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Delivery of tissue plasminogen activator and streptokinase magnetic nanoparticles to target vascular diseases

Ateke Tadayon[a], Reza Jamshidi[a], Akbar Esmaeili[b,*]

[a] Faculty of New Sciences and Technology, Semnan University, Semnan, Iran
[b] Department of Chemical Engineering, North Tehran Branch, Islamic Azad University, PO Box 19585/936 Tehran, Iran

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ABSTRACT

Thrombolytic therapy for acute myocardial infarction standardly makes use of the medications streptokinase (SK) and tissue plasminogen activator (tPA). In this study, the potential of silica-coated magnetic nanoparticles (SiO_2-MNPs) as nanocarriers clinical thrombolytic therapy was investigated. SiO_2-MNPs for use in targeted therapeutic delivery of tPA and SK were prepared using a combined technique incorporating controlled precipitation and hydrothermal methods. Response surface methodology (RSM) was employed to evaluate the efficiency of the SiO_2-MNPs. The production of SK secreted from *Streptococcus equi* was enhanced using random mutagenesis. The tPA and SK A were encapsulated by means of a silanizing agent with a surface rich in 3-aminopropyltrimethoxysilane layered around the SiO_2-MNPs. Blood clot lysis assays and fibrin-containing agarose plates were used to carry out in vitro thrombolysis testing. The optimum conditions for producing MNPs were found to be at pH = 13 and at a temperature of 75 °C for 45 min. Culture conditions of 2.75% NaCl concentration at initial pH = 7.5 for 90 s under UV resulted in maximum SK activity. The tPA/SK-conjugated SiO_2-MNPs (SiO_2-MNP-tPA-SK) increased operating stability in whole blood and storage stability in a buffer by 92%. More effective thrombolysis using magnetic targeting was indicated by a 38% reduction in blood clot lysis time achieved with SiO_2-MNP-tPA-SK compared to administering the SiO2-MNPs without guidance. The silica-coated magnetic nanocarriers developed in this study show potential for improved clinical thrombolytic therapy.

1. Introduction

Thrombotic diseases, such as ischemic stroke and myocardial infarction, are the leading causes of death and disability in developed countries (Yang et al., 2012). Used in treating myocardial infarction and pulmonary embolism, SK is a nonenzymatic protein produced by the Lancefield group C strain of β-hemolytic *Streptococcus equi* (Banerjee et al., 2004). The protein tPA functions by converting the enzyme precursor plasminogen to the enzyme plasmin, which is effective in breaking down blood clots (Bennett et al., 1991). The preferred drug employed in lysis of the fibrin clot associated with ischemic stroke is tPA, but successful treatment requires administration of a high dosage over a prolonged time period, which can lead to hemorrhage (Berger and Pizzo, 1988). In some patients successful thrombolysis is not achieved with streptokinase due to high levels of resistance acquired by previous exposure to streptococci, which can compromise the therapeutic response (Gemmill et al., 1994). Magnetic drug targeting may serve as a strategy to achieve targeted thrombolysis while reducing the hemorrhagic side effects (Wang et al., 2012).

Iron oxide MNPs can be utilized in delivery of drugs and vaccines, to magnetically induce hyperthermia in treatment of cancer, and to provide contrast in magnetic resonance imaging (Esmaeili and Hadad, 2015). Nickel MNPs are highly interesting materials due to their superparamagnetic behavior at room temperature; important results may be obtained by conjugation with an enzyme (Esmaeili et al., 2013; Verma et al., 2010). MNPs used as drug carriers are usually encased in a hydrophilic polymer shell, which provides a high-capacity functionalized surface capable of binding with drugs, inhibiting aggregation, and increasing colloidal stability (Esmaeili and Hadad, 2015). In

Abbreviations: BHI, brain-heart infusion; CCD, central composite design); FT-IR, Fourier transform infrared spectroscopy; GA, glutaraldehyde; MNP, magnetic nanoparticle; NP, nanoparticle; PS, particle size; PBS, phosphate-buffered saline; tPA, plasminogen activator; RSM, response surface methodology; SEM, scanning electron microscopy; SiO_2-MNP, silica-coated magnetic nanoparticle; SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SK, streptokinase; XRD, X-ray diffraction.

* Corresponding author. Fax: +98 21 88787204.
E-mail addresses: akbaresmaeili@yahoo.com, akbaresmaeili@iau.ac.ir (A. Esmaeili).

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contrast, MNPs with a silica shell can be readily utilized for drug loading, owing to the shell's high surface area, uniform pore size, and large amount of available pore volume (Esmaeili and Gholami, 2015; Wang et al., 2012).

The aim of this study was to develop, synthesize, and characterize a novel thrombolytic agent carrier system based on SiO_2-MNPs, which are emerging as one of the most appealing candidates for theranostic carriers. Drug carriers were generated by changing crystalline MNP parameters to obtain an optimal size, with the expectation of providing the ability to transport tPA and SK to the selective sites for local targeting and sustained drug release. Drug-carrying capacity was improved by ~45%, demonstrating that these SiO_2-MNPs may useful for treatment of thrombus (Scheme 1).

2. Experimental

2.1. Synthesis of $CuNi.Fe_2O_4$ and SiO_2-MNP-tPA-SK

2.1.1. Optimization of MNPs

Central composite design (CCD), the most popular RSM for experimental designs of this nature, and Minitab 16.2.2.0 statistical software were used to guide optimization of the MNP particle size (PS). A 3^3 factorial design was employed for the investigation. The design comprised low, medium, and high levels (−1, 0, and +1), and a total of 20 runs were carried out using reaction time (X_1), temperature (X_2), and pH (X_3) as the independent variables and particle size (Y) as the response. All experiments in the model were repeated three times. The levels used in the experiments are listed in Table 1. The set of 20 experiments is tabulated in Table 2.

