**Application of 3,6-O-sulfated chitosan modified surface as recyclable antibacterial material**

Lin Yuan[a], Min Tan[a], Hongwei Wang[a], Yanyun Wang[a], Gaojian Chen[b], Hong Chen[a]

[a] Jiangsu Key Laboratory of Advanced Functional Polymer Design and Application, College of Chemistry, Chemical Engineering and Materials Science, [b] Center for Soft Condensed Matter Physics and Interdisciplinary Research,Soochow University, 199# Ren'ai Road, Suzhou 215123, China.

**Statement of Purpose:** The extensive use of antibiotics often leads to antimicrobial or drug resistance. Therefore, increased attention has been paid to materials that can kill bacterial cells in place of antibiotics. Both chitosan and lysozyme are the natural biomacromolecules, which have been widely applied in the treatment of bacterial infection. It has been determined that the antibacterial activity of chitosan can be improved after being sulfated. A combination that maximizes the benefits of both sulfated chitosan and lysozyme was developed and evaluated in this work, which aimed to produce functional materials with excellent, durable antibacterial properties. It is based on the specific binding of 3,6-O-sulfated chitosan (3,6S-chitosan) with lysozyme, which also maintains the high specific hydrolytic activity with bacterial cell wall components, similar to that of normal lysozyme. In this study, the specific adsorption of lysozyme on the 3,6S-chitosan-modified surface was investigated, and the antibacterial effect of the modified surface was further evaluated. The regeneration of its antibacterial activity is also studied through the reversible adsorption and dissociation of lysozyme.

**Methods:** 3,6-O-Sulfated chitosan was synthesized according to the reported method. Then it was grafted to 3-aminopropyltriethoxysilane (APTES) modified silicon wafer intermediated by 4-nitrophenyl chloroformate (NPC) under a phosphate buffer at 30 °C for 24 h. Lysozyme adsorption on the surface was detected by SDS-polyacrylamide gel electrophoresis. And the antibacterial effect of the functionalized material was evaluated by the LIVE/DEAD BacLight Kit (Invitrogen, USA) with the observation of fluorescence microscope (IX71, Olympus, Japan).

**Results:** According to our previous studies, regioselectively sulfated chitosan, 3,6S-chitosan, can be easily prepared using $HClSO_3$. The structure of the sulfated product was measured by Fourier Transform Infrared Spectrometer, $^{13}$C-NMR and elemental analyzer. The results indicated that 3,6S-chitosan was successfully synthesized. Since the specific intercation occurs between 3,6S-chitosan and lysozyme, 3,6S-chitosan-grafted surface showed a significant adsorption of lysozyme. However, only a very small amount of lysozyme was adsorbed on the unmodified surface and chitosan-grafted surfaces (Figure 1). Meanwhile, the specific activity of lysozyme bound on the 3,6S-chitosan-modified surface was approximately 37,000 U/mg, close to the activity of normal lysozyme. Therefore, it resulted in the death of almost all bacterial cells attached on the surface (Figure 2). 4.0 M NaCl solution could effectively elute the adsorbed lysozyme and dead bacterial cells from the surface of the 3,6S-chitosan-modified silicon wafer. After reloaded by lysozyme, the regenerated surface regained its antibacterial activity .

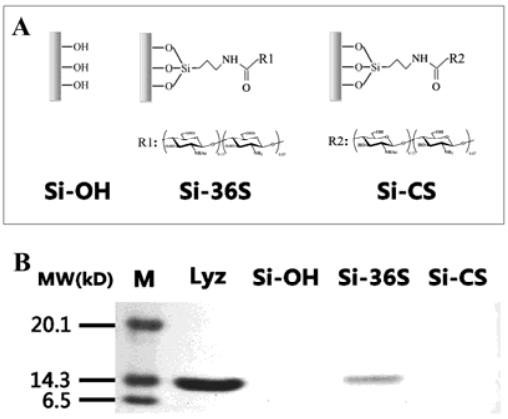


Figure 1. Modification of surfaces grafted by 3,6S-chitosan and chitosan (A) and SDS-PAGE of the lysozyme bound on the surfaces (B). M is the protein marker; Lyz is lysozyme; and Si-OH, Si-36S and Si-CS represent the surfaces after "Piranha" solution treatment, 3,6S-chitosan and chitosan modification, respectively.

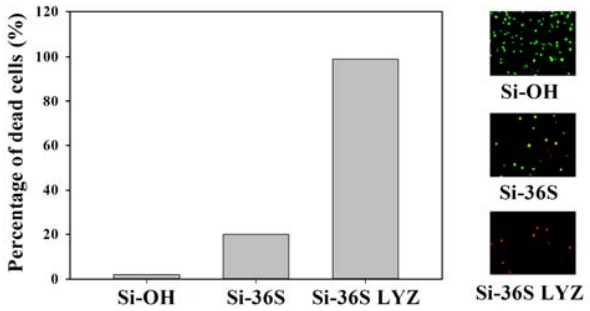


Figure 2. The antibacterial effect of modified surfaces. Si-36S LYZ represents Si-36S with lysozyme adsorption.

**Conclusions:** The 3,6S-chitosan-modified surface showed specific adsorption of lysozyme and then had the high hydrolytic activity toward bacterial cell walls, which ensured the surface's high antibacterial activity. Moreover, the regeneration of the 3,6S-chitosan-grafted surface by a simple high-salinity treatment allowed it to regain its ability to specifically bind lysozyme and kill *E. coli* cells. This research extended sulfated chitosan's range of antibacterial properties and developed a new strategy for preparing recyclable antibacterial surfaces by using 3,6S-chitosan.

**Acknowledgements:** We gratefully acknowledge the financial support by the National Science Fund for Distinguished Young Scholars (21125418), and the National Natural Science Foundation of China (21074083, 21004042 and 21104055).

**References:** Wang H, Yuan L, Zhao T, Huang H, Chen H. Chinese J Polym Sci 2012; 30: 893-899.

Yuan L, Yue Z, Chen H, Huang H, Zhao T. Colloid Surf B. 2009; 73: 346-350.