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Synthesis and Biomedical Applications of Poly((meth)acrylic acid) Brushes

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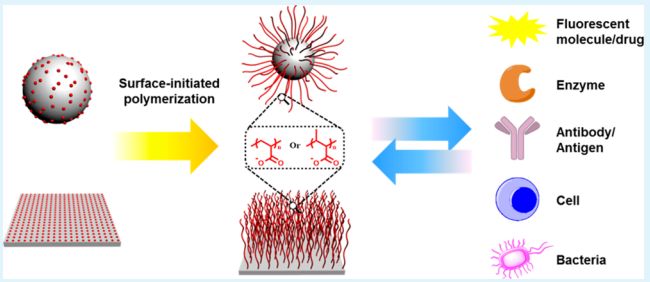
ABSTRACT: Poly((meth)acrylic acid) (P(M)AA) brushes possess a number of distinctive properties that are particularly attractive for biomedical applications. This minireview summarizes recent advances in the synthesis and biomedical applications of P(M)AA brushes and brushes containing P(M)AA segments. First, we review different surface-initiated polymerization (SIP) methods, with a focus on recent progress in the surface-initiated controlled/living radical polymerization (SI-CLRP) techniques used to generate P(M)AA brushes with a tailored structure. Next, we discuss biomolecule immobilization methods for P(M)AA brushes, including physical adsorption, covalent binding, and affinity interactions. Finally, typical biomedical applications of P(M)AA brushes are reviewed, and their performance is discussed based on their unique properties. We conclude that P(M)AA brushes are promising biomaterials, and more potential biomedical applications are expected to emerge with the further development of synthetic techniques and increased understanding of their interactions with biological systems.



KEYWORDS: *poly(acrylic acid) brushes, poly(methacrylic acid) brushes, surface-initiated polymerization, protein immobilization, biomedical application*

1. INTRODUCTION

Polymer brushes form when polymer chains are attached by one end to a surface at high density such that they are forced to adopt a stretched, brush-like morphology.[1−3] Polymer brushes offer great flexibility in regulating surface properties and incorporating functionalities via the introduction of three-dimensional (3D) architectures on conventional two-dimensional (2D) surfaces. Indeed, the past decade has witnessed great growth in this field, and a variety of polymer brushes with different compositions and morphologies have emerged.[4] These brushes have been applied broadly across many areas of research, including surface science, nanotechnology, and biotechnology.[5]

Poly(acrylic acid) (PAA) or poly(methacrylic acid) (PMAA) brushes are the simplest anionic polyelectrolyte brushes. The presence of carboxyl groups on their repeat units endows P(M)AA (PAA or PMAA) brushes with a number of unique properties: (1) the ionization of carboxyl groups results in a high swelling of P(M)AA brushes in aqueous solution, the cause of which has been theoretically predicted[6,7] and experimentally demonstrated[8,9] to be high osmotic pressure within the brushes generated by counterion localization (also termed the "Donnan effect"); (2) P(M)AA brushes, which are typical weak polyelectrolyte brushes, respond to both pH and ionic strength in a closely interrelated manner, making P(M)AA brushes a responsive system under study for fundamental research[10−20] and relevant applications;[21−27] (3) the abundant carboxyl groups on P(M)AA brushes create numerous possibilities for postmodification or bioconjugation to extend their functionality as bioactive materials;[28] and (4) in the spherical brush system, attachment of P(M)AA chains confers excellent dispersity to nanoparticles due to both electrostatic and steric stabilization effects.

This minireview will focus on recent advances in the synthesis and biomedical applications of P(M)AA brushes (including brushes containing P(M)AA segments). While P(M)AA brushes share some general features with other brush systems, we will focus on recent progress concerning synthesis strategy as well as the biomolecule immobilization and biomedical application of P(M)AA brushes. We will highlight the unique features of P(M)AA brushes and the applications that are well-served by with their unique properties. To obtain a broad view of the field, readers are directed to recent comprehensive reviews concerning the synthesis,[4] biomolecule immobilization,[28] and biomedical application of polymer brushes.[5,29,30]

2. SYNTHESIS

The synthesis of P(M)AA brushes has much in common with the synthesis of polymer brushes in general and mainly utilizes the "graft-from" and "graft-to" strategies.[31] The graft-from strategy is more commonly used because it generates polymer brushes of higher density and thickness.[4,32] With the rapid development of the surface-initiated polymerization (SIP) technique, and surface-initiated controlled/living radical polymerization (SI-CLRP) in particular, polymer brushes can

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be synthesized with an unprecedented level of control and versatility.[4,32,33] For the synthesis of P(M)AA brushes, the compatibility of carboxyl groups with the polymerization technique is an important consideration. Accordingly, when a compatibility issue exists, polymerization of a protected monomer followed by a deprotection step is usually conducted.[34] In this section we will focus on the synthesis of P(M)AA brushes via the graft-from or SIP processes. Relevant work from the past decade is summarized in Table 1, and the synthesis of P(M)AA brushes via different SIP processes is schematically illustrated in Figure 1.

2.1. Polymerization of a Protected Monomer. Of the various SIP strategies, atom transfer radical polymerization (ATRP), a representative controlled/living radical polymerization (CLRP) technique, is currently the most popular for the synthesis of P(M)AA brushes (Table 1). Nevertheless, ATRP is unable to polymerize (M)AA monomer directly because the carboxyl groups poison the catalysts by coordinating to the transition metal.[87] Consequently, *tert*-butyl (meth)acrylate (tB(M)A), a protected monomer for (M)AA, is often polymerized and then deproteced via hydrolysis or pyrolysis to recover P(M)AA brushes (Figure 1). Of the various hydrolysis catalysts developed thus far, trifluoroacetic acid (TFA) is the most widely adopted, although methanesulfonic acid (MA) seems to be the most efficient (Table 1). A kinetic study revealed that 1 min was sufficient to quantitatively convert PtBA into PAA in the presence of MA as a catalyst.[40] In addition, the *tert*-butyl group can be removed upon thermal treatment, which provides an alternative when the materials used are vulnerable to an acidic environment. On the other hand, SI-ATRP of (M)AANa in aqueous solution followed by protonation is also feasible (Table 1). This is an ideal option when both an acidic environment and thermal treatment result in undesirable effects.[66] Finally, it is worth mentioning that 2-(methacryloyloxy)ethyl succinate, a commercially available acidic monomer, can be directly polymerized by SI-ATRP to form poly(2-(methacryloyloxy)ethyl succinate) (PMES) brushes—at present, the only example of direct SI-ATRP of an acidic monomer.[88]

