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Functional Polyelectrolyte Coatings on Polymeric and Magnetic Colloidal Particles for Antifouling and Non-Toxic Bioconjugate Nanoparticles

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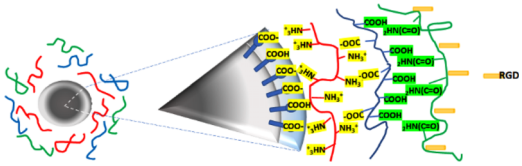
Abstract: In this paper, we used H-bonding and/or electrostatic force-driven polyelectrolyte multilayer (PEM) coatings on the surface of magnetic (paramagnetic and ferromagnetic) colloidal particles to prevent irreversible coagulation and introduce biochemical moieties. Polyacrylamide (PAAm), poly(acrylic acid) (PAA), and a cationic modified cyclodextrin polymer P(β-CyD) were used for the coating. Through the chemical groups of the PEM coating, the RGD-peptide ligand was introduced on the magnetic particles, which enhanced strong specific cell-magnetic particle interactions. P(β-CyD)-containing surface-coated magnetic particles also show great potential in cell culture. These functional PEM coatings on magnetic colloidal particles may have great potential in bio-applications including targeted intracellular drug delivery.



Keywords: surface modification, magnetic nanoparticles, polyelectrolyte multilayers, bioactive conjugate, antifouling.

1. Introduction

The development of thin-film coating technology enables unique tailoring of the functional properties of nanoparticles. Magnetic nanoparticles (MNPs) constitute a system of broad research interest encompassing such captivating aspects as superparamagnetism,[1] dipolar interactions,[2-3] ferrofluids, and rheology.[4,5] Mainly, magnetic particles offer some attractive possibilities in biomedicine, such as drug delivery targeting and diagnosis,[6] hyperthermia,[7] cell labeling, magnetic separation,[8-10] magnetic resonance imaging.[11] Furthermore, magnetic field can direct magnetic particles for drugs to specific body areas, so can be effective in treating diseases such as cancer.[12] Therefore, surface functionalization of magnetic particles with organic materials to perform biochemical modification has become a great interest.

This surface functionalization is often accomplished by coating the particles with polymers by layer-by-layer self-assembly. This method uses colloidal magnetic particles as templates to assemble polyelectrolyte multilayer films. For example, Caruso and co-workers pioneered composite hollow spheres by calcinations of polystyrene latices with magnetite nanoparticle.[13] Other groups, including us, have reported permanent binding of magnetic nanoparticles with polymers.[14-18] The primary driving force for the multilayer films on the particles is electrostatic attraction or hydrogen bonding between the oppositely charged species or H-donor/acceptor. By mixing these driving forces, many different polymers can be introduced in a surface coating on the particles.

This paper provides a detailed investigation on the construction of a novel class of colloidal magnetic particles by the layer-by-layer multilayer coatings and the interactions of the coated magnetic particles with mammalian cells. Polyelectrolyte multilayer (PEM) films were assembled in alternation with poly (acrylic acid) (PAA), polyacrylamide (PAAm), or modified β-cyclodextrin polymer (P(β-CyD)). Cyclodextrins are macrocyclic compounds of n, α-(1,4)-D-glucopyranose units where n is 6, 7, 8, designated as α, β, γ-cyclodextrins. The interior of the cyclodextrin is hydrophobic, while the exterior, which consists, on the opposite rims, of primary and secondary hydroxyl groups, is hydrophilic. Cyclodextrins have been reported as molecular receptors that bind selectively, without forming covalent bonds, ionic or molecular structures through various intermolecular interactions. Furthermore, we synthesized a cyclodextrin polymer with cationic functionality, used as a cationic polyelectrolyte in this study.

Our previous study of using PAA/PAAm multilayer coating on tissue culture plates or colloidal particles[19-21] turned the surfaces into highly non-adhesive toward cells. In contrast, a new surface functionalization with PAA/P(β-CyD) coating on the colloidal particles generates attractive cell interactions. By combining these two systems, we could tune the level of interactions of the MNPs with living cells. Biochemical contents, such as an RGD containing ligand, were also easily applied on magnetic particles with these coatings. We expect our surface-functionalized MNPs can provide great potential in various MNP based bioengineering applications.

