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Adjustment of the antibacterial activity and biocompatibility of hydroxypropyltrimethyl ammonium chloride chitosan by varying the degree of substitution of quaternary ammonium

Zhao-Xiang Peng[a], Ling Wang[b], Lin Du[a], Sheng-Rong Guo[b,*], Xiao-Qing Wang[a], Ting-Ting Tang[a,*,1]

[a] Shanghai Key Laboratory of Orthopaedic Implant, Department of Orthopaedic Surgery, Shanghai Ninth People's Hospital, Shanghai Jiaotong University, School of Medicine, Shanghai 200011, China
[b] School of Pharmacy, Shanghai Jiao Tong University, 800th Dong-chuan Road, Shanghai 200240, China

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ABSTRACT

Hydroxypropyltrimethyl ammonium chloride chitosan (HACC) was synthesised with differing degrees of substitution (6%, 18% and 44%) of quaternary ammonium by reacting chitosan with glycidyl trimethylammonium chloride. The antibacterial activities of these polymers were tested in vitro against *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus*, and *Staphylococcus epidermidis*. Mouse fibroblasts and bone marrow derived stromal cells (hMSCs) were used to investigate the biocompatibility of the HACC. The results show that the antibacterial activities of the HACC with 18% or 44% substitution were significantly higher than the others ($P < 0.05$) against all three bacteria. HACC with 6% or 18% substitution was not cytotoxic and did not interfere with the proliferation and osteogenic differentiation of hMSCs. Overall, we can make a conclusion that HACC with an 18% substitution was a potential pharmaceutical that can inhibit the growth of bacteria and has good biocompatibility with osteogenic cells.

1. Introduction

Infection is one of the most serious and devastating complications faced by millions of orthopaedic patients annually. Implant-associated periprosthetic infections often lead to implant removal and revision surgery, imposing a huge economic burden on society (Kurtz et al., 2005, 2008; Zimmerli, Trampuz, & Ochsner, 2004). Experimental models have proven that the critical dose of contaminating bacteria required to produce infection is much lower when the implant material is present at the surgical site (Cordero, Munuera, & Folgueira, 1996; Elek, 1956; Schierholz, Beuth, Rump, Konig, & Pulverer, 2001; Southwood, Rice, McDonald, Hakendorf, & Rozenbilds, 1985). Bacterial adhesion and anchorage on implant device surfaces represent a crucial initial step in this process (Campoccia, Montanaro, & Arciola, 2006). In addition, failure of bone and joint implants has been mainly attributed to bacterial infection, along with poor bonding of the implant to bone tissue (Itiravivong et al., 2003; Shi, Neoh, Kang, Poh, & Wang, 2009). The probability of successful osteointegration versus implant infection depends on competition for the device surface between osteogenic cells and bacteria (Shi, Chua, Neoh, Kang, & Wang, 2008; Zhang, Zhang, Zhu, Kang, & Neoh, 2008). Therefore, one promising strategy to prevent infections and enhance osteointegration is to develop a selective material that could be applied to the metal implant to inhibit contamination from bacteria and support the growth of osteogenic cells.

The aminopolysaccharide chitosan is a natural non-toxic biopolymer derived by the deacetylation of chitin. It has antibacterial characteristics against some bacteria, caused by the electrostatic interaction between the NH_3^+ groups of chitosan acetate and the phosphoryl groups of phospholipid components of cell membranes (Liu, Du, Wang, & Sun, 2004). Recent studies demonstrated that chitosan had good biocompatibility with bone cells and used it as a scaffold for bone tissue engineering (Heinemann, Heinemann, Bernhardt, Worch, & Hanke, 2008; Li, Ramay, Hauch, Xiao, & Zhang, 2005; Moreau & Xu, 2009; Xu & Simon, 2005). Some groups have also explored chitosan and its derivatives for orthopaedic applications with long-term antibacterial properties and osteoblast enhancing properties (Bumgardner et al., 2003; Di, Sittinger, & Risbud, 2005; Guo et al., 2008).

However, the reported antibacterial activity of chitosan is limited to acidic conditions due to its poor solubility above pH 6.5 (Li, Liu, & Yao, 2002). This restricts its use as an antibacterial agent, so studies have paid a great deal of attention to the preparation of chi-

* Corresponding authors. Tel.: +86 21 23271133; fax: +86 21 63137020.
E-mail addresses: srguo@sjtu.edu.cn (S.-R. Guo), tingtingtang@hotmail.com (T.-T. Tang).
[1] Tel.: +86 21 23271133; fax: +86 21 63137020.

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tosan derivatives whose antibacterial ability is not limited to acidic environments. For example, water-soluble quaternary ammonium salts from the reaction of chitosan with glycidyl trimethylammonium chloride have been reported as a chitosan derivative with enhanced antibacterial ability against *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* and *Candida albicans* (Qin et al., 2004). These cationic antibacterial agents have been widely used. The target site of the cation is the negatively charged cell surface of the bacteria. Polycationic biocides, including chitosan, can interact and form polyelectrolyte complexes with acidic polymers produced at the bacterial cell surface. Quaternised chitosan has been described to have a significant antibacterial activity (Sun, Wu, Dai, Chang, & Tang, 2006). However, little work has been reported on its biocompatibility with osteogenic cells, or on the effect of different degrees of substitution (DS) of quaternary ammonium on the antibacterial potency of hydroxypropyltrimethyl ammonium chloride chitosan (HACC).

Our hypothesis is that HACC with some DS of quaternary ammonium may have enhanced antibacterial ability and little cytotoxicity to mammalian cells. To test this hypothesis, we synthesised water-soluble quaternised chitosan with different DS and investigated their antibacterial ability against three of the most common bacteria in orthopaedic implant-related infections, as well as their cytotoxicity to and compatibility with the osteogenic cells.