The popularity of SI-ATRP is largely attributed to its robustness and versatility: the initiator (generally the α-bromoester derivative) can be easily immobilized on the substrate either by directly immobilizing presynthesized functional ATRP initiator (e.g., silane[14−16,25,35,38,60,89] or thiol functionalized[36,43,68]) or by conjugation of ATRP initiator (e.g., 2-bromoisobutyrate bromide (BiBB)[42,44,46,64]) to a hydroxyl- or amino-functionalized surface. The polymerization can be performed over a wide range of conditions in combination with various polymerization methods (e.g., SI-NMP[63] or SI-RAFT[80]) or surface modification techniques to produce tailor-made polymer brushes. In particular, the combination of SI-ATRP and micropatterning allows for extremely precise control over the spatial distribution and composition of planar polymer brushes.[66−69] For instance, Zhou et al. developed a generic synthetic methodology for generating multicomponent patterned brushes based on repeated initiator patterning, SI-ATRP and ATRP passivation.[68] Furthermore, the synthesis of four polymer brushes with distinctive properties on a single stamp has been demonstrated (Figure 2A). Ionov et al. reported the fabrication of responsive (or "smart") patterns with sizes that could be controlled by temperature.[69] Their synthsis was achieved through the generation of patterned thermoresponsive *N*-isopropylacrylamide (NIPAm)-tBA-AA triblock copolymer brushes via SI-ATRP, where the lower critical soluble temperature (LCST) of the copolymer was effectively tuned by changing the fraction of PtBA and PAA (Figure 2B). The presence of P(M)AA segments endowed polymer brushes with switching properties, including hydrophilicity/hydrophobicity,[63,64,69,80] positive/negative charge,[18,20,22,60,62] and thermo-/pH-responsiveness.[61] These brushes are of particular interest for regulating surface properties and responding to environmental and biological stimuli.

Contamination of metal catalyst in the SI-ATRP process is a general concern, especially when the materials generated are used in biomedical fields. In this respect, the recent development of activator regenerated by electron transfer (ARGET)-ATRP allows the reduction of metal catalyst to the order of a few parts per million, in addition to resulting in a higher tolerance to oxygen.[90−92] This ATRP variant has also been applied to the SIP process to produce brushes containing PAA segments.[44,62]

2.2. Direct Polymerization. Direct synthesis of P(M)AA brushes is highly desirable because it not only reduces the synthetic steps involved but also eliminates the possibility of incomplete deprotection and of any potential negative effects of the deprotection process. Classical conventional radical polymerization (CRP) is capable of polymerizing (M)AA directly. Despite clear weaknesses in generating narrowly distributed polymers and constructing complex architecture when compared with SI-CLRP, surface-initiated conventional radical polymerization (SI-CRP) is still used frequently because of its simplicity. A number of initiation methods are available for SI-CRP (Table 1). In addition to the traditional method mediated by radical initiator, SI-CRP can be initiated by UV irradiation,[10,70−72] which allows efficient polymerization at low temperature. Alternatively, plasma treatment provides an easy way to introduce a radical initiator to a planar substrate.[74,75]

Surface-initiated reversible addition−fragmentation chain transfer polymerization (SI-RAFT) and surface-initiated photoiniferter-mediated polymerization (SI-PIMP)—two other promising SI-CLRP techniques—are capable of producing P(M)AA brushes in a controlled manner. RAFT polymerization maintains the greatest similarity to CRP. The only difference is that RAFT chain transfer agent (CTA) is added to control polymerization via a reversible chain transfer reaction.[93] For SI-RAFT polymerization, the RAFT CTA can be immobilized either by its R group or by its Z group, where the "R-group approach" is similar to the graft-from strategy and the "Z-group approach" resembles the graft-to strategy.[33] In comparison with SI-ATRP, a clear limitation of SI-RAFT lies in difficulties with the synthesis and surface immobilization of RAFT CTA (although recently some RAFT CTAs have become commercially available[94,95]), which often involve multiple synthetic or surface derivation steps.[4] In fact, surface-immobilized RAFT CTA is often derived from its ATRP precursor.[80] With the goal of simplifying the SI-RAFT synthetic process, our group recently proposed a method for the one-pot synthesis of RAFT CTA with a silane group concomitantly incorporated in its R group.[78] Thus, immobilization of RAFT CTA was simplified as a routine silanization reaction to allow for the direct preparation of PAA brushes via the R-group approach (Figure 3).

SI-PIMP relies on the use of iniferters that decompose under UV irradiation and functions as simultaneously initiator, transfer agent, and terminator.[96] Control of the location, intensity and duration of UV irradiation offers a convenient way to adjust the polymerization kinetics and the resultant brush structures. For example, patterned gradient PMAA brushes were synthesized by gradient exposure to UV irradiation at different positions of the silicon wafer.[85] However, the process utilized an uncontrolled radical polymerization mechanism to produce a gradient of grafting density rather than brush thickness due to insufficient iniferter deactivator. Zapotoczny et al. reported the use of a combination of SI-PIMP and "dip-pen" nanolithography to prepare PMAA brushes of designated width and height, thus highlighting its strength as a method for the production of tailor-made architectures.[86]

3. IMMOBILIZATION OF BIOMOLECULES

The immobilization of biomolecules—mainly protein—tailors materials for their biomedical applications, which both endows the materials with biological functionality and facilitates the manipulation of biomolecules. In terms of immobilizing biomolecules into 3D polymer brushes, three modes have been recognized: primary interaction with the underlying substrate, secondary interaction with the outer brush sites, and ternary interaction with the inner brush sites[29,97] (Figure 4a). P(M)AA brushes, which exhibit high swelling in aqueous solutions and abundant binding sites, have the potential to bind multilayer biomolecules through ternary interaction with exceptionally high capacity. In terms of immobilization physics and chemistry, three methods have been developed for P(M)AA brushes

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Table 1. Synthesis of P(M)AA Brushes via Different SIP Processes[a]

method	polymer[b]	initiation condition	monomer	deprotection condition[c]
surface-initiated atom transfer radical polymerization (SI-ATRP)	H[15−17,20,24,25,35−55]	ATRP initiator	tB(M)A[14,16−18,22,35−38,40−44,46,47,50−52,54,56,57,59,60,63,64,68,69]	hydrolysis: TFA (6−24 h in DCM or TCM)[16,41−43,47,50,54,56,57,60]
	C[17,18,56−62]			
	M[22,63,64]			hydrolysis: MA (2−15 min in DCM)[36,37,40,46,64,69]
	G[14,61,65]			hydrolysis: ITMS (6 h in DCM)[63]
	P[66−69]			hydrolysis: HCl (reflux 5−24 h in dioxane)[14,44]
				pyrolysis: (200 °C, 0.5−2 h)[17,18,35,38,59]
			(M)AANa[15,20,24,25,39,45,49,53,55,61,62,65−67]	protonation
surface-initiated-conventional radical polymerization (SI-CRP)	H[10,70−75]	radical initiator[26,73]	AA	not necessary
	C[26]	UV irradiation[10,70−72]		
		plasma treatment[74,75]		
surface-initiated reversible addition−fragmentation chain transfer polymerization (SI-RAFT)	H[21,76−79]	radical initiator	AA	not necessary
	C[80]			
surface-initiated photoiniferter-mediated polymerization (SI-PIMP)	H[81,82]	iniferter (photoinitiation)	AA	not necessary
	C[83,84]			
	G[85]			
	P[85,86]			

[a]Relevant works from the past decade are summarized. [b]H, C, M, G, and P signify homopolymer, copolymer, mixed polymer, gradient polymer, and patterned brushes. [c]DCM, dichloromethane; TCM, trichloromethane; MA, methanesulfonic acid; ITMS, iodotrimethylsilane.

