Biocompatible chitosan-modified core-shell Fe₃O₄ nanocomposites for exigent removal of blood lactic acid

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Abstract

Excess lactic acid in blood will lead to hyperlactatemia, which is frequently detected in critically ill patients admitted to the intensive care. Reducing the blood lactic acid content using acute treatments becomes particularly important for bringing a patient out of danger. Traditional treatments often fail in case of malfunctioning of a patients’ metabolism. Herein, nanotechnology was introduced to remove blood lactic acid independent of metabolism. In this work, chitosan was employed as the shell to adsorb lactic acid, and Fe₃O₄ nanoparticles were employed as the core to enable proper magnetic separation property. Our data showed that core–shell nanocomposites (NCs) had an exigent and efficient adsorption behavior. Furthermore, they could be easily separated from blood plasma by magnetic separation. Thus, the good hemocompatibility and cytocompatibility indicated that of core–shell NCs hold great potential in lactic acid removal for emergent hyperlactatemia treatment.

1. Introduction

Lactic acid is an important product of anaerobic metabolism. Lactic acid levels in blood reflect the balance between intake and output of tissues. Glucose is decomposed into pyruvate, which is then catalyzed by lactate dehydrogenase to produce lactic acid in the absence of oxygen or under conditions of mitochondrial damage [1]. The ratio of lactic acid to pyruvate is stable, while lactic acid increases when NADH increases under hypoxic conditions. Clinically, lactic acidosis results from an acid-base balance disorder due to an excess of lactic acid. The ratio of lactic acid to pyruvate is stable, while lactic acid increases when NADH increases under hypoxic conditions. Clinically, lactic acidosis results from an acid-base balance disorder due to an excess of lactic acid. This leads to hyperlactatemia when the concentration of blood lactic acid is above 2 mmol l⁻¹ [2].

Hyperlactatemia is frequently detected in critically ill patients admitted to the intensive care [3–5]. In fact, when the patients’ blood lactic acid concentration is beyond 5 mmol l⁻¹, the mortality reaches up to 80%. Therefore, reducing the blood lactic acid content using acute treatments are of utmost importance. Clinically, to reduce blood lactic acid content, vasoactive drugs and muscle relaxants are used for tissue reperfusion [6]. In addition, mechanical ventilation is needed to supply enough oxygen [7]. Improving perfusion of the microcirculation will benefit the prognosis [8]. However, those treatments are aimed at enhancing the self-regulation of patients, and treatments would fail in case of malfunctioning of a patient’s metabolism. Therefore, developing a novel approach for exigent removal of blood lactic acid independent of metabolism regulation is highly warranted.

Direct adsorbance of lactic acid from blood to the surface of nanoparticles and subsequent separation from blood may be a sufficient method for exigent reducing the blood lactic acid content. Chitosan should be a suitable material for the removal of lactic acid as it contains active amino groups on its surface. These groups act as anchors to bind lactic acid [9]. Furthermore, chitosan is a low-cost material, biodegradable, hemocompatible, and has low-toxic effects [10–12]. These excellent properties enable chitosan to be used for lactic acid removal from blood.

Magnetic separation seems to be a promising approach to realize the separation of magnetic materials from a complex matrix [13, 14]. Coating certain materials on magnetic nanoparticles can be used for the separation of
cells [15], vesicles [16], ions [17] and other extracellular substances and subsequent removal from the blood by external magnetism. However, to our best knowledge, no studies have focused on exigent removal of blood lactic acid using magnetic separation for hyperlactatemia treatment.

Thus, in this study, chitosan was coated onto magnetic nanoparticles to prepare a novel blood lactic acid adsorbent, thereby aiming to efficiently and promptly separate lactic acid from blood plasma. The adsorption efficiency and hemocompatibility, as well as the cytotoxicity of the magnetic nanoparticles were investigated.

