Antimicrobial characteristic and mechanism of Nano-fumed silica salt grafted N,N-dimethyl-n-tetradecylamine

Cuihcng Liu[1,*], Ying Tao[2], Jianjun Gou[1], Dongchun Qin[1], Hongchun Liu[1], Shen Yan[1], Xianju Feng[1]

[1]*Clinical Laboratory, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan* 450052, *China;* [2]*The Institute of Research and Design of Enviroument, Kaifeng, Henan* 475000, *China*

Received August 20, 2008

Abstract

Objective. To investigate the antimicrobial characteristic and mechanism of Nano-fumed silica modified with quaternary ammonium compound. *Methods.* Antimicrobial characteristic and mechanism was tested by quantitative suspension test, TTC test and the test of integrity of bacterial membrane. *Results.* The result of quantitative suspension test shows that the absorption ability of Nano-fumed silica modified with quaternary ammonium group is much higher than that of Nano-fumed silica. The results for TTC and testing integrity of cell membrane prove that the antibacterial process of Nano-fumed silica modified with quaternary ammonium group kill bacteria by destroying the cell membrane to outflow DNA and RNA in the cytoplasm. *Conclusion.* The results indicate the mechanism of antibacterial activity is to absorb bacterial to its surface. [Life Science Journal. 2009; 6(1): 52 – 54] (ISSN: 1097 – 8135).

Keywords: Nano-fumed silica; quaternary ammonium salt grafted Nano-fumed silica; antibacterial mechanism

1 Introduction

Antimicrobial materials are widely used in industry, community, and private settings to prevent microbial infection[1,2]. To obtain biocidal effect without releasing biocides into the environment, antimicrobial species can be covalently coupled to the material. Previously, the authors used Nano-fumed silica as a materia: and quaternary ammonium as an antimicrobial species to covalently synthesize a new chemical, Nano-fumed silica grafted N,N-dimethyl-n-tetradecylamine (NFS-14Quas), which was proven to have antibacterial effects[3]. However, its mechanism and bactericidal characteristic are still unknown. It has been shown that the small molecule quaternary ammonium slats exert their antibacterial action by disrupting and disintegrating cell membranes. By functionalizing end groups of NFS-14Quas had been synthesized[3]. This new antibacterial material has been shown to be more potent than their small molecule counterparts. Their high potency can be attributed to their more stable action, dissolution and diffusion on the surface of the material to be protected.

*Corresponding author. Email: Hylch@tom.com

In this study, the adsorption kinetic test, TTC test and the DNA and RNA release study were used to investigate the interactions between Konjac be protected.

2 Materials and Methods

2.1 Materials

The synthesis and characterization of NFS-14Quas used in this study have been described elsewhere[3]. *Escherichia coli* 8099 (*E. coli*) were kindly provided by Dr. Kaijuan Wang, School of Public Health, Zhengzhou University.

2.2 Adsorption kinetics

The kinetic parameters for quantitative suspension methods were action time and the concentration of bacteria. The experiments were conducted as followed. 0.5 g NFS-14Quas were added to 150 ml Erlenmeyer flask containing 10^8 cfu/ml (5 ml) of *E. coli*. The resulted solution was shaken at 37 °C by a Burrell wrist action shaker in different time, and kept station for 30 minutes to obtain the mixture of three groups: NFS grafted 8.7 g, 10.7 g and 12.1 g per 100 g NFS with grafting ratio (GR) was 8.9%, 10.7% and 12.1%, respectively. 1 ml supernatant

of each of the mixtures was diluted gradient, and then 0.1 ml dilution was added to agar plate and incubated at 37 °C for 24 hours.

2.3 Testing the integrity of the cell membrane

If the bacteria membrane is compromised, the release of cytoplasm constituents of the cell such as DNA and RNA can be monitored. The amount of DNA and RNA released from the cytoplasm were evaluated by the OD value at 260 nm. The experiments were conducted as follows. The bacterial suspension was separated into several flasks. 0.5 g NFS-14Quas (GR: 8.9%, 10.7% and 12.1%) was added to each flask except the control. Samples of 1.5 ml were removed from the flasks in the period of 20 minutes. The disinfected samples were then diluted to 1 : 10, and OD at 260 nm was recorded.

