

Molecularly imprinted beads by surface imprinting

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Abstract Molecular imprinting is a state-of-the-art technique for imparting molecular recognition properties to a synthetic polymeric matrix. Conventionally, the technique is easily carried out using bulk imprinting, where molecularly imprinted polymers (MIPs) are prepared in large chunks and post-treatment processes like grinding and sieving are then required. However, this strategy tends to produce sharp-edged, irregular MIP bits with a limited scope of direct application. In addition, due to the creation of binding sites within the polymeric bulk, the issue of the hindrance of adsorbate diffusion (especially in the case of macromolecules) during template rebinding makes the MIPs prepared through this approach unsuitable for practical applications. Thus over the years, many efforts to address the limitations of conventional molecular imprinting techniques have resulted in new imprinting methodologies. Systems like suspension and precipitation polymerization, where MIPs with tunable morphologies can be prepared, have been developed. Additionally, strategies like surface imprinting have also been employed. Ultimately, both of these approaches have been combined to prepare regularly shaped surface-imprinted MIP beads. Such an approach incorporates the advantages of both methodologies at the same time. Given their desirable

physical morphologies and favorable adsorption kinetics, MIPs prepared in this manner show significant promise for industrial applications. Therefore, they will be the main focus of this review.

Keywords Molecular imprinting · Surface imprinting · Beads · Polymerization · Core-shell · Emulsion

Molecular imprinting: towards synthetic receptors

The ability of biological hosts to bind specifically to certain ligand molecules is known as molecular recognition. This process plays a profound role in biology and is the basis for many of the processes associated with life. In addition to its biological significance, this recognition property has also been widely applied in normal laboratory routines for analytical, separation and catalytic purposes. Thus, for some time now it has inspired much research into the development of synthetic equivalents to the function of biological recognition.

Today, molecular imprinting has been widely recognized as the most promising methodology for the preparation of tailor-made artificial “receptors” that can selectively bind predetermined target molecules. According to Nicholls [1], “molecular imprinting is a technique which involves the formation of binding sites in a synthetic polymer matrix that are of complementary functional and structural character to its ‘substrate’ molecule”. The principle of molecular imprinting has been widely illustrated in other reviews [2–5]. In comparison to their biological counterparts, other than possessing antibody-like molecular selectivity, the major advantages of using molecularly imprinted polymers (MIPs) include physical robustness, high strength, resistance to elevated temperatures [6] and pressures, and

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inertness to acids, bases, metal ions and organic solvents, as well as low production cost and ease of preparation.

The most common methodology for molecular imprinting is the monolithic approach, where MIPs are prepared in bulk and subsequently ground and sieved to the desired size [7–10]. Despite being a convenient approach, the system is not suited to industrial use due to poor heat dispersal. In addition, the crude post-treatment tends to produce sharp-edged, irregular polymeric bits which compromise the quality of the material, thus limiting its application in areas like chromatography and solid-phase extraction. Finally, the issue of poor accessibility of the imprinted cavities, which are often created within the polymer bulk, is a major problem with this method. This is especially important in the imprinting of macromolecules like oligosaccharides and proteins.

This review provides an account of the efforts that have been made to address the inherent limitations of the conventional molecular imprinting method. Therefore, the review focuses on the production of surface-imprinted MIP beads, which is fast becoming a standard molecular imprinting technique.

MIPs with controlled morphology

Recent research has focused on the development of molecular imprinting polymerization systems that are amenable to mass production, or on the production of MIPs with controllable shape and size distributions. The main reason for this is that MIPs with controlled morphologies have a much wider scope of application than other MIPs. In most of these works, a high degree of knowledge and understanding of common methods of preparing polymeric beads is utilized to prepare and optimize MIPs in the spherical form.

MIP beads have been commonly prepared using suspension polymerization. Templates that are suitable for this system include metal ions [11], drugs [12] and proteins [13]. The system can be adjusted to prepare relatively monodisperse MIP microspheres with sizes ranging from a few to hundreds of micrometers. The biggest advantage of this method is its excellent heat dispersion, which makes it suitable for industrial scale-up without heat transfer limitations.

