The Biological Effect of Iron Oxide and its Hydrate Nanoparticles

Q. Zhao\textsuperscript{a}, X.F. Pang\textsuperscript{b}, L.W. Liu\textsuperscript{c}, B. Deng\textsuperscript{d}

School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu 610054, China
\textsuperscript{aezhaoqiang@sohu.com, \textsuperscript{bpangxf@mail.sc.cninfo.net, \\
\textsuperscript{ciulewei@sohu.com, \textsuperscript{ddengbo@std.uestc.edu.cn}}}

Keywords: Nanobiomaterials, Biological effect, toxicology, Iron oxide

Abstract:

Iron oxide and its hydrate nanoparticles were synthesized by hydrothermal method and confirmed by infrared and SEM (Scanning Electron Microscope) et al. The dimensions of the nanoparticles are about 50-120 nm. The crystalline form of iron oxide nanoparticles is like globosity while its hydrate rod. Amino acids intermingling with the synthesized nanoparticles were crystallized to investigate the space effect of the nanoparticles. The crystalline forms of crystal are different to that of pure amino acid. The positions and width of the nanoparticles’ peaks in the infrared spectrum are changed too. Microscope observation and infrared spectrum results indicated the nanoparticles had changed the internal structure of amino acids crystal. To considerate the toxicity of the synthesized nanoparticles, MTT (3-(4,5-dimethylthiazol 2-yl)-2,5 diphenyltetrazolium bromide) assay was used to determine their cytotoxicity. The OD value (Optical Density) was used to calculated RGR\% (Relative Generation Rate) of cells, which determined the grade of cytotoxicity. The RGR of nanoparticles of iron oxide and its hydrate are about 1 to 2, which indicate they have just low toxicity.

1. Introduction

The science and technology of nanomaterials has created great excitement and expectations in the last few years. There has already been much progress in the synthesis, assembly and fabrication of nanomaterials, and, equally importantly, in the potential applications of these materials in a wide variety of technologies. Nanomaterials applied in the field of biomedicine are defined Nanobiomaterials. Of which, magnetic nanobiomaterials, with small size effect, good magnetic direction property, bio-compatibility and certain biomedical function, can be applied in the field of drug delivery, immobilization on enzymes, immunoassay and separation or classifying of cells\textsuperscript{[1-4]}, etc. It is very necessary to study on the bio-effect and toxicology of magnetic nanobiomaterials because they contact living body directly. More and more researchers think much of this problem and take lots of experiments\textsuperscript{[5,6]}. This paper are concerned on the biological effect of iron oxide and its hydrate nanoparticles which size are about 50-120 nm. The results will help us to find the proper size of magnetic nanobiomaterials for the using of biomedical engineering.

2. Experiments

2.1 The preparation of iron oxide and its hydrate nanoparticles\textsuperscript{[7]}

An exact weight of FeCl\textsubscript{3} was dissolved by distilled water and then ammonia was added quickly under stirring. The value of solution’s PH was adjusted to 10. Taking the solution into high-pressure reaction vessel for 4 hours, we obtained iron oxide nanoparticles after washing, high-speed centrifugating and vacuum drying.

An exact weight of FeCl\textsubscript{3} was dissolved by distilled water and then ammonia was added quickly under stirring. The value of solution’s PH was adjusted to 12. Taking the solution into high-pressure reaction vessel for 12 hours, we obtained iron oxide’s hydrate nanoparticles after washing, high-speed centrifugating and vacuum drying.
2.2 The interaction of nanoparticles and amino acid

The amino crystals were purchased from Sigma Ltd. The recrystal of amino acids were taken after intermingling with prepared nanoparticles. The control group did not intermingling with nanoparticles. The crystalline form was observed by microscope and compare with control group. The infrared spectrum of crystals and control group were also measured.

2.3 The cytotoxicity experiments for nanoparticles

MTT (3-(4,5-dimethylthiazol 2-yl)-2,5 diphenyltetrazolium bromide) assay with chick embryo fibroblast was taken as cytotoxicity evaluation method of iron oxide and its hydrate nanoparticles\(^8\).

3. Results and Discuss

3.1 The preparation of iron oxide and its hydrate nanoparticles

We used SEM (Scanning Electron Microscope) to measure the size of iron oxide and its hydrate nanoparticles and their scales are about 50-120 nm. The crystalline form of iron oxide nanoparticles is like globosity while its hydrate rod as shown in Fig. 1.

![Fig.1 The SEM of iron oxide (a) and its hydrate (b) nanoparticles](image)

We also measured their infrared spectrums as shown in Fig. 2. From Fig. 2, we observed the iron oxide character peak in 556 cm\(^{-1}\) and 478 cm\(^{-1}\), while no absorption observed in 556 cm\(^{-1}\) for iron oxide’s hydrate. Fig. 1 and Fig. 2 indicate we obtained the iron oxide and its hydrate nanoparticles.