2. Materials and methods

2.1. Materials
Ferric trichloride (FeCl₃), chitosan, ethylene glycol, acetic acid glacial, lactic acid (RG, Adamas-bete®), sodium hydroxide, calcium chloride dihydrate, sodium acetate trihydrate (AR), polyvinylpyrrolidone (MW = 10 000) (aladdin®), lactic acid assay kit, BCA protein quantitation kit (Nanjing Jiancheng), human umbilical vein endothelial cells (HUVEC, ScienCell), DMEM cell culture medium, penicillin-streptomycin (Hyclone), fetal calf serum (Biological Industries), CCK8 kit (Biosharp) were used following the manufacturer’s guidelines unless special requirements were given.

2.2. Synthesis of chitosan-modified core–shell Fe₃O₄ nanocomposites (Fe₃O₄@Chi NCs)
According to Jia’s approach, a one-step solvothermal method was used to prepare Fe₃O₄@Chi NCs with a certain level of magnetism, size and surface charge [18]. Briefly, 0.09 g of FeCl₃, 0.06 g of chitosan, 0.60 g of sodium acetate trihydrate, and 0.10 g of polyvinylpyrrolidone were totally dissolved in 10 ml of ethylene glycol under vigorous stirring. The mixture was transferred into a 50 ml Teflon-lined stainless steel autoclave, which was sealed and maintained at 200 °C for 8 h. After the reaction, the products were washed with 0.5% acetic acid solution and deionized water thrice, respectively. Fe₃O₄@Chi NCs were dried under vacuum.

2.3. Characterization
The morphology of Fe₃O₄@Chi NCs was observed by scanning electron microscopy (SEM, SU-70, HITACH). Fourier transform infrared spectroscopy (FTIR) spectra were determined by a fourier transform infrared spectrometer (Spectrum GX, Perkin Elmer). The zeta potential was tested by a dynamic light scattering (DLS) instrument (Zetasizer Nano S90, Malvern). The magnetization value was determined by a superconducting quantum interference device (MPMS-XL-7, Quantum Design).

2.4. The lactic acid adsorption behavior of Fe₃O₄@Chi NCs
Various masses of Fe₃O₄@Chi NCs (W:W = 1:0.5, 1:1, 1:5, 1:25, 1:50, Fe₃O₄@Chi NCs:lactic acid) were added into plasma containing high levels of lactic acid (above 3 mmol l⁻¹). Mixtures were placed in a four-dimensional rotating mixer for 0.5 h. Then, magnetic separation was used to separate the lactic acid adsorbed Fe₃O₄@Chi NCs and supernatants. The residual levels of lactic acid and protein in the supernatants were determined by a lactic acid assay kit and BCA protein quantitation kit, respectively. All the experiments were performed in triplicate.

2.5. Hemocompatibility evaluation of Fe₃O₄@Chi NCs
To evaluate the hemocompatibility of Fe₃O₄@Chi NCs, hemolysis and thromboresistant experiments were carried out. The procedures were similar as described in our previous study [19]. Released hemoglobin was detected after co-incubation of different amounts of Fe₃O₄@Chi NCs with blood and hemolysis rates were calculated. In addition, released hemoglobin was determined after co-incubation of different amounts of Fe₃O₄@Chi NCs and re-calcified blood and thromboresistant ratios were calculated.

2.6. Cytotoxicity to HUVEC
HUVEC were cultured as a monolayer in a 25 cm² cell culture flask in DMEM culture medium containing 10% (V/V) fetal calf serum and 1% (V/V) penicillin-streptomycin at 37 °C in a humidified atmosphere with 5% CO₂. Cells in the logarithmic phase were seeded into 96-well plates at a density of 5000 cells per well. After 12 h, Fe₃O₄@Chi NCs were added to the culture medium at different concentrations. After co-culturing for 0.5 h, Fe₃O₄@Chi NCs were removed. The CCK8 kit was used to determine the temporal and at the end of 24-h cellular viability. HUVEC without any treatment were supposed to show 100% viability. All experiments were performed six times.
3. Results and discussion