2. Materials and methods

2.1. Materials

Chitosan with a molecular weight of 20.0×10^4 and 91.83% N-deacetylation was purchased from Zhejiang Yuhuan Ocean Biochemistry Co. Ltd. (China). Glycidyl trimethylammonium chloride (GTMAC) was purchased from Shandong Sangong Chemical Co. Ltd. with a purity of 96%. Other chemicals used were of analytical grade.

2.2. FT-IR and ^{1}H NMR spectroscopy

IR spectra were obtained with KBr pellets against a blank KBr pellet using a Paragon 1000 FT-IR spectrophotometer. The data collection parameters employed were as follows: gain, 1; resolution, 4.0 cm^{-1}; and scans, 32.

^{1}H NMR spectra of chitosan and its derivatives in D_2O/CF_3COOD 95/5 (v/v) were obtained on a Varian Mercury Plus 400 spectrometer at room temperature. Data collection consisted of 64 acquisitions.

2.3. Preparation of 2-hydroxypropyltrimethyl ammonium chloride chitosan (HACC)

HACC was prepared by a modified method of Qin et al. (2004). Chitosan (16 g, 100 mmol) and GTMAC (7.2 g, 50 mmol) were dispersed in distilled water (240 ml) in a three-neck flask. The mixture was stirred at 80 °C with reflux. After 36 h of reaction, the yellowish reaction products were dialysed in distilled water for 72 h using a dialysis membrane with a molecular weight cut-off of 1.4×10^3 and then concentrated and lyophilised.

In order to obtain the other two HACCs with different degrees of substitution, 100 mmol and 300 mmol GTMAC were used in two similar reactions.

2.4. Determination of the DS of HACC

The DS of HACC was measured by conductometric titration of chloride ions. DS is defined as the mol ratio of bonded GTMAC per mol of glucosamine calculated from the original mass of chitosan (Sun, Du, et al., 2006) and its degree of deacetylation (DD). The formula to determine the DS of HACC is as Eq. (1):

$$DS = \frac{V \times c/1000}{C \times c/1000 + (W - V \times c \times 314/1000)/162} \times \frac{1}{DD} \times 100\% \quad (1)$$

V (ml) is the volume of $AgNO_3$ solution calculated from the inflection point where the conductivity of the solution is lowest when adding the $AgNO_3$ solution stepwise, c (mol/l) is the concentration of $AgNO_3$ solution, W (g) is the mass of HACC, and DD is the degree of deacetylation of chitosan. Quaternary glucosamine has a molar mass of 314, and glucosamine has a molar mass of 162. Dried HACC (20 mg) was dissolved in 1% (v/v) HAc (100 ml) and conductometrically titrated with $AgNO_3$ solution (0.01 mol/l). Solution conductivities were monitored with a conductivity meter with a platinum black electrode.

2.5. Estimation of water solubility

The quantitative testing of solubility was conducted using a weight method, while the pH dependence of sample water solubility was evaluated using turbidity measurements.

For quantitative testing of the solubility, the weighed chitosan or HACC samples (0.5 g) with different DS were suspended overnight in 2 ml of distilled water (pH 7.2) at 25 °C in a water bath with constant shaking. The soluble part of the sample was removed by centrifugation, and the precipitates were dried and weighed. The quantitative solubility of the sample was obtained as the concentration of dissolved sample (mg/mL). For pH dependence of the sample water solubility, the test sample (0.2 g) was dissolved in 1% HAc (100 ml). With stepwise addition of NaOH solution (1 M), the transmittance of the solution was recorded with a UV (Spectrumlab 54) spectrophotometer at 600 nm.

2.6. Testing solutions

We used the chitosan and three kinds of HACC with DS of 6%, 18% and 44% (HACC 6%, HACC 18% and HACC 44%) solution to clarify the antibacterial activity. The chitosan and HACC were extracted in 0.85% NaCl solution (2.5 mg/ml) for 24 h at 37 °C, 60 rpm, and sterilised by filtration with a 0.22 μm filter, along with 0.85% NaCl solution as a control.

For the biocompatibility test, the chitosan and HACC were extracted in α-MEM medium (2.5 mg/ml) for 24 h at 37 °C, 60 rpm, and sterilised by filtration with a 0.22 μm filter, along with α-MEM medium as a control.

2.7. Antibacterial assays

2.7.1. Preparation of bacteria

This study used bacterial strains isolated from orthopaedic implant-related infections of three patients who were undergoing treatment at the Nith People's Hospital, Shanghai Jiao Tong University (Shanghai, China): *Staphylococcus aureus* 407 (*S. aureus* 407), Methicillin-resistant *Staphylococcus aureus* 541(MRSA 541), and *Staphylococcus epidermidis* 243 (*S. epidermidis* 243) (Wang et al., 2009). To obtain inocula for the examination, we cultured the three bacteria overnight on BBL™ Trypticase™ Soy Broth (TSB, BD Biosciences, Franklin Lakes, NJ) medium at 37 °C. After two successive transfers of the test organism in TSB medium at 37 °C for 12 h, the activated culture was again inoculated into 100 ml TSB at 37 °C for 12 h. Cells were then harvested by centrifugation (8000 × *g* for 10 min). Using McFarland standards, the bacteria were suspended in testing solutions to 1×10^7 CFU/ml with 0.85% NaCl as the control.

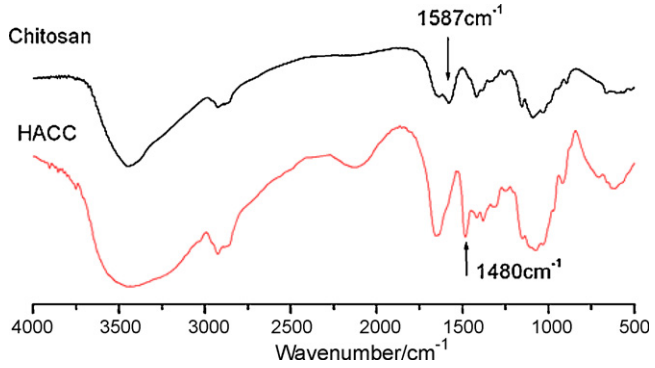

Fig. 1. FT-IR spectra of chitosan and HACC 44%.

