รายงานวิจัยฉบับสมบูรณ์

โครงการ: การสังเคราะห์พอลิเมอร์ลอกแบบจับจำเพาะเคลือบบนผิวเซลลูโลสจากแบคทีเรียที่มีคุณสมบัติยับยั้งการสื่อสารในแบคทีเรียเพื่อการประยุกต์ใช้ทางชีวการแพทย์

โดย ผศ.ดร.ธีรพล เปียฉ่ำ

มิถุนายน 2560

สัญญาเลขที่ RSA5780020

รายงานวิจัยฉบับสมบูรณ์

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ผศ.ดร.ธีรพล เปียฉ่ำ **มหาวิทยาลัยมหิดล**

สนับสนุนโดย สำนักงานกองทุนสนับสนุนการวิจัย และ มหาวิทยาลัยมหิดล
(ความคิดเห็นในรายงานนี้เป็นของผู้วิจัย สกว. ไม่จำเป็นต้องเห็นด้วยเสมอไป)

กิตติกรรมประกาศ

ผู้วิจัยขอขอบคุณสำนักงานกองทุนสนับสนุนการวิจัย (สกว.) และมหาวิทยาลัยมหิดล ที่ได้พิจารณาให้การสนับสนุนทุน พัฒนา นักวิจัย ทำให้ มีผล งานวิจัย ต่อยอดในสาขานี้เพื่อเป็นการกระตุ้นการสร้างนวัตกรรมจากเทคโนโลยีนี้ต่อไป

ขอขอบพระคุณภาคจุลชีววิทยาคลินิกและเทคโนโลยีประยุกต์ คณะเทคนิคการแพทย์ มหาวิทยาลัยมหิดล ที่เอื้อเฟื้อสถานที่อุปกรณ์ สารเคมี และเครื่องมือต่าง ๆ ตลอดจนให้การสนับสนุนการวิจัยในครั้งนี้

ธีรพล เปียฉ่ำ

Abstract

Project Code: RSA5780020

Project Title: Synthesis of Quorum quenching Molecularly Imprinted Polymer coated Bacterial Cellulose for Biomedical application

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Project Period: June 2015-June 2017

Molecular imprinting has become an attractive synthetic approach for the fabrication of novel functional polymers with pre-designed molecular target selectivity. Such molecularly imprinted polymers (MIPs) have been applied in wide range of areas such as chemical and biological sensors, solid phase extraction and drug assays owing to their inherent robustness, reusability and reproducibility. Furthermore, MIPs can also be used as tools for studies concerning antibody/receptor binding site mimicry as well as being used as antibody substitutes for biomedical applications. Herein, we prepared molecularly imprinted polymers for the purpose of binding specifically to quorum sensing molecule of microbes useful as a tool for molecular detection as well as inhibition of phenomenon regarding from quorum sensing signal communication. Furthermore, we have developed quercetin-imprinted polymer aided for applied to bind anthocyanin from the crude extract of mangosteen pericarp. The binding capacity of quercetin-MIP toward anthocyanin was approximately 0.875 mg per gram of polymer. Moreover, viral detection is a rapidly growing field owing to its increasing prevalence and ongoing evolution of viral variants and drug resistance. Therefore, this calls for effective detection, surveillance and control. Herein, we highlight and summarize the literature on the utilization of MIPs for human virus detection. Particularly, MIPs afford great potential for rapid virus detection as well as other recognition-based viral studies such as viral subtype differentiation and screening of inhibitors for drug discovery.

Keywords: Molecularly imprinted polymers, quorum sensing molecule, quercetin, anthocyanin, viral detection

บทคัดย่อ

รหัสโครงการ: RSA5780020

ชื่อโครงการ: การสังเคราะห์พอลิเมอร์ลอกแบบจับจำเพาะเคลือบบนผิวเซลลูโลสจากแบคทีเรียที่มีคุณสมบัติยับยั้งการสื่อสารในแบคทีเรียเพื่อการประยุกต์ใช้ทางชีวการแพทย์

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เทคโนโลยีพอลิเมอร์ลอกแบบจับจำเพาะได้ถูกนำมาใช้มากขึ้นด้วยคุณสมบัติที่สามารถออกแบบความจำ เพาะต่อโมเลกุลของสารที่หลากหลาย ซึ่งได้ถูกนำมาประยุกต์ใช้อย่างกว้างขวางในหลายสาขา เช่นการวิเคราะห์ตรวจหาสารทางเคมีชีวภาพ, การสกัดสารต่างๆ, การตรวจหาชนิดและระดับของยา เนื่องจากคุณสมบัติที่พอลิเมอร์ลอกแบบจับจำเพาะนี้มีความแข็งแรงทนทาน, สามารถใช้ซ้ำได้และให้ผลการทดสอบที่ดีจึงได้ถูกนำมาใช้เป็นเครื่องมือศึกษาพัฒนาและประยุกต์ใช้ทางชีวการแพทย์เสมือนการใช้งานของแอนติบอดีและรีเซฟเตอร์เทียม ผู้วิจัยจึงได้นำองค์ความรู้ด้านการสังเคราะห์นี้มาพัฒนาพอลิเมอร์ลอกแบบจับจำเพาะต่อโมเลกุลของสารสื่อสัญญาณของจุลชีพเพื่อนำมาประยุกต์ใช้ในการตรวจหาและยับยั้งการสื่อสารระหว่างจุลชีพ เพื่อนำมาประยุกต์ใช้ในการตรวจหาและยับยั้งการสื่อสารอันจะนำไปสู่การวิเคราะห์และควบคุมการแสดงออกของจุลชีพ ผู้วิจัยยังได้สังเคราะห์และประยุกต์ใช้พอลิเมอร์ลอกแบบจับจำเพาะต่อสารเคอซิตินเพื่อใช้ในการสกัดสารแอนโทไซยานินจากเปลือกมังคุดโดยพอลิเมอร์มีความสามารถในการจับกับสารแอนโทไซยานินที่ 0.875 มิลลิกรัมต่อ 1 กรัมของพอลิเมอร์

ในปัจจุบันเทคโนโลยีในการตรวจหาไวรัสมีการพัฒนาอย่างต่อเนื่องให้เหมาะกับสถานการณ์เพื่อการควบคุมการแพร่ระบาดต่างๆ รวมถึงเพื่อการรองรับการเพิ่มขึ้นของอุบัติการณ์, การกลายพันธ์อย่างรวดเร็วและการดื้อยาของไวรัส จึงมีความจำเป็นอย่างยิ่งในการพัฒนาวิธีวิเคราะห์ที่มีความสะดวกรวดเร็วและมีประสิทธิภาพ ผู้วิจัยได้รวบรวมและวิเคราะห์เทคโนโลยีทางเลือกในการใช้พอลิเมอร์ลอกแบบจับจำเพาะต่อไวรัสที่ก่อโรคในคนรวมถึงองค์ความรู้และวิธีการวิธีวิเคราะห์และเทคโนโลยีที่เกี่ยวข้องในการประยุกต์ใช้พอลิเมอร์ลอกแบบจับจำเพาะเพื่อตรวจหาไวรัสด้วยความรวดเร็ว, การแยกสายพันธุ์ชนิดของไวรัสรวมไปถึงการค้นหายาต้านไวรัสใหม่ๆ อันจะนำไปสู่การพัฒนานวัตกรรมและเป็นเครื่องมือในการตรวจวิเคราะห์ทางด้านไวรัสวิทยาต่อไป

คำหลัก: พอลิเมอร์ลอกแบบจับจำเพาะ, สารสื่อสัญญาณของจุลชีพ, เคอร์ซิติน, แอนโทไซยานิน, การตรวจหาไวรัส

เนื้อหางานวิจัยส่วนที่ 1

Quercetin-imprinted polymer for anthocyanin extraction from mangosteen pericarp.

1. Introduction

Mangosteen (*Garcinia mangostana* L.) is well known as “the queen of fruits” that is typically consumed fresh for dessert. Different parts of the mangosteen, such as the bark, fruit hull or pericarp and root, have been used for hundreds of years as medication. Due to the abundance of mangosteen consumption, there has been intense research for adding value to the residual products. For example, peel may be considered as waste but has attracted much interest in recent years from the food and pharmaceutical industries. Mangosteen pericarp is an abundant source of natural phenolic antioxidant compounds including xanthones and its derivatives, benzophenones, flavonoids, anthocyanins, tannins, etc[1]. Anthocyanins, the largest group of water soluble pigments, are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopynylium or flavylium salts [2]. It provides a broad range of biological activities including free radical scavenging activity [3], antimicrobial activity for many different pathogens [4], anti-inflamatory and anticancer activity [5]. Furthermore, it can also prevent oxidative damage to biological macromolecule such as DNA [6], protein [7] and lipid [8]. To date, there is no report of anthocyanin toxicity. Anthocyanins can be absorbed and cross the human intestinal mucosa leading to its circulation in the blood stream [9]. It can be used as a topical agent for skin UV protection [10]. Recently, anthocyanin have shown their potential health benefits in obesity control [11, 12], diabetes control [13], prevention of cardiovascular disease (CVD) [14] as well as improvement of visual and brain functions [15, 16].

These compounds are water-soluble pigments that can be extracted from many vegetables and fruits using methanol or ethanol containing small amounts of hydrochloric acid [17, 18]. There is much attention to extract and include this substance in many pharmaceutical products. One of the eminently attractive synthetic approaches of this study is the use of the molecular imprinting technology in which the natural recognition sites are created in a macromolecular matrix using a molecular template in a casting procedure. The

selected molecule for imprinting is first allowed to form self-assemble with functional monomers. The resulting complexes are subsequently captured in a rigid two or three-dimensional network following by polymerization. The imprint molecule is then removed to achieve specific recognition sites in the polymer. These novel polymers have been demonstrated to possess excellent properties for separation of many interesting compounds ranging in size from small molecules to macromolecules [19]. MIPs can bind specifically to their original and related templates and possess tolerance to mechanical stress, temperature, pH, acid-base. Owing to their robust properties, MIPs are suitable for broad range applications as separation media for chromatography and solid phase extraction[20, 21], pre-concentration and determination of trace amount of analytes [22, 23], nano-reactors for combinatorial synthesis of novel inhibitors[24], recognition elements for biosensors[25, 26], artificial receptors for drug assays[27, 28], biological receptor mimics[29], drug delivery and enzyme mimetics[30, 31]. In this study, we intend to use Quercetin-imprinted polymer (QIP) which have proven to specifically bind quercetin as well as allow cross-recognition to the structural analog, anthocyanins, from the crude extracts of mangosteen pericarp residuals.

2. Materials and Methods

2.1. Reagents and apparatus

Quercetin, 4-vinylpyridine (4-VP), ethyleneglycol dimethylacrylate (EDMA) and azobis-isobutyronitrile (AIBN) were purchased from Sigma-Aldrich. All solvents were of analytical or HPLC grade. The absorbance was measured using a UV-visible spectrophotometer (UV-1610, Shimadzu)

2.2. Preparation of Quercetin imprinted polymer

Molecularly imprinted polymers (MIPs) toward quercetin were prepared according to the bulk polymerization method. Briefly, 12 mL of pre-polymerization mixture in ethanol contained 2 mmol of quercetin, 8 mmol of 4-VP as functional monomer, 50 mmol of EDMA as cross-linking monomer and 202 mg of AIBN as the initiator. The pre-polymerization mixture was purged with argon gas for 15 min prior to subjecting it to thermal-induced

polymerization at 60°C for 24 h. The obtained monolithic polymer was ground by a mechanical mortar to produce particles of varying sizes. Sedimentation in acetone was performed to separate and collect particles with diameters around 10-25 μm. Templates were then eluted from the obtained particles using acetic acid:methanol (15%, v/v) via Soxhlet extraction. The control polymers (NIPs) were prepared in the absence of template molecules.

2.3. Binding analysis

Binding analysis was performed by incubating a fixed amount of quercetin (0.1 mg/mL) against various amounts of polymers in a 1 mL microfuge tube on a rocking table at room temperature for 12 h. Supernatants were taken after centrifugation at 12,000 rpm for 5 min prior to determining the amount of template bound via spectrophotometry at 255 nm. Analytes bound to the imprinted polymers (the adsorption capacity, *Q*) was calculated according to the following equation:

$$Q = \frac{(C_0 - C_1)V}{W}$$

where C_0 and C_1 represents the initial and the free solution analyte concentration, respectively. *V* represents the volume of solution and *W* represents the amount of the polymer.

2.4. Anthocyanin extraction from mangosteen pericarp

1 gram of freshly ground mangosteen pericarp was suspended in 1% HCl in EtOH (200mL). The mixture was subjected to sonication for 30 min, subsequently stored at 4 °C for 24 h. Then, the mixture was filtered and absorbance was measured at 540 nm. Calculation of anthocyanin pigment concentration was expressed as cyanidin-3-glucoside equivalents as follows[32]:

$$Total\ anthocyanins = O.D. \times \frac{TEV}{SW} \times \frac{DV}{SV} \times \frac{1}{E^{1\%}_{1\ cm}/10} \times 100$$

O.D. is the optical density, *DV* is the dilution volume, *SV* is the sample volume, *SW* is the sample weight, *TEV* is the total extract volume, *E* is the extinction coefficient based on a cyaniding 3-glucoside molar of 26,900 and molecular weight of 449.2. The total anthocyanin values were obtained in terms of mg of anthocyanin per 100 g of fresh-frozen mangosteen pericarp.

2.5. Evaluation of QIP for anthocyanin extraction

To 40 mg of polymer, the solution containing 0.1mg/mL of anthocyanin in EtOH:H_2O (4:1, v/v) was added. Subsequently, the mixture was incubated at room temperature for 16-18 h on a rocking table. Centrifugation was carried out at 12000 rpm for 5 minutes. Supernatant was subjected to measurement of the absorbance at 540 nm followed by calculating its % binding capacity.

