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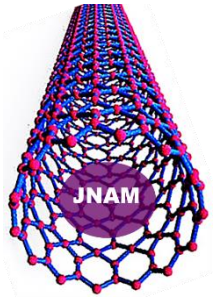

Surface-Imprinted Polymers (SIPs): Advanced Materials for Bio-Recognition

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Abstract: Imprinted polymers are selective recognition elements that grasped the attention of scientists for decades. The type of imprinted polymers relies on the template for which this polymer is tailored. For example, molecularly-imprinted polymers (MIPs) are selective for molecular templates, while ion-imprinted polymers (IIPs) are prepared using ionic templates. Recently, biological templates (bacterial cells, human cells, viruses …etc.) have been patterned on polymers in a process known as surface imprinting to give rise to surface-imprinted polymers (SIPs). This type of polymers has so many applications in different aspects of science, especially analytical chemistry and biology. This review summarizes the recent advances in SIPs technology by shedding the light on their history, synthesis protocols and applications.
Keywords: Surface-imprinted polymers; soft-lithography patterning; biosensors; bio-recognition.

1 Introduction

Imprinted polymers technology is still growing over the years. Recently, it has very wide applications from analytical chemistry to biology and medicine. The idea of imprinted polymers is based on the synthesis of polymeric materials, selective for certain molecules, cells or viruses, which are able to rebind effectively and specifically to their targets. Their preparation includes initiating the polymerization of selected monomers (several monomers give rise to a co-polymer) in the presence of the desired template for which we intend to tailor the imprinted polymer and then removing this template by using a suitable solvent/solvent-mixtures. Removal of the template from the obtained polymer results in the formation of cavities (a.k.a. recognition sites) that can selectively rebind to their corresponding template. **Fig. 1** summarizes the steps followed to synthesize an imprinted polymer for a certain template.

Polyakov is credited with doing the first practical experiment that led to the discovery of the so-called molecularly imprinted polymers (MIPs) via studying the effect of different solvents on the structure of silica pores while preparing new silica [1]. After polymerization (using H_2SO_4 as an acidifying agent/initiator), he found a correlation between the molecular weight of the solvent and the total surface area which indicates that some of the solvent molecules have been embedded in silica causing the formation of cavities which increased the surface area. In another experiment conducted by Dickey, sodium silicate was polymerized in the presence of ethyl, methyl, n-butyl and n-propyl orange dyes [1]. Removal of the dyes from the synthesized polymer resulted in a kind of selectivity in the rebinding experiments. In other words, polymerized silica (in presence of a certain dye) bound to the same dye in preference to the others. Since this moment, MIPs grasped the attention of many researchers and the first commercial trial was introduced by Merck when a nicotine filter based on nicotine-imprinted silica was patented which absorbs 10.7% more nicotine than its non-imprinted analogue [1].

Nowadays, polymer imprinting technology has been extended such that it became possible to use large templates (cells, viruses …etc.) in the imprinting process. The surface features of these templates are patterned on the synthesized polymer particles and leave specific recognition sites on it after their removal. Therefore, the resulting polymers (a.k.a. surface-imprinted polymers, SIPs) can act as recognition elements that bind specifically to their corresponding templates. To the date, SIPs have wide applications in many fields; these include but are not limited to electrochemical sensing platforms, cancer detection/diagnostics, photocatalytic degradation, optical sensors, chromatographic separation, artificial receptors/antibodies and detection of viruses and bacteria (**Fig. 1**).

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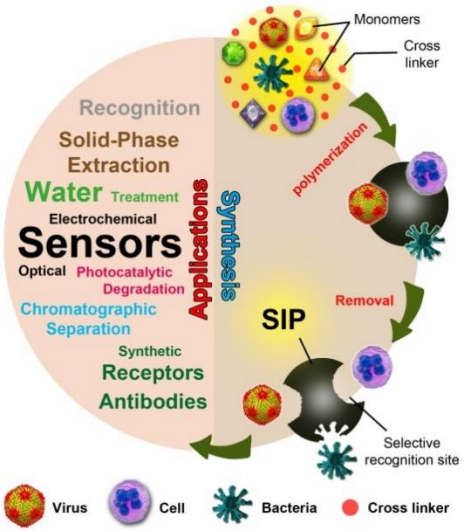

Fig. 1: Synthesis and the most common applications of imprinted polymers. If the template is a molecule or an ion, the produced polymer is known as molecularly-imprinted polymer (MIP) or ion-imprinted polymer (IIP), respectively.

In this context, we mean with SIPs the imprinted polymers prepared either on a surface of another substrate (such as chips, nanoparticles (NPs) or microbeads) or by using the surface of a particle as a template. It may include also MIPs tailored for molecules directly attached to surfaces such as cell membranes or core-shell nanomaterials.

SIPs have the advantages of easy and cost-effective synthesis, durability, high thermal and chemical stability (e.g. against organic solvents, metal ions, acids and bases), reusability [2] and resistance to high pressures [1]. In addition, they allow for specific cell detection depending not only on molecular interactions (hydrogen bonding, electrostatic attraction, van der Waals forces …etc.), but also on cell shape, size, and cell membrane functionalities [3]. These advantages make SIPs suitable for designing point-of-care testing systems [4].

2 Synthesis Protocols of SIPs

The common protocols followed to prepare an SIP for a certain template are usually limited by lots of restrictions due to the sensitivity of most of the templates to harsh conditions such as acids, free radicals, high temperature and so on. This is because most of the targets are biological particles that are usually composed of biological macromolecules such as proteins, carbohydrates and lipids. Therefore, caution should be taken while choosing the suitable synthesis protocol for your template. In this section, we give a brief description of the most common methods used to prepare SIPs.

2.1 Solution and Bulk Polymerizations

Solution and bulk polymerization are two of the principle methods in polymer synthesis. We summarize them here in a single subtitle because they are closely related to one another and the difference between them is minor.

In bulk polymerization (a.k.a. mass polymerization), the polymerizing mixture usually consists of the functional monomers and a suitable initiator, without any other additives [5]. The polymerization reaction usually requires an inert atmosphere (e.g. nitrogen or argon) and high temperature in order for the monomers to undergo the reaction [6]. Bulk polymerization has lots of advantages over other polymerization methods due to the lack of solvents and/or diluents. One advantage is that it can be used for high clarity and high molecular weight polymers. In addition, it is considered as a green method since the produced polymers require no further purification steps due to the high purity of the product. However, bulk polymerization reactions are usually very exothermic (especially at the final steps when the solution viscosity increases) and the temperature cannot be easily controlled making the polymerization reaction extremely vigorous. Therefore, this method is not favored when the monomers to be polymerized have high enthalpy of polymerization. But even when monomers with low reactivity are used, the reaction should be performed in long thin reactors supported with heat exchangers. One disadvantage of bulk polymerization is that the increase in solution viscosity during the polymerization reaction hinders the removal of common condensation reaction volatile byproducts such as water [7]. Moreover, the reaction kinetics is usually changed in case of high molecular weight (due to the product) and low monomer concentration.

Unlike bulk polymerization, solution polymerization requires the presence of a solvent or solvent blend in which the initiator, monomers and the resulting polymer are soluble. Usually, a high concentration of the monomer (≥ 70%), a minimal amount of the catalyst/initiator, and a solvent whose chain-transfer constant is low are required at the beginning of the synthesis process. Later on, more solvent and catalyst/initiator should be added to the reaction mixture so as to control the viscosity of the mixture and adjust the rate of the reaction; as the efficiency of the initiator decreases due to the continuous dilution of the monomer. In solution polymerization, the presence of the solvent which acts as a diluent makes heat dissipation much easier and controls the mixture viscosity. In addition, the initiator is not masked by the resulting polymer; therefore facilitating its removal. As a result, solution polymerization can be suitable for most of the monomers regardless of their polymerization enthalpy.