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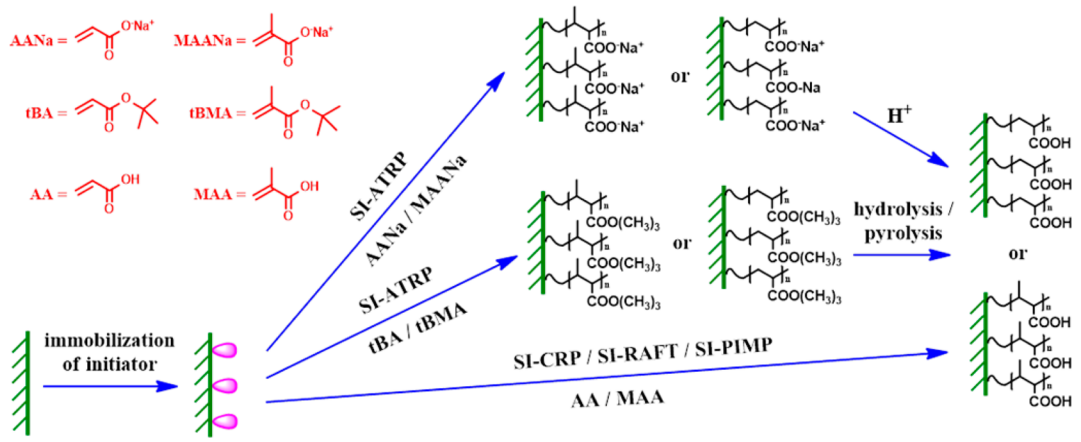

Figure 1. Illustration of the synthesis of P(M)AA brushes via different SIP processes.

thus far: physical adsorption, covalent binding, and affinity interaction (Figure 4b).

3.1. Physical Adsorption. Strong adsorption via electrostatic interaction occurs when P(M)AA brushes are mixed with protein at a low ionic strength and a suitable pH. This phenomenon was found to be universal for polyelectrolyte brushes[98−101] and is uniquely related to their ability to confine counterions. The most unusual characteristic of these brushes is that high affinity adsorption of proteins can occur even on the "wrong" side of their isoelectric point[102] when the brushes and proteins have the same charge. Two factors were found to be responsible for this issue: charge reversal[103−105] and counterion release.[106−110] The former condition occurs when a protein reverses its charge upon entry into a brush with a lower local pH, while the latter revealed that the uptake of protein was driven by the increase in entropy resulting from a concomitant release of localized counterions within brushes (Figure 5a).[107] Recently, a theoretical consideration combining both explanations has been reported.[111]

Several distinctive features are credited for this physical adsorption: (i) The proteins must enter the P(M)AA brushes to achieve a strong ternary interaction with the P(M)AA brushes, which naturally leads to a multilayer immobilization with high binding capacity (Figure 5b).[98−100,112] The binding capacity was found to increase with increasing brush thickness and grafting density and achieved higher than 30 vol % of the brush layer.[103] Notably, the immobilization (16.2 $\mu g/nm^2$) of 80 monolayers of lysozyme was reported in a planar PAA brush system.[37] A similar high protein binding capacity was also found in PMES brush systems.[88,113] (ii) The adsorption was strong enough to tolerate repeated washing in favorable conditions, while the adsorbed protein could be washed off in a well-defined manner by changing the ionic strength and pH. This endowed PAA brushes with a switching affinity for proteins modulated by ionic strength and pH.[106,114] The switching property was also observed in a poly(ethylene oxide) (PEO)/PAA mixed brush system, as the PEO segments were inherently protein-repellent.[115,116] (iii) The function of protein was largely preserved after immobilization,[46,117−120] which could be particularly attractive for downstream biomedical applications. In this regard, Fourier transform infrared (FT-IR) spectroscopy analysis revealed that a number of proteins retained their secondary structures after immobilization in both spherical and planar PAA brushes.[121,122] These properties make physical adsorption particularly suitable for protein separation and purification,[73,101] where reversible immobilization is required. The limit of physical adsorption, however, is its susceptibility to changes of ionic strength and pH, which restricts the downstream application of brush−protein complexes in many fields.

3.2. Covalent Binding. Covalent binding results in a robust and irreversible linkage, wherein a biomolecule is permanently immobilized on the material during subsequent processes.[123] Covalent binding of proteins on P(M)AA brushes can be achieved by the classical *N*-hydroxysuccinimide/carbodiimide (NHS/EDC) coupling chemistry[124] between carboxyl groups of P(M)AA brushes and amino groups of biomolecules. Compared with conventional surfaces, a higher degree of biomolecule functionalization is achieved with P(M)AA brushes due to their 3D architecture and abundant carboxyl groups. Bovine serum albumin (BSA),[37,66] (strept)-avidin,[66,78] biotin,[43] antigen/antibody,[84,125,126] acetylcholinesterase,[127] ribonuclease A (RNase A),[38] glucose oxidase,[79] folic acid,[23,42] fluorescent protein,[44] arginine-glycine-aspartic acid (RGD) peptide,[61,81,85] collagen,[45,128] galactose,[10,72,129] and silk sericin[39] were successfully immobilized on P(M)AA brushes to impart different functionalities for various purposes, including biosensing,[43,84,125−127] catalysis,[38,79] targeting,[23,42] cell adhesion, and proliferation.[39,45,61,72,81,85,128,129]

Fundamental research devoted to the NHS/EDC process has revealed unique aspects of this process for 3D P(M)AA brushes. A comparative study revealed that, while the major species obtained after activation was NHS ester for PAA brushes (as is the usual case), the major species for PMAA brushes was anhydride.[130] This result would lead to a distinct difference in composition after amidation (Figure 6A(a)). The author hypothesized that the formation of a more stable six-member-ring chair conformation hydride in PMAA brushes was responsible for this difference (Figure 6A(b)). With respect to protein immobilization, an earlier attempt to covalently immobilize BSA resulted in a low capacity (<2 monolayers) with a low dependence on brush thickness.[37] This implies that BSA preferentially adopted a secondary interaction with PAA brushes due to steric hindrance. In a subsequent study, however, covalent immobilization of RNase A achieved up to 16 monolayers with a binding capacity that increased linearly with increasing brush thickness.[38] This discrepancy was illustrated in our recent mechanism study.[131] Protein immobilization was suppressed in conventional NHS/EDC process wherein the PAA brushes' "Donnan effect" was destroyed (by NHS

