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Research and Application of Functionalized Mesoporous Silica Supported Quaternary Ammonium Salt Nano-antibacterial Materials

Jin Ziyi, Dong Jiahao, Kong Lingdong*

Jining Medical University, Rizhao, 276800, China
**Corresponding author: conlintung@163.com*

Abstract: *Objective: Nano-mesoporous silica has the superior physical properties and special ordered mesoporous structure.It can be used to prepare a surface material with antibacterial activity. Methods: Nano-mesoporous silica has been prepared by TEOS as the silicon source and CTAB as template. In this study, mesoporous silica was synthesized by sol-gel method. In the last, amino surfactants were used as carriers to bind to long chain organic surfactants by surface modification. The mesoporous silica functionalized with long chain quaternary ammonium salt was characterized by infrared spectroscopy, laser particle size and Zeta potential test, and its antibacterial effect was tested. The results show that amino groups are introduced to the surface of mesoporous silica after removing the template, and then dodecyl hydroxyethyl dimethyl ammonium bromide is bound to the surface of mesoporous silica. The combined product has good water solubility and its particle size is about 240 nm.Zeta potential test shows that it can be ionized and has positive charge in pure water. The antimicrobial effect test showed that the material could inhibit the growth and reproduction of DH5α Escherichia coli obviously at room temperature(P<0.05), and had certain antimicrobial activity.*

Keywords: *Mesoporous silica; Antibacterial material; Long chain quaternary ammonium salt; Surface modification*

1. Introduction

Quaternary ammonium salts are organic antimicrobial materials[1], which have the ability to adsorb negatively charged bacteria and can play a role in inhibiting bacterial growth[2], and are widely used in various fields due to their simple and inexpensive preparation process and their wide antimicrobial range[3]. However, common quaternary ammonium salts have low chemical activity, mostly exist in the free state when they act, and are relatively toxic and irritating[4]. Therefore, in order to improve the antimicrobial effect of quaternary ammonium salts and to make the antimicrobial of quaternary ammonium salts with long-lasting effect, mesoporous silica was chosen as a carrier to make a quaternary ammonium salt surface-modified nano-silica microspheres. Silica itself has the advantages of large specific surface area, controlled morphology, high stability, good biocompatibility, and the surface is easily modified by active groups[5], and when combined with quaternary ammonium salts, it can not only improve the bactericidal efficacy of quaternary ammonium salts, but also reduce the irritation of quaternary ammonium salts themselves[6].

2. Synthesis of Long-chain Quaternary Ammonium Salt Combined with Functionalized Silica Nano-antibacterial Materials

2.1. Mesoporous Silica Synthesis

Weigh 0.2 g of CTAB, dissolve in pure water, add 80 ul of triethanolamine, accelerate the dispersion by ultrasonication and reflux at 95°C for 1 h. Add 500 ul of TEOS to the three-necked vial in three portions at 30 min intervals and continue to heat and stir for 1 h. After the reaction, add 45 ml of anhydrous ethanol to the three-necked vial, elute by ultrasonication at 28°C and centrifuge. The above product was added to the single-necked vial, and the dissolution was accelerated by ultrasonication

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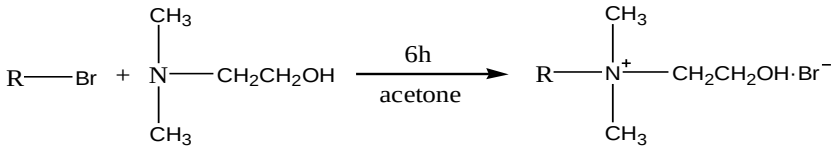
with methanol-sodium chloride solution, and the reaction was heated and stirred for 3 h at 40°C. After the reaction, the mesoporous silica 0.1852 g was obtained by centrifugation.

2.2. Amino Modification of Mesoporous Silica

Weigh 0.15 g of MSN, 25 ml of dimethyl sulfoxide into a single-necked vial, add 270 ul of APTES, add 3~4 drops of triethylamine, heat and stir at 110°C for 5h, centrifuge at the end of the reaction, add 30 ml of anhydrous ethanol to dissolve the substrate and elute, centrifuge for 20 min and dry under vacuum for 24h to obtain 0.8653 g of amine-modified mesoporous silica, recorded as NH_2-MSN.

2.3. Quaternary Ammonium Synthesis

Weigh 24 g of dodecyl bromide, 13.7 g 46 ml of N,N-dimethylethanolamine and acetone into a single-necked flask, the mixed solution was heated and stirred at 63 ℃ for 6 h. Immediately after the reaction, the solution was placed in an ice bath at 5 ℃ to precipitate crystals, methanol-acetone mixed solution was added, and the solution was recrystallized in an ice bath at 35 ℃ under ultrasonic acceleration and dried in an electric drying oven for 12 h. Finally 21.37 g of dodecyl hydroxyethyl dimethyl ammonium bromide was obtained, which was recorded as DHDAB.