2.7.2. Determining the antibacterial activity of CS and HACC

Ten millilitres of the original cell suspension of the three bacteria, with bacterium age of 16 h, separately and with concentrations of about 10^7 CFU/ml was added into several clean tubes. Immediately after the solution and the bacteria suspension were mixed, a 0.1 ml sample of each suspension was taken to determine the bacterial numbers by serial dilutions with triplicate plating on Todd-Hewitt agar plates (countable range: 30–300 cfu/plate), then incubated the test tubes at 37 °C in a shaking incubator operating at 100 cycles/min. Two plates were used per testing solution. After incubation for 24 h at 37 °C, the surviving colonies were counted, and the mean number of the cells on duplicate plates was calculated. The number calculated expressed the antibacterial potency of the samples. Plates showing no growth were incubated for an additional 24 h to allow for detection of slow-growing colonies. The numbers were used as the starting value in each testing solution. After 15 min, 2, 4, 6, 8 and 12 h, the bacteria were incubated by the methods described above, and the mean numbers of surviving cells were calculated. The antibacterial efficiency of the samples for different incubation times was modified using Eq. (2) according to Chung and Chen (2008):

$$\text{Antibacterial efficiency}\,(\%) = \frac{(\text{initial cell number} - \text{cell number after treatment})}{\text{initial cell number}} \times 100\% \quad (2)$$

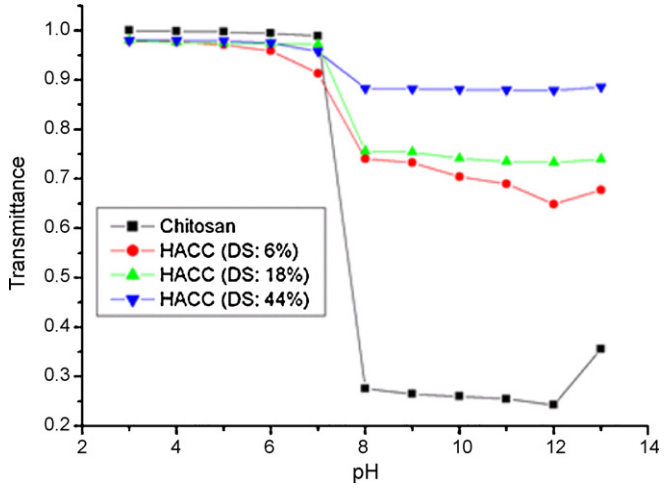

Fig. 3. pH dependence of water solubility of chitosan and HACC; the concentrations of chitosan and HACC are 0.2% (w/v), the molecular weight and degree of deacetylation of chitosan are 2×10^5 and 91.8%, respectively.

2.8. Cytotoxicity testing

L929 cells (mouse fibroblasts) were provided by the Chinese Academy of Sciences (Shanghai) and grown in α-MEM (GIBCO, Grand Island, NY, USA) supplemented with 10% FBS (Hyclone, Tauranga, New Zealand) and antibiotics (penicillin 100 U/ml, strep-

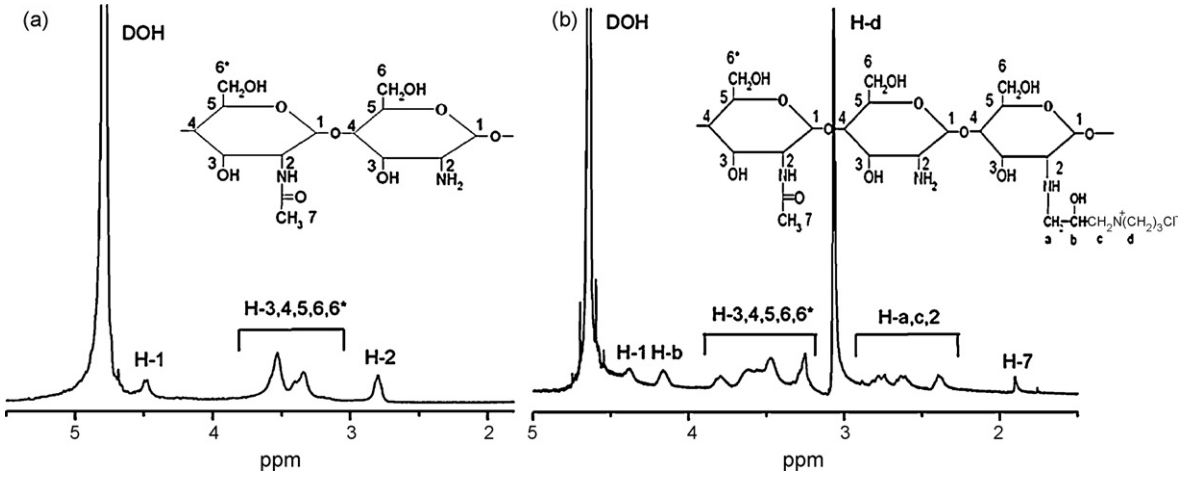

Fig. 2. ^{1}H NMR spectrum of chitosan (a) and HACC 44% (b).

Table 1
DS and Solubility of HACC.

Reaction	Feed mol ratio of *n* (chitosan)/*n* (GTMAC)	DS of HACC	Solubility (mg/ml)[a]
I	100 mmol/50 mmol	6%	2.5
II	100 mmol/100 mmol	18%	15.4
III	100 mmol/300 mmol	44%	40.2

[a] The solubility of chitosan was 0 mg/ml.

