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Application of Polydopamine in Biomedical Microfluidic Devices

Pojchanun Kanitthamniyom and Zhang Yi*

Singapore Centre for 3D Printing, School of Mechanical and Aerospace Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798; Email: yi_zhang@ntu.edu.sg; Tel: +65 67905590

Abstract Polydopamine (PDA) is a bioinspired material with tremendous potential for applications involving surface modifications. By simply immersing the substrate in the dopamine monomer solution, we are able to apply a hydrophilic and biofunctional PDA coating that adheres strongly to any surface, including (super)hydrophobic surface, with unprecedented ease. Using PDA, almost any materials can be immobilized on the surface in a single step by mixing them with the dopamine monomer solution. This review provides a comprehensive coverage of the applications of PDA in the device fabrication, surface modification, and biofunctionalization of biomedical microfluidic devices. While discussing the advantages and limitations of PDA, we pay special attention to its unique properties that specifically benefit biomedical microfluidic devices. We also discuss other potential applications of PDA beyond the current development. Through this review, we hope to promote PDA and encourage a broader adoption of PDA by the microfluidic community.

Keywords Polydopamine; Biomedical; Microfluidic; Surface modification; Surface functionalization

1. Introduction

Bivalvia Mollusca, or mussel in common terms, is well known for its unique ability to attach strongly to a wide variety of surfaces regardless of their physical and chemical properties (Dreyer et al. 2013; Ho and Ding 2013; Kim et al. 2011; Lee et al. 2007b; Liu et al. 2014; Lynge et al. 2011; Madhurakkat Perikamana et al. 2015; Salazar et al. 2016). Such a strong adhesion is a result of mussel adhesive proteins, which are rich in 3,4-dihydroxy-L-phenylalanine (DOPA) and lysine. This naturally occurring phenomenon has inspired the development of a novel coating material known as the polydopamine (PDA). PDA is the polymerized form of catechol such as DOPA or dopamine (Ball 2014). Similar to its naturally occurring counterpart secreted by the mussel, PDA is able to adhere strongly to almost all types of surfaces. A PDA coating can be applied with great ease by simply immersing the substrate in the solution of dopamine monomers which polymerize spontaneously in an alkaline environment and form a dark brown or black layer of PDA adherent to the substrate (Salazar et al. 2016). Even though the structure of PDA is still not well understood, it has been generally accepted that the reaction starts with the oxidation of dopamine followed by the cyclization and rearrangement, which yields an important intermediate called 5,6-dihydroxyindole (DHI) to initiate the polymerization (d'Ischia et al. 2014; d'Ischia et al. 2009; Liebscher et al. 2013). Lee *et al.* first demonstrated this

simple PDA coating approach by immersing several types of substrates into a solution containing 2mg/mL dopamine monomers in 10 mM Tris at pH 8.5 (Lee et al. 2007b). Subsequently, this facile approach has been widely adopted to modify the surface of many materials with PDA, including noble metals (Liang et al. 2011; Sileika et al. 2011), metals with native oxide surfaces (Dong et al. 2015), oxides (Lee et al. 2007b), semiconductors (Liu et al. 2013c), ceramics (Lee et al. 2007b), and synthetic polymers (Ling et al. 2016).

One of the most remarkable feature of PDA is its ability to adhere robustly to (super)hydrophobic substrates which have low surface energies. Consequently, PDA provides an easy tool to functionalize (super)hydrophobic surfaces, which are difficult to modify by traditional means due to their strong resistance to adhesion. PDA contains many hydrophilic functional groups such as amino, carboxyl, and hydroxyl groups (Zhang et al. 2013a). Once it adheres to the hydrophobic surface, PDA makes the surface hydrophilic. The hydrophilic PDA coating has been demonstrated on a variety of materials with strong hydrophobicity including graphene (Yan et al. 2013), gold (Kim et al. 2011), Teflon (Shen et al. 2015), and anodic aluminium oxide (AAO) (Kang et al. 2010). Because of its diverse functional groups, PDA can be protonated or deprotonated depending on the pH, thereby exhibiting zwitterionic properties. For example, in the low pH environment, the amino group protonates and displays positive charges. But in the high pH environment, the phenolic group deprotonates and displays negative charges (Liu et al. 2011; Yu et al. 2010). This hydrophilic PDA coating is also biocompatible, which is an important consideration for biomedical materials. Ku *et al.* showed that PDA could be applied to various types of cells such as fibroblasts, osteoblasts, neurons, and endothelial cells without showing cytotoxicity (Ku et al. 2010b). Many other investigations also proved that PDA-coated surface promoted cell adhesion and cell proliferation with low cytotoxic effect (Gao et al. 2013; Liu et al. 2013d; Postma et al. 2009). PDA does not only form a highly hydrophilic and biocompatible coating but also grafts many functional groups and enables a wide range of chemical conjugations to the surface (Chen et al. 2014). For example, the conjugation of trypsin to the cellulose paper by PDA makes the paper enzymatically active (Lee et al. 2009). The conjugation of oligonucleotides or aptamers immobilizes these probes to the solid substrate for biosensing (Fan et al. 2016; Qiang et al. 2015). PDA also exhibits strong ion chelation and redox capability (Liang et al. 2017). It is able to reduce metal