3. Result and discussion

QIP was prepared using quercetin as template, 4-VP as functional monomer and EDMA as the cross-linking monomer (Figure 1). The choice of functional monomer is crucial for maintaining the stability of template-monomer complexes during the imprinting processes as well as pertinent in stabilizing the binding site and affinity. 4-VP was selected as a functional monomer owing to the H-bond acceptor property from the pyridine nitrogen. Such functional moiety of 4-VP is complementary to quercetin, which contained 5 H-donor sites from hydroxyl groups. Previously, systemic investigation on the influence of monomer and crosslinking monomer on the properties of MIPs for quercetin had been carried out [33].

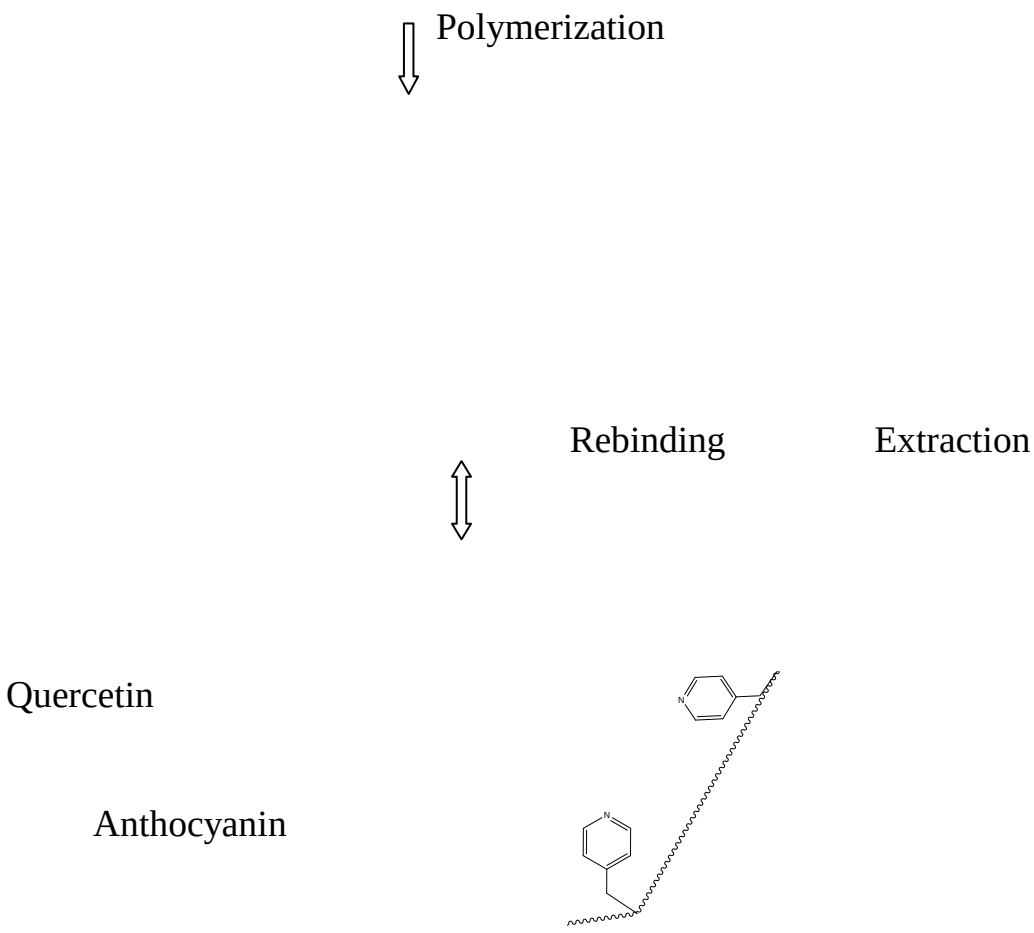

Fig. 1. QIP synthetic scheme

The suitable ratio of quercetin:4-VP:EDMA that afforded high specificity of MIPs for quercetin was 1:4:20, respectively. Meanwhile, MAA-containing polymer showed lower binding affinity for the template. Therefore, MIPs in this study were prepared using the molar ratio of 1:4:50. The binding performance of QIP was examined by varying the amount of polymers using a fixed amount of template. The binding analysis was performed in the same solvent used

during polymerization (EtOH). Using 80 mg of polymer incubated against 0.1 mg/mL quercetin, the result is shown in Figure 2. It can be observed that the QIP can bind to quercetin (44.8%) significantly greater than that of the control polymer (14.8%) by 3 folds of magnitude. To further enhance the binding performance of QIP, a small amount of H_2O were added to the rebinding solvent as follows: ethanol:H_2O(3:2) and ethanol:H_2O(4:1) in order to promote the interaction via π-π stacking in the polar binary solvents mixture. Such findings were in accordance with the results from our previous reports [34, 35] in which the binding performance of QIP and its control polymer in solvent mixture of EtOH:H_2O (4:1,v/v) afforded good rebinding selectivity towards quercetin where MIP afforded 53.9% binding to its template molecule, which is 4.5 folds higher than that of the control polymer (12.5%) at 80 mg polymer. The calculated binding capacity of QIP was approximately 1 mg/g of polymer. For rebinding analysis in ethanol:H_2O(3:2) there was significant increases in non-specific binding as represented for 80 mg of both QIP and control polymer in which binding to the template was 69.6% and 46.2% respectively. Furthermore, the binding specificity of QIP was tested against tocopherol succinate, tocopherol nicotinate and 8-hydroxyquinoline.

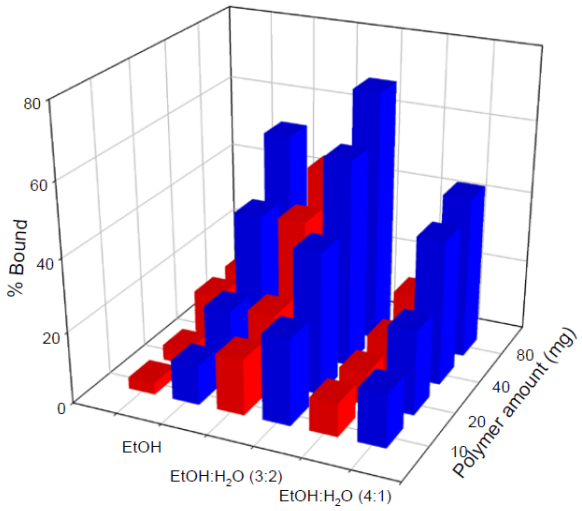

Fig. 2. Rebinding experiment of QIP and control polymer toward their template with optimization of binding solvent.

To 40 mg of QIP, each of various analyses (0.1 mg/mL) was added. QIP could bind quercetin up to 40% whereas it could bind tocopherol succinate, tocopherol nicotinate and 8-hydroxyquinoline at less than 5%. Results indicated that QIP displayed high selective recognition toward its template than those of the other analytes as shown in Figure 3.

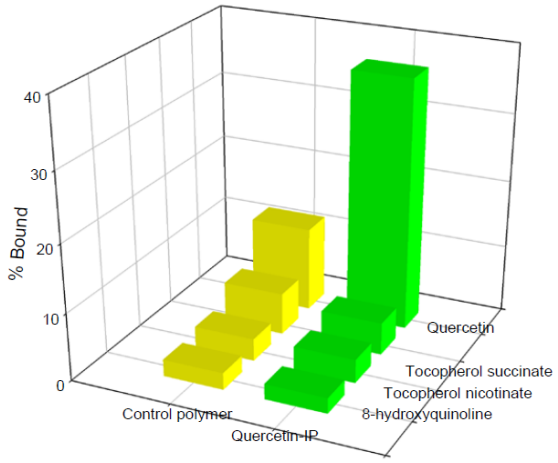

Fig. 3. Binding specificity of QIP.

Anthocyanins were extracted from mangosteen pericarp using 1% HCl in cold ethanol incubation at 4 °C for 24 hours. The anthocyanin contents were calculated using the previously described formula. The total anthocyanin content in acidified ethanol over the durations of 1, 2 and 24 hours was found to be 47.07, 54.42 and 73.7 mg/g respectively, in the ethanol extract. Anthocyanin containing crude extract from the mangosteen pericarp was calculated for preparing the concentration of 0.1mg/mL prior to binding analysis with QIP. Results indicated that 40 mg of QIP possessed high cross-binding property toward anthocyanin in which it could bind anthocyanin up to 35% whereas the control polymer could bind only 4% (Figure 4).

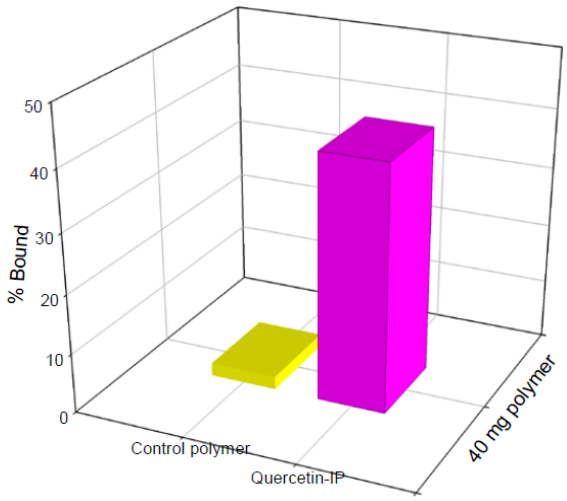

Fig. 4. Rebinding experiment of QIP toward anthocyanin.

The calculated binding capacity of QIP toward anthocyanins was approximately 0.875 mg/g polymer. This result indicated that QIP has great potential for future application in separation and extraction of anthocyanins from mangosteen pericarp. QIP has been reported to be useful for pre-concentration and clean-up of catechin and structurally related compounds from several natural products [33]. It has been used to extend their selectivity toward quercetin analogues for drug discovery development [36]. This study demonstrates the great potential of MIP technology for the development of highly specific functional material for separation and concentration.

4. Conclusion

The prepared QIP showed good rebinding and selective recognition for quercetin as compared to the control polymer. The resultant MIP displayed specificity toward the quercetin template molecule over those of other analytes such as tocopherol succinate, tocopherol nicotinate and 8-hydroxyquinoline. Furthermore, QIP was evaluated for its cross-recognition against anthocyanin from the mangosteen pericarp extract. High specific binding of QIP could be attributed to its high affinity for related compounds as anthocyanin. This MIP holds great potential for the pre-concentration and separation of flavonoids and related analogs from fruits and vegetables extract.

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เนื้อหางานวิจัยส่วนที่ 2

Molecularly Imprinted Polymer for Human Viral Pathogens Detection

1. Introduction

Today, there is an apparent upsurge in the emergence and re-emergence of infectious diseases that have a major impact on the health of humans, animals and plants. In humans, infectious diseases are responsible for approximately 27% of annual deaths incurred throughout the world [1]. This reflects the combined impact of rapid demographic, environmental, social, technological and other changes in our society and habitat. Thus, there is an inherent need to develop novel diagnostic techniques so as to keep pace with the ever changing health problems that may emerge in the future. Several detection tools are available on the market based on the concept of antigen-antibody and/or receptor-ligand interactions. In spite of their many advantages, such biomolecules possess inherent flaws in terms of stability and usage. Although a variety of bioassays and biosensors have been developed, there is still a need for low cost, disposable or reusable biosensors that are capable of rapid detection and accurate identification of a wide range of pathogens. The utilization of the molecular imprinting technology for the development of molecularly imprinted polymers (MIPs) as biorecognition elements provide a real alternative to antibodies owing to their inherent robustness and reproducibility [2]. Molecular imprinting has made it possible to produce tailor-made artificial receptors that are capable of binding specifically to template molecules of interests. The molecular imprinting process essentially involves three main steps: (i) self-assembly of template and functional monomer molecules, (ii) polymerization of template-monomer complex with cross-linking monomers and (iii) template removal to unveil a binding cavity that is specific to the imprint molecule. These novel polymers have been demonstrated to possess excellent properties for the separation of many interesting compounds ranging from small molecules to macromolecules. MIPs can bind specifically to the original as well as related template molecules while also possessing tolerance to mechanical stress, temperature, pH, acid-base, etc. Owing to their robust properties, MIPs are suitable for a wide range of applications such as separation media for chromatography and solid phase extraction [3, 4] nanoreactors for the

combinatorial synthesis of novel enzyme inhibitors [5, 6], recognition elements for biosensors [7], artificial receptors for drug assays [8, 9], biological receptor mimics [10], drug delivery [11] and enzyme mimetics [12]. A wide range of templates have been imprinted such as folic acid [13], 5-fluorouracil [14], glutathione [15], gramine [16], hydrazone [17], human prostate cancer cell lines [18], matrine [19], paclitaxel [20], (S)-2-(acrylamido) propanoic acid [21], theophylline [22], uric acid [23], valganciclovir [24]. Many viruses possess a high mutation rate thereby giving rise to new variants. A change in the antigenic epitope on the surface protein would possibly create new variants, which in turn may cause reinfection. Therefore, effective detection and control is of urgent need for proper clinical management. The inherent robustness and reproducibility of MIPs facilitate the fabrication of tailor-made receptors for rapid detection as to keep pace with emerging viral infections. This review article focuses on the surrounding literature of MIP utilization for the detection of viral human pathogens.

