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Design of Size-Tunable Molecularly Imprinted Polymer for Selective Adsorption of Pharmaceuticals and Biomolecules

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Abstract

The tunable and nano-sized materials have recently been received much attention due to the unique physical and chemical properties which are different from bulk materials. Moleculary imprinted polymer (MIP) nanoparticles with special functions provide opportunities for transformative approaches for a wide of variety applications such as imaging, adsorbent, therapeutic reagents, drug delivery vehicles, sensors, toxin neutralization and enzyme inhibition. In this review, the principle, fabrication approaches, parameters to affect affinity and selectivity of MIP were summarized. The application of MIPs for removal of pollutants as well as the separation of macrobiomolecules was also introduced. Through the tuning processes, MIPs can satisfy the needs of simple, fast, cost-effective and robust. In addition, MIPs are easily to be integrated with the diagnosis and separation system, which have instinct advantages in separation, drug delivery, therapeutic and diagnosis fields. Contents shown in this review clearly show that MIPs are highly potential materials for facile design for not only separating the molecules from small molecules to biomolecules by precipitation polymerization but can also be used for imaging, cell tissue engineering and antidotes.

Keywords: Molecularly imprinted polymers (MIPs); Polymerization; Imprinting; Separation; Drug delivery

Introduction

Polymer materials have been explored for decades and applied in a wide variety of fields such as environment, energy and biomedical purposes. They have many properties such as easy to synthesis, low cost, facile incorporation functional group into resultant polymer and robustness. Recently, the synthesis and exploitation of polymer nanomaterials with novel functionality have been paid much attention in many fields including physics, chemistry, biology, biomedical, pharmaceutical and engineering. Nanomaterials often have unique physical and chemical properties which are different from those of bulk materials. In addition, nanomaterials possess extremely high surface-to-volume ratios which can afford a high capacity for a wide of applications such as adsorption, drug delivery, energy storage and support.

Among the polymeric materials developed, molecularly imprinted polymers (MIPs) are one of the most attractive materials for bioanalytical and biomedical applications. Molecular imprinting technology (MIT) is a promising method to fabricate polymer receptors with nano-size. Although the technique is not very new [1], MIT has been paid much attention in recent years because of the importance of MIPs in novel biomedical applications. Figure 1 shows the fundamental concept of molecularly imprinting process [2]. The print molecule (template) is mixed with the functional monomer selected to interact with specific functionality of the template in step (i). Generally, the template is used to guide the assembly of functional monomers. The template-monomer complex may be formed by non-covalent or covalent associations in step (ii). The complex polymerizes with an excess of cross-linking monomer after adding the initiator in step (iii). A polymerization reaction is then employed to fix the pre-assembled binding groups around the template. Ethylene glycol dimethacrylate (EGDMA) or divinylbenzene (DVB) are commonly used cross-linking monomer. In step (iv), the template is removed from the resultant polymer by solvent extraction or chemical treatment. The polymer retains a memory for the original template. Imprinted materials can be considered as analogues to enzyme in that binding sites are constrained by three-dimensional scaffold, contains oriented functional groups and can only accommodate guest molecules that fit closely within the cavities [3]. Since the MIT can be tunable to fabricate a wide variety of MIPs for different purposes, this review would focus on the introduction to the principle of MIT, approaches to fabricate MIP nanoparticles, parameters to affect affinity and selectivity, and the selection of template. Finally, the application of MIPs for removal of pollutants as well as the separation of macro biomolecules would also be discussed.

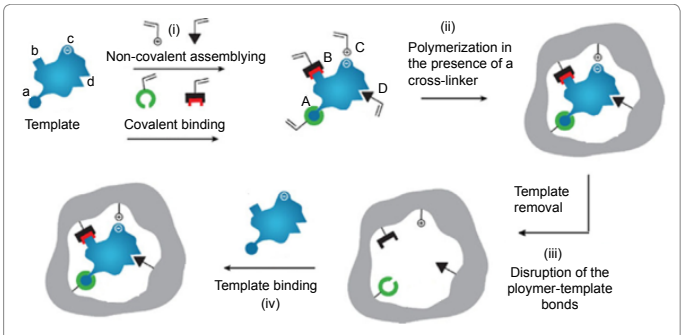

Figure 1: Illustration of molecular imprinting technology processes [2].

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Strategies of imprinting

Functional monomers interact with the template in the solutions. The interaction between template and the imprinting polymer includes covalent bonding, non-covalent bonding, and semi-covalent imprinting. Table 1 represents the advantages and disadvantages of imprinting method.

Covalent imprinting: Wulff and his colleagues reported the first covalent imprinting in 1977 [4]. One of the keys factors for successful producing covalent imprinting is the choice of the covalent linkage which connects the functional monomer with a template. These linkages must be stable and reversible. They must be sufficiently stable and be kept intact during the polymerization, but should be easily cleaved later under mild conditions without damaging the imprinting effects [5]. The commonly used linkages include boronic acid esters, acetals, ketals, Schiff bases, disulfide bonds and coordination bonds [6].

