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Reactive magnetic poly(divinylbenzene-co-glycidyl methacrylate) colloidal particles for specific antigen detection using microcontact printing technique

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ABSTRACT

Epoxy-functionalized magnetic poly(divinylbenzene-co-glycidyl methacrylate) colloidal particles (mPDGs) were prepared by co-polymerization of 1,4-divinylbenzene and glycidyl methacrylate monomers. The reaction was conducted by batch emulsion polymerization in the presence of an oil in water magnetic emulsion as a seed. The chemical composition, morphology, iron oxide content, magnetic properties, particle size and colloidal stability of the prepared magnetic polymer particles were characterized using Fourier transform infrared spectroscopy, transmission electron microscopy, thermal gravimetric analysis, vibrating sample magnetometry, dynamic light scattering, and zeta potential determination, respectively. The prepared mPDGs were immobilized on a self-assembled monolayer of 3-aminopropyltriethoxysilane (APTES)/octadecyltrichlorosilane (OTS), which were patterned on glass using microcontact printing technique, forming mPDGs–APTES/OTS reactive surface. This construction (mPDGs–APTES/OTS) was used as a solid support for immunoassay. The immobilized magnetic particles were bioconjugated with monoclonal anti-human IL-10 antibody to provide specific and selective recognition sites for the recombinant human IL-10 protein (antigen). Fluorescence microscopic examination was carried out to follow this immunoassay using fluorescently labeled anti-human IL-10 antibody. The results obtained proved the successful use of mPDGs–APTES/OTS microcontact printed surfaces in an immunoassay, which can be exploited and integrated into microsystems in order to elaborate medical devices (e.g. biosensors) which could provide rapid analysis at high sensitivity with low volumes of analyte.

1. Introduction

Over the last decade magnetic nanoparticles/functional polymer hybrid materials, forming functional magnetic polymer nanoparticles or microspheres (FMPLs), have attracted the attention of researchers in various areas, especially in the biomedical field [1]. The promising features of polymer particles, including a high specific surface area, narrow size distribution and versatility in surface functionality, make them convenient for wide use as carriers for biomolecules (proteins, antibodies, antigens, etc.) [2]. In addition, a major advantage of using magnetic nanoparticles is their rapid separation upon applying even a low strength magnetic field in a one step process. Accordingly, the coupling of biomolecules to magnetic field stimuli-responsive carriers can be exploited to achieve rapid, simple, and specific separation of biomolecules under the effect of an external magnetic field, avoiding difficult and time-consuming separation processes, like filtration and centrifugation, which limit the automation of biomedical diagnosis [3,4]. Nowadays FMPLs are used not only for in vitro diagnostic applications, such as the separation and purification of biomolecules, probes for DNA/RNA hybridization and identification, and protein and nucleic acid detection, but also for in vivo therapeutic applications, such as targeted drug delivery, magnetic resonance imaging (MRI) as contrast agents, and in hyperthermia [5–19].

In the field of in vitro diagnosis the highly sensitive detection and accurate analysis of biological molecules in human fluid samples are essential for early detection, treatment, and management of diseases. In some cases diagnosis in the early stages of infection is a critical factor in obtaining optimal results in terms of therapy and in improving the chances of survival, as in the case of cancer [20]. Recently the conjugation of magnetic polymer particles with antibodies has been extensively studied, because it combines the properties of the magnetic polymer particles with the ability of antibodies to specifically and selectively recognize antigens [21]. Bioconjugation can take place by physical adsorption (at the isoelectrical point of the antibody via electrostatic interaction), by direct covalent linkage between the surface of the nanoparticle and

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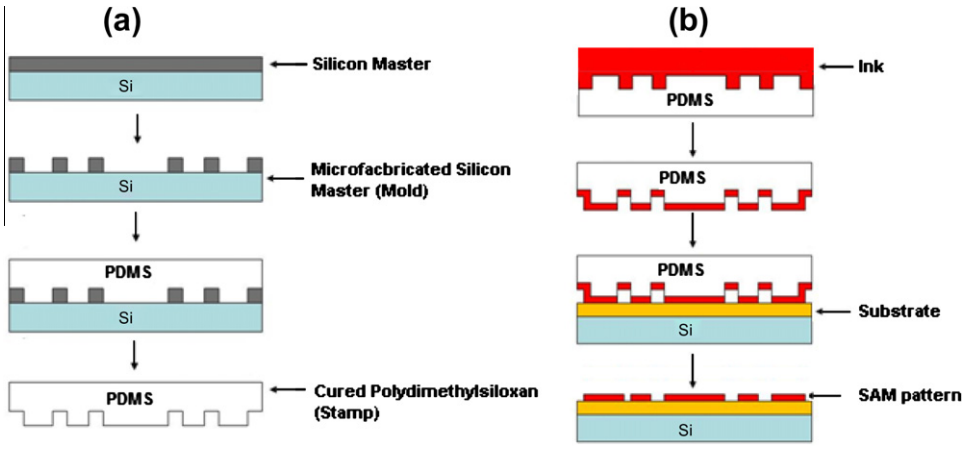
the antibody, or by using adapter molecules [22–25]. Covalent linkage is advantageous compared with physical adsorption in that it prevents the competitive displacement of the adsorbed antibodies by blood components, which occurs for adsorbed antibodies. In addition, the interaction between biomolecules and reactive particles is strongly dependent on the colloidal and surface properties of the dispersion and on the physico-chemical properties of the biomolecules. Thus the synthesis process should be adapted to prepare structured colloidal particles bearing a reactive shell with well-defined properties [1,26,27].