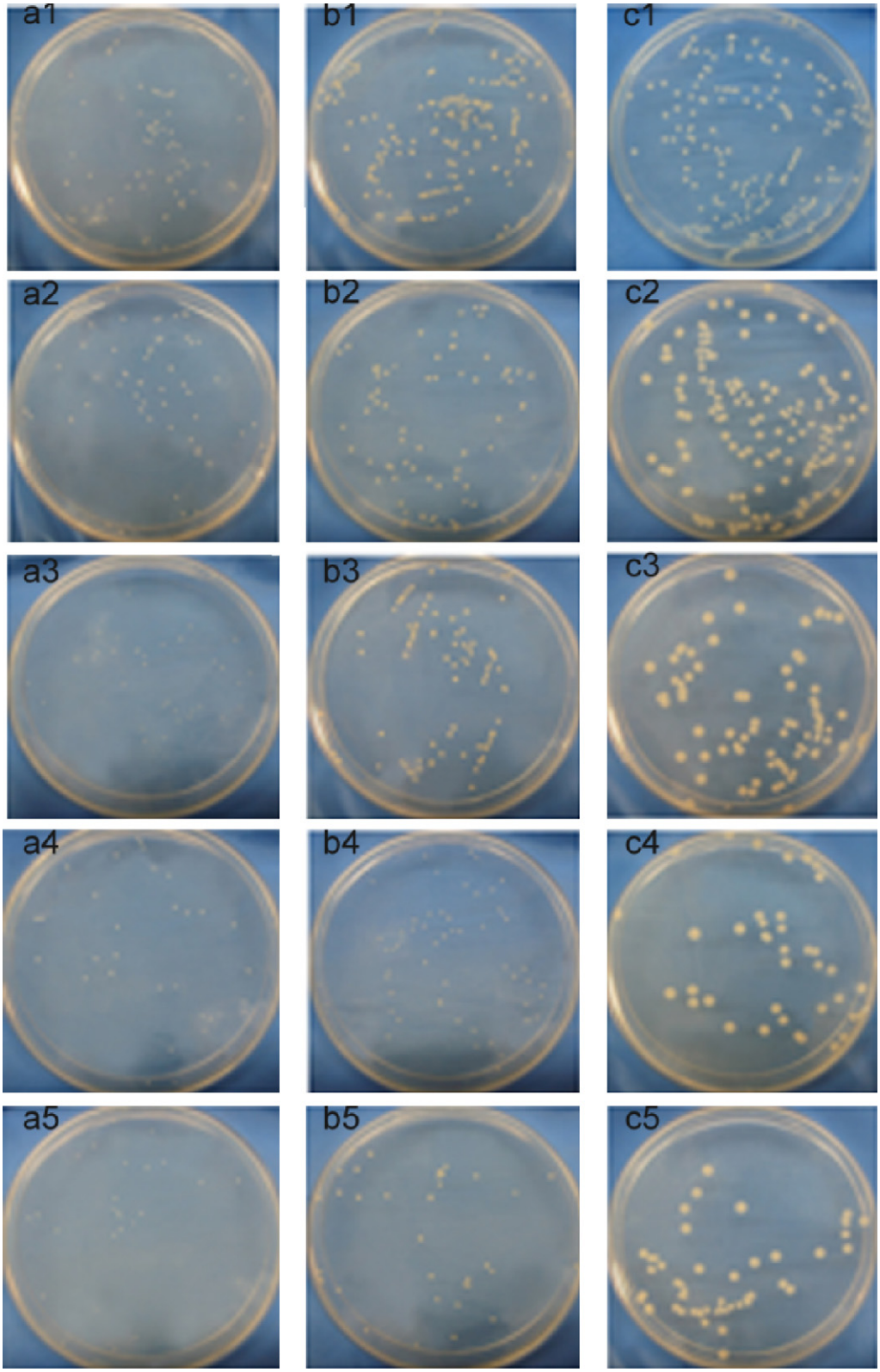

Fig. 4. Representative images of the cell growth in different solutions after 12 h incubation. Antibacterial efficacy of the samples (1) Control, (2) Chitosan, (3) HACC 6%, (4) HACC 18% and (5) HACC 44% against (a) *S. aureus*, (b) MRSA and (c) *S. epidermidis*.

tomycin 100 μg/ml; Hyclone, Logan, UT, USA) in a 37 °C humidified atmosphere with 5% CO_2.

According to the ISO standards (1992) of in vitro cytotoxicity evaluation, L929 cells (10^3) were seeded in a 96-well plate with 150 μl testing solution for 48 h, along with a control with cells grown in the α-MEM medium and five duplicates for each sample. Succinate dehydrogenase activity was then determined by adding 20 μl of a 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, M-2128, Sigma) to each well, followed by 3 h of incubation at 37 °C. Medium and MTT were