($R=C_{12}H_{25}$)

2.4. Long Chain Quaternary Ammonium Salt combined with MSN-NH2

Measure 30 ml of anhydrous methanol into a conical flask as the reaction solvent, weigh 0.2 g of MSN-NH_2 and 1.2 g of DHDAB into the conical flask, and accelerate the dissolution by ultrasonication at 29°C. The dissolved solution was stirred at room temperature for 36 h. After the reaction, the solution was centrifuged and dried in a vacuum drying oven for 12 h to obtain 0.2254 g of long-chain quaternary ammonium salt-bound mesoporous silica, which was recorded as DHDAB@MSN-NH_2.

3. Antimicrobial Effectiveness Testing

Weigh 0.2 g of DHDAB@MSN-NH_2, dissolved it in 10 ml of methanol, 3 ml was taken and put on the slide with a dropper and dried in an electric drying oven for 6 h. The antibacterial plate covered with antibacterial material was obtained. The slides covered with mesoporous silica were prepared in the same way. Weigh 12.5 g of LB medium powder, add 500 ml of pure water and stir well, dispense the liquid medium into 100 ml culture flasks and autoclave them for 15 min at 121°C. Add 100 ul of matured DH5α E. coli into each culture flask and incubate them in an incubator overnight at 37°C. Antibacterial plates as well as slides covered with mesoporous silica were added to the culture flasks, and the flasks were placed in an oscillating incubator for overnight incubation. The OD values of each group of cultures were tested at 600 nm on the 1st, 2nd and 3rd days after E. coli inoculation, respectively, and the differences in their antimicrobial effects were compared.

4. Results and Discussion

4.1. Infrared Characterization

4.1.1 Infrared Characterization of Dodecyl Hydroxyethyl Dimethylammonium Bromide (DHDAB)

As shown in Figure 1,3348 cm^{-1} is the absorption peak of hydroxyl group, 1469 cm^{-1} is the absorption peak of C-N, 2916 cm^{-1} is the absorption peak of C-H 720 ~ 780 cm^{-1}, and the vibration absorption peak formed by dodecyl long chain appeared. Since dodecyl hydroxyethyl dimethyl

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ammonium bromide contains not only hydroxyl group but also C-N and alkyl long chain, it can be proved that this compound is the DHDAB used for surface modification of MSN.

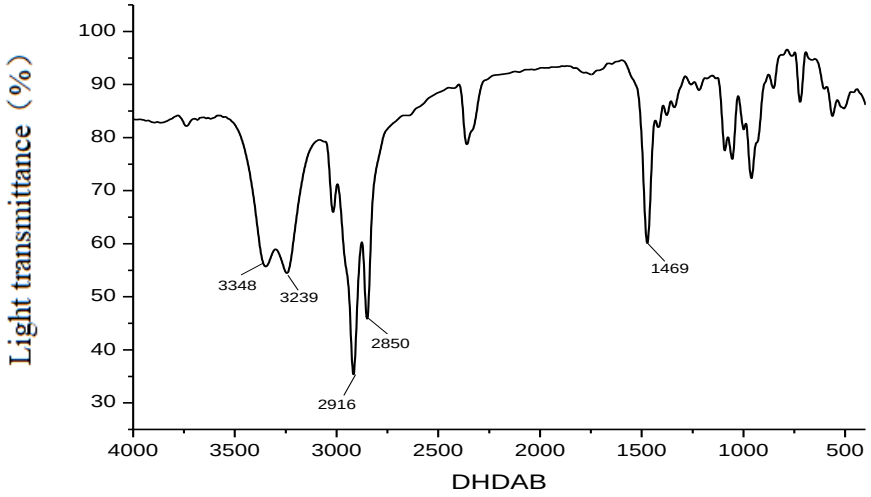

Figure 1: Infrared Spectrum of DHDAB

4.1.2 Infrared Characterization of DHDAB@NH2-MSN

Figure 2: Infrared Spectrum of DHDAB@MSN-NH_2

As shown in Figure 2, the stronger and wider stretching vibration peak at 1084 cm^{-1} is an asymmetric Si-O-Si absorption peak, and the symmetric stretching vibration peak of Si-OH at 798 cm^{-1}, both peaks are characteristic absorption peaks of SiO_2. The absorption peak of methylene at 2931 cm^{-1} is due to the presence of methylene in the 3-aminopropyltriethoxysilane used in the amino modification, and the vibration absorption peak of NH_2 at 1483 cm^{-1}, both of which prove that the amino group has been successfully modified on the surface of MSN. Both of them prove that the amino group has been successfully modified to the surface of MSN. The methyl absorption peak at 2863 cm^{-1} is due to the presence of methyl in DHDAB, which proves that DHDAB was successfully bonded to the mesoporous silica.

