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Magnetic Nanoparticles for Plasmid DNA Adsorption via Hydrophobic Interaction

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This study aims the preparation of magnetic poly(2-hydroxyethylmethacrylate-N-methacryloyl-L-phenylalanine), [poly(HEMA-MAPA)] nanoparticles for plasmid DNA separation on the basis of hydrophobic interactions. Magnetic nanoparticles will be produced emulsion polymerization of 2-hydroxyethylmethacrylate (HEMA) and N-methacryloyl-L-phenylalanine (MAPA) monomers. Nanosized particles including hydrophobic groups stemmed from polymerizable derivative of phenylalanine aminoacid were evaluated to offer surface area that is enough for the higher capacity DNA purification than commercial micronsized sorbents for DNA purification.

Keywords: Genomics, Nanoparticles, pDNA purification, Poly(HEMA), Phenylalanine.

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1. INTRODUCTION

The identification of human genes and the associated phenotype has rapidly increased over the last decade with the human genome project. As more human gene sequences are placed on databases, with their function, regulation and expressed product identified, combined with the recent advances in molecular and cell biology, significant improvements in our understanding of disease mechanisms as well as in our ability to prevent, diagnose and treat human disease have been achieved, and new therapeutic perspectives have become available [1]. Recent developments in therapeutic approaches, such as DNA vaccination and gene therapy have fostered the development of large-scale pDNA purification processes [2].

The need for quick bacterial plasmid DNA (pDNA) preparation methods, free of protein, RNA, salts, and enzyme inhibitors, has increased with the flood of molecular protocols requiring highly purified genetic template [3]. Chromatography, being a high-resolution method, is often the preferred purification technique due to its ability to provide the product purity required of gene therapy agents [4]. Of the different chromatography modes for plasmid purification available, anion-exchange chromatography (AEC), together with size-exclusion chromatography, can be considered the real workhorse, although, such techniques as, hydrophobic chromatography, affinity chromatography and thiophilic chromatography are also used frequently [5]. The purification of pDNA by hydrophobic interaction chromatography (HIC) explores differences in hydrophobicity between pDNA, single-stranded nucleic acid impurities and endotoxins. HIC of pDNA constitutes an advance over the more common AEC [6]. In fact, HIC is able to separate pDNA from lipopolysaccharides, denatured genomic DNA, RNA and denatured pDNA. Furthermore, separation of pDNA isoforms is possible with some stationary phases [7].

Many matrices sold on the market have been developed primarily for much smaller proteins. The implication of this is that plasmids bind largely only to the outer surface of the beads, resulting in a low plasmid-binding capacity. The plasmid-binding-capacity challenge has been tackled by the development of new chromatographic supports [4]. One way is to increase the surface available by perforating the beads with large flow pores able to accommodate plasmids [8]. This approach has proven successful and has resulted in matrices with good plasmid DNA-binding capacities. An important consideration in creating such superpores is that they provide sufficient pore flow. This is accomplished mainly by use of a high pore-diameter to bead-diameter ratio. The pore flow is essential since it allows the plasmids to be transported to the point of interaction much faster than is possible with diffusion, which is exceptionally slow due to the size of the plasmids. Another matrix-development approach is the use of small non-porous supports. Since no pores are present in this case, sufficient pore flow is a non-existing problem. The particles involved can still have sufficient binding surface to allow for a satisfactory plasmid capacity [9].

This study aims the preparation of magnetic poly (2-hydroxyethylmethacrylate-N-methacryloyl-L-phenyl-alanine), [poly(HEMA-MAPA)] nanoparticles for plasmid DNA separation on the basis of hydrophobic interactions. Magnetic nanoparticles will be produced emulsion polymerization of 2-hydroxyethyl methacrylate (HEMA) and N-methacryloyl-L-phenylalanine (MAPA) monomers. Nanosized particles including hydrophobic groups stemmed from polymerizable derivative of phenylalanine aminoacid will be evaluated to offer surface area that is enough for the higher capacity DNA purification than commercial microspherical sorbents for DNA purification.

2. EXPERIMENTAL

2.1 Synthesis of Functional Monomer

Applied procedure for synthesizing the functional comonomer, N-methacryloyl-L-phenylalanine (MAPA), was reported elsewhere (Fig. 1) [10,11]. It can be given as briefly; L-phenylalanine (5.0 g) and $NaNO_2$ (0.2 g) were dissolved in 30 ml of K_2CO_3aqueous solution

(5%, w/v). The solution was cooled to 0 °C. Methacryloyl chloride (4.0 ml) was slowly poured into this solution under nitrogen atmosphere. This solution was then stirred magnetically at room temperature for 2 h. At the end of this chemical reaction period, the pH of the solution was exactly adjusted to 7.0 and subsequently the solution was extracted with ethyl acetate. The aqueous phase was evaporated in a rotary evaporator. The residue (i.e., MAPA) was crystallized from ether and cyclohexane.

The MAPA was characterized by ^{1}H-NMR measurement. The determined characteristic peaks are as follows: [^{1}H NMR ($CDCl_3$)] 2.84 (t, 3H, CH_3), 3.05 – 3.19 (m, 2H, CH_2), 4.80 – 4.85 (m, 1 H, methyne), 5.24 (s, 1H, vinyl H), 5.56 (s, 1H, vinyl), 6.24 (σ, 1H, NH), 7.04 – 7.20 (m, 5H, aromatic), 10.07 (s, 1H, OH).

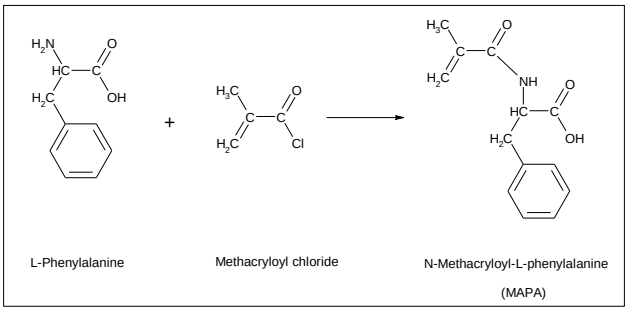

Fig. 1 – Synthesis of L-phenyalanine based functional monomer

3. RESULTS AND DISCUSSION

In this study, we prepared magnetic poly(2-hydroxy-ethylmethacrylate-N-methacryloyl-L-phenylalanine), [poly(HEMA-MAPA)] nanoparticles for plasmid DNA purification from *E. coli* lysate. Magnetic nanoparticles were produced emulsion polymerization of 2-hydroxyethyl methacrylate (HEMA) and N-methacryl-oyl-L-phenylalanine (MAPA) monomers. Magnetic nanoparticles in size 53 nm having surface area as 2052 m^2/g were characterized by atomic force and transmission electron microscopy, and Fourier transform infrared spectroscopy. Resonance magnetic field (H_r) and g factor, which shows magnetic properties, of the magnetic nanoparticles determined by electron spin resonance spectroscopy is about 3212 G and 2.03, respectively. The effective factors such as pH, initial DNA concentration, temperature, salt type and concentration etc. on adsorptive properties of the magnetic nanoparticles were evaluated. Maximum adsorption capacity was determined as 788.1 mg/g at pH 5.50 and 25 °C for 2h incubation period. Magnetic nanoparticles were also used for plasmid DNA purification from *E. coli* lysate under optimal condition. The results shows that the magnetic nanoparticles can be used for plasmid DNA purification successfully depending on the result obtained from agarose gel electrophoresis. As last step, the reusability of the magnetic nanoparticles were evaluated and no significant decrease was determined after tenth adsorption-desorption-regeneration cycle.

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