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Superparamagnetic microsphere-assisted fluoroimmunoassay for rapid assessment of acute myocardial infarction

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ABSTRACT

Rapid assessment of acute myocardial infarction (AMI) was successfully demonstrated using an improved superparamagnetic polymer microsphere-assisted sandwich fluoroimmunoassay to detect two early cardiac markers—myoglobin and human heart-type fatty acid binding protein (H-FABP). This assay used a preparation of superparamagnetic poly(styrene-divinylbenzene-acrylamide) microspheres, glutaraldehyde-coupled capture antibodies (monoclonal anti-myoglobin 7C3 and anti-H-FABP 10E1) grafted onto the polymer microspheres, and a sequential sandwich fluoroimmunoassay using detection antibodies (FITC-labeled anti-myoglobin 4E2 and FITC-labeled anti-H-FABP 9F3). Characterization of the polymer microspheres by TEM, SEM and Fourier transform infrared spectroscopy (FT-IR) showed that the microspheres were uniformly round with an average diameter of 1.12 μm, and had a Fe_3O_4-polymer core–shell structure (shell thickness was about 84 nm) with 0.22 mmol/g amino groups on their surfaces. The magnetic behavior of the Fe_3O_4-polymer microspheres was superparamagnetic (M_s = 13 emu/g, H_c = 13.1 Oe). Fluorescence images of the post-immunoassay microspheres recorded using a confocal laser-scanning microscope showed that the average fluorescence intensity was correlated with the concentration of cardiac markers, in agreement with the results obtained by an F-4500 FL spectrophotometer; this indicated that the fluoroimmunoassay could be used to semi-quantitatively detect both myoglobin and H-FABP. The detection limit was 25 ng/mL for myoglobin and 1 ng/mL for H-FABP.

1. Introduction

Acute myocardial infarction (AMI), a severe cardiovascular disease, remains one of the leading causes of mortality in both developing and developed nations (Ryan et al., 1996; Sala et al., 2005; Condon and McCarthy, 2006; Yang and Zhou, 2006). The length of the interval between onset and treatment is a major determinant of the outcome of AMI (Chen et al., 2005). However, patients with chest pain who are admitted to an emergency department are usually diagnosed based on medical history, physical examination and electrocardiography (ECG) (Ahmar and Lefkovits, 2008; Spiers, 2007). Often diagnosis will be inconclusive for a number of possible reasons: (1) Pulmonary embolism can also produce chest pain (Wolf et al., 2004). (2) Transient left ventricular apical ballooning syndrome may mimic ST-elevation AMI with a simultaneous chest pain (Parodi et al., 2007). (3) Non-ST-elevation myocardial infarction is especially difficult to diagnosis by ECG (Schindler et al., 2007). (4) Approximately one quarter of all myocardial infarctions can be silent, without chest pain or other symptoms (Kannel, 1986).

In order to address these phenomena and to improve the accuracy and efficiency of detection, a number of cardiac marker proteins, such as total CK and isoenzymes (Gillum et al., 1984), C-reaction protein (Brunetti et al., 2006), cardiac troponins (Hamm et al., 1997), myeloperoxidase (Cheng et al., 2008), myoglobin (Matveeva et al., 2005; Mair et al., 1992), and fatty acid-binding protein (FABP) (O'Regan et al., 2002; Glatz et al., 2002) have been employed to diagnose AMI. Of these markers, the protein used in an assay should be one that is present in measurable concentrations in the early post-AMI stage and that also has a high clinical specificity. In this instance, the use of proteins that are smaller in size would lead to earlier detection. Studies have shown that myoglobin (17.8 kDa) and FABP (15 kDa) are two relatively small proteins that show elevated levels in the blood stream shortly after an acute myocardial infarction. Additionally, a combined analysis of these two markers can specifically discern an injury to the myocardium after AMI onset (Van Nieuwenhoven et al., 1995; Wodzig et al., 1998;

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Hillis et al., 2003). Therefore, the two are highly suitable for use in a rapid screening test for early AMI diagnosis, providing high sensitivity and a high negative predictive value (Yang and Zhou, 2006).

Detection of a disease using biomarkers usually is accomplished by an immunoassay based on the reaction between an antibody to the biomarker and the antigen (biomarker) itself (Hillis et al., 2003; Tanasijevic et al., 1999). In a critical situation, such as that imposed by a suspected AMI, early and quick diagnosis is essential. However, conventional immunoassay, in addition to often requiring skilled and somewhat subjective interpretation, is laborious, expensive, consumes large quantities of reagents, and involves a prolonged development time. Standard immunoassay is therefore not suitable for rapid measurement, and is difficult to apply for point of care testing (van der Voort et al., 2004).

Recently, superparamagnetic microsphere-assisted fluoroimmunoassay has attracted considerable interest in clinical diagnosis because of its unique properties (Haik et al., 2002). First, antibody immobilized on the surface of the microspheres with functional groups can be readily used for immunoassay (Bergervoet et al., 2008); in comparison with other microspheres (Wang and Zhang, 2006; Martins et al., 2006), antigens captured by magnetic microspheres can be very easily separated from a reaction system simply by applying an external magnetic field rather than centrifugation. Second, the three-dimensional structure of the microspheres increases the surface-to-volume (S/V) ratio, so that more target proteins can be bound and analyzed (Lim and Zhang, 2006). This can also decrease the detection time and the consumption of specimen (Bergervoet et al., 2008). Several superparamagnetic microsphere-based fluoroimmunoassay procedures have been reported (Bergervoet et al., 2008), including one based on alkaline phosphatase research (Haik et al., 2002), but none of these are sufficiently developed for rapid detection of early AMI biomarkers. In the present paper, we describe an improved magnetic polymer microsphere-assisted sandwich fluoroimmunoassay for the analysis of the cardiac markers myoglobin and H-FABP.