ions for electrodeless metal plating (Lee et al. 2007a). Lee *et al.* demonstrated Ag plating on the PDA-modified surface by immersing the PDA-coated substrate in the Ag⁺ solution. The PDA exhibits sufficient reductive capability for Ag⁺ hence does not require exogenous reducing agents. As a broad-spectrum antibiotic, the Ag antimicrobial coating has been applied using the same process with the assistance of PDA (Sileika et al. 2011). Besides Ag, PDA is used to synthesize and immobilized Au particles on the surface of microfluidic channel (Liang et al. 2011; Wood et al. 2013). PDA is also used to co-immobilize functional molecules for surface activation. A one-pot surface modification protocol has been developed by immersing the substrates in the solution containing both the functional molecules and dopamine monomers (Kang et al. 2012). As dopamine monomers polymerize, PDA and the functional molecules are co-deposited onto the surface. This simple one-pot protocol grafts a variety of functional groups onto the surface in addition to the intrinsic groups carried by PDA. For instance, molecules with tertiary amine group are co-immobilized with PDA for subsequent bio-silicification. Initiator molecules are co-deposited with PDA for surface-initiated atom transfer radical polymerization reactions.

Due to its unrivaled advantages, PDA has been widely

adopted as a simple surface modification technique for numerous applications (Lee et al. 2010; Liu et al. 2014; Salazar et al. 2016; Wang et al. 2015). Among these applications, the utilization of PDA in biomedical microfluidic devices has drawn a lot of attention recently (Kim et al. 2013b; Shi et al. 2014; You et al. 2012). Microfluidics is a well-established platform that is able to precisely control a minute amount of fluids. It is a valuable tool for both fundamental biological research and translational clinical applications. Due to its small scale, biomedical microfluidic devices substantially reduce the sample volume and reagent consumption, thereby acquiring a maximal amount of information from a limited amount of precious samples (Whitesides 2006). The small scale allows a high degree of parallelization for high-throughput assays without significantly increasing the device footprint. Furthermore, biomedical microfluidic devices match well with the scale of biological entities and provide a spatially and temporally controlled microenvironment for biological interactions. At the system level, biomedical microfluidic devices are integrated into a lab-on-a-chip system (also known as the micro total analysis system) to streamline complex assays for automated sample-to-answer tests.

Applications of PDA in Biomedical Microfluidic Devices

Device Fabrication and Surface Modification

Biofunctionalization

Fig. 1 Applications of PDA in biomedical microfluidic devices. Dopamine monomers spontaneously polymerize in the alkaline environment and form the PDA that adheres strongly to almost any surface, including the super(hydrophobic) surface. PDA renders the surface hydrophilic and introduces many functional groups for bioconjugation. This review covers two main functions of PDA in biomedical microfluidic devices. In the first section, we mainly focus on the use of PDA for device fabrication and surface modification (left panel). In the second section, we examine how convenient and efficient it is to biofunctionalized the microfluidic device using PDA (right panel). Insets adopted and modified from Ref.(Liang et al. 2011; Wood et al. 2013), (Kim et al. 2013b; Shi et al. 2014; You et al. 2012), (Feng et al. 2015) with the permission from American Chemical Society, Royal Society of Chemistry, Elsevier, John Wiley and Sons.

Post-fabrication surface modification is often required to functionalize the microfluidic device for biomedical applications (Gomez 2008; Sackmann et al. 2014). PDA has compelling advantages for surface modification of microfluidic devices over some traditional approaches. First, PDA introduces functional groups for bioconjugation. Molecules used as ligands, primers or enzymes can be directly conjugated to designated regions of biomedical microfluidic devices via the covalent bond to PDA. Second, thin metal layers can be deposited in microfluidic channels via PDA-assisted electrodeless plating. Biomolecules can self-assemble on the metal layer via interactions such as gold-thiol bonds (Liang et al. 2011). Third, the liquid phase reaction used to coat PDA is able to access the interior of biomedical microfluidic devices which often have complex microscale architectures and are inaccessible by conventional surface treatment methods such as dip coating, spin coating or gas plasma treatment. Fourth, PDA is applied under a mild reaction condition. The polymerization process takes place in a slightly alkaline aqueous environment at room temperature and atmospheric pressure. Such a mild reaction condition does not denature biomolecules, neither does it damage the microfluidic devices. This “green” coating protocol does not impose any potential hazard to the users and the environment.

To recognize the great potential of PDA, we would like to review the latest development of biomedical microfluidic devices that incorporate PDA for various purposes. These biomedical microfluidic devices are categorized into two groups based on the role of PDA – the ones use PDA for device fabrication and modification, and the ones use PDA for biofunctionalization as shown in Fig. 1 We will identify the advantages as well as the limitations of PDA for its intended functions. In particular, we will emphasize the unique advantages of PDA that specifically benefit biomedical microfluidic devices. We will also discuss other possible applications of PDA for biomedical microfluidic devices beyond the current development. We hope to introduce and promote PDA to the microfluidic community through this review.

