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Adsorption and separation of amyloid beta aggregates using ferromagnetic nanoparticles coated with charged polymer brushes

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Amyloid beta (Aβ) protein aggregates, which include fibrils and oligomers, are neurotoxic and are considered to cause Alzheimer's disease. Thus, separation of these Aβ aggregates from biological samples is important. Herein, we report the use of strongly ferromagnetic few-layer graphene-coated magnetic nanoparticles (C/Co), which were functionalized with cationic polymer, poly[3-(methacryloyl amino) propyl] trimethylammonium chloride (polyMAPTAC), C/Co@polyMAPTAC, for the adsorption and magnetic separation of Aβ aggregates. Fast adsorption (~ 1 min) of Aβ fibrils and oligomers onto the particles was observed. Interestingly, the Aβ monomer was not captured by the particles, suggesting that binding to Aβ molecules is toxic species-selective. Selective adsorption was also observed in the presence of serum albumin protein. We also showed that C/Co@polyMAPTAC could reduce the cytotoxicity of the Aβ aggregate solutions. This study should be useful for further elucidation of the application of nanoparticle adsorption in mediating Aβ toxicity.

Introduction

Amyloid beta (Aβ) protein is a 39- to 43-amino acid polypeptide that is the primary constituent of senile plaques and cerebrovascular deposits in Alzheimer's disease (AD) [1, 2]. There are six negatively charged residues and three positively charged residues in the peptide, yielding a net charge of -3, with isoelectric point of about 5.5 [3]. This amphiphilic peptide spontaneously forms fibrillar aggregates in aqueous solutions at or below the physiological pH. Self-association occurs faster for the longer, more hydrophobic forms of Aβ (i.e. $Aβ_{1-42}$ versus $Aβ_{1-40}$) [2]. These Aβ fibrils are cytotoxic, and their physiological presence and tissue deposition are associated with AD. Recent studies also indicated that soluble oligomeric Aβ aggregates are more toxic and cause AD [4].

By virtue of their unique physical and structural properties, nanoparticles (NPs) are increasingly suggested for the adsorption and extraction of complex compounds in biomedicine, though there is limited understanding of the relationship between the physicochemical properties of nanomaterial and its interaction with target biological molecules [5, 6]. Magnetic NPs have attracted much attention for application as rapid separation agents to purify or remove target compounds [5, 7]. Various applications such as gold extraction [8], blood purification [9], and water purification [10] have been successfully demonstrated with the aid of various surface modifications (i.e. antibodies, cyclodextrins and polymers).

Much work is being pursued to investigate the interaction between NPs and Aβ, most of which focused on studying the effect of NPs on the Aβ aggregation process: the potential for NPs to inhibit the formation of Aβ amyloid fibrils, to slow down the progress of the disease, or to promote the assembly of proteins in vitro by assisting the nucleation process [11]. For example, gold NPs have been shown to bind Aβ and inhibit aggregation [12]. However, not much attention has been paid to the application of NPs for the removal of toxic proteins such as Aβ aggregates, including oligomers and fibrils.

In this study, strongly magnetic NPs were functionalized with poly [3-(methacryloyl amino) propyl] trimethylammonium chloride (polyMAPTAC) and employed for adsorption and separation of Aβ aggregates. C/Co@polyMAPTAC particles are ferromagnetic carbon-coated cobalt NPs functionalized with a highly charged polymer that is prepared via surface-initiated atom-transfer radical polymerization [13]. Although metal-based nanomagnets usually have high agglomeration tendency in solution, particularly under salt-rich buffered circumstances, polyMAPTAC could efficiently stabilize magnetic particles in buffer solution through the introduction of a highly charged, sterically bulk polymers. Because of the positively charged polymer immobilized on the surface, we anticipated that they

could capture negatively charged Aβ aggregates. Herein, we demonstrated that C/Co@polyMAPTAC could capture Aβ aggregates, including fibrils and oligomers. This allows the separation of Aβ aggregates by the application of a magnetic field. Interestingly, the Aβ monomer was not captured by the NPs, suggesting that the binding of C/Co@polyMAPTAC to the Aβ molecules is toxic species-selective. This selectivity was still observed in the presence of bovine serum albumin (BSA). We also showed that adsorption and separation of the Aβ aggregates could reduce the cytotoxicity of the Aβ aggregate solutions. This study should be useful for further elucidation of the application of NP adsorption in mediating Aβ toxicity.

Materials and methods

Materials

C/Co@polyMAPTAC was synthesized as described previously[13]. Briefly, the carbon-coated cobalt NPs (C/Co) were produced in a one-step process by reducing flame spray synthesis [14], and functionalized with polyMAPTAC via surface initiated atom transfer radical polymerization[13].

