Reflux Precipitation Polymerization: A New Platform for the Preparation of Uniform Polymeric Nanogels for Biomedical Applications

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Reflux precipitation polymerization (RPP) represents an effective approach that enables to prepare various types of polymeric nanogels with precise control over the morphology and structure. Owing to facile loading or modification by a variety of functional moieties, rationally designed nanogels pose the possibility to attain a platform for tailoring functional properties that could be widely used for various biomedical applications, such as multifunctional drug delivery, enrichment of functional peptides, separation of specific proteins, as well as detection of circulating tumor cells. This feature article highlights RPP as a promising polymerization strategy that provides access to facile generation of modular nanostructures or multifunctional properties in a diverse range of biomedical applications, proving that RPP has great potential to become one of the most attractive polymerization techniques in polymer chemistry.

1. Introduction

1.1. Emergence of Reflux Precipitation Polymerization

Hydrogels consist of hydrophilic, 3D interconnected polymer networks that enable absorbing a large amount of water, exhibit swelling behavior, and maintain the network structure.[1] It is well known that when the hydrogel particles are confined to nanoscopic dimensions, they will be named as nanogels. Owing to their hybrid features of nanosized range and hydrogel, nanogels have attracted tremendous attention as a multifunctional nanostructure for versatile applications in pharmaceutical and biomedical field.[2] Traditional fabrication of nanogels commonly undergoes macromolecular precursor interaction or monomer polymerization. In the past two decades, many methods have been developed to prepare nanogels. According to the crosslinking pattern of hydrogel, those approaches can be divided into physical crosslinking and chemical crosslinking. The physical crosslinking, commonly involved in the formation of nanogels from precursor polymers, includes hydrogen bonding, hydrophobic interaction, and electrostatic interaction.[3] However, the chemical crosslinking often generated during the synthetic process of nanogels from monomers leading to covalent bonds, thus can be utilized to form novel polymeric architectures and endows extensive application potentials to nanogels.[4]

Precipitation polymerization is a commonly used synthetic approach for fabricating monodisperse nanogels,[5] which involves the initiation and polymerization in the homogeneous solution by mixing with monomers and initiators, followed by the precipitation of generated polymeric chains out of the continuous medium due to the low solubility in the selective reaction media, and then forming the uniform particles. Unlike emulsion polymerization[6] or inverse suspension polymerization,[7] precipitation polymerization can take the advantage of preparing neat polymeric nanogels free of any surfactant or stabilizer. Furthermore, distillation precipitation polymerization (DPP), as a type of modified precipitation polymerization, undergoes the reactive condition in distilling state resulting in the formation of polymeric particles.[8] To solve the problem that the distillation process occasionally led to the unstable of dispersed system, our group developed the reflux precipitation polymerization (RPP) by replacing the distilling reaction apparatus with refluxing reaction apparatus.[9] During the polymerization process, the polymers with critical chain length enable to form well-defined polymeric nanogels and precipitate from the poor solvent without adhesion to the reaction flask inner wall. In that case, RPP approach might be a superior synthetic approach for the preparation of nanogels in terms of high colloidal stability and desirable reaction efficiency.[10] Additionally, RPP could easily be scaled up due to the simple reaction apparatus and polymerization process. As an efficient method to prepare nanogels with uniform structure and size without any surfactant, there has been increasing interest in using RPP to prepare functional nano- or microgels for diverse biomedical applications in the last few years (Figure 1).

1.2. Scope

The intention of this article is to highlight the use of RPP as an efficient engineering tool for the production of acrylic...
monomer-based multifunctional polymeric nano- or micro-
structures, focusing on its applications in biomedical fields,
such as drug delivery, adsorption/purification, cell detection,
and so on. The Section 2 introduces the RPP and describes
advantage and disadvantage characteristics of RPP. The Sec-
tion 3 gives an overview of the various nanogels that have been
prepared using this technique with particular emphasis on
the synthetic strategies and biomedical applications, especially
in drug delivery field. The Section 4 focuses on the synthesis
and adsorption/purification applications of hybrid microgels
combining inorganic materials with functional polymer shells
prepared by RPP. In Section 5, the combination of RPP with
surface-enhanced Raman scattering (SERS) is implemented for
tumor cell detection. Finally, the possible further applications
of RPP in various fields are presented in Section 6.