Another common method of preparing spherical MIP particles is by precipitation polymerization, which was first proposed by Ye et al. [14]. Starting with a very dilute monomer solution, highly cross-linked polymeric microgels precipitate out after polymerization due to their low solubility in the solvent. This approach offers an efficient methodology for preparing MIP beads in high yield that can be used for a wide range of applications. However, as the

monomer needs to be soluble in the solvent while the resulting MIP gel beads should not be, careful optimization of the system in terms of the monomer type and concentration is required. In addition, aggregation of the MIP beads could occur, resulting in poor particle yields. This technique can also be employed to prepare MIP nanospheres [15]. These nanobeads can either be used directly, as in capillary electrochromatography [16], or they can be loaded onto a membrane [17] for template extraction.

Besides the methods mentioned above, other less common approaches for spherical MIP preparation include two-step swelling and polymerization [18], miniemulsion polymerization [19] and novel micelle formation based on diblock copolymers [20].

Application of surface imprinting to MIPs

Surface imprinting has been widely studied as one approach to improving the performance of MIPs, as it solves the problems of limited mass transfer and template removal often associated with the traditional technique of molecular imprinting. This improvement is especially valuable when imprinting macromolecules, for which diffusion limitation is the major issue. One common approach to surface imprinting is the creation of thin MIP films on suitable substrates, such as gold and carbon. An advantage of this approach is the direct formation of a thin film that can be applied to sensor electrodes like the quartz crystal microbalance (QCM) [21, 22] and surface plasmon resonance (SPR) [23]. Various methodologies used to prepare such MIP films include spin-coating [21] and surface-initiated atom transfer radical polymerization (ATRP) [23]. One major issue with this approach is controlling the MIP film thickness, as it should be sufficiently thin for rapid mass transfer. This is especially important in sensor applications, where high sensitivity and short response time are desired.

An alternative approach to surface imprinting that is currently being studied is the water-in-oil (W/O) emulsion method, which is very similar to conventional bulk imprinting. While grinding into particles and sieving are still required post-treatment for this W/O emulsion method, the difference is that an emulsion stabilizer such as *N*-D-glucono dioleoyl glutamate ($2C_{18}\Delta^9GE$) [24] is required to create the W/O emulsion. In addition, interfacially active functional monomers like 1,12-dodecanediol-*O,O'*-diphenyl phosphonic acid (DDPPA) [25], phenyl phosphonic acid monododecyl ester (n-DDP) [26] or dioleoyl phosphoric acid (DOLPA) [27] will have to be synthesized, depending on the type of template to be imprinted. With its interfacial activity, the functional monomer can interact with the template molecule at the oil–water phase boundary, thus creating imprinted sites

at the interface upon polymerization. However, strict requirements of the functional monomer in terms of its interfacial activity, rigidity and the template–monomer affinity [25] have largely limited the application of the technique. To date, this technique has been successfully applied to imprint amino acids [28], metal ions [26, 29] and nucleotides [24].

The best of both worlds: molecularly surface-imprinted beads

It can be seen from the above that being able to control the morphology of the resulting MIPs increases the applicability of molecular imprinting to separation and greatly enhances the possibility of it being used for industrial mass-production, while surface imprinting greatly widens the scope of application, especially in the field of bioanalytical chemistry. Therefore, the next logical step was to incorporate the advantages of both approaches in order to develop surface-imprinted MIP beads. One of the more prominent examples of this approach is the work by Kempe et al. [30]. In this work (Fig. 1), silica particles