$A\beta_{1-42}$ peptide was purchased from Peptide Institute (Osaka, Japan). RPMI 1640 medium was purchased from Sigma (St. Louis, MO, USA). Mouse monoclonal antibodies to Aβ (6E10 and 4G8) were purchased from Abcam (Cambridge, UK). HRP conjugated with anti-mouse immunoglobulin G (IgG) was obtained from R&D Systems (Minneapolis, MN, USA). The enhanced chemiluminescence (ECL) detection kit was purchased from GE Healthcare (Little Chalfont, UK). Cell Proliferation Kit (MTT) was obtained from Roche (Basel, Switzerland).

Aβ monomer, fibril and oligomer preparation

The seed-free Aβ solutions were prepared as described previously [15]. Briefly, the Aβ peptide (1.25 mg/mL) in a 0.1% ammonia solution was ultracentrifuged at 100,000 rpm for 3 h at 4°C to obtain a seed-free Aβ solution, which was referred to as Aβ monomer in this study. Then, the supernatant was collected and stored as aliquots at -80°C. For Aβ fibril formation, the Aβ monomer stock (180 μM) was thawed, diluted with phosphate buffered saline (PBS) to 25 μM, and incubated at 37°C for 48 h. After incubation, the samples were diluted to the appropriate concentrations for the use in the experiments. Aβ oligomers were prepared as described previously with minor modifications [16]. In brief, Aβ stock solution (2 mM) was prepared by dissolving the lyophilized peptide in 100 mM NaOH followed by sonication in water bath for 30 s. The oligomerization reaction was initiated by diluting the stock solution in PBS (45 μM final Aβ concentration), and the mixture is incubated at room temperature overnight. Oligomer formation was confirmed by Native-PAGE/Western blot method as shown below (Fig. 3(a)) and TEM analysis (ESI, Fig. S1), indicating that the size of the produced oligomer is approximately 100-200 kDa in molecular weight and 70 nm in size.

Adsorption and separation of Aβ samples with C/Co@polyMAPTAC

The NPs were dispersed in Milli-Q water and subjected to ultrasonication using UP50H Ultrasonic Processor (Hielscher Ultrasound Technology, Germany) for 1 min before use. This NP dispersion (50 μg/mL) was mixed with 12.5 μM of the Aβ samples (monomers, fibrils and oligomers), and swirled gently for 1 h at room temperature. The particles were collected using a strong $Nd_2Fe_{14}B$ magnet (B~0.5 T) on the tube wall, and the supernatant solution was transferred into a new sample tube for analysis. The extent of adsorption and separation of Aβ samples was estimated from the amount of Aβ sample in the supernatant by dot blot analysis for the monomers and fibrils, and by native-PAGE/Western blot analysis for the oligomers, as described below.

To estimate the adsorption capacity of C/Co@polyMAPTAC for the Aβ samples, 12.5 μM of the Aβ samples (monomers or fibrils) were incubated with particles at various concentrations (1, 5, 10, 20, 30, 50, 75, 150, and 300 μg/mL) for 1 h at room temperature. The Aβ adsorption capacity was calculated based on the minimum amount of particles needed to collect all (> 95%) of the Aβ samples in PBS solution.

For the adsorption kinetics study, a dispersion of particles (50 μg/mL) was incubated with 12.5 μM of the Aβ samples (monomers and fibrils). The suspensions were swirled gently at room temperature for different length of time, and the NPs were removed using a magnet.

The efficiency of Aβ adsorption onto C/Co@polyMAPTAC was calculated based on the difference in the amount of Aβ in the PBS solution before and after adsorption, using the intensities of the dots obtained by the dot blot assay or native PAGE/Western blotting assay as described below. The Aβ solution without mixing with the particle dispersion was used as the 100% control.

The adsorption of Aβ samples (8 μM monomers, oligomers and fibrils) to C/Co@polyMAPTAC (50 μg/mL) was also estimated in the presence of 0.1% (1 mg/mL) BSA. The adsorption of Aβ samples (12.5 μM) to C/Co@polyMAPTAC (50 μg/mL) in phosphate buffer at various pH (6.4, 7.0, 8.0, 9.0) was also examined. The adsorption was quantified using the dot blot assay as described below.