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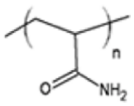
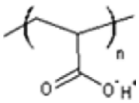
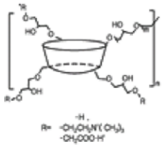
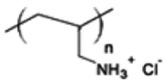
2. Results and discussion

We prepared the PEM films comprised of PAA, PAAm, and cationic β-CyD polymer, P(β-CyD). The chemical structures and molecular weight information for these polymers are listed in Table 1, and the synthetic method and characterization of P(β-CyD) was reported in our previous publication.[22] The magnetic colloidal particles coated with fluorescent-dye-labeled PAA/PAAm multilayers are uniformly coated with the H-bonded multilayers. For the investigation of PEM deposition on magnetic particles, we utilized two types of them, carboxylated paramagnetic polystyrene particles (PS-COOH) and ferromagnetic Fe_3O_4 iron oxide nanoparticles (F-ION).

Table 1. Chemical structures and molecular weights of the polymers used for multilayer thin film assembly

Notation of polyelectrolytes	Chemical structure	Molecular weight
Poly(acrylamide), PAAm		5,000,000
Poly(acrylic acid), PAA		100,000
Cationic-β-cyclodextrin polymer, Poly(β-CyD)		~7,885
Poly(allylamine hydrochloride), PAH		150,000

We measured surface zeta (ζ)-potentials of bare PS-COOH and F-ION magnetic colloidal particles in deionized water with a concentration of 0.03% and obtained the zeta (ζ)-potential values as -5.8 and -24.4 mV, respectively. Therefore, we chose an oppositely positive-charged polymer, poly(allylamine hydrochloride) (PAH), for the first layer deposited on the negative-charged surface of MNPs at pH6.0. When PAH chains were adsorbed onto the surface of MNP particles, the ζ-potential of the surface of particles turned to a positive value. In the subsequent deposition of PAA, the ζ-potential of the MNP reversed back to negative. These alternating values of the surface zeta potentials of MNP demonstrate successful recharging of the particle surface with each polyelectrolyte deposition, as summarized in Figure 1.

Then non-ionized PAAm chains could be assembled on the surfaces of particles with PAA top layer coating forming the PAA/PAAm multilayers by H-bonding interactions (Figure 1(a)-(b)). Either using PS-COOH or F-ION particles, the surfaces of magnetic particles were converted into negative charges as PAA was adsorbed; however, PAAm deposition did not turn ζ-potentials to positive values amide groups in PAAm are not ionizable in a wide range of pH remaining uncharged. Therefore, attractive forces between PAAm and PAA for their deposition onto the particles should be hydrogen bonding interactions. So, we chose the deposition pH conditions of both polyelectrolytes as pH 3.0, where many carboxylic acid groups of PAA remained un-ionized, reacting as H-donor to PAAm. Since ζ-potentials of the particles remained negative values in the overall deposition process, it was helpful to prevent the particles from flocculating. As PAA/PAAm coated PS-COOH and F-ION showed a similar trend in their ζ-potentials (Figure 1(a)-(b)), both MNPs were well dispersed in an aqueous solution as assembled PAA/PAAm film was coated. However, due to their different magnetic property, paramagnetic vs. ferromagnetic, unmodified F-IONs were more challenging to pre-

