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Biofunctionalization of magnetic poly(glycidyl methacrylate) microspheres with protein A: Characterization and cellular interactions

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ABSTRACT

Monodisperse poly(glycidyl methacrylate) (m-PGMA) microspheres which show superparamagnetic behaviour were synthesized by dispersion polymerization. Bioligand protein A was covalently immobilized onto glutaraldehyde activated microspheres (3.12 mg protein A per gram of microspheres). Cell culture studies denoted that 61% of total L929 mouse fibroblasts were bound to the m-PGMA microspheres while 84% of total cells were bounding to the protein A immobilized (m-PGMA-PrA) microspheres. Interactions between m-PGMA-PrA microspheres and L929 cells were stronger than that of m-PGMA microspheres due to the non-specific interactions between protein A and cell surface. The cells interacted with m-PGMA-PrA microspheres keep their round form rather than attaching to the tissue culture polystyrene (TCPS) surface. In conclusion, this study consists a basis for the fractionation of blood lymphocytes bearing IgG antibodies on their surfaces by using protein A immobilized m-PGMA microspheres.

1. Introduction

Nano- and micron-size magnetic polymeric particles have been used in a wide range of biomedical applications, for example specific cell labeling [1], cell separation [2], affinity chromatography [3], hyperthermia [4], targeted drug delivery [5], contrast agents for MRI [6] and diagnostics [7]. These applications require the magnetic particles to fulfill some properties such as colloidal stability, uniform size or narrow size distribution, high and uniform magnetite content, superparamagnetic behaviour and enough surface functional groups for coupling of active bioligands [8]. There are variety of methods to produce magnetic polymeric microspheres. These are emulsion polymerization [9], multistep swelling polymerization [10], solvent evaporation [11], etc. [12,13]. Contrary to these methods, which are either very complicated or induce broad particle size distribution, dispersion polymerization was suggested as an alternative method to produce micron-size magnetic particles [14]. Its main advantage is in its simplicity: it is a single-step technique which yields monosize particles under suitable reaction conditions [15]. Horak et al. have prepared glycidyl methacrylate (GMA) based magnetic microspheres by using dispersion polymerization for different purposes such as polymerase chain reactions (PCR) applications [16], immunomagnetic separation and detection of *Salmonella* cells [17], DNA diagnostics [18] and genomic DNA isolation [19]. GMA is one of the monomer which exhibits some significant advantages with its multifunctional epoxy groups as potential carrier matrix, i.e. easy and stable covalent linkages with different groups such as amino, thiol and phenolic ones under mild experimental conditions [20]. As GMA is polymerized, the reactive oxirane ring and aldehyde group allow direct coupling of enzymes and proteins on the surface of the supports, whereas enzyme and protein immobilization onto supports containing amino groups can be easily achieved by means of glutaraldehyde [21].

Magnetic cell separation is one of the most effective process for many clinical and immunological applications [22,23]. This technology explains using magnetic beads or magnetic colloids–antibody systems to specifically bind the target cells and isolate them from a cell suspension. Then, magnetic beads–cell complex is separated by using a suitable magnetic separator [24]. Among the current cell separation techniques, immunomagnetic cell separation has particularly become a popular tool for the isolation of target cells from cell suspensions by using immunomagnetic microspheres and has proven to be a more rapid and simple technique, in comparison to fluorescence-activated cell sorting (FACS) [25]. Microspheres used in immunomagnetic separation possess uniform spherical shape and superparamagnetic properties. Dynabeads® were the first commercial magnetic microspheres used for cell separation applications [26]. They are monodispersed polystyrene microspheres specifically designed with antibodies according to the applications. They have been used in a wide variety of

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immunoisolation studies. The circulating human endothelial cells were successfully separated by Dynabeads® coated with anti-endothelial antibodies [27]. Highly pure human deriving endothelial cells from different organs of the same 12-week-old embryos were isolated by using Dynabeads® coated with CD 31+ or CD 34+ specific endothelial antibodies [28]. Another mostly used commercial magnetic cell separation system is developed by Miltenyi Biotech. While Dynabeads® are micron-sized microspheres, Microbeads® (Miltenyi Biotech, Germany) are submicroscopic magnetic particles which are approximately 50 nm in size and composed of iron oxide and dextran. They have been widely used for the isolation of CD 34+ and CD 133+ cells from different sources on both small- and clinical-scales [29]. Kekarainen et al. optimized an immunomagnetic cell sorting protocol to purify CD 34+, CD 133+ and Lin-hematopoietic stem cells from fresh and cryopreserved cord blood by using magnetic cell sorting system (MACS, Miltenyi Biteoch, Germany) [30]. Sieben et al. tested different types of magnetic particles which specifically functionalized via antibodies for the tumor cell separation from peripheral blood and established a very effective method with respect to different parameters [31].