Dot blot assay

The dot blot assay was performed as described previously[15, 17]. Aβ monomers or fibrils (6 μL) were spotted onto nitrocellulose membrane (0.22 μm, Whatman, GE Healthcare). After blocking with 5% skim milk in Tris-buffered salin containing 0.01% Tween20 (0.01% TBST) for 1 h at room temperature, the membrane was incubated with anti-Aβ (6E10, 1:2000) at 4°C overnight, followed by a secondary antibody (1:2000) for 1 h at room temperature. Proteins were visualized using the ECL plus blotting detection system according to the manufacturer's instructions. Luminescence was detected in a LAS4000 mini luminescent Image Analyzer (Fujifilm, Tokyo, Japan), with the

Image Reader Las4000 software. ImageJ software was used for quantification of the dot intensity.

Native-PAGE and Western blotting

The Aβ oligomer samples (5 μL) from the supernatant after magnet separation were mixed with 5 μL native-PAGE sample buffer and then applied to native-PAGE using a Tris-glycine 10–20% gradient precast gel (Wako, Osaka, Japan). A HMW native marker kit (GE Healthcare) comprising thyroglobulin (669 kDa), ferritin (440 kDa), catalase (232 kDa), lactate dehydrogenase (140 kDa) and albumin (66 kDa) was used as a molecular mass marker. The Aβ monomer alone was also subjected to this test as a control. Following transfer to the nitrocellulose membrane, the membrane was blocked overnight at 4°C with 5% skim milk in 0.01% TBST. After washing using 0.01% TBST, the membrane was incubated with anti-Aβ (4G8, 1:2000), followed by incubation with the secondary antibody as described above. The proteins were visualized as described above.

Transmission electron microscopy (TEM)

The Aβ samples mixed with C/Co@polyMAPTAC were dropped onto a carbon-coated TEM grid, and the grid was allowed to air-dry. Samarium acetate (2.5%) was used to stain the Aβ oligomer sample. Samples were observed at an excitation voltage of 80 kV using a JEM-1230 transmission electron microscope (JEOL, Tokyo, Japan).

Cytotoxicity assay

Rat PC12 cells (ATCC, Manassas, VA) were cultured on poly-D-lysine-coated dishes in RPMI 1640 medium (Sigma) containing 10% heat-inactivated horse serum, 5% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified 5% CO_2 incubator at 37°C. The medium was replaced every 3 days.

The viability of the PC12 cells was determined using the Cell Proliferation Kit I (MTT) [18] according to the manufacturer's instructions. The PC12 cells (40,000/well in 80 μL culture medium) were cultured overnight in 96-well plates coated with poly-D-lysine. Aβ samples (12.5 μM monomers, oligomers and fibrils) were incubated with 50 μg/mL C/Co@polyMAPTAC for 1 h, and the supernatants after magnetic separation were used as Aβ samples with particle treatment. Then Aβ samples (0.5 μM) were added to the cells, and the cells were incubated at 37°C for 24 h. For the measurements, the absorption values at 550 nm were determined using a Tecan microplate reader (Männedorf, Switzerland). The viability of the cells exposed to PBS was used as the 100% viability control.