2. Introduction to Reflux Precipitation Polymerization

Precipitation polymerization was first reported in 1993, taking
advantages of the absence of any surfactant or stabilizer.[11]
However, it is hard to prepare hydrophilic polymer particles
with well-defined spherical shape and narrow size distribution
through conventional precipitation polymerization.[12] In this
regard, DPP has been developed that can be utilized for the fab-
crication of hydrophilic poly(methacrylic acid) (PMAA) polymer
particles; the difference between DPP and conventional precipi-
tation polymerization is the distillation of the solvent during
polymerization. The typical apparatus for DPP is a two-necked
flask attached with a fractionating column, Liebig condenser,
and receiver for the collection of distilled solvent.

Although DPP allows fast fabrication process (1–2 h) and
easier operation, distilling certain solvent volume within the
stipulated time cannot be guaranteed under the common condi-
tion. Besides, the resulting polymeric particles easily adhered on
the inner wall of the flask might be out of the ongoing polym-
erization system, leading to inhomogeneous polymerization and
unsatisfied particle size distribution. These factors might also
impede the possible large-scale production of polymer particles.[9]

Inspired by the precursor DPP, our group developed a
more efficient synthetic approach for precipitating poly-
meric nanogels from polymerization system, namely RPP. In
RPP, refluxing was employed to agitate the reaction system
instead of distilling by simplifying the reaction apparatus into
a single-necked round-bottom flask equipped with a spherical
condenser. If needed, mechanical stirring can also be used in
this system. Thus, RPP allowed having advantages such as
handy manual operation and more stable polymerization pro-
cess. In comparison with the previous precipitation polymeriza-
tion technique, RPP not only facilitates the formation of mono-
disperse particles, but also is appropriate to freely control the
reaction time. Besides, a wide range of functional monomers
could be used for the preparation of the polymer nanogels by
3. Polymeric Nanogels for Drug Delivery

With the aim of guiding a nonspecialist, this section will first depict the functional mechanism and significance of nanogels in drug delivery for cancer therapy, and then summarize the various biodegradable polymeric nanogels that have been prepared using the RPP approach in the application of theranostic cancer therapy. The biodegradability is mainly due to the properties of different crosslinkers; so this section is divided into two: the nanogels with crosslinker that contains disulfide bond and the nanogels with crosslinker that contains metal ions.

Many important cancer therapeutic agents are small, hydrophobic compounds with intrinsic natures such as poor water solubility, undesirable pharmacokinetics, nonspecific distribution, and rapid clearance that compromises their treatment efficacy.[13] Driven by the need for the alternative drug formulations and accelerated by the progression of material science, a wide range of nanotechnology-based drug delivery systems have been designed for improving drug therapeutic potentials and reducing side effects; these drug delivery systems include liposomes, micelles, polymeric nanoparticles,[14] carbon nanotubes,[15] graphene oxides,[16] metallic nanoparticles,[17] and so on.

Nanogels have received considerable attention as a crosslinked polymeric nanoparticle in drug delivery field given their favorable properties such as excellent drug-loading capacity, high flexibility, and biocompatibility,[18] the nanoscale dimension endows nanogels with numerous advantages, including targeting tumor tissue via enhanced permeability and retention (EPR) effect,[19] protecting payloads from premature biodegradation, and reducing unwanted side effects. The hydrogel nature such as porosity and swelling property enable to achieve the feasibility of high drug-loading capacity and controlled drug release. As a novel and efficient preparation method, RPP combines precise control of the nanogel structure with a facile introduction of multiple functional groups. The resulting nanogels can be used as passively or actively targeted vehicles to deliver therapeutic or diagnostic agents to the tumor sites. Therefore, nanogels prepared by RPP have been explored as anticancer-agent carriers for diagnostic, therapeutic or theranostic applications due to their tailoring properties and capability to efficiently encapsulate different classes of bioactive compounds.

