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Detection of Waterborne Viruses Using High Affinity Molecularly Imprinted Polymers

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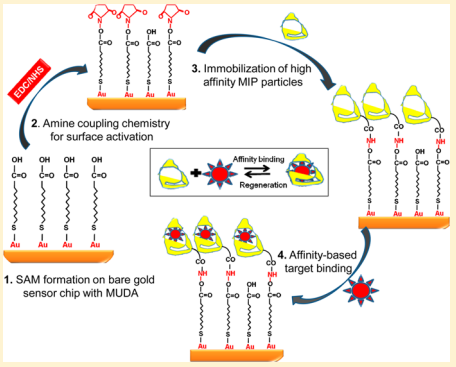
ABSTRACT: Molecularly imprinted polymers (MIPs) are artificial receptor ligands which can recognize and specifically bind to a target molecule. They are more resistant to chemical and biological damage and inactivation than antibodies. Therefore, target specific-MIP nanoparticles are aimed to develop and implemented to biosensors for the detection of biological toxic agents such as viruses, bacteria, and fungi toxins that cause many diseases and death due to the environmental contamination. For the first time, a molecularly imprinted polymer (MIP) targeting the bacteriophage MS2 as the template was investigated using a novel solid-phase synthesis method to obtain the artificial affinity ligand for the detection and removal of waterborne viruses through optical-based sensors. A high affinity between the artificial ligand and the target was found, and a regenerative MIP-based virus detection assay was successfully developed using a new surface plasmon resonance (SPR)-biosensor which provides an alternative technology for the specific detection and removal of waterborne viruses that lead to high disease and death rates all over the world.



Contamination of the water sources due to viruses is one of the big reasons for waterborne diseases which lead to hundreds of thousands of deaths each year, particularly for children under five years old.[1] Hepatitis A, hepatitis E, norovirus, and rotavirus are the major causes of waterborne diseases in humans.[2−4] They are enterically transmitted, infectious in low doses, stable in the environment for extended periods of time, and difficult to study. Moreover, removal of viruses from water requires specialized analytical methods and experienced technicians.[1−5]

Viruses have diverse mechanisms of action and consist of a virion containing DNA or RNA genome encapsulated in a protein coat. The function of viral nucleic acid is to carry the genetic information required to program the synthetic machinery of the host cell for viral replication whereas the capsid protects the nucleic acid from nucleases and aids in the attachment of the virus to the host cell. They need a host organism to reproduce, and many of them are pathogenic. Moreover, viruses are highly resistant to acidic conditions, high temperatures, disinfectants, and pressure. They are intracellular parasites incapable of reproducing outside of the host's cells.[6] Once they infect the host cells they direct the production of vast quantities of virus progeny which are excreted by the infected organism and which pose a threat to other healthy organisms.[7]

The human enteric viruses enter the general water supply through contaminated sewage waste. The viruses are so prevalent in sewage since the infected people excrete the viruses in enormous quantities. The people infected with these viruses can excrete 10^5 to 10^{11} virus particles per gram of feces. Moreover, the effective diagnosis of viruses is difficult since the morphological identification is problematic and the diagnosis is generally achieved when the virus has spread to other individuals.[8,9] The detection limit of analytical techniques is also insufficient for researchers to conclude that a sample is completely free of viruses.[10−12] The currently available diagnostic tests cannot offer complete assurance that a sample is 100% free of viruses.[13] Biosensors have recently provided highly sensitive, reliable, easy-to-use, and cost-effective methods for virus recognition and detection.[14,15] However, these works rely on natural antibodies which are difficult to produce and highly expensive and require the ethical approvals for production with long-term processes.

Due to these reasons, we aim to develop a MIP-based affinity system to remove the bacteriophage MS2 from water sources using high affinity between MIP nanoparticles and the model virus. This study presents many novelties with MIP production technique, SPR-2 microfluidics system, and usage of MIP-based affinity materials for the detection of biological agents from water sources through sensor technology. Moreover, a regenerative MIP surface could be developed for the first time in this research work. The results provide a new and promising technology for the purification of water sources from biologically toxic agents such as viruses, bacteria, and fungi.