Non-covalent imprinting: The non-covalent imprinting method was first reported by Masbach and his colleagues which showed that covalent linkages between functional monomers and templates were not necessarily required for molecular imprinting [7,8]. The interactions include hydrogen-bonding, electrostatic, apolar, hydrophobic and other non-covalent bonding [9,10]. The major interaction in non-covalent imprinting is hydrogen-bonding.

Semi-covalent imprinting: Whitcombe et al., (1995) have first reported the semi-covalent imprinting in 1995 [11]. The method combines the advantage of covalent bond with anyone of non-covalent bonds. This method involves the covalent attachment during the polymerization and hydrogen-bond formation during the recognition, which could overcome the disadvantages of covalent imprinting.

The polymerization method

Traditionally, MIPs are prepared by bulk polymerization. This method is, by far, the most widely used for imprinting because of its simplicity and universality. The bulk polymerization is processed by mixing all the components including functional monomer, cross-linker, template and initiator together and then initiates the polymerization reaction by irradiation or by heat. The resultant polymer monoliths are required to be crushed, ground, and sieved to a suitable particle size for the particular purposes such as chromatography and support. However, after these treatments, the morphology of particles is irregular and the binding sites may be crushed to lose the recognition ability. Besides that, there are many disadvantages for bulk polymerization. First of all, bulk polymerization cannot be prepared safely on more than 100 g due to the exothermic reaction during the polymerization. In addition, polymer particles fabricated in bulk polymerization are wasted up to 70% during the processes of grinding and sieving [12]. Furthermore, timing consuming and labor intense are drawbacks. Besides these disadvantages, bulk polymerization has limitation to prepare nano-sized MIPs. Therefore, several polymerization methods including precipitation, core-shell approaches and emulsion polymerization have been developed to fabricate MIP nanoparticles [13-31].

Items	Covalent	Non-covalent	Semi-covalent
Synthesis of monomer-template conjugate	necessary	unnecessary	necessary
Polymerization conditions	rather free	Restricted	rather free
Removal of template after polymerization	difficult	Easy	difficult
Guest – binding and guest-release	slow	Fast	Fast
Structure of guest-binding site	clearer	Less clear	Less clear

Table 1: Advantages and disadvantages of imprinting method [5].

Precipitation polymerization: This synthesis procedure of precipitation polymerization is based on the method developed by Ye et al., [13]. The particle size synthesized by precipitation polymerization from very dilute monomer solutions is submicron scale (0.3-10 μm). Phase separation occurs during the cross-linking process when the polymeric chains grow more and more insoluble in an organic or aqueous medium. The point of phase separation depends on the nature of MIPs, volume of porogen used and amount of cross-linker employed. In more dilute reaction system, it forms dispersion of macrogel particles in the solvent [14]. It has the advantages that the particle size, shape and morphology can be easily controlled and the polymerization can be performed efficiently in good yield. In addition, precipitate polymerization generates uniform microspheres without any stabilizer or complicated operations [15].

Core-shell approaches: Two common methods, emulsion polymerization and grafting approaches, can be used to fabricate core-shell MIP nanoparticles [2]. The emulsion polymerization is two-step method, the formation of monodisperse seed latex and the creation of molecularly imprinted shell by emulsion polymerization. At first, seed latex can be prepared from a large variety of materials, such as styrene, divinylbenzene, alkyl acrylate, and methacrylate ester-based seeds have all been reported [16-18]. In recent years, magnetic cores are exploited as the core material for recoverable purpose [19-21]. The seed latex particles are monodispersed and vary in size from approximately 30 nm to more than 1 μm in diameter. In the second step, the seed is mixed with another the monomer before the polymerization. The size, morphology, physico-chemical properties of the core-shell polymer particles can be controlled by the composition, structure of monomers and the reaction conditions.

Another method to fabricate MIP core-shell nanoparticles is grafting of the MIP membrane on the seed nanoparticles by controlling living radical polymerization. Atom-transfer radical polymerization (ATRP) [22] and reversible addition-fragmentation chain-transfer polymerization (RAFT) [23] are two common approaches by controlled living radical polymerization. ATRP is a method of forming carbon-carbon bond through transition metal catalyst (e.g., Cu) [22]. RAFT polymerization uses thiocarbonylthio compounds such as dithioesters, thiocarbomates and xanthates to mediate the polymerization through reversible chain-transfer process [23]. Whatever ATRP or RAFT polymerization, the catalyst or thiocarbonylthio compounds required to initiate polymerization might disrupt the interaction between the template and functional monomer and have to be removed from the resultant polymer nanoparticles.

Besides these two methods, the simplest method is to deposit MIPs membrane on core surface by two steps. First, the core materials are modified by functional groups which the structures end by double bonds. In second step, the core functionalized by double bond was directly mixed with the polymerization solution, including the functional monomer, the cross-linker, the template and initiator with following polymerization. MIPs are coated on core surface to form core-shell structure. Table 2 summarizes the approaches to fabricate the MIP core-shell nanoparticles.

