Abstract—The different particle sizes’ nano silica spheres are synthesized by the TEOS hydrolysis method. The XRD patterns testified that the sample is single phase and its strongest peak is significantly broadened. From the photos of SEM we can see that the samples’ surfaces have a good dispersion and uniform spherical morphology. The Hela cell proliferating activity is assessed by MTT assay, combined with the cell morphology and Annexin Hoechst 33342 staining are employed to determine apoptosis in Hela cells. Results shows that while the Hela cell treated with different concentrations of 200nm nano silica, cytocompatibility becomes better with the lower concentrations of nano silica. 24h cells stained photograph shows that under the concentrations of nano silica 50μg/ml the cell structure is normal and nucleus is complete.

Keywords—TEOS hydrolysis method; nano silica; biocompatibility

I. INTRODUCTION

Nano silica is a kind of inorganic materials with nanoscale size holes, the aperture size in a range from several nanometers to tens of nanometers. Nano silica itself can show the Quantum-Size Effect and Macroscopic Quantum Tunneling Effect when the radius of the silica particles reach nanoscale. The nano silica has a broad application prospect in terms of drug controlled-release and sustained-release carrier, because of its small particles, specific surface area, high surface reactivity, active center and high adsorption features. The preparation of SiO₂ nanoparticles mainly includes physical methods and chemical methods. The physical method is the mechanical crushing method in general. The smaller micron grade products can obtained by grinding the SiO₂ aggregates in use of the super jet mill or Simoloyer mill. Chemical methods include ion exchange method, precipitation method, chemical vapor deposition method, liquid phase method and sol-gel method, etc. Compared with the physical methods, chemical methods can produce ultrafine SiO₂ particles[1-9].In 1992, the silica zeolite M41S which can be used in gene transfection and drug carrying was found in the research team led by Dr. Kresgebo in their study, and their report was published in the journal Nature of the year. Under some special conditions, the silica zeolite M41S can effectively regulate the permeability of nano silicon dioxide carrier capsule wall for drug release based on certain stimulation mechanism, the release rate can be adjusted through the rate of diffusion through the wall of the nanocarrier guest drug molecules. In 1983, the concept of biocompatibility was proposed[7], and further recognized SmIgG’B lymphocytes, HepG hepatoma cells, WA systemic lupus erythematosus cells and leukemia cells ,etc[8,9]. In recent years, the research of nano silicon dioxide developed rapidly, after 2005, Vallet-Regi etc, systematically studied the influence of different sizes, mesoporous structure, surface area and mesoporous volume to the drug-loading capacity and the drug release rate of nano-silica[10]. Since 2007, our country have done a lot of research on Hela toxicity of SiO₂[11],and later, the research of the magnetic functionalization and fluorescent markers of functional nano-silica as targeted transport and biomarkers materials has always been rising. From 2012 to 2014, our country has explored nano-silica compound used as gene transfection carrier[12], and has a number of studies in the SiO₂ particle size and its toxicity to different cells[14,15]. In this experiment, we prepared the nano-silica with TEOS hydrolysis method, studied its in vitro cytotoxicity, and looked forward to having more applications in medicine.

II. EXPERIMENT

A. The preparation of nano silica spheres

Nano silica spheres were synthesized through ethyl silicate hydrolysis method. The starting materials were TEOS (92.9%-93.6%), CH2CHOH (>99.0%), NH4OH (>99.0%). All the reagents used were analytical grade. TEOS: CH2CHOH (1:4) mixed solution A, 500 r/min stir 4h by constant temperature magnetic stirrer to get emulsion, and slowly add distilled water (distilled water: solution = 7:1) in A solution to get solution B. Using NH4OH adjust pH of solution B to get solution C, then placed in drying oven, 80 ℃, 48h drying to get the solid A. Finally, using muffle furnace 580 ℃ sintering 4h get product.

Rigaku D/Max-2400 X-ray diffract meter was employed to check the phase of the phosphor powder using CuKα radiation under room temperature. The wavelength is 1.54056 x 10⁻⁴ um. The scanning electron microscopy (SEM) images of the nanoparticles were obtained by using SEM (JEM-2100F Electron Microscope/JEOL Co.200 kV). Measure and adjust pH value of solution B using the Sartorius pH meter adjustment. The solution C dried by the electric blast oven. Solid A heated by muffle furnace.

B. MTT cell experiment

Selection of logarithmic phase Hela, 0.25% trypsin digestion, made into single cell suspension, the density of every hole 3 x 10³ vaccinated in 96-well plates (corning Inc,
NY, USA). Experiment is divided into blank control group and experimental group, the experimental group added a solution containing nano silica concentrations were in complete medium 50μg/ml, 40μg/ml, 30μg/ml, 20μg/ml, 10μg/ml; the blank in the control group only complete medium. The 96-well plates placed 37℃, 5% CO2, in the water-saturated culture incubator, remove 24h, to which was added 20μL (5mg · ml⁻¹) of the MTT, placed in an incubator after 4h reaction, remove quickly dump hole liquid, add DMSO 150μL, oscillating reaction 10min, measuring wavelength of 570nm absorbance values in ELISA analyzer (Bio-Rad, Hercules, CA, USA) on MTT assay procedure was repeated three more times. Hela cell activity measured by the OD value in laboratory. Calculation of Hela growth inhibition rate by the public (1) to calculate, all the experiment is repeated three times.