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EXPERIMENTAL DETAILS

Reagents and Chemicals. *N*-Isopropylacrylamide (NIPAm), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), ammonium persulfate (APS), acrylic acid (AAc), 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), *N*,*N*′-methylenebis(acrylamide) (BIS), *N*-*tert*-butylacrylamide (TBAm), phosphate buffered saline (PBS) tablets, tris(hydroxymethyl)aminomethane (TRIS), glutaraldehyde (GA), 3-aminopropyltrimethyloxysilane (APTMS), 1,1-mercaptoundecanoic acid (MUDA), TWEEN 20 (polyoxyethylenesorbitan monolaurate), cysteamine, methanol, ethanol, toluene, and acetone were purchased from Sigma-Aldrich (U.K.). *N*-(3-Aminopropyl) methacrylamide hydrochloride (APM) was purchased from Polysciences Inc. (Pennsylvania). Double-distilled ultrapure water produced by a Millipore Direct-Q 3 UV (Millipore; Molsheim, France) was used for analysis. All chemicals and solvents were analytical or HPLC grade and were used without further purification.

Apparatus and Equipment. An Optima MAX-XP ultracentrifuge (Beckman Coulter) was used to purify the bacteriophage MS2 culture medium. An automated reactor (HEL Ltd., Hertfordshire, U.K.) was used to produce the MIP nanoparticles from the bacteriophage MS2 virus template. Glass beads were obtained from Sigma-Aldrich (U.K.). A Retsch AS200 shaker (Retsch Inc.) was used to agitate ceramic beads with the glass beads. A Zetasizer Nano (Nano-S) from Malvern Instruments Ltd. (Malvern, U.K.) was used to determine the size of the nanoparticles by dynamic light scattering (DLS). A Scanvac Coolsafe freeze-dryer (LaboGene, Denmark) was used to determine the yield of nanoparticles. Gold-coated sensor chips (SIA Kit Au, Biacore) were purchased from GE Healthcare Biosciences AB, Sweden. A Biacore 3000 SPR system (BIAcore, Sweden), as well as an SPR-2 sensor system (Sierra Sensors, Germany), was used to monitor the binding interactions (association and dissociation) between the MIP nanoparticles and their templates. A Grant sonicator (Patterson Scientific, Luton, U.K.) was used to dissolve solutions thoroughly and was used as required.

Preparation of Bacteriophage MS2. A suspension of the host organism (*Escherichia coli*, NCIMB 9481) was prepared by inoculating 60 mL of sterile tryptone soya broth (TSB) with a 10 μL loopful of *E. coli* from a 20 h old culture grown on a tryptone soya agar (TSA) (Biomerieux) plate. This was incubated (120 rpm) for 150 min at 37 ± 2 °C. The MS2 suspension was prepared by inoculating 4 × 10^{11} plaque forming units (pfu) into the TSB and incubating for 3 h (120 rpm) at 37 ± 2 °C and centrifuged at 2000 *g* (2 × 20 min) to remove the cell debris.[16] The supernatant was filtered through a 0.2 μm filter and ultracentrifuged on a 3 mL 20% w/v sucrose/PBS mixture at 100 000 *g* for 19 h at 4 °C, to pellet the bacteriophage. The supernatant was discarded, and the virus pellet was resuspended in 1 mL of PBS. This was further purified by $CsCl_2$ ultracentrifugation using 0.55 g $CsCl_2$/g of solution for >24 h at 123 302 *g*; the resulting band of concentrated virus was removed using a syringe and dialyzed twice for 24 h into PBS using a Slide-a-lyser dialysis cassette (20 000 MWCO).[17]

MS2 Plaque Assay. A TSA plate was inoculated with *E. coli* and incubated at 37 ± 2 °C for 19−20 h, after which a 10 μL loopful from the plate was transferred to a 10 mL sterile nutrient broth in a glass universal bottle, which, after mixing, was incubated at 37 ± 2 °C for 260 min. Meanwhile, stoppered bottles containing 3 mL volumes of soft bacteriophage agar (1 L consists of 4 g Agar, 25 g nutrient broth and 1 L of sterile water) were heated for at least 120 min at 90 to 100 °C and then stored at 60 ± 2 °C until required. The bottles were then cooled to 45 °C. Serial dilutions of the MS2 suspension in PBS (100 μL) were added to the soft agar followed immediately by 60 μL of the *E. coli* 9481 suspension. After mixing, the soft agar was poured immediately on TSA plates (in duplicate) that were then incubated at 37 ± 2 °C overnight.[16] The clear plaques were counted, and the total phage recovery was calculated.

Preparation of Microbeads for MIP Nanoparticle Synthesis. The glass beads were thoroughly washed with acetone under vacuum using a large Buchner funnel, flask, and vacuum pump. The beads were left in the funnel under vacuum until completely dry. The beads were subsequently incubated in a 5% v/v glutaraldehyde solution in PBS (pH 7.2) for 2 h, after which they were rinsed with double-distilled water under vacuum with the Buchner funnel and flask. Bacteriophage MS2 templates (2 mL) were then immobilized by incubating the glass beads with a 50 mL solution of the template in PBS (pH 7.2) overnight at 4 °C. The beads were rinsed with water under vacuum before being dried. The beads were packed in the automatic reactor in a dry state or placed in a sealed bottle at 4 °C for storage. Because of the low concentration of virus available to bind to the glutaraldehyde, it was thought that they might be exposed glutaraldehyde species remaining on the beads which need to be blocked with ethanolamine. A 50 mL solution of ethanolamine (0.1 mM) in PBS (pH 7.4) was used to incubate the glass beads for 15 min. The glass beads were then washed with double distilled water before being placed in the automatic reactor.