4.2. Particle Size Characterization

Appropriate amounts of MSN-NH_2 and DHDAB@MSN-NH_2 were weighed and dissolved in pure water, ultrasonically dispersed and then tested using a laser particle sizer.

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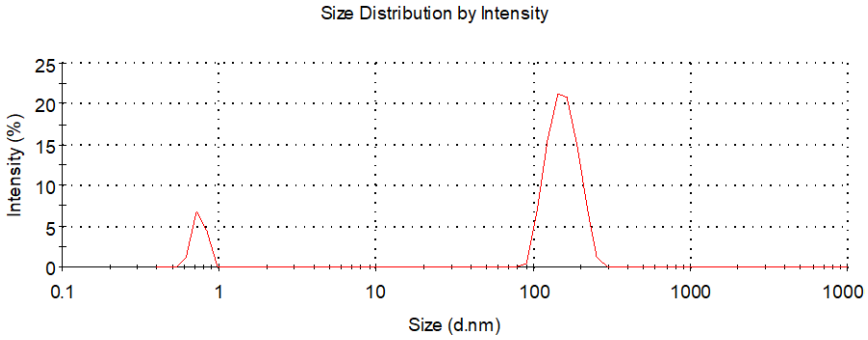

Figure 3: Particle Size Distribution of MSN-NH_2

As shown in Figure 3, the range of particle size distribution of MSN-NH_2 is approximately 156.5nm.

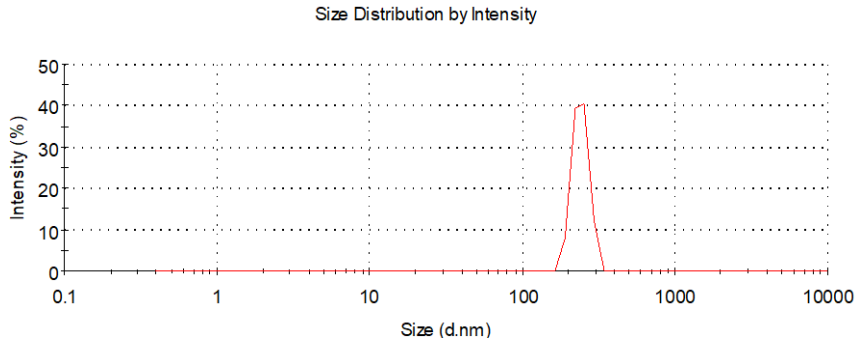

Figure 4: Particle Ssize Distribution of DHDAB@MSN-NH_2

As shown in Figure 4, the range of particle size distribution of DHDAB@MSN-NH2 is approximately 240nm.

Table 1: Particle Size Values for MSN-NH_2 and DHDAB@MSN-NH_2

	MSN-NH_2			DHDAB@MSN-NH_2		
	Peak 1	Peak 2	Peak 3	Peak 1	Peak 2	Peak 3
Size(d.nm):	156.5	0.7500	0.000	240.9	0.000	0.000
Intensity (%)	87.7	12.3	0.0	100.0	0.0	0.0
Width(d.nm):	33.71	0.6717	0.000	28.53	0.000	0.000
Z-Average(d.nm):	115.1			240.9		
PDI	0.335			0.375		
Intercept	0.611			0.873		

As shown in Table 1, the PDI of both the amino-modified MSN and the DHDAB-bound MSN were less than 0.4 after dispersion in aqueous solution, which indicated that they were well dispersed in water, and the particle size of the MSN without DHDAB was 156.5 nm and the particle size of the bound MSN was 240.9 nm. The successful incorporation of the long-chain quaternary ammonium salt DHDAB on the final product synthesized in this experiment can be demonstrated by comparing the two.

4.3. ZETA Potential

As shown in Table 2, the potential value of the amino-modified MSN in water after ionization is 28mv, which indicates that it is positively charged. The reason is that the amino group on the surface of MSN replaces the silicon hydroxyl group originally distributed on the surface of MSN as well as inside the pore channel, and the amino group is positively charged after ionization in water, so it can also prove the success of this experimental modification. The potential value of DHDAB in pure water is positive due to the presence of quaternary ammonium cations, so the potential value of DHDAB@MSN-NH_2 produced by the combination of the two is also positive in pure water because it

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contains not only positively charged amino groups but also quaternary ammonium cations, so its potential value is greater than the first two at the same mass concentration.