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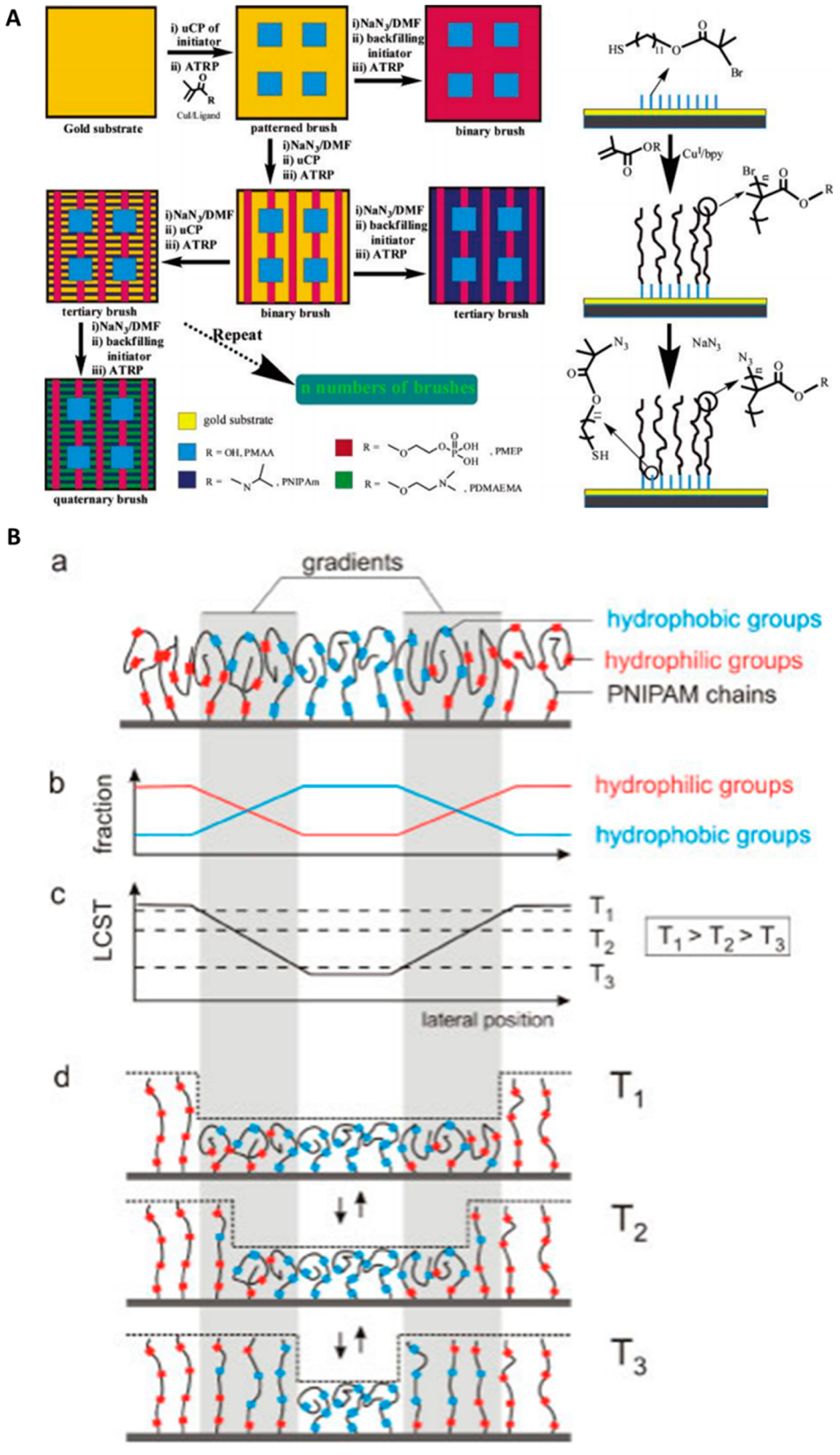

Figure 2. (A) Synthesis of multicomponent patterned brushes: an illustration of the synthetic procedure comprising repeated patterning of initiator, SI-ATRP, and passivation of ATRP active end group. Reproduced with permission of ref 68. Copyright 2006 American Chemical Society. (B) Concept of temperature-induced size-controlled patterns. (a and b) Lateral gradients of thermoresponsive copolymer with varied hydrophilicity or hydrophobicity were formed by SI-ATRP of NIPAm with different fractions of AA and tBA. (c) Variations in hydrophilicity led to a gradual change of LCST of the copolymer. (d) Conformational changes of the copolymer induced by temperature resulted in a surface with size-controlled patterns. Reproduced with permission from ref 69. Copyright 2011 John Wiley and Sons.

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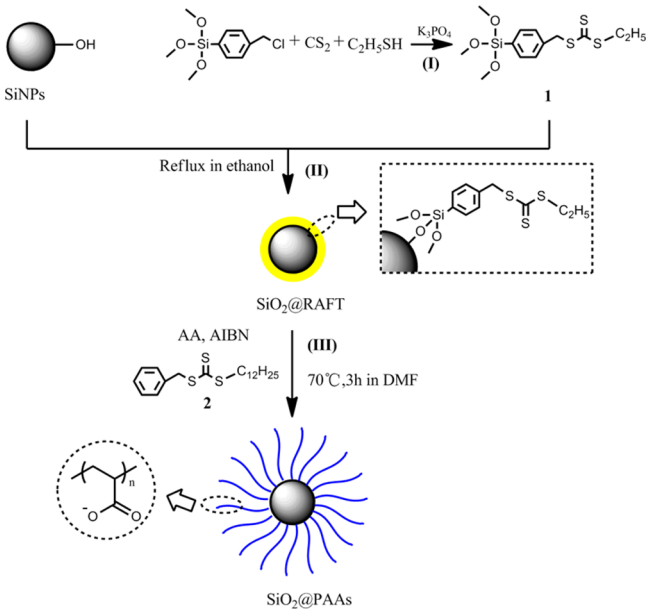

Figure 3. Synthetic route to PAA brushes via SI-RAFT. The SI-RAFT process was simplified by a one-pot synthesis of silane-functionalized RAFT CTA and by the immobilization of the R group through a routine silianization reaction. Reproduced with permission from ref 78. Copyright 2013 Elsevier.

activation) and the pH of conjugation buffer was unfavorable to electrostatic interaction. However, in a modified conjugation buffer (pH = 5.0), the protein binding capacity increased gradually with the hydrolysis of the NHS ester, thus clearly demonstrating that electrostatic interaction plays a dominant role in the covalent conjugation of protein (Figure 6B(a)). Accordingly, a "chemical conjugation after electrostatic entrapment" (CCEE) method was developed, wherein chemical conjugation was performed after the uptake of protein utilizing the unique Donnan effect of PAA brushes. A linear increase in the binding capacity versus brush thickness with an ultrahigh capacity of up to 4.2 mg of BSA/(mg of brushes) was realized for the CCEE method, much higher than that achievable by NHS/EDC process (Figure 6B(b)).[131] In a more recent work, we leveraged the CCEE method to achieve covalent immobilization of horseradish peroxidase (HRP) with an improved retention of enzyme activity.[132] These results highlighted the critical influence of morphology and neighbor groups on the chemical reaction in 3D materials. Considering the wide application of covalent binding processes, more fundamental research is needed to thoroughly illuminate this topic.

3.3. Affinity Interaction. One type of affinity interaction is based on the coordination bond between chelated metal ions on the materials and histidine residues on the proteins. Initially used in affinity chromatography,[133,134] it has also found wide applications in biosensing[24,135] and protein purification.[136,137] Dai et al. first transferred this method into the PAA brush system.[37] PAA brushes were modified with NTA to form immobilized chelates with Cu^{2+}, which were used to capture the protein through an NTA-Cu^{2+}-histidine coordination bond. A high immobilization capacity was achieved for a number of proteins. Subsequently, Cullen et al. employed both the NHS/EDC process and the affinity interaction method to immobilize RNase A.[38] The results showed that while the metal-ion affinity interaction provided a higher binding capacity, covalent coupling was better for maintaining the activity of the immobilized enzyme, thus highlighting the importance of binding chemistry for enzyme binding capacity and activity. One unique aspect of the metal-ion affinity interaction is that the immobilization is stable in aqueous solutions, while the immobilized protein can be recovered by washing with ethylenediaminetetraacetic acid (EDTA). Taking advantage of this property, a reusable biosensing platform has been developed based on selective immobilization of His-tagged protein in NTA modified PMAA brushes.[24]

Another type of affinity interaction utilizing the (strept)-avidin−biotin interaction was reported by Dong et al. on a patterned PAA brush system,[66] wherein an avidin was first attached to PAA brushes via the NHS/EDC process to specifically capture biotinylated biomolecules. Immobilization through the (strept)avidin−biotin affinity interaction is expected to better preserve protein functionality than direct covalent conjugation.