Synthesis of MIP Nanoparticles. The procedure has been adapted.[18] In 98 mL of H_2O, the following monomers were dissolved: 39 mg of NIPAm (2.14×10^{-4} mol, 53% mol), 2 mg of BIS (1.3×10^{-5} mol, 2% mol), 33 mg of TBAm (2.60×10^{-4} mol, 40% mol), and 2.2 μL of AAc (2.24×10^{-5} mol, 5% mol, d = 1.051 g mL^{-1}). TBAm was dissolved in 2 mL of ethanol and then added to the aqueous solution. This resulted in the 100 mL of the polymerization mixture required for the MIP synthesis which was placed into a labeled bottle. The total monomer concentration in the polymerization mixture was 6.5 mM. The solution was degassed under vacuum and sonicated for 10 min, and then purged with N_2 for 30 min (to remove atmospheric oxygen). After this, the container with the polymerization mixture was connected to one of the pumps of the automatic synthesizer, and 80 mL of it was injected into the reactor vessel containing 60 g of template-derivatized glass beads. *N*-(3-Aminopropyl) methacrylamide hydrochloride (54 mg) was used as additional monomer to functionalize primary amine groups to the surface of the MIPs nanoparticles, and it was dissolved in 3 mL of water. The vial was agitated and connected to an injection valve of the automated reactor. This monomer was used to help form the MIP around the bacteriophage because the virus has a higher molecular weight than the molecules normally imprinted with the automatic synthesis method. The mixture of APS and TEMED was used as the initiator for MIP synthesis. APS (180 mg) was dissolved in 3 mL of water and placed in a 5 mL glass vial. Then, 90 μL of TEMED was added to the vial. The vial was agitated and connected to the initiator tube of the automated reactor. The polymerization was started by adding 800 μL of the initiator. The polymerization solution immersed the bulk of glass beads, and then the polymerization was carried out at room

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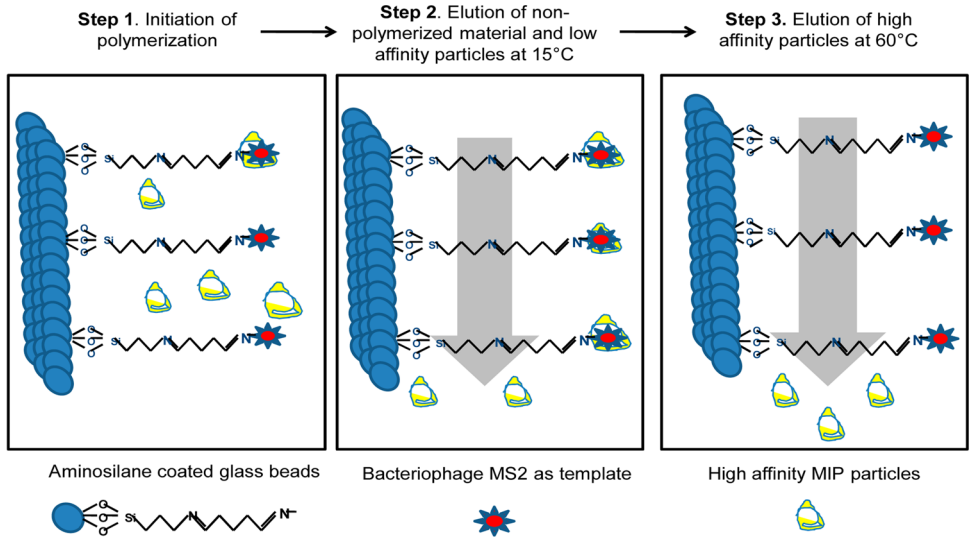

Figure 1. Principles of the high affinity molecularly imprinted polymer production using a novel solid-phase synthesis method.

temperature for 2 h. After this time, the reactor temperature was adjusted to 15 °C, and the remaining polymerization mixture was discarded. Two subsequent washing steps (50 mL of double-distilled water at 15 °C) were performed. Then, the high affinity nanoparticles were eluted from the affinity media by passing three fractions of 50 mL of double-distilled water at 60 °C. QB phage and vancomycin were also imprinted using the completely same recipe to obtain control MIP receptors.