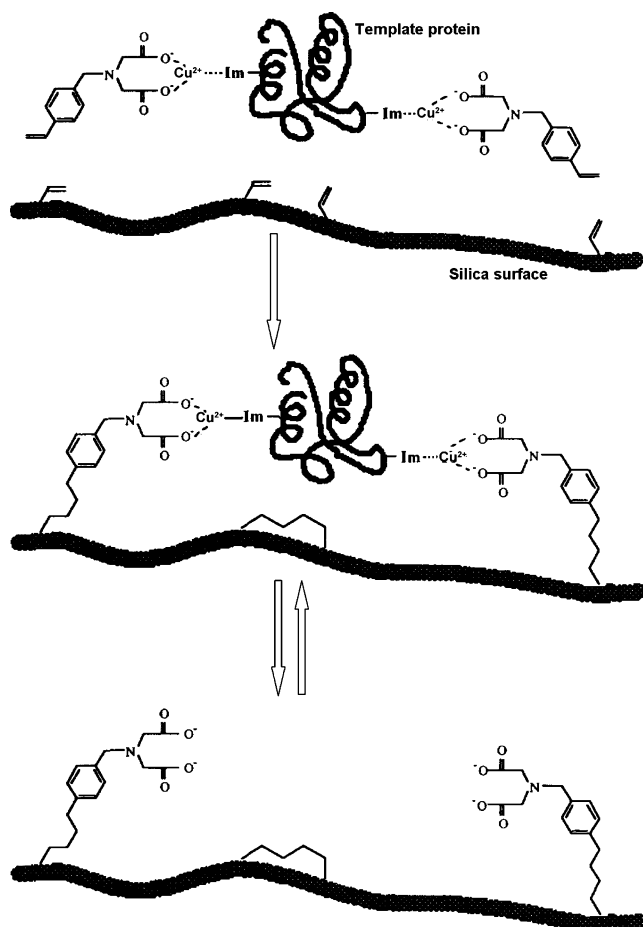


Fig. 1 Molecular imprinting of ribonuclease A (with exposed imidazole groups) on methacrylate-derivatized silica particles (adapted from [30])

were first derivatized with methacrylate groups. A metal-chelating monomer, *N*-(4-vinyl)-benzyl iminodiacetic acid (VBIDA), was then synthesized and the subsequent addition of copper ions during the polymerization allowed the template ribonuclease A (RNase A) to interact with VBIDA through metallic coordination. After template removal, complementary binding sites were left on the silica particle surface. These imprinted beads can be employed in high-performance liquid chromatography (HPLC) as the regular bead morphology allows for a better flow pattern in the column packings as compared to that obtained using the irregularly shaped particles obtained from the conventional monolithic imprinting process. This produces a better peak shape and resolution overall and also minimizes the problem of peak broadening. Additionally, macromolecular templates of protein have been successfully imprinted using this method of surface imprinting, thus overcoming the issue of limited diffusion.

The following sections provide a more detailed account of the production of surface-imprinted spherical MIP particles, highlighting some of the more recent approaches that have been employed.

Pore-filling, with dissolution of support beads

One methodology used to prepare surface imprinted spherical particles is to fill the pores of the support beads with the imprinting mixture, and then polymerize and dissolve the support. One of the most pioneering works that uses this approach is that by Yilmaz et al. [31]. In their work, the targeted template, a theophylline derivative, was first immobilized onto the aminopropyl-derivatized silica particles through the formation of amide bonds. Following that, the imprinting mixture—which includes the functional monomer, trifluoromethylacrylic acid (TFMAA), and the crosslinker, divinylbenzene (DVB)—was used to fill the pores of the template-immobilized silica particles. Upon the completion of the polymerization process, the silica support was dissolved with aqueous hydrofluoric acid (HF), thus creating the imprinted binding sites on the surface of the remaining polymer. The advantage of this approach lies in the creation of regularly shaped imprinted polymeric particles with a narrow pore size distribution. Expanding on this concept, Titirici et al. imprinted against peptide using hierarchical imprinting (Fig. 2) [32]. They synthesized the template peptide of interest on a porous silica support through solid-phase peptide synthesis to immobilize the template on the support beads. The silica pores were then filled with a mixture of monomers (methacrylic acid, MAA, and ethylene glycol dimethacrylate, EDMA) and initiator with subsequent polymerization and the final dissolution of the silica porous support with aqueous ammonium hydrogen difluoride (NH₄HF₂) solution. In