3.1. Synthesis of Biodegradable Polymeric Nanogels by Disulfide Crosslinker

Tumor tissues have many distinct characteristics, including hypoxic acid microenvironment and elevated intracellular glutathione level, which offers tremendous opportunities in developing smart nanoformulated drug carriers that are able to release therapeutic cargos under specific external stimuli.[20] The smart stimuli-responsive nanogels have been considered as promising alternatives to overcome the limitations of small-molecule chemotherapeutics which release the therapeutic payloads in response to specific stimuli arising from biomedical relevant changes, leading to both enhanced therapeutic efficiency and reduced side effects.[21] The difference of glutathione level between normal tissue and tumor tissue is considered as one of the most important physiological changes in cancer biology, thus introducing redox-sensitive disulfide bonds that could be cleaved by intracellular glutathione as a classic strategy to achieve triggered drug release in targeted cancer therapy.[22]

Our group has developed a series of multifunctional PMAA nanogels with bis(acryloyl)cystamine (BACy) as the crosslinker that contains disulfide bonds and endows these nanogels with redox-responsive and biodegradable properties. Jin et al. fabricated...
a new type of redox/pH dual stimuli-responsive biodegradable PEGylated PMAA nanogels crosslinked by BACy (Figure 3),[23] the particle sizes of nanogels could be regulated from 194 to 709 nm according to the dynamic light scattering (DLS) measurement by changing the key RPP reaction parameters, including the amount of monomer, crosslinker and initiator, reaction time, and reaction temperature. After further surface decoration by polyethylene glycol (PEG), the DLS data showed that all the z-average sizes of the nanogels increased about 100 nm, indicating successful surface modification. These PEGylated nanogels could be simultaneously loaded doxorubicin hydrochloride and paclitaxel with high drug-loading capacities due to their regional hydrophobic nature; this capability of effective incorporation of both hydrophobic and hydrophilic agents could open possibilities for synergistic drug combination therapies.

Negatively charged carboxyl groups in PMAA can not only favor the encapsulation of anticancer drug doxorubicin via electrostatic interaction with the positively charged amino groups on doxorubicin, or encapsulation of cisplatin via the chelation with platinum atom in cisplatin,[24] but also facilitate the further modification on nanogel surface using the carbodiimide-mediated coupling reaction. For instance, the hydrophilic PEG coated on the surface of nanogels enable prolonging their systemic circulation time and decreasing immunogenicity, while the folic acid grafted onto the surface of nanogels as a targeting moiety contributed to active targeting toward the tumor cells that overexpress folate-receptors.[25] Interestingly, due to the presence of amide bonds in the crosslinker (BACy), similar to the molecular structures in protein, the resulting nanogels could also be degraded by protease, providing their potential application in normal tissue and organs.

Although the existing PMAA nanogels prepared via RPP have demonstrated their capacities for efficient-targeting drug delivery performance, some critical issues remain to be elucidated in future clinical applications, including 1) more details about biocompatibility and biotoxicity of these nanogels, which might influence the biological features of immune cells when killing tumor cells in tumor tissue, 2) the quantitative analysis of drug biodistribution according to in vivo antitumor experiments, and 3) the integration of different microenvironment stimuli and their synergistic effect.

Hollow-structured particles have been well studied owing to their unique structural features and great potential for applications in various areas.[26] The hollow chamber is capable of imbedding therapeutics or imaging contrast agents in the core by encapsulation through the outer shell. RPP as a facile approach can fabricate core-shell structured nanogels, followed by a selective dissolving of non-crosslinked inner polymeric cores to form the hollow nanostructure. Li and coworkers developed a new type of hollow PMAA nanobubbles with well-defined structural properties for theranostic application (Figure 4). The non-crosslinked PMAA core was synthesized via RPP, followed by coating of a layer of crosslinked PMAA and removal of the non-crosslinked PMAA core by ethanol etching. The sizes of nanobubbles could be tailored from ≈50 to ≈750 nm by modulating the diameter of the non-crosslinked PMAA core with the corresponding monomer ratio and concentration. The resulting hollow PMAA nanobubbles were loaded with doxorubicin in outer layer as an anticancer therapeutic and perfluorohexane in inner cavity as an ultrasound contrast agent.[27] In addition, in the RPP system, certain metallic nanostructures can serve as seeds for uniform coating by polymeric networks to give a more complicated configuration. Using this method, Yang et al. prepared a novel kind of biodegradable triple-functional yolk–shell particles. The poly(glutamic acid)-stabilized Fe3O4 nanocluster seeds were consecutively coated with a layer of non-crosslinked PMAA and a layer of BACy-crosslinked PMAA. The subsequent ethanol etching provides a metallic core within a hollow cavity surrounded by a biodegradable polymeric outer shell.[28] After co-loading of doxorubicin as the anticancer drug and perfluorohexane as the acoustic sensitive, the resulting yolk–shell particles can be utilized as ultrasound/MR dual-modality imaging contrast agents and therapeutic carriers. These biodegradable hollow particles have the capability of simultaneous controlled delivery of anticancer therapeutics and monitoring

![Figure 3. Preparation of redox/pH dual stimuli-responsive biodegradable PEGylated PMAA nanogels for dual-drug loading. Reproduced with permission.[23] Copyright 2015, Elsevier B.V.](image-url)
of therapeutic effects. However, despite their promising theranostic functions, it should be noted that the hollow-structured polymer particles might induce a perturbation of the intracellular environment, which evokes the cellular safeguard mechanism that may limit the therapeutic effect of the drug-loaded particles in tumor cells.\(^{[29]}\) This drawback can be overcome by suitable surface modification.