Protein A is a cell wall associated protein domain exposed on the surface of the gram-positive bacterium *Staphylococcus aureus*. It has high affinity to immunoglobulin G (IgG) from various species, for instance human, rabbit and guinea pig. Therefore researchers have been frequently using protein A immobilized magnetic polymeric sorbents for IgG separation [32,33]. These sorbents can also be used for animal cell separation. Molday et al. used ferromagnetic iron dextran microspheres conjugated to protein A for specifically separating red blood cells (RBC) by means of high gradient magnetic chromatography [34]. Before separation procedure, red blood cells were incubated with rabbit antihuman RBC serum. Thus protein A conjugated microspheres can specifically bind to the treated cells. Moreover, Thomas et al. applied immunomagnetic selection for isolation of acrosome-reacted *Bovine Spermatazoa* using rabbit antibodies to goat-anti-immunoglobulin-coated magnetic microspheres (IgG-MAG, Collaborative Research Inc., USA) or protein A magnetic microspheres (PA-MAG, Polysciences Inc., USA) [35]. It was shown that PA-MAG microspheres were much more effective than IgG-MAG as a tool for selecting *spermatozoa*. On the other hand, Kumar et al. [36] investigated the capture of human acute myeloid leukemia KG-1 cells expressing the CD34+ surface antigen. In addition, the fractionation of human blood lymphocytes were evaluated on polyvinyl alcohol (PVA)–cryogel beads and dimethyl acrylamide (DMAAm) monolithic cryogel. Protein A was chemically coupled to the reactive PVA cryogel beads and epoxy-derivatized monolithic cryogels through different immobilization techniques. In the same study, either lymphocytes were treated with goat anti-human IgG or protein A was used for coupling of anti-CD34 antibodies, recognizing the cell surface receptors for KG-1 cells and anti-IgG [36].

The goal of this study was to produce cost effective, monodisperse magnetic PGMA microspheres bearing protein A and to investigate their non-specific interactions with IgG negative L929 mouse fibroblasts. As discussed above, the specific interactions between IgG and protein A have been studied extensively. It will be possible to develop more effective magnetic cell separation processes by taking into account the non-specific interactions. Owing to this aim, m-PGMA microspheres were synthesized by dispersion polymerization in the presence of iron oxide nanoparticles. Non-magnetic PGMA microspheres were also synthesized by using the same procedure but in the absence of iron oxide nanoparticles. These microspheres were characterized by Fourier transform infrared (FTIR) spectroscopy, vibrating sample magnetometer (VSM) and scanning electron microscope (SEM). Cytotoxicity of the microspheres onto L929 fibroblastic cells were evaluated by using 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT) test. Magnetic PGMA microspheres were activated by glutaraldehyde chemistry. Bioligand protein A was then covalently immobilized onto these glutaraldehyde activated microspheres. Protein A immobilized magnetic poly(glycidyl methacrylate) (m-PGMA-PrA) microspheres were directly used to investigate their interactions with L929 fibroblast cells in a tube-based magnetic cell separation system. The cellular interactions were observed by optic microscope and SEM.

2. Experimental

2.1. Materials and methods

Protein A from *Staphylococcus aureus*, ferric chloride ($FeCl_3{\cdot}6H_2O$), steric stabilizer poly(vinylpyrrolidone) (PVP K 30, M_w = 40,000), glutaraldehyde solution (25% in H_2O), boric acid (H_3BO_3) and phosphate-buffered saline (PBS) tablets were purchased from Sigma (St. Louis, MO, USA). Ferrous chloride ($FeCl_2{\cdot}4H_2O$) and the monomer glycidyl methacrylate (GMA) were purchased from Aldrich (USA). Initiator 2,2′-azobisisobutyronitrile (AIBN) was purchased from Acros Organics (USA). Ammonia solution (25%), acetone and hydrochloric acid solution (HCl, 37%) were purchased from Merck (Germany). Ethanol (96%) was obtained from Aklar Kimya (Turkey). Methanol was received from BİRPA® (Turkey). All the water used in experiments were ultrapure water which is obtained from Barnsted EasyPURE™ system. All the chemicals were used as received.

2.2. Synthesis of iron oxide nanoparticles

Superparamagnetic iron oxide nanoparticles were synthesized according to the Massart's procedure [37]. An aqueous solution of ferric chloride (40 mL; 4.093 g $FeCl_3{\cdot}6H_2O$) and ferrous chloride (10 mL; 2.595 g $FeCl_2{\cdot}4H_2O$ in 2 M HCl) was added to ammonia solution (500 mL, 0.7 M) and the mixture was stirred. The Fe_3O_4 precipitate was isolated from the solution by magnetic separation, washed with water three times and then transferred to the ethanol solution.

2.3. Synthesis of magnetic poly(GMA) microspheres

Magnetic poly(glycidyl methacrylate) (m-PGMA) microspheres were synthesized via dispersion polymerization [38]. Polymerization was performed in a magnetic driven, sealed cylindrical reactor (volume: 250 mL) equipped with a temperature control system. A recipe used for the dispersion polymerization of glycidyl methacrylate (GMA) is given in Table 1. Iron oxide nanoparticles (0.05 g) synthesized before were added in 68 g of aqueous ethanol solution (96%) and the resulting medium was sonicated for 30 min at 80% power within an ultrasonic water bath (Sonorex Super 10 P, Bandelin, Germany). PVP (2.4 g) was dissolved in this black magnetite–ethanol dispersion. Then, a solution of 0.24 g of AIBN in 12 g of GMA was poured into the reactor and the reactor was purged with

Table 1
Recipe used for the preparation of m-PGMA microspheres by dispersion polymerization.*

Material	Weight (g)
GMA	12.0
PVP	2.4
Ferrofluid**	0.05
AIBN	0.24
Ethanol	68.0

* 70 °C.
** Dry weight.