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ARTICLE

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In this study, a series of waterborne biodegradable polyurethanes with antibacterial and biocompatible properties were developed. To obtain these polyurethanes, lysine-derivative gemini quaternary ammonium salts (GQAS) chain extenders with different hydrophobic alkyl chain length (named EGn, n=8, 12, 16, the hydrophobic alkyl chain length of GQAS) were designed and synthesized. Then, waterborne biodegradable polyurethanes (PCLPUn) were prepared using isophorone diisocyanate (IPDI), poly (ε-caprolactone) (PCL), poly (ethylene glycol) (PEG), L-lysine and EGn. Antibacterial activities of these EGn and PCLPUn emulsions were evaluated by minimal inhibitory concentration (MIC) method, and antibacterial and antifouling functionalities of PCLPUn film surfaces were confirmed by contact-active antibacterial and culture-based method using both Gram-positive and Gram-negative bacteria. In vitro degradation and cytotoxicity of these obtained polyurethanes were also systematically investigated. These results indicated that the PCLPUn films owned good antibacterial and antifouling abilities, biodegradable, and good biocompatibility, notably, PCLPU12 shows the best antibacterial activity and cytocompatibility. Such antibacterial materials could be degraded to non-toxic components, and potentially be widely used in medical and environmental applications, especially as coatings of biodegradable surgical equipment and medical implants.

Introduction

Infection related to implant biomaterials remains the most severe and devastating complications in clinical, such as urinary catheters, intravascular catheters, [1-4] biomedical devices,[5] and orthopaedic implants.[6] Despite much advanced sterilization and aseptic techniques had made to minimize the possibilities of biomaterials associated infection during the surgery, unfortunately, it is still unable to eradicate completely. As this infection happened, implant removal and substitution, with attendant medical risks and complications, is often inevitable. Therefore, integrating implants with good anti-infection activity during the service period is of great importance. The positively charged broad spectrum antibacterial agents quaternary ammonium salts (QAS) with good environmental stability and biological activity,[7] which are not easily forming resistance,[8-11] have been already applied in many commercial products, such as deodorants, mouth and hair rinse products, contact lens solutions, and so on. Moreover, numerous researches concerning QAS antibacterial materials have been made and demonstrated constantly[12-14]. Of particular note is the fact that QAS antibacterial surfaces are apt to adhesion of killed microorganisms on them due to positive charge of QAS, which could block these functional groups and trigger immune responses and inflammatory reactions.[15] As a result, these antibacterial materials as implants cannot fulfill both requirements of biocompatibility and non-fouling ability. It is worth noting that these surfaces constructed from biodegradable polymers could be gradually decomposed and degraded under enzymatic attack, which could polish the attached living organisms.[16-19] Thus, biodegradable antibacterial materials with good biocompatibility that could maintain anti-infection effect in the serving time, and then degrade to non-toxic small molecules seem to be attractive materials to fight infection of biomaterials.[20]

In addition, the biological activity of QAS compounds depends on the nature of the organic groups attached to nitrogen positive ion (N^+), the number of N^+ present, and the counter ion.[21, 22] Gemini quaternary ammonium salts (GQAS) possessing two hydrophobic hydrocarbon chains and two hydrophilic quaternary ammonium groups have much stronger surface activity and permanent excellent antibacterial activity than single-chain QAS[23, 24]. It has been shown that an increase of alkyl chain length of QAS is followed by an increase in antimicrobial activity of the compound.[15, 25-29] Hydrophobic alkyl chain length of QAS not only affects antibacterial activity, but also has an effect on materials biocompatibility.[29]

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Therefore, development of biocompatible antibacterial materials need to investigate the effect of hydrophobic alkyl chain length of QAS on their antibacterial activity and biocompatibility.

In this study, to develop biocompatible antibacterial and antifouling GQAS waterborne polyurethanes, of lysine-derivative chain extenders containing GQAS side chains consisted of two hydrophilic head groups and two hydrophobic alkyl tails (denoted as EGn, n=8, 12, 16, the length of GQAS hydrophobic alkyl tails) were designed and synthesized., Then, a series of biocompatibility degradable antibacterial waterborne polyurethanes (named as PCLPUn, n=8, 12, 16, the length of GQAS hydrophobic alkyl tails) were prepared using isophorone diisocyanate (IPDI), poly (ethylene glycol) (PEG), poly (ε-caprolactone) diols (PCL), L-lysine and EGn as primary materials. Antibacterial and antifouling abilities of these obtained polyurethanes were determined by minimal inhibitory concentration (MIC), contact-active antibacterial and a culture-based antibacterial and antifouling assay. In vitro extensive degradation and cytotoxicity analysis studies on the obtained PCLPUn samples were also performed to study the effects of different hydrophobic alkyl chain of GQAS on degradation and biocompatibility of PCLPUn.

