then serially diluted by DMEM cell culture mediums. Final concentration of DMSO in test solutions was not higher than 1%. HGF cells were seeded into 96-well plate at a density of 1 $\times$ 10^4 cells per well in 200 µL growth media and incubated for 24 h. After removal of the culture medium, cells were exposed to 200 µL solutions containing monomers prepared as described above for 24 h. Exposure of the cells was stopped by discarding of monomer solutions and cell viability was immediately recorded using MTT assay. Briefly, at the end of experiment, medium was changed whereas containing MTT (0.5 mg mL^{-1} in DMEM, sigma) and incubated for 1.5 h at 37°C and 5% CO_2. Then all medium was removed from each well and 200 µL of DMSO solution was added with 10 min shaking at room temperature. Optical densities were read at 490 nm on a multiwell spectrophotometer (Biotek Instruments, Burlington) against a lysis buffer blank. Wells contained same density of cells but no monomer served control group. Cell survival rates were expressed as optical density readings (OD readings). Morphological alteration of the cells was observed directly by phase contrast microscope and photographed by camera (Olympus, Japan). All experiments were carried out in six replicates for each monomer concentration. Each experiment was repeated a minimum of three times.

Polymerization of DMAEMA-PMDPM Salt

DMAEMA-PMDPM salt monomers were synthetized as previous. There were four groups for the experimental synthesis of the polymers: For the first two groups, camphoroquinone (CQ, Sigma-Aldrich Chemical Co.) and N,N-dimethylaminoethyl methacrylate (DMAEMA, Sigma-Aldrich Chemical Co.) were mixed in a proportion CQ/DMAEMA equal to 1 by weight (0.5% and 1%, respectively). In the other two groups, only CQ was mixed into monomers and the concentrations were 0.5% and 1% by weight, respectively. About 0.5% and 1% w/w CQ could dissolve completely in liquid monomer and color the monomer in light yellow uniformly. Type of the polymerization reaction was photo-initiation and light-emitting diode (Spectrum® 800, Dentsply; output power: 450 mW/cm_2) was used as the light source. In order to know the process of polymerization reaction more clearly and determined appropriate amount of CQ as well as the best exposure time, the degree of conversion of DMAEMA-PMDPM salt was measured by Fourier transformation infrared spectroscopy (FT-IR, FTIR-8400s, Shimadzu, Kyoto, Japan). A small drop of each sample was placed between two translucent polyethylene films, which were pressed between two KBr crystals for measurement. In DMAEMA-PMDPM salt, the peak around at 1600 cm^{-1} assigned to aromatic C···C bond was used as an internal standard because the position and the intensity of this peak is unchanged during polymerization. The intensity of the peak around at 1640 cm^{-1} was referred to the aliphatic carbon double bonds in DMAEMA-PMDPM salt. DC was calculated according to the formula $DC = (A_0 - A_t)/A_0 \times 100$, where A_0 is the absorption of the peak at 1640 cm^{-1} when time is equal to zero and A_t is the absorption at exposure time t ($t = 20$, 40, 60, 80, and 100 s).[24,25] Every concentration group contained three specimens.

Statistical Analysis

For MTT assay, results were expressed as mean ± SD. Median lethal concentration (LC50) of monomer solutions were calculated by Probit analysis (SPSS17.0 software).[26] DC of experimental synthesis groups were analyzed with two-way classification ANOVA and the differences among groups were analyzed by LSD-t test (least significant difference t-test). Statistical analyses were performed by SPSS 17.0 software at a significance level of 0.05.

RESULTS

Synthesis and Characterization of DMAEMA-PMDPM Salt Monomer

The chemical structure of the raw materials DMAEMA, PMDPM and product were shown in Figure 1. Product (DMAEMA-PMDPM salt) was colorless and transparent liquid at room temperature. Product was water-soluble. Density was 1.2064 g mL^{-1} and pH value was 7.12. Reaction was indicated to be successful, based on the FTIR spectra of raw materials and product (Figure 2). For PMDPM, FTIR showed the stretching vibration of O—H and C—H were in 3500 and 2900 cm^{-1} region. In the spectra of DMAEMA-PMDPM salt, position of O—H moves to 3300 cm^{-1} and the stretching vibration of N—H and C—N appeared in 3100 and 1250 cm^{-1} region, respectively. This showed formation of the $—NH^+$ and $—CH_2—NH^+$.

