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Sterilization on dextran-coated iron oxide nanoparticles: Effects of autoclaving, filtration, UV irradiation, and ethanol treatment

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ABSTRACT

Dextran-coated magnetic iron oxide nanoparticles (DMNPs) have attracted significant attention for their many applications in biomedical area such as diagnostic magnetic resonance imaging (MRI), and gene therapy. For biomedical applications, nanoparticles must be sterile, and several terminal sterilization methods can be used. However, very little is known regarding the effect of sterilization methods on the properties of DMNPs. It is crucial to find out whether the DMNPs are affected by the sterilization process. This paper reported the influences on the physicochemical properties of DMNPs from four different methods: autoclaving, sterile filtration, UV irradiation and chemical sterilization with ethanol. In addition, cell viability was also studied. Our results indicate that filter sterilization changes the iron oxide/dextran ratio in DMNPs sample significantly. Besides, the autoclaving and ethanol treatment affected the polydispersity index of DMNPs sample. Thus, caution must be taken when choosing an appropriate method to perform sterilization for DMNPs.

1. Introduction

Magnetic iron oxide nanoparticles used in biomedical area are usually coated with organic or inorganic materials to improve their solubility, stability and biocompatibility [1]. One of the most commonly used coating agents is dextran. Dextran-coated magnetic iron oxide nanoparticles (DMNPs) have attracted significant attention for their applications in diagnostic magnetic resonance imaging (MRI) and gene therapy [2,3]. For biomedical applications, the nanoparticles must be sterile. Terminal sterilization and aseptic processing are the two major sterility methods. In the research area, sterilization of the nanoparticles is usually carried out after fabrication. However, very little is known regarding the effect of sterilization methods on the properties of DMNPs.

In the terminal sterilization, several methods can be used, such as autoclaving, sterile filtration, UV irradiation, and chemical sterilization. Among these methods, autoclaving is a commonly used sterilization method in many biomedical researches [4–7]. Sterile filtration can only be used for nanoparticles with sizes smaller than its pore size; otherwise it will lead to the clogging of sterilization filters [8]. Chemical sterilization with ethylene oxide or formaldehyde can cause serious toxicological problems due to the chemical residues [9]. The applications of DMNPs in *in vitro* experiments and MRI contrast reagents both require prior sterilization. The influences of sterilization procedure on the intrinsic properties of DMNPs need to be investigated. Herein, we describe the effects of the most commonly used sterilization techniques (including autoclaving, sterile filtration, UV irradiation and ethanol treatment) on the physicochemical properties of DMNPs.

2. Experiments

2.1. Materials

The non-sterile dextran-coated magnetite nanoparticles in water supplied by Liquid Research Limited were used in this work. The dextran (m.w. 40,000 Da) was coated to the iron oxide nanoparticles via a general standard co-precipitation method. This

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method has been reported widely in literature [10–12]. The human cervical cancer cell line (HeLa cells) and immortalized retinal pigment epithelial cell line (RPE cells) were incubated in our laboratory. Dulbecco's Modified Eagle's Medium (DMEM), Kaighn's modified Ham's F12 medium (F12K) and fetal bovine serum (FBS) were purchased from Invitrogen.

2.2. Sterilization methods

DMNPs were divided into five aliquots of equal volume and sterilized by four different techniques: autoclaving, sterile filtration, UV irradiation and ethanol treatment. Autoclaving treatment was performed at 121 °C for 20 min using autoclave sterilizer (NOVA3, Tuttnauer). In sterile filtration process, original DMNPs (10 mg/ml) were filtered through a 0.22 μm filter. UV irradiation was carried out at room temperature for 12 h. In ethanol treatment, 1 ml non-sterile DMNPs (10 mg/ml) were mixed with 9 ml 75% ethanol on a vortex mixer for 15 s. It was kept at room temperature for 30 min. Then the precipitate was centrifuged at 3800 rpm for 10 min and air-dried in biosafety cabinet. The pellet was re-suspended in 1 ml sterilized DI water to form colloidal solution at the same concentration of 10 mg/ml as the original DMNP sample. One aliquot was kept as a control for comparison.