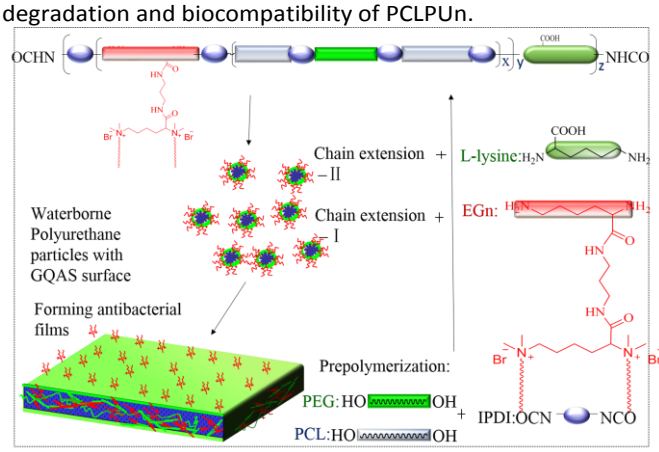

Figure 1. Schematic structure of gemini quaternary ammonium salt waterborne polyurethane with antibacterial surfaces.

Table 1. Theoretical composition, the molecular weight, and MIC results (μg/mL) of the PCLPUn samples.

Samples	Molar ratio of IPDI/ PEG/ PCL/ EGn/ Lysine	Chain extender EGn	EGn (wt%)	Mn (g/mol)	Mw/ Mn	MIC (μg/mL)	
						S,aureus	E.coli
PCLPU0	2:0.33:0.67:0.5:0.5	0	0	62419	1.41	>1000	>1000
PCLPU8	2:0.33:0.67: 0.5:0.5	EG8	14.40	91912	2.06	100	400
PCLPU12	2:0.33:0.67: 0.5:0.5	EG12	15.83	55265	1.53	45.7	91.4
PCLPU16	2:0.33:0.67: 0.5:0.5	EG16	17.41	101085	1.52	48.1	192.4

Experimental section

Materials

Unless otherwise noted, all chemical reagents and reagent grade solvents obtained from commercial suppliers were used without additional purification. 1, 3-Propane diamine, 1-Bromooctane, 1-bromododecane and 1-Bromohexadecane was purchased from Aladdin reagent. Isophorone diisocyanate (IPDI) (BASF) was redistilled under vacuum before use. Poly (ethylene glycol) (PEG, molecular weight1450, Dow Chemical) and poly (ε-caprolactone) diols (PCL, molecular weight 2000, Dow Chemical) were dehydrated at 80-90 ºC under vacuum for 2 h before use. L-Lysine and N, N, N', N'-tetramethyl-L-lysine ethyl ester (LE) was used as received. $(Boc)_2$ lysine was synthesized using a conventional manner of preparing peptides in our laboratory. Lipase from Pseudomonas cepacia (Lipase PS, ≥30 U/mg) was purchased from Sigma.

Characterization

Proton nuclear magnetic resonance (^{1}H NMR, 400 MHz) spectra were recorded on a Bruker AV II-400 MHz spectrometer in DMSO-d6. Gel permeation chromatography (GPC) was performed by Waters-1515 using N, N-dimethylformamide (DMF)/LiBr as eluent and polymethyl methacrylate (PMMA) as reference. The sample concentration was 2-3 mg·mL^{-1}, and the flow rate was 1.000 mL·min^{-1} at 40 ºC. Scanning electron microscopy (SEM) pictures were taken using a SEM instrument (Inspect F, FEI Company) at an accelerated voltage of 15 kV.

Synthesis of antibacterial waterborne polyurethanes

To obtain a novel series of biodegradable waterborne PCLPUn with long-term contact-active antibacterial and antifouling properties, the lysine-derivative GQAS monomer containing two primary amine groups with different hydrophobic alkyl chain length (EGn, n=8, 12 and 16, the length of GQAS hydrophobic alkyl tails) were first designed and synthesized. The synthesis details (synthesis route: Scheme S1) and ^{1}H NMR information (Figure S1) are provided in the Supporting Information. Followed by, biodegradable antibacterial and antifouling PCLPUn based on IPDI, PEG, PCL and the chain extender lysine and EGn were synthesized using two-step polymerization. The feed ratios were shown in Table 1, which were decided by considering both the antibacterial and biocompability of the polyurethanes by our previous work.[30] First IPDI and 0.1% stannous octoate were added to the stirred

and dried PEG and PCL at 70-75ºC under a dry nitrogen atmosphere. After reaction for 1h at 70-75 ºC, the reaction mixture was cooled down to room temperature, and then chain extender EGn was added in the mixture with continuous stirring for 20-30 min. Finally, the prepolymer was poured into L-lysine aqueous solution to emulsify with high-speed stirring (600 rpm) and ultrasound (200W) simultaneously, dilute sodium hydroxide solution was added dropwise into aqueous emulsion to neutralize the carboxyl groups of L-lysine under room temperature for 2h. Polyurethane without GQAS (PCLPU0) as a control was also prepared with a similar process as described above.