### 3.2. Synthesis of Biodegradable Polymeric Nanogels by Metal Ion Crosslinker

Cisplatin-based therapy is a common strategy for the treatment of various cancers but provides limited clinical benefit partly due to the presence of glutathione,\(^{[30]}\) the over-secreted glutathione in tumor tissue can detoxify cisplatin via chelating with Pt(II) to form a less toxic conjugate which is subsequently exported out of the cell. For the purpose of improving the therapeutic efficacy of cisplatin, Zhang et al. synthesized dimethacrylate-Pt(IV) as monomer and prepared a delicate glutathione-responsive nanogel with a high platinum drug loading of 60.8% (Figure 5).\(^{[31]}\) By means of RPP, the prodrug dimethacrylate-Pt(IV) self-crosslinked into nanogels with uniform spherical morphology and narrow size range with an average particle size of 76.2 nm as measured by transmission electron microscope (TEM) and 112.3 nm as measured by DLS. The stabilizer TPGS1000 was then coated onto nanogels to prolong blood circulation time and facilitate EPR effect to achieve tumor targeting. Since the Pt–O bonds in dimethacrylate-Pt(IV) can undergo reducing elimination and be broken to release Pt(II) compound cisplatin, these nanogels are able to dissociate in high bio-reducing intratumoral environment and release pharmacologically active cisplatin. At the same time, the detoxifying glutathione molecules are consumed and oxidized into GSSG forms which have less ability of detoxifying cisplatin, improving cytotoxicity and therapeutic potential of released cisplatin.

Inspired by the natural zinc finger protein structure in which a zinc ion is chelated by two thiol groups,\(^{[32]}\) another metal-ion containing complex, dimethacrylate-Zn(II), was successfully synthesized and employed to copolymerize with MAA to fabricate a new kind of Zn(II)-crosslinked biodegradable polymeric nanogel for tumor-targeted delivery of doxorubicin.\(^{[33]}\) The crosslinker dimethacrylate-Zn(II) endowed the resulting nanogels with glutathione/pH dual stimulus responsive drug-release
capability. After arriving to the targeted sites and encountering environmental triggers, the zinc ions can be removed from the nanogel network by thiol group of glutathione via coordination substitution, as well as protonated carboxyl group via acid cleavable metal chelation, resulting in subsequent nanogel degradation and doxorubicin release. Following this conception, many different kinds of metal ions could be used in this gel system.

4. Polymeric Composite Nanogels for Adsorption/Purification Application

In this section, various polymeric composite nanogels with specific binding properties prepared by RPP for adsorption/purification application will be summarized. The particle size is not necessarily restricted to nanometer scale for in vitro applications; herein, polymeric composite microgels have also been prepared by RPP for the same purpose. Based on different target biomolecules, this section is divided into three: polymeric composite particles (in this part, we call the composite nanogels or microgels as composite particles) for glycopeptides enrichment, polymeric composite particles for phosphorylated biomolecules separation, and polymeric composite particles for histidine-rich protein enrichment. The preparation strategies and functional characteristics of these polymeric composite particles will be discussed.