2.3. DMNP characterization

Following the sterilization process, the characterizations of the DMNP samples were performed. TEM (Philip Tecnai G2 20 S-TWIN) operating at 200 kV accelerating potential was used to observe the size and morphology of nanoparticles. To prepare the nanoparticle samples, dilute drops of suspension were allowed to dry slowly on carbon-coated copper grids. The Fourier transform infrared (FT-IR) spectra were obtained with a Shimadzu FTIR-8300 spectrometer using KBr pellets. The hydrodynamic sizes of the DMNPs in DI water were measured by dynamic light scattering (DLS) using a Malvern Zetasizer 3000 (Malvern, UK). The magnetic property measurement of the lyophilized samples was carried out at room temperature by using a vibrating sample magnetometer (Lakeshore, VSM 7400). The iron oxide composition was determined by using thermal gravimetric analysis (TGA, Perkin–Elmer TGA-7). The mass loss from lyophilized sample was monitored under N_2 at temperatures from 50 °C to 600 °C at a rate of 50 °C/min.

2.4. Cell viability test

HeLa cells were maintained in DMEM medium supplemented with 10% FBS. RPE cells were maintained in DMEM/F12K (50:50) medium supplemented with 10% FBS. The cells were grown and maintained in 5% CO2 humidified incubator at 37 °C. DMNPs were dispersed in the cell culture medium by vortex for 15 s at room temperature. In the treatment groups, HeLa cells and RPE cells were cultured in medium in the presence of 0.40 mg/ml DMNPs. After 24-h culturing, the cells were observed under an optical microscope with phase contrast, and the cell numbers were counted by haemocytometer. Cells incubated in culture medium without the DMNPs served as the control group in each experiment.

3. Results and discussion

The dextran in the colloids is transparent under TEM observation, and the iron oxide cores of the DMNP samples were imaged by TEM. The DMNP samples used in our experiment consist of approximately 5 nm magnetite cores (Fig. 1), which is very similar to the core sizes of Ferumoxides (4.96 ± 0.14 nm) and Ferumoxtran (5.85 ± 0.09 nm) [13]. No significant core size change was found under the TEM observation of the DMNP samples after autoclaving (Fig. 1b), filtration (Fig. 1c), UV irradiation (Fig. 1d) and ethanol treatment (Fig. 1e).

The stability of DMNPs in biological media is provided by the dextran layer on their surfaces. Therefore, any alteration or any degradation of the dextran shell during the sterilization process is undesirable. The stability of dextran coating around magnetite cores after sterilization process was studied using FT-IR spectroscopy. Fig. 2 shows the spectra for the DMNPs samples. The bands at ~3400 cm^{-1} (broad) and ~1620 cm^{-1} are due to the characteristic νO–H stretching and δO–H deformation modes of dextran

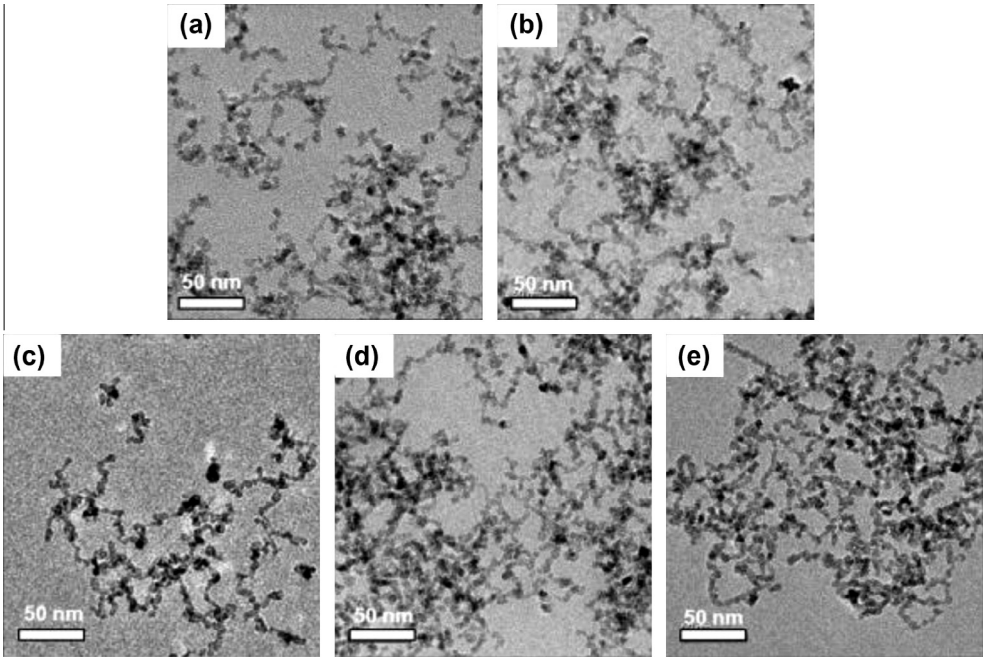

Fig. 1. TEM images of (a) control dextran-coated magnetic iron oxide nanoparticles, and dextran-coated magnetic iron oxide nanoparticles after (b) autoclaving, (c) filtration, (d) UV irradiation, and (e) ethanol treatment.