DLS Size Analysis of MIP Nanoparticles. To verify the size of the synthesized nanoparticles, the eluted fractions were sonicated for 20 min, then filtered through 1.2 μm glass fiber syringe filters and analyzed in 3 cm^3 disposable polystyrene cuvettes at 25 °C using a Zetasizer Nano (Nano-S).

TEM Imaging of MIP Nanoparticles and Bacteriophage MS2. TEM images of MIP nanoparticles were taken using a Philips CM20 transmission electron microscope to confirm the nanoparticle size and quality of the production using a polymer solution. For the bacteriophage MS2 virus, it was placed into a centrifuge cartridge (30 kDa limit). The cartridge was loaded with 5−10 mL of double distilled water and centrifuged (3 min at 2500 g) repeatedly until all the PBS was completely removed and replaced with pure water (approximately 7−8 centrifugation runs). The PBS salts would render the TEM image useless, and therefore, the virus must be placed in a water medium. The sample was then prepared by filtering 5 μL through a 1.2 μm glass fiber syringe filter and depositing the filtrate on a silicon chip attached to a TEM holder, and leaving them to dry overnight in a fume hood.

MIP Nanoparticle Immobilization on MUDA Coated SPR-2 Sensor Chips. The surface of the chip was activated with a 100 μL injection of EDC/NHS solution (0.05 M of NHS and 0.2 M of EDC). Three injections (100 μL) of MIP nanoparticles were deposited on the surface. A 100 μL injection of ethanolamine (0.1 mM) was added to block MIP free areas on the surface to prevent nonspecific binding of the analyte on the chip. 100 μL of each dilution of bacteriophage MS2 (least concentrated to the most concentrated) was sequentially injected to the sensor.

Kinetic data has been analyzed and manipulated using BIAevaluation Software v4.1 (Biacore, Sweden) and SPR-2 analyzer (Sierra Sensors, Germany). The MIP-nanoparticle immobilization and virus binding around the particles on the sensor chip surface were confirmed using a scanning electron microscopy (SEM) technique. For this, MIP-nanoparticles were initially immobilized on the chip surface and then dried prior to taking SEM image. The chip was then incubated with MS2 virus and visualized to see the MS2 attached nanoparticles.

RESULTS AND DISCUSSION

Automatic Solid-Phase MIP Nanoparticle Synthesis. This new synthesis technique (Figure 1) was developed by the Cranfield University Biotechnology Group to produce high affinity MIP nanoparticles performed in the presence of templates (bacteriophage MS2) immobilized on beads. By using different temperatures, elution of low and high affinity nanoparticles can be separated. High temperatures (60 °C) are sufficient to successfully disrupt the binding interactions between the high affinity nanoparticles with its template while low temperatures (15 °C) are sufficient to elute low affinity nanoparticles because their binding interactions with the template are weak and easily broken.[19,20]

The MIP for the virus was produced six times, and the quality and uniformity of the synthesized particles were determined for each time using dynamic light scattering (DLS) analysis. DLS measures Brownian motion and relates this to particle size. Brownian motion is the random movement of particles due to the bombardment of the solvent molecules which surround them. The larger the particle the slower the movement as it requires more force to move compared to a smaller particle. The size of the nanoparticles that are synthesized in different times ranged from 205 to 238 nm with DLS technique (Table 1). Polydispersity indexes (PDIs) of the measurement were determined which provide an indication about the accuracy of the measurement, the lower PDI the more accurate the measurement. The DLS spectrum also indicates the uniformity and quality of the separate productions. Transmission electron microscopy (TEM) was also conducted to check the purity and production quality of the nanoparticles in the solution. A clean image was obtained with the expected particle size which is ∼200 nm (Figure 2a). TEM analysis was also performed on bacteriophage MS2 after *E. coli* culture and purification. The TEM image illustrates the bacteriophage as small spheres (Figure 2b). After the characterization and quality determination of the polymer

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Table 1. Mean and Standard Deviation of the MIP Nanoparticle Diameter with Polydispersity Readings Obtained Using Dynamic Light Scattering[a] and Kinetic Data Analysis To Determine Affinity between MIP Nanoparticles and MS2 Phage Using Biacore and SPR-2 Sensor Systems

nanoMIPs	diameter (nm)	polydispersity (PDI)	dissociation constant (K_D), M
batch 1	238 ± 16	0.417 ± 0.01	1.14×10^{-9}
batch 2	229 ± 10	0.392 ± 0.01	3.00×10^{-9}
batch 3	217 ± 8	0.311 ± 0.02	3.34×10^{-9}
batch 4	230 ± 11	0.295 ± 0.02	2.33×10^{-9}
batch 5	214 ± 9	0.333 ± 0.01	1.30×10^{-9}
batch 6	205 ± 6	0.327 ± 0.01	1.32×10^{-9}

[a]Polydispersity index (PDI) of the synthesized MIP nanoparticles provides an indication about the accuracy of the measurement, the lower PDI the more accurate the measurement. Each batch represents a separate production of MIP nanoparticles. DLS measurements were taken 10 times for each batch, and standard deviations were calculated.