2. Influenza

Seasonal influenza, more commonly known as the flu, is an acute viral infection caused by the influenza virus. The virus spreads through an infected individuals cough thereby allowing dispersal of infected droplets into the air with the ability to infect those in the vicinity. The virus can also be transmitted through direct contact with an infected person. In addition, influenza causes an estimated 3 to 5 million cases globally with around 250,000 to 500,000 deaths annually [25]. Furthermore, the influenza virus can be classified into 3 types: A, B and C. Type A influenza viruses can be further divided into subtypes according to the combination of virus surface proteins (i.e. several isoforms of both hemagglutinins and neuraminidases) such as H1N1 and H3N2 subtypes, etc. Molecular imprinting polymer was first applied to screen influenza A virus by Wangchareansak and coworkers [26, 27] as summarized in Figure 1. This appreciated work combined MIPs and QCM for proof-of-concept of screening protocols for influenza virus subtypes including H5N1, H5N3, H1N1, H1N3 and H6N1. Influenza virus surface antigens are made up of glycoproteins hemagglutinin (HA) and neuraminidase (NA) that play a role in the subtype classification. MIPs were made for each subtype of Influenza A virus whereby each MIP possessed a good recognition property towards its original viral template. Furthermore, the template sharing the same neuraminidase domains as H1N3 and H5N3 can be differentiated by its own MIP, suggesting that the

hemagglutinin domain contribute more to the selectivity property in this case. H5N1-MIP has been found to bind strongly to virus containing N1 rather than those of virus containing N3. Their finding suggests that both the H and N domain play important roles in molecular recognition of MIP. Furthermore, 5 different MIPs have been possessed as their recognition profile which are offered as molecular fingerprints. This report has opened a new feasibility providing an alternative rapid way to screen influenza A virus subtypes in unknown samples with detection limits as low as 105 particles/ml. Moreover, Wangchareansak et al. has also used the influenza virus-MIPs as a novel polymer for identifying molecular binding to the influenza virus H5N1. These MIPs were used to facilitate identification of inhibitors that can bind to, and inhibit the function of virus upon inducing a conformational change. Thus, the inhibitor treated virus is expected to have a reduced or inhibited binding to the H5N1 specific MIPs.

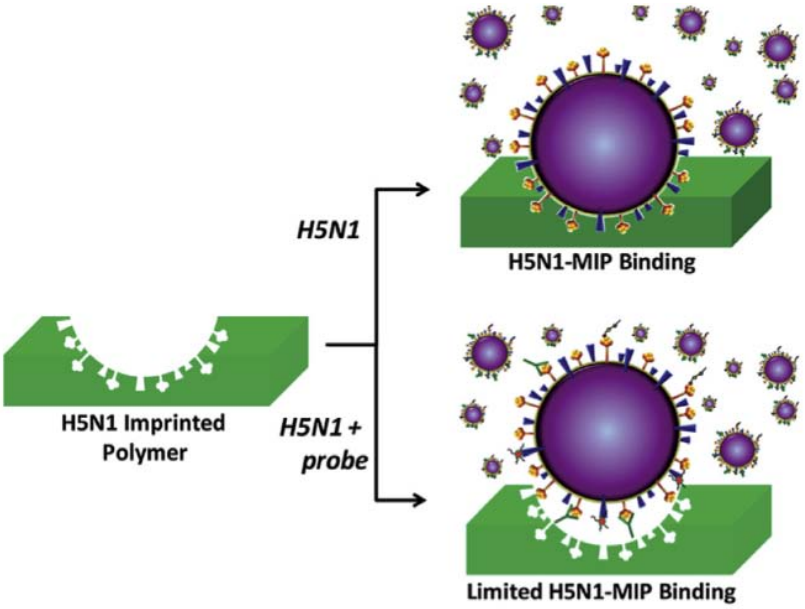

Fig. 1. Schematic representation of the binding of H5N1 virus to the imprinted polymer in the absence (right, top) and presence (right, bottom) of probe molecules (e.g. H5 antibody, Oseltamivir, Sialic acid, GlcNAc13 and H1 antibody). The binding of probe molecules to the virus causes significant conformational change which consequently, leads to limited binding of the virus with the imprinted polymer.

3. Dengue virus

Dengue is one of the fast emerging and evolving diseases in many parts of the world. Dengue is a mosquito-borne viral infection caused by dengue virus.

Annually, an estimated 50 million dengue infections and 500,000 individuals are hospitalized due to dengue infection having a fatality rate of about 5 percent in some areas [28]. The infection can cause severe clinical problems due to non-specificity of clinical presentations leading to misdiagnosis of the disease. An effective vaccine development for the prevention of dengue is currently underway. Therefore, early detection is still urgently required for clinical diagnosis and patient management. Tai and coworkers [29, 30] developed MIP for the recognition of Dengue virus protein NS1 (nonstructural protein 1). NS1 is normally found in the blood specimen during the viremia phase. It is found on the infected cell surface or as secreted NS1 in the blood. The linear 15-mer peptides derived from NS1 of JEV (Japanese encephalitis virus) was chosen as the template (Thr-Glu-Leu-Arg-Tyr-Ser-Trp-Lys-Thr-Trp-Gly-Lys-Ala-Lys-Met) according to its consensus linear epitope mapping of dengue virus NS1 toward antibody D2/8-1. This epitope-mediated imprinting based MIP was coated on QCM. The MIP-grafted 15-mer peptide chip could bind to pentadecapeptide, purified and unpurified NS1 proteins with a nanomolar range dissociation constant (K_D) of 0.6, 0.04 and 0.09 nM, respectively. The results indicated a strong polymer-template interaction from the multi-point attachment of the NS1 protein to the 15-mer peptide chip, which was comparable to the monoclonal antibody immobilized chip (K_D = 0.05 nM). Furthermore, the MIP-QCM has been applied for dengue virus detection from patients serum samples using the flow injection system. This MIP-QCM retained their specificity towards NS1 protein in serum and possessed high sensitivity detection down to the μM range with a short operation time of 20-30 minutes/sample. Therefore, this technique could be used to detect the 4 serotypes of dengue virus whereby a pretreatment sample was not needed for this novel sensor.

4. Japanese Encephalitis Virus

Japanese encephalitis virus (JEV) is one of the main causes of viral meningitis is Asia and Australia. Just like Dengue, Japanese encephalitis (JE) is also a mosquito-borne viral disease. The annual incidence of human cases has been reported to be about 50,000 to 175,000. In addition, JE not only has a high mortality rate (25-30%) but also reported that 50% of surviving patients suffer from neuropsychiatric sequelae. JE is considered as the most frequent viral encephalitis associated with fatal or severe outcomes [31] The JEV recognition MIP has been frabicated based on fluorescent resonance energy transfer (FRET)

[32]. The JEV recognition MIP was coated on silica microspheres which contained the fluorescent dye, pyrene-1-carboxaldehyde (PC). The FRET phenomenon can be enhanced upon virus binding as energy donor while PC acted as an energy acceptor. The novel FRET based virus detection MIP possessed high sensitivity detection of 9.6 pM at ambient temperatures. The specificity of this MIP was evaluated and found to exert an imprinting factor of 2.12. In addition, the virus MIP displayed a good selectivity towards the JEV over those of other viruses including Hepatitis A virus (HAV), Leprosy virus (LV) and Rabies virus (RV). Furthermore, its application with real samples has been demonstrated using a diluted human serum with a resulting recovery of nearly 100%. JEV-imprinted magnetic silicon microspheres were successfully invented by He et al. [33]. These microspheres exhibited excellent binding selectivity towards JEV with an imprinting factor of 2.95. Moreover, the virus-MIP possessed a detection limit as low as 0.32 nM. Furthermore, great selectivity on JEV using JEV-imprinted magnetic silicon microsphere has been demonstrated over other viruses (LV, RV). This novel MIP microsphere exhibited good recovery upon testing with virus spiked samples. Thus, JEV-imprinted polymer has great potential as a tool for application on JEV analysis and disease diagnosis.

5. Human Immunodeficiency Virus (HIV)

HIV primarily targets the immune system of patients, making them susceptible against other infections and certain cancers. HIV can be transmitted via various types of body fluids from an infected person, such as blood, breast milk, semen and vaginal secretions. HIV is one of the main causes of mortality and morbidity in the world. HIV has infected about 75 million people around the world and an estimated of 37 million people are currently living with the virus [6]. Lu et al. [34] has developed a HIV-1 related glycoprotein 41 (gp41) bio-imprinting sensors based on the epitope imprinting technique as summarized in Figure 2.

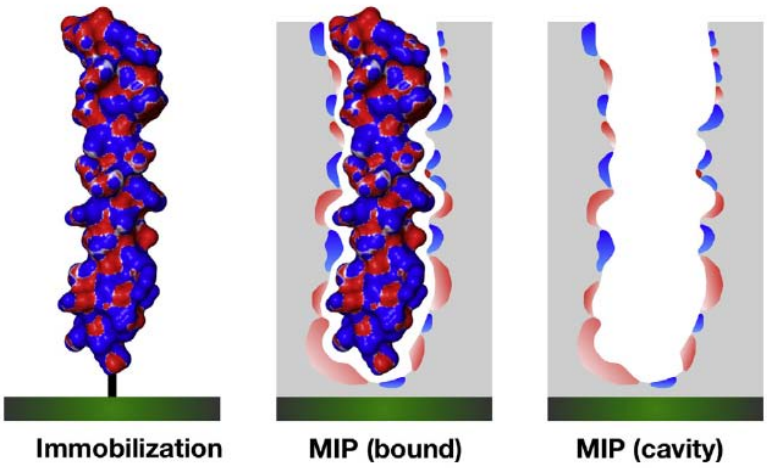

Fig. 2. Cartoon illustration summarizing the immobilization of a template protein onto a surface, formation of a molecularly imprinted polymer around the template protein and finally the elution of the template protein to reveal an empty cavity that can accommodate an incoming protein.

This glycoprotein is located on the viral coat of HIV-1. HIV-1 gp41 plays a significant role in membrane fusion upon glycoprotein 120 (gp120) binding towards CD4 cells. This membrane fusion allowing for the activation of gp41 may provide strategies for vaccine and antiviral drug development. Synthetic mimicking of a 35 amino acid residue (aa 579-613) was used as a template due to its property as a major immune-dominant region containing antibodies that recognized around 98% of AIDS patients. In this work, dopamine was used as a functional monomer. Polydopamine possessed high stability, hydrophilicity and biocompatibility. The synthetic peptide was embedded into the polydopamine during induction of the polymerization process. Hydrogen bonding, ionic bonding and hydrophobic interactions may play a role in the possible interaction between the peptide and dopamine. The hydrophobic MIP film grafted on QCM has been fabricated. The MIP coated QCM exerted a good specific affinity toward its template peptide. Upon a 100 ng/mL injection of template, the maximal frequency shifts of MIP-coated QCM sensor was 15.13 Hz whereas MIP-coated QCM sensor displayed only 1.799 Hz. The calculated KD of this MIP was 3.17 nM, indicating a high affinity of the MIPs towards the template molecule. The selectivity of the MIP-coated QCM has been investigated against the peptide with bovine serum albumin (BSA), two and eleven mutated residues (2M-peptides, 11M-peptide). The result indicated that the BSA and 11M-mutated peptide displayed a much lower frequency response as compared to the template peptide. Whereas the 2M-peptide displayed a similar frequency response to the original template peptide. Thus the display of frequency attained

by the 2M-peptide in comparison with the 11-M peptide could be due to the possible reason that it has only two mutated amino acids difference than the original template. furthermore, the whole molecule of HIV-1 gp41 has been tested to display a linear frequency shift in the range from 5 ng/mL-200 ng/mL with a detection limit of 2 ng/mL. This detection limit is comparable to the reports from the ELISA method. The recovery performance of MIP-coated QCM towards HIV-1 gp41 urine spiked sample was in the range of 86.5-94.1%. This novel MIP-coated QCM was successfully fabricated and applied for monitoring HIV-1 gp41 in urine samples with high sensitivity and selectivity, suggesting its potential application in the future. Khaled Seidi and Farajzadeh [35] proposed nanomagnet-based detoxifying machines using MIPs as viral capture for complementary approach in HIV therapy. Furthermore, the magnetic MIP behaves as a capture probe for the HIV-1 antibody that has already been invented [36]. These magnetic-MIPs have characteristically displayed a good advantage as a tool in combination with immunoassay. This MIP-combined immunosensor has also provided a low cost, simple and high sensitivity tool, which is suitable for the early diagnosis of HIV infected patients.

6. Hepatitis A virus

Hepatitis A is a liver disease caused by the Hepatitis A virus (HAV). The most common mode of infection of the disease is via contaminated food and water with feces of an infected person. Even though hepatitis A does present with chronic liver disease which is also seen in hepatitis B and C, the debilitating symptoms and sudden liver failure that accompany hepatitis A can cause death of the patient. Hepatitis A is mostly spread via the fecal-oral route and close contact with an infected person. Yang et al. [37] has applied a molecular imprinting strategy for the direct detection of the hepatitis A virus. HAV is a significant human pathogen which cannot be distinguished from other types of hepatitis viruses upon clinical presentation. Therefore, direct detection with high specificity and sensitivity plays a crucial role for accurate diagnosis. Yang developed a novel core-shell molecularly imprinted nanoparticles based on polydopamine (PDA) capped with SiO_2. This HAV-imprinted SiO_2@PDA nanoparticles possessed hydrophilic, biocompatibility and specificity recognition properties towards the HAV template. The novel resonance light scattering (RLS) technology has been employed for specific recognition and detection based on the remarkable advantage of high sensitivity and

convenience in operation by using simple fluorescence spectrophotometer. The enhancement of light scattering of HAV-imprinted SiO_2@PDA nanoparticles was correspondingly related to the increasing of HAV concentration. The selectivity of HAV-imprinted SiO_2@PDA nanoparticles were tested against HAV, rabies virus, a mixture of measles and rubella viruses and Japanese encephalitis virus. The binding selectivity were in the following order: HAV was set as 100% > rabies virus (16.4%) > Japanese encephalitis virus (12.9%) > the mixture of measles and rubella viruses (9.6%) as shown in Figure 3.