4. BIOMEDICAL APPLICATIONS

In comparison with conventional surfaces, P(M)AA brushes exhibit a number of unique properties with respect to their regulated structure, flexible 3D architecture, high-density negative charge, responsiveness, and rich carboxyl groups. These properties are expected to provide superior performance in a variety of biomedical applications. Based on the aforementioned developments in synthesis and biomolecule immobilization, some promising areas of application have been identified.

4.1. Enzyme Immobilization. Enzymes are functional proteins with high catalytic efficiencies. Thus, the principle of protein immobilization by P(M)AA brushes can be directly applied to enzyme immobilization. P(M)AA brushes have been shown to be superb carriers for enzyme immobilization via electrostatic interaction, which provided increased binding capacity and improved activity compared with conventional carriers.[117,119,120,138] Utilizing these properties, Kudina et al. demonstrated a versatile biocatalysis enzymogel system by immobilizing cellulase (CEL) in 100 nm spherical PAA brushes.[46] The catalytic efficiency was improved dramatically by the enhanced loading capacity, enzyme activity, contact area, and enzyme mobility (Figure 7) of the system. The enzymogel shows potential for biofuel and biomedical production because immobilized CEL converts cellulose into glucose efficiently. Xu et al. developed a magnetic spherical PAA brush platform to achieve efficient enzyme immobilization with easy recycling, which holds great promise for enzyme catalysis, separation, purification, and reuse.[139] Immobilization of RNase A was also achieved by covalent conjugation and affinity interaction (see section 3.3).[38] Here, because the RNA substrate of RNase A is a macromolecule, its diffusion barrier of the substrate would increase with increasing enzyme binding capacity. To achieve the highest overall catalytic efficiency of the brush−enzyme complex, the enzyme binding capacity had to be optimized. The author further demonstrates the feasibility of using RNase A-functionalized beads in plasmid DNA purification.

4.2. Controlled Release and Delivery. A smart system for controlled release is generated when responsive polymers are grafted on the surface of porous particles to act as molecular gatekeepers.[5,140−142] In particular, PAA brushes with a core of mesoporous silica (MSN) nanoparticle have been shown to be an effective pH-responsive system, wherein the uptake and release of guest molecules (e.g., drugs, fluorescent molecules, or DNA) by an MSN nanocontainer is regulated by surface PAA chains in response to pH (Figure 8).[21] Alternatively, drugs can

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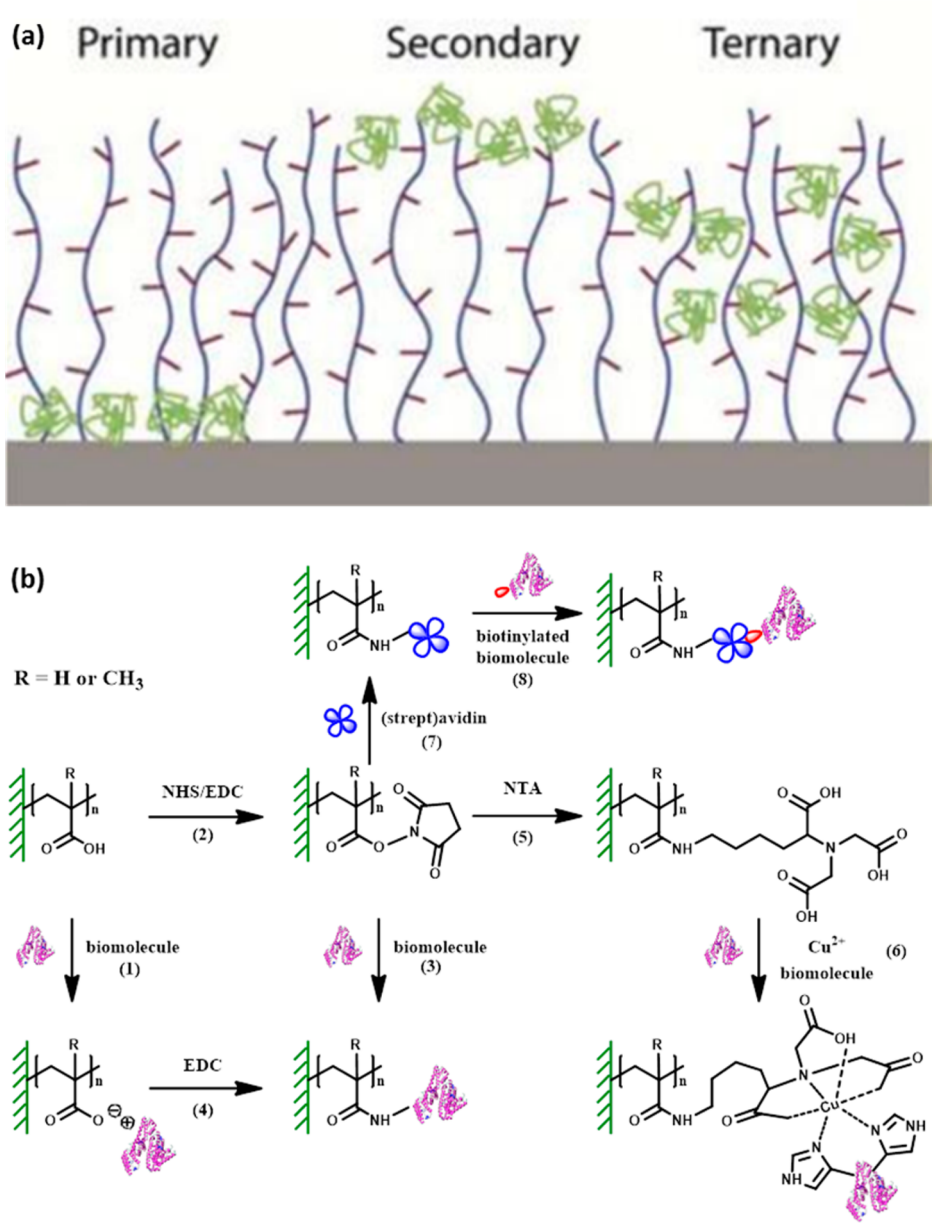

Figure 4. (a) Modes of immobilization of biomolecules into 3D brushes. Reproduced with permission of ref 29. Copyright 2014 American Chemical Society. (b) Immobilization methods of biomolecules to P(M)AA brushes: (I) Physical adsorption, step 1; (II) covalent immobilization via *N*-hydroxysuccinimide/carbodiimide (NHS/EDC) coupling chemistry, steps 2 and 3, and chemical conjugation after electrostatic entrapment (CCEE) method, steps 1 and 4; (III) affinity interaction via metal−ion interaction, steps 2, 5, and 6, and (strept)avidin−biotin interaction, steps 2, 7, and 8. See text for detailed explanations.