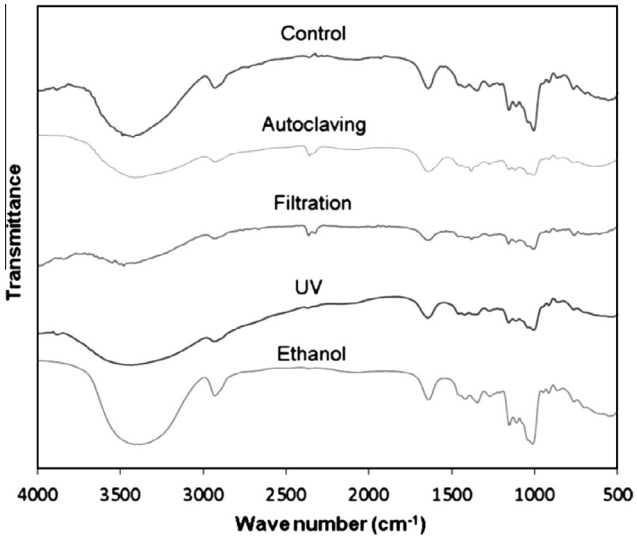

Fig. 2. FT-IR spectra of dextran-coated magnetic iron oxide nanoparticles after sterilization by using autoclaving, filtration, UV irradiation, and ethanol treatment. The sample with no treatment served as control.

Table 1
Hydrodynamic size of dextran-coated magnetic iron oxide nanoparticles after sterilization in DI water.

Samples	Mean diameter[a] (nm) and polydispersity index[b]
Control	152.7 [0.22]
Autoclaving	155.0 [0.23]
Filtration	131.6 [0.22]
UV irradiation	153.6 [0.22]
Ethanol treatment	152.1 [0.21]

[a] Mean particle size results from three measurements, weighted by intensity.
[b] Polydispersity index values (P.I.), given in brackets, range from 0 to 1; a higher value indicates a less homogeneous nanoparticle size distribution.

hydroxyl groups and physically absorbed water. The bands in the ~2900 cm^{-1} and 1240–1460 cm^{-1} regions are assigned to dextran νC–H and δC–H vibrational modes. The bands in the range ~1040 cm^{-1} and ~1010 cm^{-1} correspond to dextran νC–O vibrations. The characteristic α-glucopyranose ring deformation modes are evident at ~910 cm^{-1}, ~840 cm^{-1} and ~750 cm^{-1} regions. Compared to the FT-IR spectra of the control sample, all these four treatments did not alter or destroy the dextran shell in the DMNP samples. Unfortunately, the characteristic absorption bands of Fe–O vibration modes cannot be distinguished easily. That might be due to the relatively small amount of iron oxide cores compared to the dextran molecules in our DMNP samples.

The hydrodynamic size of the DMNPs in DI water was measured by DLS. The average hydrodynamic sizes weighted by intensity (*Z*-average size) and polydispersity index (P.I.) values of these samples are reported in Table 1. In the case of DMNP samples after autoclaving, UV irradiation and ethanol treatment, no significant difference was found on their average sizes when comparing to the control. However, the DMNP sample after filtration showed much smaller hydrodynamic size of around 131.6 nm than the control one (152.7 nm). The decrease of hydrodynamic size should be due to the loss of nanoparticles with larger sizes, which cannot pass thorough the pore size of the sterile filter during filtration treatment. The size distribution of the DMNP samples is expressed in the polydispersity index (P.I.), which corresponds to the variance of the size distribution of the nanoparticles. The DMNP samples after filtration and UV irradiation displayed the size distribution with a P.I. value of 0.22 as same as the control. However, the P.I.