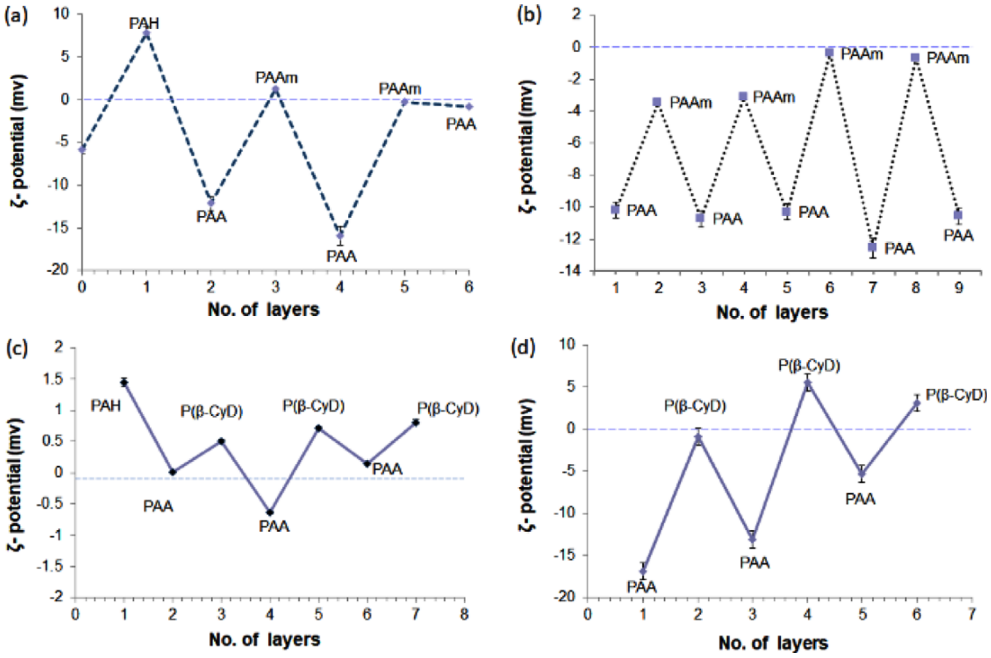


Figure 1. Zeta(ζ)-potentials of magnetic colloidal particles coated with $(PAA/PAAm)_m$ on (a) PS-COOH and (b) F-ION. Zeta potentials of (c) $(PAA/P(b\text{-}CyD))_n$ and (d) $[(PAA/PAAm)_3 + (PAA/P(b\text{-}CyD))_n]$ coating applied on PS-COOH. A PAH layer was applied as the first layer on the particles, and pH 3.0 was used for all polymer dipping conditions.

vent coagulation so, we utilized paramagnetic PS-COOH particles for further biochemical modification than F-IONs.

The assembled PAA/PAAm multilayer film is not stable at high pH due to its nature of H-bonding interaction. Therefore, they should be crosslinked if they are used other than low pH conditions. More detailed PAA/PAAm multilayer deposition condition was explained in our previous publication.[19-21,23]

We also tried to coat PS-COOH particles with a P(b-CyD) having cationic functional groups, synthesized in our lab for the counterpart of PAA deposition driven by electrostatic interactions. To compare with PAA/PAAm system, we used the same deposition conditions for PAA/P(β-CyD) multilayers, and it was confirmed as obtaining the alternative surface charges on the magnetic particles using micro-electrophoresis (Figure 1(c)). The more minor changes in ζ-potentials obtained from PAA/P(β-CyD) compared to PAA/PAAm may be due to the lower molecular weight P(β-CyD) than PAAm. Because the amount of a polyelectrolyte in a layer is determined by the amount of oppositely charged polyelectrolyte from the previously deposited layer in the layer-by-layer process, small molecular weight P(β-CyD) could make the amount of the subsequent layer deposition decrease. Furthermore, since the initial layers of the multilayer film significantly influence film stability than the later deposition steps, the resulting multilayer film thickness increased only a little when we used P(β-CyD) as the initial layer.

Therefore, we have tried to fabricate a combinative coating in which PAA/P(β-CyD) film deposited after a PAA/PAAm multilayer was initially formed on the magnetic particles (Figure 1(d)). In this case, the larger incremental ζ-potentials from P(β-CyD) were obtained, with the aid of a large ζ-potential from the pre-deposited PAA layer surface, than the direct deposition of PAA/P(β-CyD) coated one.

The magnetic colloidal particles were uniformly coated with this combined multilayer system, and we confirmed that by fluorescent dye labeling technique, which we have used for PAA/PAAm multilayer-coated colloids in the previous study.[22] Figure 2 presents fluorescence microscopy images of PS-COOH colloidal particles coated with FITC dye-labeled [$(PAA/PAAm)_3$+ $(PAA/P(\beta\text{-CyD}))_2$] multilayers.