Antimicrobial Test

The MIC and MBC values of the monomer determined for two species of oral bacteria are listed in Table I. The antimicrobial activity of DMAEMA-PMDPM salt for *S. mutans* was better than *C. albicans*. For both the species, DMAE-CB showed the best bactericidal activity, at the same time, the function of DMAE-CB to these two bacterial species was similar.

The time–kill curves for DMAEMA-PMDPM salt against *Candida albicans* presented in Figure 3. Higher concentrations of DMAEMA-PMDPM salt led to a rapid decrease in bacterial numbers. Survival of *Candida albicans* was not observed after incubation with 78.8 mg mL^{-1} (4× MBC) of the monomer solution for 120 min, and with 19.7 mg mL^{-1} (1× MBC) of the monomer solution for 960 min.

Cytotoxic Test of Monomer

Cultured HGF cells were elongated and spindle-shaped in appearance [Figure 4(a)]. Cell morphology in group exposed to 1.206 µg mL^{-1} DMAEMA-PMDPM salt monomer solution for 24 h was similar to the control group [Figure 4(b)]. For group exposed to 12.06 µg mL^{-1} DMAEMA-PMDPM salt monomer solution, many HGF cells became round, however the cell

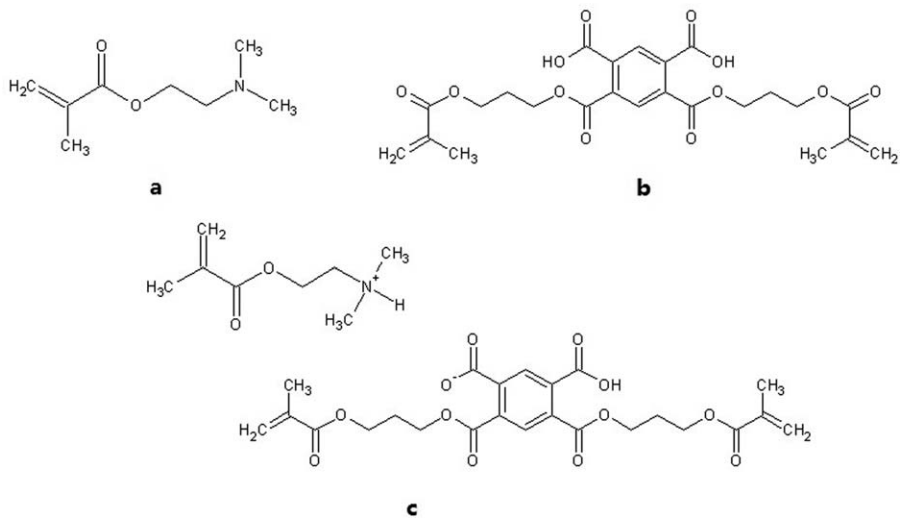

Figure 1. Chemical structure of the raw materials DMAEMA, PMDPM, and product DMAEMA-PMDPM: (**a**) Structure of DMAEMA; (**b**) Structure of PMDPM; (**c**) Structure of DMAEMA-PMDPM salt.

density did not decreased [Figure 4(c)]. Exposure to 1206 μg mL^{-1} DMAEMA-PMDPM salt monomer solution resulted in remarkable pulp cell retraction. The cell density decreased dramatically and disrupted cells and cell fragments could be observed in microscope [Figure 4(d)]. As Figure 5 illustrating, the cell viability of different kind and concentrations monomer solutions was determined as optical density readings. LC50 of DMAEMA-PMDPM salt and Bis-GMA monomer were 51.62 and 39.90 μg mL^{-1}, respectively.