2.2 Free-Radical Polymerization

Free-radical polymerization (FRP) can be considered a subtype of bulk or solution polymerization depending on the presence or absence of the solvent/diluent. The main characteristic property that distinguishes this type of polymerization from the other types is that the catalyst must be a free-radical initiator and the monomer must be a vinyl monomer [8]. The most commonly used initiators often belong to two classes of compounds; namely, peroxides (inorganic (e.g. potassium persulfate, $K_2S_2O_8$) or organic (e.g. *tert*-butyl hydroperoxide and Luperox®)) and azo compounds (e.g. 2,2'-azobis-isobutyronitrile, AIBN). Ideally, a free-radical initiator decomposes at the polymerization temperature while remains stable at ambient temperature. The selection of a suitable initiator depends on the used solvent/monomer system because the rates of decomposition of all initiators (k_d) are strongly affected by changes in the solvent/monomer mixture. For instance, the *"cage effect"* results in secondary reactions such as the regeneration of the decomposed initiator from its radicals. This effect originates due to increasing the viscosity of the medium [9]. The initiator *"half-life"* (the time required to reduce the original initiator content of a solution by 50% at a given temperature, denoted $t_{1/2}$) is usually used as an important indicator for the initiator's activity. FRP is an economic way to get the desired polymer from vinyl monomers because the purity of the reactants is not very urgent. In addition, the reaction does not require rigorous exclusion of air, moisture and other impurities. The versatility and synthetic ease of polymers via FRP makes the process the most important in the industrial scale. Despite these advantages, FRP has the disadvantage of producing polydisperse materials with limited control over macromolecular weight and architecture [10].

The mechanism of FRP of vinyl chloride monomers is depicted in **Fig. 2**.

2.3 Self-Assembly

In this approach, a solution containing the template, monomers, initiator (if necessary) and the cross linker is prepared. The presence of the template in close proximity to the monomers during the cross-linking process facilitates the occurrence of certain kinds of interactions between the monomers and the functional groups of the template (e.g. covalent and/or noncovalent interactions). After completion of the cross-linking process, the template units can be removed by excessive washing, leaving the specific active sites in the polymer which are capable of rebinding with the template particles. Although the steps of preparation used in the self-assembly technique are very similar to those of molecular imprinting, large templates can be used in order to prepare SIPs. This approach is usually used when a very thin layer of SIP is to be deposited on the surface of a microchip or for coating nano- or microbeads with an SIP [11]. In this case, it is preferred to use monomers that have the ability to undergo self-polymerization such as dopamine (DOP). One disadvantage of self-assembly is that self-polymerizable monomers are not always available because the nature of the template itself dictates the properties of the monomer to be used; and therefore the range of monomers available for use is narrowed [4]. Researchers from Stony Brook University prepared potentiometric sensors for the detection of cancer biomarkers and viruses based on SIPs [12]. They used proteins and poliovirus particles to imprint self-assembled thiol monolayers. An aqueous suspension of the templates was mixed with its alkanethiols counterpart, and then the resulting mixture was applied to the surface of a gold substrate for 2 h. Thereafter, the template particles were peeled off via rinsing with deionized water.

2.4 Molding on Solid Substrates

The first step in the molding process is the immobilization step during which the template particles are fixed on a solid substrate (**Fig. 3**). Thereafter, a mixture containing the suitable polymer is prepared and deposited on the top of the template layer. Polymer molecules then start to assemble themselves around the template particles and interact with their accessible functional groups (e.g. –COOH, $–NH_2$, –OH and others) during the cross-linking step. A curing step follows cross linking after which the formed polymer is removed and the template particles are washed off with a suitable solvent or buffer solution. The removal of the template particles results in a very thin flexible layer of the polymer decorated with a huge number of specific template-recognition sites. [4].

Any polymer can be used to form the recognition layer, but it is more favorable to use monomers/polymers that can be cross linked at room temperature such as chitosan or polyacrylamide [13,14]. Molding provides the advantage of saving time and costs as the whole synthesis process may take less than an hour. In the literature, a large number of solid substrates are used from which glass, aluminium,

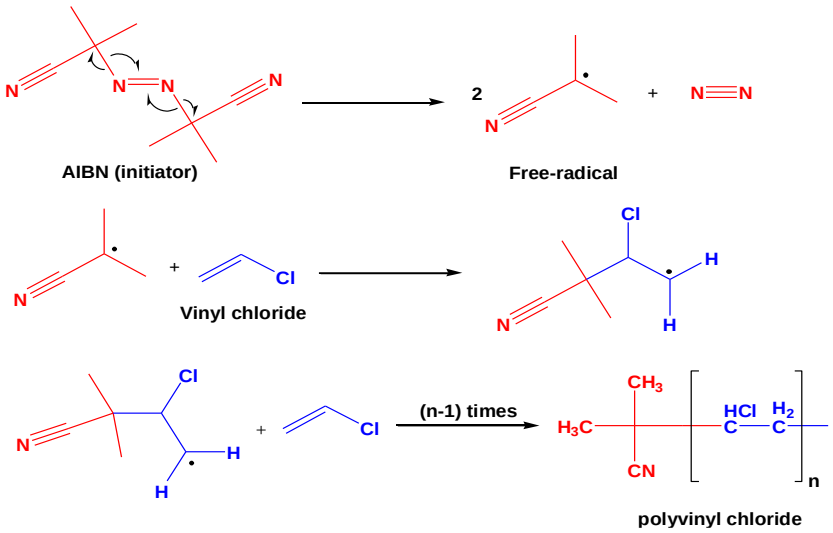

Fig. 2: The mechanism of free-radical polymerization of vinyl chloride monomer with AIBN radical initiator.

indium tin oxide (ITO) and silica scaffolds are very common [14,15].

2.5 Stamping

As described in **Fig. 3 c**, the simplest technique for stamping is to make imprints for large templates (bacterial cells, human cells, proteins, viruses …etc.) on a polymeric monolayer (polyurethane (PU), epoxy, etc.). First, the template is immobilized on a solid substrate such as polydimethylsiloxane (PDMS) scaffolds (shown as a black layer in **Fig. 3 c**) and the resulting structure is used as a stamp to imprint a polymeric monolayer deposited on another solid support with the template pattern. Second, the stamp is removed leaving behind the template units attached to the polymeric monolayer (red). Finally, the template units must be washed off using a suitable washing solution so as to expose the active recognition sites of the formed SIP. This process is known as *"micro-contact"* stamping.

The stamping technique is also known as *"soft-lithography patterning"* which includes other types of stamping in addition to the micro-contact stamping shown in **Fig. 3 c**. The name *"soft-lithography"* is derived from the well-known photo-lithography technique used by physicists to make very tiny patterns on solid substrates such as silicon wafers. However, soft-lithography does not use light for making the desired patterns. The process was first described by Bain and Whitesides (1989) [16]. The size of the imprinted template features ranges from 30 nm to 100 µm [17]. It is highly recommended that the template-containing suspension be with high concentration/cell-count so as to give high-density patterns because researchers found that the density of the imprinted patterns strongly affects the measured signals particularly in sensor applications [18]. The advantage of stamping/soft-lithography is that it not only allows for the translation of the geometrical features of a wide range of templates, but also it allows for the translation of their chemical fingerprints in the form of reversible non-covalent chemical interactions between the functional groups of the polymer and those of the template units. Therefore, more specificity and selectivity can be attained via soft-lithographic patterning compared to the other surface-imprinting techniques. **Fig. 4** shows the different methods used for surface patterning with soft lithography and the most common up-to-date types of this approach. The most common types of soft-lithography include micro-contact printing (MCP) [19], micro-molding in capillary (MMIC) [20], replica molding (RM) [21], micro-transfer molding (MTM) [22], solvent-assisted micro-molding (SAMM) [23], phase-shifting edge lithography (PSEL) [24], decal transfer lithography (DTL) [25], nano-skiving (NS) [26], dip-pen lithography (DPL) [27] and nano-transfer printing (NTP) [28].

