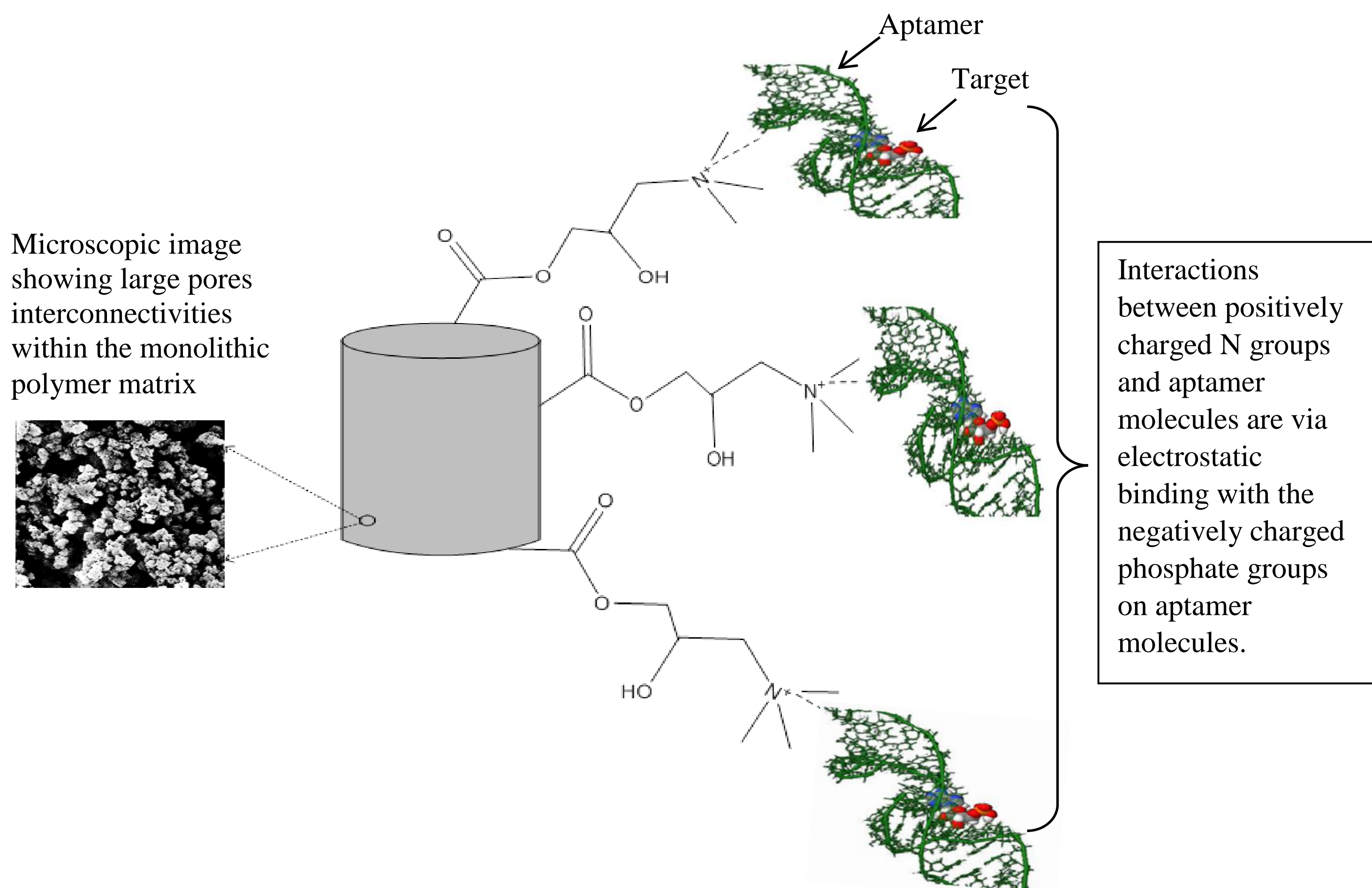
*Graphical Abstract

GRAPHICAL ABSTRACT



Sample of a proposed macro-porous polymer with functionalised aptamer bonded to a target

*Highlights

Highlights

- Aptameric binding have superior properties over other affinity binding techniques.
- Oligosorbents are hydrodynamically unsuitable for rapid biomolecular screening.
- High throughput binding is possible using monoliths with convective mass transport.