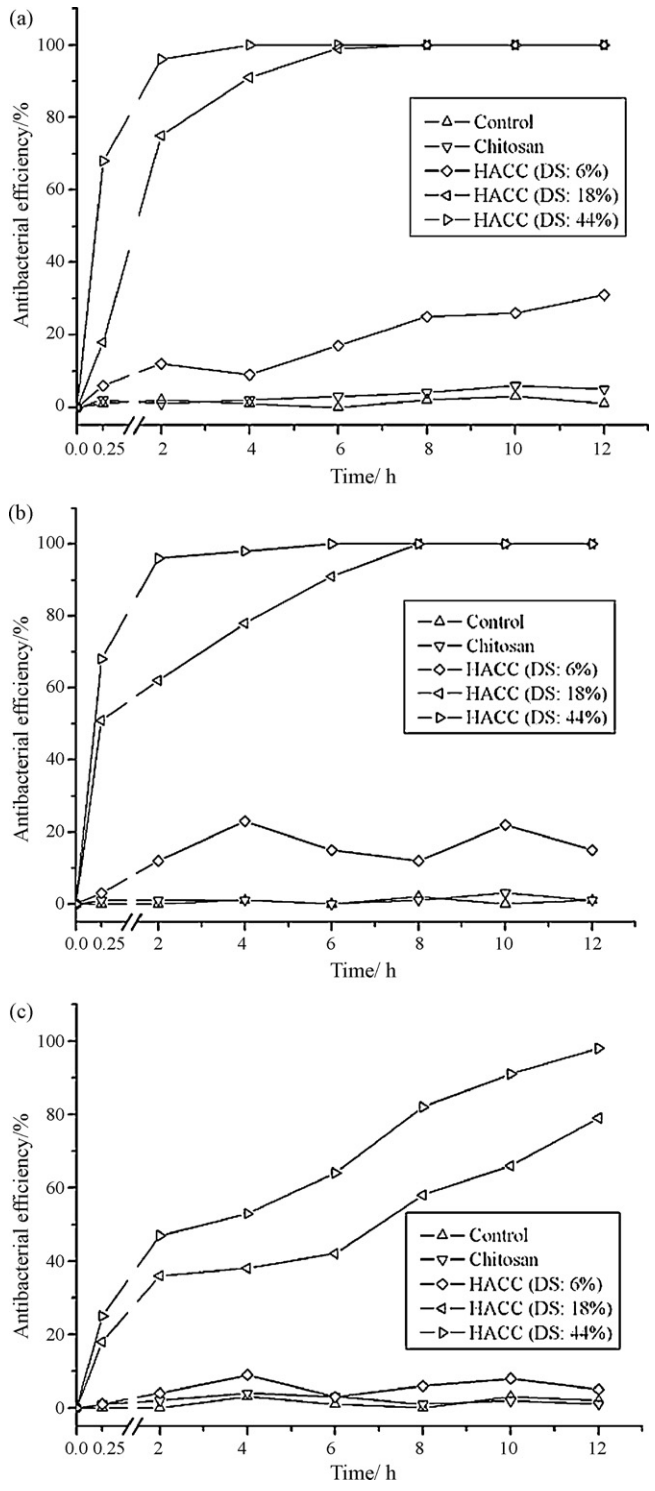

Fig. 5. Antibacterial efficiency of the samples against (a) *S. aureus*, (b) MRSA, and (c) *S. epidermidis*. Experimental conditions: 1.39 mg/ml of chitosan, 2.5 mg/ml of HACC 6%, HACC 18% and HACC 44%; initial pH: 7.45.

removed. The formazan product was then solubilised in 100 μl 0.04 mol l/l HCl in isopropanol. The amount of this dye was quantified by measuring the absorption at 570 nm using an automated plate reader (PerkinElmer). Extracts were rated as severely (<30%), moderately (30–60%), slightly (60–90%), or not cytotoxic (>90%) based on the activity relative to control values.

2.9. Evaluation of the proliferation and differentiation of human bone marrow stromal cells (hMSCs)

2.9.1. Cell culture

The hMSCs were isolated and expanded using a modification of standard methods as described previously (Pittenger et al., 1999). The donor was healthy without metabolic disease, inherited illnesses, or other diseases that may affect the current study. Cells were grown in complete Alpha Minimum Essential Medium (α-MEM; GIBCO, Grand Island, NY, USA) supplemented with 10% foetal bovine serum (FBS; Hyclone, Tauranga, New Zealand) and antibiotics (penicillin 100 U/ml, streptomycin 100 μg/ml; Hyclone, Logan, UT, USA) in a 37 °C humidified atmosphere with 5% CO_2. A previous report (Sun, Du, et al., 2006; Sun, Wu, et al., 2006) characterised the phenotype of these cells. Cells at a passage from P2 to P4 were used for these experiments.

2.9.2. Proliferation of hMSCs

The MTT assay was done according to the manufacturer's instructions (Sigma–Aldrich). Briefly, 1×10^3 hMSCs were seeded in a 96-well plate with 150 μl of testing solution as described above, plus α-MEM medium solution as a control. At each time point, MTT solution was added to each well, and plates were incubated for 3–4 h; subsequently, dimethyl sulphoxide (DMSO, Sigma–Aldrich) was added to the wells for 5 min. The plates were then read at 570 nm using an automated plate reader (PerkinElmer).

2.9.3. Osteogenic differentiation of hMSCs

Cells seeded at a density of 10^4 cells/cm^2 were used to evaluate the effect of chitosan or HACC on the osteogenic differentiation of hMSCs. After 24 h, the medium was changed to one of the following media: normal growth medium without osteogenic induction supplement, osteogenic induction medium [α-MEM supplemented with 10% FBS, 0.1 μM dexamethasone (Sigma), 50 μM ascorbate acid (Sigma), and 10 mM β-glycerophosphate sodium (Sigma)], osteogenic induction medium containing chitosan, HACC 6%, HACC 18% and HACC 44%. These media were renewed every 3 days throughout the study period.

2.9.4. Alkaline phosphatase activity (ALP)

After 2 weeks of osteogenic induction in 6-well plates ALP staining was accomplished according to the procedures provided by the manufacturers of the ALP staining kit (Renbao, Shanghai, China). In short, hMSCs were fixed on day 14 using buffered formalin for 30 s. After two washes with distilled water, cells were stained with a staining reagent for 30 min. Stained cells were photographed using an Olympus digital camera. The level of ALP activity was also determined after 2 weeks. ALP activity was determined following the procedures as mentioned in our previous article (Sun, Du, et al., 2006; Sun, Wu, et al., 2006). All experiments were conducted in triplicate.

2.9.5. Alizarin red staining

After 4 weeks of osteogenic induction in 6-well plates, hMSCs were fixed in 10% formalin and incubated with 1% Alizarin Red S (Sigma–Aldrich Co., St Louis, MO, USA) solution for 10 min at room temperature for Alizarin Red S staining. Stained cells were photographed using an Olympus digital camera.