Results and discussion

Adsorption and separation of Aβ fibrils by C/Co@polyMAPTAC

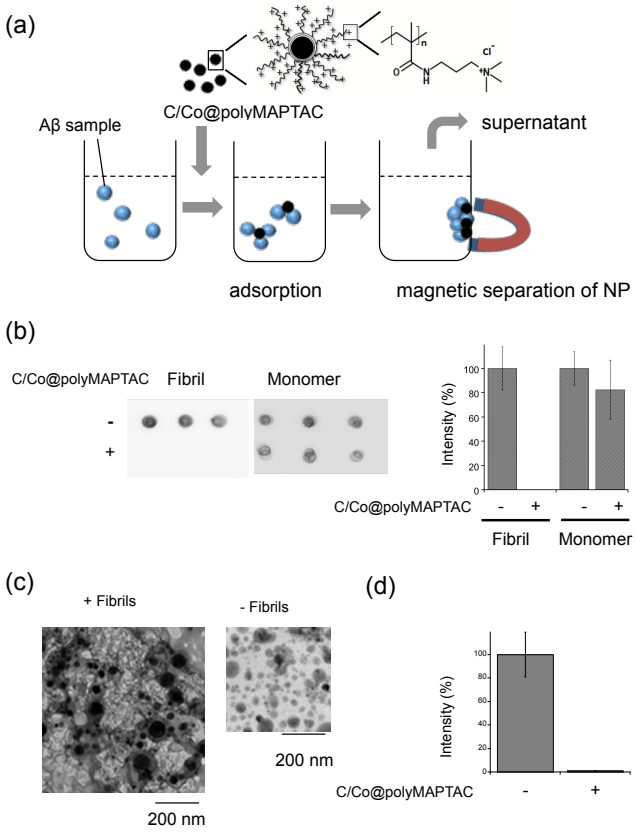

Figure 1 (a) Adsorption and separation of Aβ aggregates with C/Co@polyMAPTAC. (b) Dot blot assay to estimate the selective separation of the Aβ fibrils by C/Co@polyMAPTAC (Left). After incubation of 12.5 μM Aβ samples (fibrils and monomers) with particles (50 μg/mL) and following magnetic separation, supernatants (6 μL) were spotted on the membrane (three spots). Proteins were stained with an anti-Aβ antibody. The relative intensity indicates the amount of fibrils and monomers left in the supernatant after magnetic separation (right). The intensities of the samples without particles were normalized to 100%. (c) TEM images of the Aβ fibrils incubated with C/Co@polyMAPTAC (left) and. C/Co@polyMAPTAC only sample (right, Tecnai F30 ST, FEI, 300 kV). The scale bar = 200 nm. (d) Adsorption and separation of Aβ fibrils with C/Co@polyMAPTAC in cell medium analyzed by dot blot assay. The intensities of the samples without particles were normalized to 100%.

The adsorption and separation of the Aβ fibrils by C/Co@polyMAPTAC was examined by detecting the amount of Aβ sample in the supernatant by dot blot assay using anti-Aβ (Fig. 1a) after magnetic separation of the particles. The Aβ sample solutions (monomers or fibrils) were incubated with 50 μg/mL particles. After collecting the NPs using the magnet, the supernatant was applied to the nitrocellulose membranes for dot blot assay. As shown in Fig. 1b, no fibrils were left in the supernatant, indicating that the particles could successfully adsorb and separate Aβ fibrils. Interestingly, the amount of Aβ monomers did not decrease after they were mixed with the particles, suggesting selective binding of C/Co@polyMAPTAC to the fibrils. The TEM image (Fig. 1c) also supported the interaction between the particles and the fibrils, as densely bound particles were found on the fibrils. This would explain

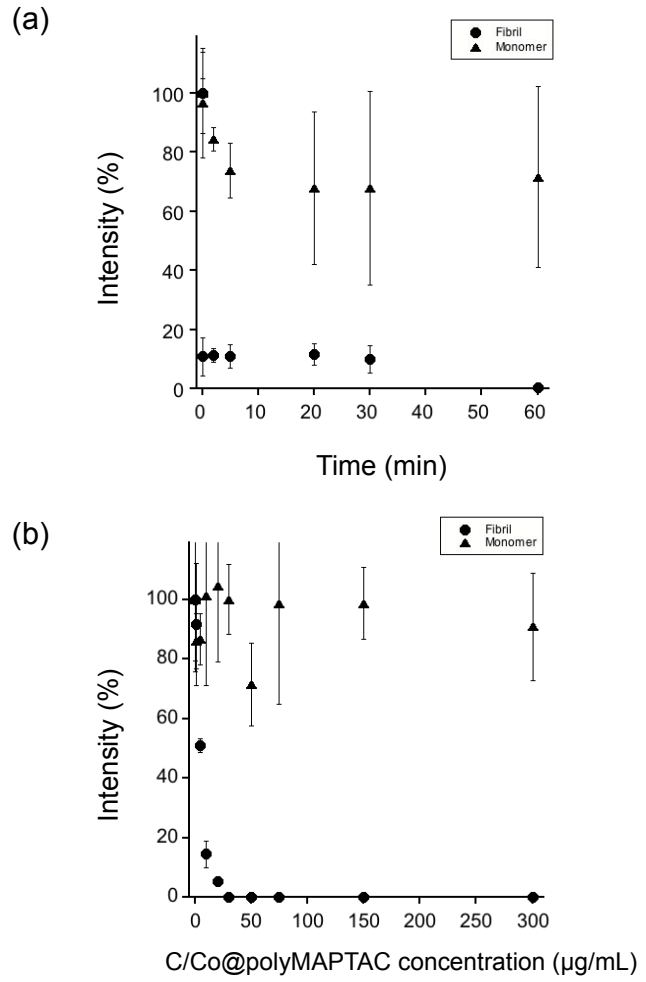

Figure 2 Adsorption efficiency of Aβ fibrils and monomers onto C/Co@polyMAPTAC. (a) Time course assay. Relative intensity obtained by dot blot assay against the supernatant after various incubation times (from 0 to 60 min) with particles (50 μg/mL) and following magnetic separation. (b) Relative intensity obtained by dot blot assay against the supernatant of Aβ samples incubated with various amounts of particles (from 0 to 300 μg/mL) for 1 h and following magnetic separation. The intensities of the samples without C/Co@polyMAPTAC were normalized to 100%.