2. Experimental

2.1. Reagents and materials

The micro BCA™ protein assay kit and EZ-label™ FITC protein labeling kit were obtained from Pierce Biotechnology (Rockford, IL). Anti-myoglobin 7C3, anti-myoglobin 4E2, anti-H-FABP 10E1, anti-FABP 9F3, myoglobin and human H-FABP were received from Hytest Ltd. (Turku, Finland). Bovine serum albumin (BSA) and divinylbenzene (DVB) were purchased from Sigma–Aldrich (St. Louis, MO). All solvents and other chemicals were purchased from local commercial suppliers and were of analytical reagent grade, unless otherwise stated. Deionized (DI) water (Milli-Q, Millipore, Bedford, MA) was used to prepare aqueous solutions.

2.2. The assay principle

The assay principle is explained in Scheme 1. Magnetic poly(styrene-DVB-acrylamide) microspheres with active amino groups were first synthesized by dispersion polymerization of styrene and acrylamide in the presence of sodium oleate-coated magnetic Fe_3O_4 nanoparticles, using DVB as a cross-linking agent. Next, the capture antibody was covalently linked with glutaraldehyde to the surface of the microspheres. Upon addition of a sample containing antigen (H-FABP or myoglobin), the antigen occupies the antigen-binding site of the antibody, and is subsequently assayed using FITC-labeled detection antibody.

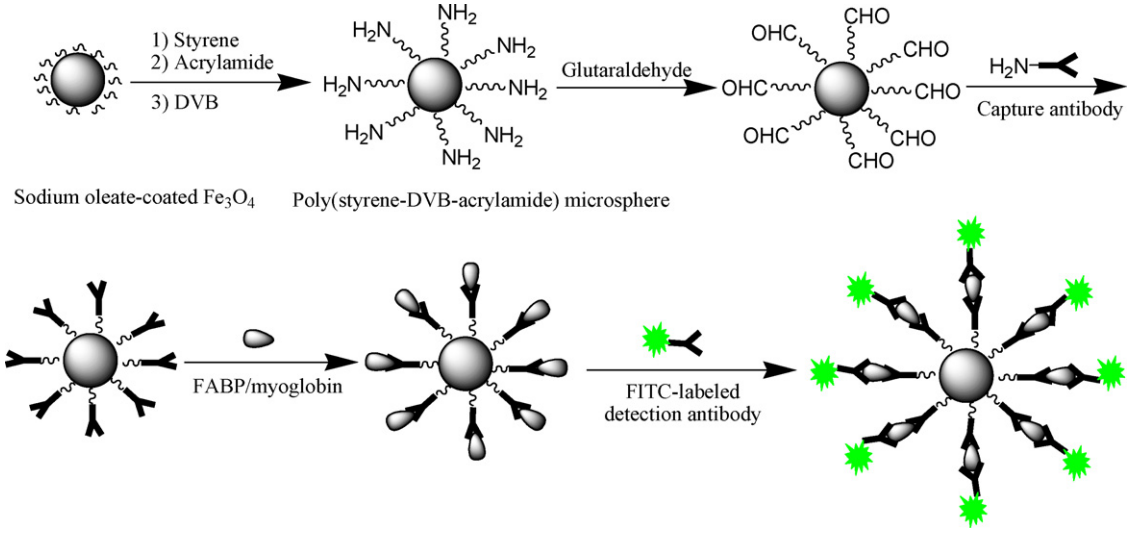
2.3. Synthesis of magnetic poly (styrene-DVB-acrylamide) microspheres

2.3.1. Synthesis of sodium oleate coated-iron oxide nanoparticles

Sodium oleate-coated iron oxide nanoparticles were prepared following the methods reported previously (Sauzedde et al., 1999; Shen et al., 1999; Iwasaki et al., 2008). Briefly, 7.5 mL of 0.12 M $FeCl_2$ and 0.2 M $FeCl_3$ solution were first prepared using DI water, transferred to a 100 mL three-necked flask, and stirred together under nitrogen. The solution temperature was raised to 55 °C over 15 min, and then 7.2 mL of NaOH solution (3.0 M) was added rapidly; the reaction mixture was vigorously stirred and incubated for 40 min. The iron oxide dispersion that formed was then heated to 65 °C and stirred slowly while adding 30 mL of sodium oleate solution (0.36 M) dropwise. The mixture was then stirred at 90 °C for 30 min. After cooling to room temperature, the iron oxide nanoparticles were precipitated with a magnetic field.

2.3.2. Synthesis of magnetic polymer microspheres

Magnetic poly(styrene-DVB-acrylamide) microspheres were prepared by dispersion polymerization, using a typical recipe



Scheme 1. Schematic representation of the principle of the synthesis of poly(styrene-DVB-acrylamide) microspheres, immobilization of capture antibody onto the microspheres, and the fluoroimmunoassay procedure.