The antibacterial surfaces coated PCLPUn films were prepared through casting the PCLPUn emulsions on surfaces of siliconized culture dishes and dried at room temperature for 2 d, then put into an oven at 60 ºC for 2 d, followed by 60 ºC under vacuum for 2 d. The films were cut in sheets with 1×1 cm in size and approximately 0.5 mm thickness for physicochemical characterization. PCLPU0 was used as a control. Certain polyurethane films (1×1 cm, 0.5 mm thickness) were immersed in water in a horizontal laboratory shaker (110 rpm, 37 ºC) for 60s, and then dried at 60 ºC under vacuum for 2 d before testing.

Antibacterial studies

MIC studies: E. coli (ATCC 25922; Gram-negative), S. aureus (ATCC 6538; Gram-positive) were used for antibacterial activity assessments by the MIC method. The MIC was quantified using a broth microdilution method according to national committee for clinical laboratory standards (NCCLS). The nutrient media were prepared from the Mueller-Hinton broth (Hangzhou microbial reagent) dissolved in distilled water and sterilized in an autoclave at 121 ºC, 103 kPa for 20 min. Cultures in the nutrient media were grown overnight at 37 ºC and diluted to 10^6-10^7 cells·mL^{-1}. Each well of a sterile 96-well plate was inoculated with 100 µl of media. EG8, EG12, and EG16 as controls, of which the initial concentrations were 1024 mg·mL^{-1}, while the directly obtained waterborne PCLPUn and PCLPU0 emulsions were diluted 4-10 times before the test. Using this starting concentration, 10 serial two-fold dilutions of them were made in 96-well plate. Then, 100 µl of diluted bacteria (10^6-10^7 cells·mL^{-1}) were added into each well. One well on each plate also had a positive control without any antibacterial agents, while a negative control containing only media. After incubation at 37 ºC, 110 rpm for 16-18h, 5µl triphenyltetrazolium chloride (TTC, 5mg·mL^{-1}) was added into each well of the plate for half an hour before the results were read.

Culture-based antibacterial and antifouling assay: To eliminate the influences of water-soluble antibacterial moieties onto these PCLPUn films evaluation of their antibacterial and antifouling activities, all the films were immersed in water in a horizontal laboratory shaker (110 rpm, 37 ºC) for 6h before the antibacterial test. The antibacterial and antifouling activities of these films were assessed against both E. coli and S. aureus according to shaking flask methods.[31, 32] Each PCLPUn film (1.0×1.0 cm, 0.50 mm thickness) was placed in a well of 24-well plate and sterilized under UV overnight. PCLPU0 and commercial thermoplastic polyether polyurethane witout PEG (TPEU, 0.65mm thickness, Dongguan City grindlays film Products Company) were used as controls. E. coli and S. aureus strains were cultured in the nutrient broth (NB), grown overnight at 37 ºC and diluted to 10^7 cells·mL^{-1}. This bacteria liquid (2 mL) was added into each well with one film. The 24-well plate was then placed in a constant temperature incubator at 37 ºC, 110 rpm for 2 d. After that, the films were taken out and rinsed three times with sterile deionized water, then placed in another tube and 2 mL of sterile water was added. The bacteria were detached in an ultrasonic cleaner for 5 min and diluted serially to proper concentration then counted by the method of agar dish. All the reported values were replicated at least three times.

Contact-active antibacterial activity: Samples were prepared through casting the emulsions (50µl) onto cover glasses (1.5×1.5 cm^2 region) and dried. Then these samples were immersed in water at 37 ºC for 6h and dried in vacuum oven. The coated glass slide samples were sprayed with an aqueous suspension of S. aureus (1×10^6 cells·mL^{-1}) and air-dried for 10 min, then incubated under nutrient broth agar (0.8% agar) at 37 ºC for 24 h. Bacterial colonies grew from the individual cells and stained red with 3 mL 5mg·mL^{-1} TTC.

Degradation test

PCLPUn and PCLPU0 films were prepared through casting the polyurethane emulsions on surfaces of siliconized culture dishes and dried at room temperature for 2 d, then put into an oven at 60 ºC for 2 d, followed by 60 ºC under vacuum for 2 d. The films were cut in sheets with 1×1 cm in size and approximately 0.5 mm thickness (without washing before the test) for the degradation test.