2.4 TTC test

Activity of dehydrogenase can be a direct indicator of microbial activity. One of the most frequently used methods is based on the use of TTC as an artificial electron acceptor for several dehydrogenases. The TTC is water-soluble, and nearly all microorganisms reduce it to triphenylformazan (TF). The liquid sample (L) experiments were conducted as follows. A mixture of 90 ml normal saline, 0.5 g NFS-14Quas, and 10 ml *E. coli* (10^9 cfu/ml) was added to one flask. The control flask used the same mixture except that 0.5 g NFS was instead of 0.5 g NFS-14Quas. Both the control and the experiment were processed in exactly the same way. The mixture was shaken at 37 °C for 30 minutes, and was then kept stationary for 30 minutes to separate the supernatant liquid from the solid phase. Two milliliters supernatant liquid was added into a 150-ml flask. Two milliliters glucose solution (1 mol/L) and 2 ml TTC solution (1.0000 g/L) was added to the flask and allowed to react for 30 minutes. Two drops of H_2SO_4 were added to terminate the reaction. The hydrolysis reaction product (TF) was extracted for 2 hours at 30 °C with toluene. The TF concentration was then determined spectrophotometrically at 492 nm[5].

The solid sample (S) experiments were conducted as follows. The solid of NFS (S) and NFS-14Quas (S) was filtered and dissolved in 2 ml normal saline. It was then treated with the supernatant.

3 Results and Discussion

3.1 Adsorption kinetic study

Figure 1 showed the adsorption kinetic curve of NFS-14Quas and NFS. As seen from the figure, 0.5 g NFS showed a little adsorption during the 60 minutes. In contrast, NFS-14Quas (GR: 8.9%, 10.7% and 12.1%) dose-dependently absorbed *E. coli*. During the first 5 minutes, NFS-14Quas could drastically decrease from 10^9 to 10^5. However, further incubation with NFS-14Quas could decrease the number of bacteria but not so drastically as the first 5 minutes. This is very interesting, which implies that bacteria adsorption of NFS-14Quas is fast and can be saturated.

3.2 Testing the integrity of the cell membrane

The bacterial membrane serves as a structural component that may become compromised during a biocide challenge such as exposure to a cationic biocide. Therefore, the release of intracellular components such as large molecules, DNA, RNA, and other materials is a good indicator of membrane integrity. Since these nucleotides have strong UV absorption at 260 nm, they are described as 260-nm absorbing materials[6]. Using a UV-VIS spectrophotometer, it is very easy to detect any 260-nm absorbing material.

The UV-VIS study on the release of 260-nm absorbing materials upon addition of 0.5 g NFS-14Quas (GR: 8.9%, 10.7% and 12.1%) to an *E. coli* suspension was shown in Figure 2. The ratio of OD of a bacterial suspension with NFS-14Quas to a bacterial suspension with NFS was plotted versus time. Upon addition of the NFS-14Quas, the OD of the *E. coli* suspension at 260 nm increased. Increasing the amount of NFS-14Quas also causes the ratio of OD to rise, suggesting that the leakage of DNA, RNA, and other materials correlates well with growth inhibition and a bacteriostatic effect (Figure 2).

3.3 TTC test

After incubation with NFS and NFS-14Quas (GR: 8.9%, 10.7% and 12.1%), *E. coli* either exists in the supernatant

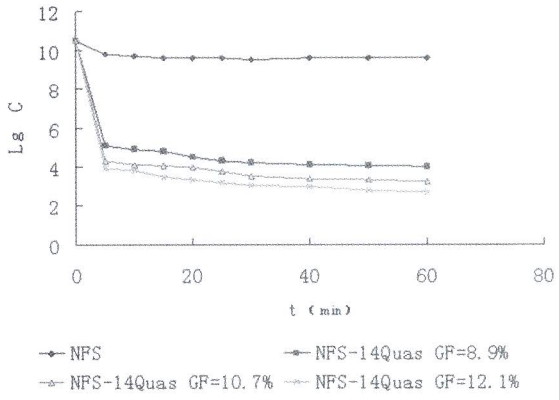

Figure 1. Absorption kinetics curve of NFS-14Quas. C: the concentration of *E. coil.*