With the preliminary successful applications for drug delivery in terms of the preparation of monodisperse PMAA nanogels, the RPP approach has been expanded to the fabrication of various types of composite nanogels or microgels with well-defined architecture, controllable sizes, low dispersity, and versatile surface functional groups for adsorption separation and purification application. 2-hydroxyethyl methacrylate (HEMA), 1-vinyl imidazole (VIM), 4-vinylphenylboronic acid, and many other monomers were also exploited for the synthesis of various nanogels via RPP.[17] The versatile functional nanogels could provide ideal platforms that not only offer feasible ways for recognizing the small organic molecules, but also demonstrate the capabilities of enriching various peptides and proteins. In one study of purifying the natural flavonoid molecule luteolin, the poly(vinyl imidazole) (PVIM) was coated onto magnetic Fe₃O₄ core with desirable morphology via RPP to immobilize Zn(II), the immobilized Zn(II) can serve as binding site for luteolin (Figure 6).[34] In another research, for the purpose of protein enrichment and immobilization, Zhang and co-workers reported the combination of RPP and RAFT polymerization for the production of a novel kind of nanosponge. Carboxyl-containing RAFT reagent was used to mediate the growing of ultrastiff poly(acrylic acid) shell on the poly(hydroxyethyl methacrylate)-coated Fe₃O₄ magnetic supraparticle core with ≈20 nm thickness of intermediate poly(hydroxyethyl methacrylate) layer. After RAFT polymerization of poly(acrylic acid), the thickness of the polymeric layer increased to around 100 nm. The nanosponge contains negatively charged net-like shells and can almost enrich all kinds of protein.[35] Additionally, in comparison with free enzyme, the higher catalytic activity of immobilized enzyme on this nanosponge implicates broad potential utilization in real applications. Generally speaking, it is notable that the specific separation and enrichment of peptides or proteins with certain functional groups such as glycosyl and phosphoryl in biomedical acquirement. In this regard, RPP can be utilized to synthesize various particles to meet the specific requirements due to their tailored surface functionalization.

4.1. Polymeric Composite Particles for Glycopeptides Enrichment

As one of the important protein post-translational modifications, the glycosylation shows great significance in various biological processes, including signaling transduction, cellular recognition, and carcinogenesis. The abnormal glycosylation is involved in the progress of many diseases where glycosylated peptides or proteins served as detection biomarkers for diseases, especially for cancer.[16] However, the low abundance of glycopeptides in complex biological samples and the interference from vast non-glycopeptides make it difficult for further investigation of glycosylation.[37] These obstacles underscore the need for the development of efficient method to enrich glycopeptides. So far, various techniques have emerged for
glycopeptides enrichment and separation, including hydrophilic interaction chromatography (HILIC), phenylboronic acid chemistry, hydrazide chemistry, among others.

Magnetic nanoparticles have multiple merits such as fast magnetic separation, easy surface modification, and biocompatibility.\(^3\) RPP can facilitate the uniform polymer coating on the magnetic nanoparticle surface, and can be employed for facile construction of a core-shell hybrid platform combing magnetic properties and glycopeptides enrichment functionality. Magnetic nanoparticles can be modified with specific functional groups in order to capture glycopeptides through chemical bonding. To date, benzoboroxole-functionalized,\(^4\) hydrazide-functionalized,\(^5\) and alkoxyamine-functionalized\(^6\) magnetic core-shell hybrid particles have been prepared via RPP-mediated surface coating, respectively, which exhibited many promising capabilities for glycopeptides enrichment such as quick enrichment process and large enrichment capacity.

On the other side, magnetic nanoparticles have also been endowed with physicochemical interaction-mediated glycopeptides enrichment capabilities. HILIC is frequently employed for the separation of glycopeptides because of the strong hydrophilic nature of glycan in glycopeptides. Compared with chemical-bonding approach, HILIC could capture glycopeptides without damaging the structure of glycan and provide integrated structure information of glycopeptides.\(^7\) Zhao et al. reported the combination of RPP with HILIC for the development of a kind of magnetic zwitterionic-hydrophilic material, the resulting hybrid particle showed high detection sensitivity and selective enrichment of glycopeptides, relying on hydrophilic interactions (Figure 7).\(^8\) In this study, a thick layer of poly(glycidyl methacrylate) (PGMA) network was coated on the Fe\(_3\)O\(_4\) nanocore and aminated for the in situ decoration of Au nanoparticles which can immobilize plenty of amphoteric L-cysteine through Au–S bonds, along with the L-cysteine providing zwitterionic sites and hydrophilic nature for the capture of glycopeptides. In a similar study, zwitterionic polymer, poly(2-(methacryloyloxy)ethyl)-dimethyl-(3-sulfo propyl) ammonium hydroxide (PMSA), was coated on magnetic nanoparticles via an intermediate layer of SiO\(_2\) through RPP,\(^9\) the resulting Fe\(_3\)O\(_4\)@SiO\(_2\)@PMSA nanoparticles showed high detection sensitivity, large binding capacity, and satisfied enrichment recovery in glycopeptides enrichment. In addition, ionic liquid, as another novel hydrophilic material for glycopeptides enrichment, represents a class of organic salts containing organic/inorganic anions, showing favorable properties such as good solubility and thermal stability.\(^10\) Jiao and coworkers chose 2-(methacryloyloxy)ethyl trimethylammonium chloride (MAC) monomer as ionic liquid and synthesized hydrophilic PMAC-modified Fe\(_3\)O\(_4\) nanocores via one-step RPP,\(^11\) the resulting nanoparticles demonstrated high detection sensitivity (10 fmol), large binding capacity (100 µg mg\(^{-1}\)), and satisfied enrichment recovery (approximately 82%) in glycopeptide enrichment, relying on the hydrophilicity and electrostatic interaction between PMAC layer and glycopeptides. Furthermore, they also showed their potential application in glycopeptides enrichment of tumor cell exosome proteins.

