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Magnetic microparticles post-synthetically coated by hyaluronic acid as an enhanced carrier for microfluidic bioanalysis

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ABSTRACT

Iron oxide based particles functionalized by bioactive molecules have been utilized extensively in biotechnology and biomedicine. Despite their already proven advantages, instability under changing reaction conditions, non-specific sorption of biomolecules on the particles' surfaces, and iron oxide leakage from the naked particles can greatly limit their application. As confirmed many times, surface treatment with an appropriate stabilizer helps to minimize these disadvantages.

In this work, we describe enhanced post-synthetic surface modification of superparamagnetic microparticles varying in materials and size using hyaluronic acid (HA) in various chain lengths. Scanning electron microscopy, atomic force microscopy, phase analysis light scattering and laser diffraction are the methods used for characterization of HA-coated particles. The zeta potential and thickness of HA-layer of HA-coated Dynabeads M270 Amine were −50 mV and 85 nm, respectively, and of HA-coated p(GMA-MOEAA)-NH_2 were −38 mV and 140 nm, respectively. The electrochemical analysis confirmed the zero leakage of magnetic material and no reactivity of particles with hydrogen peroxide. The rate of non-specific sorption of bovine serum albumin was reduced up to 50% of the naked ones. The coating efficiency and suitability of biopolymer-based microparticles for magnetically active microfluidic devices were confirmed.

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1. Introduction

Magnetic iron oxide particles in sizes ranging from nanometre to micrometre scales have been put to use in a number of areas: biotechnology, therapeutics, and *in vitro* and *in vivo* clinical diagnostics [1–3]. Many automatic immunoanalysers or multiplex systems in clinical laboratories utilize superparamagnetic micro- or nanoparticles with high specific surface area as a solid phase for creating efficient reaction conditions. Another rapidly developing field of application for magnetic particles is microfluidics [1,2]. In this field, such prerequisites as narrow particle size distribution, colloidal and reaction stability, and excellent superparamagnetic behaviour are necessary. To integrate the suspension of magnetic particles into microfluidic devices, it is necessary to eliminate any instability of magnetic particles during analytical steps caused by deviations from physiological reaction conditions (e.g. pH, ionic strength and density of particles). The tendency of particles to agglomerate and adhere to the inner surfaces of microfluidic devices and non-specific adsorption of biomolecules are significantly limiting factors in such routine applications [4–7]. It is well known that spontaneous aggregation of particles is accompanied by loss of their superparamagnetic behaviour [7]. Moreover, the hydrophilic or hydrophobic character of such particles affects the activity of biomolecules, resulting in a decrease or total loss of their activity due to their potential denaturation [6].

To minimize all the aforementioned disadvantages, post-synthetic coating with an appropriate stabilizer of natural or synthetic origin

Abbreviations: AFM, atomic force microscopy; BCA, bicinchoninic acid; BSA, bovine serum albumin; COC, cyclo-olefin copolymer; COP, cyclo-olefin polymer; CTAB, cetyl trimethyl-ammonium bromide; EDC, 1-ethyl-3(3-dimethylaminopropyl)carbodiimide hydrochloride; HA, hyaluronic acid; LSV, linear sweep voltammetry; MES, 2-(N-Morpholino)ethanesulphonic acid; OPD, o-phenylenediamine; p(GMA-MOEAA)-NH_2, poly([glycidylmethacrylate-(methacryloyloxy)ethoxy]acetic acid)amine; PALS, phase analysis light scattering; PMMA, polymethylmethacrylate; SEM, scanning electron microscopy; sulpho-NHS, N-hydroxysulphosuccinimide sodium salt.

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(e.g. using polymers, surfactants or other biomolecules with a hydrophilic nature) is one of the ways to overcome these obstacles. Such commonly used polymer stabilizers as poly(ethyleneglycol), poly(vinyl)alcohol and poly(lactic acid) and such carbohydrates as dextran, chitosan and starch have been successfully applied [3,4,6,7]. Another potential low-fouling modifier is hyaluronic acid (HA). Among such surface stabilizers, HA has received much attention due to its unique characteristics [8].

HA is a naturally occurring, linear, non-sulphated glycosaminoglycan consisting of a repeating disaccharide unit of β-1,3-N-acetylglucosamine linked to β-1,4-glucuronic acid. Among its other attributes, HA possesses highly hydrophilic and polyanionic characteristics under physiological conditions [8]. Its viscous solutions have unusual rheological properties and are exceedingly lubricious [9,11]. The naturally occurring locations and molecular mass of HA polymer chains (which can exceed even 10^6 Da) are the key factors affecting HA's broad spectrum of biological activities and features [12–14]. Namely high molecular mass extracellular HA is an important structural element, acts as a signalling component [12,13] and exhibits antiangiogenic properties whereas HA oligosaccharides support angiogenesis [13,14]. HA fragments also induce inflammatory, tumour growth and metastasis [13,14] and they are able to activate transcription factor like NF-κB, but high molecular mass HA has an inhibitory effect [12]. High molecular mass HA does not activate the inflammatory or proliferative genes [13]. HA is known for such excellent biological features as high biocompatibility, biodegradability, low immunogenicity, and high tolerance for the human body's immune system [9,15].