2. PDA for Microfluidic Device Fabrication and Surface Modification

2.1 PDA-Facilitated Surface Modification of Microfluidic Devices

The surface properties of microfluidic components are crucial for their functions. Due to a limited selection of materials that are compatible with the microfabrication process, biomedical microfluidic devices often require post-fabrication surface modification in order to achieve desired surface properties. Polydimethylsiloxane (PDMS) is one of the most commonly used materials for biomedical microfluidic devices. PDMS is hydrophobic with a static water contact angle of about 120°. As a result, a high pressure is required to pump aqueous solution through PDMS-based microfluidic channels. Although the oxygen plasma treatment renders PDMS hydrophilic, the effect is only temporary. Furthermore, it is less effective in modifying the internal structures of biomedical microfluidic devices. PDA provides a simple and permanent way of converting a (super)hydrophobic surface into a

hydrophilic surface (Jiang et al. 2011; Lee et al. 2007b). Coating PDMS with PDA reduces the static water contact angle from about 120° to 20°–100°, depending on the thickness of the PDA coating which form by autoxidation process that controlled by oxygen diffusion in solution.(Yang et al. 2013). Jun *et al.* applied PDA to a droplet microfluidic device to generate uniform oil-in-water (O/W) droplets (Jun et al. 2016). The hydrophilic coating by PDA prevented the oil droplet from sticking to the surface of the microfluidic channel, thereby stabilizing the droplet and leading to a more uniform size distribution.

In addition to PDMS, several other moldable polymers have been explored for the fabrication of biomedical microfluidic devices using the soft lithography process. Examples include perfluoro alkoxy (PFA) and fluorinated ethylene propylene (FEP). PFA and FEP are melt-processible and optically transparent which are desired properties for biomedical microfluidic devices (Ren et al. 2011). Both materials belong to the Teflon family, a type of amorphous fluorinated polymers with low surface energy (Resnick 1989). Microfluidic devices composed entirely of PFA and FEP have been fabricated using a molding process (Ren et al. 2011). Because of the low surface energy of PFA and PEP, the microfluidic devices are highly resistant to surface wetting and modification. PDA is used to modify the surface property of Teflon-based devices by flowing the dopamine monomer solution through the microfluidic channel. The resulted surface is highly hydrophilic and biocompatible. Sandwich immunoassays and long-term cell culture are successfully performed on the PDA-coated Teflon device (Shen et al. 2015). The PDA-enabled biofunctionalization will be discussed in more details in the following sections.

Fig. 2 (a) The creation of PDA gradient by controlling the dopamine monomer concentration using the “Christmas Tree” concentration gradient generator. (b) The water static contact angle changes from about 110° in Zone 4 (Z4) where PDA is the thinnest to about 30° in Zone 1 (Z1) where PDA is the thickest. Adapted from Ref. (Shi et al. 2014) with the permission from American Chemical Society.

The liquid phase reaction allows us to apply a PDA gradient to the biomedical microfluidic devices. Because the surface hydrophilicity is positively related to the thickness of the PDA coating, the surface wettability changes along the PDA gradient. Two methods have been demonstrated to create the PDA gradient. The first method uses a “Christmas tree”(Dertinger et al. 2001) microfluidic gradient generator to generate a concentration gradient of dopamine monomer solution (Shi et al. 2014). The solution of high dopamine monomer concentration deposits PDA at a higher rate and *vice versa*. The static water contact angle changes from about 120° to about 30° as the thickness of PDA increases along the gradient. The other method restricts the oxygen concentration instead of the dopamine monomer concentration in order to create the PDA gradient (Yang et al. 2013). The PDA polymerization process is an oxygen-dependent reaction as no PDA formation is observed in the absence of the oxygen (Kim et al.

2013a). Naturally, an oxygen concentration gradient would form along the depth of the solution as a result of the diffusion under atmospheric pressure. In a deeper solution where the oxygen concentration is lower, PDA polymerizes at a slower rate and results in a thinner PDA coating. The PDA gradient on a silicon substrate created using this approach exhibits static water contact angles ranging from about 110° to 30° along the gradient (Jiang et al. 2011). The PDA gradient has a great potential for microfluidic control and bioconjugation. Besides controlling the dopamine and oxygen concentration, other reaction parameters, such as temperature and pH, can also be explored to create a PDA gradient in the future.

In addition to hydrophilic surface modification, the PDA chemistry can be used to deposit a hydrophobic coating using a perfluorinated dopamine derivative (Hong et al. 2014). The perfluorinated dopamine derivative (f-DOPA) contains a dopamine moiety and a long fluorocarbon tail. Similar to the formation of PDA, f-DOPA undergoes oxidative self-polymerization and adheres to a wide range of surfaces. The fluorocarbon tail of f-DOPA makes the surface hydrophobic with a static water contact angles above 150° and a water sliding angle of about 7° . This application shows the versatility of the PDA chemistry in surface modification. With properly designed dopamine derivatives, one may add any desired property to any surface using the simple one-step PDA chemistry.