Spherical nanoparticles of which the mean diameter was roughly 150 nm were synthesized by the solvothermal method. SEM analysis showed that the established nanoparticles were of good dispersion, and that no significant agglomeration occurred (figure 1(a)). Therefore, a relatively larger specific surface area would be present for the better adsorption performance [20]. The FTIR spectrum of those nanoparticles was presented in figure 1(b). There were two intense bands around 3410 cm\(^{-1}\) and 1620 cm\(^{-1}\), assigned to stretching vibrations and bending vibrations of N–H bond, respectively. The adsorption peak around 3400 cm\(^{-1}\) also represented the existence of associated carboxyl groups. The FTIR spectrum showed a weak adsorption peak around 1070 cm\(^{-1}\), resulted from the stretching vibrations of C–O groups. And the typical absorption peak of Fe–O around 580 cm\(^{-1}\) could also found in figure 1(b). Taken together, the FTIR results indicated that chitosan was successfully coated on the surface of Fe\(_3\)O\(_4\) nanoparticles. Fe\(_3\)O\(_4\)@Chi NCs were synthesized, and the zeta potential of Fe\(_3\)O\(_4\)@Chi NCs in a neutral solution was determined. The results showed that the zeta potential was \(-10.7 \pm 4.12\) mV (figure 1(c)), indicating that lactic acid with positive electricity could be adsorbed by electrostatic interactions. For their use in biomedical applications, Fe\(_3\)O\(_4\)@Chi NCs must have superparamagnetic properties. To realize magnetic separation, Fe\(_3\)O\(_4\)@Chi NCs should be easily gathered by external magnetism. Therefore, the magnetization value of Fe\(_3\)O\(_4\)@Chi NCs was determined by a superconducting quantum interference device, and the magnetization curve was shown in figure 1(d). Their saturation value was 53.78 emu g\(^{-1}\). And there was no hysteresis and their remanence as well as coercivity were zero, indicating Fe\(_3\)O\(_4\)@Chi NCs were superparamagnetic. In our previous studies, we showed that superparamagnetic nanoparticles performed well in magnetic guidance studies both in vitro [21–23] and in vivo [19]. Similarly, Fe\(_3\)O\(_4\)@Chi NCs could be conveniently gathered and guided by a magnet in a tubular structure (figure 1(d) insert) which not only showed good magnetic responses in vitro, but also revealed their potential application in a blood vessel.

To realize exigent removal of lactic acid, lactic acid must be effectively adsorbed by Fe\(_3\)O\(_4\)@Chi NCs in a short time. Thus, the adsorption efficiency of Fe\(_3\)O\(_4\)@Chi NCs on lactic acid in plasma at various concentrations within half an hour was investigated. After adsorption, magnetic separation was used to separate lactic acid loaded Fe\(_3\)O\(_4\)@Chi NCs and supernatants. The residual concentration of lactic acid in supernatant was determined and the adsorption rate was calculated. Overall, the adsorption rate increased with the increment of the mass of Fe\(_3\)O\(_4\)@Chi NCs. The lactic acid adsorption behavior was concentration dependent (figure 2). When the ratio reached 1:1, the adsorption rate was beyond 30%, thereby indicating that Fe\(_3\)O\(_4\)@Chi NCs could realize efficient adsorption in a short period, which would be promising for use at intensive care units [24]. More importantly, differing from the traditional lactic acid lowering methods, Fe\(_3\)O\(_4\)@Chi NCs directly adsorbed lactic acid. Therefore, patients could avoid the metabolic burden from lactic acid clearance [25].
After adsorption, the FTIR spectrum of lactic acid loaded \( \text{Fe}_3\text{O}_4@\text{Chi} \) NCs was tested to investigate the underlying mechanism of action of lactic acid adsorption. The amino groups in chitosan were protonated, when the chitosan shell came in contact with lactic acid [26]. In addition, protonated amino groups could react with the carboxyl groups of lactic acid to form ammonium lactate. This was confirmed by the FTIR spectrum (figure 3), which showed that the adsorption peak around 1575 cm\(^{-1}\) was significantly enhanced, thereby indicating the formation of amide groups. Covalent binding was observed between chitosan and lactic acid. Furthermore, the intense band resulting from stretching vibrations of C–O blueshifted from 1070 cm\(^{-1}\) to 1150 cm\(^{-1}\) due to the hydrogen-bond interaction between the amino group of chitosan and the carboxyl group of lactic acid. Both the bonding interaction and electrostatic interaction between \( \text{Fe}_3\text{O}_4@\text{Chi} \) NCs and lactic acid resulted in efficient adsorption. Together, the results showed the successful adsorption of lactic acid using \( \text{Fe}_3\text{O}_4@\text{Chi} \) NCs. The novel lactic acid lowering method was free from medicine, which would reduce the side effect of traditional therapy.