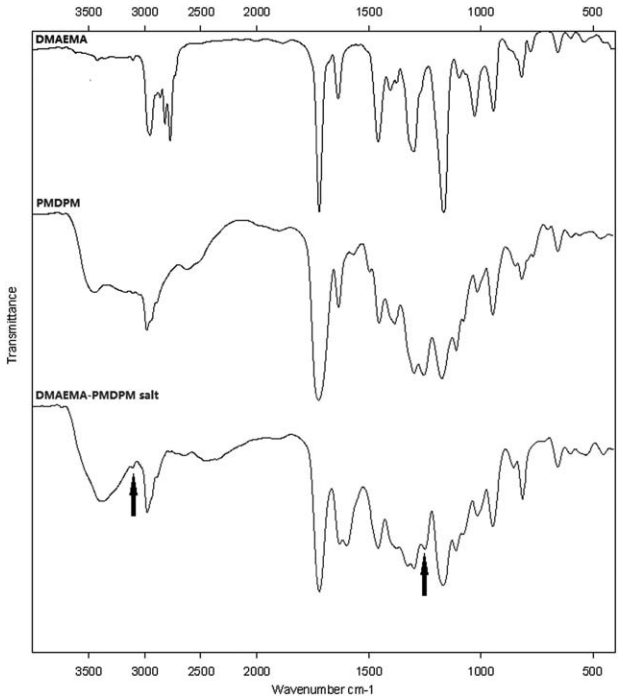

Figure 2. FTIR spectra of DMAEMA, PMDPM, and DMAEMA-PMDPM salt. The *arrows* indicated the stretching vibration of N—H and C—N appeared in 3100 and 1250 cm^{-1} region. This showed formation of the $—NH^+$ and $—CH_2—NH^+$.

Polymerization of DMAEMA-PMDPM Salt

The physical character of mixture changed from liquid to solid under the light irradiation. As Figure 6 showing, DC of DMAEMA-PMDPM salts increased with the growth of exposure time for all the groups. DC of groups contained larger amount of CQ/DMAEMA was higher in general. However, the differences became smaller with the increase of irradiation time and was no statistical significance at 100 s ($P > 0.05$). It was noticed that for groups with same amounts of CQ contained DMAEMA and no DMAEMA, DC of them had no statistical difference when they received the same irradiation time. Additionally, DC values of all treatment groups were over 90% at exposure time of 100 s. For groups with 1% w/w CQ, DC got 90% at 60 s and was nearly unchanged at 80 and 100 s. The absorbance curves of uncured monomer contained 1% w/w CQ and exposure to 60 s light irradiation were recorded as the Figure 7 showing.

DISCUSSION

In this study DMAEMA-PMDPM salt was synthetized by using acid-alkali neutralizing reaction and the synthesis was proved to be successful by FTIR. This hydrophilic monomer had some antimicrobial activities to *S. mutans* and *C. albicans*. The results of MTT assay indicated that DMAEMA-PMDPM salt monomer had a similar cytotoxicity to human gingival fibroblasts with Bis-GMA monomer. This new monomer has application

Table I. MIC&MBC Values of the Materials Determined for Two Species of Oral Bacteria

	MIC/MBC (mg mL^{-1})	
Bacteria strains	DMAEMA-PMDPM salt	DMAE-CB
Streptococcus mutans (ATCC 25175)	0.039/0.078	0.0024/0.0048
Candida albicans (ATCC 90028)	9.844/19.700	0.0024/0.0094

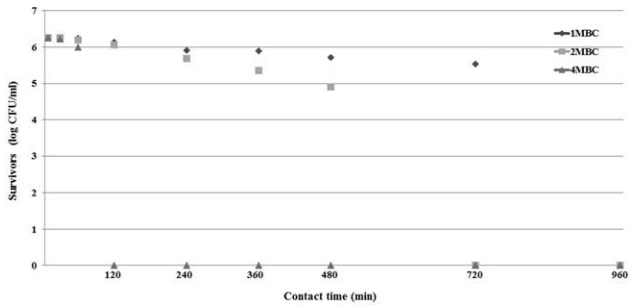

Figure 3. Time–kill curves for DMAEMA-PMDPM salt at different concentrations against *C. albicans*.

prospects in the preparation of novel antifungal denture base and soft liner materials.

PMDPM is organic acid and DMAEMA is organic base, DMAEMA-PMDPM salt formed by acid-alkali neutralizing reaction and the synthesis was proved to be successful by FTIR. The pH value of products was neutrality indicated that salts were generated. This method of synthesis was convenient and nearly no impurity substance formed. DMAEMA-PMDPM salt had hydrophilic carboxyl group in its structure. Different from DMAE-CB and MAE-HB,[18,27] the new monomer is liquid and hydrophilic. Additionally, its water solubility is excellent. It might be miscible with most resin monomers and has good process ability because of the liquid character. Both of the cation and anion parts of the new monomer are organic structure. It has one methacrylate end group in the cation part and two in the anion part. This kind of structure might provide material greater likelihood of crosslink formation.[28] Therefore lower leachable levels might be obtained while maintaining surface antimicrobial activity.