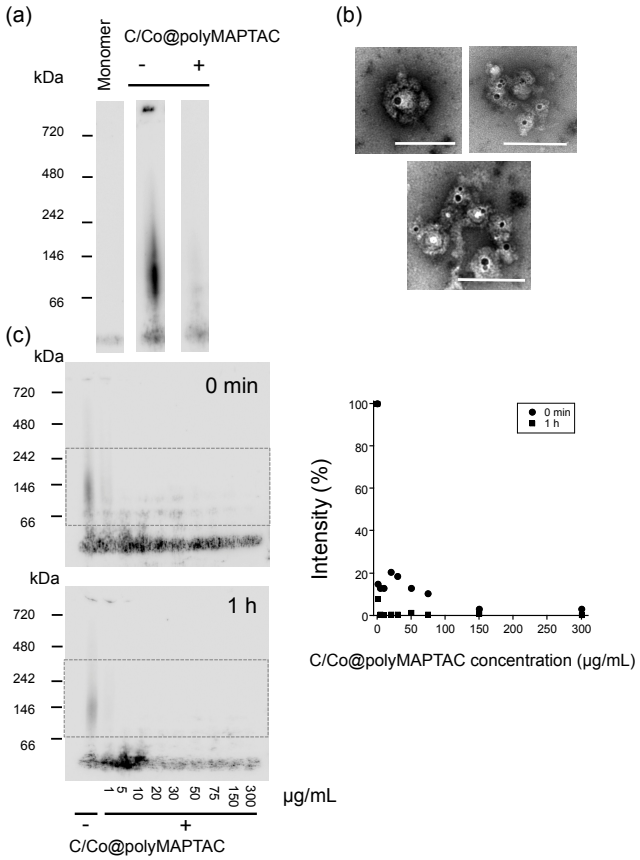

Figure 3 (a) Native PAGE/Western blot analysis of adsorption and separation of Aβ oligomers by C/Co@polyMAPTAC. The Aβ oligomer (12.5 μM) was incubated with C/Co@polyMAPTAC (50 μg/mL) for 1 h. The supernatant after magnetic separation was subjected to the analysis. (b) Transmission electron micrograph of Aβ oligomers incubated with particles (black dots). The scale bar = 200 nm. (c) Adsorption efficiency of Aβ oligomers to particles. The Aβ oligomers were incubated with increasing amounts of NPs (from 0 to 300 μg/mL) for less than 1 min (left, upper)) and for 1 h (left, bottom). The relative intensities (0 min (circle) and 1 h (square)) were obtained from the membrane image (square area) using ImageJ software. The intensities of the samples without NP were normalized to 100%.

how fibrils are collected from the solution by application of C/Co@polyMAPTAC. The binding of C/Co@polyMAPTAC to the $A\beta_{1-40}$ fibrils was also confirmed by dot blot assay and STEM analysis (ESI, Fig. S2a), indicating that the particles can bind both $A\beta_{1-40}$ and $A\beta_{1-42}$ fibrils. Energy dispersive X-ray spectroscopy (EDX) analysis also indicates the presence of cobalt only in the round black spots bound on the $A\beta_{1-40}$ fibrils. This further confirms the binding of the particles to the fibrils (ESI, Fig. S2b).

Characterization of the binding of C/Co@polyMAPTAC to Aβ fibrils

The adsorption efficiency of C/Co@polyMAPTAC to Aβ fibrils was estimated by incubating the Aβ samples with particles at different time durations (from 0 min (several seconds for mixing) to 60 min) (Fig. 2a). A rapid process of fibril adsorption onto NP was observed, which reached approximately 85% adsorption within 5 min and 100% within 60 min. This result suggests that it required a relatively short time to achieve equilibrium with high adsorption efficiency. In contrast, less than 25% adsorption of the monomer was observed within 60 min, which is consistent with the previous result (Fig. 1b). The adsorption of fibrils (12.5 μM) onto particles (50 μg/mL) in the cell medium (D-MEM cell medium with 10% FBS) was confirmed by dot blot assay (Fig. 1d), suggesting that the capture of fibrils by C/Co@polyMAPTAC occurs under physiological conditions.