Emulsion polymerization: Emulsion polymerization, one kind of radical polymerization, starts with an emulsion incorporating water, monomer, and surfactant. Surfactants are required in emulsion polymerization. In an oil-in-water emulsion (also called inverse emulsion), the most common type, the reaction system is characterized by the emulsified monomer droplets (10 nm to 10 m) dispersed in the continuous aqueous phase with the aid of an oil-in-water surfactant at

Core	Template	Results	Ref.
CdSe/ZnS	Uracil	[1] Fabrication of MIPs based sensors [2] The emission intensity increases to several folds when the template binds to the recognition sites of the cross-linked polymeric matrix.	24
Fe3O4/Au	Estradiol (E2)	[1] The magnetic molecularly imprinted polymers were successfully used for detecting E2 in milk power with good sensitivity, selectivity, reproducibility and efficiency. [2] The linear range of the imprinted polymer for E2 was 0.025-10 $\mu molL^{-1}$ with the limit of detection of 2.76 $nmolL^{-1}$	25
Fe_3O_4/SiO_2	Folic acid	[1] The maximum adsorption capacity of molecularly imprinted polymer was 8 mg g^{-1} [2] The recovery were in the range of 95%-104%	26
Fe_3O_4/SiO_2	Clozapine	[1] The magnetic molecularly imprinted based on $Fe_3O_4@SiO_2$ were regenerated and their adsorption capacity in the eighth use showed about an 6.67% loss. [2] The inta and inter-day precision value were less than 6% and 4%, respectively.	27
Fe_3O_4	Pesticides (Chlorpyrifos, Diazinon and Phosalone)	[1] The maximum adsorption capacity for phosalone, diazinon, and chlorpyrifos by Langmuir equation was 196.07, 192.30 and 172.41 mg g^{-1} [2] After four adsorption-desorption cycles, the molecularly imprinted polymers maintained its adsorption capacity without significant loss.	28
Fe_3O_4/SiO_2	Estradiol	[1] Reversible addition fragmentation chain transfer polymerization to comprise iron oxide core, a fluorescent dye layer, and a MIP layer in one system [2] It can be reused for at least up to 5 times without significant loss of magnetic moment and signal intensity	29
Divinylbenzene styrene	Caffeine/(S)-propranolol/ theophlline	[1] Emulsion polymerization [2] K_d: caffeine MIP 200 $\mu mol/dm^{-3}$, (S)-propranolol 30 $\mu mol/dm^{-3}$, theophlline 390 $\mu mol/dm^{-3}$	30

Table 2: Summary of MIPs core-shell materials in recent years.

the beginning of polymerization. The droplets are like mini reactor for the polymerization reaction. The particle size is controlled to an extent by the amount (and type) of surfactant present. In addition, when larger amount of surfactant stabilizes a larger interfacial area, smaller particle size is produced. Other parameters, including the initiator concentration and the ratio of functional monomer phase to the continuous phase, also can be adjusted to control the particle size [31]. However, the addition of surfactants may disturb the complex between functional monomer and the template during the polymerization, especially for non-covalent imprinting.

The synthetic parameters affected sensitivity

Solvents (porogens): The solvent serves first function in dissolving all the components in the polymerization. It serves as the second important function in creating the pores in macroporous polymer [32]. For this reason, it is quite common to refer to the solvent as the "porogen". The solvent can control the morphology and the total pore volume. The porosity and surface area are related with the mass transfer of the substrate. Porogens with low solubility phase separate early and tend to form larger pores and materials with lower surface areas. Conversely, porogens with higher solubility phase separate later in the polymerization providing materials with smaller pore size distributions and greater surface area [33]. In addition, the choice of solvent is more critical in molecular imprinting technology, especially in non-covalent method. The creation of binding sites is corresponding to the formation of the monomer/template complex during pre-polymerization and polymerization steps. The polarity of the porogen affects the formation of the monomer/template complex. Less polar solvents such as chloroform or benzene can facilitate polar non-covalent interaction such as hydrogen bonding or bridging of ionic salts. More polar solvents enhance dissociate the non-covalent interactions in pre-polymer step.

Some studies showed that the solvent optimized when the template binds on MIPs is the same as the porogen used in the polymerization [34,35]. The mechanism of template recognition by MIPs originates from two factors. One is the shapes of templates and another is the spatial positioning of the functional groups in the polymer [36]. A swelling process can affect the distance between the groups and the orientation in the polymer. In order to recreate and maintain these shapes and