Before being used in clinical applications, biomaterials need to be rigorously evaluated for biocompatibility [27–29]. In this study, the hemocompatibility and cytotoxicity of \( \text{Fe}_3\text{O}_4@\text{Chi} \) NCs were studied as the initial evaluation for biocompatibility. Table 1 listed the hemolysis rates and thromboresistant ratios of \( \text{Fe}_3\text{O}_4@\text{Chi} \) NCs. The hemolysis rate of every experimental group was below 5% in standard ISO 10993-4. This showed that \( \text{Fe}_3\text{O}_4@\text{Chi} \) NCs didn’t cause hemolysis at these concentrations. In addition, the thromboresistant ratios of \( \text{Fe}_3\text{O}_4@\text{Chi} \) NCs were above 40% after 0.5 h, indicating the good anticoagulant activity. Chitosan performed
well in ionic adsorption studies [30, 31]. In this study, blood clotting was reduced, which could be ascribed to the adsorption of calcium ions by Fe₃O₄@Chi NCs [32]. Furthermore, due to the good adsorption effect of chitosan, plasma proteins might be adsorbed, which might lead to side effects. The BCA test was used to determine protein adsorption. Except for the 1:0.5 group, less than 10% of plasma proteins was adsorbed by Fe₃O₄@Chi NCs (figure 4). Combined, the results showed that Fe₃O₄@Chi NCs were of good hemocompatibility at certain concentrations.

Table 1. Hemolysis and thromboresistant ratios of Fe₃O₄@Chi NCs.

| Concentration (mg ml⁻¹) | Hemolysis ratio (%) | Thromboresistant ratio (%) |
|-------------------------|---------------------|----------------------------|
| 0.0054                  | 0.15 ± 0.05         | 42.64 ± 0.45               |
| 0.0108                  | 0.24 ± 0.13         | 49.31 ± 1.56               |
| 0.0504                  | 0.67 ± 0.18         | 53.34 ± 2.19               |
| 0.27                    | 0.98 ± 0.22         | 74.48 ± 2.38               |
| 0.54                    | 1.17 ± 0.09         | 77.66 ± 1.81               |

Figure 4. The residual rate of proteins after adsorption.

Figure 5. The cytotoxicity of Fe₃O₄@Chi NCs to HUVEC.
Fe$_3$O$_4$@Chi NCs were expected to be used in vivo as the targeting lactic remover in the blood vessel. Therefore, the growth inhibition effect on HUVEC using various concentrations of Fe$_3$O$_4$@Chi NCs was performed. Figure 5 showed that the viability of HUVEC treated with Fe$_3$O$_4$@Chi NCs at all tested concentrations was above 90% (figure 5), indicating that there was nearly no toxicity to cells [33]. Fe$_3$O$_4$ nanoparticles were biocompatible and have often been used as drug carriers and for imaging studies in vivo [34–36]. Moreover, chitosan has also been used as a biocompatible material for various biomedical applications [37]. Accordingly, Fe$_3$O$_4$@Chi NCs would be a safe lactic acid remover in the blood vessel at these concentrations tested.

4. Conclusion

In conclusion, chitosan-modified core–shell Fe$_3$O$_4$ NCs were used to adsorb excessive lactic acid from plasma. These NCs showed to present an exigent and efficient adsorption behavior. Moreover, Fe$_3$O$_4$@Chi NCs can easily be separated from plasma using magnetic separation. Furthermore, they are highly hemocompatible and cytocompatible. Consequently, Fe$_3$O$_4$@Chi NCs hold great potential in lactic acid removal for emergent hyperlactacemia treatment.

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