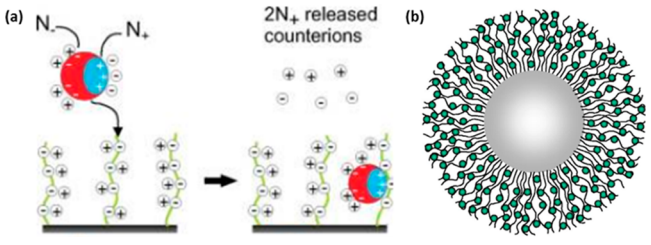

Figure 5. (a) Illustration of the counterion release mechanism leading to physical adsorption of protein into P(M)AA brushes. Reproduced with permission from ref 107. Copyright 2010 American Chemical Society. (b) Schematic representation of multilayer protein immobilization in spherical PAA brushes. Reproduced with permission from ref 112. Copyright 2004 American Physical Society.

be loaded in the brush layer,[23] and PAA brushes can be further conjugated with a targeting agent to enable targeted delivery.[42] By combining these two elements, a multifunctional, thermal- and pH-dual-responsive platform for controlled drug release and targeted delivery was developed using PNIPAm-PAA copolymer brushes as the carrier;[23] this system exhibited great potential for cancer therapy. Additionally, Motornov et al. demonstrated that mixed polyanionic/polycationic (PAA-poly-(2-vinylpyridine)) brushes possessed switching properties for controlling the permeability of positive and negative ions as a function of pH.[143] This in principle could be used to develop a smart delivery system for drugs containing positively and negatively charged species.

4.3. Biosensing. Recent advances in nanotechnology provide numerous examples of how well-designed and finely

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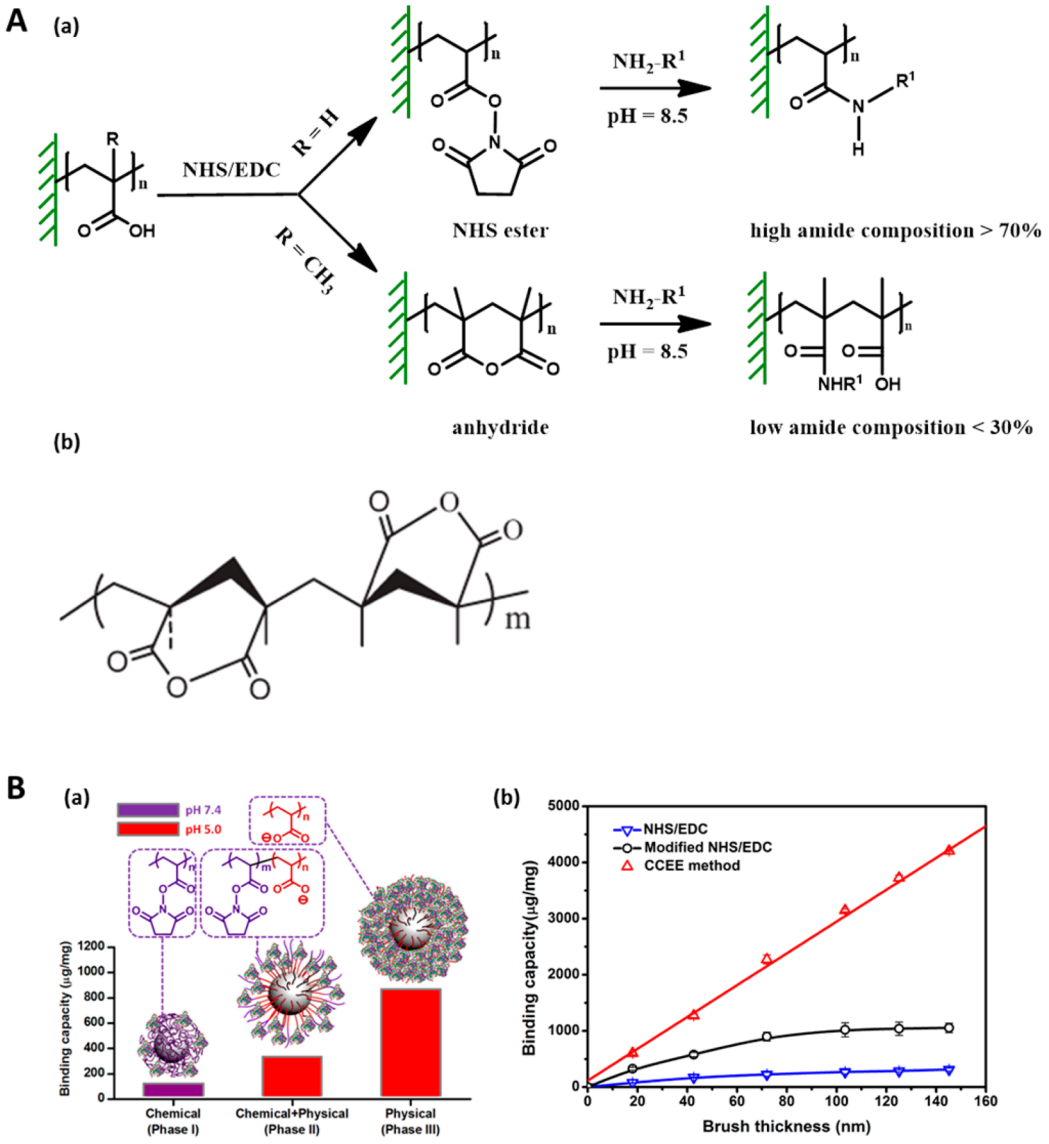

Figure 6. (A) Comparative study on NHS/EDC process for PAA and PMAA brushes: (a) Difference major species obtained for PAA and PMAA brushes after activation, which led to different amide composition; (b) proposed six-member-ring chair conformation of PMAA anhydride. Reproduced with permission from ref 130. Copyright 2011 American Chemical Society. (B) Mechanism study on covalent immobilization of proteins in PAA brushes: (a) Illustration on the brush structure and protein binding capacity obtained in difference processes and conditions; (b) covalent immobilization of BSA in PAA brushes with different thicknesses via CCEE method, conventional NHS/EDC method (pH = 7.4), and modified NHS/EDC method (pH = 5.0). Reproduced with permission from ref 131. Copyright 2014 American Chemical Society.

tuned nanostructures contribute greatly to the enhanced performance of biosensors.[144−147] As a representative example, P(M)AA brushes were recognized as an ideal candidate due to their ability to immobilize multilayer biomolecules.[37,43,126] To maximize the advantage of their 3D architecture, the morphology of brushes and their immobilization capacity are two critical parameters to optimize the accessibility of the immobilized biomolecules on the interior.[43] Patterned PAA brushes, with large space between PAA brush "islands", were better than both unpatterned PAA brushes and conventional 2D surfaces in immobilizing immunoglobulin G (IgG) and its subsequent recognition by anti-IgG antibody due to steric effects.[125] Meanwhile, P(M)AA brushes are inherently protein-resistant under physiological conditions,[43,110,114,125,135] which is particularly attractive for the reduction of biosensor background signals. Another advantage lies in the robustness and flexibility of the brush platform for the simultaneous regulation of surface properties and the incorporation of multifunctionality. Ma et al. developed interesting dual-functional two-layer PEGMA-PAA brushes, wherein the upper PAA layer enhanced antibody immobilization and the bottom PEGMA layer suppressed nonspecific adsorption.[84] Hess et al. fabricated a biosensing system by growing PDMAEMA-PAA block copolymer brushes on a graphene transistor. The PAA block was used to immobilize an enzyme, while the PDMAEMA block response to pH change was induced by the enzymatic reaction to generate a signal.[127]