2.6 Mini-Emulsion Polymerization

Mini-emulsion polymerization is a technique very close to bulk polymerization for surface imprinting. However, this technique provides a more convenient way for the preparation of core-shell imprinted particles. Firstly, the core particle is produced in a solution to be used as a scaffold of good mechanical stability and then the SIP shell is then produced on the surface of the pre-synthesized particles (core). Usually silica nanoparticles can act as a good core for the preparation of such core-shell surface-imprinted particles while DOP is a suitable self-polymerizable functional monomer. Mini-emulsion polymerization has the advantage of adding both lipophilic and lipophobic functional groups to the surface of the same particle. Such functionalized amphiphilic particles can be applied to the extraction of hydrophobic substances from

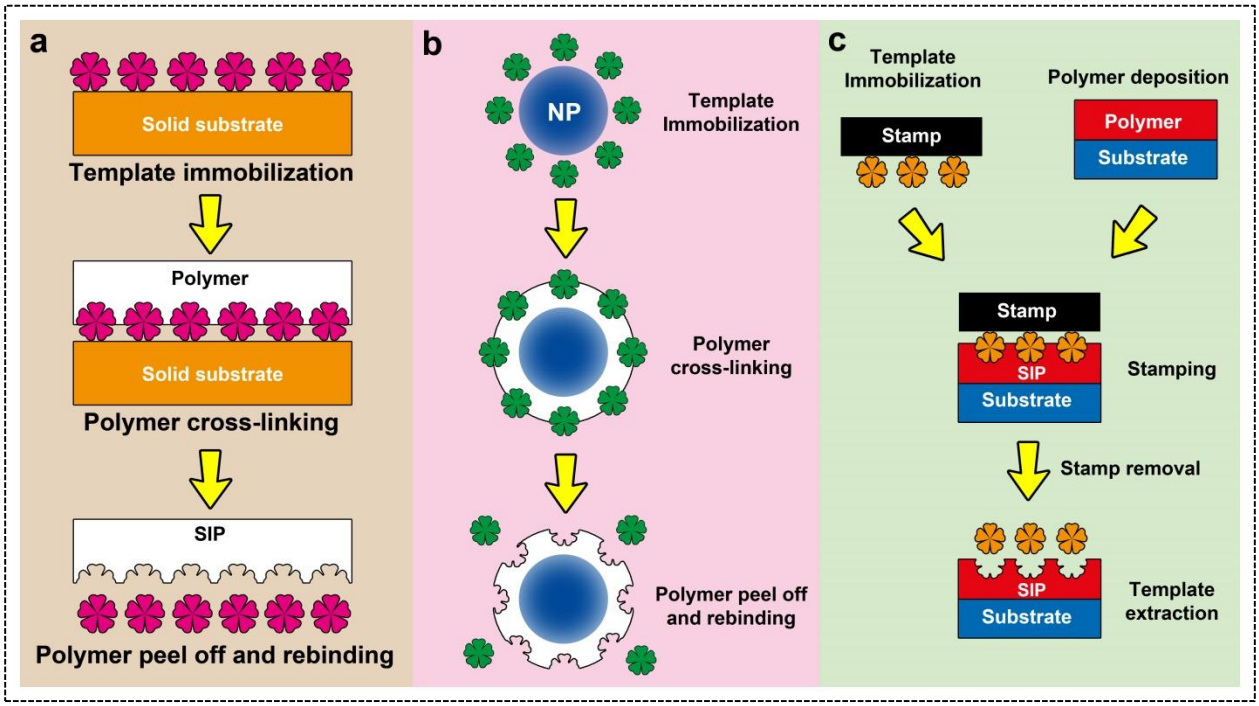

Fig. 3: Illustration of the molding (a and b), and stamping (c) methods for the synthesis of SIPs. Panel b shows the molding process on a surface of a pre-synthesized nanoparticle (NP). The process depicted in panel c is known as microcontact stamping in which the template is attached to a solid substrate (e.g. polydimethylsiloxane, PDMS) and used as a stamp to imprint the red polymer monolayer (e.g. polyurethane or epoxy) in order to form the desired SIP. The blue substrate may be any solid support such as glass or silicon.

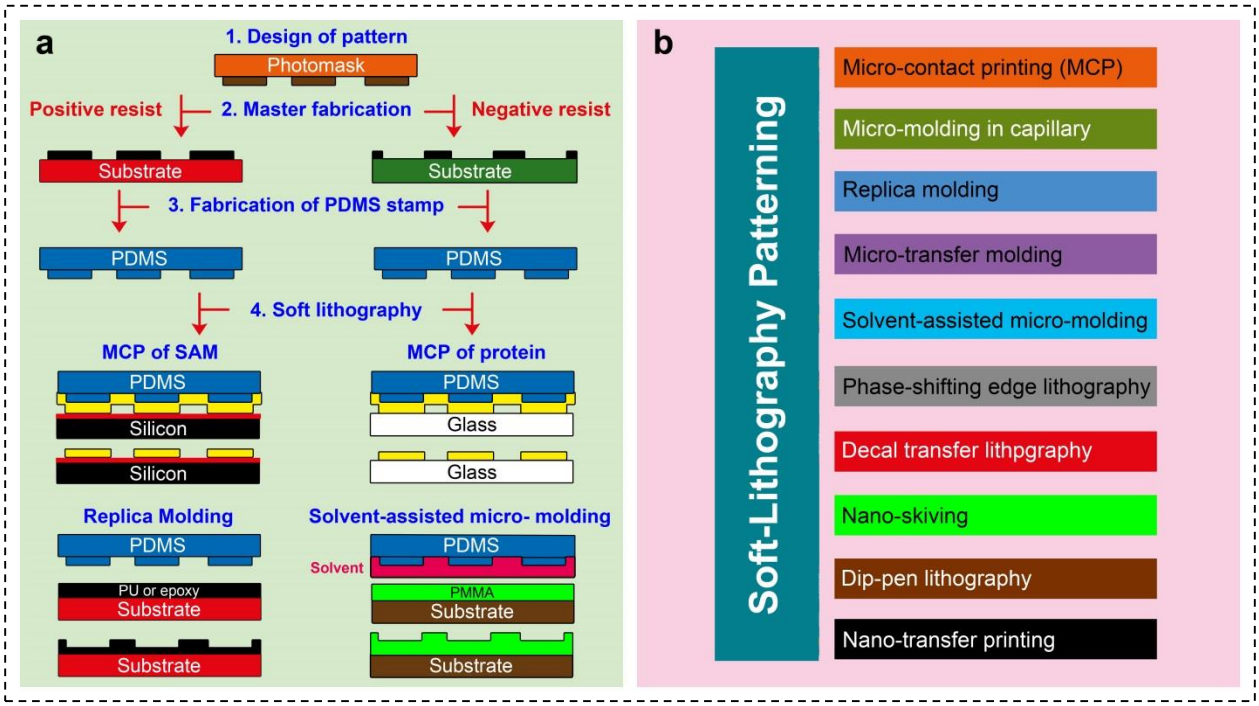

Fig. 4: Soft-lithography patterninig of solid surfaces showing the main steps of soft-lithoghraphy imprinting (a), and its common types (b).

aqueous/polar solutions and *vice versa*. In 2002, Asua published a very interesting review article summarizing the preparation and polymerization of monomer mini-emulsions and reviewing their industrial applications [29].

Zhao et al. proposed a surfactant-free mini-emulsion polymerization technique for synthesizing core-shell silica nanoparticles (polystyrene@SiO_2) in a single step [30]. A year later, the same group synthesized poly(methyl methacrylate) (PMMA) coated silica nanoparticles through a one-pot synthesis strategy [31]. Both methods depend on two processes, an emulsification step of a mixture of polyethoxysiloxane (PEOS) and styrene monomers in water followed by heating to initiate the polymerization reaction. A more recent method was applied by Arai et al. for the preparation of polyacrylate@SiO_2 [32]. An acrylic surfactant was used for covering the precursor silica nanoparticles so as to facilitate the adsorption of acrylic monomers. Thereafter, the polymerization reaction was initiated in order to form a very thin film of polyacrylate on the surface of the silica nanoparticles.

Scaffolds other than silica particles and coatings other than the above-mentioned polymers can be used for the mini-emulsion-polymerization based synthesis of core-shell particles. For example, Bertuoli et al. succeeded to deposit a polyaniline (PANi) thin layer (~4 nm thickness) on the surface of polymeric core nanoparticles composed of PMMA, poly(butyl acrylate) (PBA) and poly(acrylic acid) (PAA) [33]. The resulting particles, having semiconducting properties as revealed by UV-vis and conductivity measurements, are thermally stable and have good mechanical properties. Therefore, these particles show enhanced protection to phosphatized carbon steel and can be applied as an anti-corrosion material. Development continues to prepare surface-imprinted core-shell particles which have diverse applications in many areas of research.

2.7 Grafting

This technique was invented in an attempt to avoid the disadvantages of bulk imprinting [34]. This is because bulk imprinting technique results in large clumps of the polymer which must be crushed before employment in the subsequent application. This crushing/grinding process leads to the destruction of the polymer recognition sites and reduces the recovery of the template units. However, in grafting-based surface imprinting, the polymerization reaction takes place while the template units are attached to the surface of a solid support such as spherical micro-/nanoparticles or beads leading to the formation of a very thin layer of the SIP. In this case, the functional groups of the template units undergo chemical interaction with those of the polymer units that have been already grafted on the solid support surface. In other words, the template units do not undergo any kind of interaction with the solid support (**Fig. 5**).