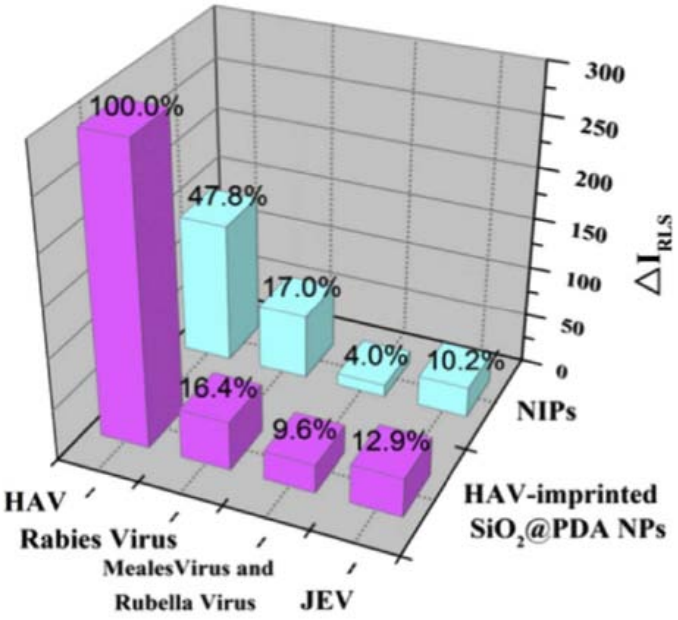

Fig. 3. Binding selectivity of hepatitis A virus (HAV) imprinted SiO_2 nanoparticles (SiO_2@PDA) towards HAV and three other viruses consisting of rabies virus, Japanese encephalitis virus (JEV) and a mixture of measles virus and rubella virus.

This finding indicated that an excellent selectivity was obtained. Furthermore, the HAV-imprinted SiO_2@PDA nanoparticle was successfully used for the determination of HAV in real sample serum and possessed the detection limit of 8.6 pM. This novel method combined a promising technology of MIP and RLS and may open up a potential alternative way for MIPs-based virus detection.

7. Hepatitis B virus

Hepatitis B is a serious liver disease caused by the Hepatitis B virus (HBV). HBV infection is the most common chronic viral infection in the world, with an estimated rate of infection of around 2 billion people with more than 350 million people serving as chronic carriers of the virus [38]. HBV infection is highly contagious and can transmit from mother to child or through exposure to infected blood. It is also spread by various body fluids such as saliva, vaginal, seminal and menstrual fluids. The hepatitis B vaccine is the mainstay of HBV prevention. An effective developed immunity against HBV require levels of hepatitis B surface antibody (HBsAb 10 mIU/mL). Uzan et al. [39, 40] developed a HBsAb imprinted poly(hydroxyl-ethyl-methacrylate-N-methacryloyl-L-tyrosine methylester film on the surface plasmon resonance (SPR) sensor chip for diagnosis of HBsAb in human serum and HBsAb imprinted PHE-MAT particle for HBsAb purification. The prepared HBsAb-imprinted PHEMAT SPR chip displayed a good relevance of HBsAb detection which was comparable to the ELISA method (AxSYM immunoassay system, Abbott Laboratory, Illinois, USA) with a R2 value of 0.9969 linearity. The chip retained the ability to detect the HBsAb from human serum with 99.7% precision in the range of 0-120 mIU/mL. The maximum detection limit of this sensor chip was 208.22 mIU/mL with KA and KD values of 0.015 mIU/mL and 66 mIU/mL, respectively. HbsAb negative human serum was also tested with the PHEMAT SPR chip where no significant response was observed. Therefore, the HbsAb-imprinted PHEMAT SPR chip provides a potential use for diagnosis of HBsAb from human serum.

8. Adenovirus

Human Adenoviruses (HAdVs) can cause a plethora of clinical diseases including conjuctivitis, gastroenteritis, hepatitis, myocarditis and pneumonia. Globally, 5-7% of respiratory tract infections among children are ascribed to HAdV [41, 42]. Altintas et al. [43] has fabricated Adenovirus specific-MIP nanoparticles immobilized on a surface plasmon resonance (SPR). In comparison to the MIP-based adenovirus assay, the direct and sandwich assays using natural antibodies have also been developed. MIPs were produced using glass beads as solid support for the adenovirus template followed by the

components for MIPs synthesis to obtain adenovirus-MIP nanoparticles with a size of approximately 260 nm (Figure 4).

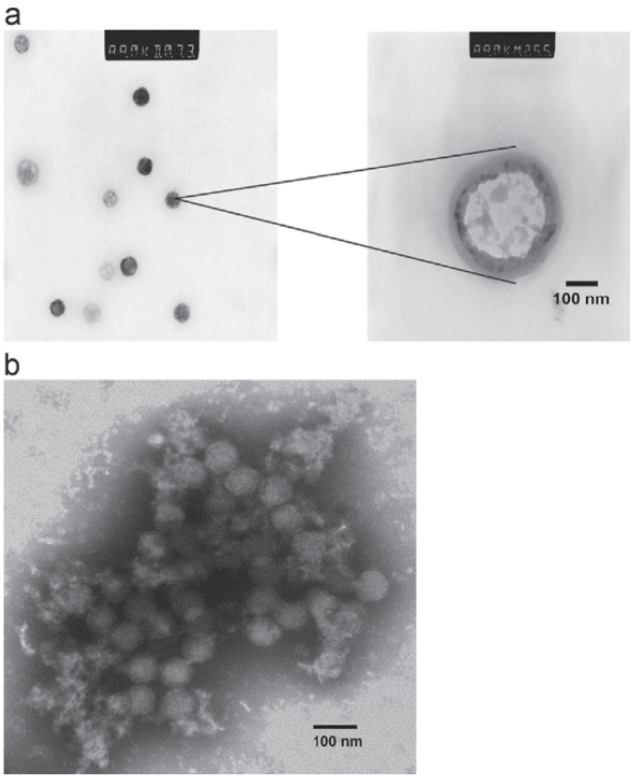

Fig. 4. Transmission electron microscope images of adenovirus imprinted polymer (a) and adenovirus (b).

The MIPs beads were immobilized on the SPR chip via glutaraldehyde coupling. The immobilized MIPs obtained a good reproducibility for signal detection in a concentration range of 0.02-20 pM with the detection limit of 0.02 pM. The binding affinity was calculated with a K_D of 3.10×10^{-11} using the Biacore 3000 software. In addition, the binding specificity of adenovirus-MIP was tested against the MS2-phage and vancomycin whereby non-specific binding was found in a very low level against the adenovirus-MIP. Furthermore, the adenovirus polyclonal antibody was used for direct and sandwich assays as a comparison to the adenovirus-MIP in terms of sensitivity. The K_D were calculated as 2.30×10^{-12}, 3.10×10^{-11} and 1.41×10^{-10} M, respectively, with the following order: sandwich antibody assay> MIP > direct antibody assay. The detection limit of the sandwich antibody assay was found to be the most sensitive as compared to MIP and direct antibody assay with 0.008, 0.02 and 0.3 pM, respectively. The overall results demonstrated the excellent achievement of all assay types and confirmed the suitability of the MIPs-based sensors for the

detection of virus, which exerted high sensitivity and specificity as well as was easy-to-use.

9. Picornaviruses

Picornaviruses are non-enveloped, RNA viruses consisting of over 50 species in the family that can be divided among 29 genera. These viruses can reside in mammals and birds and can cause many diseases including paralysis, meningitis, hepatitis and poliomyelitis [44]. MIPs for the smallest RNA virus, picornaviruses has been developed using human rhinovirus (HRV) as a representative. Stamp imprinting procedure has been used as a template for virus-patterned memorizing geometries feature. More than 100 different serotypes of HRV were reported. Jenik et al. [45] has developed HRV-imprinted polymer coated on QCM for rapid analysis on fast frequency response shift as well as reversible non-covalent interaction behavior. The HRV2-imprinted sensor showed a frequency decrease of -750 Hz upon injection of 30 μg/mL of virus suspension whereas the non-imprinted reference channel was decreased only at -100 Hz. The frequency decrease of the sensors was dependent on the analyte concentration in linear concentration. The sensor also had the ability to distinguish between native and denatural forms of the virus. Furthermore, different serotypes of HRV in surface chemistry (HRV14, HRV2 and HRV1A) can be selectively recognized by its own HRV template origin over other species (Figure 5).

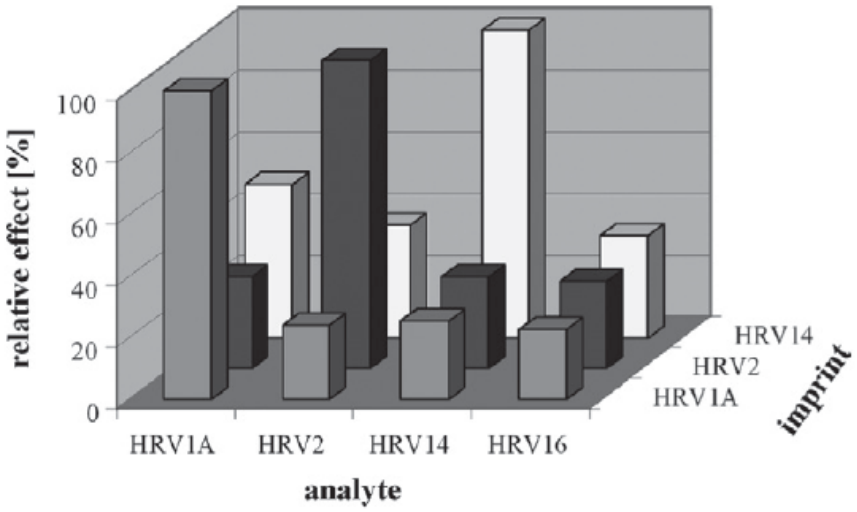

Fig. 5. Cross-selectivity of imprinted polymers against various serotypes of HRV.

Imprinted polymers showed stronger rebinding to its own template. This finding confirmed the properties of this novel MIP in which it can be distinguished based upon surface chemistry even though the geometry features of template were the same. The smallest picornavirus, foot and mouth disease virus (FMDV), serotype Manissa has been evaluated for binding to HRV2-imprinted sensor. Although the geometry of FMDV was not exactly fitting due to its small size (25 nm), the surface chemistry is nearly the same to HRV. Hence, the sensor can response according to its non-covalent interactions in terms of cross-sensitivity. This work has opened up applications of MIPs for developing MIP-based virus sensors.

10.Technical remarks

Many types of pathogenic viruses have been imprinted to investigate the feasibility of various practical applications (Table 1). Various types of transducers have been utilized for fast signal recognition as well as increased sensitivity of detection such as QCM and SPR. Stamp imprinting and epitope-mediated imprinting have been favorably employed for governing sensing elements. Functional nanoparticles support is more straightforward upon the binding sites, which are located on imprinted surfaces for fast recognition and installation of the sensing system phenomenon such as FRET, RLS and ECL. Most of the virus template can be imprinted and can investigate binding properties in an aqueous environment.

Table 1
Summary of experimental conditions for the molecular imprinting of human viral pathogens.

Virus	Template	Functional monomer[a]	Crosslinker[b]	Porogenic solven[c]	Format	Transducer[d]	Adsorption solvent	Ref.
Influenza virus	H5N1, H5N3, H1N1, H1N3, H6N1	Am, MAA, MMA, NVP	DHEBA	DMSO	SI	QCM	PBS pH 7.2	[26,27]
Dengue virus	15-mers peptide from JEV NS1	Am, AA, BAm	EDMA	Acetonitrile, Phosphate buffer pH 4.0	EmI	QCM	PBS pH 4.0	[29,30]
Japanese encephalitis virus	Freeze dried JEV vaccine	APTES	TEOS	Ultrapure water	MIPs coated Fe_3O_4@SiO_2 microspheres		Ultrapure water	[33]
	Freeze dried JEV vaccine	APTES	TEOS	Ultrapure water	PC-modified silica microspheres	FRET	Ultrapure water	[32]
Human immunodeficiency virus	HIV-1 gp41	PDA	PDA	Tris-HCl buffer pH 8.5	EmI	QCM	Tris-HCl pH 7.0	[34]
	HIV-1 antibody	APBA	APBA	Phosphate buffer pH 8.0	Magnetic MIPs	ECL	Phosphate buffer pH 8.0	[36]
Hepatitis A virus	HAV2	PDA	PDA	Tris-HCl buffer pH 8.5	PDA-coated SiO_2 nanoparticles	RLS	Tris-HCl buffer pH 6.2	[37]
Hepatitis B virus	Hepatitis B surface Ab	MAT, HEMA	EDMA	Toluene	PHEMAT particles		0.9% NaCl	[39]
	Hepatitis B surface Ab	MAT, HEMA	EDMA	MOPS buffer pH 6.0	PHEMAT particles	SPR	Deionized water	[39]
Adenovirus	Adenovirus	NIPAM, AA	MBAm, TBA, APTES	Ethanol	MIP nanoparticles	SPR	PBS pH 7.4	[43]
Picornavirus	HRV2, HRV14, HRV1A	PU, BPA	Phloroglucinol	Tris-HCl, THF	SI	QCM	Tris-HCl buffer pH 7.2	[45]

[a] Am: acrylamide; AA: acrylic acid; APBA: 3-aminobenzeneboronic acid; APTES: 3-aminopropyl triethoxysilane; BAm: N-benzylacrylamide; BPA: bisphenol A; HEMA: hydroxyethyl methacrylate, MAA: methacrylic acid; MAT: *N*-methacryloyl-L-tyrosine methyl ester; MBAm: N,N′-methylenebisacrylamide; MMA: methylmethacrylate; NIPAM: N-isopropylacrylamide; NVP: N-vinylpyrrolidone; PDA: polydopamine; PU: polyurethane; TBA: N-tert-butylacrylamide; TEOS: tetraethoxysilicane.
[b] DHEBA: N,N′-(1,2-dihydroxyethylene) bisacrylamide; EDMA: ethylene glycol dimethacrylate.
[c] DMSO: dimethylsulfoxide; THF: tetrahydrofurane.
[d] SI: stamp imprinting; EmI: epitope mediated imprinting; QCM: quartz crystal microbalance; PC: pyrene-1-carboxyaldehyde; FRET: Förster resonance energy transfer; ECL: electrochemiluminescence.

11.Conclusion

Molecular imprinting is an attractive technology used to create selective recognition sites within a macromolecular polymer network. One of the major advantages of MIPs is their robustness, high selectivity, long-term stability and cost effectiveness which cannot be obtained by using fragile biomolecules. MIPs have preliminarily been used for selective and sensitive viral detection as well as applied for viral subtype differentiation. Moreover, MIPs have been introduced as a novel media for the screening of inhibitors for drug discovery that may be used in the combat against viral infections. The manifold of advantages brought about by the molecular imprinting technology is anticipated to find its home in the frontlines of future viral studies.