![Fig. 2 The infrared spectrum of iron oxide and its hydrate nanoparticles](image)

3.2 The bio-effect and toxicity of nanoparticles

3.2.1 The interaction between amino acid molecule and nanoparticles

We mixed amino acid and prepared nanoparticles into distilled water to be saturated solution and then stood it in watch glass for recrystal. The control group did not mix nanoparticles. The crystalline forms of glutamic acid and glycine crystals intermingling with or without nanoparticles are shown in Fig. 3. It is seen great different between the crystalline form of amino acid intermingling with nanoparticles and without. The infrared spectrums of glutamic acid and glycine...
crystals intermingling with or without nanoparticles are shown in Fig. 4. The infrared spectrum of amino intermingling with nanoparticles are changed. Some adsorption peak’s position and peak’s area are changed. It is also seen some peak disappeared or appeared. All of these indicate the interaction between nanoparticles and amino acid molecule.

The microscope of glutamic acid crystal
×100

The microscope of Glu-Fe$_2$O$_3$ crystal
×100

The microscope of glycin crystal
×100

The microscope of Gly-Fe$_2$O$_3$ crystal
×100

Fig. 3  The microscope of amino acid crystals intermingling with and without nanoparticles

![Infrared Spectrum of Glutamic Acid and Glycin](image)

a. Glutamic acid

b. Glycin

Fig. 4  The infrared spectrums of glutamic acid and glycin crystals intermingling with and without nanoparticles

3.2.2 The cytotoxicity of nanoparticles

We used MTT assay to evaluate the cytotoxicity of nanoparticles. MTT colorimetric method is a kind of way to check the states of activity and grow of the cell. In the mitochondrion of cell, the externally applied yellow tetrazolium salt MTT is reduced and become, under the action of the dehydrogenase of amber acid, as blue insoluble formazan form, which is deposited in the cell after this reaction. But dead cell has not this function. The dimethylsulfoxide can further resolve the blue
insoluble formazan. The quantity of the blue insoluble formazan is proportional with the number of cell participated. The number of cell can be obtained indirectly through measuring the strength of absorption of the light with determined wavelengths, the strength can be measured by enzymic-immunoassay instrument and spectrophotometer. Thus we can check the number and activity of the cell or of biological factors in the cell and the toxicity of the cell, etc. The advantages of this way is fast, accurate, and have higher sensitivity and very good repeatability.

Statistical methods were used to deal with experimental data. The results were expressed as Mean±SD. RGR(Relative Generation Rate) was calculated to evaluate toxicity degree.

$$\text{RGR} = \frac{\text{OD value of experimental group}}{\text{OD value of control group}} \times 100\%$$

The results are listed in the table 1. It is seen high RGR of iron oxide and its hydrate nanoparticles, and the score of cytotoxicity are 1 to 2 degree.

<table>
<thead>
<tr>
<th>Groups</th>
<th>OD Value(X±SD)</th>
<th>RGR(%)</th>
<th>Score</th>
<th>OD Value(X±SD)</th>
<th>RGR(%)</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%T1</td>
<td>0.823±0.072</td>
<td>81.6</td>
<td>1</td>
<td>0.981±0.075</td>
<td>73.5</td>
<td>2</td>
</tr>
<tr>
<td>50%T1</td>
<td>0.903±0.052</td>
<td>89.6</td>
<td>1</td>
<td>1.109±0.049</td>
<td>83.1</td>
<td>1</td>
</tr>
<tr>
<td>25%T1</td>
<td>0.943±0.083</td>
<td>93.6</td>
<td>1</td>
<td>1.228±0.041</td>
<td>92.1</td>
<td>1</td>
</tr>
<tr>
<td>100%T2</td>
<td>0.810±0.044</td>
<td>80.4</td>
<td>1</td>
<td>0.996±0.046</td>
<td>74.7</td>
<td>2</td>
</tr>
<tr>
<td>50%T2</td>
<td>0.893±0.067</td>
<td>88.6</td>
<td>1</td>
<td>1.191±0.047</td>
<td>89.3</td>
<td>1</td>
</tr>
<tr>
<td>25%T2</td>
<td>0.943±0.116</td>
<td>93.6</td>
<td>1</td>
<td>1.261±0.050</td>
<td>94.5</td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>1.008±0.105</td>
<td>100</td>
<td>0</td>
<td>1.334±0.760</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

*T1 is soakage solution of iron oxide nanoparticles and T2 that of its hydrate nanoparticles

4. Conclusions

Iron oxide and its hydrate nanoparticles about 50-120 nm affect amino acid crystallization and cell growth slightly. It may be predicted that the effect of smaller nanoparticles will be greater for their greater nano-effect. To obtain lower toxicity magnetic nanobiomaterials with specific function, our study will focus on the preparation of difference size of them and their bio-effect in living body in the future.

References

Nanoscience and Technology
10.4028/www.scientific.net/SSP.121-123

The Biological Effect of Iron Oxide and Its Hydrate Nanoparticles
10.4028/www.scientific.net/SSP.121-123.735

DOI References
doi:10.1021/1097-4628(20010307)79:10<1847::AID-APP130>3.0.CO;2-I