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A review on immobilised aptamers for high throughput biomolecular detection and screening

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Abstract

The discovery of Systematic Evolution of Ligands by Exponential Enrichment (SELEX) assay has led to the generation of aptamers from libraries of nucleic acids. Concomitantly, aptamer-target recognition and its potential biomedical applications have become a major research endeavour. Aptamers possess unique properties that make them superior biological receptors to antibodies with a plethora of target molecules. Some specific areas of opportunities explored for aptamer-target interactions include biochemical analysis, cell signalling and targeting, biomolecular purification processes, pathogen detection and, clinical diagnosis and therapy. Most of these potential applications rely on the effective immobilisation of aptamers on support systems to probe target species. Hence, recent research focus is geared towards immobilising aptamers as oligosorbents for biodetection and bioscreening. This article seeks to review advances in immobilised aptameric binding with associated successful milestones and respective limitations. A proposal for high throughput bioscreening using continuous polymeric adsorbents is also presented.

Keywords: Aptamers; Biosensing; Screening; Immobilisation; Oligosorbents

1.0 Introduction

Nucleic acids are thread-like polynucleotides contained in all living and non-living cells, and are essential to all known forms of life. Since the pioneering of nucleic acid research by Friedrisch Meister in 1868, there has been an ever-growing development and understanding of nucleic acids and their applications. One of such developments is the discovery of aptamers from synthetic sequence of nucleotide linkages [1, 2].

Aptamers are *in vitro* chemically synthesised oligonucleotides with high specificity and sensitivity towards a specific target. The length of aptamers is usually in the region of 35-100 nucleotides, with a unit size being equal to the size of its base [2]. The advent of aptamers began in the year 1990 when three different laboratories of G. F. Joyce, J.W. Szostack and L. Gold reported independently on a novel *in vitro* selection technique in successive months [3-5]. The technique allows for the selection of a specific sequence of nucleic acids from a large pool of different sequences of DNA and RNA against any target, and was termed as the systematic evolution of ligands by exponential enrichment, SELEX [5]. An infinite array of targets exist for specific binding to aptamers. Some of the aptamer binding targets reported in literature include numerous variant molecular apatopes (binding site of a target by an aptamer) of small organic molecules such as ethanolamine, protein molecules, whole cells, metal ions such as K^+, lipids and sugar moieties [1, 6, 7]. Also, aptamers can be biomolecularly engineered to improve their bioavailability and biostability [7, 8]. The biostability of aptamers has been reportedly achieved by engineering 2′-amino-modified, 2′-fluoro-modified pyrimidines, 2′-methyl pyrimidines, 4′-thio pyrimidines or 3′–3′ linked dinucleotide caps into the aptamer [8]. Notably, the chemical structure of an aptamer is made up of 4 bases of nucleotides whereas protein markers such as antibodies are made up of 20 bases of amino acids. Antibodies have a vast number of combinatorial parings which allows

for specific molecular recognition of targets [9, 10]. However, aptamers are highly specific due to their singular function of binding to their cognate molecules [9-11].

Over the past decade, there have been interesting developments in the application of immobilised aptamers as biomolecular devices for binding of target molecules. These new applications, unlike conventional technologies, such as protein nanopores [12, 13], solid phase extraction [14], and liquid chromatography [15], have several advantages. These advantages include higher sensitivity, longer life span, absence of matrix effect, higher throughput, and absence of sample pre-treatment. This is attributed to the molecular recognition ability of aptamers to selectively bind to their specified targets and differentiate between enantiomeric targets [16]. One of such new aptamer-based chromatographic application has been referenced to as oligosorbents [16].

Protein nanopores are useful for biosensing, single molecule recognition and detection of nucleic acids. Through structure-directed genetic engineering and chemical modifications, the characteristics of protein nanopore can be enhanced [12]. Protein nanopores have fixed pore sizes which are mostly invariable for different target sizes, and possess fragile lipid bilayers [12]. Several attempts to circumvent these setbacks have led to the fabrication of synthetic nanopores in micropipettes with immobilised antibodies and subsequently with aptamers as probes [13].