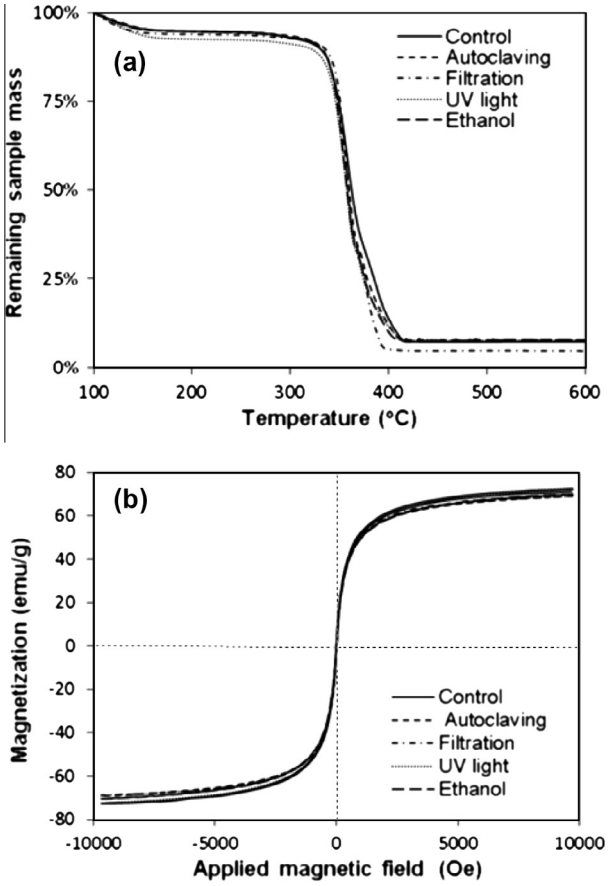

Fig. 3. (a) TGA results and (b) VSM results of dextran-coated magnetic iron oxide nanoparticles after sterilization by using autoclaving, filtration, UV irradiation, and ethanol treatment. The sample with no treatment served as control.

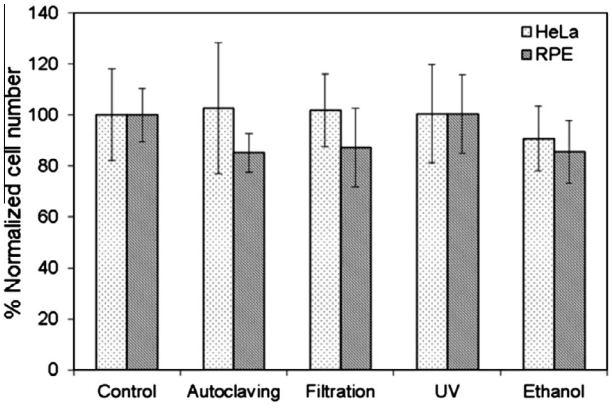

Fig. 4. Determination of relative cell numbers of HeLa cells and RPE cells after 24-h treatment with 0.40 mg/ml sterilized DMNPs samples. The control cells were treated with no DMNPs. (n = 5, no significant difference compared to control group of each cell line.)

value of the samples after autoclaving and ethanol treatment showed a slight increase (0.23) and a slight decrease (0.21), respectively. The alteration on the hydrodynamic size of dextran-coated iron oxide nanoparticles has also been observed by K.G. Paul and his co-workers before [11]. The detailed explanation about the influence from autoclaving and ethanol on the size distribution of dextran-coated iron oxide nanoparticles needs further study.

The heating curve and magnetic hysteresis curve of the lyophilized DMNP samples were measured by TGA and VSM, as

Table 2
General description of the physicochemical consequences of the different sterilization methods on the dextran-coated magnetic nanoparticles.

	TEM	FTIR	DLS (mean diameter)	DLS (polydispersity index)	TGA	VSM	Cell viability
Autoclaving	√	√	√	+	√	√	√
Filtration	√	√	×	√	×	√	√
UV irradiation	√	√	√	√	√	√	√
Ethanol treatment	√	√	√	−	√	√	√

[√] Non-detectable alterations, [+] slight increase, [−] slight decrease and [×] strong alteration.

shown in Fig. 3. The TGA results indicated that only sterilization by filtration affect the weight percentage of iron oxide core in DMNP sample. The weight percentage of iron oxide core in DMNP sample after filtration decreased to 4.7%, which is much less than the weight percentage of iron oxide core in control sample (7.8%). That means the loss of iron oxide cores was more than the loss of dextran in the filtration procedure, which resulted in a decreased weight percentage of iron oxide in final DMNP sample. The weight percentage of iron oxide core in DMNP samples after autoclaving, UV filtration and ethanol treatment has a same value of 7.8% as control. In the VSM results, all the samples displayed a superparamagnetic behavior, with similar saturation magnetization (Ms) values of iron oxide cores around 70 emu/g. The Ms values are less than the Ms for bulk Fe_3O_4 (92 emu/g) and the Ms for bulk -Fe_2O_3 (74 emu/g) [14], and are comparable to the Ms reported for the iron oxide nanoparticles [15]. There are no obvious influences observed from these four sterilization methods on the magnetic behavior of DMNP samples.