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Outputs จากโครงการวิจัยที่ได้รับทุนจาก สกว**.**

ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

1. **Piacham T***, Isarankura-Na-Ayudhya C, Prachayasittikul V. Quercetin-imprinted polymer for anthocyanin extraction from mangosteen pericarp. Mater Sci Eng C. 2015; 51:127-31.
2. Malik AA, Nantasenamat C, **Piacham T***. Molecularly imprinted polymer for human viral pathogens detection. Mater Sci Eng C. 2017;77:1341-8.

Materials Science and Engineering C 51 (2015) 127–131

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Quercetin-imprinted polymer for anthocyanin extraction from mangosteen pericarp

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ARTICLE INFO

Article history:
Received 22 November 2014
Received in revised form 31 January 2015
Accepted 25 February 2015
Available online 26 February 2015

Keywords:
Molecularly imprinted polymer
Anthocyanin
Mangosteen pericarp
Quercetin

ABSTRACT

Molecular imprinting is a facilitative technology for the production of artificial receptors possessing great endurance with high specificity toward target molecules of interest. The polymers are commonly applied for separation or analysis of substances of interest. In this study, we prepared molecularly imprinted polymers for the purpose of binding specifically to quercetin and related compounds. Quercetin was used as the template molecule, 4-vinylpyridine (4-VP) as the functional monomer, ethylene glycol dimethacrylate (EDMA) as the cross-linking monomer, azobisisobutyronitrile (AIBN) as the polymerization initiator and ethanol as the porogenic solvent. Such 4-VP-based imprinted polymer was found to bind the template molecule greater than that of the control polymer with an approximate 2 folds higher binding using 20 mg of polymer in the optimal solvent, ethanol: water (4:1 v/v). Quercetin-imprinted polymer (QIP) was found to bind well against its template; approximately 1 mg/g polymer. In addition, QIP was applied to bind anthocyanin from the crude extract of mangosteen pericarp. The binding capacity of quercetin-MIP toward anthocyanin was approximately 0.875 mg per gram of polymer. This result indicated that quercetin-MIP showed its specific binding to quercetin and related compound particularly anthocyanin. In conclusion, we have demonstrated the successful preparation and utilization of molecularly imprinted polymer for the specific recognition of quercetin as well as structurally related anthocyanins from the mangosteen pericarp with enhanced and robust performance.

1. Introduction

Mangosteen (*Garcinia mangostana* L.) is well known as "the queen of fruits" that is typically consumed fresh for dessert. Different parts of the mangosteen, such as the bark, fruit hull or pericarp and root, have been used for hundreds of years as medication. Due to the abundance of mangosteen consumption, there has been intense research for adding value to the residual products. For example, peel may be considered as waste but has attracted much interest in recent years from the food and pharmaceutical industries. Mangosteen pericarp is an abundant source of natural phenolic antioxidant compounds including xanthones and its derivatives, benzophenones, flavonoids, anthocyanins, and tannins [1]. Anthocyanins, the largest group of water soluble pigments, are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopynylium or flavylium salts [2]. It provides a broad range of biological activities including free radical scavenging activity [3], antimicrobial activity for many different pathogens [4], anti-inflammatory and anticancer activities [5]. Furthermore, it can also prevent oxidative damage to biological macromolecule such as DNA [6], protein [7] and lipid [8]. To date, there is no report of anthocyanin toxicity. Anthocyanins can be absorbed and cross the human intestinal mucosa leading to its circulation in the blood stream [9]. It can be used as a topical agent for skin UV protection [10]. Recently, anthocyanin has shown their potential health benefits in obesity control [11,12], diabetes control [13], prevention of cardiovascular disease (CVD) [14] as well as improvement of visual and brain functions [15,16].

These compounds are water-soluble pigments that can be extracted from many vegetables and fruits using methanol or ethanol containing small amounts of hydrochloric acid [17,18]. There is much attention to extract and include this substance in many pharmaceutical products. One of the eminently attractive synthetic approaches of this study is the use of the molecular imprinting technology in which the natural recognition sites are created in a macromolecular matrix using a molecular template in a casting procedure. The selected molecule for imprinting is first allowed to form and self-assemble with functional monomers. The resulting complexes are subsequently captured in a rigid two or three-dimensional network following polymerization. The imprint molecule is then removed to achieve specific recognition sites in the polymer. These novel polymers have been demonstrated to possess excellent properties for separation of many interesting compounds ranging in size from small molecules to macromolecules [19]. MIPs can bind specifically to their original and related templates and possess tolerance to mechanical stress, temperature, pH, and acid–base. Owing to their robust properties, MIPs are suitable for broad range applications as separation media for chromatography and solid phase extraction [20,21],

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http://dx.doi.org/10.1016/j.msec.2015.02.051
0928-4931/

pre-concentration and determination of trace amount of analytes [22, 23], nano-reactors for combinatorial synthesis of novel inhibitors [24], recognition elements for biosensors [25,26], artificial receptors for drug assays [27,28], biological receptor mimics [29], and drug delivery and enzyme mimetics [30,31]. In this study, we intend to use Quercetin-imprinted polymer (QIP) which has proven to specifically bind quercetin as well as allow cross-recognition to the structural analog, anthocyanins, from the crude extracts of mangosteen pericarp residuals.

2. Materials and methods

2.1. Reagents and apparatus

Quercetin, 4-vinylpyridine (4-VP), ethyleneglycol dimethylacrylate (EDMA) and azobis-isobutyronitrile (AIBN) were purchased from Sigma-Aldrich. All solvents were of analytical or HPLC grade. The absorbance was measured using a UV–visible spectrophotometer (UV-1610, Shimadzu).

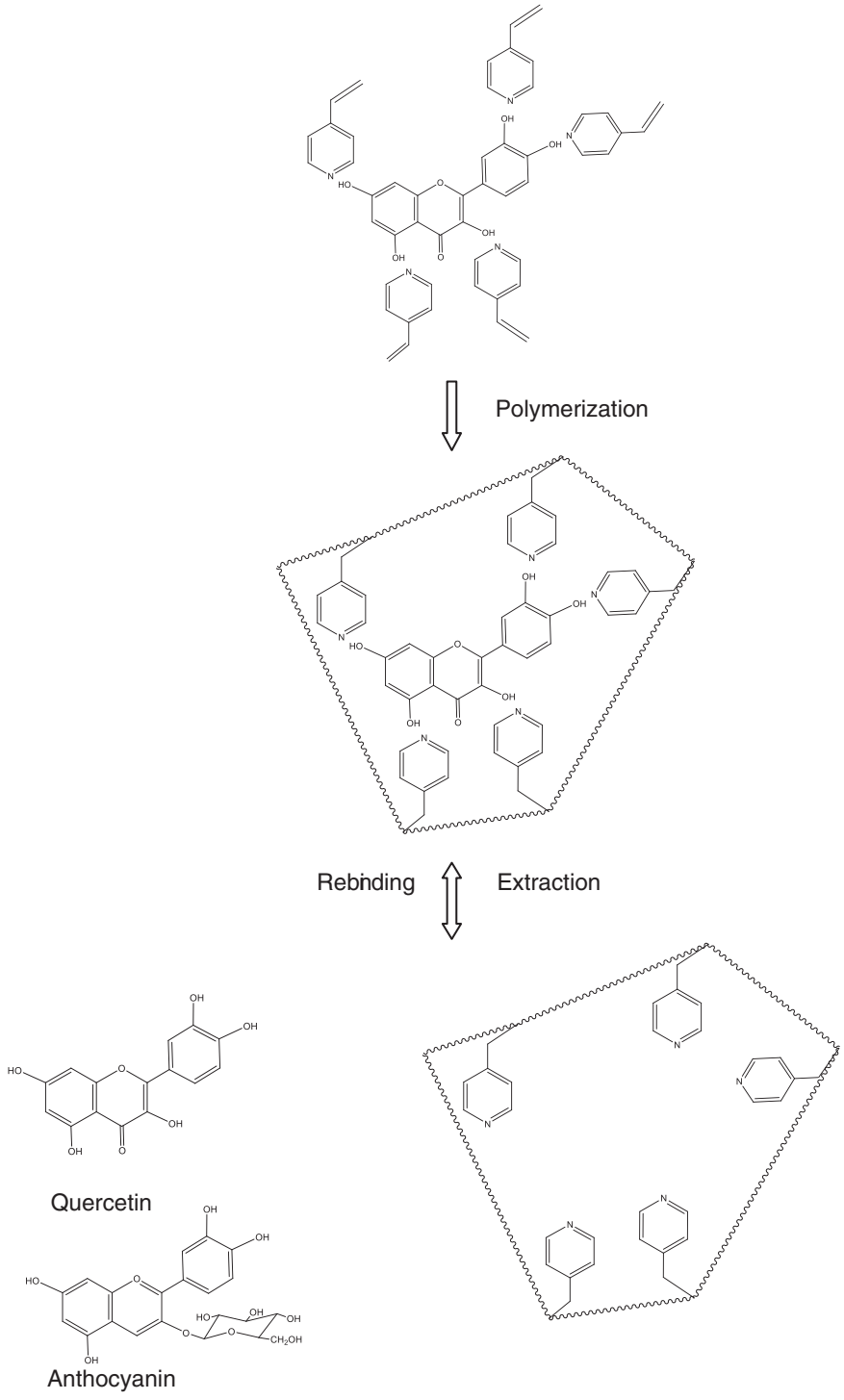

Fig. 1. QIP synthetic scheme.

2.2. Preparation of quercetin imprinted polymer

Molecularly imprinted polymers (MIPs) toward quercetin were prepared according to the bulk polymerization method. Briefly, 12 mL of pre-polymerization mixture in ethanol contained 2 mmol of quercetin, 8 mmol of 4-VP as functional monomer, 50 mmol of EDMA as crosslinking monomer and 202 mg of AIBN as the initiator. The pre-polymerization mixture was purged with argon gas for 15 min prior to subjecting it to thermal-induced polymerization at 60 °C for 24 h. The obtained monolithic polymer was ground by a mechanical mortar to produce particles of varying sizes. Sedimentation in acetone was performed to separate and collect particles with diameters around 10–25 μm. Templates were then eluted from the obtained particles using acetic acid:methanol (15%, v/v) via Soxhlet extraction. The control polymers (NIPs) were prepared in the absence of template molecules.

2.3. Binding analysis

Binding analysis was performed by incubating a fixed amount of quercetin (0.1 mg/mL) against various amounts of polymers in a 1 mL microfuge tube on a rocking table at room temperature for 12 h. Supernatants were taken after centrifugation at 12,000 rpm for 5 min prior to determining the amount of template bound via spectrophotometry at 255 nm. Analytes bound to the imprinted polymers (the adsorption capacity, Q) were calculated according to the following equation:

$$Q = \frac{(C_0 - C_1)V}{W}$$

where C_0 and C_1 represent the initial analyte concentration and the free solution analyte concentration, respectively. V represents the volume of solution and W represents the amount of the polymer.

2.4. Anthocyanin extraction from mangosteen pericarp

1 g of freshly ground mangosteen pericarp was suspended in 1% HCl in EtOH (200 mL). The mixture was subjected to sonication for 30 min, subsequently stored at 4 °C for 24 h. Then, the mixture was filtered and absorbance was measured at 540 nm. Calculation of anthocyanin pigment concentration was expressed as cyanidin-3-glucoside equivalents as follows [32]:

$$\text{Total anthocyanins} = O.D. \times \frac{TEV}{SW} \times \frac{DV}{SV} \times \frac{1}{E^{1\%}_{1\ cm}/10} \times 100.$$

$O.D.$ is the optical density, DV is the dilution volume, SV is the sample volume, SW is the sample weight, TEV is the total extract volume, and E is the extinction coefficient based on a cyaniding 3-glucoside molar of 26,900 and molecular weight of 449.2. The total anthocyanin values were obtained in terms of milligrams of anthocyanin per 100 g of fresh-frozen mangosteen pericarp.

2.5. Evaluation of QIP for anthocyanin extraction

To 40 mg of polymer, the solution containing 0.1 mg/mL of anthocyanin in EtOH:H_2O (4:1, v/v) was added. Subsequently, the mixture was incubated at room temperature for 16–18 h on a rocking table. Centrifugation was carried out at 12,000 rpm for 5 min. Supernatant was subjected to measurement of the absorbance at 540 nm followed by calculating its % binding capacity.

3. Result and discussion

QIP was prepared using quercetin as template, 4-VP as functional monomer and EDMA as the cross-linking monomer (Fig. 1). The choice of functional monomer is crucial for maintaining the stability of template–monomer complexes during the imprinting processes as well as pertinent in stabilizing the binding site and affinity. 4-VP was selected as a functional monomer owing to the H-bond acceptor property from the pyridine nitrogen. Such functional moiety of 4-VP is complementary to quercetin, which contained 5H-donor sites from hydroxyl groups. Previously, systemic investigation on the influence of monomer and crosslinking monomer on the properties of MIPs for quercetin had been carried out [33]. The suitable ratio of quercetin:4-VP:EDMA that afforded high specificity of MIPs for quercetin was 1:4:20, respectively. Meanwhile, MAA-containing polymer showed lower binding affinity for the template. Therefore, MIPs in this study were prepared using the molar ratio of 1:4:50. The binding performance of QIP was examined by varying the amount of polymers using a fixed amount of template. The binding analysis was performed in the same solvent used during polymerization (EtOH). Using 80 mg of polymer incubated against 0.1 mg/mL quercetin, the result is shown in Fig. 2. It can be observed that the QIP can bind to quercetin (44.8%) significantly greater than that of the control polymer (14.8%) by 3 folds of magnitude. To further enhance the binding performance of QIP, a small amount of H_2O was added to the rebinding solvent as follows: ethanol:H_2O(3:2) and ethanol:H_2O(4:1) in order to promote the interaction via π–π stacking in the polar binary solvent mixture. Such findings were in accordance with the results from our previous reports [34,35] in which the binding performance of QIP and its control polymer in solvent mixture of EtOH:H_2O (4:1,v/v) afforded good rebinding selectivity toward quercetin where MIP afforded 53.9% binding to its template molecule, which is 4.5 folds higher than that of the control polymer (12.5%) at 80 mg of polymer. The calculated binding capacity of QIP was approximately 1 mg/g of polymer. For rebinding analysis in ethanol:H_2O(3:2) there were significant increases in non-specific binding as represented for 80 mg of both QIP and control polymer in which binding to the template was 69.6% and 46.2% respectively. Furthermore, the binding specificity of QIP was tested against tocopherol succinate, tocopherol nicotinate and 8-hydroxyquinoline. To 40 mg of QIP, each of various analyses (0.1 mg/mL) was added. QIP could bind quercetin up to 40% whereas it could bind tocopherol succinate, tocopherol nicotinate and 8-hydroxyquinoline at less than 5%. Results indicated that QIP displayed high selective recognition toward its template than those of the other analytes as shown in Fig. 3.