Degradation of PCLPUn in vitro was evaluated as weight loss of these polyurethane films (10×10 mm in size, with approximately 0.5 mm thickness) in phosphate buffer solution alone (PBS, pH = 7.4) and enzyme medium (PBS with lipase PS, 0.3 mg/ml), then the vials were incubated with shaking at 37 ºC.[33-35] PCLPU0 was used as a control. The samples were taken out at a predetermined time and washed with distilled water three times, dried in vacuum oven at 25 ºC to a constant weight. The residual weight was calculated as:

$$\text{Weight loss}\,(\%) = \frac{m_0 - m_t}{m_0} \times 100\%$$

Where m_0 represents the initial weight of the films, and m_t is the dry weight of these films degraded at various times. The morphology of these PCLPUn surfaces degraded was examined using a scanning electron microscope microscopy.

Biocompatibility of PCLPUn

All obtained films (PCLPUn, PCLPU0 films and latex rubber film) were immersed in water in a horizontal laboratory shaker (110 rpm, 37 ºC) for 60s, and then dried at 60 ºC under vacuum for 2 d before biocompatibility test.

Cytotoxicity of PCLPUn extracts

Methylthiazoletetrazolium (MTT) assay was performed to evaluate cytotoxicity of these polyurethane extracts, and latex rubber (Microflex Corp., Sparks, NV) extract was used as a positive control. All polyurethane films and latex rubber film

were gamma irradiation sterilized for 30 min, followed by another 30 min of UV irradiation. The samples were immersed into complete culture medium at the ratio of 100 mg/mL and incubated at 37 °C for 24 h. The culture medium was then filtered through a 0.2μm membrane filter, and then aseptically diluted by 5, 50 and 100 times using the culture medium. Meanwhile, 1000 fibroblast cells/ well (100 μL/well) were plated in 96-well plates and incubated for 12 h to allow the cells to attach. Various concentrations of polyurethane extract media were then added into the cultured fibroblast cells (100 μL/well), and the 96-well plates were incubated at 37 °C in a humidified 5% CO_2 atmosphere for 24-96 h, followed by the addition of 20μL of MTT solution (5 mg/mL, PBS). Then the media were removed and the insoluble form azan crystals were dissolved in 150 μL of dimethylsulfoxide (DMSO). The optical density (OD) was measured at 492 nm with a Microplate reader (Model 680, Bio Rad Corp.). The cell viability was normalized to that of fibroblasts cultured in the culture media with negative control.

$$\text{Cell viability}(\%) = \frac{\overline{\text{OD}}_{\text{Sample}}}{\overline{\text{OD}}_{\text{Negative}}} \times 100\%$$

Cytotoxicity of PCLPUn degradation products

The cytotoxicity of PCLPUn and PCLPU0 degradation products was carried out by completely degrading polymers and exposing them to cultured cells. Sample discs (150 mg) were placed in 1.5 mL of NaOH solution (1 mol/L) and incubated at 80°C for approximately 14 d to completely degrade. The solution was filtered through a 0.2μm membrane filter and the pH was adjusted to 7.4 with HCl (1 mol/L). The solution was filtered again for sterilization and then diluted by 2, 4, 10, 100, 1000, and 10000 times with culture media. Sterile PBS was similarly diluted with media as a control. The solutions were added to the cells cultured in 96-well plates (100 μL/well), which were then incubated at 37 °C, 95 % relative humidity, and 5 % CO_2 for 24 h. Cell viability was evaluated employing MTT assay as afore described. In addition, cytocompatibility of EGn was also assessed. Solutions of gemini in culture media were prepared with concentrations ranging from 1 to 1×10 $^{-4}$ mg/mL, and the cytotoxicity was tested similarly as the degradation solutions.

Statistical data analysis.

The results for the antibacterial test, protein and adsorption cytotoxicity experiments are expressed as mean values± standard deviations (SD, n=3 for each analysis). Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, version 17.0) software. Statistical comparisons were made by Student'st-test or one-way analysis of variance (ANOVA). In all cases, $p<0.05$ was considered as statistically significant.

Results and discussion

Synthesis and characterization of waterborne PCLPUn

To obtain new biocompatible materials with good antibacterial and antifouling properties, the lysine-derivative of GQAS monomers containing two primary amine groups and different lengths of hydrophobic alkyl chain as chain extender (EGn) were first designed and synthesized. Their synthesis details, structures, and ^{1}H NMR information are provided in the Supporting Information (Scheme S1 and Figure S1). Followed by, a series of waterborne biodegradable polyurethanes containing various GQASs have been prepared (Scheme 1). PCLPU0 without GQAS chain extender was also prepared for comparison. The characteristic peaks at 3.99 (-COO*CH$_2$*-), 2.27 (-*CH$_2$*COO-), 1.55 (-*CH$_2$*CH$_2$CH$_2$-), and 1.29 (-CH$_2$*CH$_2$*CH$_2$-) ppm are assigned to methylene protons of the PCL blocks in ^{1}H NMR spectra (Figure 2). The peaks at 3.49 ppm are assigned to methylene protons (-*CH$_2$ CH$_2$*O) of PEG units. The peak at 3.81 ppm is ascribed to terminal methylene groups (-NHCOO-*CH$_2$*-) in PEG and PCL. The peaks at 3.00-3.19 ppm, originated from methyl and methylene groups of N positive ions of these GQAS chain extenders, can only be found in PCLPUn samples. The ^{1}H NMR spectra show that all gemini chain extenders (EG8, EG12, EG16) have been copolymerized into chains of these polyurethanes, as compared to that of PCLPU0 (Figure 2). Number molecular weights (Mn) of these resulting PCLPUn and PCLPU0 determined by GPC range from 55265 to 101085 presenting monodisperse and narrow molecular weight distributions (Table 1). More detailed information on bulk structures of these polyurethanes (DSC and FTIR results) is presented in the Supporting Information (Figure S2 and Figure S3). These results demonstrate that these waterborne antibacterial biodegradable polyurethanes have been successfully synthesized.