As shown in Figure 2, the particles are uniformly coated with the H-bonded and electrostatic multilayers. Furthermore, the PEM-coated particles did not coagulate, mainly exist as well-dispersed single particles, which may be due to the repulsive forces from negative charges generated from PAA dominating

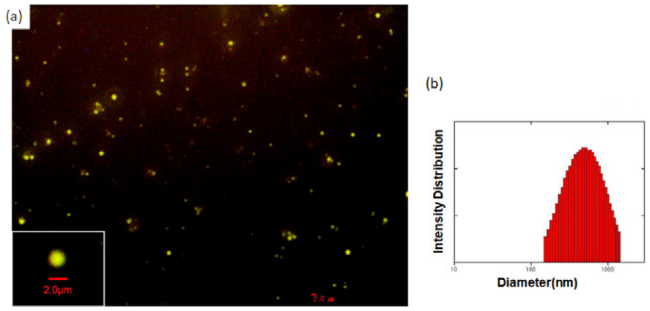


Figure 2. Fluorescence microscope image (a) and size distribution of magnetic colloidal particles coated with [$(PAA/PAAm)_3$ + (PAA/ P(β-CyD))$_2$]. The scale bar is 2 μm.

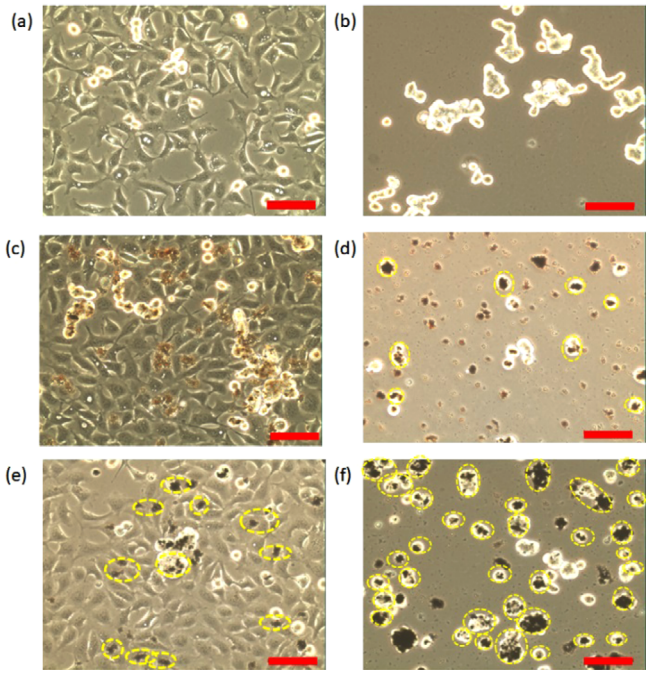


Figure 3. Phase-contrast optical microscope images of HEK293 cells cultured on (a) bare TCPS, (b) $(PAA/PAAm)_3$-coated TCPS, (c) bare TCPS with $(PAA/PAAm)_3$-coated PS-COOH particles, (d) with (PAA/PAAm)$_3$-coated PS-COOH particles on a $(PAA/PAAm)_3$-coated TCPS. 293 cells responded to the RGD-conjugate $(PAA/PAAm)_3$-coated PS-COOH particles on (e) bare TCPS and (f) $(PAA/PAAm)_3$-coated TCPS substrates (scale bar, 100 μm). Yellow circles indicate where the particles are attached to cells.

the multilayer system.

PAA/PAAm H-bonded multilayer coating showed the most excellent cellular adhesion resistance (bio-inert coating effect). We used this film on colloidal particles to check whether we could find the same colloid system. Figure 3 shows the cellular interactions (a) without and (b) with PAA/PAAm coated on a tissue culture grade PS (TCPS) substrate. Without PAA/PAAm coating, HEK293 cells were attached to the substrate and proliferated (Figure 3(a)), while PAA/PAAm thin film exhibits cell adhesion resistance, showing all the cells floating in the media forming spheroids (Figure 3(b)). PAA/PAAm film-coated colloids also did not show strong interaction with cells in terms of adhesion (Figure 3(c)-(d)). However, after we modified the PAA/PAAm film-coated colloids with RGD ligand (an oligopeptide of GRGDSPC, see the method in Supporting) conjugated on the surface of the colloids, they became highly interactive to the cells (Figure 3(e)-(f)). RGD motif is a peptide unit found in adhesive proteins such as fibronectin; therefore, it has been used for cell adhesive surface modification. Especially in the case of PAA/PAAm thin-film coated substrate, the RGD ligand conjugated magnetic particles were attached and engulfed by the floating spheroidal cells. As indicated in yellow circles in Figure 3f and Figure 1S (in Supporting information), most of the spheroidal cells and the magnetic particles are gathered together, showing strong interaction.