The adsorption capacity of C/Co@polyMAPTAC towards Aβ fibrils was estimated by examining the effect of dosage of particles (0 to 300 μg/mL) on the adsorption of a fixed amount of Aβ fibrils (12.5 μM). As shown in Fig. 2b, the amount of adsorbed fibrils increased with increasing particle concentration. The fibril removal efficiency reached 95% after adding 30

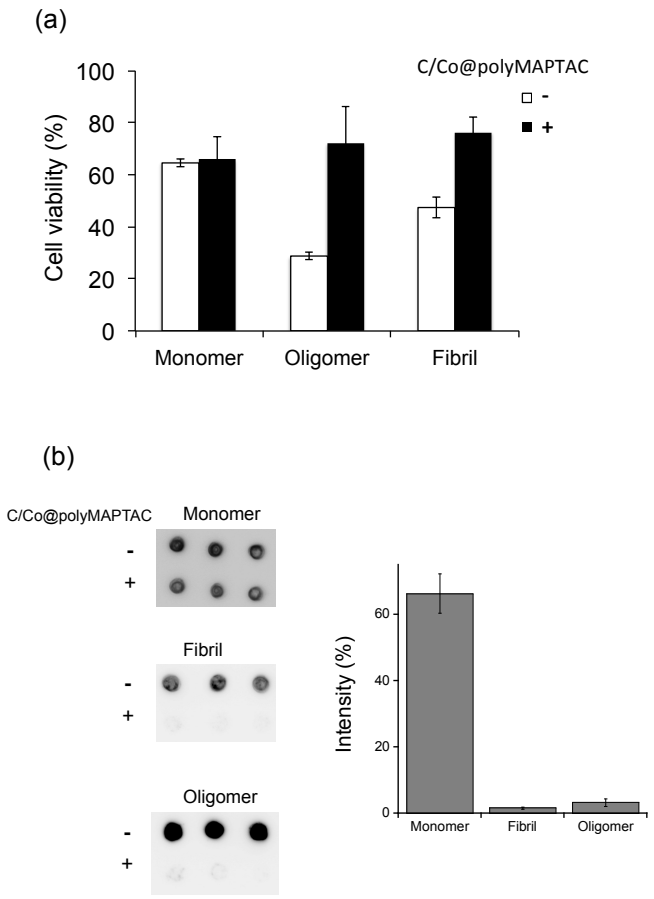

Figure 4 (a) Cytotoxicity of Aβ oligomer and fibril solutions before and after C/Co@polyMAPTAC treatment. Aβ samples (0.5 μM monomers, oligomers and fibrils) with or without particle treatment (50 μg/mL, 1 h) were added to rat PC12 cells. Cell viability was measured using the MTT method. (b) Adsorption of Aβ samples onto C/Co@polyMAPTAC in the presence of BSA. Aβ samples (8 μM monomers, oligomers and fibrils) were incubated with C/Co@polyMAPTAC (50 μg/mL) in the presence of 0.1% (1 mg/mL) BSA. The adsorption was quantified using the dot blot assay.