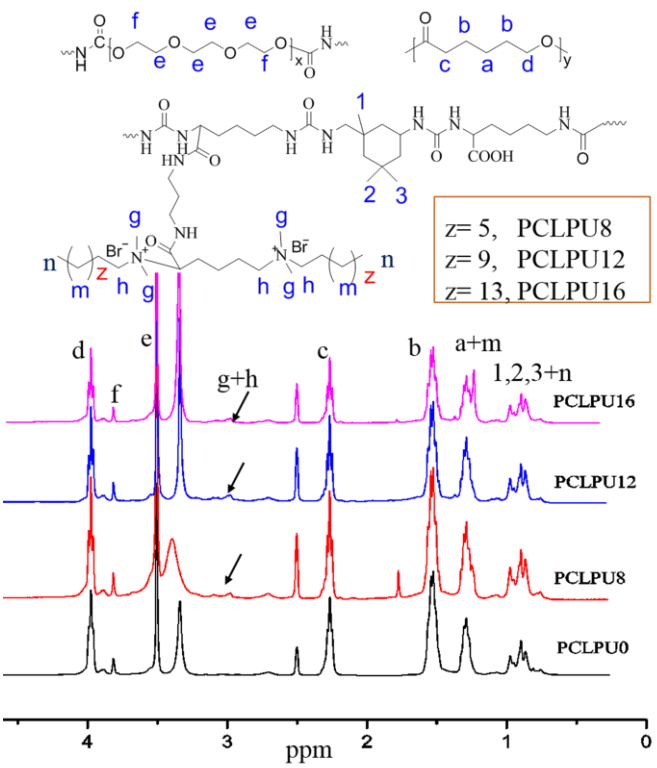

Figure 2. The structures and ^{1}H NMR spectra of PCLPUn samples recorded in DMSO-d6.

Antibacterial and antifouling activities of obtained waterborne PCLPUn films

To verify antibacterial properties of these biodegradable waterborne PCLPUn, we first measured MICs of these polyurethane emulsions against suspensions of ubiquitous infectious bacteria, including Staphylococcus aureus (S. aureus; gram-positive), Escherichia coli (E. coli; gram-negative), using EGn as controls. The MICs of EGn were in the 4-16 μg·mL^{-1} range for S. aureus, and 8-64 μg·mL^{-1} for E. coli, and EG12 showed more efficient antibacterial activity than EG8 and EG16 (Table S1), since the length of hydrophobic alkyl chain had a strong impact on the antibacterial activity.[25, 36, 37] Biodegradable waterborne polyurethanes containing GQAS were in the 45.7-100 μg·mL^{-1} range for S. aureus, 91.4-400 μg·mL^{-1} for E. coli (Table1), indicating that these polyurethanes have good antibacterial activities referred to our previous work.[30] In particular, PCLPU12 shows the best antibacterial efficiency in these polyurethanes owing to chain extender EG12, as described above. Similarly, these GQAS polyurethanes had better antibacterial activity to gram-positive bacteria than those to gram-negative bacteria,[32] since gram-negative bacteria have very sophisticated outer cell walls to effectively keep out antibacterial agents.[22]