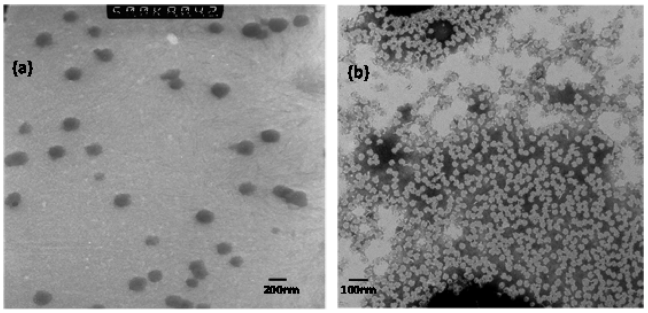

Figure 2. (a) Characterization of MIP-nanoparticles in solution using transmission electron microscopy (TEM). (b) Characterization of bacteriophage MS2. Bacteriophage MS2 was characterized after it was cultured and produced in *E. coli* culture using TEM technique. The small dots represent the phage alone.

nanoparticles, the amount of the MIP particles was also calculated after the freeze-dried particles which appeared as a white, slightly opaque transparent compound in tubes. The total yield for each solid-phase production of MIPs was around 23.1 mg that shows the considerable yield amount to be acceptable and indicated effective nanoparticle synthesis.

Plaque Assays. The concentration of MS2 used to generate MIP particles was determined to be $>10^{13}$ pfu mL^{-1}. The results of the plaque assay of nanoparticles incubated with MIPs for 30 and 60 min indicated that the bacteriophage MS2 was noninfectious within the MIP nanoparticle and illustrated that the MIP nanoparticles were nontoxic to *E. coli*. The absence of the phage in the nanoparticles indicated that the MIP nanoparticles were being efficiently formed with the template remaining attached to the glass beads and not leaking into the synthesized nanoparticles. This is one of the most important factors in MIP design.[21,22] Template removal is critical in MIP imprinting, but the polymer network and the affinity of the MIP to the template make removal difficult. Removal is important because if the template remains in the MIP, fewer cavities will be available for rebinding which decreases the MIP efficiency. Additionally, if template leaking or bleeding occurs during analytical experiments, errors will result. The aim of template removal is to allow the cavities to be available for template rebinding while maintaining their conformation.[22]

SPR-Based Affinity Analysis of MIP. A novel SPR-based sensor was employed in this study for the first time for specific recognition and detection of viruses (Figure 3). Amine coupling chemistry is used to attach biomolecules strongly to

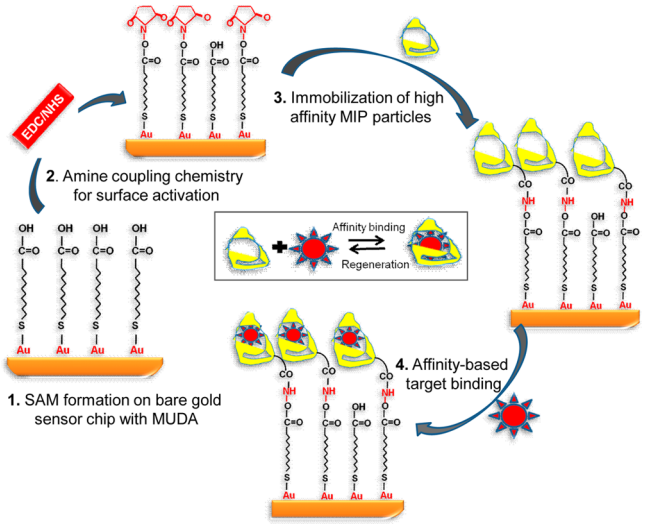

Figure 3. Affinity-based sensor assays for virus detection.

the gold sensor surface. The principle of this method relies on the activation of carboxyl groups on the sensor surface by an EDC/NHS mixture which creates reactive succinimide esters.[23,24] For this, the sensor chips were initially coated with mercaptoundeconoic acid (MUDA) to obtain self-assembled monolayer (SAM) on the surface and protect the immobilized compound from degradation and get rid of signal decrease during the assays. The surface was then activated using the mixture of EDC/NHS prior to immobilization of MIP nanoparticles to the activated surface.