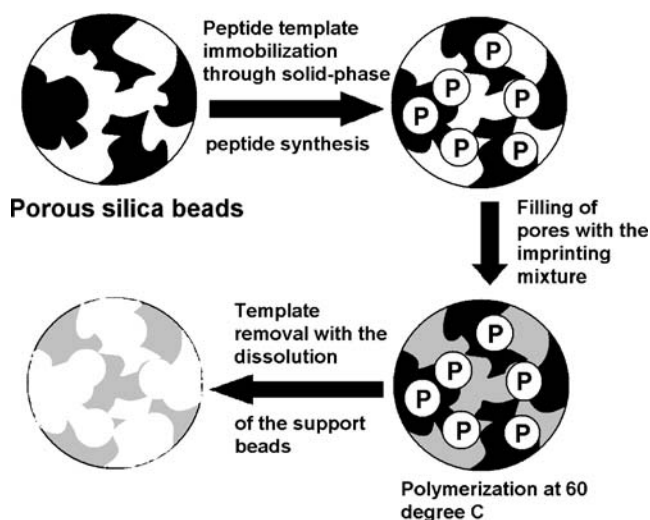


Fig. 2 Schematic representation of the technique of hierarchical imprinting (adapted from [32])

addition to the selectivity demonstrated towards the immobilized template peptide, the resulting imprinted material also displayed preferential binding towards larger peptide molecules that are structurally related to the template. This example is an illustration of epitope imprinting, as previously proposed by Rachkov et al. [33].

Surface-imprinted beads prepared using this methodology can be applied in HPLC [34] and catalysis [35]. However, this approach has not found widespread application, probably due to the extra effort required for template immobilization.

Core-shell structure MIP beads

In the preparation of surface-imprinted MIP beads, one of the most common strategies is to use preformed support beads for the deposition of imprinted polymers. This imparts a core-shell structure to the final imprinted polymer. The most common type of material used as support beads is silica, as seen in the above section, mainly due to its stability, favorable physical properties and its ease of derivatization. Other support materials include polystyrene, chitosan or other types of polymers, depending on the requirements of the application.

Emulsion polymerization

The most direct way of creating core-shell MIP particles is through the process of core-shell emulsion polymerization. This approach is a two-stage process where core or seed particles of suitable materials are prepared in a first step. The core particles primarily provide mechanical stability to the resulting core-shell MIP particles. In addition, it can sometimes be used as the locus of template immobilization or as a means of incorporating desirable properties, such as

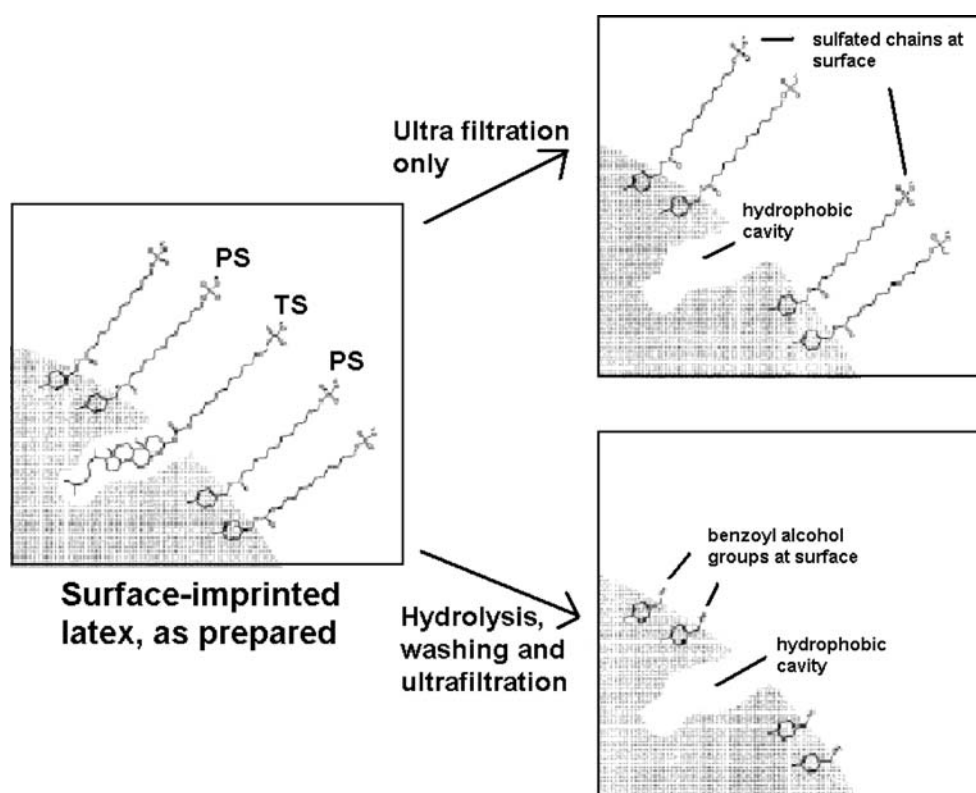
superparamagnetic and drug-releasing properties, into the MIP particles. The second step then involves the creation of an imprinted shell on the seed particles in an emulsion polymerization. A surfactant like sodium dodecyl sulfate (SDS) is often required to stabilize the emulsion.