In this study, the bactericidal activity of DMAEMA-PMDPM salt monomer was examined by MIC&MBC test and time–kill study *in vitro*. *S. mutans* and *C. albicans* were selected to determine MIC&MBC of the new monomer. *S. mutans* and *C. albicans* are bacteria and fungi, respectively.[29,30] Although *S. mutans* is not closely related to stomatitis, in order to know more about antimicrobial activity of the new monomer, *S. mutans* was used to evaluate the function to bacteria, and *C. albicans* represented fungi. Methacryloxylethylcetyl dimethyl ammonium chloride (DMAE-CB) was selected to be positive control group in the MIC&MBC test. It also is an antimicrobial QAS monomer. It was reported previously that this monomer had obvious antibacterial activities.[31,32] That was the reason why DMAE-CB was chosen for the control in the test.

In aqueous environment, QAS exist as amphiphilic cations. The positively charged sited (N^+) of QAS can attract the negatively charged microorganism and then damage the cell membrane and finally lead to the death of microbe. The activity depends both on the character of the polar heads (size, electric charge distribution) and hydrocarbon chains (length, saturation, multiple chains).[33] The results showed that the antimicrobial

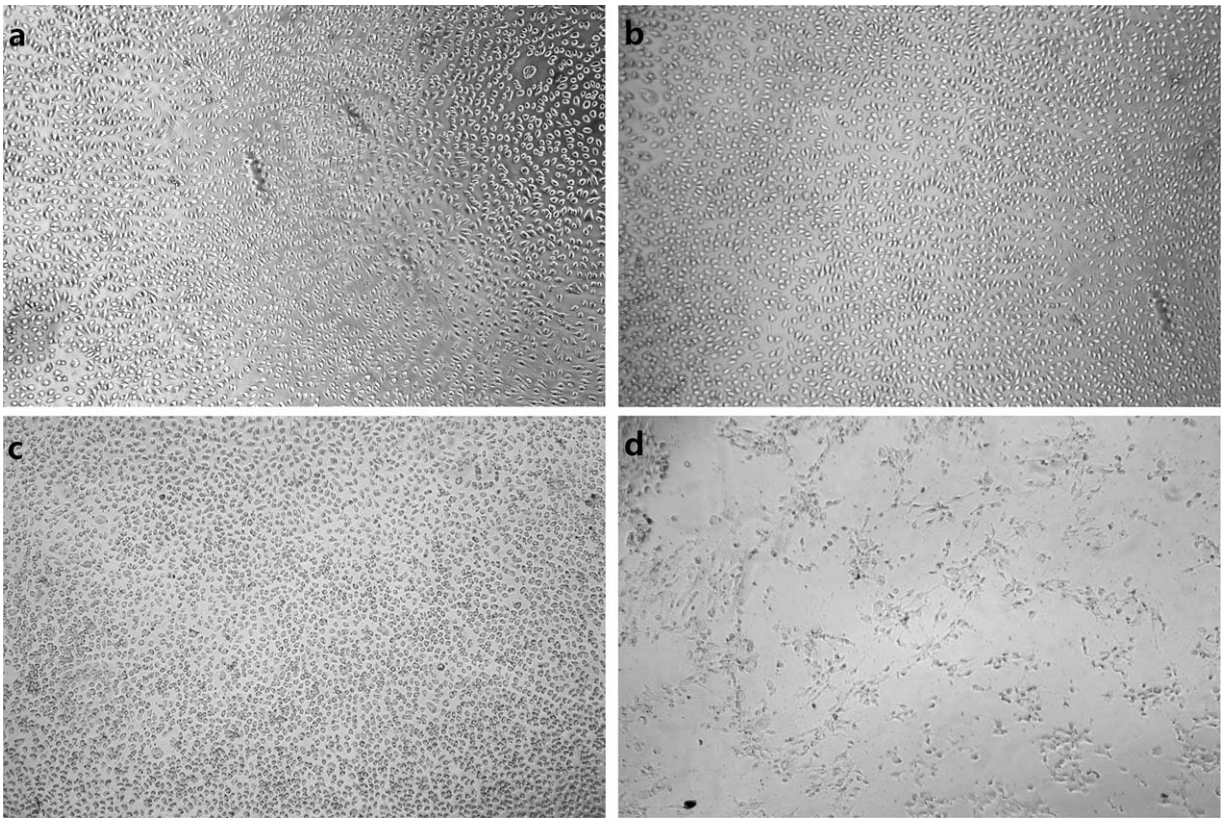

Figure 4. Morphological changes of HGF cells following exposure to DMAEMA-PMDPM salt solutions with different concentrations for 24 h. (**a**) Control group, (**b**) Exposure to 1.206 g mL^{-1} DMAEMA-PMDPM salt monomer solution, (**c**) Exposure to 12.06 g mL^{-1} DMAEMA-PMDPM salt monomer solution, (**d**) Exposure to 1206 g mL^{-1} DMAEMA-PMDPM salt monomer solution.