Anthocyanins were extracted from mangosteen pericarp using 1% HCl in cold ethanol incubation at 4 °C for 24 h. The anthocyanin contents

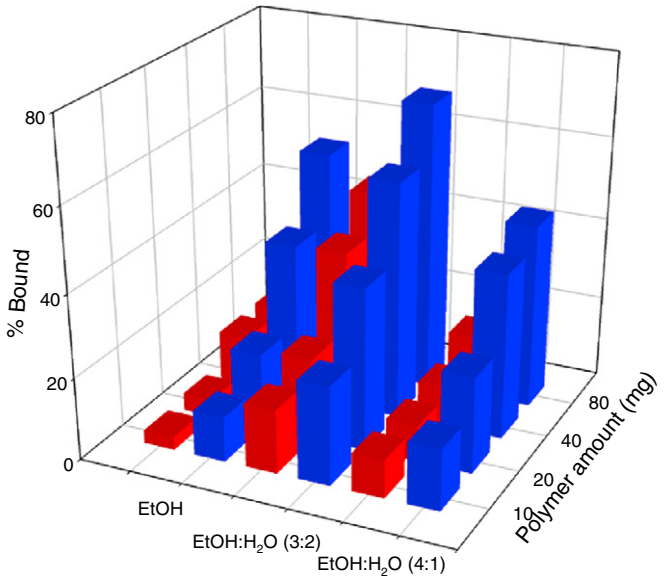

Fig. 2. Rebinding experiment of QIP and control polymer toward their template with optimization of binding solvent.

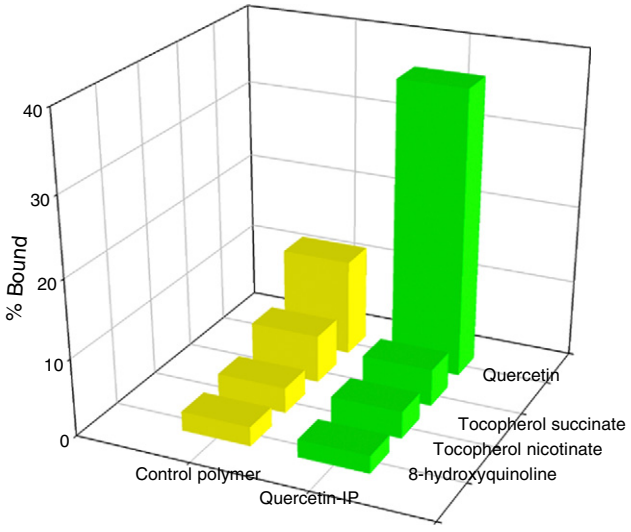

Fig. 3. Binding specificity of QIP.

were calculated using the previously described formula. The total anthocyanin content in acidified ethanol over the durations of 1, 2 and 24 h was found to be 47.07, 54.42 and 73.7 mg/g respectively, in the ethanol extract. Anthocyanin containing crude extract from the mangosteen pericarp was calculated for preparing the concentration of 0.1 mg/mL prior to binding analysis with QIP. Results indicated that 40 mg of QIP possessed high cross-binding property toward anthocyanin in which it could bind anthocyanin up to 35% whereas the control polymer could bind only 4% (Fig. 4). The calculated binding capacity of QIP toward anthocyanins was approximately 0.875 mg/g polymer. This result indicated that QIP has great potential for future application in separation and extraction of anthocyanins from mangosteen pericarp. QIP has been reported to be useful for pre-concentration and clean-up of catechin and structurally related compounds from several natural products [33]. It has been used to extend their selectivity toward quercetin analogs for drug discovery development [36]. This study demonstrates the great potential of MIP technology for the development of highly specific functional material for separation and concentration.

4. Conclusion

The prepared QIP showed good rebinding and selective recognition for quercetin as compared to the control polymer. The resultant MIP displayed specificity toward the quercetin template molecule over those of other analytes such as tocopherol succinate, tocopherol nicotinate and 8-hydroxyquinoline. Furthermore, QIP was evaluated for its cross-recognition against anthocyanin from the mangosteen pericarp extract. High specific binding of QIP could be attributed to its high affinity for related compounds as anthocyanin. This MIP holds great potential for the pre-concentration and separation of flavonoids and related analogs from fruit and vegetable extracts.

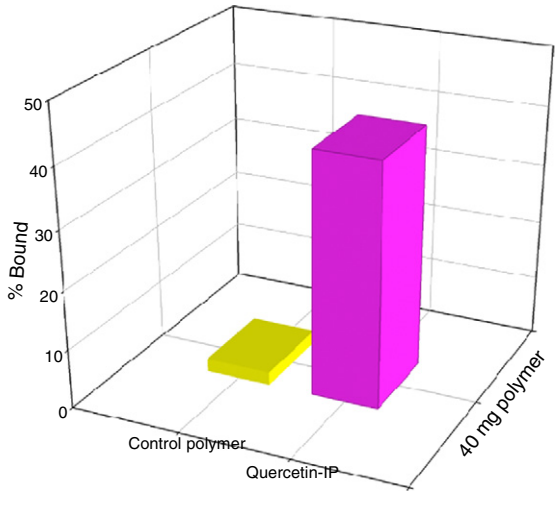

Fig. 4. Rebinding experiment of QIP toward anthocyanin.

Acknowledgments

This work was supported by the TRF Research Career Development Grant from the Thailand Research Fund (Grant No. RSA5780020) as well as the Office of the Higher Education Commission and Mahidol University under the National Research Universities initiative. We thank Dr. C. Nantasenamat for linguistic advice from the Center of Data Mining and Biomedical Informatics, Faculty of Medical Technology, Mahidol University.

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Materials Science and Engineering C 77 (2017) 1341–1348

Contents lists available at ScienceDirect

Materials Science and Engineering C

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Review

Molecularly imprinted polymer for human viral pathogen detection

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ARTICLE INFO

Article history:
Received 7 December 2016
Received in revised form 22 March 2017
Accepted 23 March 2017
Available online 27 March 2017

Keywords:
Virus
Viral detection
Molecular imprinting
Molecularly imprinted polymer
MIP

ABSTRACT

Molecular imprinting has become an attractive synthetic approach for the fabrication of novel functional polymers with pre-designed molecular target selectivity. Such molecularly imprinted polymers (MIPs) have been applied in wide range of areas such as chemical and biological sensors, solid phase extraction and drug assays owing to their inherent robustness, reusability and reproducibility. Furthermore, MIPs can also be used as tools for studies concerning antibody/receptor binding site mimicry as well as being used as antibody substitutes for biomedical applications. Viral detection is a rapidly growing field owing to its increasing prevalence and ongoing evolution of viral variants and drug resistance. Therefore, this calls for effective detection, surveillance and control. Herein, we highlight and summarize the literature on the utilization of MIPs for human virus detection. Particularly, MIPs afford great potential for rapid virus detection as well as other recognition-based viral studies.

Contents

1. Introduction

Today, there is an apparent upsurge in the emergence and re-emergence of infectious diseases that have a major impact on the health of humans, animals and plants. In humans, infectious diseases are responsible for approximately 27% of annual deaths incurred throughout the world [1]. This reflects the combined impact of rapid demographic, environmental, social, technological and other changes in our society and habitat. Thus, there is an inherent need to develop novel diagnostic techniques so as to keep pace with the ever changing health problems that may emerge in the future.

Several detection tools are available on the market based on the concept of antigen-antibody and/or receptor-ligand interactions. In spite of their many advantages, such biomolecules possess inherent flaws in terms of stability and usage. Although a variety of bioassays and biosensors have been developed, there is still a need for low cost, disposable or reusable biosensors that are capable of rapid detection and accurate identification of a wide range of pathogens. The utilization of the molecular imprinting technology for the development of molecularly imprinted polymers (MIPs) as biorecognition elements

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http://dx.doi.org/10.1016/j.msec.2017.03.209
0928-4931/

provide a real alternative to antibodies owing to their inherent robustness and reproducibility [2]. Molecular imprinting has made it possible to produce tailor-made artificial receptors that are capable of binding specifically to template molecules of interests.

The molecular imprinting process essentially involves three main steps: (i) self-assembly of template and functional monomer molecules, (ii) polymerization of template-monomer complex with cross-linking monomers and (iii) template removal to unveil a binding cavity that is specific to the imprint molecule. These novel polymers have been demonstrated to possess excellent properties for the separation of many interesting compounds ranging from small molecules to macromolecules. MIPs can bind specifically to the original as well as related template molecules while also possessing tolerance to mechanical stress, temperature, pH, acid-base, etc. Owing to their robust properties, MIPs are suitable for a wide range of applications such as separation media for chromatography and solid phase extraction [3,4] nanoreactors for the combinatorial synthesis of novel enzyme inhibitors [5,6], recognition elements for biosensors [7], artificial receptors for drug assays [8,9], biological receptor mimics [10], drug delivery [11] and enzyme mimetics [12]. A wide range of templates have been imprinted such as folic acid [13], 5-fluorouracil [14], glutathione [15], gramine [16], hydrazone [17], human prostate cancer cell lines [18], matrine [19], paclitaxel [20], (S)-2-(acrylamido) propanoic acid [21], theophylline [22], uric acid [23], and valganciclovir [24]. Many viruses possess a high mutation rate thereby giving rise to new variants. A change in the antigenic epitope on the surface protein would possibly create new variants, which in turn may cause reinfection. Therefore, effective detection and control is of urgent need for proper clinical management. The inherent robustness and reproducibility of MIPs facilitate the fabrication of tailor-made receptors for rapid detection as to keep pace with emerging viral infections. This review article focuses on the surrounding literature of MIP utilization for the detection of viral human pathogens.

2. Influenza

Seasonal influenza, more commonly known as “the flu”, is an acute viral infection caused by the influenza virus. The virus spreads through an infected individuals cough thereby allowing dispersal of infected droplets into the air with the ability to infect those in the vicinity. The virus can also be transmitted through direct contact with an infected person. In addition, influenza causes an estimated 3 to 5 million cases globally with around 250,000 to 500,000 deaths annually [25]. Furthermore, the influenza virus can be classified into 3 types: A, B and C. Type A influenza viruses can be further divided into subtypes according to the combination of virus surface proteins (i.e. several isoforms of both hemagglutinins and neuraminidases) such as H1N1 and H3N2 subtypes.

Molecular imprinting polymer was first applied to screen influenza A virus by Wangchareansak et al. [26,27] as summarized in Fig. 1. This appreciated work combined MIPs and QCM for proof-of-concept of screening protocols for influenza virus subtypes including H5N1, H5N3, H1N1, H1N3 and H6N1. Influenza virus surface antigens are made up of glycoproteins hemagglutinin (HA) and neuraminidase (NA), that play a role in the subtype classification. MIPs were made for each subtype of Influenza A virus whereby each MIP possessed a good recognition property towards its original viral template. Furthermore, the template sharing the same neuraminidase domains as H1N3 and H5N3 can be differentiated by its own MIP, suggesting that the hemagglutinin domain contribute more to the selectivity property in this case. H5N1-MIP has been found to bind strongly to virus containing N1 rather than those of virus containing N3. Their finding suggests that both the H and N domain play important roles in molecular recognition of MIP. Furthermore, 5 different MIPs have been possessed as their recognition profile which are offered as molecular fingerprints. This report has opened a new feasibility providing an alternative rapid way to screen influenza A virus subtypes in unknown samples with detection limits as low as 105 particles/mL. Moreover, Wangchareansak et al. has also used the influenza virus-MIPs as a novel polymer for identifying molecular binding to the influenza virus H5N1. These MIPs were used to facilitate identification of inhibitors that can bind to, and inhibit the function of virus upon inducing a conformational change. Thus, the inhibitor treated virus is expected to have a reduced or inhibited binding to the H5N1 specific MIPs.

3. Dengue virus

Dengue is one of the fast emerging and evolving diseases in many parts of the world. Dengue is a mosquito-borne viral infection caused by dengue virus. Annually, an estimated 50 million dengue infections and 500,000 individuals are hospitalized due to dengue infection having a fatality rate of about 5% in some areas [28]. The infection can cause severe clinical problems due to non-specificity of clinical presentations leading to misdiagnosis of the disease. An effective vaccine development for the prevention of dengue is currently underway. Therefore, early detection is still urgently required for clinical diagnosis and patient management. Tai and coworkers [29,30] developed MIP for the recognition of Dengue virus protein NS1 (nonstructural protein 1). NS1 is normally found in the blood specimen during the viremia phase. It is found on the infected cell surface or as secreted NS1 in the blood. The linear 15-mer peptides derived from NS1 of JEV (Japanese encephalitis virus) was chosen as the template (Thr-Glu-Leu-Arg-Tyr-Ser-Trp-Lys-Thr-Trp-Gly-Lys-Ala-Lys-Met) according to its consensus linear epitope mapping of dengue virus NS1 towards antibody D2/8-1. This epitope-mediated imprinting based MIP was coated on QCM. The MIP-grafted 15-mer peptide chip could bind to pentadecapeptide, purified and unpurified NS1 proteins with a nanomolar range dissociation constant (K_D) of 0.6, 0.04 and 0.09 nM, respectively. The results indicated a strong polymer-template interaction from the multi-point attachment of the NS1 protein to the 15-mer peptide chip, which was comparable to the monoclonal antibody immobilized chip (K_D = 0.05 nM). Furthermore, the MIP-QCM has been applied for dengue virus detection from patients' serum samples using the flow injection system. This MIP-QCM retained their specificity towards NS1 protein in serum and possessed high sensitivity detection down to the μM range with a short operation time of 20–30 min/sample. Therefore, this technique could be used to detect the 4 serotypes of dengue virus whereby a pretreatment sample was not needed for this novel sensor.