The bacteriophage MS2 samples prepared in the serial concentration range 0.33−27 pmol were then injected to the sensor system separately. Two separate channels of SPR-2 system were used for the affinity assays, and the nearly same response was achieved from both spots which indicates the reliability and sensitivity of our sensor assay (Figure 4a,b). Blue and red lines in the sensorgrams indicate two separate sensing spots which provide duplicate sample detection at the same time. Each bacteriophage MS2 sample was injected to the sensor for 4 min, and a continuous binding assay was performed from low to high concentration (Figure 4a). After this, a regenerative MIP surface was aimed to be developed, and this was achieved for the first time without losing the activity of the MIPs against the target (Figure 4b). The regeneration protocol was optimized as 1 min injection of 25 μL with 0.1 M HCl and 20 mM NaOH, respectively. The regression analysis was performed for overall results, and correlation coefficient was found as 0.99 for both assay types (continuous and regenerative) with minimal standard errors (3 ± 3 and 7 ± 5RU, respectively) (n = 10). The specificity of the binding between the MS2 phage and MIP surface was also checked using QB-phage as negative control (Figure 4c), and non-specific binding was recorded as 1 ± 1RU (n = 3). The immobilization of the MIP nanoparticles on sensor surface and bacteriophage MS2 binding were also confirmed using the scanning electron microscopy (SEM) technique (Figure 5). The affinity between the artificial ligand (MIP nanoparticles) and bacteriophage MS2 was investigated using Biacore and SPR-2 systems. The analyses were performed using Biacore

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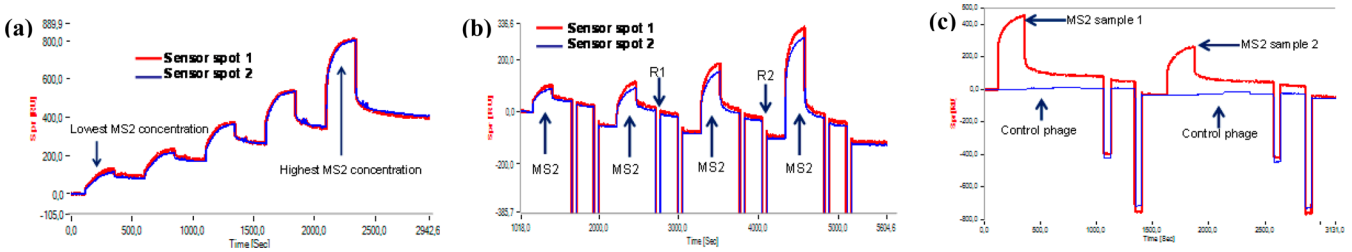

Figure 4. Real-time sensorgrams of the (a) continuous and (b) regenerative virus binding assays. MIP surface was regenerated using 0.1 M HCI (R1) and 20 mM NaOH (R2) as regeneration solutions for subsequent virus samples tests. (c) Cross-reactivity test using QB phage as control; blue line shows QB-phage binding on MS2-MIP whereas red line represents the MS2-phage binding on MS2-MIP.