4.2. Polymeric Composite Particles for Phosphorylated Biomolecules Separation

Protein phosphorylation is an important regulator of signaling pathways that involves most cellular events such as proliferation, differentiation, and apoptosis. Thus, the purification and subsequent mass spectrum analysis of peptide or protein carrying a phosphate group is of great significance in analytical chemistry. Due to the low abundance of phosphopeptides in biological samples, the enrichment procedure prior to mass spectrum analysis is necessary.\(^12\)

Metal oxide affinity chromatography is one usual practice for phosphopeptide enrichment; a commonly used metal oxide (titanium dioxide) possesses Lewis acid and ion exchange properties and serves as the anchoring site for the capture of phosphopeptides via forming multidentate bonds.\(^13\) Ma et al. designed a novel kind of rattle-like composite microspheres containing a mesoporous titanium dioxide core, an

![Figure 7. Schematic illustration of the preparation procedure of zwitterionic-HILIC magnetic Fe\(_3\)O\(_4\)@PGMA@Au-L-cys material. Reproduced with permission.\(^1\) Copyright 2017, Elsevier B.V.](image-url)
intermediated hollow space, and a poly(N-isopropyl acrylamide-co-N,N’-methylenebisacylamide) [P(NIPAM-co-MBA)] polymeric shell via two-step RPP for specific phosphopeptidome extraction (Figure 8).[49] The outer polymeric shell provides size-exclusion effect against high molecular weight proteins, while mesoporous titanium dioxide inner core is responsible for the selective enrichment of the low molecular weight phosphopeptides. With the aid of these unique features, this rattle-like microsphere showed excellent promise for the one-step selective extraction of the phosphopeptides from complicated biological samples.

Immobilized metal affinity chromatography (IMAC) is another widely used method for phosphopeptide enrichment and especially suitable for multiphosphorylated peptide purification. The metal ions are commonly immobilized to a fixed phase such as silica via chelation effect.[50] Wang and coworkers developed a new type of Ti⁴⁺ dotted polymeric microgels using the chemical tagging technique which took advantage of the reactivity of the phosphate group (Figure 9).[10] In this system, they prepared monodisperse poly(ethylene glycol methacrylate phosphate) (PEGMP) microgel via RPP, then immobilized Ti⁴⁺ onto the microgel surface via coordination reaction between Ti⁴⁺ and phosphate groups. The resulting PEGMP-Ti⁴⁺ microgel showed high selectivity toward phosphoproteins and exhibited great potential for phosphoprotein enrichment.

4.3. Polymeric Composite Particles for Histidine-Rich Protein Enrichment

Histidine-tag, as one of the most common affinity tags in protein purification field, possesses a short sequence that shows little effect on the protein functions. Thus, his-tagged recombinant proteins are often genetically designed and expressed for the convenience of further purification and enrichment.[51] Besides, in biomarker-related clinical diagnosis field, some native proteins, such as serum albumin and hemoglobin, also contain high abundance of histidine, and could hamper the detection of relatively low-abundant blood biomarkers. IMAC is also a reliable method for selective separation of his-tagged recombinant proteins or histidine-rich native proteins attributed to the strong interaction between the immobilized transition metal ions and electron donor histidine. Zhang et al. prepared a novel kind of Ni²⁺ immobilized crosslinked PVIM layer coated Fe₃O₄ magnetic core via RPP, combining the advantages of IMAC and magnetic microspheres (Figure 10).[52] The resulting flower-like magnetic microspheres not only showed efficiency in his-tagged recombinant protein purification, but also exhibited remarkable performance for removal of histidine-rich native proteins from the complex real sample.