Recently, Lowdon et al. proposed a method for grafting polished aluminium plates with MIP-receptor layers [35]. The polished aluminium plates were functionalized with silyl groups via hydroxylation followed by silylation. The MIP-coated plates were used as sensors for the detection of a new group of structurally-related psychoactive drugs; namely, diphenidine, 2-methoxphenidine, ketamine and phencyclidine.

IIPs play crucial roles in environmental chemistry particularly for the removal of heavy metals from wastewater in water-treatment plants. Recently, researchers from Northwest Minzu University prepared activated carbon (AC) particles grafted with an IIP selective for Pb(II) ions [36]. The process involved treatment of the carboxylated AC with a solution containing $Pb(NO_3)_2$ (template) and ethylenediamine (monomers). Epichlorohydrin was used as the cross-linker and N,N-dicyclohexylcarbodiimide was used as the condensation agent. This system was tested as a sorbent for the removal of Pb(II) ions from waste water samples and proved to have a high selectivity to Pb(II) over other heavy metals (Zn(II), Ni(II), Co(II) and Cu(II)).

3-Monochloropropane-1,2-diol (3-MCPD), a common carcinogenic food-processing contaminant, was successfully determined with the aid of a MIP@CDs filter paper sensor [37]. First, the filter paper (a solid support with hydroxyl groups) was activated via immersion in 5 M hydrogen peroxide. Second, CDs functionalized with amino groups were synthesized via a hydrothermal method utilizing citric acid as a carbon source and ethylenediamine as an aminating agent. Furthermore, the purified functionalized CDs were introduced to the solid support (filter papers) by negative pressure where the CDs adsorbed on the paper surface via electrostatic attraction of amino groups on the CDs surface and the filter-paper hydroxyl groups. Thereafter, a MIP layer was built on the filter paper surface via a simple FRP step using MAA as a functional monomer and EGDMA as a cross-linker. The proposed sensor was successfully applied to the spectrofluorometric determination of 3-MCPD in real food samples with high percentage recoveries (97.2 - 105.3%, %RSD < 5.6) and a very low detection limit (0.6 ng/mL).

2.8 Electro Polymerization

When very thin layers of SIPs are needed particularly for electrochemical sensing applications, electropolymerization is favored over the previously-mentioned synthesis methods [38]. Electro polymerization provides a facile and effective method for depositing conductive polymers (polyaniline, polypyrrole …etc.) on the surfaces of electrodes. The process involves the generation of free radicals in the

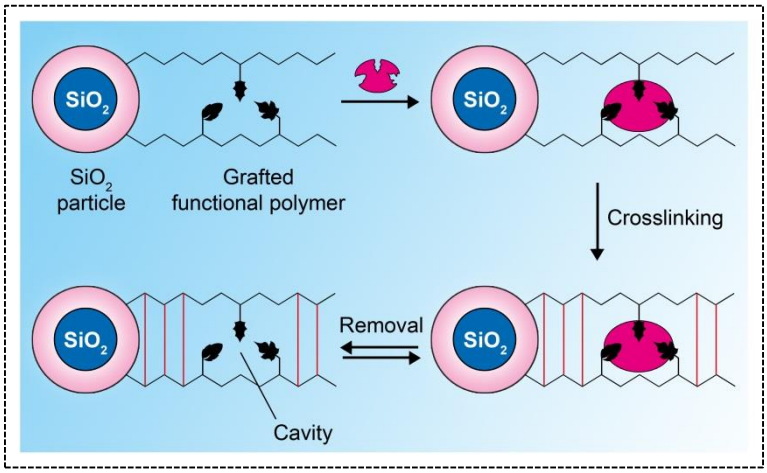

Fig. 5: Schematic showing the steps of grafting-based surface imprinting on the surface of a silica particle.

electrochemical processes taking place at the electrode surface. One of the advantages of electro polymerization is that the polymeric films are usually formed in the form of very small nanoparticles with uniform size and shape. In addition, this method is very effective while very small areas are to be coated with the desired polymer [39]. Caution should be taken while selecting the applied potential used for electro polymerization, the potential should be high enough to oxidize the monomer molecules and initiate their polymerization. On the other hand, it should not be too high so as to avoid corrosion/dissolution of the electrode material. The process is usually performed in a three-electrode electrochemical cell (the working electrode to be coated, an inert counter electrode (usually Pt) and a reference electrode (SCE or Ag/AgCl)). Electro polymerization can be subdivided into potentiostatic, potentiodynamic and Galvanostatic. The solution used for electro polymerization usually contains the monomers, solvent and the supporting electrolyte as well as the template to be imprinted. After polymer formation, the template is removed by washing to uncover the recognition sites. This method is suitable for imprinting all templates except for electrically active ones because the identity of the template may be changed when high voltage is applied.

3. SIPs as Recognition Elements

3.1 Heat-transfer Sensors

Heat-transfer sensors depend on measuring the heat-transfer through a certain kind of materials in order to detect/determine an analyte in a sample. Although this kind of sensors is not common in analytical chemistry, it is widely used in MIP- and SIP-based sensing strategies. In 2012, Grinsven et al introduced a Heat-Transfer Method (HTM) for biosensing when they found that molecular brushes of single-stranded DNA grafted into synthetic diamond raises the heat-transfer resistance as it behaves like a thermally insulating layer (**Fig. 6 A**) [40]. The HTM sensor is composed of a SIP-coated aluminum chip connected to a copper block which is heated and its temperature is kept constant using a thermocouple and a temperature-control unit forming a feedback loop. The SIP surface and the target sample -placed in a liquid measuring chamber- are heated too, and then heat is transferred to the copper block. The transmitted temperature is monitored in time by a second thermocouple. Binding of cells, bacteria, viruses or biological molecules results in a concentration-dependent increase in thermal resistance at the solid-liquid interface which could be registered as a decrease in the transmitted temperature [41].

HTM can have various applications for the detection of cancer cells; even with the existence of other non-target cells. For instance, cell-imprinted polyurethane coupled with HTM was able to detect ovarian cancer cells as they over-express mucin-1 (MUC1) cell-surface-associated protein which may display O-linked glycosylation on its extracellular domain. Ovarian cell-SIP could bind to both target and non-target cells; however, washing could eliminate non-target cells as they do not form strong bonds with the SIP. Therefore, after washing, thermal resistance significantly changed with cells of over-expressed MUC1 but approximately did not change with other cells [3]. SIP-based heat-transfer sensors were also able to detect breast-cancer cells and to assess the quality of their culture. Thermal resistance decreases as non-target cells contaminate the culture of breast cancer cells as they do not express the same functional groups leading to weaker bonds with the imprints [42].

There are some limitations in the previously mentioned design of the HTM sensor; the large copper heat sink coupled to flow cell led to a significant heat loss and obvious fluctuations in temperature causing a high noise in the signal reaching the functional interface. Therefore, Stilman et al. suggested some modifications to the HTM sensor geometry in order to decrease power consumption and heat loss, and to increase the sensor