2.2 PDA-Enabled Digital Microfluidic Platform

PDA has inspired the development of a new digital microfluidic platform that drives droplets with the gravity and guides their movement with the surface tension. Digital microfluidics manipulates fluids in the form of discrete droplets on a hydrophobic surface using magnetic force, electrowetting or other mechanisms (Choi et al. 2012; Jebrail et al. 2012; Zhang and Nguyen 2017). Unlike conventional microfluidics where fluids are enclosed in microchannels, digital microfluidics handle droplets on an open surface. These droplets are self-formed, self-contained, and serve as virtual reaction chambers for many biomedical applications (Chiou et al. 2013; Miller and Wheeler 2008; Ng et al. 2015; Ng et al. 2012; Pipper et al. 2007; Pipper et al. 2008; Shah et al. 2009; Shamsi et al. 2014; Zhang et al. 2011; Zhang et al. 2013b; Zhang and Wang 2013). To achieve a comprehensive droplet control, hydrophilic patches are introduced to trap the droplets on the hydrophobic substrate such as a Teflon-coated surface (Zhang and Wang 2013). These hydrophilic patches are created by etching Teflon with the oxygen plasma through a shadow mask. However, this method is very resource-demanding. PDA provides a simple alternative to pattern hydrophilic patches on the (super)hydrophobic substrate for droplet manipulation. Instead of etching the Teflon using the oxygen plasma, a hydrophilic PDA layer is deposited on top of the hydrophobic substrate through the lithographically patterned photoresist followed by a lift-off process which shows in fig. 3a (You et al. 2012). The resulted hydrophobic substrate with hydrophilic PDA patterns is used to guide the movement of the droplets driven by the gravity. The substrate is placed on an inclined surface. Once dispensed on the substrate, the droplets roll down the slope and follow the

hydrophilic paths defined by PDA as shown in Fig. 3. A hydrophobic substrate with a Y-shaped hydrophilic PDA path was used to synthesize gold nanoparticles on a digital microfluidic platform (You et al. 2012). Two reactants in the form of droplets were dispensed to the two arms of the Y-shaped path. As they rolled down the slope along the path, two droplets merged at the Y-junction. The synthesis took place as the merged droplet rolled down the stem of the Y-shaped path. Later, the same group demonstrated this concept on a slippery liquid-infused porous surface (Wong et al. 2011) that functioned as the hydrophobic substrate for digital microfluidics (You et al. 2014). In this case, PDA was first used to synthesize nanostructured TiO_2 to create a superhydrophobic substrate. Next, PDA was used to pattern the hydrophilic path to guide the droplet movement on the substrate.

Both examples discussed above rely on lithographically defined photoresist to pattern the hydrophilic features on the hydrophobic substrates. This process is expensive and resource-demanding. We believe that the coating process can be simplified by depositing PDA through a stencil with desired patterns. The stencil can be made by cutting, screen-printing or other less expensive methods.

Fig. 3 Pattern hydrophilic path on a (super)hydrophobic surface using PDA. (a) The hydrophilic path is created by depositing PDA through a lithographically defined mask. (b) The hydrophilic PDA path guides the movement of the water droplet as it rolls down the hydrophobic substrate. Adopted from Ref. (You et al. 2012) with the permission from John Wiley and Sons.

2.3 PDA-Aided Fabrication of PDMS Chip

PDMS is one of the most popular polymeric materials used for biomedical microfluidic devices due to its low cost and simple fabrication process. Its excellent properties, such as gas permeability, biocompatibility, nontoxicity, and optical transparency among others (Duffy et al. 1998; Mata et al. 2005), make PDMS-based microfluidic devices well-suited for a lot of biomedical applications such as cell culture and biosensing. The fabrication of a PDMS device involves the casting of PDMS over a lithographically patterned mold. A silane coating is often applied to prevent PDMS from sticking

to the mold. PDA has been used to replace silane as the anti-adhesion coating (Chen et al. 2014). The highly hydrophilic PDA layer repels PDMS from the surface, thereby enabling easy PDMS demolding. Compared to the toxic silane, PDA is much safer and easier to prepare with a simple dip-coating process. Furthermore, the PDA coating is highly durable. Comparing with the normal silane coating which can last only several demolding, PDA coating can be used around 30 times. The PDA coating should be promoted to the microfluidic community to replace silane during the fabrication of PDMS-based devices in the future. Doing so will reduce the pollution to the environment and prevent the hazards to users.