2.1.2. Effect of reaction time

To study the effect of different reaction times on the properties of MNPs, after preparation a mixture of salts of Fe, Ni, and Cu, the mixture was stirred in an oil bath under a nitrogen atmosphere and tested after 30, 45, and 60 min.

2.1.3. Effect of temperature

To determine the optimum temperature for the oil bath, the mixture was stirred in the bath under a nitrogen atmosphere at 50 °C, 75 °C, and 100 °C, and the resulting PS measured for each increment.

2.1.4. Effect of pH

The pH of the mixture was adjusted to 8, 10, and 13, as determined with a pH meter calibrated prior to each measurement, by dripping a 1.2 M NaOH aqueous solution into the mixture under magnetic stirring. The resulting PS was measured for each pH.

Table 1
Factors and levels used in the factorial design.

Factor / Level	−1	0	+1
Reaction time (min) (X_1)	30	45	60
Temperature (°C) (X_2)	50	75	100
pH (X_3)	8	10.5	13

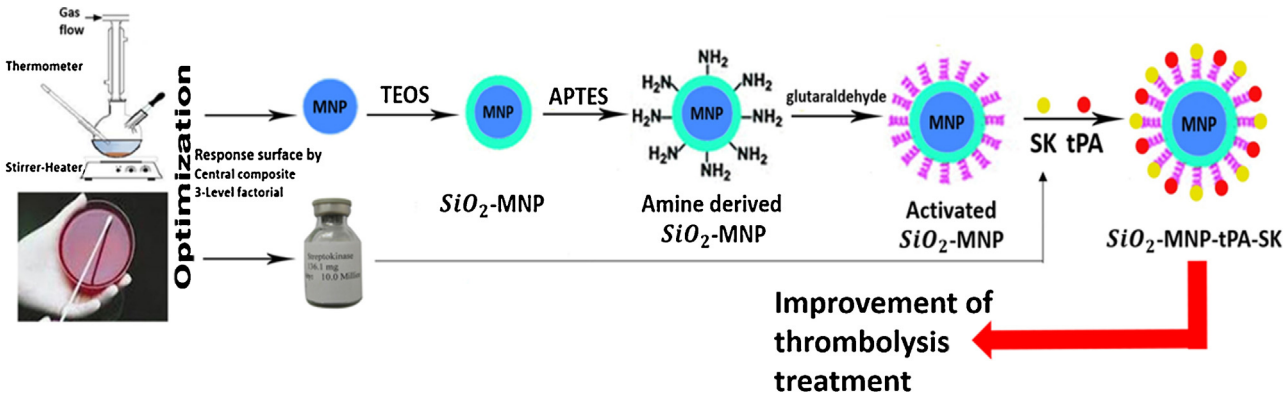
2.1.5. Synthesis of $Cu_{0.5}Ni_{0.5}Fe_2O_4$ NPs

$Cu_{0.5}Ni_{0.5}Fe_2O_4$ was obtained by a combined technique utilizing controlled precipitation and hydrothermal methods (Bingölbali et al., 2015). $FeCl_3{\cdot}6H_2O$, $CuCl_2{\cdot}2H_2O$, and $NiCl_2{\cdot}6H_2O$ (Cu:Ni:2Fe) were mixed in water at room temperature under magnetic stirring at 1000 rpm for 15 min. Next, the mixture was placed in an oil bath heated to 80 °C under a nitrogen atmosphere with constant stirring at 500 rpm for 40 min. Following this, 1.2 M NaOH solution was added drop-by-drop to the solution. After this procedure the mixture was transferred to a Teflon-lined autoclave and heated at 250 °C for 12 h. The product was rinsed three times in water, magnetically decanted to separate the particles, and dried at 90 °C for 15 h in a vacuum oven. Finally, the MNPs were heated at 800 °C for 2 h in an oven.

2.1.6. Synthesis of SiO_2-MNP-tPA-SK

For synthesis of SiO_2-MNPs, ethanol, water, and tetraethyl orthosilicate were mixed in a two-neck flask and stirred magnetically for 30 min while being warmed at 40 °C in a water bath. NH_4OH was stirred into the solution and allowed to react for 30 min. The MNPs were added to the solution and stirred for another 6 h. The SiO_2-MNPs were separated by magnetic decantation, washed with ethanol three times, and dried at 90 °C in a vacuum oven.

The amine-modified SiO_2-MNPs were prepared by washing the SiO_2-MNPs in ethanol and water for 30 min with an ultrasonic processor (Bandelin SONOREX RK 514H, capacity 18.7 L, 600 W). (3-Aminopropyl) triethoxysilane and dimethylformamide were added to the solution, which was then shaken in an incubator for 2 h. The SiO2-MNPs were rinsed with phosphate-buffered saline (PBS) three times, isolated with a magnet, and stored in PBS. Finally, the SiO_2-MNP suspension was mixed with glutaraldehyde (GA) in PBS and shaken at 30 °C. To prepare the activated SiO_2-MNPs, the suspension was washed three times with PBS. SiO_2-MNPs were mixed with tPA and SK for 6 h at 15 °C using a rotor at 7 rpm, rinsed with PBS, and isolated magnetically.



Scheme 1. Strategy of experimentation.