Several extensive reviews existing on the use of surface based aptasensors for target detection cover aptazymes, acoustic, optical, molecular beacon aptasensors, electrochemical, cantilever-based, signalling aptamers, proximity ligation and extension, and nanoparticle-conjugated aptasensors [17-20]. Also, Deng et al. (2014) reported a review on the use of thrombin targets as models for the development of aptameric assays, which included various affinity separation and screening assays [21]. In addition, the development of oligosorbents

and applications as superior assays over conventional immunosorbents has also been critically discussed recently [22]. All these reviews have only focussed on the application of aptameric binding as a form of biomarking for target detection. There has been no comprehensive review reports on the application of aptameric binding for high throughput bio-separation and screening of molecular/cellular species by convective mass transport using macroporous continuous polymers (called monoliths)**.** Macroporous continuous polymers can possess convective mass transfer ability to enable rapid target adsorption. Also, there are limited reports discussing the inherent limitations for oligosorbents in the literature. Current oligosorbent formats have only relied on the immobilisation of aptamers on particulate adsorbents. These adsorbents are disadvantaged with slow diffusive mass transport mechanism due to their small particle pores, thus hindering the achievement of high throughput screening.

Herein, this article reports on recent advances in the application of immobilised aptamers as polymeric aptasensors for biomolecular detection and screening including milestones covered and their associated limitations. A theoretical insight into modifiable features and constraints of both oligosorbents and aptamer-monoliths to enhance performance is presented. A proposal for enhanced bioscreening technology using monolithic supports with convective mass transport mechanism for high throughput applications is also presented.

2.0 An Overview of Aptamer-Target Interaction Mechanisms

Aptamers are readily generated from SELEX for a specific target. The main idea behind SELEX is to simulate the evolution of synthetic oligonucleotides as the natural process of evolution is slow and complex [1]. SELEX, which is an *in vitro* selection process, is very rapid and can be modified for different targets. The entire SELEX process is simplified to be an iterative cycle of partitioning and amplification [23]. A library of nucleic acids with

defined sequences is randomised and incubated against a specified target for binding and selection. Nucleic acid molecules that do not bind to the target are eluted by employing techniques such as magnetic separation, filtration and affinity chromatography. Selected nucleic acids are amplified and enriched several times by adjusting binding parameters such as pH and buffer composition until the best binding ligand dominates the population of sequences. The selected and most specific binding ligand amongst the pool of sequences usually has the smallest dissociation constant, K_d, often in the millimolar-picomolar range or even less [23-26].

Aptamers possess the inherent ability to form secondary structures which enables them to detect and bind onto specific targets [27]. Aptamers, with the aid of their secondary structural conformation, are able to distinguish between variant targets or enantiomers based on the conformational differences in the structures of target molecules [28-30]. Some of these structural differences include the presence or absence of a hydroxy group, a methyl group or the D- enantiometric configuration against the L- enantiometric configuration [28]. Rationally, aptamers can be modified to conformational switching with their ligand binding abilities preserved. In the absence of a target, the thermodynamically stable conformation of the aptamer is maintained [27]. In the presence of the target, the affinity between the duo induces binding to form an aptamer-target complex. After binding, an adaptive trapping of the target by the aptamer occurs. This results in the formation of stabilised tertiary structures from the secondary structures of the aptamer [23]. Such structural changes in the conformations of aptamers can be characterised through the use of circular dichroism, quartz crystal microbalance, interferometer and isothermal titration calorimetry [31]. Hianik *et al.,* (2007) studied the effect of immobilisation, ionic concentration, aptamer configuration, and pH on the interactions between thrombin and its binding aptamer with an electrochemical indicator and a quartz crystal microbalance [32]. It was observed that the linear aptamer had a

faster change in frequency and a higher steady state frequency value than their molecular beacon aptamers. This observation was linked to the bulky structure of the molecular beacons and their high molecular weights of 10576 Da and 6791Da [32].

Aptamer-target interactions are based on affinity binding. The binding between the aptamer and the target molecule existing within the tertiary structure (aptamer-target complex) is non-covalent. The concept of aptamer-target binding and the existence of non-covalent bonding is illustrated in Figure 1. The non-covalent interactions can be as a result of hydrogen bonding, hydrophobic interaction, aromatic stacking, electrostatic interactions, and van der Waals interactions [23, 30, 34]. Aptamers either fold around small molecules such as ethanolamine into their nucleic acid structures or are encapsulated into the structures of large molecules such as proteins [35]. Research advancements have led to the development of aptamers for multiple variant target binding. With such multi-targeted aptamers, the interactions between the aptamer and the targets can be any of the above-mentioned non-covalent bonding types, or a combination, depending on the interacting moieties [11, 36]. It has been reported that aptamers do not only bind to their cognate molecules, but also inhibit their biological functions by interfering with the catalytic site of the molecule, ligand-receptor recognition sites, or through the induction of allosteric effects [29, 37, 38]. In terms of binding performance and specificity, no distinguishing features have been reported for RNA and DNA aptamers to date. However, RNA aptamers are easily expressed within the same cell, whereas DNA molecules are much more stable [1]. RNA and DNA aptamers have different sequences and patterns of folding towards the same target molecule [39].