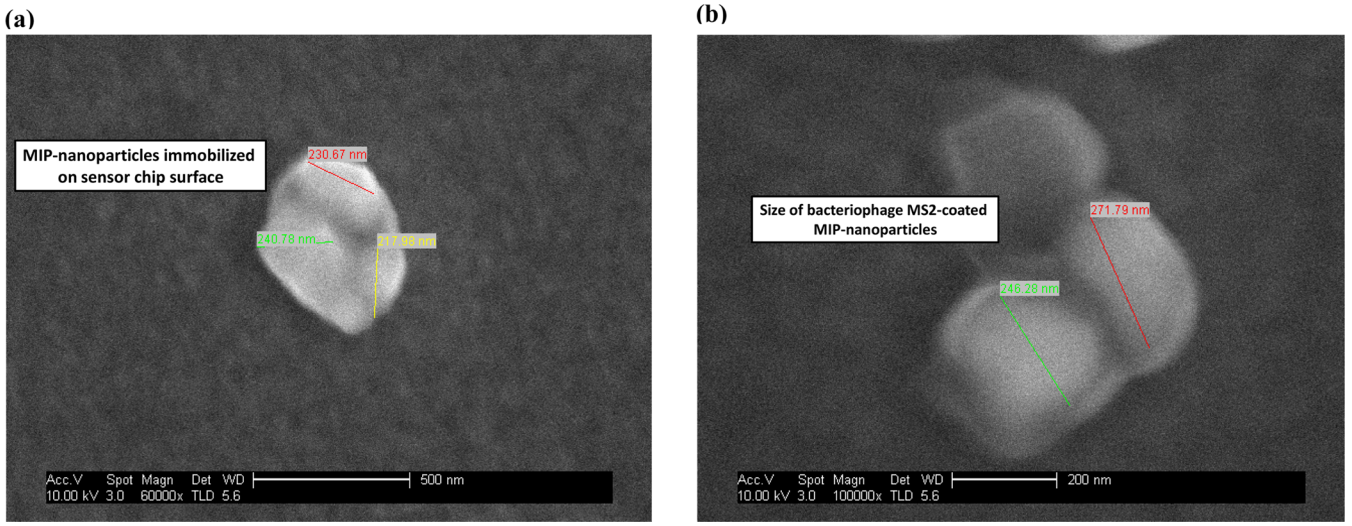

Figure 5. Size of the particles indicates (a) the affinity ligand immobilization on sensor surface and (b) the layer around the particles present the analyte binding. The immobilization of MIP-nanoparticles and the binding of target analyte (bacteriophage MS2) on sensor assay were confirmed using scanning electron microscopy (SEM) technique after obtaining real-time sensorgrams from SPR-2 biosensor.

3000 software and SPR-2 analyzer. The affinity between the MIP particles and bacteriophage MS2 was found to be quite high which has a crucial importance for the removal of viruses from water samples using a MIP-based approach. The comparative analysis of both sensing systems gave similar results for affinity according to the Langmuir binding model (Table 1). QB-phage and vancomycin were also imprinted as control MIP receptors. MS2 capture test was conducted on these control MIP-receptors and also on the target surface. Due to the completely different structure of MS2 phage from vancomycin, vancomycin-MIP did not interact with MS2 phage whereas QB-phage showed a certain level of binding against MS2 phage due to the structure and size similarity (Figure 6).

The different techniques for the production and manufacture of MIP nanoparticles were reviewed in recent years, and the extensive list of manufacturing techniques such as precipitation and emulsion polymerization was also reported.[25,26] However, there was no evidence in the scientific literature for our automated manufacturing method for producing MIP nanoparticles or commercial exploitation of the technology. This computer controlled production technique for MIP nanoparticles was built by our research team at Cranfield University. The technique was superior to all those appearing in the literature because of the inherent benefits of the automated process. This process enables the fabrication and purification of high affinity MIP nanoparticles which have a uniform particle size distribution. The method was also proven to be applicable to a wide variety of templates.[27,28] Our MIP production

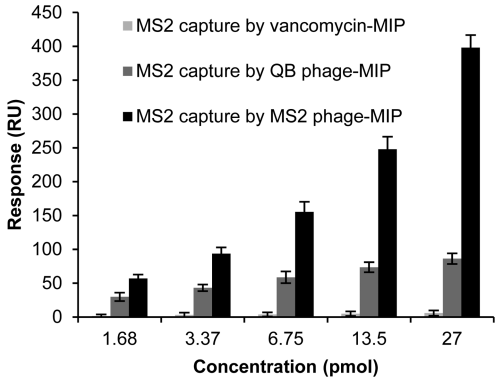

Figure 6. Comparison of affinity binding between two control MIPs (QB phage-MIP and vancomycin-MIP) and target MIP (MS2 phage-MIP) against MS2 phage. The three different MIPs were immobilized on separate sensors, and MS2 phage samples were injected onto the sensors. The results indicate 6 separate experiments including 2 different sensor chips and 3 replicates for each virus concentration on each sensor surface. $n = 6$ with standard deviations.

method provides many advantages including the following: (1) the synthesized MIPs in the presence of immobilized template can be fractionated in accordance to their affinity allowing collection of the particles with very narrow distribution of high affinity binding sites, (2) MIP nanoparticles were easily separated from the template, nonpolymerized monomers, and initiator using temperature-controlled elution step, (3) the

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synthesis process is fully automatic, and (4) the required time for the synthesis and purification of nanoparticles was very short when compared with that of other protocols in the literature.