These strong cell-magnetic particle interactions were also observed in the case of P(β-CyD)-coated surfaces. Figure 4 shows cellular interactions with magnetic particles having combinative PEM coatings comprised of P(β-CyD) outermost layers. Even

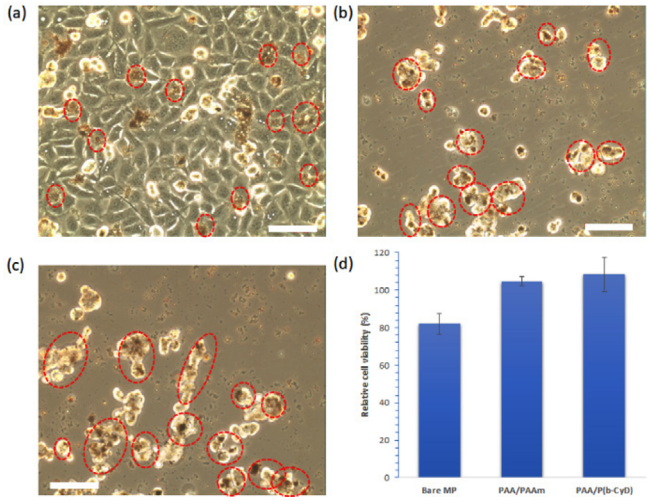


Figure 4. Phase contrast optical microscope images of HEK293 cells with [$(PAA/PAAm)_3$ + $(PAA/ P(\beta\text{-CyD}))_n$]-coated PS-COOH particles. (a) particle coating n=1 for $(PAA/ P(\beta\text{-CyD}))_n$ on bare TCPS, (b) particle coating n=1 for $(PAA/ P(\beta\text{-CyD}))_n$ on $(PAA/PAAm)_3$-coated TCPS, (c) particle coating n=2 for $(PAA/ P(\beta\text{-CyD}))_n$ on $(PAA/PAAm)_3$-coated TCPS substrates (scale bar, 100 μm). (d) MTT assays of HEK293 cells with uncoated and multilayer-coated magnetic particles (TCPS as a standard). Red circles indicate where the particles are attached to cells.

underlying PAA/PAAm multilayer film presents non-adhesive and suitable dispersive property, P(β-CyD) top coating altered the surface as the attractive surface for cellular interaction. The more P(β-CyD) containing surface exhibits, the stronger interactions with cells. Figure 4(c), which has more bilayers (n=2), shows more connected cell aggregates than Figure 4(b) (n=1).

MTT assays of these multilayer-coated magnetic particle samples support that they are non-toxic as the relative cell viability of PAA/PAAm and PAA/P(b-CyD) samples were obtained much better results than bare ones (Figure 4(d)). All MTT data were calculated as relative cell viability (%) compared to the value of TCPS as control. Based on the results, our anti-fouling PAA/PAAm and functional PAA/P(β-CyD) coated magnetic colloids might be a new potent material interacting with living cells without toxicity. They even have versatile modification properties such as introducing bioconjugate like RGD with simple chemistry or delivering bioactive chemicals using an interior pocket of CyD. Therefore, we plan to study the effect of drug incorporated P(β-CyD) containing multilayer in further investigation near future.

In conclusion, PEM films of electrostatic and H-bonded assembly were applied on magnetic particles, and their influence on cell culture was studied. Weak polyelectrolyte multilayers such as PAA/P(β-CyD) and PAA/PAAm were assembled successfully on colloidal particles. The deposited PEM films on their surface properties, such as cellular adhesions, were characterized. PAA/PAAm thin films are cell resistant. Cellular interactions with PEM films changed to an excellent adhesive surface for cell adhesion after P(β-CyD) layers were added to the film. RGD ligand-coated particles generated much stronger specific interaction with cells than the particle without RGD. These functional PEM coatings on magnetic colloidal particles may have a great potential in bio-applications, including targeted intracellular drug delivery.

Supporting information: Experimental procedure for the synthesis of PEM films on magnetic particles is provided. The materials are available *via* the Internet at http://www.springer.com/13233.

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