So far, the most reliable technique for PDMS bonding relies on the oxygen plasma treatment. The treatment converts the surface siloxane groups (Si-CH_3) to silanol groups (Si-OH) which covalently bond PDMS to the glass or silicon. This oxygen plasma treatment method is quite efficient, but the process is difficult to control. Under-treatment does not generate enough silanol groups for covalent bonding. Over-treatment would convert the silanol group to inorganic silicon oxide which adversely affects the bonding strength (Hillborg et al. 2000). PDA is inspired by the strong adhesion of mussels to the rock. It is, therefore, sensible to use PDA as an adhesive for chip bonding. However, research in this area is still lacking. The only protocol that uses PDA for PDMS chip bonding still requires the oxygen plasma treatment. The PDMS is grafted with hydroxyl groups by the oxygen plasma treatment, which covalently bonds to the PDA layer deposited on top of a poly(ethylene glycol) methacrylate (PDMP)-coated glass slide (Kim et al. 2013b). The bonding strength reaches around 60 kPa (8.7 psi) which is much lower than the bonding strength when PDMS is directly bonded to the glass using the oxygen plasma treatment (Lu et al. 2007).

3. PDA for Microfluidic Device Biofunctionalization

To fulfil their biomedical functions, microfluidic devices are frequently conjugated with biomolecules (Lee et al. 2009), bioactive compounds, and biocompatible materials (Hong et al. 2011). These bio-functional additives play an essential role in the interaction with biological entities of interest, including molecules, cells and other intracellular or extracellular components. Because of the many applications of biomedical microfluidic devices, the biofunctionalization process often requires introducing a wide range of materials using different conjugation chemistries (Kim et al. 2011). PDA, with its strong adhesion, active functional groups and reductive capability, offers a simple and universal solution to functionalizing microfluidic devices for biomedical applications.

3.1 Functionalization with Oligonucleotide Using PDA

Oligonucleotides are frequently immobilized on a solid substrate as target-specific probes for the detection of DNA/RNA targets based on sequence-complementary hybridization. In recent years, a special class of oligonucleotides known as the aptamer has been discovered. Aptamers have the ability to recognize molecular targets

(Bunka and Stockley 2006). They are the nucleic acids equivalent of antibodies with enormous potentials for diagnostics and therapeutics. Aptamers are identified by an iterative selection process during which oligonucleotides with high binding affinity to the target are enriched after each selection cycle (Tuerk and Gold 1990). Once identified, aptamers can be synthesized chemically with much reduced cost compared to antibodies. More and more biomedical microfluidic devices incorporate aptamers as the target recognition element for biosensing (Jolly et al. 2016; Shin et al. 2016; Zhao et al. 2016). To covalently couple the oligonucleotides to the microfluidic device, the surface requires silane treatment which is hazardous. PDA offers a surface-independent and user-friendly way of immobilizing oligonucleotides in biomedical microfluidic devices. The most straightforward approach is to directly link an amine-terminated oligonucleotide to the surface via the PDA-assisted covalent coupling. Wood *et al.* used this approach to create a microarray for surface plasma resonance (SPR)-based DNA sensing (Wood et al. 2013). The 5' terminal of the oligonucleotide probe was labeled with a primary amine group, which would covalently bind to the PDA as illustrated in Fig. 4. Zhang *et al.* used the same approach to fabricate an electrochemical sensor on a screen-printed carbon electrode via PDA (Zhang et al. 2015). The sensor used a sandwich hybridization assay and gold nanoparticle-labelled reporter probes to identify the target of interest. Loget *et al.* micropatterned amine-terminated nucleotides on a PDA microarray for electrochemical sensing. The PDA array was patterned using electrodeposition instead of pH-triggered spontaneous PDA polymerization (Loget et al. 2013).

Fig. 4 Immobilization of DNA oligonucleotide as the sequence complementary probes on a PDA microarray for biosensing. The change in reflectivity (ΔR) is positively correlated to the amount of DNA target (%D) that hybridizes to the probe immobilized on the PDA microarray. Adopted from Ref. (Wood et al. 2013) with the permission from American Chemical Society.

PDA seems to react only with the terminally labeled amine group of the oligonucleotides. Although the nucleotide bases contain primary amine groups, several studies suggested that they did not form a covalent bond with PDA (Della Vecchia et al. 2013; Ye et al. 2011). In these studies, fluorescently labeled DNA probes or aptamers were mixed with PDA nanospheres. The PDA functioned as an excellent fluorescent quencher and formed a smart sensor together with the DNA adsorbed on its surface. In the presence of the target, the DNA probes or aptamers bound to the target through Watson-Crick base pairing or structural recognition. Once they bound to the

target, the DNA probes were released from the PDA nanospheres and restored their fluorescence. Such a reversible adsorption of the oligonucleotide to PDA strongly suggests that the interaction between the PDA and oligonucleotide bases is non-covalent.