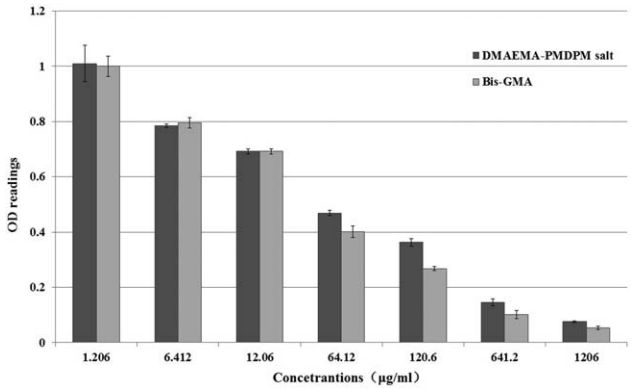

Figure 5. Effects of different concentrations of DMAEMA-PMDPM salt and Bis-GMA monomers on the cell viability of the HGF cells. The results are expressed as OD readings. The bar represents the standard deviation of the mean.

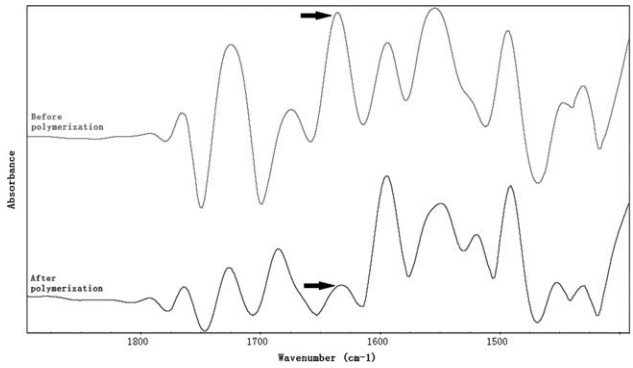

Figure 7. Absorbance curves of uncured monomer contained 1% CQ and exposure to 60 s light irradiation were measured by FTIR. The *arrows* indicated spectra change during the photo-polymerization process. The intensity of the peak around at 1640 cm^{-1} referred to the aliphatic carbon double bonds decreased dramatically after polymerization.

property of the new monomer is lower than DMAE-CB, especially for *C. albicans*. There may be some reasons for this phenomenon. *C. albicans* is bigger than *S. mutans* (the size of *C. albicans* is about 2∼4 μm and 0.5∼1 μm for *S. mutans*). The cell wall and membrane structure of *C. albicans* is more complex than *S. mutans* and the electric charge distributions of these two species are different also.[20] The large mismatch between DMAEMA-PMDPM salt chain and membrane structure of microorganism might be a reason for weaker antimicrobial function to *C. albicans*. DMAE-CB had equal activity to *S. mutans* and *C. albicans* and the antimicrobial activity was stronger than DMAEMA-PMDPM salt. It was found that the bactericidal activities of the QAS were closely related to the substituted lipophilic chain in polar heads. The hydrophobic chain length between 12 and 18 carbons processed an excellent antimicrobial activity.[33,34] DMAE-CB has a long lipophilic chain with 16 carbons according to the previous studies,[17] but chain length of DMAEMA-PMDPM salt is very short, so this might be the reason why DMAEMA-PMDPM salt has a higher MIC/MBC. The anionic part of QSA monomer could affect the bactericidal activity but the law is not very clear now. Therefore the anionic part of DMAEMA-PMDPM salt might influence the antimicrobial function by the effect on distribution of electric charges but the mechanism is not very clear also. However, the new monomer is liquid and has three polymerizable groups. We can infer that the monomer could incorporate in resin with bigger amounts, and the high antimicrobial monomer content might enhance bactericidal activity of materials in applications.