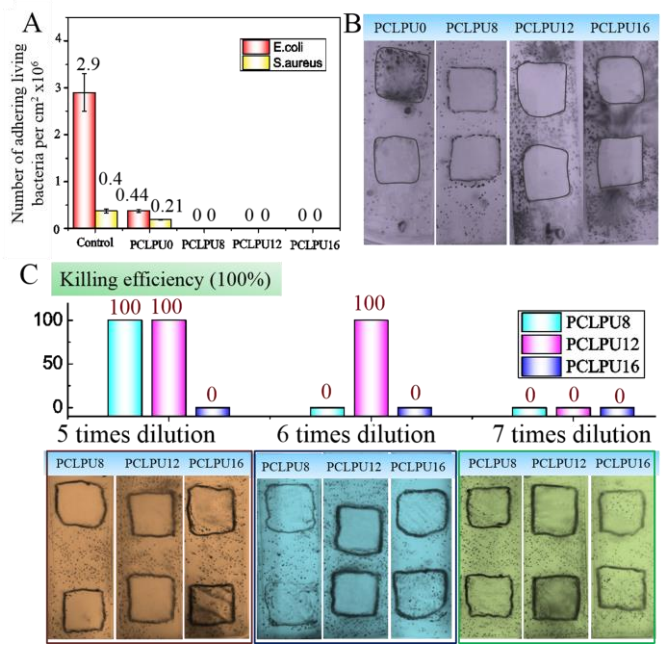

Figure 3. A. Antibacterial and antifouling activity of PCLPUn films: live bacteria attached on surfaces with and without GQAS and PEG. B. Contact-active antibacterial and antifouling activity of PCLPUn films. C. Killing efficiency and contact-active antibacterial activity of PCLPUn films with various GQAS contents, which were obtained via changing the weight ratios of PCLPUn and PCLPU0.

The antifouling and antibacterial potential of these films were also evaluated by protein adsorption and shake-flask method,[32, 38] and the results were illustrated in Figure 3 and Figure S5. Nonspecific protein adsorption is a key factor in materials surface fouling, because this surface that has the ability to inhibit nonspecific protein adsorption will some degree resist bacterial adhesion under no antibacterial moiety condition.[39, 40] Herein, the adsorption of protein BSA on PCLPUn and TPEU films surfaces is shown in Figure S5. The amount of protein adsorption on PCLPU0 surfaces is about 0.17 μg/cm^2, while the adsorbed protein on the commercial TPEU surfaces is higher to 1.31μg /cm^2, owing to the rearrangement of PEG and –COOH repulsion of PCLPU0 to reduce the adsorption of proteins from solution after contact with water.[41, 42] However, the introduction of GQAS in polyurethanes had a remarkable increase of protein adsorption on these surfaces compared to that on surfaces of PCLPU0, because the transition from a negatively charged surface to positively charged surface could enhance electrostatic interaction between BSA and the surface (BSA, negatively charged at pH 7)[43]. In addition, the amount protein adsorption of PCLPUn samples was increased with the length increases of these GQAS hydrophobic alkyl chains, since these longer hydrophobic alkyl chains are more likely to enhance the protein-surface hydrophobic interactions.[44] Interestingly, the increased protein adsorption on surfaces of gemini quaternary ammonium salts polyurethanes would not much affect their antifouling and antibacterial activities. The culture-based antibacterial and antifouling results are shown in Figure 3A. The density of live bacteria attached on control surfaces (without PEG) was 2.90×10^6 CFU/cm^2 E. coli and 4.0×10^5 CFU/cm^2 S. aureus, of which E. coli and S. aureus was about 6.6 and 2 times higher than those on PCLPU0 surfaces (4.4×10^5 CFU/cm^2 live E. coli and 2.1×10^5 CFU/cm^2 live S. aureus), respectively, indicating that PEG and carboxy groups could endow antifouling properties for materials surfaces,[45, 46] distinctly, more needs antibacterial activities for these surfaces. Thus, there was no live E. coli or S. aureus cells detected on all surfaces of PCLPUn containing different GQAS. A glass slide spreading method was also employed to investigate the contact-active antibacterial activity of these obtained PCLPUn films,[47] and using PCLPU0 as a negative control. All PCLPUn films containing GQAS show excellent contact-active antibacterial activity that no bacteria colonies were observed on their surface (Figure 3B). To further test which GQAS chain extender introduced can make a better antibacterial activity to polyurethanes, a series of PCLPUn films with different GQAS content (5, 6, 7 times dilution) were prepared by changing the weight ratios of PCLPUn and PCLPU0. After 6 times dilution, PCLUP12 still has good contact-active antibacterial activity. The results show that PCLPU12 has the best contact-active antibacterial activity (Figure 3C), and the antibacterial activity of PCLPU16 is relatively the weakest among these obtained PCLPUn films. The contact-active antibacterial mechanism of QAS polymers is that the positively charged quaternary ammonium salts on the polyurethane surface first interact with the negatively charged phospholipid head groups of the bacterial cellular membrane, causing general perturbation of the lipid bilayer. The long hydrophobic alkyl chains of QAS then pierce the membranes of these surface-attached bacteria, forming holes that cause cytoplasm leakage, lysis, and death.[48] Therefore, the antibacterial activity is heavily impacted by their hydrophobic alkyl chains and cellular membrane thickness of bacteria,[37, 49, 50] the optimal alkyl chain of QAS could achieve the best antibacterial activity.