3.2 Functionalization with Proteins Using PDA

The covalent coupling of proteins to PDA occurs over a wide pH range via different amino acids (Lee et al. 2009). In addition to the terminal amine, PDA reacts with the side chain of amino acids. At low pH, PDA mainly reacts with the imidazole in the histidine side chain. At high pH, PDA mainly reacts with the primary amine group in amino acids such as lysine. Proteins such as antibodies are often functionalized onto microfluidic biosensors to detect specific molecular targets using the sandwich immunoassay (Eteshola and Leckband 2001). PDA is used to covalently conjugate antibodies to biomedical microfluidic devices. Shen *et al.* treated a whole-Teflon chip with PDA and conjugated the anti-Rabbit antibody to measure the concentration of rabbit IgG using a fluorescent immunoassay (Shen et al. 2015). Although this simple model assay does not have clinical significance, it serves as a proof-of-concept. Feng *et al.* created a protein microarray by flowing PDA through a microfluidic network. The protein microarray analyses a large number of different samples in parallel through surface-immobilized ligand molecules. Feng group, First, injected the dopamine solution into the microchannel. The bottom of the microchannel was perforated and functioned as a stencil that defined the pattern of the microarray. As it flowed through the microchannel, the dopamine solution made contact with the substrate through the perforation and deposited PDA onto the substrate. Capture antibodies were then immobilized on the microarray via PDA for subsequent immunoassays as shown in Fig. 5. (Feng et al. 2015). Another work by Zhang *et al.* used a more intricate approach to immobilize and orient antibodies using PDA for the detection of exosomes (Zhang et al. 2016). In this work, the microfluidic channel was first coated with a layer of graphene oxide via the electrostatic interaction. The graphene oxide layer was covalently conjugated with protein G via the PDA coating. Antibodies that recognize particular exosome markers were coupled to the surface via the protein G that bound to the Fc region of the antibodies. Because of the improvement of exosome immune-capture and the effective suppressing of non-specific exosome absorption, the sensitivity of graphene oxide with PDA configuration was higher when compared back-to-back with the cases where only polydopamine is used and where graphene oxide and polyethylene glycol (PEG) are used to immobilize antibodies.

Fig. 5 (a) Schematic illustration showing the fabrication method of the PDA microarray through a microfluidic network. (b) Schematic illustration of immunoassay by immobilizing capture antibodies on the microarray using PDA. Adopted from Ref.(Feng et al. 2015) with the permission from Royal Society of Chemistry.

Enzyme is another class of important proteins that are often studied in microfluidic systems. Cheng *et al.* conjugated trypsin to microfluidic channels using PDA to create a bioactive surface for rapid proteolysis (Cheng et al. 2015). In this study, A PDMS microfluidic channel was first coated with PDA, which resulted in a nanostructured coating consisting of PDA nanospheres. Trypsin was then covalently linked to the microfluidic channel via the PDA coating. This device digested proteins that continuously flowed through the microfluidic channel. It significantly shortened the digestion from an overnight process to 2 minutes. Rivera *et al.* used the same approach to immobilize trypsin on silica and titania surface (Rivera and Messersmith 2012). A similar digestion system using magnetic particles in microfluidic channels was also demonstrated (Cheng and Zheng 2014). The trypsin was conjugated to the magnetic particles using PDA to facilitate the recovery of trypsin for reuse. In another work, an interesting enzyme-immobilized microfluidic reactor for the biosynthesis of ester wax was demonstrated (Bi et al. 2017). The microfluidic reactor used PDA to functionalize the polytetrafluoroethylene (PTFE) microtube for the conjugation of *Candida antarctica* lipase B. Up to 8 layers of the lipase were coated using a layer-by-layer adsorption process in conjunction with polyethyleneimine. The microfluidic reactor prepared ester wax in 45 minutes with a yield of 95%. The PDA-assisted enzyme conjugation is robust enough to sustain 144 hours of reaction while retaining 83% of its initial activity.

Other proteins with specialized functions are also introduced to biomedical microfluidic devices using PDA. A PDMS microfluidic extracorporeal oxygenator for infant blood perfusion was covalently conjugated with antithrombin-heparin (ATH) using PDA as the bonding agent to minimize the potential of the life-threatening blood clotting (Leung et al. 2015). PDA increased the ATH loading on the PDMS surface. With PDA, the microfluidic device retained about 70% ATH on its surface after 72 hours. This performance was much better than that with the device without the PDA coating, which could only retain less than 30% of ATH. PDA also helped to immobilize bovine serum albumin (BSA) in a microfluidic capillary electrochromatography chip (Liu et al. 2013b), (Liu et

al. 2013a). PDA simplified the protocol for the preparation of the protein stationary phase in the capillary electrochromatography microfluidic device. The separation of chiral tryptophan, threonine, and the glycine-phenylalanine dipeptide were successfully performed using this device.

3.3 Promoting Cell Adhesion and Cell Patterning Using PDA

PDA is a biocompatible material. By strongly adhering to the surface and modifying the surface property, PDA by itself promotes cell attachment to biomedical microfluidic devices. PDA has been shown to promote tissue growth *in vitro*. For example, coating artificial scaffold with PDA enhances the development of cartilage tissue and promotes the growth of human endothelial cells by prolonging the stability of cells adhesion (Chuah et al. 2015a; Ku and Park 2010; Tsai et al. 2011). More importantly, the PDA coating reduces the inflammatory response to poly-L-lactic acid and the immunological responses of the blood (Hong et al. 2011), thereby minimizing the cytotoxic effect and foreign body reaction of the implanted biomaterials (Liu et al. 2013d).