2.10. Statistical analysis

Differences between groups were examined for statistical significance using analysis of variance (ANOVA), and $P < 0.05$ was considered significant. All experiments were performed in triplicate.

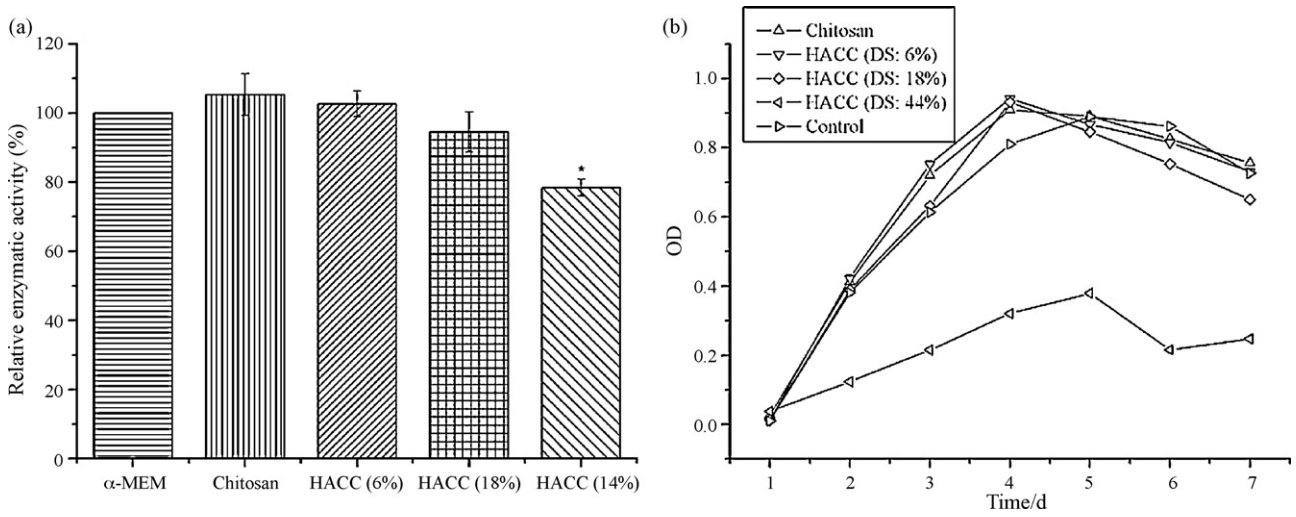

Fig. 6. (a) MTT-reducing activity relative to control after 48 h exposure of L-929 cells to extracts made from 24 h test samples. Data are means ± SD ($n = 5$). *Denotes significant differences from control values with $P < 0.05$. (b) MTT results of hMSCs showed that the proliferation of hMSCs in the sample solution of the HACC 44% was significantly lower ($P < 0.01$) than that in other solutions from day 2 to day 7, while no significant difference was found among other groups ($P > 0.05$). Experimental conditions: 1.39 mg/ml of chitosan, 2.5 mg/ml of HACC 6%, HACC 18% and HACC.

3. Results

3.1. Preparation and characterisation of HACC

The IR spectrum of HACC (Fig. 1) shows evidence of the introduction of the quaternary ammonium salt group to the chitosan backbone. A peak at 1480 cm^{-1} was assigned to the C–H bending of the trimethylammonium group. The N–H bending (1587 cm^{-1}) of the primary amine becomes weak due to the partial change of the primary amine of chitosan to the secondary amine. In addition, the HACC spectrum shows a broader band than chitosan at around 3400 cm^{-1} due to the increased number of hydroxyl groups (Lim & Hudson, 2004).

Fig. 2 shows the ^1H NMR spectra of chitosan and HACC. There are obvious differences between the spectra of the two substances. An obvious characteristic peak of hydrogen (3.21 ppm) of trimethylammonium groups is present in the spectrum of HACC, but absent from the spectrum of chitosan.

The DS of the HACC was measured by conductometric titration of Cl^- with 0.01 M aqueous $AgNO_3$ solution. As listed in Table 1, HACC 6%, HACC 18% and HACC 44% were successfully synthesised by adjusting the feed mol ratio of chitosan to GTMAC.

3.2. Water solubility of HACC

As shown in Table 1, the solubility of HACC increases with the increased DS of HACC. The native chitosan was water-insoluble due to its strong intermolecular hydrogen bonds. As the reaction occurred, the introduction of quaternary ammonium groups with some steric hindrance and a good hydration capacity greatly reduced the intermolecular (Kurita, 2001) and intramolecular hydrogen bonds of chitosan. After quaternisation, chitosan became a water-soluble cationic. The good solubility of HACC can be attributed to the statement mentioned above.

Fig. 3 shows the pH dependence of the water solubility of chitosan and HACC. At low pH (pH < 6.0), the transmittance is close to 100% not only for the chitosan solution but also for the HACC solutions. This indicates that the chitosan and HACC molecules have good solubilities in acidic conditions. When the pH increases from 7.0 to 8.0, the transmittance of the chitosan solution rapidly drops, and the solution becomes opaque. In contrast, the transmittance of HACC solutions slowly decreases, with a smaller decrease when the DS of HACC increases from 6% to 44%. These results suggest that HACC has a better solubility in basic conditions than chitosan, and that the solubility increases with increasing DS of HACC. There is an increasing transmittance from pH 12.0 to 13.0 for all the solutions, especially chitosan. This could be due to the fact that the solubility of chitosan is improved when the hydroxy groups of chitosan are alkalised as the alkoxide form (Kurita, 2001).