4. Japanese encephalitis virus

Japanese encephalitis virus (JEV) is one of the main causes of viral meningitis is Asia and Australia. Just like Dengue, Japanese encephalitis (JE) is also a mosquito-borne viral disease. The annual incidence of human cases has been reported to be about 50,000 to 175,000. In addition, JE not only has a high mortality rate (25–30%) but also reported that 50% of surviving patients suffer from neuropsychiatric sequelae. JE is considered as the most frequent viral encephalitis associated with fatal or severe outcomes [31].

The JEV recognition MIP has been fabricated based on fluorescent resonance energy transfer (FRET) [32]. The JEV recognition MIP was coated on silica microspheres which contained the fluorescent dye, pyrene-1-carboxaldehyde (PC). The FRET phenomenon can be enhanced upon virus binding as energy donor while PC acted as an energy acceptor. The novel FRET based virus detection MIP possessed high sensitivity detection of 9.6 pM at ambient temperatures. The specificity of this MIP was evaluated and found to exert an imprinting

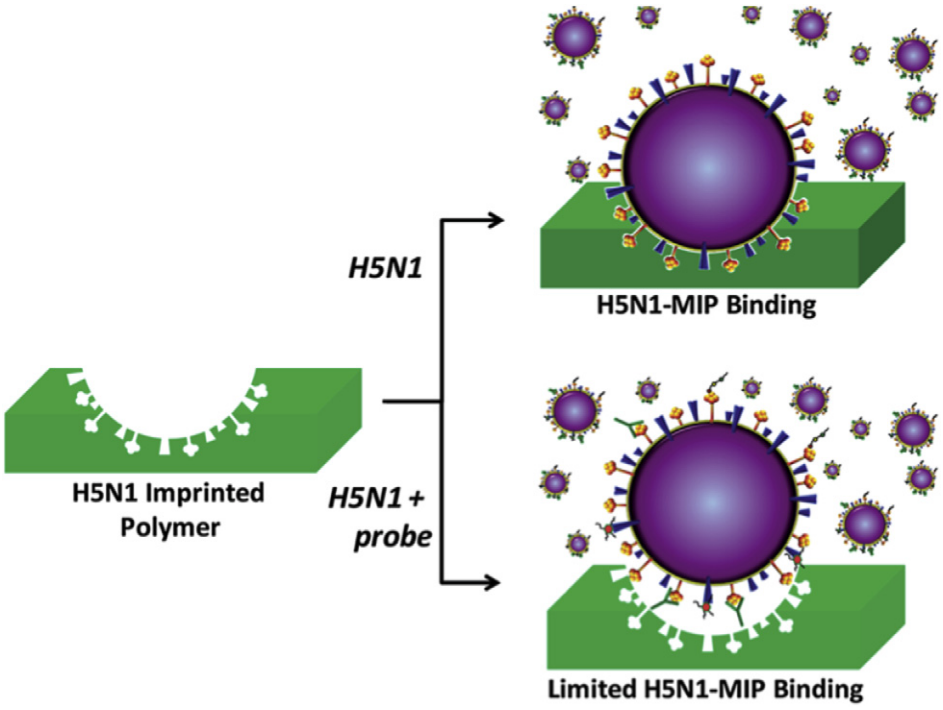

Fig. 1. Schematic representation of the binding of H5N1 virus to the imprinted polymer in the absence (right, top) and presence (right, bottom) of probe molecules (e.g. H5 antibody, Oseltamivir, Sialic acid, GlcNAc13 and H1 antibody). The binding of probe molecules to the virus causes significant conformational change which consequently, leads to limited binding of the virus with the imprinted polymer.
Source: Reprinted from [26] with permission from the Royal Society of Chemistry.

factor of 2.12. In addition, the virus MIP displayed a good selectivity towards the JEV over those of other viruses including Hepatitis A virus (HAV), Leprosy virus (LV) and Rabies virus (RV). Furthermore, its application with real samples has been demonstrated using a diluted human serum with a resulting recovery of nearly 100%. JEV-imprinted magnetic silicon microspheres were successfully invented by He et al. [33]. These microspheres exhibited excellent binding selectivity towards JEV with an imprinting factor of 2.95. Moreover, the virus-MIP possessed a detection limit as low as 0.32 nM. Furthermore, great selectivity on JEV using JEV-imprinted magnetic silicon microsphere has been demonstrated over other viruses (LV, RV). This novel MIP microsphere exhibited good recovery upon testing with virus spiked samples. Thus, JEV-imprinted polymer has great potential as a tool for application on JEV analysis and disease diagnosis.

5. Human immunodeficiency virus (HIV)

HIV primarily targets the immune system of patients, making them susceptible against other infections and certain cancers. HIV can be transmitted via various types of body fluids from an infected person, such as blood, breast milk, semen and vaginal secretions. HIV is one of the main causes of mortality and morbidity in the world. HIV has infected about 75 million people around the world and an estimated of 37 million people are currently living with the virus [6].

Lu et al. [34] has developed a HIV-1 related glycoprotein 41 (gp41) bio-imprinting sensors based on the epitope imprinting technique as summarized in Fig. 2. This glycoprotein is located on the viral coat of HIV-1. HIV-1 gp41 plays a significant role in membrane fusion upon glycoprotein 120 (gp120) binding towards CD4 cells. This membrane fusion allowing for the activation of gp41 may provide strategies for vaccine and antiviral drug development. Synthetic mimicking of a 35 amino acid residue (aa 579–613) was used as a template due to its property as a major immune-dominant region containing antibodies that recognized around 98% of AIDS patients. In this work, dopamine was used as a functional monomer. Polydopamine possessed high stability, hydrophilicity and biocompatibility. The synthetic peptide was embedded into the polydopamine during induction of the polymerization process. Hydrogen bonding, ionic bonding and hydrophobic interactions may play a role in the possible interaction between the peptide and dopamine. The hydrophobic MIP film grafted on QCM has been fabricated. The MIP coated QCM exerted a good specific affinity towards its template peptide. Upon a 100 ng/mL injection of template, the maximal frequency shifts of MIP-coated QCM sensor was 15.13 Hz whereas MIP-coated QCM sensor displayed only 1.799 Hz. The calculated K_D of this MIP was 3.17 nM, indicating a high affinity of the MIPs towards the template molecule. The selectivity of the MIP-coated QCM has been investigated against the peptide with bovine serum albumin (BSA), two and eleven mutated residues (2M-peptide, 11M-peptide). The result indicated that the BSA and 11M-mutated peptide displayed a much lower frequency response as compared to the template peptide. Whereas the 2M-peptide displayed a similar frequency response to the original template peptide. Thus the display of frequency attained by the 2M-peptide in comparison with the 11-M peptide could be due to the possible reason that it has only two mutated amino acids difference than the original template. furthermore, the whole molecule of HIV-1 gp41 has been tested to display a linear frequency shift in the range from 5 ng/mL to 200 ng/mL with a detection limit of 2 ng/mL. This detection limit is comparable to the reports from the ELISA method. The recovery performance of MIP-coated QCM towards HIV-1 gp41 urine spiked sample was in the range of 86.5–94.1%. This novel MIP-coated QCM was successfully fabricated and applied for monitoring HIV-1 gp41 in urine samples with high sensitivity and selectivity, suggesting its potential application in the future. Khaled Seidi et al. [35]proposed nanomagnet-based detoxifying machines using MIPs as viral capture for complementary approach in HIV therapy. Furthermore, the magnetic MIP behaves as a capture probe for the

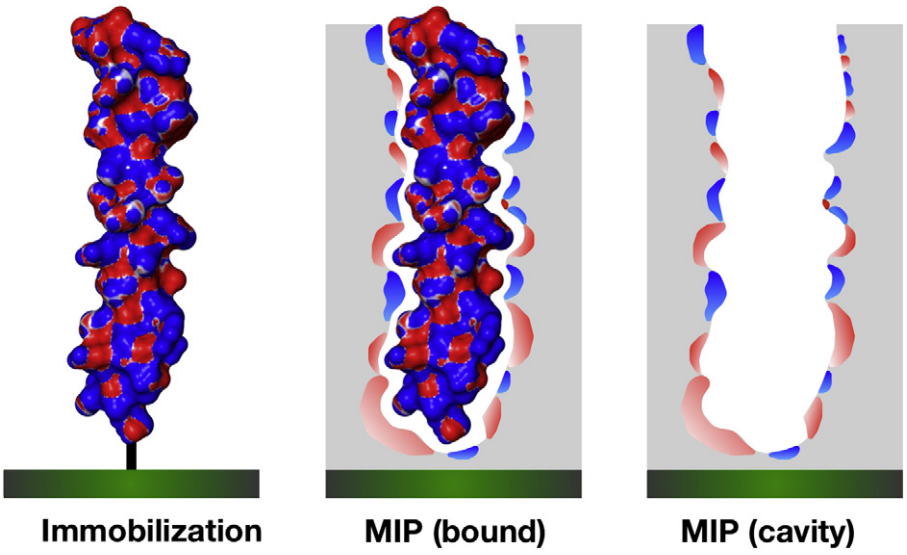

Fig. 2. Cartoon illustration summarizing the immobilization of a template protein onto a surface, formation of a molecularly imprinted polymer around the template protein and finally the elution of the template protein to reveal an empty cavity that can accommodate an incoming protein.

HIV-1 antibody that has already been invented [36]. These magnetic-MIPs have characteristically displayed a good advantage as a tool in combination with immunoassay. This MIP-combined immunosensor has also provided a low cost, simple and high sensitivity tool, which is suitable for the early diagnosis of HIV infected patients.

6. Hepatitis A virus

Hepatitis A is a liver disease caused by the Hepatitis A virus (HAV). The most common mode of infection of the disease is via contaminated food and water with feces of an infected person. Even though hepatitis A does present with chronic liver disease which is also seen in hepatitis B and C, the debilitating symptoms and sudden liver failure that accompany hepatitis A can cause death of the patient. Hepatitis A is mostly spread via the fecal-oral route and close contact with an infected person.

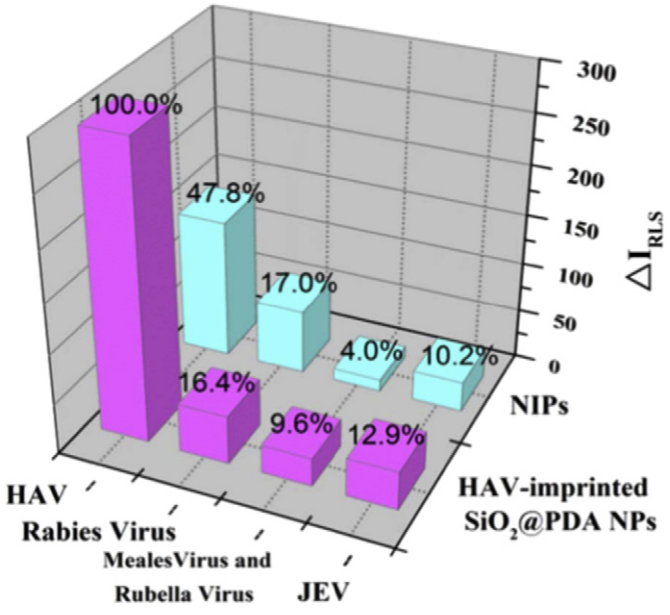

Fig. 3. Binding selectivity of hepatitis A virus (HAV) imprinted SiO_2 nanoparticles (SiO_2@PDA) towards HAV and three other viruses consisting of rabies virus, Japanese encephalitis virus (JEV) and a mixture of measles virus and rubella virus.
Source: Reprinted from [37] with permission from Elsevier.

Yang et al. [37] has applied a molecular imprinting strategy for the direct detection of the hepatitis A virus. HAV is a significant human pathogen which cannot be distinguished from other types of hepatitis viruses upon clinical presentation. Therefore, direct detection with high specificity and sensitivity plays a crucial role for accurate diagnosis. Yang developed a novel core-shell molecularly imprinted nanoparticles based on polydopamine (PDA) capped with SiO_2. This HAV-imprinted SiO_2@PDA nanoparticles possessed hydrophilic, biocompatibility and specificity recognition properties towards the HAV template. The novel resonance light scattering (RLS) technology has been employed for specific recognition and detection based on the remarkable advantage of high sensitivity and convenience in operation by using simple fluorescence spectrophotometer. The enhancement of light scattering of HAV-imprinted SiO_2@PDA nanoparticles was correspondingly related to the increasing of HAV concentration. The selectivity of HAV-imprinted SiO_2@PDA nanoparticles were tested against HAV, rabies virus, a mixture of measles and rubella viruses and Japanese encephalitis virus. The binding selectivity were in the following order: HAV was set as 100% > rabies virus (16.4%) > Japanese encephalitis virus (12.9%) > the mixture of measles and rubella viruses (9.6%) as shown in Fig. 3. This finding indicated that an excellent selectivity was obtained. Furthermore, the HAV-imprinted SiO_2@PDA nanoparticle was successfully used for the determination of HAV in real sample serum and possessed the detection limit of 8.6 pM. This novel method combined a promising technology of MIP and RLS and may open up a potential alternative way for MIPs-based virus detection.