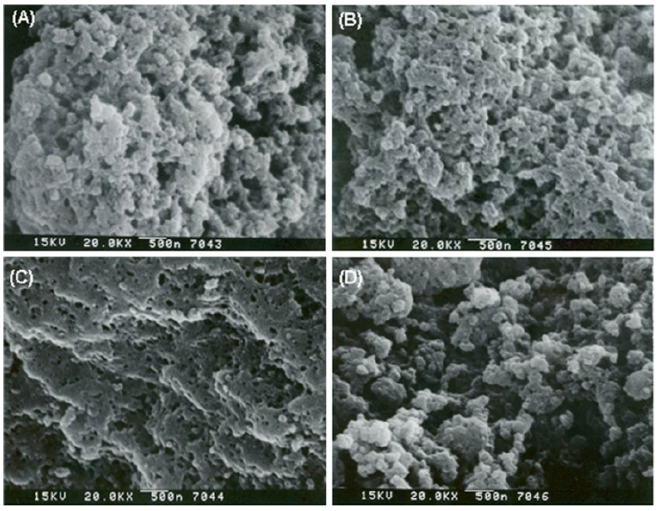

Figure 2: Scanning electron micrographs (SEM) of MIPs prepared in different porogenic solvents. (a) Acetonitrile before the removal of template, (b) acetonitrile after the removal of template, (c) chloroform before the removal of template and (d) chloroform after the removal of template [35].

distance parameters, the optimum rebinding conditions require the same or similar, salvation conditions utilized in polymerization. Lee and Doong have used two porogenic solvents, acetonitrile and chloroform, to prepare MIPs for detection of 17ß-estradiol [35]. After the removal of template by methanol, the MIP structures prepared by both solvent became loose (Figure 2). However, the increase in porosity after the removal of template was also observed, presumably attributed to the removal of template and un-reacted reagents including excess functional monomers, cross-linkers, and linear or branch chain of the functional monomer polymerized with cross-linkers without the connection to network polymers.

Temperature: In principle, MIPs are synthesized by cross-linking complexes of the template and functional monomers. In non-covalent method, the template forms the complex with the functional monomers before the polymerization. The amount of monomer/template complexes is relative to the recognition sites and performance. Temperature plays

an important role in forming monomer/template complexes. The lower temperature polymerization is favorable to the preparation of MIPs based on non-covalent imprinting. High temperature can disrupt the hydrogen bond and electrostatic interaction. Temperature also affects reaction completeness, reaction rates, and polymer structure, such as pore structure and swelling properties. These factors can influent the recognition sites and performance. Lower polymerization temperature is advantageous to stabilize the monomer/template complexes. Higher polymerization temperature is favor to complete the reaction. The affinity and specificity of MIPs are significantly improved by optimizing the polymerization temperature. The temperature also affects the morphology. Pore volume and surface area increase at increasing temperature [37]. The polymer will "memorize" the temperature developed during polymerization [35].

Pressure: Sellergren group shows that the preparation of MIPs at high pressure (1000 bar) can enhance the selectivity [38]. It is because that pressure affects the kinetics of polymerization, the boiling temperature of solvent and the rigidity of polymer. Pressure can affect the kinetics of polymerization reaction which is faster at high pressure as compared with ambient pressure. The boiling temperature of the solvent used as a porogen in the polymerization reaction is affected by the pressure. The polymer made at ambient pressure was more susceptible to the temperature than the polymer at high pressure [39].

The ratio of functional monomer to the template: The formation of the complex between the template and functional monomer with following polymerization is a critical step to create binding sites, especially for non-covalent bond imprinting. The ratio of functional monomer to the template is corresponding to the number of the complex formation. The study shows the ideal ratio to achieve high affinity is 2 to 4 in non-covalent imprinting [33]. Besides, the content of cross-linkers related with hardness of MIPs is concerned due to the maintenance of binding structures.

The selection of the template

Small molecules imprinted polymers: Molecular imprinting has been applied to small molecule templates in the areas of separations [40-42], drug delivery [43,44] and sensors systems [45-47]. In separation purpose, MIPs are usually applied in solid phase extraction [48,49], liquid chromatography [50-52] and capillary electro chromatography [53,54]. This part is most close to practical realization. Companies that produce product based on MIPs include MIP Technologies AB (Lund Sweden), POLYIntell (Val de Reuil, France), MIP Solutions Inc. (Kettle Falls, Washington, USA) and Semorex (Fenwood, NJ, USA). Due to high chemical stability and low toxicity, MIPs have advantages to be utilized for drug delivery and antidote [43,44]. In addition to high compatibility with transducers and long-term stability in harsh conditions, MIPs are proper materials to substitute recognizable elements in sensor systems.

Biomolecularly imprinted polymers: Biomolecular targets such as peptides and proteins are the most successful affinity tools used in both fundamental and applied research, such as diagnostics, purification and therapeutics [55,56]. Nonetheless, they have inherent disadvantages which include poor chemical, physical, long-term stability, batch-to-batch variability and high cost. MIPs are appropriate materials to substitute biomolecules. Table 3 shows the comparison of biomolecules with MIPs [57]. However, imprinting biomolecules is a challengeable work due to the inherent properties of proteins, such as size, complexity and conformational instability.