To date, many surface-imprinted beads have been successfully fabricated with the core-shell emulsion polymerization method [36–38]. One notable work is that by Pérez et al. [39], where cholesterol surface-imprinted submicron particles (50–100 nm) were successfully prepared. The MIP nanoparticles were composed of MMA or styrene core particles (30–40 nm) and imprinted shells with functional monomers and EDMA. The significance of this work is the application of a carbonate ester sacrificial spacer; the utilization of this in molecular imprinting had already been illustrated in the work by Whitcombe et al. through the conventional bulk imprinting approach [40]. In this technique, the template cholesterol is covalently linked to the functional monomer through the synthesis of cholesteryl (4-vinyl)phenyl carbonate (CVPC), and this was used in the imprinting polymerization reaction. Upon completion, the template was removed by hydrolysis of the ester bonds with aqueous sodium hydroxide (NaOH) solution in methanol. The subsequent rebinding of the template molecule to the MIP would only require non-covalent interactions. This semicovalent approach allows the creation of homogeneous binding sites with covalent imprinting, while it also takes advantage of the fast rebinding kinetics of noncovalent adsorption.

In another work, Pérez and coworkers prepared surface-imprinted beads for cholesterol imprinting [41] using core-shell emulsion polymerization with a polymerizable surfactant, pyridinium 12-(4-vinylbenzyloxycarbonyl)dodecanesulfate (PS), and pyridinium 12-(cholesteryloxycarbonyloxy)dodecanesulfate (TS), which acts as both a surfactant and a template. The second stage of the core-shell emulsion polymerization was carried out in the presence of PS and TS (Fig. 3). With TS, the template molecules were effectively constrained to the W/O phase boundary during the emulsion polymerization, thus successfully creating hydrophobic binding sites for cholesterol on the imprinted bead surfaces. This was demonstrated by flocculation experiments using poly(ethylene glycol) (PEG)-bis-cholesterol. Additionally the use of PS and TS enabled the creation of beads with two different surface functionalities, and helped to differentiate the hydrophobic imprinted cavities from other surfaces without the binding sites. This further enhanced the selectivity of the surface-imprinted beads.

Core-shell emulsion polymerization is a straightforward strategy for creating core-shell surface-imprinted beads. Moreover, the system is highly suited to industrial applications. However, due to the complexity of the polymerization system and the use of surfactants, there

Fig. 3 Schematic illustration of the use of novel synthesized TS and PS for the preparation of surface-imprinted core-shell submicrometer particles (adapted from [41]). Different post-imprinting treatments were adopted for template removal as well as the creation of beads with different surface functionalities



may be issues of template–system incompatibility. This can affect the scope of application of this imprinting methodology.

Grafting of imprinted polymer

Besides emulsion polymerization, another method of fabricating core-shell structured surface-imprinted MIP beads is to directly polymerize the monomers with the templates on the support spherical particles, thus creating a film of MIP shell on the core support material. With this method, there may be issues regarding the locus of MIP film formation and possible film detachment after repeated use. To address these problems, the grafting methodology was devised.