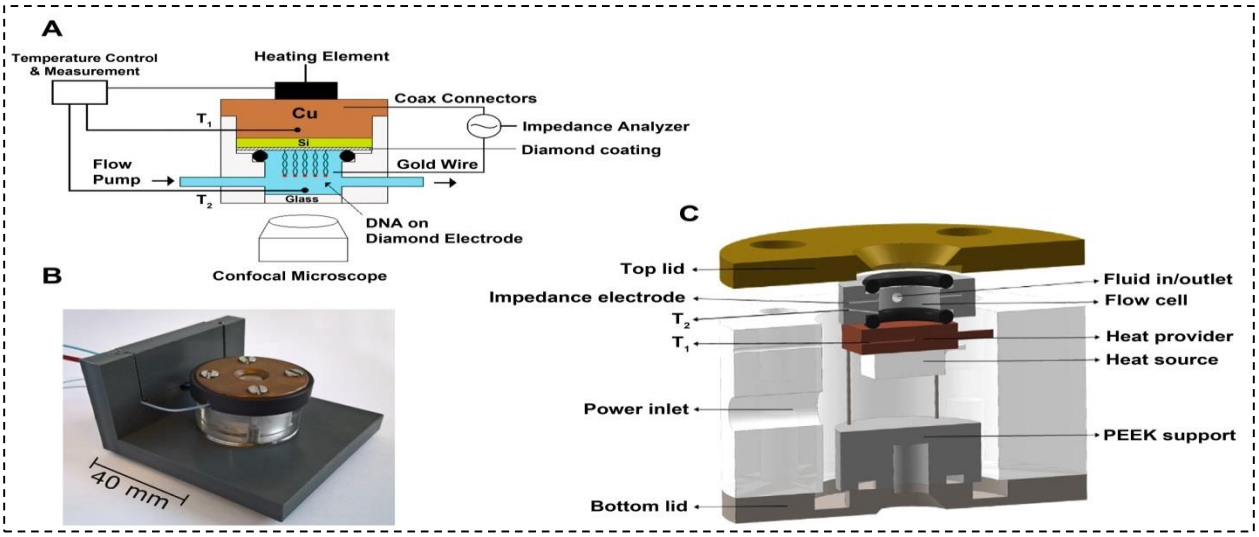

Fig. 6: Schematic representation of a thermal flow cell used for thermal monitoring of DNA denaturation through a HTM (A) and the heat-transfer setup modified by Stilman et al. (B) and (C). Heat flow at the solid-liquid interface is monitored by a thermocouple. Panel (A) was adapted with permission from (B. Van Grinsven, N. Vanden Bon, H. Strauven, L. Grieten, M. Murib, K.L. Jiménez Monroy, S.D. Janssens, K. Haenen, M.J. Schöning, V. Vermeeren, M. Ameloot, L. Michiels, R. Thoelen, W. De Ceuninck, P. Wagner, Heat-transfer resistance at solid-liquid interfaces: A tool for the detection of single-nucleotide polymorphisms in DNA, ACS Nano. 6 (2012) 2712–2721. doi:10.1021/nn300147e). Copyright (2012) American Chemical Society. Panels (B) and (C) were adapted with permission from (B. Van Grinsven, K. Eersels, M. Peeters, P. Losada-Pérez, T. Vandenryt, T.J. Cleij, P. Wagner, The heat-transfer method: A versatile low-cost, label-free, fast, and user-friendly readout platform for biosensor applications, ACS Appl. Mater. Interfaces. 6 (2014) 13309–13318. doi:10.1021/am503667s). Copyright (2014) American Chemical Society.

sensitivity (**Fig. 6 B** and **C**) [43]. The authors changed the dimensions of the copper block to be of the same dimensions of the aluminum chip to minimize heat loss. Moreover, the temperature inside the flow cell is monitored near to the sensor surface, ensuring that the relative contribution of the functional interface to the overall thermal resistance increases. The results indicated that the sensor could quantitatively detect *E. coli* by higher sensitivity, lower noise, and lower power consumption by a factor of two. Furthermore, the authors performed computational simulations using COMSOL Multi-physics software to study the influence of the flow cell dimensions on heat transfer indicating the sensitivity of the device. The simulations depict that decreasing the height of the measuring cell and making the lid of the system with a good thermal conductor will lead to a better homogeneity of the heat flow; so, further modifications to the geometry based on the results of the simulations are recommended. In a further study performed by the same group, the modified HTM sensor was used to detect *E. coli* on a contaminated surface and to measure its concentration by forming a calibration curve. The calibration curve showed a linear increase of the phase shift with increasing the bacterial concentration then reached a plateau when the concentration was greater than 10^5 CFU/mL. The results were compared to that of gold standard culture-based assay and showed good agreement in the linear part. However, further modifications are recommended to enhance sensitivity [44].

In 2017 a novel detection method based on SIP and Thermal Wave Transport Analysis (TWTA) was introduced in which two thermal waves propagate through an SIP sensor. The first one is propagating above the SIP in a measuring chamber while the second one is propagating in a copper chip located below the SIP layer. The phase shift between these two waves is measured using a thermocouple, and this shift indicates whether there is something bonded to the SIP layer. In this case, the measured shift is due to the delay of the propagating wave when passing through the sample, and this coincides with the increase in thermal resistance noticed in the HTM methods. However, TWTA shows better sensitivity and response time than HTM by a factor of 2, because HTM is usually associated with a lower signal-to-noise ratio. The first SIP-TWTA sensor was able to differentiate between 9 different strains of bacteria and to detect *E. coli* in urine with a detection limit of 3×10^4 CFU/mL [45].

Further research was done on the same TWTA sensor introduced by Heidt et al. in order to compare its ability to diagnose urinary tract infection compared to the classical microbiology techniques. The results showed that the sensor was reproducible over the studied samples containing different concentrations of *E. coli* and was not influenced by some variables such as gender and age. The sensor was able to detect bacteria that fit the previously established dynamic range by classical microbiological UTI tests (10^4–10^5 CFU/mL). Moreover, the SIP sensor was

advantageous over the classical tests due to its fast response, so it can be used to test fresh urine samples that have the highest percentage of live bacteria, unlike the slow classical tests whose accuracy is seriously affected by the higher ratios of dead bacteria present in old urine samples [46].

In summary, heat transfer can be an effective signal when integrated with SIP, but it needs further modification to increase the sensitivity either by optimizing the geometry or by improving the sensing approach. Moreover, computational simulations can be beneficial to reach the optimum setup.

3.2 Electrochemical Sensors

SIP was incorporated within electrochemical sensors; however, most polymers used in SIP are not good electrical conductors, so that a composite of the polymer and carbonaceous nanomaterials could be utilized in order to enhance the conductivity. Generally, a sensing layer of SIP is deposited over the working electrode; this is done through several steps: First, the prepolymer gel is prepared, then mixed with the carbon nanomaterial (e.g graphene oxide (GO) or carbon nanotubes (CNTs)) with the suitable percentage forming the so called *Polymer Matrix Composite* (PMC). Second, the PMC is coated onto the working electrode. Finally, the target molecule or cell solution is added and the polymerization proceeds over the electrode [47,48].

SIP-electrochemical sensors were used to detect different viruses. Tancharoen et al. [47] used SIP-electrochemical sensor for the detection of Zika virus (ZV). The sensor was made of a polymer and GO, and it was characterized with cyclic voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS). It was found that as the load/titer of ZV increases, the electric resistance decreases and the produced current increases. In other words, ZV enhanced the heterogeneous charge transfer at the electrode surface. The detection limit was 2×10^{-3} PFU/mL in 10% serum, but lower in buffer (2×10^{-4} PFU/mL). This lowering in the detection limit in pure synthetic media may be attributed to the presence of other components in serum which affect the sensitivity of the sensor [47].

Another study by Navakul et al. [48] used a similar system for the detection of Dengue virus (DENV), a type of viruses famous for causing Dengue hemorrhagic fever. It was found that DENV increases the charge-transfer resistance (R_{ct}) hence decreasing the flowing current at the electrode surface. The detection limit of this method is 0.12 PFU/mL [48]. Other researchers used a layer of multi-walled CNTs over the working electrode covered with a layer of chitosan (instead of mixing them together) for the detection of human immunodeficiency virus p24 (HIV-p24). In this case, binding of HIV led to a higher resistance. This sensor was very effective as the detection limit reached 0.083 pg/mL which is significantly lower than the other techniques [49].

Electrochemical SIP sensors can be considered as versatile tools for the detection of bacteria. For instance, Golabi et al. developed a sensor to detect *Staphylococcus epidermidis* based on polymerized 3-aminophenylboronic acid (3-APBA). The advantage of using this polymer is the presence of boronic acid group which specifically interacts with *cis*-diol in the bacterial cell wall allowing the formation of a polymeric network and presenting both morphological and chemical recognition abilities. Moreover, its interaction with the *cis*-diol is reversible; thus facilitating the bacterial removal after the imprinting process. The results obtained from EIS showed that binding of bacteria to the SIP led to a decrease in R_{CT} and a low detection limit was attained (10^3 CFU/mL). However the selectivity to *Staphylococcus epidermidis* was not very high as the SIP bonded to other types of bacteria with significant amounts. This is attributed to the disability of the boronic acid group to distinguish among different bacterial cell walls. Therefore, using poly(3-APBA) resulted in a low detection limit but decreased the selectivity of the sensor [50].

Recently, Lahcen et al. introduced the first electrochemical sensor based on spore-imprinted polymer for *B. cereus* spores (bacterial spores) [51]. The sensor was built using electro polymerization of pyrrole over a carbon electrode in the presence of the spores. The sensor detected the spores with concentrations in the range of 10^2 to 10^5 CFU/mL.