The cytotoxicity study of the DMNPs after these four sterilization methods was carried on the HeLa cells and RPE cells. The cell viabilities were determined by cell numbers counting using haemocytometer. The total cell numbers of the HeLa cells and the RPE cells were normalized to the corresponding control groups. As shown in Fig. 4, no significant cell number decrease was found on the HeLa and RPE cells after 24-h treatment with the DMNP samples sterilized by autoclaving, filtration, UV irradiation, and ethanol treatment. Thus, the autoclaving, filtration, UV irradiation and ethanol processes on the DMNP samples did not induce additional toxic effects in the cell culture experiments.

4. Conclusion

This study indicates that caution must be taken when choosing a suitable method to sterilize the dextran-coated magnetic iron oxide nanoparticles. The stability and physicochemical characteristics (Table 2) of the DMNPs should be maintained. All autoclaving, filtration, UV irradiation and ethanol treatments on DMNPs did not alter its core size, dextran shell, magnetic behavior and induced no additional toxic effects in the cell culture experiments. However, the filtration procedure could decrease the mean dynamic size and the weight percentage of iron oxide cores in sample significantly. The size distribution (P.I. value) of samples has detectable alterations under autoclaving and ethanol treatment. We show in this study that treatment with UV irradiation seems to be the most suitable method for the DMNPs.

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References

[1] S. Laurent, D. Forge, M. Port, A. Roch, C. Robic, L. Vander Elst, R. Muller, Chemical Reviews 108 (2008) 2064–2110.
[2] J. Dobson, Gene Therapy 13 (2006) 283–287.
[3] J.S. Weinstein, C.G. Varallyay, E. Dosa, S. Gahramanov, B. Hamilton, W.D. Rooney, L.L. Muldoon, E.A. Neuwelt, Journal of Cerebral Blood Flow & Metabolism 30 (2009) 15–35.
[4] H.L. Rodríguez-Luccioni, M. Latorre-Esteves, J. Méndez-Vega, O. Soto, A.R. Rodríguez, C. Rinaldi, M. Torres-Lugo, International Journal of Nanomedicine 6 (2011) 373.
[5] E. Memisoglu-Bilensoy, A.A. Hincal, International Journal of Pharmaceutics 311 (2006) 203–208.
[6] M. Latorre, C. Rinaldi, PR Health Sciences Journal 28 (2009).
[7] L.C. Gerhardt, G.M.R. Jell, A. Boccaccini, Journal of Materials Science: Materials in Medicine 18 (2007) 1287–1298.
[8] Y.N. Konan, R. Gurny, E. Allémann, International Journal of Pharmaceutics 233 (2002) 239–252.
[9] P. Sommerfeld, U. Schroeder, B.A. Sabel, International Journal of Pharmaceutics 164 (1998) 113–118.
[10] J. Lodhia, G. Mandarano, N. Ferris, P. Eu, S. Cowell, Biomedical Imaging and Intervention Journal 6 (2010) e12.
[11] K.G. Paul, T.B. Frigo, J.Y. Groman, E.V. Groman, Bioconjugate Chemistry 15 (2004) 394–401.
[12] K.G. Neoh, L. Tan, E.-T. Kang, Magnetic Core - Polymer Shell Nanoparticles: Synthesis and Biomedical Applications, Nanotechnologies for the Life Sciences, Wiley-VCH Verlag GmbH & Co. KGaA2007.
[13] C.W. Jung, P. Jacobs, Magnetic resonance imaging 13 (1995) 661–674.
[14] T. Yogo, T. Nakamura, W. Sakamoto, S. Hirano, Journal of Materials Research 15 (2000) 2114–2120.
[15] M. Morales, T.K. Jain, V. Labhasetwar, D. Leslie-Pelecky, Journal of Applied Physics 97 (2005) 10Q905.