Traditional molecularly imprinted polymers (for small molecules) tend to be relatively dense networks in order to retain the binding sites created during the polymerization. Generally, the materials have small pore for diffusion of the template into and out of the matrix. This property leads the problem for biomolecules, such as proteins and antibodies. Biomolecules are entrapped in the network after polymerization and cannot be easily removed to create the binding sites. In addition, they cannot diffuse facilely into the MIPs to find the binding sites. Besides, the polymerization conditions employed for small molecules may lead to the different conformation of biomolecules from those found in the natural environment. Therefore, it may create the recognizable sites which are specific to alternate state. Following re-binding step in natural environmental, MIPs showed non-specific bindings to the template. Some studies shows that three most common biomolecules template (bovine serum albumin, lysozyme, and bovine hemoglobin) in the presence of several common monomers (acrylamide, methacrylic acid, aminophenylboronic acid, acrylic acid, and N-isopropylacrylamide) and crosslinkers (N,N'-methylenebisacrylamide and ethylene glycol dimethacrylate) at relevant polymerization concentrations reveal the conformational change detected by circular dichroism [58,59]. Solvent is also an important issue for imprinting biomolecules. Biomolecules are often insoluble or unstable in aprotic organic solvents generally used for small molecule imprinting. These situations lead biomolecules imprinting to less success than small molecule imprinting. Therefore, only less than 2% of published work in the area of biomolecules imprinting (Figure 3).

There are four approaches, including bulk imprinting, particle-based imprinting, surface imprinting and epitope imprinting, to fabricate biomolecules imprinted polymers [57]. The removal template to create recognizable sites is a critical problem for biomolecules due to slow mass transfer of large biomolecules from the interior of polymer network, especially bulk imprinting. Bulk imprinting is the imprint of whole macromolecule during the polymerization. As described in previous sections, the processes are time- and labor-intensive due to the requirement of grind and sieve. Therefore, precipitation and emulsion polymerization are utilized to fabricate MIPs nanoparticles (particle-based imprinting) [60-65]. Surface imprinting is the imprint of the template located at or very near surface of polymer. It can be achieved by either synthesizing a thin film using similar approaches as the bulk imprinting or by attaching the template on the surface of substrates with following polymerization. Core-shell approaches are

Parameters	Biomolecules	MIPs
Binding affinity	High affinity /specific	Varies
Generality	One receptors per analyte	MIPs can be developed for any template
Robustness	Limited stability	Stable in the harsh conditions (pH, temperature, ionic strength, solvents)
Cost	Expensive	Inexpensive
Storage	Days at room temperature	Long term storage without loss in performance (several months to years)
Synthesis/preparation	Time-intensive	Facile
Sensor integration	Poor compatibility with transducer surface	Fully compatible

Table 3: The comparison of biomolecules with MIPs [55].

generally applied for surface imprinting. Surface imprinting facilitates diffusion of biomolecules in and out of MIPs to minimize the template size concerns. Table 4 summarizes current researches in biomolecules imprinting field by surface imprinting [60-65].

Besides surface imprinting, epitope imprinting has the advantage to mitigate the issue about the size of the template. It is the imprint of a short polypeptide during the polymerization and then to recognize the whole polypeptides composed of the certain polypeptides. This technique attempts to more closely mimic the specific interaction between an antibody and antigen. Nishino et al., developed surface imprinting strategy to overcome the mass transfer issue [66]. They immobilized target peptide epitopes on a glass surface then fabricated a polymer thin film on the surface. The thin film harvested by the separation from the glass surface had a high affinity and selectivity for the epitopes and proteins containing the epitopes. The surface imprinting methods solved the mass transfer issue in the target removing process and target rebinding process. However, the materials have limited binding sites due to the surface to volume ratio of the resulting material. Surface imprinting on nanoparticles has intrinsic advantages over bulk counterparts. Nanoparticles (NPs) offer the potential for higher capture capacity and a greater number of end-use applications, such as therapeutic purposes The NP format expands opportunities for applications such as synthetic substitutes for natural antibodies [67-69]. Zeng et al., developed a strategy to create synthetic polymers NPs with antibody-like affinity for a hydrophilic peptide by inverse micro emulsion polymerization [70]. The peptide target was coupled with fatty acid chains of varying chains to orient the imprint peptide at the interface of the water and oil domains during polymerization. The polymer NPs generated by this method had nanomolar affinity and high specificity to target peptides (Table 5).