Bone marrow stromal cell (BMSC) is an unspecialized stem cell with self-renewal and multilineage differentiation ability. It is an important cell source for stem cell therapy and regenerative medicine (Wei et al. 2013). BMSCs are often required to be expanded over 3 weeks (Chuah et al. 2015a). Although PDMS is a good material for microfluidic cell culture system with its high biocompatibility and gas permeability, it is not well-suited for long-term cell culture beyond three weeks because its surface hydrophobicity leads to poor cell attachment (Chuah et al. 2015b; Fuard et al. 2008). Although the oxygen plasma treatment and protein coating improve the cell attachment, the effect is transient and often ends up with cells detachment and aggregation in long term. Chuah *et al.* stabilized the BMSCs culture by coating the PDMS microfluidic chip with PDA (Chuah et al. 2015a). The PDA-modified PDMS surface did not only improve the cell adhesion and proliferation, but also improved the multipotency of the BMSCs during the long-term culture.

The one-step liquid phase deposition of PDA enables us to spatially pattern the PDA on a substrate by flowing the dopamine solution through a microfluidic network. Ku *et al.* patterned a PDMS substrate with PDA and cultured three types of cells, including fibrosarcoma HT1080, mouse preosteoblast MC3T3-E1, and mouse fibroblast NIH-3T3 (Ku et al. 2010a). All cell types showed a strong preference for the PDA-coated regions on the substrate (Ku et al. 2010a). All cells proliferated along the patterned PDA strips and maintained normal cell morphologies. Several research groups used a similar approach to pattern the PDA on various types of substrates using micro contact printing to control the cell attachment (Beckwith and Sikorski 2013; Chien et al. 2012; Chien and Tsai 2012; Sun et al. 2012). In these studies, a PDMS stamp was coated with PDA that was later transferred onto a substrate coated with a polyethylene glycol (PEG), polystyrene (PS) or poly(vinyl alcohol) (PVC). The micro printed PDA determined the spatial pattern of cells on the substrate.

One may also control the cell seeding density in the microfluidic channel by varying the thickness of the PDA coating. Shi *et al.* used a microfluidic concentration gradient generator to create a thickness gradient of PDA across a microfluidic chamber as shown in Fig. 6. (Shi et al. 2014). The hydrophilicity of the surface was directly related to the PDA thickness. The thicker the PDA was, the more hydrophilic the surface became. When seeded with human adipose mesenchymal stem cells, different cell seeding densities were observed along the PDA thickness gradient. Interestingly, regions with thicker PDA show a smaller number of adherent cells. As the thickness of PDA decreased, the cell number dropped drastically.

Fig. 6 Cell seeding density changes along the PDA gradient. The largest number of adherent cell is observed in Zone 1 (Z1) where PDA is thickest, and the smallest number of adherent cell is observed in Zone 4 (Z4) where PDA is thinnest. Adopted from Ref. (Shi et al. 2014) with the permission from American Chemical Society..

3.4 Metallization Using PDA

PDA has sufficient reductive capacity to plate noble metals on any surface. More reactive metals such as copper can also be plated by PDA with additional exogenous reducing agents. PDA is found to form stable covalent bonds with metals via two hydroxyl groups (Weinhold et al. 2006). PDA has been used to synthesize and immobilize gold nanoparticles on the surface of a microfluidic channel. Liang *et al.* treated the microfluidic surface by flowing a solution containing both the dopamine monomer and HAuCl_4 through the microfluidic channel (Liang et al. 2011). The PDA adhered to the surface and reduced the gold ion to form gold nanoparticles. The surface after the treatment, which composed of a PDA matrix with uniformly dispersed gold nanoparticles, suppressed electroosmotic mobility and non-specific adsorption of amino acids. Five amino acids were successfully separated in the PDA-treated microfluidic channel using capillary electrophoresis. Silver is another commonly used metal in biomedical applications for its broad-spectrum antibacterial activities. (Lara et al. 2011) Sileika *et al.* deposited silver on the surface using PDA as an antimicrobial coating and protected

the silver layer with PEG for sustained Ag release (Sileika et al.

2011). Although it was only demonstrated on a flat surface, this example showed the great potential of PDA to introduce Ag as an antimicrobial coating, a process that can be readily translated onto a microfluidic platform as shown in Fig. 7.

Fig. 7 Ag antimicrobial coating by PDA-assisted electrodeless plating. Polydopamine has two functions. It both reduces the Ag^+ to form the Ag coating and conjugates polyethylene glycol (PEG) to the silver layer for sustained Ag release. The one with both Ag and PEG show the best antimicrobial property. Adopted from Ref.(Sileika et al. 2011) with the permission from American Chemical Society.