7. Hepatitis B virus

Hepatitis B is a serious liver disease caused by the hepatitis B virus (HBV). HBV infection is the most common chronic viral infection in the world, with an estimated rate of infection of around 2 billion people with more than 350 million people serving as chronic carriers of the virus [38]. HBV infection is highly contagious and can transmit from mother to child or through exposure to infected blood.

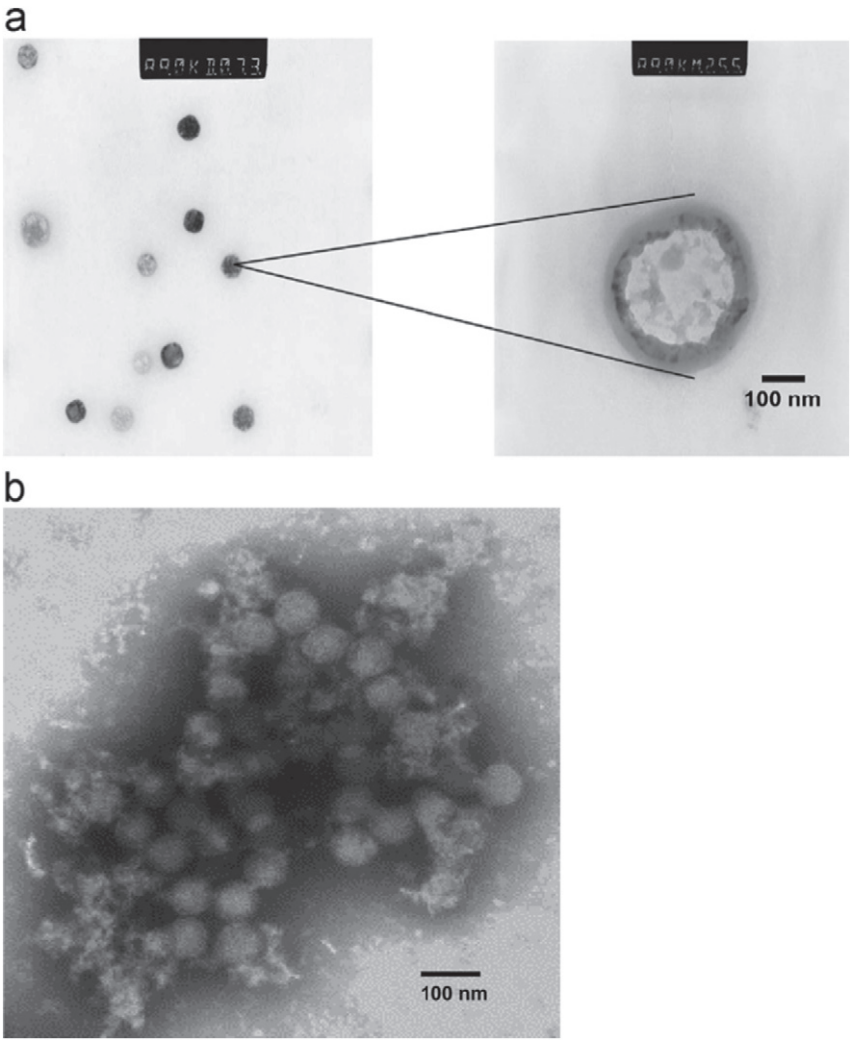

Fig. 4. Transmission electron microscope images of adenovirus imprinted polymer (a) and adenovirus (b).
Source: Reprinted from [43] with permission from Elsevier.

It is also spread by various body fluids such as saliva, vaginal, seminal and menstrual fluids. The hepatitis B vaccine is the mainstay of HBV prevention. An effective developed immunity against HBV require levels of hepatitis B surface antibody (HBsAb $\geq$ 10 mIU/mL). Uzun et al.[39,40] developed a HBsAb imprinted poly(hydroxylethyl-methacrylate-*N*-methacryloyl-L-tyrosine methylester film on the surface plasmon resonance (SPR) sensor chip for diagnosis of HBsAb in human serum and HBsAb imprinted PHEMAT particle for HBsAb purification. The prepared HBsAb-imprinted PHEMAT SPR chip displayed a good relevance of HBsAb detection which was comparable to the ELISA method (AxSYM immunoassay system, Abbott Laboratory, Illinois, USA) with a R2 value of 0.9969 linearity. The chip retained the ability to detect the HBsAb from human serum with 99.7% precision in the range of 0–120 mIU/mL. The maximum detection limit of this sensor chip was 208.22 mIU/mL with K_A and K_D values of 0.015 mIU/mL and 66 mIU/mL, respectively. HbsAb negative human serum was also tested with the PHEMAT SPR chip where no significant response was observed. Therefore, the HbsAb-imprinted PHEMAT SPR chip provides a potential use for diagnosis of HBsAb from human serum.

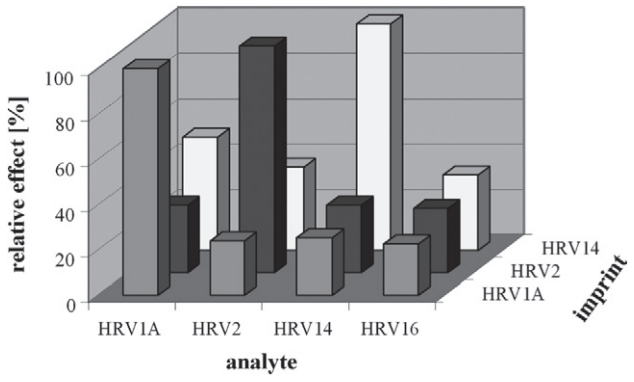

Fig. 5. Cross-selectivity of imprinted polymers against various serotypes of HRV. Imprinted polymers showed stronger rebinding to its own template.
Source: Reprinted from [45] with permission from the American Chemical Society.

8. Adenovirus

Human adenoviruses(HAdVs) can cause a plethora of clinical diseases including conjuctivitis, gastroenteritis, hepatitis, myocarditis and pneumonia. Globally, 5–7% of respiratory tract infections among children are ascribed to HAdV [41,42]. Altintas et al. [43] has fabricated Adenovirus specific-MIP nanoparticles immobilized on a surface plasmon resonance (SPR). In comparison to the MIP-based adenovirus assay, the direct and sandwich assays using natural antibodies have also been developed. MIPs were produced using glass

Table 1
Summary of experimental conditions for the molecular imprinting of human viral pathogens.

Virus	Template	Functional monomer[a]	Crosslinker[b]	Porogenic solven[c]	Format	Transducer[d]	Adsorption solvent	Ref.
Influenza virus	H5N1, H5N3, H1N1, H1N3, H6N1	Am, MAA, MMA, NVP	DHEBA	DMSO	SI	QCM	PBS pH 7.2	[26,27]
Dengue virus	15-mers peptide from JEV NS1	Am, AA, BAm	EDMA	Acetonitrile, Phosphate buffer pH 4.0	EmI	QCM	PBS pH 4.0	[29,30]
Japanese encephalitis virus	Freeze dried JEV vaccine	APTES	TEOS	Ultrapure water	MIPs coated Fe_3O_4@SiO_2 microspheres		Ultrapure water	[33]
	Freeze dried JEV vaccine	APTES	TEOS	Ultrapure water	PC-modified silica microspheres	FRET	Ultrapure water	[32]
Human immunodeficiency virus	HIV-1 gp41	PDA	PDA	Tris-HCl buffer pH 8.5	EmI	QCM	Tris-HCl pH 7.0	[34]
	HIV-1 antibody	APBA	APBA	Phosphate buffer pH 8.0	Magnetic MIPs	ECL	Phosphate buffer pH 8.0	[36]
Hepatitis A virus	HAV2	PDA	PDA	Tris-HCl buffer pH 8.5	PDA-coated SiO_2 nanoparticles	RLS	Tris-HCl buffer pH 6.2	[37]
Hepatitis B virus	Hepatitis B surface Ab	MAT, HEMA	EDMA	Toluene	PHEMAT particles		0.9% NaCl	[39]
	Hepatitis B surface Ab	MAT, HEMA	EDMA	MOPS buffer pH 6.0	PHEMAT particles	SPR	Deionized water	[39]
Adenovirus	Adenovirus	NIPAM, AA	MBAm, TBA, APTES	Ethanol	MIP nanoparticles	SPR	PBS pH 7.4	[43]
Picornavirus	HRV2, HRV14, HRV1A	PU, BPA	Phloroglucinol	Tris-HCl, THF	SI	QCM	Tris-HCl buffer pH 7.2	[45]

[a] Am: acrylamide; AA: acrylic acid; APBA: 3-aminobenzeneboronic acid; APTES: 3-aminopropyl triethoxysilane; BAm: N-benzylacrylamide; BPA: bisphenol A; HEMA: hydroxyethyl methacrylate, MAA: methacrylic acid; MAT: *N*-methacryloyl-L-tyrosine methyl ester; MBAm: N,N′-methylenebisacrylamide; MMA: methylmethacrylate; NIPAM: N-isopropylacrylamide; NVP: N-vinylpyrrolidone; PDA: polydopamine; PU: polyurethane; TBA: N-tert-butylacrylamide; TEOS: tetraethoxysilicane.
[b] DHEBA: N,N′-(1,2-dihydroxyethylene) bisacrylamide; EDMA: ethylene glycol dimethacrylate.
[c] DMSO: dimethylsulfoxide; THF: tetrahydrofurane.
[d] SI: stamp imprinting; EmI: epitope mediated imprinting; QCM: quartz crystal microbalance; PC: pyrene-1-carboxyaldehyde; FRET: Förster resonance energy transfer; ECL: electrochemiluminescence.

beads as solid support for the adenovirus template followed by the components for MIPs synthesis to obtain adenovirus-MIP nanoparticles with a size of approximately 260 nm (Fig. 4). The MIPs beads were immobilized on the SPR chip via glutaraldehyde coupling. The immobilized MIPs obtained a good reproducibility for signal detection in a concentration range of 0.02–20 pM with the detection limit of 0.02 pM. The binding affinity was calculated with a K_D of 3.10×10^{-11} using the Biacore 3000 software. In addition, the binding specificity of adenovirus-MIP was tested against the MS2-phage and vancomycin whereby non-specific binding was found in a very low level against the adenovirus-MIP. Furthermore, the adenovirus polyclonal antibody was used for direct and sandwich assays as a comparison to the adenovirus-MIP in terms of sensitivity. The K_D were calculated as 2.30×10^{-12}, 3.10×10^{-11} and 1.41×10^{-10} M, respectively, with the following order: sandwich antibody assay > MIP > direct antibody assay. The detection limit of the sandwich antibody assay was found to be the most sensitive as compared to MIP and direct antibody assay with 0.008, 0.02 and 0.3 pM, respectively. The overall results demonstrated the excellent achievement of all assay types and confirmed the suitability of the MIPs-based sensors for the detection of virus, which exerted high sensitivity and specificity as well as was easy-to-use.

9. Picornaviruses

Picornaviruses are non-enveloped, RNA viruses consisting of over 50 species in the family that can be divided among 29 genera. These viruses can reside in mammals and birds and can cause many diseases including paralysis, meningitis, hepatitis and poliomyelitis [44]. MIPs for the smallest RNA virus, picornaviruses has been developed using human rhinovirus (HRV) as a representative. Stamp imprinting procedure has been used as a template for virus-patterned memorizing geometries feature. More than 100 different serotypes of HRV were reported. Jenik et al. [45] has developed HRV-imprinted polymer coated on QCM for rapid analysis on fast frequency response shift as well as reversible non-covalent interaction behavior. The HRV2-imprinted sensor showed a frequency decrease of −750 Hz upon injection of 30 μg/mL of virus suspension whereas the non-imprinted reference channel was decreased only at −100 Hz. The frequency decrease of the sensors was dependent on the analyte concentration in linear concentration. The sensor also had the ability to distinguish between native and denatural forms of the virus. Furthermore, different serotypes of HRV in surface chemistry (HRV14, HRV2 and HRV1A) can be selectively recognized by its own HRV template origin over other species (Fig. 5). This finding confirmed the properties of this novel MIP in which it can be distinguished based upon surface chemistry even though the geometry features of template were the same. The smallest picornavirus, foot and mouth disease virus (FMDV), serotype Manissa has been evaluated for binding to HRV2-imprinted sensor. Although the geometry of FMDV was not exactly fitting due to its small size (25 nm), the surface chemistry is nearly the same to HRV. Hence, the sensor can response according to its non-covalent interactions in terms of cross-sensitivity. This work has opened up applications of MIPs for developing MIP-based virus sensors.

10. Technical remarks

Many types of pathogenic viruses have been imprinted to investigate the feasibility of various practical applications (Table 1). Various types of transducers have been utilized for fast signal recognition as

well as increased sensitivity of detection such as QCM and SPR. Stamp imprinting and epitope-mediated imprinting have been favorably employed for governing sensing elements. Functional nanoparticles support is more straightforward upon the binding sites, which are located on imprinted surfaces for fast recognition and installation of the sensing system phenomenon such as FRET, RLS and ECL. Most of the virus template can be imprinted and cab investigate binding properties in an aqueous environment.

11. Conclusion

Molecular imprinting is an attractive technology used to create selective recognition sites within a macromolecular polymer network. One of the major advantages of MIPs is their robustness, high selectivity, long-term stability and cost effectiveness which cannot be obtained by using fragile biomolecules. MIPs have preliminarily been used for selective and sensitive viral detection as well as applied for viral subtype differentiation. Moreover, MIPs have been introduced as a novel media for the screening of inhibitors for drug discovery that may be used in the combat against viral infections. The manifold of advantages brought about by the molecular imprinting technology is anticipated to find its home in the frontlines of future viral studies.

Acknowledgments

This work was supported by the TRF Research Career Development Grant from the Thailand Research Fund (Grant No. RSA5780020) as well as the Faculty of Medical Technology, Mahidol University. This work is also partially supported by the Office of Higher Education Commission and Mahidol University under the National Research University Initiative. The authors would like to thank Dr. Nalini Schaduangrat from the Center of Data Mining and Biomedical Informatics, Faculty of Medical Technology, Mahidol University for linguistic advice.

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