Spherical PAA brushes can also be used as novel labels in biosensors. Our group[132] demonstrated an ultrasensitive ELISA system in which conventional enzyme-labeled antibody was replaced with functionalized spherical PAA brushes (Figure 9). The inner and outer spaces of the PAA brushes were selectively modified with HRP and antibody via CCEE and NHS/EDC processes, respectively, thus imparting the dual functionality of recognizing an analyte and generating a signal. As a highly

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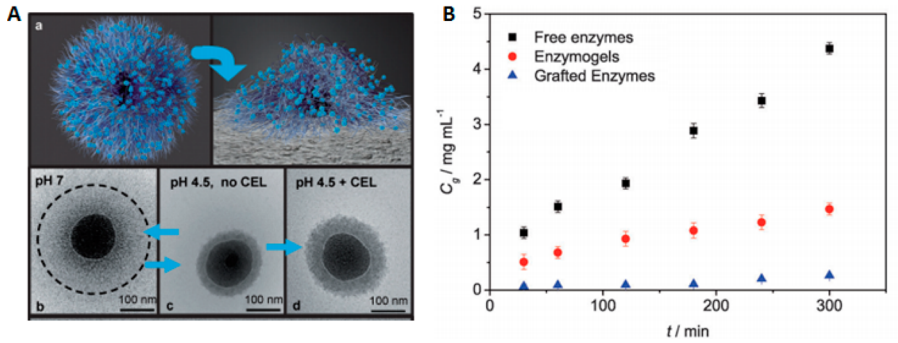

Figure 7. (A) Enzyme-loaded nanospherical PAA brushes (enzymogel) for efficient biocatalysis: (a) Schematic representation of enzymogel morphology in solution and when spread on a solid substrate (spreading of the polymer brushes provided enhanced contact area with the substrate); (b–d) cryo-TEM of PAA brushes(b) in the swollen and (c) in the shrunken states and (d) after loading with CEL. (B) Catalysis kinetics of glucose shown as glucose concentration (C_g) versus time (t) using free enzymes, enzymogel, and CEL grafted to silica particles (grafted enzymes). Reproduced with permission from ref 46. Copyright 2014 John Wiley and Sons.

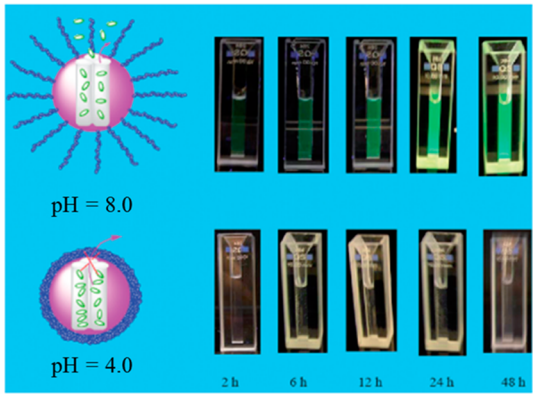

Figure 8. Smart pH-responsive controlled release system that uses MSN as a container and PAA brushes as molecular gatekeepers. Fluorescent molecules were encapsulated in MSN under acidic conditions when PAA brushes were collapsed, and released under alkaline conditions when PAA brushes were stretched. Reproduced with permission from ref 21. Copyright 2009 Royal Society of Chemistry.

efficient enzyme carrier, the PAA brushes afforded a dramatic amplification of signal, which was converted into a 267-fold improvement in detection sensitivity. The amplification effect was higher than that of conventional silica nanoparticles of similar size[148−150] and was comparable to other functional particle labels with enhanced enzyme loading capacities (e.g., mesoporous silica particles,[151] hollow particles,[152,153] or micrometer-sized magnetic beads[154]). The PAA brush labels are expected to be a versatile signal amplifier in a variety of biosensing platforms.

4.4. Cell Adhesion and Proliferation. Cell adhesion and proliferation is a central issue for a variety of modern biotechnologies, including tissue engineering and the development of cell-based sensors.[155] P(M)AA brushes are generally considered to be cell-resistant[61,85,156] but can be functionalized by the addition of extracellular matrix (ECM) proteins (e.g., collagen[45,128,156,157]) or peptides (e.g., RGD[61,81,85]) to achieve cell-adhesive properties. Galactose, a ligand specific to the hepatocyte asialoglycoprotein receptor, has also been conjugated to P(M)AA brushes to promote hepatocyte adhesion.[72,129,158] The behaviors, functions, and morphologies of cells are greatly influenced by the topologies and surface properties of their substrates.[155] In this respect, P(M)AA brushes are an excellent model material due to their well-defined 3D architecture and abundance of functional groups for the attachment of cell-adhesive agents.

With the refinement of surface modification and polymerization techniques, the architecture of P(M)AA brushes and their subsequent functionalization can be precisely tailored to provide an excellent substrate for the study of cell–material interactions. Surface-immobilized RGD with a density gradient was produced via covalent conjugation to gradient PMAA, and enhanced cell adhesion was obtained with increasing RGD density in a spatially controlled manner (Figure 10).[85] PNIPAm-PAA copolymer brushes with gradient in PAA thickness, synthesized by SI-ATRP, were also used as a substrate for the adhesion of HepG2 cells after RGD functionalization. In this case, optimal PAA thickness was obtained as a consequence of competition between cell-repellent PAA and cell-adhesive RGD.[61] This is consistent with an earlier report that a thin PAA brush performed better than a thick one for cell-adhesion and proliferation after immobilization of collagen.[156] The introduction of thermoresponsive PNIPAm block provided a convenient control for cell adhesion and detachment under different temperatures.[61,129] Selective functionalization of RGD inside PMAA brushes was also achieved by a chain extension step after immobilization, which induced a marked difference in the morphology of the adhered human osteoblasts compared with RGD immobilized on the tops of the brushes.[81] Interestingly, Chiang et al. discovered that the normally cell-repellent PAA brushes became cell-adhesive when patterned at a subcellular dimension to a suitable thickness.[67] This study indicated that the observed adhesion was associated with fibronectin that was secreted by the cells and absorbed by the brushes. Cellular response to these tailor-made 3D surfaces is an interesting and significant topic of research for both basic science and biomedical applications.