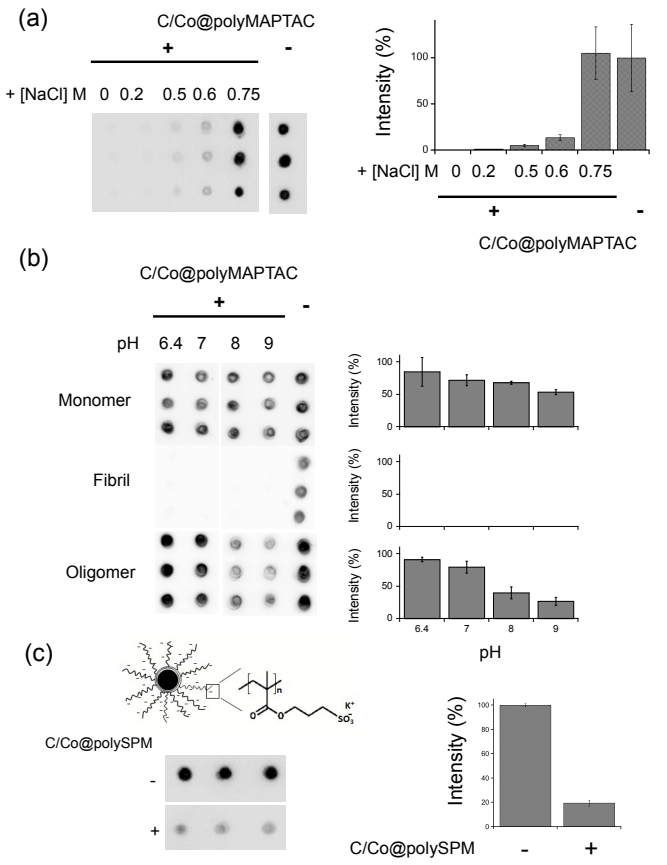

Figure 5 (a) Adsorption of C/Co@polyMAPTAC onto Aβ fibrils at different salt concentrations. Aβ fibrils (12.5 μM) were incubated with NPs (50 μg/mL) in PBS buffer with additional salt (from 0 to 0.75 M NaCl) for 1 h. The supernatants after magnetic separation were subjected to dot blot assay. The relative intensities were shown as a bar graph (right). The intensities of the samples without particles were normalized to 100%. (b) Adsorption of Aβ samples to C/Co@polyMAPTAC at various pH (6.4, 7.0, 8.0, 9.0). Aβ samples (12.5 μM monomers, fibrils and oligomers) were incubated with NPs (50 μg/mL) in phosphate buffer at various pH (6.4, 7.0, 8.0, 9.0). The adsorption was quantified using the dot blot assay as described above. The relative intensities were shown as a bar graph (right). The intensities of the samples without particles were normalized to 100%. (c) Adsorption of the Aβ fibrils to the negatively charged magnetic NP, C/Co@polySPM. Aβ fibrils (12.5 μM) were incubated with C/Co@polySPM (100 μg/mL) for 1 h. The amount of unbound Aβ fibrils in the supernatants was analyzed by dot blot assay (left). The intensity of the sample without NPs treatment was normalized to 100%.

μg/mL adsorbent. When the particle concentration was above 50 μg/mL, no fibrils were left in the supernatant after incubation for 1 h. The weight ratio between the maximum the adsorbed fibril and C/Co@polyMAPTAC is calculated to be around 2:1. In contrast, Aβ monomer showed no significant decrease in adsorption with increasing NP dose (Fig. 2b); less than 20% of the Aβ monomer was associated with particles, which is also consistent with our previous result, showing that Aβ monomers do not interact significantly with C/Co@polyMAPTAC.

Characterization of Aβ oligomer adsorption

Recent studies revealed that Aβ oligomers are considered to cause AD [4], and hence it is important to remove Aβ oligomers. Characterization of Aβ oligomer adsorption was carried out by native-PAGE/Western blot method because the prepared soluble oligomer was actually a mixture of monomers, oligomers and a small amount of fibrils. As shown in Fig. 3a, soluble oligomers were clearly removed by incubation with C/Co@polyMAPTAC, while the monomer was left in the supernatant. It is notable that fibrils, which were observed at the well of the gel, were also removed, supporting the previous result (Fig. 1). These results indicate that oligomer and fibril could be selectively extracted by the particles. TEM observation also supported the association of particles with the Aβ oligomers (Fig. 3b). Interestingly, the number of bound NP to Aβ oligomers was smaller compared to that to Aβ fibrils (Fig. 1c), suggesting that binding of NP to Aβ oligomers is more efficient.

To further study the adsorption of Aβ oligomers onto C/Co@polyMAPTAC, Aβ oligomers (12.5 μM) were incubated

with various amounts of NPs (0 to 300 μg/mL), and the supernatant after magnet separation was applied to the native PAGE followed by Western blot assay. As shown in Fig.3c, soluble oligomer species were reduced to 15% in less than 1 min by a small amount of particles (1 μg/mL). After 1 h incubation, the Aβ oligomers were completely removed by 5 μg/mL C/Co@polyMAPTAC. The weight ratio between the maximum adsorbed oligomer and particles is calculated to be around 10:1 after 1 h incubation, which is 5 times higher than that for fibril adsorption, supporting more efficient binding of NP to oligomers.

Affinity of C/Co@polyMAPTAC to Aβ aggregates (fibrils and oligomers) was estimated using adsorption ratio (%) on various NP concentrations (ESI, Fig. S3). The obtained affinities (K_D= 0.87 μM for fibrils and 12 nM for oligomers) also support stronger affinity to oligomers.

Cytotoxicity of Aβ aggregates solution was reduced with C/Co@polyMAPTAC

Aβ aggregates, including soluble oligomers and fibrils, have been identified as toxic species [19]. Herein, we examined whether the cytotoxicity of these Aβ aggregates solution could be reduced by particle treatment. As shown in Fig. 4a, cell viability after the addition of Aβ aggregates solution increased significantly upon particle treatment, indicating reduced cytotoxicity. This was because both Aβ oligomers and fibrils were removed from the supernatant by adsorption on C/Co@polyMAPTAC. In contrast, no change of cytotoxicity in the Aβ monomer was observed, consistent with the weak-adsorption of the particles for the Aβ monomer. This result also implies that there is a possible medical application of this material in the removal of toxic amyloid aggregates from biological samples.

Selective adsorption of Aβ aggregates in the presence of BSA

To further confirm feasibility of C/Co@polyMAPTAC in bio-application, the adsorption of Aβ samples in the presence of BSA was estimated. BSA is negatively charged in PBS (similar isoelectric point with human serum albumin (pI=4.6~4.9) [20]). As shown in Fig. 4b, more than 98% of oligomers and fibrils were separated from the solution including 0.1% BSA, while more than 60% monomers were left. This experiment supports the selective adsorption of C/Co@polyMAPTAC to the toxic Aβ aggregates even in the competitive biological components such as BSA.