The aforementioned biological and physicochemical characteristics of this fascinating biomolecule have increased the interest in its safe utilization within the fields of modern biotechnology and biomedicine. The premise is that the HA-based and HA-coated materials gain the unique properties of HA. Its potential is certainly broad and versatile. HA is a beneficial structural component and basic building block of such HA-based carriers and biomaterials as HA-nanoparticles, hydrogels and scaffolds, and thus it offers great promise for diagnostics and even for therapy [10]. As already proven, HA also serves as an effective stabilizer for surface treatment and coating of a variety of medical devices and materials, including the likes of catheters [16], guidewires [17], superparamagnetic nanoparticles for in vivo imaging [18,19], various types of artificial replacements such as dental, orthopaedic implants [20,21] or neuronal implants [22], and microfluidic channels [23].

The post-synthetic surface modification of superparamagnetic microparticles by HA was the aim of this study. Hyaluronic acid was chosen with respect to its unique biological and physicochemical characteristics contributing tissue integrity and cell communication. We assume that HA imparts qualitatively new surface properties to the modified particles mainly for protein analysis performed in magnetically active microfluidic devices. No paper has yet been published describing such post-synthetic modification of magnetic microparticles for bioapplications. We have clearly demonstrated increased colloidal stability of HA-coated microparticles in aqueous solutions, their suppressed adhesion to the various materials used in microfluidic devices and reduced non-specific sorption of biomolecules.

2. Materials and methods

2.1. Chemicals

Hyaluronic acid (molecular weight = 10 kDa and 26 kDa) was a product of Contipro Group s.r.o (Dolní Dobrouč, Czech Republic). Oligo-HA4, fluorescein hyaluronic acid (≈800 kDa), hyaluronan biotin sodium salt (≥700 kDa), 2-(N-Morpholino)ethanesulphonic acid (MES), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), bovine serum albumin (BSA), o-phenylenediamine (OPD), (+)-hydrazide biotin, streptavidin-peroxidase polymer (STR-HRP), cetyl trimethyl ammonium bromide (CTAB), and 30% hydrogen peroxide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Streptavidin-conjugated fluorescent dye Alexa Fluor 488 was obtained from Life Technologies (Carlsbad, CA, USA). N-hydroxysulphosuccinimide sodium salt (sulpho-NHS) was obtained from Fluka (Buchs, Switzerland), and a Micro BCA protein assay kit was purchased from Thermo Fisher Scientific (Rockford, IL, USA). All other chemicals were of reagent grade and produced by PENTA (Chrudim, Czech Republic).

2.2. Magnetic particles

Dynabeads M-270 Amine magnetic particles (polystyrene, 2.8 μm) were purchased from Life Technologies (Carlsbad, CA, USA) and SiMAG-Amine magnetic microparticles (silica, 0.75 μm) from Chemicell GmbH (Berlin, Germany). Magnetic particles of poly([glycidylmethacrylate-(methacryloyloxy)ethoxy]acetic acid)amine (p(GMA-MOEAA)-NH_2) (4.5 μm) were obtained from the Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic (Prague, Czech Republic) [24].

2.3. Coating of magnetic particles by HA

One milligramme of magnetic particles with $-NH_2$ functional groups was washed 5 times with MES buffer (0.1 M, pH 6.0). Subsequently, carboxylic groups located on HA chains were activated. Ten milligrammes of EDC and 1.7 mg of sulpho-NHS dissolved in 400 μL of MES buffer (0.1 M, pH 6.0) were successively added to HA (from 0.1 to 500 nM according to the molecular weight of the HA chain) dissolved in 200 μL of MES buffer (0.1 M, pH 6.0). The solution of activated HA was then added immediately to the pre-washed particles and the mixture was stirred for 3 h at room temperature. Particles modified by HA were washed 3 times with MES buffer (0.1 M, pH 6.0), then 3 times with 1 M NaCl in the same buffer and 5 times with phosphate buffer (0.1 M, pH 7.0) to remove all unreacted or non-specifically adsorbed molecules. Phosphate buffer (0.1 M, pH 7.0) with sodium azide as a preservative was used as a storage solution.

2.4. Methods applied for characterizing HA-coated particles

2.4.1. Zeta potential measurement of magnetic microparticles

The electrophoretic mobilities and zeta potentials were determined by electrophoresis and phase analysis light scattering (PALS) using a ZetaPALS instrument (Brookhaven Instruments Corporation, Holtsville, NY, USA) equipped with Pd electrodes at 25 °C. The suspension of pre-washed naked/HA-coated magnetic particles in redistilled water at a concentration of 0.05 mg mL^{-1} was analysed. The zeta potential was calculated from the electrophoretic mobility using the Smoluchowski equation as the median from 42 experimental values.

2.4.2. Size measurement of magnetic microparticles

The size of the naked/HA-coated magnetic microparticles was measured by laser diffraction using the MasterSizer 2000 particle size analyser (Malvern Instruments Ltd., Malvern, UK). The magnetic carriers were thoroughly washed with redistilled water and ultrasonicated for 10 min. To analyse hydrodynamic diameter, 10 mL of magnetic carrier suspension was used at a concentration of 0.05 mg mL^{-1}. These samples were injected directly into the measuring cell. The laser obscuration was between 5% and 10%, and every sample was measured three times. The average particle size and its distribution were evaluated by red light (He–Ne laser with wavelength 633 nm) and blue diode (466 nm) on the basis of Fraunhofer bending.

2.4.3. SEM and AFM measurement

The naked/HA-coated magnetic microparticles were prepared for atomic force analysis (AFM) and scanning electron microscopy (SEM) imaging by spin-coating on the glass substrate according to a procedure described previously [25,26]. Gold-coated samples were visualized using the SEM instrument Jeol JSM 5500 LV (JEOL Ltd., Tokyo, Japan).

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