Table 2: Zeta Potential Values for$MSN\text{-}NH_2$, DHDAB, and DHDAB@$MSN\text{-}NH_2$

	$MSN\text{-}NH_2$			DHDAB			DHDAB@$MSN\text{-}NH_2$		
	Peak1	Peak2	Peak3	Peak1	Peak2	Peak3	Peak1	Peak2	Peak3
Mean(mv)	28.4	0.00	0.00	24.7	0.00	0.00	30.1	0.00	0.00
Area(%)	100.0	0.0	0.0	100.0	0.0	0.0	100.0	0.0	0.0
Width(mv)	6.81	0.00	0.00	7.78	0.00	0.00	5.57	0.00	0.00
Zeta Potential (mv):	28.4			24.7			30.1		
Zeta Deviation (mv):	6.81			7.78			5.57		
Conductivity (mS/cm):	0.0146			0.0269			0.0164		

4.4. Absorbance Test

On the 1st, 2nd and 3rd days after inoculation, the culture solution was taken from each flask and diluted 10 times (on a sterile table), and each parallel group and blank group were tested three times at 600 nm and the average values were taken, and the detailed data are shown in Table 3.

Table 3: OD values of the cultures in each culture flask on days 1, 2 and 3 after inoculation with bacteria

	Group I				Group II				Group III
	Parallel 1	Parallel 2	Parallel 3	MEAN	Parallel 1	Parallel 2	Parallel 3	MEAN	
Day1 (24h)	0.163	0.172	0.175	0.170	0.181	0.171	0.184	0.179	0.189
Day2 (48h)	0.158	0.166	0.158	0.161	0.276	0.219	0.278	0.258	0.254
Day3 (72h)	0.897	0.919	0.915	0.910	1.453	1.402	1.476	1.444	1.494

There was almost no difference in absorbance between group II and the blank group on each day after adding the slides covered with NH_2-MSN, however, after comparing with group I, it could be found that the OD value of the culture solution after 10 times dilution was significantly lower than that of groups II and III on the first day (48h incubation) and the second day (72h incubation) after adding the antibacterial plates, so it could be proved that the DHDAB@$MSN\text{-}NH_3$ prepared in this experiment had a significant antibacterial effect.

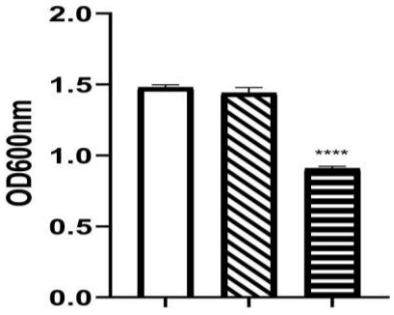

Figure 5: Difference in DH5α E. coli content in culture medium of each test group, (P<0.0001)

After the data were processed by GraphPad Prism, it was concluded that there was a significant difference (P<0.0001) in the E. coli content in the culture medium of group I (DHDAB@$MSN\text{-}NH_2$) compared to group III (blank control) proving that DHDAB@$MSN\text{-}NH_2$ had significant antibacterial activity, while group II ($MSN\text{-}NH_2$) compared to group III (blank control), the E. coli was less different (P>0.05), indicating that NH_3-MSN hardly affected the activity of E. coli.

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5. Conclusion

After infrared spectroscopy and laser particle size testing, it was shown that N,N-dimethylethanolamine and bromododecane synthesized dodecyl hydroxyethyl dimethyl ammonium bromide (DHDAB) through the substitution reaction of haloalkanes with tertiary amines, and DHDAB combined with amino surface-modified mesoporous silica to form a novel antibacterial material, which showed a significant inhibition against DH5aE. coli after comparison with the blank group inhibitory effect ($P<0.001$).

Acknowledgements

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References

[1] Liu Chengkun, Jiang Zhiwei, Mao Xue, Wu Hong. Research progress of common antibacterial materials [J].Journal of XI'an Polytechnic University, 2020, 349(2): 37-46.
[2] Wang Xiaojuan. Preparation and application of polysiloxane quaternary ammonium salt antibacterial agent [J].Textile Dyeing and Finishing Journal, 2018, 40(05):8-11.
[3] Yin Shanshan. Antibacteral modification of polyamide 6 fiber by synthetic long chain quaternary ammonium salt [D].Zhejiang University of Science and Technology, 2020.
[4] Chen Yining, et al. Application of Quaternary Ammonium Salt in Antibacterial Materials [J]. WESTLEATHER, 2013, 35(08):13-16+21.
[5] Feng Yin, Panwar N, Tng Djh, et al. The application of mesoporous silica nanoparticle family in cancer theranos- tics [J].Coordination Chemistry Reviews, 2016, 319: 86-109.
[6] Xie Yu, Zhang Changhui, Xu Xuan. Recent Development of Polisilicone Quaternary Ammonium Salt Antimicrobial Agent [J]. Technology and Development of Chemical Industry, 2008(04):25-28+34.