The glass beads are an effective and efficient affinity media to immobilize the template around them prior to the synthesis of the MIP nanoparticles. The automatic synthesis of the nanoparticles by the automatic reactor allowed for the fast and efficient production of the nanoparticles. This allows MIP synthesis to be reproducible as the conditions are kept constant for each production. The yield of the nanoparticles was decent, and it is considered that further optimization would improve it further. To our knowledge, there are no published papers on the production of MIPs nanoparticles specific for bacteriophages. This proves the novelty and innovation that offers real value to the MIP research community. This research work can be used as a base for pursuing innovative MIP research in the imprinting of biological targets such as important pathogenic viruses hepatitis A, hepatitis E, rotavirus, adenovirus, and norovirus. There were a variety of articles on MIP production specific for viruses.[29,30] These articles were well-documented projects of successful scientific work which were able to fully characterize the MIPs produced.[31] The results of our research also gave better improvement for the affinity. Jenik et al. reported the creation of MIPs by stamp imprinting which reversibly bound to two types of picornaviruses (human rhinovirus and foot and mouth disease virus) by almost an order of magnitude more than the control MIPs.[32] However, our research provides a regenerative sensing for virus detection. Hoshino et al. reports the production of nanoparticles specific to the cytotoxic peptide melittin which demonstrated effective capture of the peptide in the bloodstream of mice. This is a significant result and advancement in the MIP imprinting field because of their use *in vivo*.[33] In the current study, the nanoparticles were nontoxic to *E. coli* cells which was a good result as it suggests they can be used in cells safely which may have potential applications in the future. Guerreiro et al. report the production of soluble nanoparticles which were separated first by size and then subsequently by affinity to obtain a high affinity fraction which displayed affinity (K_D: 6.6×10^{-8}) to its template.[34] The separations of the nanoparticles bear similarities to the separation of the nanoparticles in this research by affinity which was expected as both sets of research were conducted at Cranfield University. This research acquired better affinity (K_D: 3.22×10^{-9}) than the work from Guerreiro's article which indicates that the MIP technology has been improving by the group.

The affinity separation in this study was temperature-controlled which allowed the low affinity nanoparticles and the high affinity nanoparticles to be separated. This coupled with their automatic synthesis probably explains the improvement in affinity. A general protocol for creating MIPs specific for epitopes of protein templates was also reported. This approach has advantages since it does not require the whole protein or virus and that binding interactions to the epitope may be stronger than those for the whole protein.[35] Because it only requires a portion of a template it would be easier to acquire or produce a high purity epitope solution which would result in the production of more homogeneous, higher affinity MIP nanoparticles.

Chianella et al. reports the development of a MIP for use in a solid-phase extraction cartridges to preconcentrate (Microcystin-LR) a toxin for sensor analysis where MIPs were used to capture a toxin from water.[36] This can be extended to the removal and capture of an analyte (for example a virus) from water.

CONCLUSIONS

MIP nanoparticles were synthesized and integrated on a surface plasmon resonance (SPR-2) sensor chip with microfluidics for the automated capture and analysis of viruses. An *E. coli* bacteriophage (MS2) grown in house, isolated, and purified was used as a model pathogenic virus. The bacteriophage was attached to silica microbeads and used as the template for MIP synthesis utilizing a novel automated solid-phase method to produce nanoparticles with high affinity binding sites. The synthesized nanoMIPs size ranged from 200 to 230 nm measured using dynamic light scattering (DLS) with a Zetasizer Nano. TEM images of the nanoparticles illustrated their spherical structural morphology. Affinity analysis performed using SPR-2 biosensor functionalized with the nanoMIPs using amine coupling chemistry with a serial dilution of virus solutions (0.33 pmol −27 pmol) resulted in virus capture and detection in real-time. The developed procedure was able to bind the virus and regenerate the MIP surface for sequential analysis of the virus in solution. The mean affinity between nanoMIP and bacteriophage MS2 was found to be $\sim 3 \times 10^{-9}$ M. The detection limit was also calculated as plaque-forming units (pfu), and it was determined as 5×10^6 pfu mL^{-1}. This concentration was the lowest amount that gave the significant response on the sensor with respect to background. Cross reactivity analysis using the SPR sensor with other virus or analyte (QB phage, vancomycin) indicated the suitability of the nanoMIP as an artificial receptor for specific virus detection. This work confirmed the suitability of the nanoMIPs SPR sensor for the detection of viruses. Moreover, a regenerative MIP surface has been developed for the first time to enable the use of the nanoparticles for extended time periods for viruses capture and analysis. The developed affinity MIPs of the current research will be used to create membrane filters which provide a separation−filtration system for waterborne viruses to purify the water sources. The further improvements of our novel solid-phase MIP production will lead to obtaining more stable and easy-to-use technology and provide a promising combined technology with biosensor technology.

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Author Contributions

Z.A. and M.G. performed the experiments. Z.A. and A.G. produced the MIP nanoparticles. Z.A performed all data analysis and characterization studies. S.P. provided the polymer laboratory facilities. K.-A.T. and J.W. provided the microbiology facilities. Z.A. and I.E.T supervised the project and prepared the manuscript.

Notes

The authors declare no competing financial interest.

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