In general, magnetic polymers consist of a magnetic core surrounded, in the ideal case, by a polymer shell. Regarding the magnetic core, iron oxides such as magnetite (Fe_3O_4) or its oxidized form maghemite (γ-Fe_2O_3) are preferred in biomedical applications because they are superparamagnetic, safe, easily prepared, and biocompatible [28]. On the other hand, the polymer shell has several aims, e.g. it prevents iron oxide release from the particles and enables covalent attachment of target biomolecules (drugs, proteins, antibodies, etc.) via reactive functional groups on their surfaces [29]. Moreover, the functionality of the polymer matrix plays an important role in its affinity and selectivity towards the desired biomolecules. Amongst the reactive functional monomers glycidyl methacrylate (GMA) is widely used as a precursor in the synthesis of reactive functional polymers [30] because GMA contains a polymerizable double bond and a reactive epoxy group in its chemical structure, which are useful for chemical grafting of any biomolecule containing reactive functional groups (–NH_2, –OH, –COOH, etc.) or can be chemically modified to desired functional groups having an affinity for metal ions, gases and proteins [2,31–38].

In the last decade the encapsulation or coating of iron oxide nanoparticles with reactive polymer shells has attracted the attention of scientists in various industrial and biotechnological fields. Thus numerous interesting approaches have been developed to prepare these hybrid particles, including classical heterogeneous polymerization processes using emulsions, suspensions, dispersions, mini-emulsions, inverse emulsions or inverse microemulsions, as well as some multi-step synthesis procedures [26]. Recently, two major strategies leading to submicron particles have been reported, one using a mini-emulsion polymerization technique and another based on the use of oil in water (o/w) magnetic emulsion droplets as the seed in emulsion polymerization. Mini-emulsion polymerization has been explored for the preparation of submicron hybrid latex particles [39–45]. However, this has various undesirable limitations, such as a heterogeneous distribution of magnetic nanoparticles in the polymer matrix, a low magnetic material content in the polymer particles and, finally, a small particle size, which lead to poor magnetic separation under a classical magnetic field [46]. The use of o/w magnetic emulsion droplets as the seed in emulsion polymerization was first reported by Montagne et al. [47]. This new approach is based on the transformation of magnetic emulsion droplets into magnetic latex particles by emulsion polymerization using hydrophobic monomers such as styrene. The magnetic seeds used were submicron droplets of a highly stable magnetic emulsion and the particle size distribution of the magnetic polymer emulsion was controlled by the size distribution of the initial magnetic emulsion. Homogeneous encapsulation of iron oxide nanoparticles was also efficiently achieved using a styrene/1,4-divinylbenzene (DVB) monomer mixture in the presence of potassium persulfate (KPS) as initiator. In addition, the magnetic polymer produced had a high iron oxide content (~60 wt.%) with a high carboxylic surface charge density [47].

Recently the patterning of specific protein molecules (antibodies, antigens, etc.) on surfaces has become of paramount importance in biotechnological applications, such as diagnostic immunoassays and biosensor technology, which require the use of fewer reagents and less sample solution. Therefore, different patterning techniques to immobilize biomolecules on surfaces with high precision have been developed [48–51]. Microcontact printing (μCP) is considered to be one of the most applicable techniques in this area, because it can be used in the creation of reactive surfaces with specific selectivity and allows the controlled deposition of molecules that can interact in specific ways. In addition, it is very effective, fast, inexpensive, easy to use, and requires no special laboratory equipment, so it has potential applications ranging from analysis of biochemical mixtures to molecular scale electronic components [52–56]. μCP is considered a form of soft lithography that uses the relief patterns on a master stamp made of polydimethylsiloxane (PDMS) to form patterns of self-assembled monolayers (SAMs) of ink (bioaffinity ligand) on the surface of a glass substrate through conformal contact. Scheme 1 shows the μCP procedure, which consists of four steps; preparation of the master, creation of the stamp, inking the stamp, and, finally, applying the stamp to the substrate (contact printing).

Based on the aforementioned literature, the present work aims to elaborate new reactive epoxy-functionalized mPDGs using seed emulsion polymerization of DVB and GMA monomers in the presence of Fe_3O_4 magnetic emulsion droplets as the seed. The key parameters of mPDGs for use in in vitro diagnostic applications, including chemical composition, morphology, particle size, colloidal stability and magnetic properties, were investigated.



Scheme 1. General procedure of microcontact printing. (a) Creation of the polydimethylsiloxane (PDMS) master using a silicon pattern, pouring and curing the PDMS and then peeling it away from the substrate. (b) Ink is poured over the PDMS stamp and left to dry, followed by conformal contact with the glass substrate, with the pattern being left behind.