Adsorption mechanism of C/Co@polyMAPTAC for Aβ aggregates

In this study, strongly magnetic carbon-coated cobalt NPs that were functionalized with positively charged polymers were employed to capture Aβ aggregates. We anticipated that the charge interaction between the particles and the negatively charged Aβ aggregates is important. To confirm this idea, fibril adsorption in the presence of salt was examined. Various amounts of NaCl (200, 500 and 750 mM) were added to the reaction buffer, and the amount of unbound Aβ fibrils in the supernatant was estimated (Fig. 5a). As shown in the figure, the binding of C/Co@polyMAPTAC to the Aβ fibrils decreased in the presence of higher concentrations of salt, confirming that electrostatic interaction is important for adsorption.

The adsorption of Aβ samples to C/Co@polyMAPTAC at different pH (6.4, 7, 8, 9) was also examined (Fig. 5b). The result showed that Aβ fibrils were mostly eliminated from the solution at all pH values, while more than 80% of Aβ monomers remained in the solution, supporting the selective adsorption of Aβ fibrils to C/Co@polyMAPTAC at various pH. Interestingly, more monomers were captured at higher pHs, possibly due to the increasing negative surface charge of monomers with the more basic environment. The Aβ oligomers were also captured more at higher pH. These results support the importance of electrostatics interaction for adsorption.

Next, we determined whether the same type of magnetic NPs, functionalized with a negatively charged polymer, poly(3-sulfopropylmethacrylate) (C/Co@polySPM) [21], could also capture Aβ fibrils. The Aβ fibrils (12.5 μM) were incubated with 50 μg/mL C/Co@polySPM for 1 h at room temperature; the amount of Aβ fibrils in the supernatant was estimated as described above. Interestingly, negatively charged NP could also capture Aβ fibrils (Fig. 5c). Aβ oligomers could also be adsorbed (ESI Fig. S4). These results imply that Aβ aggregates could be captured by NPs coated with both positively and negatively charged polymer brushes. A possible explanation is that locally distributed charge in the Aβ aggregates could be crucial for adsorption to the charged NP. Moores et al. demonstrated that there are strongly charged regions in Aβ aggregates [22]. The β-sheet structure of the Aβ monomer has a positively charged region on either side, and the negatively charged regions are dispersed through the remainder of the peptide. In contrast, when several β-sheet monomers are stacked together, strongly positively/negatively charged regions, were formed within the aggregate, creating a quadrupole moment [22]. Therefore, it is plausible that Aβ aggregates with stacked β-sheet structures could be captured by both positively and negatively charged polymer surfaces. This would also explain that Aβ monomer binding to NP is less efficient because of the weaker charge distribution.

Conclusions

In conclusion, we demonstrated the use of strongly magnetic few-layer graphene-coated cobalt NPs functionalized with a charged polymer, polyMAPTAC, for the adsorption and extraction of toxic Aβ aggregates, including fibrils and oligomers. Interestingly, the Aβ monomer was not captured by C/Co@polyMAPTAC, suggesting that binding of the particles to the Aβ molecules is toxic species-selective. The selectivity was also observed in the presence of competitive proteins such as BSA. We also showed that the particles reduce the

cytotoxicity of the Aβ aggregate solutions. This study indicates the potential application of ferromagnetic nanomaterials in disease diagnosis as well as treatment of diseases by finding and removing these toxic species from biological fluid such as blood and dialysis buffer.

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Notes and references

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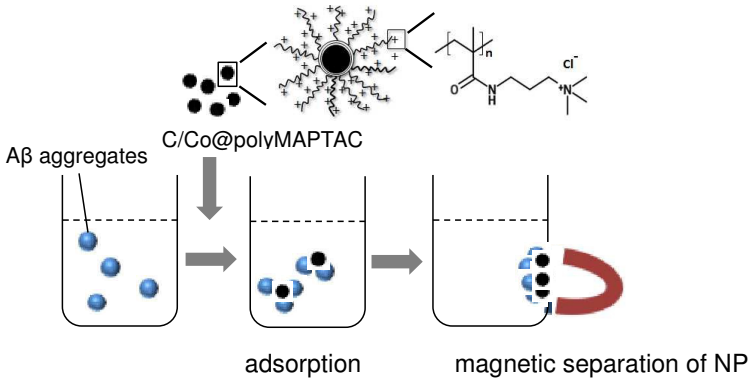

Adsorption and separation of toxic Aß aggregates (fibrils and oligomers) using ferromagnetic nanoparticles functionalized with cationic polymer (C/Co@polyMAPTAC) was demonstrated.