Degradation properties of PCLPUn

From what has been discussed before, a polymer surface with biodegradable property could be gradually decomposed and degraded and polish the attached living organisms.[16-19] Thus investigation of degradation properties of these antibacterial PCLPUn films is of great importance. The hydrolytic and enzymatic degradation characteristics of obtained PCLPUn have been preliminarily explored in vitro. The degradation rates of the obtained PCLPUn films were determined by measuring their weight losses during degradation (Figure 4). For both hydrolytic and enzymatic degradation, weight loss ratios of all polyurethanes increased sharply in the first day, and then slowed down afterward, possibly due to the good solubility and relatively small-molecular-weight components leaching out from these polyurethane matrix at the initial phase of degradation.[51] The degradation rates of these polyurethanes observed in Lipase PS were higher than those in PBS, indicating that PCLPUn are facilely degraded in enzymatic solution by the acceleration of lipase PS to PCL-based polymers biodegradation.[33, 52, 53] It is valuable to note that the degradation rates of PCLPUn had an increase in lipase PS than that in PBS. Also, the enzymatic degradation rate of PCLPU12 is the highest in all samples. The probable reason is that more hydrophilic surfaces of these polyurethanes are more liable to be accelerated degradation by lipase PS (Figure S4). The good hydrophilicity is due to the higher migration ratio of PEG and gemini quaternary ammonium on PCLPU12 surfaces,[51] which will be investigated in our further work.

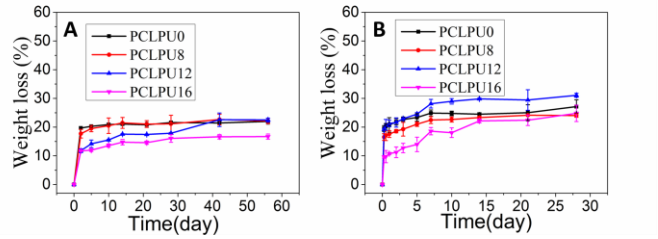

Figure 4. In vitro hydrolytic and enzymatic degradation profile of PCLPUn and PCLPU0 films. A: Hydrolytic degradation in PBS; **B**: Enzymatic degradation in lipase PS. Error bars represent means ± standard deviation for n=3.

To further investigate the degradation mechanism under different conditions, SEM was used to observe the surface morphology changes of all PCLPUn samples. Figure 5 shows the SEM images of non-degraded and degraded PCLPUn films surfaces in PBS and lipase PS media. After 28 day of hydrolytic degradation, these samples show rough and porous surfaces, even cracks to channel-like structures (Figure 5, a1-d1), which are attributed to the leaching of water-soluble oligomers or monomers formed after hydrolytic degradation from amorphous areas of these film surfaces into the surrounding media.[51,54] However, enzymatic degradation shows a superficial erosion over the whole surfaces and all samples exhibit rough surfaces without any porous or crack structure under lipase PS condition (Figure 5, a2-d2), especially bacterial enzyme, [34] because enzymes adsorb onto the surface before initiating polyurethane hydrolysis.[55] These results are in good agreement with the commonly recognized fact of superficial erosion in the case of enzymatic degradation.[56] More importantly, this degradation pattern can give PCLPUn films antifouling property in bacterial growth environment by releasing the attached bacteria with the whole superficial erosion degradation, and further studies are currently being carried out in our group to investigate the antifouling mechanism.

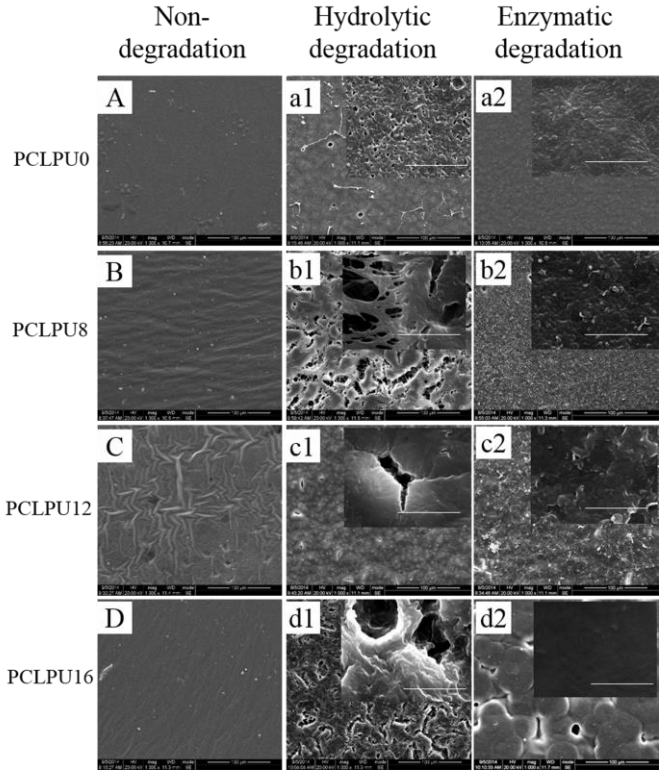

Figure 5. Superficial SEM micrographs of PCLPUn films: A-D: Non-degraded samples; a1-d1: Samples degraded in PBS medium for 28 days; a2-d2: Samples degraded for 28 days in lipase PS. Bar=100 μm. The enlarge images bar= 10μm.