In the “grafting to” approach, the support particles, which are usually made of silica, are initially vinylated to create residual double bonds on the surface. Subsequent free-radical polymerization of the imprinting mixture (template, functional monomer, crosslinker and initiator) in the presence of the surface-modified silica beads will enable copolymerization of the imprinting mixture and the surface double bonds, thus grafting a MIP coating onto the core surface. The applicability of this technique has been demonstrated in various works [42–44]. Nevertheless, there are some concerns about this technique. For example, although the graft copolymerization resulted in good attachment of the imprinted coating onto the support particles, uncontrolled bulk polymerization can occur

simultaneously. In addition, there may be difficulties in controlling the thickness of the resulting MIP surface.

In contrast to the “grafting to” approach, the “grafting from” methodology enables the MIP coating to be formed locally at the surface. Sulitzky et al. [45] first modified the silica particle surface with an azoinitiator, 4,4'-azobis(4-cyanopentanoic acid) (ACPA) via (3-amino-propyl)trimethoxysilane (APS) and glycidoxypentyltrimethoxysilane (GPS). Subsequently, an imprinting mixture that consisted of MAA, EDMA and the template L-phenylalanine anilide was stir-mixed with the initiator-modified silica beads, and imprinting polymerization was initiated. By confining the initiating radicals onto the surfaces of the support silica particles, and in the absence of chain transfer species, polymer chain propagation will take place mainly on the surface, with minimal polymerization occurring in the solution. A very thin film of MIP (average film thickness: 0.8 nm) was successfully created on the silica surfaces. Similar approaches were used in other studies [46–49], with some [47, 48] employing the use of iniferter-immobilized silica rather than an initiator to further minimize chain propagation in the solution. To gain better control of the grafting process in terms of the final imprinted film thickness, and to prevent gelation of the solution, Titirici et al. [50] employed a reversible addition–fragmentation chain transfer (RAFT)-mediated polymerization on azoinitiator-modified silica to prepare surface-imprinted, core-shell structured composite beads.

The “grafting from” strategy is an effective and feasible way of preparing surface-imprinted beads. However, due to the chemical complexities involved, more investigations will be needed to determine its applicability as a possible general imprinting methodology for other types of templates.

Template-mediated lithography

A novel template-mediated lithographic approach for the preparation of surface-imprinted particles had been developed by Aherne et al. [51]. This work focused on bacteria imprinting (Fig. 4). First, a water-soluble poly(amine) and a diacid chloride in a dispersed organic phase were allowed to react in a stirred suspension of bacteria. Due to the propensity of microorganisms to partition at the water–oil phase boundary, and the capability of the diacid chloride to form covalent bonds between the nucleophilic groups in the bacterial cell walls and poly(amine), a polyamide shell layer was thus synthesized under and around the template bacteria, forming a microcapsule with the template bacteria “trapped” at the surface. Secondly, polymerization was photoinitiated to polymerize diacrylate monomers in the