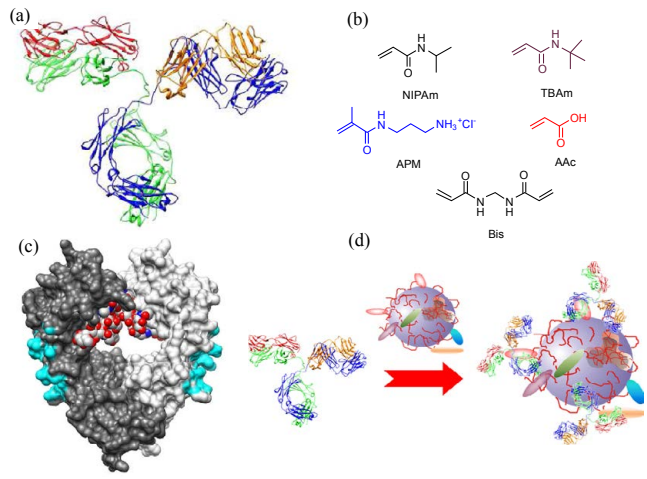

Figure 3: The processes for the preparation of synthetic MIPs for detection of macrobiomolecules of IgG. (a) The structure of IgG with heavy chains in blue and green and light chains in orange and red, (b) monomers used for synthesis of MIP NPs, (c) protein A binding site shown on IgG1–Fc dimer and (d) illustration of detection process of IgG [71].

The engineered synthetic polymer nanoparticles can also be formulated with an intrinsic affinity to a specific domain of a large biomacromolecule. It is noteworthy that the MIPs are usually formed by a kinetically driven process and the sequence of functional monomers in the polymer chain is not controlled. A previous study has used an iterative process to improve affinity by optimizing the composition and proportion of functional monomers [71]. They reported a lightly cross-linked (2%) *N*-isopropyl acrylamide (NIPAm) synthetic MIPs with diameters of 50–65 nm which can incorporate hydrophobic and carboxylate groups that binds with high affinity to the Fc fragment of IgG. In addition, a computational analysis was used to identify potential NP–protein interaction sites. Candidates include a NP binding domain that overlaps with the protein A–Fc binding domain at pH 5.5. The computational analysis supports the inhibition experimental results and is attributed to the difference in the charged state of histidine residues. Affinity of 3.5–8.5 nm MIPs to the Fc domain at pH 5.5 is comparable to protein A at pH 7.

Applications

MIPs are the tailor-made synthetic receptors with the high ability of recognition and binding of target molecules and have recently been demonstrated as the effective materials for a wide variety of applications including separation, solid phase extraction, drug delivery and sensor fabrication. Lee et al., have prepared the MIP particles by precipitation polymerisation to selectively and rapidly capture acetaminophen, a commonly used analgesic and antipyretic drug [75]. By adjusting the ratio of cross-linker and functional monomer, the particle size of MIPs changed from 177 nm to 2.8 mm when the ratio increased from 0.43 to 12.8 (Figure 4). In addition, the adsorption equilibrium of acetaminophen by MIPs can be reached within the first 30 min because of the surface imprinting characteristics and small particle sizes. In addition, the maximum adsorption capacity of acetaminophen and the adsorption constant, well-fitted by Langmuir equation, were 0.35

Templates	Comments	Ref
Albumin	[1] MIP membrane was coated on QCM chip by UV polymerization [2] Imprinting factor is 8 and MIPs present selectivity.	60
BSA	[1] UV polymerization [2] These polymers exhibited selective recognition of the template relative to competing proteins with up to 2.9 times more BSA adsorbed than either glucose oxidase or bovine hemoglobin	61
Lysozyme	[1] A novel monomer, acryloyl-β-CD, to offered hydrophilic exteriorand hydrophobic cavity, was used to self-assemble with the template protein through hydrogen interaction and hydrophobic interaction. [2] The column packed with lysozyme imprinted silica beads could effectively separate lysozyme from the mixture.	62
Cyt. C	[1] Thermal polymerization was used in D.I. water. BSA attached to mica surface for imprinting. [2] Imprinting factor is up to 4.1.	63
BHb	[1] Thermal polymerization in PBS on core-shell superparamagnetic NP. NPs can be recovered by external magnetic field. [2] The maximum number of imprinted cavities on the surface was 2.21x 10^{18} g^{-1}.	64
CEA	[1] Novel self-assembled monolayers surface MIP for variety of cancer biomarkers. [2] The CEA assay was both sensitive (detection range 2.5–250 ng/mL) and specific (no cross-reactivity with hemoglobin; no response by a non-imprinted sensor).	65

BHb: Bovine hemoglobin, Cyt C: cytochrome C, CEA: carcinoembryonic antigen, BSA: bovine serum albumin.

Table 4: Summary of biomolecule imprinted polymer by surface imprinting [55,56].

Templates	Comments	Ref
Angiotensin II, SA (octapeptide)	[1] IF affected by pH, ionic strength of PBS and % acetonitrile in aqueous loading solution [2] MIPs column: detection limit of 8 pmol and a response that is linear ($r^2 > 0.99$) over the concentration range 0.4–20 μM.	72,73
15 mer peptide for dengue virus protein	[1] Fabrication of MIP membrane on QCM chip by UV polymerization [2] The thickness is 70 nm. The selectivity for 15-mer peptide and parent protein in patient samples represents.	74
Epitopes for Cyt c, ADH and BSA	[1] C-terminus nonapeptide of Cyt c, ADH and BSA were used as the imprinting peptides. [2] Specificity to target protein. When it changed one amino acid to imprint, it shows no binding	66
Nanopeptide sequence for GPF	[1] A method for the fabrication of synthetic polymer NPs with surface binding sites for hydrophilic peptides by inverse emulsion polymerization [2] By surveying peptides with fatty acid chains of varying length, conditions were found to achieve success peptide imprinting at the droplet interface	71

Cyt C: cytochrome C, BSA: bovine serum albmin, ADH: alchol dehydrogenase, GPF: green fluorescent protein.