Biocompability of PCLPUn films

The biocompatibility of the PCLPUn films were carried out by evaluating the cytotoxicity of their extracts and degradation liquor by MTT assay, which was frequently applied to screen polymer cytotoxicity for its reliability and sensitivity, and L929 mouse fibroblasts are used in cytotoxicity assays. The cytotoxicity of GQAS chain extenders were also performed through MTT assay (Figure S6) and GQAS chain extender with longer hydrophobic alkyl chain has a higher toxicity.

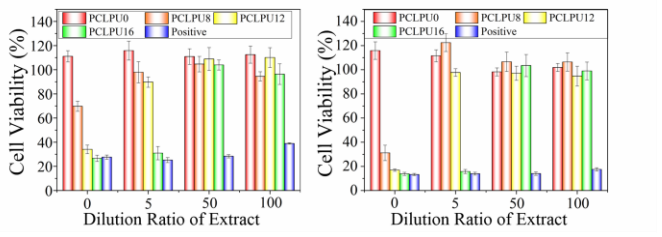

Figure 6. Cell viability measured by MTT assay after 24 h (A) and 72 h (B) of incubation with serial dilutions of samples extracts. Error bars represent means ± standard deviation for n=3. Statistical significance: $p < 0.05$

The extraction procedures were carried out to simulate clinical conditions to evaluate the release of leachable toxic components from these PCLPUn matrix without damaging their physicochemical or mechanical properties. The effect of these extract concentrations on the proliferation of L929 mouse fibroblasts is assessed, as shown in Figure 6. These extracts of PCLPUn samples show an increasing cytotoxicity against fibroblasts with GQAS hydrophobic alkyl chain increasing. However, more than 90% cell viability could be retained after 5 times dilution with a 24 or 72 h incubation for PCLPU8 and PCLPU12, suggesting that GQAS polyurethanes containing shorter hydrophobic alkyl chain length of GQAS are able to have good biocompatibility.

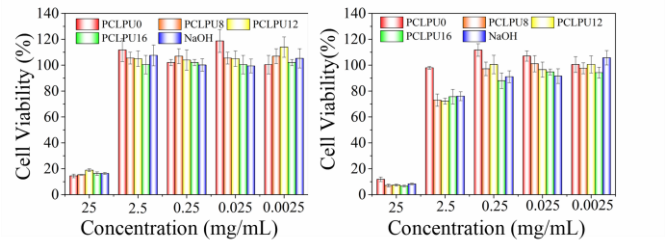

Figure 7. Cell viability measured by MTT assay after 24 h (A) and 72 h (B) of incubation with different concentration of PCLPUn degradation products. Error bars represent means ± standard deviation for n=3. Statistical significance: $p < 0.05$.

To further evaluate biocompatibility of these PCLPUn samples, an accelerated degradation in a strong basic solution was carried out to completely decompose the polymers and yield the maximum release of degradation products. All these polyurethanes degradation solutions were performed using the MTT assay and these results showed no apparent inhibition effect for the L929 fibroblasts when the degradation solutions are diluted to 0.25mg/mL (Figure 7 and Figure S7). The lowered cell viability at a high concentration is probably caused by the inherent toxicity existing in most polycations[51] and large amounts of sodium chloride in the degradation solutions. The blank control sample (NaOH+HCl) without dilution shows high cytotoxicity can confirm this phenomenon. Therefore, in view of clinical usage and the low degradation ratio of the samples, these polyurethanes and their degradation products can potentially meet safety requirements of biomedical applications. In addition, the effect of GQAS content on antibacterial and biocompability was further studied in our ongoing works.

Conclusions

In summary, a series of antibacterial and antifouling waterborne biodegradable polyurethanes containing GQAS have been successfully synthesized using PCL, PEG, lysine and IPDI by a facile polymerization strategy. The incorporation of different hydrophobic alkyl chain length GQAS has significant impacts on the properties of these waterborne polyurethanes, including antibacterial activities, antifouling, biodegradability, and biocompatibility. The obtained PCLPUn films could be degraded via a superficial erosion mechanism in lipase PS, thus this degradation pattern is favorable for these surfaces owning good antifouling ability in bacterial growth environment to release the attached bacteria accompanied by the layer degradation of PCLPUn films. Hence, these waterborne, especially PCLPU12, with the best antibacterial activity and the relatively good biocompability, could potentially be applied as coatings in implant surfaces and tissue engineering scaffold to prevent biomaterials related infection.

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