3.3. Antibacterial assays

The surviving colonies of the *S. aureus*, *S. epidermidis* and MRSA strains incubated with the test samples at different fixed incubation periods were counted on Todd-Hewitt agar plates (Fig. 4). The antibacterial efficiencies were calculated using Eq. (1) and are shown in Fig. 5. These results show that the antibacterial efficiencies of HACC 18% and HACC 44% against each kind of bacterium were significantly higher ($P < 0.01$) than those of the other solutions. The antibacterial efficiencies against *S. aureus* or MRSA for HACC 6% was significantly higher ($P < 0.05$) than the control solution. The antibacterial efficiency of chitosan against each kind of bacterium is not different from the control solution ($P > 0.05$).

3.4. Cytotoxicity

Extracts of Chitosan, HACC 6% and HACC 18% were rated non-cytotoxic. The extract of HACC 44% was rated slightly cytotoxic (Fig. 6(a)).

3.5. Proliferation and differentiation of hMSCs

The proliferation of hMSCs in the sample solution of the HACC 44% was significantly lower ($P < 0.01$) than that in other solutions from day 2 to day 7, while no significant difference was found among the other groups (Fig. 6(b)). Fig. 7(A) shows the positive ALP staining in the Chitosan, HACC 6%, HACC 18% and osteogenic induction medium treated cells and negative staining in the HACC 44% treated cells after a 14 days culture. The ALP activity of the hMSCs was measured with the pNPP assay and normalized on the basis of protein content per disk. As shown in Fig. 7(B), HACC 44% treated cells presented lower activity compared with the Chitosan, HACC 6%, HACC 18% or osteogenic induction medium treated cells ($^{\#}P < 0.01$), furthermore, we found that HACC 6% and 18% enhanced

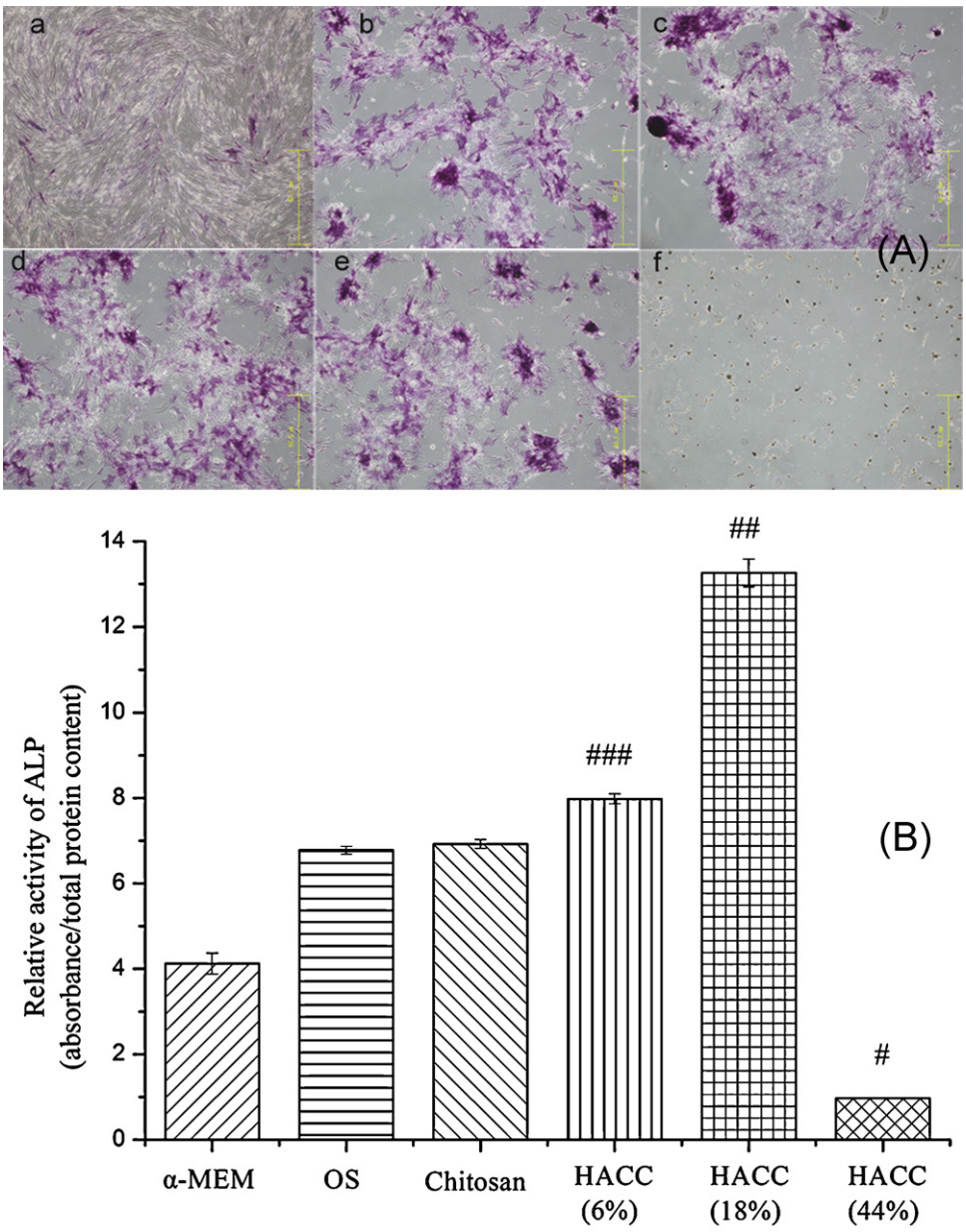

Fig. 7. (A) Positive ALP staining in the (b) positive control (OS only); (c) Chitosan; (d) HACC 6%; (e) HACC 18% treated cells, and negative ALP staining in the (a) negative control (α-MEM medium) and (f) HACC 44% treated cells after a 14 days culture. (B) After a 14 days culture in osteogenic medium, HACC 44% treated cells presented lower ALP activity compared with the Chitosan, HACC 6% and HACC 18% or osteogenic induction medium treated cells ($^{\#}P < 0.01$), HACC 6% and HACC 18% enhanced the ALP activity of hMSCs compared with the Chitosan or osteogenic induction medium ($^{\#\#\#}P < 0.05$, $^{\#\#}P < 0.01$).

the ALP activity of hMSCs compared with the Chitosan or osteogenic induction medium ($^{\#\#\#}P < 0.05$, $^{\#\#}P < 0.01$).