4.4. Polymeric Composite Particles for Molecular Recognition

Molecularly imprinted polymers (MIPs) as artificial antibodies are novel carriers synthesized by imprinting of a template molecule over a polymer, which will attract increasing interest to substitute for natural antibodies due to their favorable properties, such as low cost, high stability, and facile preparation.[53]
RPP can be employed to prepare MIPs with a typical process of mixing the functional monomers, template molecules, and crosslinker monomers sufficiently, and accelerating polymerization under the refluxing condition, followed by the removal of the template molecules. Chen and coworkers used RPP for the first time to fabricate uniform MIP microspheres, and demonstrated that molecular imprinting at high temperature is feasible when electrostatic interactions played main roles in the imprinting process. However, some vulnerable template molecules, such as proteins, might not be suitable for RPP approach due to the high reaction temperature.

As mentioned previously, RPP represents as a multifunctional approach to fabricate a variety of composite polymeric micro- or nanogels for recognizing small biomolecules such as polypeptides and proteins. Furthermore, the resulting nanogels via RPP may try to show their potential applications in detecting the even “bigger object.” The following section will describe a representative polymer particle prepared by RPP that has been demonstrated to have the potential for tumor cell detection.

5. Polymeric Composite Particles for Cell Detection

Circulating tumor cells (CTC) belongs to a subset group of aggressive cancer cells that are shedding from the primary tumor site, circulating in the blood stream, and acting as seeds for the development of fatal distant metastasis. The detection and evaluation of CTC in blood stream could provide information about tumor biology and have a significant prognostic value in clinical practice. However, there still remain challenges in accurate CTC analysis due to their extremely low abundance in blood. Nanostructures with distinct structural and functional features hold strong promise in CTC capture and identification. Li et al. designed SERS probe that equipped a spherical suspension chip as a novel strategy for CTC capture, recovery, and detection. The folate-modified PMAA shell coated magnetic supraparticle core was prepared and mixed with SERS probe/folate comodified microsphere to identify and extract interested folate receptor-expressing tumor cells from biological samples, taking advantage of Raman enhancing signal originating from the SERS probe. Thus, the combination of RPP and SERS allowed for the synthesis of novel probes that grafted from a polymer particle surface, indicating a useful tool to improve the sensitivity as well as contribute to clinically viable CTC detection.

6. Conclusion and Future Perspectives

In this article, we summarized recent advances in RPP as a novel synthetic platform for the preparation of uniform polymeric microgels or nanogels and demonstrated their promising potential in biomedical applications. Compared to conventional precipitation polymerization, RPP not only facilitates the synthesis of narrowly distributed particles, but also is convenient for manipulation and application of multiple functional monomers, including methacrylic acid, glycidyl methacrylate, HEMA, VIM, and other related monomers. Owing to the convenient process and pure system, RPP has proven to be an outstanding technique for the fabrication of multiple functional microgels or nanogels with precisely controlled structures, modular functionalities, and desirable payload encapsulation.

Therefore, the RPP platform opens a route for its future applications in various areas as follows: 1) Photonic crystal—A photonic crystal with a periodic optical nanostructure can be achieved by the uniform monodisperse polymer particles. RPP could be introduced to synthesize these polymer opals, followed by further compression between two pieces of polyethylene terephthalate (PET) foil in order to obtain a photonic crystal film, which can be further processed to prepare various optical films for potential applications in automobile, architectural, and other decorative areas. 2) Gene delivery vehicles—When a cationic acrylic monomer such as 2-(diethylamino) ethyl methacrylate is employed in RPP, the synthetic cationic polymer nanogels from RPP could combine with plasmid or small interfering RNA to form complexes, exhibiting promising potential in gene delivery field.

RPP is a novel and robust polymerization technique for the preparation of versatile functional materials. Perhaps one of the best features of RPP is the preparation of complex architectures in a facile manner as indicated by the continuous growth in a number of publications and citations, demonstrating the feasibility of this novel polymerization technique. Furthermore, the
combined use of RPP with other strategies is of great interest for the next generation of novel multifunctional nanostructures to meet requirement in multidisciplinary applications, such as co-delivery system of antigen or adjuvant for immunotherapy, and catalyst carriers for chemical manufacturing, holding great promise for the fabrication of even more advanced functional materials.

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Conflict of Interest

The authors declare no conflict of interest.

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