Table 5: Summary of biomolecule imprinted polymer by epitope imprinting.

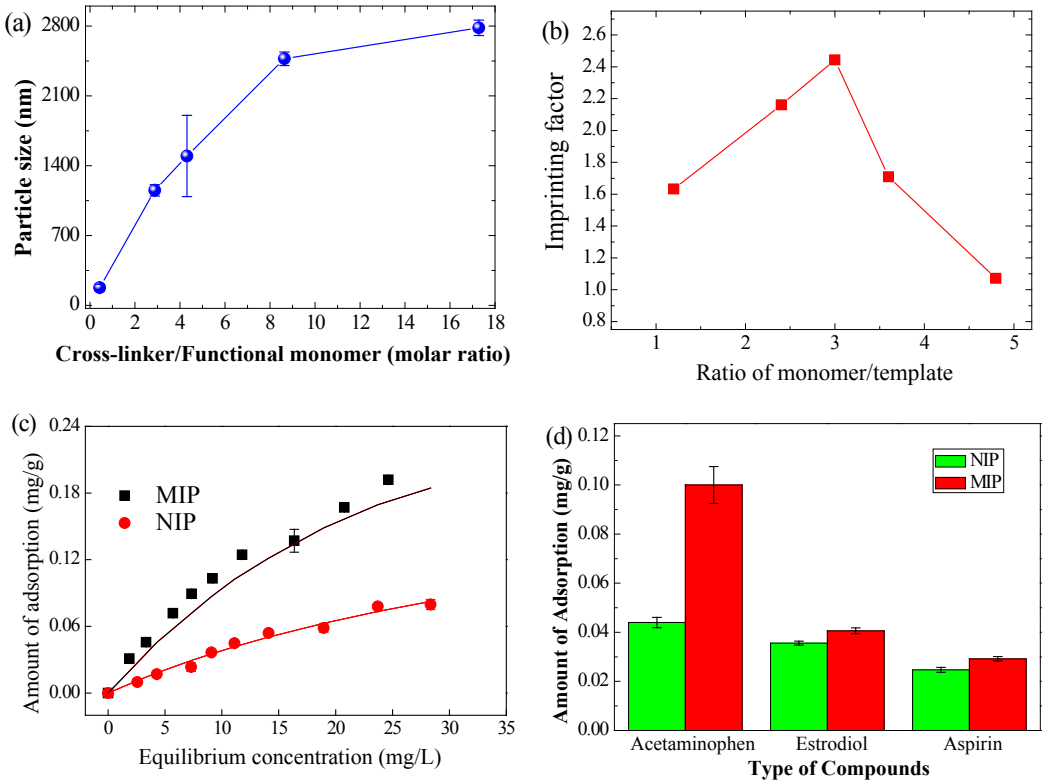

Figure 4: The (a) particle size as a function of C/F ratio of the acetaminophen-imprinted polymers (A-MIPs), (b) the imprinting factor of A-MIPs synthesised at various ratios of functional monomer to template ranging from 1.2 to 4.8, (c) adsorption isotherm of A-MIPs and non-imprinted polymers (NIP) toward acetaminophen adsorption and (d) the selective adsorption of acetaminophen, estradiol and aspirin on A-MIPs and NIPs [75].

mg/g and 0.045 L/mg. In addition, the MIPs exhibited the excellent selectivity to acetaminophen. The MIPs prepared in chloroform also found to have a higher adsorption capacity towards 17ß –estradiol than that in acetonitrile. When using capacity factor values to understand the selectivity of MIPs for similar structures of compounds, the estradiol-templated MIPs show good selective toward the templates and the rebinding ability from selective recognition sites of MIPs decreased in the order 17ß-estradiol >testosterone >benzo[a]pyrene >progesterone >phenol, clearly showing that MIPs can be used to selectively adsorb as well as to separate chemicals with similar structures.

Pang et al., reported a novel system for harvesting cell sheets which relied on poly(*N*-isopropyl-acryamide) (PNIPAAm)-based MIP hydrogel layer with thermo-responsive affinity toward specific biomolecules by redox-initiated polymerization [76]. Molecular imprinting methodology was to introduce the cell-adhesive peptide Arg-Gly-Asp-Ser (RGDS) onto a thermo-responsive cell culture substrate, which was innovatively used as a highly efficient novel system for harvesting cell sheets. With the reversible thermo-responsive specific recognition sites, the imprinted hydrogel could recognize and bind RGDS molecules at a common temperature for cell culture (37°C), and then rapidly release RGDS when the temperature was lowered. The imprinted thermo-responsive hydrogel proved to not only promote cell adhesion during cell culture, but also facilitate cell detachment in cell sheet harvest process. This method solved the long-standing problem in cell sheet technology.