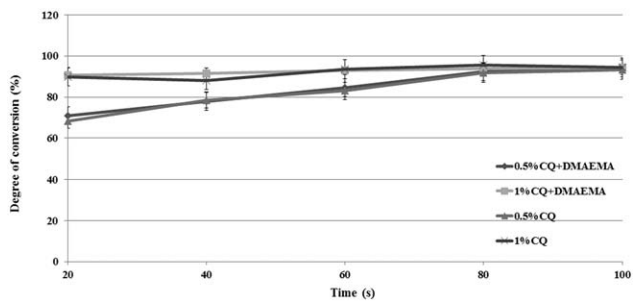

Figure 6. DC of experimental resins at 100s exposure determined by FTIR. The bar represents the standard deviation of the mean.

We expect that the new monomer could be used for forming a layer of antimicrobial coating on the surface of denture base or developing a new kind of antimicrobial soft lining materials. This material has some properties like hydrogel due to its hydrophilic groups and ionic bond. Denture base with this type of material on the soft tissue surfaces might enhance the denture's retention for absorbing water.[35] It may be acted as drug delivery for releasing antimicrobial and antiphlogistic agents which could have some therapeutic action to oral infection. In order to get a balance between hydrophilic, liquid character and antimicrobial activity of the monomer, this design was made and further modification should be carried out for getting the aim above.

Candida albicans is closely related to denture stomatitis and other oral mucosal infections.[3] In order to evaluate the performance of using DMAEMA-PMDPM salt to prepare antifungal coating, denture base resins and soft lining materials better, *C. albicans* was used for the time–kill study. The time–kill curves indicated that the sterilization speed of the solution increased with the growth of the monomer concentration. It could be observed from the curves that the bactericidal activity was not so effective in the initial stage when monomers acted with *C. albicans*, but increased suddenly at a certain time, and the monomer solution killed nearly all fungus in a short time. This phenomenon showed that the action between DMAEMA-PMDPM salt monomer and *C. albicans* might be slow and present as an accumulative action. When the damage reaches a certain level, fungus begin to die. In addition, the slow fungicidal function might be a reason for high MIC&MBC of DMAEMA-PMDPM salt monomer to *C. albicans*.

MTT assay is one of the cytotoxicity test methods, which could be used to evaluate the biocompatibility of materials. The MTT test has been used extensively to assess cytotoxicity of dental materials. It indicates the effects on cell viability by alterations of mitochondrial dehydrogenase activities.[23] Bis-GMA is widely used in resin materials, so it was chosen as the control group in

this study. The TC50 value for Bis-GMA was 39.90 μg mL^{-1} and this result is similar to Reichl's research (EC50 of Bis-GMA was 0.087 mmol L^{-1} in this study).[22] TC50 of DMAEMA-PMDPM salt was 51.62 μg mL^{-1} and from the result that Figure 6 showed. It can be deduced that the cytotoxicity of DMAEMA-PMDPM salt and Bis-GMA was similar. Some previous researches presented that for common resin monomer and orders of the cytotoxicity of the monomers were ranked by TC50 was Bis-GMA > TEGDMA > DMAEMA > HEMA.[22,23] Although the cytotoxicity of DMAEMA-PMDPM salt is higher than many other dental resin monomers, they can polymerize with resins. It could be speculated that the amount of DMAEMA-PMDPM salt monomer leaching from the dental materials would be very small. The toxicity of materials containing DMAEMA-PMDPM salt might be low. Additional studies are needed to clarify the bactericidal effect and biocompatibility of the polymer and resin material containing DMAEMA-PMDPM salt monomer.

DMAEMA-PMDPM salt polymers were synthesized by photo-polymerization process. The polymerization activity of was evaluated by the measurement of DC to DMAEMA-PMDPM salt monomer during the reaction process. According to the results, most of C═C double bonds can be consumed after polymerization. In this study, CQ and DMAEMA were selected as initiator and accelerator respectively for the synthesis of poly-DMAEMA-PMDPM salt and the type of reaction was free-radical polymerization. CQ is an excellent photo-initiator that absorbs over a wide spectrum of wavelengths from 360 to 510 nm. The CQ/DMAEMA photo initiator system is one of the most commonly used system in current photo activated dental materials. The concentration of them in the matrices was around 1%.[36] The light source chosen is used for cure of commercial dental photo-cured materials commonly and it emits radiation predominantly in the 400-500 nm range, where also CQ can absorb. Additionally, because DMAEMA-PMDPM salt monomer is liquid, it is speculated that the polymerization methods of the new monomer might like most liquid resin monomers. For this reason, solvent need not to be used in the synthesis of polymers. Generally, the method for acquiring DMAEMA-PMDPM salt polymers is simple and effective, and it could make the chair-side process to be possible.