3.0 Aptamer Immobilisation

Aptamer based biosensors are referred to as aptasensors. The application of immobilised aptamers as bio-receptors in the development of biosensors has several benefits. Generally,

aptamers are immobilised on solid surfaces to enhance their structural stability, prolong the life span of the sensor, and for real-time recognition applications [40].

3.1 Development of Immobilised Aptamers for Biodetection and Screening

Centi *et al*. (2008) reported using the thrombin aptamer as a model to study the detection limit for binding immobilised and free aptamers towards a single protein target. Immobilised aptamers detected the target proteins in solution with a detection limit of 430nM [41]. Free aptamers in solution could not detect at this limit, and this was attributed to steric hindrance [41]. However, a further improvement in detection (175nM) was achieved with an immobilised aptamer in a sandwich assay format. Binding studies were carried out with a surface plasmon resonance technique [41].

Aptamer immobilisation is significant to explore different applications of aptameric binding with targets. Aptamers immobilise at high densities owing to their small molecular size, thus enhancing their ability to probe target molecules [14]. Various methods of immobilising aptamers on solid supports have been recently reported. These include physical adsorption, covalent bonding immobilisation, self-assembly, immobilisation by polymerization, coupling by affinity reactions immobilisation, and polynucleotides–nanoparticles hybrids [42]. The afore-mentioned immobilisation chemistries are chiefly achieved through modifications of the 3' and/or 5' ends of the aptamer with an appropriate functional group [43]. However, the binding characteristics of aptamers under immobilised conditions could vastly be affected by the physicochemical properties of the binding surface, resulting from a possible induced structural dislocation of active and/or binding sites of the aptameric molecule, and this may affect the K_d value. K_d is the fundamental parameter characterising the binding strength, hence K_d for an immobilised aptamer may be different from that of the same aptamer in free solution. Under immobilised conditions, K_d might increase because of restricted mobility

towards the formation of unique secondary structures for access to active group on the aptamer, or decrease as a result of enhanced molecular interaction between the aptamer and the target. However, research report investigating the possible biophysical differences between the binding features of immobilised and free aptamers are minimal.

Conspicuously, the key design criteria essential for the development of an effective immobilised aptameric sensor for high throughput screening applications include: (i) real-time rapid detection with high sensitivity and selectivity through the achievement of high ligand density immobilisation of aptamers on adsorbents with convective mass transport; (ii) engineering of the immobilisation chemistry through the introduction of spacers and/or surface modifying agents to maintain the binding performance of aptamer under immobilised conditions; (iii) capacity to withstand complex environment as with field samples such as water bodies, food matrices, agricultural soil and plants, and human or animal samples; (iv) capacity for multiple bioaffinity interactions and simultaneously monitor different molecular species; (v) a wide range of possibilities for developing signal transduction and amplification mechanisms; (vi) ability to regenerate to enable routine and repeated use. Techniques for target elution and regeneration are dependent on the type of stationary support and the immobilisation chemistry. This is important to maintain the physicochemical properties of the adsorbent and the maximum ligand density. Common regeneration methods include the use of chaotropic reagents, temperature effect or DNA enzyme digestion [14]. Peyrin *et al.*, (2009) discussed the selection of operating conditions for bioseparation of targets in a chromatographic context based on differences in binding association and dissociation constants [44]. They classified aptameric assays as type 1 and type 2 where the former represents aptamers with moderate to high affinity constant, and the latter for aptamers with low affinity constants. Key operating conditions affecting aptamer conformations for optimal

throughput in type 1 and 2 assays include ionic concentration, pH of the mobile phase, addition of organic modifiers and operating temperatures [22, 44].