\[ 1 - \frac{A_{490\text{ (test)}}}{A_{490\text{ (control)}}} \times 100\% \]  

(1)

C. Hoechst 33342 cell staining to observe the influence of nano-silica to Hela apoptosis

The Hela cells 1×10³ per well were seeded in 96-well cell culture plate and the cells were divided into control and experimental groups. After being adherent cells, added the blank control group to the complete medium; the experimental groups were having a concentration of 50μg · ml⁻¹, 40μg · ml⁻¹, complete culture 30μg · ml⁻¹, 20μg · ml⁻¹, 10μg · ml⁻¹ SiO₂ nanoparticles base cultured 24h. After incubation, abandon culture absorption and clean three times with PBS buffer, each time for 5 minutes. Observed under inverted phase contrast microscope (push around- DP72).

D. Statistical analysis

Using SPSS 20.0 statistical software for statistical analysis. Pairwise comparisons using t test, P <0.05 was considered statistically significant

III. RESULT AND DISCUSSION

A. The sample analysis of the XRD and surface photograpy

Fig.1 shows the XRD pattern of the samples. As can be seen from the figure, all samples are single phase, the strongest peak position are same with the standard JCPDF NO 75 - 0923, with the increase of pH, the width of the diffraction peak change constantly.

![Figure 1.XRD of nano silica sample](image)

From the figure we can know that the sample particle size is about 200 nm. We can see that sample have good dispersibility, smaller particle size and a better morphology, it is because that we improved the experiment process to reduce the concentration of reaction system, adjusted the pH and did the ultrasonic dispersion, so that the morphology and particle size of the silica balls changed when they formed.

![Figure 2.The best sample's TEM photo](image)

B. The analysis of nano-silica sample for Hela cells toxicity

Figure 3 shows that Hela cell survival rate of different concentrations of SiO₂ group were higher than 89%, after 24 h, 48 h period nano silica spheres and Hela increased as the concentration of SiO₂ training to 48 h. There was significant difference in comparison (P <0.05), between the control group 50μg·ml⁻¹ and 40μg·ml⁻¹, 30μg·ml⁻¹, 20μg·ml⁻¹ group and 10μg·ml⁻¹ group compared with blank control group had no statistical difference (P > 0.05). The nano silica spheres had no strong stimulating effect on the growth of Hela. At the concentration of 10μg·ml⁻¹, the comparison with the control group, and its influence is more weak, indicating that the cell has good cell compatibility.
Figure 3. MTT assay in different concentrations of 200nm SiO2 combined with Hela cells.

Figure 4 shows the photo which was the Hela cells affected by the different concentrations of nano silica ball for 24 h. The photo was observed by an inverted microscope. It is observed that Hela cells were morphologically normal structure, continued to show a long spindle and no large areas of abnormal death. It is possible to observe a 10% -15% of the cells of the bubble formation of apoptotic bodies, off the wall of death, at a nano-silica concentration of 50\(\mu\)g·ml\(^{-1}\). At the 10\(\mu\)g·ml\(^{-1}\) concentration of the nano-silica, the 97 percent of Hela cells are normal cell structure, and there are no significant difference with the control group.

Figure 4. The photo which was the Hela cells affected by the different concentrations of nano silica for 24 h.

Figure 5 shows the Hela cells’ stained photos in different concentrations of SiO\(_2\) after 24h. Dyed the Hela cells with Hoechst 33342. Form the photo we can see that the Hela cells treated with different concentrations SiO\(_2\) ball after 24h, the concentrated nucleus and small, round apoptotic bodies can be seen occasionally at a concentration of 50\(\mu\)g / ml. Hela structure is normal, nuclear is complete, and the cells dispersed normally when the concentrations is less than 30\(\mu\)g/ml. It follows that SiO\(_2\) in low concentrations have no apparent toxicity on Hela cells.

Figure 5. Hela cells’ stained photos in different concentrations of SiO\(_2\) after 24h.

IV. CONCLUSION

Series samples are synthesized by TEOS hydrolysis method, all samples are single phases and particle sizes are approximately 200 nm and surface morphology are spherical. Factors that affect the particle size of the sample are magnetic stirring rate and pH of the reaction solution. But the big size sample have reunion phenomenon, this is because the reactant concentration of the system was too high and the samples have not been treated by ultrasonic dispersion after generated. We confirmed through the experiment, the inhibition of nano silica to the proliferation in vitro of the Hela cells is weak. Cultured the Hela cells 48h at these concentrations of nano silica:50\(\mu\)g/ml,40\(\mu\)g/ml, 30\(\mu\)g/ml, 20\(\mu\)g/ml and 10\(\mu\)g/ml. Nano silica cultured Hela cells 48h, the survival rate of each concentration group was more than 89%. Observed by inverted phase contrast microscope, Hela structure is normal, no abnormal death. MTT cytotoxicity assay showed that the data of 24h/48 h confirmed that the nano silica has smaller influence on the growth of Hela, has good biological compatibility and can be used as potential controlled release drug carrier.

REFERENCES

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