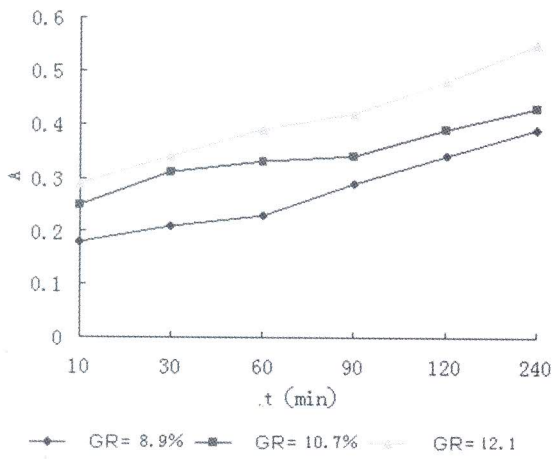

Figure 2. The release of absorbing material at 260 nm from *E. coil* with addition of NFS-14Quas with different LR and exposure for different period of time.

Table 1. The absorbance NFS-14Quas of different LR and *E. coli* after exposure for different period of time

Sample		The OD of TF after exposure for different time in killing *E. coil*	
		30 minutes	60 minutes
NFS	L	0.70	1.01
	S	0.98	0.99
NFS-14Quas (8.9%)	L	0.52	0.35
	S	0.49	0.32
NFS-14Quas (10.7 %)	L	0.41	0.30
	S	0.39	0.27
NFS-14Quas (12.1%)	L	0.35	0.31
	S	0.30	0.19

Note: The temperature was 19 – 22 °C. The results were means of triplicate tests. L: The supernatant liquid of *E. coil* after exposure of NFS or NFS-14Quas. S: The surface of NFS or NFS-14Quas *E. coil* after exposure NFS or NFS-14Quas.

liquid or adheres to the surface of NFS and NFS-14Quas (Table 1). The bacteria that adheres to the surface of NFS and NFS-14Quas is either alive or killed by the cationic group of NFS-14Quas, which was demonstrated by the TTC test. The principle of the TTC test is that only live bacteria produce active dehydrogenase. Dehydrogenase transforms TTC to TF, which can be quantified by UV-VIS absorbance[6] when *E. coli* is incubated with NFS, the quantity of TF in the liquid phase is higher than that in solid phase (Table 1). This can be explained in two ways. One possibility is that NFS absorbs little bacteria or does not absorb at all. The other is that NFS is not bactericidal. On the contrary, in the NFS-14Quas experiment, both the amount of live bacteria in the supernatant liquid and the bacteria adhering to the solid surface were very low, which suggests that the bacteria absorbed by NFS-14Quas then were killed by NFS-14Quas.

4 Conclusion

This study proves that NFS-14Quas is able to absorb *E. coli* to its surface and then kill the bacteria by destroying their membrane. These results also provide further evidence that NFS-14Quas is a useful medicinal material for preventing infection.

References

1. Ebi N, Imazato S, Noiri Y, Ebisu S. Inhibitory effects of resin composite containing bactericide-immobilized filler on plaque accumulation. Dental Materials 2001; 17(6): 485 – 91.
2. Huang RH, Du YM, Zheng LS, Liu H, Fan LH. A new approach to chemically modified chitosan sulfates and study of their influences on the inhibition of *Escherichia coli* and *Staphylococcus aureus* growth. Reactive & Functional Polymers 2004; 59(1): 41 – 51.
3. Xu X, Li SR, Jia FY. Synthesis and antimicrobial activity of Nano-fumed silica derivate with N,N-dimethyl-n-hexadecylamine. Life Science Journal 2006; 3(1): 59 – 62.
4. Alef K, Nannipieri P. Methods in Applied Soil Microbiology and Biochemistry. Academic Press. New York, NY, 1995; 228 – 9.
5. Federica A, Anabela C, Tiziana C, Roberta G, Marco R, Antonella I. Optimisation of a microbial bioassay for contaminated soil monitoring: bacterial inoculum standardisation and comparison with Microtox® assay. Chemosphere 2003, 53(8): 889 – 97.
6. Chen CZS, Stuart LC. Interactions between dendrimer biocides and bacterial membranes. Biomaterials 2002; 23(16): 3359 – 68.