Fig. 8 also shows the same positive staining of alizarin red S in the Chitosan, HACC 6%, HACC 18% and steogenic induction α-MEM medium treated cells and negative staining in the HACC 44% treated cells after a 28 days culture.

4. Discussion

Both chitosan and small molecular quaternary ammonium salts have good antibacterial activities (Belalia, Grelier, Benaissa, & Coma, 2008; Jakimiak et al., 2006). In order to combine their antibacterial activities and to improve the poor solubility of chitosan, quaternised chitosan was synthesised. GTMAC, a small molecular quaternary ammonium with an epoxy group, was chosen as a quaternisation reagent because of its ease of reaction with the nucleophilic amino groups of chitosan.

HACC was synthesised with three different degrees of substitution (6%, 18%, 44%). Our ultimate goal is to find a new antibacterial biomaterial that can be used in the prevention of orthopaedic implant-associated infection. According to Campoccia et al. (2006), *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* contribute to 66% of disastrous orthopaedic implant-related infections. Therefore, we used these three bacteria to investigate the antibacterial properties of HACC with different degrees of substitution in vitro. For the sustained release and efficacy of the disinfectants, the HACC concentration should be as low as possible. The solubility of HACC increases with the DS, and in this case, we chose the 2.5 mg/ml as an appropriate concentration for test-

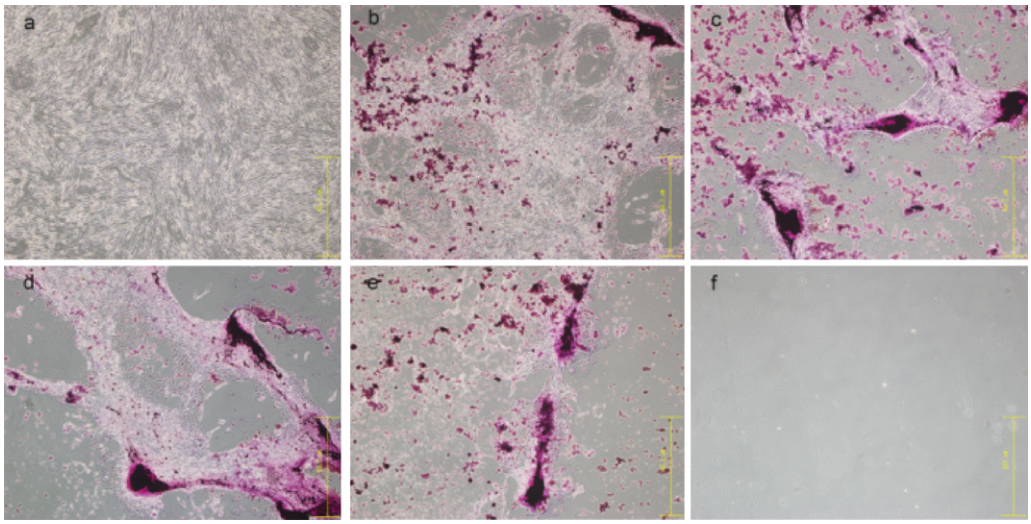

Fig. 8. Positive staining of alizarin red S in the (b) positive control (osteogenic induction α-MEM medium); (c) Chitosan; (d) HACC 6%; (e) HACC 18% treated cells, and negative alizarin red S staining in the (a) negative control (α-MEM medium) and (f) HACC 44% treated cells after a 28 days culture.

ing their antibacterial activity and biocompatibility at a pH of 7.45.

These results demonstrate that the water-soluble HACC has an enhanced antibacterial activity against the three bacteria compared with chitosan. The DS of HACC was found to affect the antibacterial activity of the prepared chitosan derivatives with GTMAC. The antibacterial activity of HACC 18% and HACC 44% was significantly higher ($P < 0.01$) than that of others. Therefore, the increased DS of the chitosan derivatives could improve the antibacterial activity of the HACC.

The osteointegration of orthopaedic implants is very important to the functional reconstruction of the damaged tissue (Borsari et al., 2009; Landor et al., 2007). Because of this, the implant surface needs to have low cytotoxicity and good biocompatibility with bone cells. Chitosan is structurally similar to glycosaminoglycan and has many desirable properties as a tissue engineering scaffold (Chua, Neoh, Shi, & Kang, 2008; Cooney, Petermann, Lau, & Minteer, 2009; Kim et al., 2008; Shi et al., 2009; Twu, Chang, & Ping, 2005), but Dunne et al. (2008) reported that it had no antimicrobial effect and reduced mechanical performance in gentamicin-loaded bone cement. There have not yet been any reports about the potential use of HACC in this field. Based on our data, chitosan and HACC 6% and HACC 18% were rated as not cytotoxic, and the HACC 44% was rated as slightly cytotoxic. We further investigated the effect of HACC on the proliferation and osteogenic differentiation of hMSCs. We found that the HACC 44% inhibited the proliferation as well as osteogenic differentiation. These results indicated that the HACC 44% had poor biocompatibility with bone cells, although its antibacterial activity was the best.

5. Conclusions

The antibacterial activity of HACC can be adjusted with changes to the DS. The HACC 18% had increased antibacterial activity, was noncytoxic to L-929 cells and had good biocompatibility with osteogenic cells. Thus, it is a potential antibacterial coating material and HACC 18% is being studied as antibacterial coating on titanium implants in our group.

Acknowledgements

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