4.5. Antibacterial Surfaces. Materials with antibacterial surfaces are attractive in the food industry and for sanitary materials, household products, and medical supplies. Thus far, only a few studies of the application of P(M)AA brushes in constructing antibacterial surfaces exist. It has been reported that negative P(M)AA brushes themselves prevent the adhesion of bacteria to some extent.[39,159] Alternatively, P(M)AA brushes could be further functionalized with an antibacterial agent to improve their antibacterial properties.[41,74,75,159] For instance, a PAA brush of modified cellulose paper was decorated by silver nanoparticles via *in situ* reduction of Ag^+ entrapped in the PAA brush matrix, and subsequently exhibited an improved inhibition of *Escherichia coli* (*E. coli*) compared to pristine PAA brushes or cellulose paper alone.[41]

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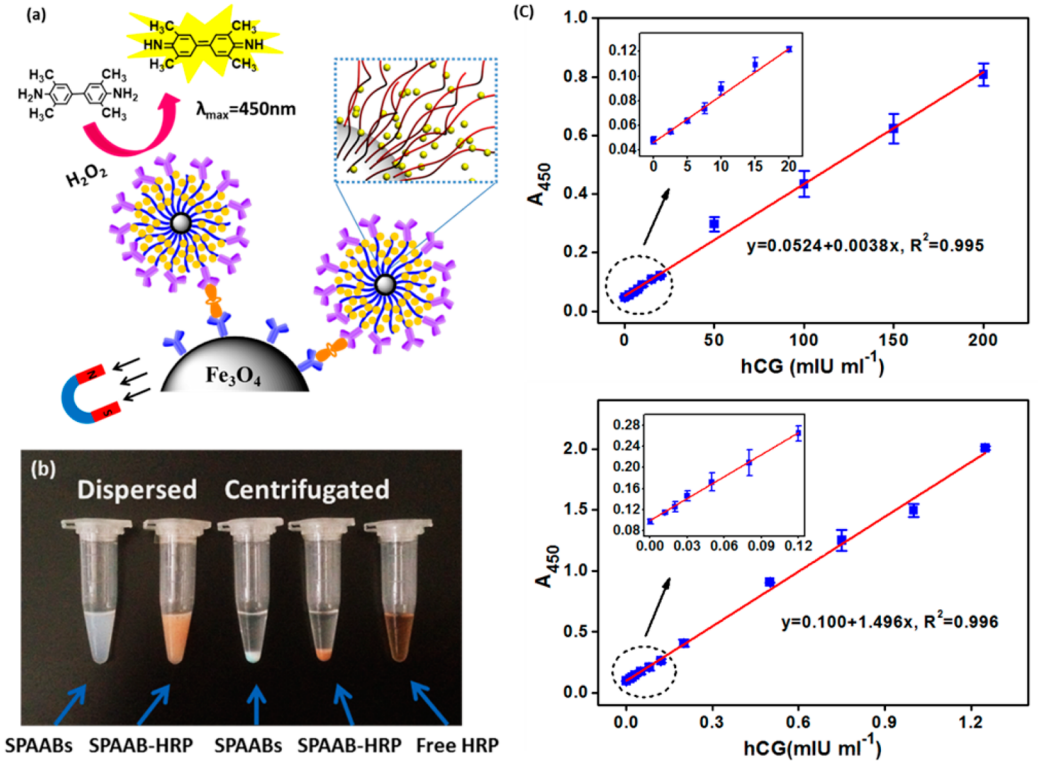

Figure 9. Brush-amplified ELISA with enzyme- and antibody-functionalized spherical PAA brushes (SPAABs) as labels: (a) Schematic illustration; (b) photograph of SPAABs before and after HRP loading (the characteristic color of HRP was evident in the SPAAB-HRP complex); (c) detection profile of human chorionic gonadotrophin by conventional ELISA (upper) and brush-amplified ELISA (lower) systems. Reproduced with permission from ref 132. Copyright 2014 American Chemical Society.

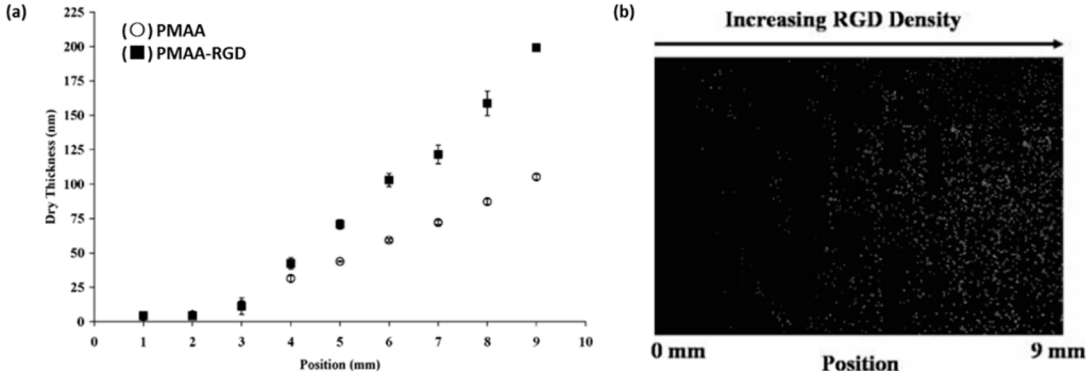

Figure 10. RGD-functionalized density gradient PMAA brushes for cell adhesion: (a) ellipsometric dry thickness versus the position of the PMAA brushes before and after RGD immobilization; (b) cell culture image of a PMAA-RGD-modified film (the cells appear as small white dots on the black background). Reproduced with permission from ref 85. Copyright 2006 American Chemical Society.

5. CONCLUSION AND OUTLOOK

Following early theoretical and experimental studies, the past decade's research has rapidly improved P(M)AA brushes by focusing on their synthesis, properties, biomolecule immobilization, and relevant biomedical applications. Notably, state-of-the-art SI-CLRP and surface modification techniques have allowed the construction of very complex brush architectures. Hence, recent research interest has focused on copolymer or mixed polymer brushes containing P(M)AA segments with various morphologies to generate multifunctionality and "smart" properties. In this review, we have presented representative examples, but these brushes possess unlimited potential for development. Thus, we anticipate the emergence of more exquisitely designed P(M)AA brush architectures and a more profound understanding of their structure−property relationships. Novel properties can be obtained by a proper integration of unique responsiveness, high charge density, and rich functional groups properties of P(M)AA segments. Despite these encouraging advances, synthesis of P(M)AA brushes by direct SIP of unprotected (M)AA remain a challenge. Although a number of SIP techniques are available to polymerize (M)AA directly, the protected monomer method is still frequently used to improve control of the polymerization process and compatibility with the solid substrate. In addition, synthesis of very thick (M)AA brushes remains a difficult task.

From the viewpoint of biomedicine, current platforms have already benefited greatly from the precise structure control afforded by P(M)AA brushes and from insights into their

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interactions with biomolecules. In particular, great efforts have been devoted to clarifying the mechanism of the interactions between P(M)AA brushes and proteins. In contrast, the interactions between brushes and with nucleic acids have received limited attention. Additionally, more research must be conducted to illuminate the behavior of P(M)AA brushes (or brushes containing P(M)AA segments) when interacting with cells, bacteria, or other complex biological units. Superior performance has been observed for well-designed P(M)AA brush systems with switching properties when in contact with biological media; for example, high-density protein immobilization/antifouling, and cell-adhesive/antibacterial properties. More examples of such smart and multifunctional material platforms are expected to be available in the near future with the rapid development of synthetic techniques. However. the robustness of these newly emergent systems must be improved to meet the demands of their biomedical applications. This requires deeper insight not only into materials chemistry and physics but also into the biological response of the brushes in a highly complex biological environment. Research into these topics is just beginning, and the acquisition of novel material properties with respect to biomedical applications requires the joint effort of several different fields.

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Notes

The authors declare no competing financial interest.

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