Kunath *et al.,* reported the application of MIP nanoparticles labeled by fluorescent for cell and tissue imaging [77]. Glucuronic acid which is abundant on the surface of cells such as keratinocytes in

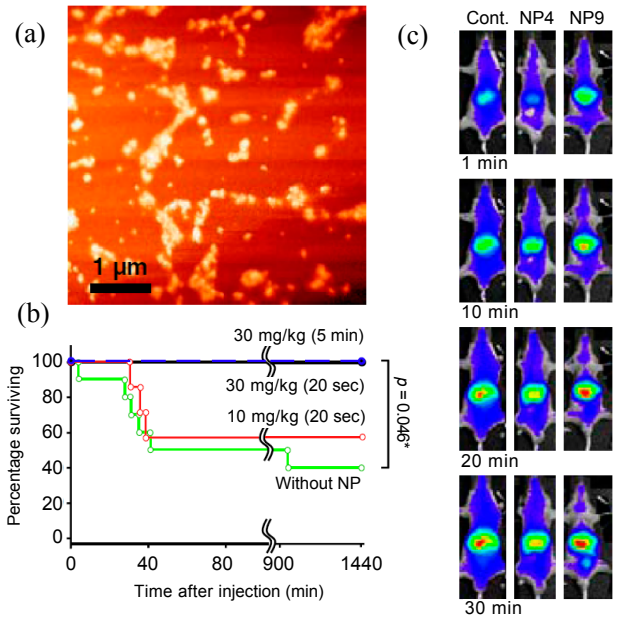

Figure 5: Interaction between melittin and MIP synthesized with various feed ratios of tert-butyl groups (TBAm) and carboxylic acids. (A) AFM image of MIP NPs. (b) Survival rates of mice over a 24 h period after intravenous injection of 4.5 mg kg^{-1} melittin (green). 30 mg kg^{-1} of melittin imprinted NPs (red), not imprinted NPs (gray) was systemically administrated via a tail vain 20 seconds after melittin injection. P Values are calculated by the Wilcoxon test. (c) Fluorescent images of Cy5-melittin (70 min after injection) in livers from mice with various doses of Cy5-melittin and molecularly imprinted nanoparticles [81].

form of hyaluronan as part of the glyocalix was chosen as the target for epitope imprinting. This strategy demonstrated that MIPs as "plastic antibodies" have a great potential for bioimaging and multiple labels are more easily implemented for staining several targets. Panagiotopulou et al., reported a novel photopolymerization method to coat quantum dots (QDs) with molecularly imprinted polymer shells by using the visible light emitted from QDs excited by UV light [78]. Glucuronic and *N*-acetylneuraminic acid were imprinted, respectively, on QDs to recognize hyaluronic acid and sialylated glycoproteins and glycolipids on keratinocytes and demonstrate the multiplexed detection and imaging of glycosylations in cells.

Hoshino et al., designed a novel synthetic NPs that bind and suppress the activity of a toxic peptide. Melittin, a 26 amino acid peptide isolated from bee venom, is a representative of membrane-damaging toxins [67]. It was selected as the target to demonstrate the concept by neutralizing toxin. A small library of *N*-isopropylacrylamide (NIPAm) based copolymer nanoparticles incorporating combinations of functional monomers were prepared by precipitation polymerization. *N*-tert-Butylacrylamide (TBAm), acrylamide (AAm), *N*-(3-aminopropyl) methacrylamide hydrochloride (3-APM) and acrylic acid (AA) were used as hydrophobic, hydrophilic, positively charged and negatively charged functional monomers, respectively. These functional monomers were used to mimic antibodies, like 23 amino acids in nature. In vitro experiment, Hoshino et al., reported that NPs can capture and clear Melittin in bloodstream of living mice. High binding capacity and selectivity of NPs comparable to nature antibodies were achieved by combining a functional monomer optimization strategy and imprinting process. The mortality and peripheral toxic symptoms of melittin were significantly diminished and the injection of NPs can increase survival rate. The melittin·MIPNP complexes are then cleared from the blood by the mononuclear phagocytic system in the liver (Figure 5) [79-81]. These results provide a platform to design plastic antidotes and reveal the potential and possible limitations of using synthetic polymer nanoparticles as plastic antidotes.

Conclusions

In this review, we have given a short introduction from the development of MIPs to the applications in selective adsorption of small molecules of pharmaceuticals and separation of macromolecules. MIPs have been developed to satisfy the need for simple, fast, cost-effective and robust purposes. In addition, MIPs are easily to be integrated with the diagnosis and separation system. It is obvious that MIPs (artificial antibodies) have instinct advantages in separation, drug delivery, therapeutic and diagnosis fields. MIPs show high potential not only in these areas but also in imaging, cell tissue engineering and antidotes. We believe that molecular imprinting is a promising technique in the future.

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