3.3 Optical Sensors

Imprinting technology presented a great value in the applications of optical sensing in the last few years. Rico-Yuste and Carrasco published a recent review article dealing with the development of novel optical sensors based on imprinted polymers combined with other materials during the last three years [52]. The literature review summarized over there depicts that about 315 out of 420 publications were devoted to the development of MIP-based optical sensors. Optical sensors can be categorized based on the transduction mechanism used for measuring the changes in optical properties of the analytical system. Examples for optical transduction mechanisms include Surface Plasmon Resonance (SPR), Surface-Enhanced Raman Spectroscopy (SERS), fluorescence, interferometry, electrochemiluminescence (ECL) and Reflectometric Interference Spectroscopy (RIFS).

Recently, progress has been done in the application of $[Ru(bpy)_3]^{2+}$ as an ECL material. In one of the studies, $[Ru(bpy)_3]^{2+}$ was incorporated into a mixture of materials containing MWCNTs and TiO_2 NPs. The tripartite nanocomposite was used for modification of Nafion-film-

based ECL electrode whose surface was modified with bisphenol A-imprinted polymer. This sensor was successfully applied for the sensitive determination of bisphenol A in water samples [53]. Similar systems have been developed in which SIPs were prepared on the surface of silica NPs and used for ECL detection of different kinds of analytes [54,55]. The following are the most common types of SIP-based optical sensors.

3.3.1 Resonance-Light Scattering (RLS)

Cells and viruses have complicated space structure, large size, and easily denatured protein components, so their detection using SIP-RLS sensors is challenging and uncommon [56]. However, there were some attempts to detect some viruses using SIP-RLS setups. In 2016, Yang et al. formed SiO_2 NPs coated with poly-DOP imprinted with hepatitis-A virus (HAV), and obtained the RLS spectra for SiO_2 NPs before and after rebinding to HAV, free SiO_2 NPs, and the free HAV viruses. The spectra depicted that the maximum intensity of RLS was reached at 323 nm in all cases; however, the highest intensity was with the HAV bonded to SIP@SiO_2 because binding of the viruses results in the formation of a complex of a larger volume [56].

Despite the limited use of RLS sensors, they have higher sensitivity than their fluorescence counterparts. This was demonstrated by Cai's group who developed several SIP fluorescence sensors for detecting Japanese Encephalitis Virus (JEV), but their sensitivity needs more improvements and the adsorption equilibrium time was too long [57–59]. They proposed a sensor in which an SIP was built over Fe_2O_3 NPs coated with SiO_2. The results showed that as the concentration of JEV increases, RLS intensity increases; moreover, the sensor was able to detect JEV selectively with a detection limit of 1.3 pM within 20 min [60]. Therefore, further studies are needed to develop more SIP sensors based on RLS to give lower detection limits and rapid analytical results.

3.3.2 Surface-Plasmon Resonance (SPR) Sensors

SPR sensors are sensitive techniques that detect changes in the surface plasmon resonance wave of gold nanochip when a target molecule adheres to its surface. The surface of the gold chip is modified with SIPs in order to be able to bind to the target analyte [61]. SPR was used to detect bacteria, viruses and some biomolecules such as hormones. Some studies modified the gold chip by reacting gold with organosulfur molecules such as allyl mercaptan and mercaptoundeconoic acid to form S-Au bonds from one side and another bond with the SIP from the other. For instance, the SPR gold chip was modified with SIP NPs for the detection of bacteriophage MS2. The detection limit of the sensor was 5×10^6 PFU/mL. This work can be extended to be used in filtration to capture waterborne viruses [62]. Moreover, SIP-SPR was able to detect adenovirus with a detection limit of 8.08×10^6 PFU/mL [63]. For the detection of bacteria, polymers containing histidine may be used to enhance the selectivity by creating recognition regions for amino acids present on the bacterial cell wall [64,65]. Yilmaz et al. created an SPR sensor to detect *E. coli* which showed a nearly linear response to concentration; moreover, it had a short response time (113 s) within a concentration range of 0.5-3 McFarland [65]. Furthermore, an SIP-SPR sensor was developed to selectively target *Salmonella paratyphi*, and the detection limit was about 2.5×10^6 CFU/mL, so further research is needed to enhance the detection limits of SPR sensors [64].

3.4 Quartz Crystal Microbalances

Quartz Crystal Microbalance (QCM) is an extremely sensitive mass balance that can detect changes in mass in micro- and nanogram levels. The idea of this type of sensors is based on a quartz disc which acts as a piezoelectric material oscillating at a specific frequency when a specific voltage is applied. One of the factors affecting the frequency is the mass, so when a mass is added to the quartz disk, the frequency of oscillation changes. Incorporation of QCM within SIP systems can detect the target cells/molecules with high sensitivity and selectivity [66,67].

At first, bacteria were detected using QCM whose resonator is coated with antibodies, avidins or peptides to chemically bind to the bacterial surface [68,69]. However, physical interaction is also an important factor to enhance the attachment of bacteria by using imprinted structures on the polymer coating of the resonator. The earlier trials on polymer imprints on QCM to detect *E. coli* were performed using patterned polystyrene substrate with different hierarchical aligned structures of 2 µm or 250 nm rod-like shapes. They also aligned the 2-µm structure with further parallel or perpendicular 250-nm features. The 2-µm structure which is of comparable size to bacterial cells could entrap more bacteria than their 250-nm features counterparts [70]. This supports the idea of having surfaces with the same shape of bacteria enhances their entrapment.

In further studies, different types of cells were imprinted on polymer-coated QCMs, and many studies recommended polyurethane to be used with QCM due to its good adherence to the QCM crystal [71–73]. Furthermore, surface-imprinted polyurethane-QCM sensors were able to detect bacteria [73], yeast cells [72], pollens [74], and viruses [18]. However, some studies recommended other materials for SIP-QCM sensors. For instance, a study was conducted to choose a polymer that gives the best entrapment; this was done by coating QCM with four different polymers: two different concentrations of polyurethanes; ready-to-use Epon 1002F and poly (vinyl alcohol)/N- ethyl-4(4′-formylstyryl) pyridinium methosulfate acetal. *E. coli* structures were stamped over

each surface. The authors recommended using Epon 1002F as it has high sensitivity compared to polyurethane (LOD = 1.4×10^7 CFU/mL) and signal intensities (2.8−0.3 kHz); moreover, Epon 1002F is one of the ready-to-use epoxy resins which have the advantages of easy day-to-day commercial reproducibility and the short time of imprint fabrication [75]. In another study, Tokonami et al. imprinted *Pseudomonas aeruginosa*, *Acinetobacter calcoaceticus*, *Escherichia coli*, *and Serratia marcescens* bacteria -each bacterium separately- on QCM coated with polypyrrole (PPy) film by electropolymerization of pyrrole using a solution containing pyrrole and the target bacteria. Washing off bacteria was conducted by over-oxidization of the PPy to expel the negatively charged bacteria from the polymer texture. This way of washing removed about 89% of bacteria leaving empty cavities for detection. The resonance frequency response of QCM was high (1.8-5.5 kHz) when the sensor was applied to the target bacterial solution; however, no significant changes were observed with other types of bacteria. The sensor could detect bacteria within a concentration range from 10^3 to 10^9 CFU/mL [76]. In conclusion, polyurethane can be used to coat QCM with high adherence, but epoxy resins are easier and faster. In addition, bacteria can be removed effectively from PPy by over-oxidization.

3.5 Microfluidic Devices

Microfluidic devices applications are devoted to two aspects; namely, separation of specific cells from a complex liquid such as blood, and sensing of target cells/molecules. Modification of microfluidic systems with SIPs is thought to enhance the separation efficiency and the sensitivity of the associated analytical method. Typically, separation is achieved via attraction of target cells by the flowing liquid; however, large biological particles such as viruses and human/bacterial cells cannot easily diffuse through bulk SIP and only the surface of the polymeric layer is exposed to the solution containing the target particles. So, microfluidics solve this problem by providing long channels of high surface-to-volume ratio coated with SIP where the solution containing the particles of interest flows and they can be trapped in the cavities of applied the SIP. Microfluidic chips can have different designs according to the desired application. Most of these systems are made of PDMS because it is easily patterned using soft lithography. The chip is provided with a pump to allow the solution to enter the channel, a reservoir at the outlet of the channel and coupled with an analytical instrument or electrical circuit if needed [77] (**Fig. 7**).