FTIR spectra was selected to determine the DC of DMAEMA-PMDPM salt polymers in this study. The amount of double vinyl bonds remaining in the sample exposed to irradiation is shown by the intensity of the peak around at 1640 cm^{-1} referring to the C═C. The degree of conversion was directly related to the decrease of 1640 cm^{-1} absorption on the FTIR spectra. This method was used widely in the DC measurement of resin monomers.[25] For the synthesis of poly-DMAEMA-PMDPM salt, DC could reflect the degree of polymerization and report the yield of the polymers.

The results of DC measurement indicated that the effectiveness of adding 1 wt % CQ was better than adding 0.5 wt % CQ. DC of materials containing 1 wt % CQ was nearly 90% at 80 s but only about 70% for materials containing 0.5 wt % CQ. Polymerizations of the dental resin monomers rely on the radical polymerization reaction. In order to set off this reaction, small amounts of initiator are required, which will be consumed during the polymerization reaction. Initiators possess atomic bonds with low dissociation energy and these radicals will set off the radical polymerization reaction.[28] Therefore bigger amounts of CQ can produce more free radicals for setting off reaction and the rate of polymerization would be more rapid in 1 wt % CQ groups. Additionally, longer exposure time could provide more energy for the generation of free radicals under a fixed irradiance.[37] So DC of all groups increased with the growth of irradiation time. For 1 wt % CQ groups, DC reached at 90% around after receiving 20 s irradiation but nearly unchanged when having longer exposure time. For 0.5 wt % groups they also got similar DC after receiving 80 s irradiation. It might be speculated that the final DC was around 90% for DMAEMA-PMDPM salt under this experimental conditions, adding more CQ or extending irradiation time over 100 s could not help for the increase of DC.

Compared with other dental resin monomers like Bis-GMA, TEGDMA and UDMA, DC of DMAEMA-PMDPM salt were higher. It was reported that the limiting DC of Bis-GMA, TEGDMA, and UDMA were all below 80%.[24] The monomers in dental resins photo-polymerize thanks to a radical polymerization reaction which the chain transfer was involved.[28] The results showed that DMAEMA-PMDPM salt had higher polymerization reactivity. This high value may be due to the labile hydrogen atoms of —NH^{+}— groups, which greatly favor chain transfer reactions. Additionally it is noted that groups without DMAEMA as an accelerator also presented a similar polymerization activity to groups containing CQ. DMAEMA as amines are efficient hydrogen donors, and are extensively used for co-initiator. Photo-initiator interacts with a second molecule (co-initiator/accelerator) to produce free radicals and the reaction rate can be increased by adding accelerator.[28] However the polymerization activity of DMAEMA-PMDPM salt itself was strong, small amount of free radicals generated by CQ could trigger rapid reaction. Therefore DMAEMA-PMDPM salt monomer could polymerize with a high speed without any accelerator.

CONCLUSIONS

In summary, DMAEMA-PMDPM salt can be synthesized successfully by simple acid-alkali neutralizing reaction. It was liquid and hydrophilic monomer with three polymerizable end groups. This monomer had antimicrobial functions to *S. mutans* and *C. albicans* but the activity to *C. albicans* was weaker. Solution of 4× MBC concentration monomer can reach the fungicidal result to *C. albicans* in 2 h. The cytotoxicity of DMAEMA-PMDPM salt to HGF cells was similar to Bis-GMA, but it is speculated that the new monomer could incorporate into the materials and the opportunity of its escaping from the matrix is rare. So the toxicity of materials containing DMAEMA-PMDPM salt might be small. Moreover DMAEMA-PMDPM salt monomer could polymerize with a high speed without any accelerator or solvent. This new monomer is liquid and expected to have similar solubility to common dental resins. Therefore it may be mixed into materials with higher

concentration. It also might have lower monomer leachability due to multiple vinyl groups and high polymerization efficiency. Its hydrophilic character might give DMAEMA-PMDPM salt polymers a property like hydrogel. The antibacterial coatings on denture base formed by surface grafting technique and soft liners could be expected to improve the adsorptive power under the action of saliva and could be used as a drug delivery for carrying some anti-inflammatory and antiseptic agents. DMAEMA-PMDPM salt has potential for preparing antimicrobial coatings on denture base and soft lining materials, as well as other biomedical applications. Further studies need to be done for its application.

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