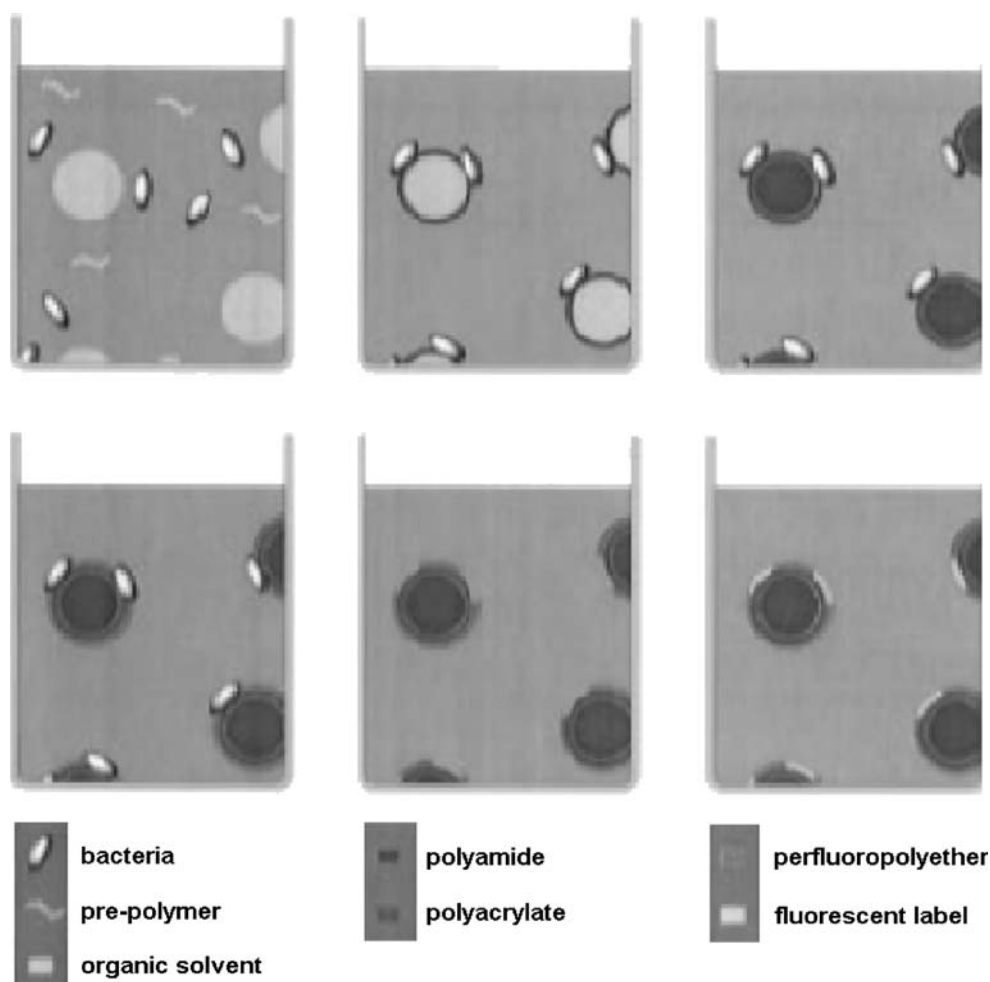
dispersed organic phase, producing the core portion of the material. This was followed by treatment with diisocyanato-terminated perfluoropolyether. With the template bacteria trapped on the particle surface, the aim was to shield the remaining amino acid groups on the surface where the template is absent. Finally, the bacteria were removed with acid hydrolysis, thus resulting in the formation of imprinted binding cavities which were functionally and structurally complementary to the template bacteria. This produced imprinted beads with a multilayered, core–shell structure. In another work, the same approach was applied but spores were used as the template [52].

Note that, other than the works mentioned above, no other related works have been published to the best knowledge of the author. Therefore, although this technique may be suitable for imprinting microorganisms, it may not be generally applicable to other templates of interest.

Another methodology

Another approach to preparing surface-imprinted spherical particles was an extension of the work by Yoshida et al. to

Fig. 4 Schematic representation of the bacteria-mediated lithographic imprinting technique [51]



prepare surface-imprinted polymer using W/O emulsion [26]. The same research group [53] made use of trimethylolpropane trimethacrylate (TRIM) and DDDPA as the crosslinker and functional monomer respectively. Zinc ion surface-imprinted microspheres were then prepared with water-in-oil-in-water (W/O/W) multiple emulsions. The use of DDDPA helped to align the template and ensure the formation of imprinted sites on the polymer surface. The key factor for the successful fabrication of spherical particles is the composition of the external aqueous phase in the W/O/W emulsion. The ionic strength should be sufficient to prevent shrinkage of the emulsion during the imprinting polymerization. In addition, the choice of a water-soluble surfactant is crucial to the stabilization of the spherical W/O emulsion. Imprinted spheres prepared through this approach with controllable sizes that range from 10 to 100 μm are morphologically well-defined and could be applied directly in HPLC. Nevertheless, to date, only the imprinting of zinc ion has been successfully demonstrated with the technique.