The first attempt to integrate microfluidic devices with SIPs in order to separate different strains of cyanobacteria was presented by Schirhagl et al. in 2011. The chip was composed of two layers of PDMS, a top layer containing the channel where the bacterial suspensions of different strains flow and a bottom layer having the surface-imprinted PDMS. Although the suspension contains bacterial species having large morphological similarities, high specificity was obtained and the achieved separation efficiency was between 80 and 90%. Improving the separation can be attained by orienting the imprints parallel to the liquid flow-direction, and adjusting the pH to a more acidic value [78].

By implementation of a circuit or incorporation of an analytical technique, microfluidic devices can be used for detection of lots of analytes. For instance, Birnbaumer et al. combined a microfluidic chip with a microcircuit connected to a bioimpedance spectroscopy device to detect Tobacco Mosaic Virus (TMV). The binding of the virus to the surface of the detection system caused an increase in the measured impedance signal. The detection limit of the microfluidic device was lower than that of ELISA; furthermore, microfluidic sensors have faster response times and can be reused by pumping a solution by 50-fold increase in fluid velocity to wash any residues of the virus [79].

3.6 Bacterial Removal and Photo-Thermal Killing

Although SIP-based bacterial photo-thermal killing is not very well-known in the literature, a paper published by Roy et al. has shed the light on this application [80]. Therein, the authors used a composite of Ag-ZnO bimetallic NPs and GO for imprinting *E. coli* bacteria and to participate in their laser-light induced photo-thermal killing. In addition, the developed system was used as a sensor for detecting *E. coli* as few as 10 CFU/mL. The amount of bacteria which can be captured by the SIP-based system is about 98% of 10^5 CFU/mL concentrated solution of *E. coli*. It was found that, using a 16 cm^2 area of polymer-modified glass plate was able to kill 10^5 CFU/mL of the bacteria within 5 min. This area of research is very promising and lacks more work and investigations.

3.7 Imaging

Sometimes, clinical and forensic investigations of biological samples require the use of imaging techniques to enable the investigator to see what is happening inside a specific tissue or what is present in a certain sample. SIPs proved their applicability in this aspect due to their specific binding to their templates, especially when coupled with other nanostructures such as quantum dots (QDs) or magnetic nanoparticles. These nanocomposite materials facilitate imaging of cells, proteins …etc. through fluorescence and/or Surface-Enhanced Raman Spectroscopy (SERS).

Past studies reported fluorescent materials incorporated with SIPs targeting cell-wall monosaccharides. Shinde et al. fabricated NPs consisting of silica coated with an SIP incorporated with nitrobenzoxadiazole (NBD) as a fluorescent material.

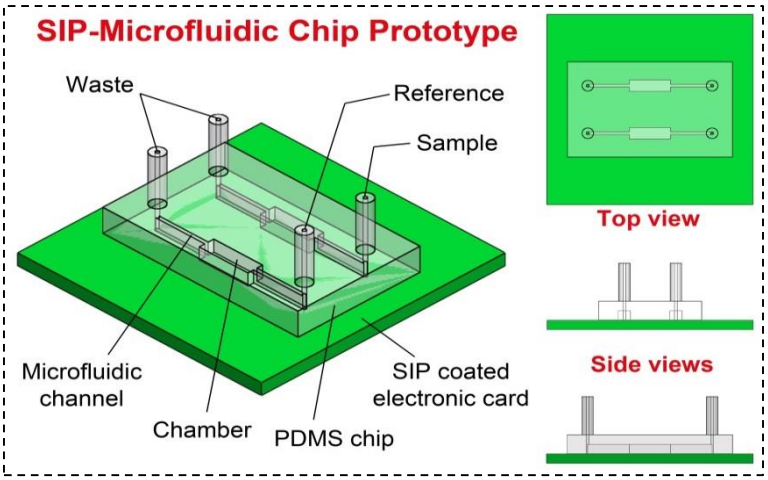

Fig. 7: Schematic representation of SIP-based microfluidic system integrated with an electronic circuit chip for signal transduction and data collection.

The SIP was designed to selectively bind to sialic acid (SA) which is located at the terminal position of the sugar chains of the cells and is over-expressed on metastatic cancer cells. The proposed system was applied to different chronic lymphocytic leukemia cell lines using flow cytometry and fluorescence microscopy. The results showed that the SIP can form suitable antibody for SA which is known to have limited anti-bodies [81,82]. Another study by Wang et al. used fluorescein isothiocyanate (FITC) doped silica NPs as a fluorescent agent. These NPs were then coated with tetraethyl orthosilicate polymer imprinted with SA (**Fig. 8 A**). Confocal microscopic images showed that the walls of human hepatocellular carcinoma cells (HepG-2) and mammary cancer cells (MCF-7) were stained with the fluorescent dye, while their normal analogues could not retain the dye [83].

Quantum dots (QD) are also used as powerful fluorescent agents in SIP-based systems. The advantages of using QDs are: (i) size-tunable light emission, (ii) low photo-bleaching leading to high brightness, (iii) possible multiplexing and (iv)long-term photo-stability. For instance, Panagiotopoulou et al. coated InP/ZnS QDs with an SIP directly built upon them via a novel *in situ* photopolymerization method in which the QDs were used as internal light sources [84]. The reported QDs emitted green and red lights and were used for labelling human keratinocytes. Red QDs targeted N-acetylneuraminic acid (NANA), and green QDs targeted glucuronic acid (GlcA) and they were used together for multiplexing to strengthen the fluorescence signal. Kerationcytes were stained with larger amounts of the QDs when compared to other cells [84]. Although this system is innovative and very promising, it needs further development as the selectivity to the target cells needs to be enhanced.

Hyaluronic acid (HA), a common cancer biomarker, was also targeted for imaging certain kinds of cancer cells. Its expression is either intra- or para-cellular, and its intracellular detection is crucial for diagnosis of cancer in its early stages; however, it is challenging due to masking by other HA-binding macromolecules. Rangel et al. were able to detect intra- and extracellular HA with the aid of an SIP on the surface of glass beads (**Fig. 8 B**) [85]. Further research is needed for using the whole cancer cells as templates for polymer imprinting, as this may lead to more selective labeling.

4 Concluding Remarks

Surface imprinting is an emerging technology in polymer sciences which has vast applications in the recent years especially in analytical chemistry, medical diagnostics and drug delivery. In this review, we shined a light on the recent advances in the preparation, utilization and applications of SIPs as an attempt to present an integrated, concise and reliable source of information for the readers interested in enriching their background or using this type of advanced materials in their work. For more clear explanation, we provided the readers with examples from the literature devoted to this technology. Herein, some informative and helpful tips can be given to researchers interested in this area of expertise based on the work published in the literature.

- First of all, caution should be taken while synthesizing an SIP for biological templates such as cells or proteins because these templates are sensitive to harsh conditions of temperature, pH, chemicals …etc. Therefore, the concept *"not all synthesis protocols are suitable for all biological templates"* should be kept in mind.
- When bulk polymerization is the synthesis protocol of choice, it is important to decide whether inert atmosphere such as N_2 or Ar is required. This condition is usually used in case of bulk polymerization in order to prevent oxidation processes and other side reactions.