Modifying the surface with metals greatly improves the biofunctionality of microfluidic devices. For examples, thiol-terminated oligonucleotides and proteins can easily adsorb to the microfluidic surface *via* the thiol-gold interaction. Small molecules of interest can be coupled to the microfluidic device via the self-assembled monolayer on the gold film(Wang et al. 2015). Coating the microfluidic device with silver, as discussed above, makes the device resistant to bacteria(Lara et al. 2011). A nickel thin film in the microfluidic channel can be used to purify the recombinant protein in a continuous flow. Functionalizing the microfluidic surface with metals using PDA has compelling advantages over the conventional e-beam deposition or sputtering (Wöllenstein et al. 2003; Zou et al. 2008). The conventional approaches are unable to deposit metals on the interior surface of a microfluidic channel. The highly directional deposition by e-beam and sputtering can only deposit metal on an open surface before the chip bonding. Furthermore, the conventional methods are resource-demanding, requiring highly specialized equipment in a controlled environment. In contrast, PDA is coated using a wet chemistry. The dopamine solution can easily access the interior surface and deposit PDA after chip bonding. This straightforward and user-friendly PDA modification protocol can be easily adopted by any laboratories.

4. Conclusion and Outlook

Although the polymerization mechanism and the exact structure of PDA are still under debate, its exceptional properties, such as strong adhesion, simple coating protocol, and versatile biofunctionality, are well understood and appreciated. More and more researches are adopting PDA for various purposes. In this review article, we have discussed the two main functions of PDA in biomedical microfluidic devices. In the first section, we mainly focus on the use of PDA for device fabrication and surface modification. In the second section, we examine how convenient and efficient it is to biofunctionalized the microfluidic device using PDA.

The single-step liquid phase PDA coating protocol is uniquely suited for biomedical microfluidic devices. It gives PDA unrestricted access to the interior surface of both open and closed microfluidic components. PDA is able to coat microfluidic channels with convoluted architectures by simply flowing the dopamine solution through the microfluidic network. Conventional surface modification approach, such as oxygen plasma, cannot modify the interior surface effectively even for devices made of gas permeable materials. One

example worth highlighting is the PDA-modified Teflon surface for digital microfluidics. Because of its ability to adhere to the (super)hydrophobic surface like Teflon, PDA can easily pattern hydrophilic regions on the hydrophobic surface to guide the droplet movement. The PDA coating on Teflon has opened many possibilities for more intricate digital microfluidic platforms.

In most cases, PDA coats the entire microfluidic channel indiscriminately. The coating is accomplished by constantly flowing the dopamine solution through the microfluidic channel continuously or incubating the channel with the dopamine solution. In both methods, all surfaces are in contact with the solution and coated with PDA. But the mis-coated regions may cause nonspecific adsorption of biomolecules, which leads to undesirable outcomes. On a flat surface, the patterning of PDA in specific regions has been demonstrated by using a lithographically defined mask or guiding the flow of dopamine solution with a microfluidic network. In microfluidic devices, we could use hydrodynamic focusing or discrete microfluidics to restrict the flow of dopamine solution to specific regions where the PDA coating is coveted. The precise patterning of PDA in microfluidic channels could further improve the performance of biomedical microfluidic devices.

PDA has been demonstrated for PDMS chip bonding, but the process is not more advantageous than the conventional oxygen plasma-enabled bonding. We wish PDA would function like a glue and stick PDMS to the glass. Unfortunately, PDA layers are unable to bond with each other. An intermediate layer is applied to enhance the adhesion, and the oxygen plasma treatment is still required. Nonetheless, we believe that PDA has the potential to simplify the bonding process. New PDA derivatives have been developed as adhesives. For example, copolymer made of PEG and dopamine (or other catechol) have been used as a surgical glue (Lee et al. 2011). These dopamine-based glues could be used for device bonding.

We have seen how PDA-functionalized biomedical microfluidic devices can immobilize oligonucleotides, proteins, cells, and metal layers on the surface with unprecedented ease. In other research fields, PDA has been used in a broader scale. Using PDA, almost any materials can be immobilized on the surface in a single step by mixing them with the dopamine monomer solution. Furthermore, we can coat reaction initiators or primers using PDA and perform more sophisticated surface functionalization subsequently. For example, molecules with tertiary amine groups are co-immobilized with PDA for subsequent silica coating through the silicification process. Many other applications of PDA, such as energy storage (Zhang et al. 2013b), nanoparticle sensors (Salazar et al. 2016), drug delivery (Liu et al. 2013c), and water treatment (Gao et al. 2013; McCloskey et al. 2010), have been discussed in detail in several review articles (Ho and Ding 2014; Kim et al. 2011; Liu et al. 2014; Lynge et al. 2015; Lynge et al. 2011; Madhurakkat Perikamana et al. 2015; Salazar et al. 2016).

PDA has been gaining popularity in recent years. Groups in different disciplines are adopting PDA as a useful tool for surface modification and molecule conjugation in their

research. Nonetheless, PDA has not yet received its fair share of attention from the microfluidic community. This review provides an extensive coverage of PDA applications in the field of microfluidics, but the total number of relevant work is dwarfed by PDA-related works in other fields. Besides providing a comprehensive review of PDA and its unique properties beneficial for biomedical microfluidics, this review also aims to promote PDA to the microfluidic community in the hope of a broader adoption of PDA for applications in biomedical microfluidic devices and beyond.

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