A novel one-step preparation

It can be seen from the above that the normal strategy employed for the preparation of surface-imprinted spheres involves the use of preformed support beads or the synthesis of novel functional monomers. Our group has developed a novel methodology with only a one-step reaction for the preparation of surface-imprinted nanoparticles using proteins as templates without the need for any support particles. In this work (Tan CJ, Bai R, Tong YW, 2007, submitted to *Anal Bioanal Chem*), miniemulsion polymerization was employed as the imprinting system. Due to its amphiphilic nature, there is a strong propensity for water-soluble proteins to partition at the water–oil interface [54]. With the template proteins aligned at the surfaces of the micelles, upon polymerization and subsequent template removal, binding sites were created on the surfaces of the imprinted nanoparticles (Fig. 5). Since protein molecules are inherently fragile and sensitive to external conditions, the main challenge of this approach to protein imprinting is the possible denaturation of the template protein molecules by protein–surfactant interactions and other reaction conditions. Thus, an extensive study of template protein structural stability under different parameters

like temperature, homogenization, and surfactant system has been carried out [55]. The system was carefully optimized to ensure its compatibility for protein imprinting, and surface-imprinted nanoparticles around 40 nm in size were successfully fabricated. These demonstrated good selectivity towards the template ribonuclease A (RNase A) in aqueous batch and competitive rebinding tests. The nanospheres also displayed favorable adsorption kinetics and the protein loading achieved was higher than those seen in comparative works. The application of miniemulsion polymerization to molecular imprinting was previously been illustrated in the work of Tovar et al. [19] for the imprinting of amino acids. On the other hand, we extended this to the imprinting of macromolecular protein molecules and proposed a mechanism for the imprinting process.

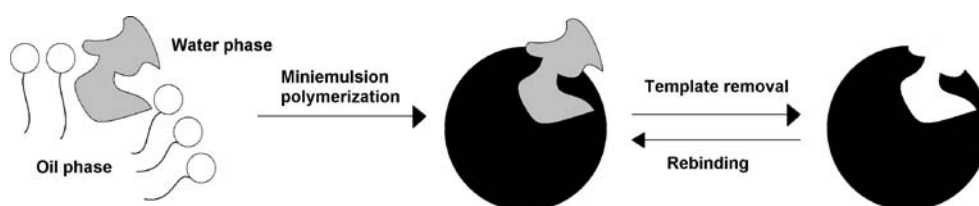
One excellent advantage of this approach to protein imprinting is the possible concurrent incorporation of desirable properties into the imprinted spheres during the imprinting process. Our group demonstrated this by preparing superparamagnetic surface-imprinted spheres for RNase A using miniemulsion polymerization [56]. This will further widen the scope of applications for this material, for example in the fields of bioanalytical chemistry and bioseparation.

Nevertheless, this approach has only been successfully applied to the surface imprinting of proteins. Its applicability as a feasible imprinting system for other types of templates remains unknown and is currently the focus of our research.

Conclusions

Molecular imprinting is a viable technique for the creation of synthetic material for molecular recognition. The past decade has witnessed an increasing amount of interest in this field of research. To date, with continuous efforts and resources being directed into many different studies, the technique of molecular imprinting has reached a certain degree of maturity in terms of our understanding of the imprinting mechanism as well as the development of various systems for MIP preparation, at least in the laboratory context. It is foreseeable that research efforts will increasingly be steered towards developing imprinting systems that are suitable for large-scale production of MIPs.

Fig. 5 The application of the amphiphilic nature of protein template molecules to surface imprinting through miniemulsion polymerization (Tan CJ, Bai R, Tong YW, 2007, submitted to *Anal Bioanal Chem*)



Futhermore, from the perspective of practical applications, approaches that can produce MIPs with controllable and homogeneous morphologies may slowly replace the conventional imprinting methodology in order to extend the scope of industrial applications. It is the authors' belief that surface-imprinted MIP spheres, with their regular, tunable sizes and fast rebinding kinetics for macromolecules, can fulfill the needs of industry, and could well become the main form of MIP material in the future.

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