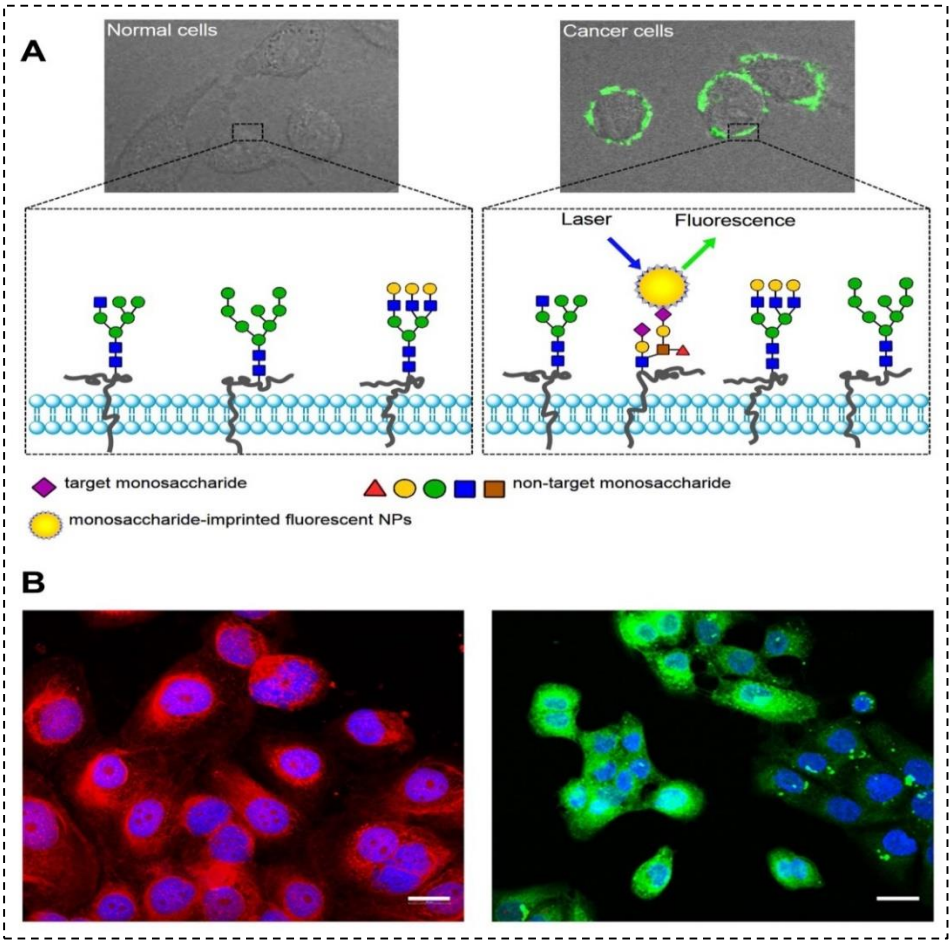

Fig. 8: (A) Schematic representation showing the targeting of cancer cells with NPs imprinted with monosaccharides (Reprinted from [83]). (B) Confocal fluorescence microscope images of fixed human keratinocytes depicting extracellular, intracellular and nuclear labeling by red and green fluorescence SIP-based NPs (Reprinted from [85]).

- Bulk polymerization is not favored when the monomers are of high polymerization enthalpy. In this case the polymerization reaction becomes very vigorous due to the uncontrollable reaction temperature especially at the end of the polymerization process when the viscosity of the mixture increases rapidly. This condition may lead to vigorous explosions.
- In the first steps of solution polymerization a high concentration of the monomers (≥ 70%) is required, while more solvent and initiator must be added to the reaction mixture after certain period of time in order to control the solution viscosity and prevent initiator passivation resulting from dilution of the monomer concentrations.
- The initiator in FRP is sensitive to the used solvent and monomers; since the initiator activity (in terms of dissociation rate, k_d) can be influenced by changes in the type of solvent and monomers.
- When SIPs are to be tailored for living cells, viruses, proteins …etc., usually FRP is the best choice amongst other synthesis protocols since it can be performed at low temperatures because high temperatures cause denaturation for such biological templates. Another alternative is to use photo polymerization or self-polymerizing monomers such as DOP.
- It is necessary to perform a rebinding assay and to study the surface adsorption phenomena for the prepared SIPs. Surface adsorption can be investigated with one of the common adsorption models such as Langmuir or Freundlich isotherms.
- Solid substrates used in the stamping and molding techniques require polymers with low surface energy such as PDMS. In this case, the adhesion force between the immobilized template and the polymer surface decreases to the extent that allows for easy removal of the template.
- According to the literature, electro polymerization requires the optimization of the number of CV cycles used to build up the desired polymer on the electrode surface. Different numbers of CV cycles give rise to different polymeric layer thicknesses (usually in the

Table 1: Some of the recently published SIP-based analytical methods in order to give the readers an overview about the types of analytical techniques that can be coupled with SIPs as well as their sensitivity.

Sensor type / analytical method	Target/Analyte	Signal	Detection limit	Characterization techniques	Year of publication	References
SIP-HTM sensor	*E. coli* in urine	TWTA	2×10^4 bacteria/mL	Microscopy, Heat transfer	2019	[46]
Magnetic-SIP RLS sensor	Japanese encephalitis virus	RLS	1.3 pM	FTIR, AFM	2019	[60]
Imaging method	Kerationcytes	FM	-	DLS, Fluorimetry, FM	2019	[85]
Voltammetric sensor	Zika virus	Electric current	2×10^{-3} PFU/mL	CV, SEM, EIS	2018	[47]
Voltammetric sensor	*B. cereus* spores	Electric current	10^2 CFU/mL	CV	2018	[51]
HTM sensor	*E. coli*	Heat transfer	2.10×10^4 CFU/mL	COMSOL simulations, Heat transfer	2017	[43]
HTM sensor	*E. coli* from a contaminated surface	Heat transfer	10^4 CFU/mL	Heat transfer	2017	[44]
TWTA sensor	*E. coli* in urine	TWTA	3×10^4 CFU/mL	TWTA	2017	[45]
QCM sensor	*E. coli*	QCM	1.4×10^7 CFU/mL	Light microscope, AFM	2017	[75]
SPR sensor	*S. paratyphi*	SPR	2.5×10^6 CFU/mL	SEM, Ellipsometry	2017	[64]
Voltammetric sensor	Dengue virus	Electric current	0.12 PFU/mL	CV, EIS, AFM	2016	[48]
Voltammetric sensor	Human immune-deficiency virus p24	Electric current	0.083 pg/mL	CV, DPV, SEM	2016	[49]
EIS sensor	*S. epidermidis*	Impedance	10^3 CFU/mL	EIS, SEM, FM	2016	[50]
RLS sensor	Hepatitis A Virus	RLS	8.6 pM	TEM, FESEM	2016	[56]
Flow cytometry and FM	leukemic cells	Imaging	-	Flow cytometry, FM,	2016	[82]
SIP-NPs	human hepatoma carcinoma cells (HepG-2) and mammary cancer cells (MCF-7)	Imaging	-	Fluormetry, FM, TEM	2016	[83]
SIP-QDs based FM	Kerationcytes	Imaging	-	TEM, DLS, Fluorimetry, FM	2016	[84]
SPR sensor	bacteriophage MS2	SPR	5×10^6 PFU/mL	TEM, SEM	2015	[62]
SPR sensor	Adeno virus	SPR	8.08×10^6 PFU/mL	TEM	2015	[2]
SPR and QCM	*E. coli*	SPR	1.54×10^6 CFU/mL	SEM, AFM	2014	[65]
HTM sensor	Ovarian cancer cells	Heat transfer	5×10^5 cells/ mL	FM	2014	[3]
QCM sensor	*E. coli*	Frequency	1.6×10^8 bacteria/mL	AFM, Light microscope	2014	[73]
QCM sensor	*E. coli*, *P. aeruginosa*, *A. calcoaceticus* and *S. marcescens*	Frequency	10^3	SEM, FM, Light icroscope	2013	[76]

DPV: Differential pulse voltammetry **FESEM:** Field-emission scanning electron microscope **FM:** Fluorescence microscopy **DLS:** Dynamic light scattering.

micrometer scale) which strongly influences the resulting current signal.

- One of the most important issues to be mentioned here is that a non-imprinted polymer (NIP) should be prepared simultaneously with the SIP in order to calculate the imprinting factor. Sometimes, imprinted polymers do not make significant differences when compared with their non-imprinted counterparts.
- Although non-conducting SIPs improve the selectivity of electrochemical sensors, they may result in an observable decrease in the electrochemical signal leading to lowering the sensor sensitivity. In this case, the use of conducting polymers is recommended. Another solution for this problem is to use conducting nanostructures (e.g. metallic nanoparticles or

carbonaceous nanomaterials) for the modification of the sensor in order to increase its sensitivity.

- The area of bacterial capturing and photo-thermal killing using SIPs is not well studied and needs more investigations in the near future. This may lead to important breakthroughs in fields such as water treatment and hazards mitigation.

Table 1 summarizes some of the recently published SIP-based analytical methods in order to give the readers an overview about the types of analytical techniques that can be coupled with SIPs as well as their sensitivity.

5 Future Prospects

Imprinted polymers have grasped the attention of the scientific community for decades especially after the SIPs had evolved. It is expected that this technology will show tremendous growth in the near future with regard to the methods of their synthesis and applications. So far, the techniques used for characterization of imprinted polymers are too limited in a manner that does not provide clear experimental evidences for the interaction of the templates with their recognition sites. However, molecular modeling (e.g. MM and QM calculations) plays a significant role in unearthing these interactions. We expect that state-of-the-art technologies such as 4D electron microscopy and femtochemistry will act as turning points in understanding and interpreting MIPs/SIPs phenomena in the future, hence allowing for more creativity and innovations in this area of science. Moreover, the application of imprinted polymers in imaging and medical diagnostics is expected to grow rapidly in the coming few years as this area of application has not been waded yet.

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