The development of porous polymeric biosensensors with immobilised aptamers has proven to be more effective than antibody formats. By flanking aptamers with two primer sequences, they are able to report the detection of targets without being labelled, unlike antibodies [45]. Two main assay configurations for aptamers are in existence and are a function of the size of the target, namely, single site binding and double sites binding [19, 30]. The single site binding configuration is often employed for molecules small in size, whereas double sites binding is employed for large molecules [19].

4.0 Current Applications of Aptamers

The multifarious applications of aptamers is as a result of the ability to form stable three-dimensional structures, have a low dissociation constant (k_d), and undergo chemical modifications of its sugar backbone with amino/fluoro groups when necessary. Modifications of the sugar backbone extend the aptameric half-life and project other functionalities. Prior to chemical modifications, aptamers usually last for less than 10mins in biological fluids [28, 29, 34]. Table 1 shows some advantageous properties of aptamers for different process parameters.

4.1 Aptamers for Medical Applications

Sensors for clinical diagnosis are expected to be highly specific, easy to read, sensitive, accurate, have good performance under varying physiological conditions, stable and fast. These requirements are achievable with aptamers as probes for detection [1, 48]. For example, in generating aptamers for a target protein, it is not significant to know the molecular signature (number or type) of the protein pre-generation [25, 49]. This makes it easier to probe new pathogenic species with minimal knowledge of their biomolecular

framework. Several methods of generating aptamers against protein targets exist. Some of these methods are Primer-free SELEX, Toggle SELEX, *In vivo* SELEX, Cell SELEX and Genomic SELEX [1, 10, 50]. Pathogenic processes have a unique protein or enzymatic synthesis and activity that can be detected with the use of aptameric probes with high specificity. Fang *et al.* (2010) elaborated on the significance of aptamer generation by cell-SELEX for the molecular recognition of cancerous cells [49]. An aptamer-modified microfluidic device for cell enrichment has also been developed to separate viable and non-viable cells from a large cellular environment with little or no sample pre-treatment [48, 49]. Other promising approaches reported in literature for cell detection are flow cytometry analysis, aptamer-functionalized nanoparticles for biosensing, and histological examination.

Application of aptasensors is essential in the detection of food and water-borne diseases in complex matrices [51-53]. Several other successes have been notably achieved by the application of aptameric recognition for the detection of toxins in the lectin family [6], Lup-an-1 food allergen [54], prions [55], variant strains of *Escherichia coli* [56], and Trypanosomes [57]. Mann *et al.* (2005) reported the successful identification of an immobilised aptamer suitable for binding ethanolamine; one of the smallest compounds ever detected by an aptamer [7]. Ethanolamines are of clinical and environmental concerns due to their disease causing effects [7]. Minunni *et al.* (2004) demonstrated the reproducibility, specificity and reusability of aptasensors through the use of an RNA^{TAT} aptamer for the detection of HIV-Tat protein [58].

The success of therapeutic analysis is measured by the capacity to target specific infected cells amongst healthy cells [37, 59]. This synchronises with the aims of modern molecular therapy, which seeks to avoid conventional trial-and-error targeting with low specificity [49]. Aptameric binding of targets is a significant component of modern molecular therapies in the development of high sensitivity and rapid molecular or cellular targeting systems for disease

treatment. The following features make aptamers effective therapeutic markers: high specificity and efficiency, non-immunogenic, non-toxic, and non-recalcitrance after being tested at high dosages of 10 mg/kg daily for 90 days in rats; administration through intravenous or subcutaneous injection; and economical to develop [34]. Therapeutic aptamers function by either inhibiting target molecules or as receptor agonists [1]. Advances in modern therapeutic research have led to the invention and acceptance of Macugen, which is a vascular endothelial growth factor binding aptamer [40, 59] with the potential of providing new therapeutic pathways to prevent cardiovascular diseases. Other successful work carried out include: ARC1779 which has an antithrombotic activity and currently undergoing clinical trials[29]; AS1411, formerly known as AGRO100, which is a cancer aptamer and undergoing clinical trials [59], NOX-E36, an aptamer for diabetes [59], and significant findings made in preclinical studies against cancer targets by modulating apoptosis in organisms [59].

Suggestions are being made to improve healthcare delivery through the establishment of Point-of-Care Testing (POCT) approach [60]. The use of aptasensors for POCT will help save time, increase productivity, and avoid huge capital investments required for establishing several fixed structures for lab diagnosis. Alternatively, aptasensors can be routinely applied in door-to-door domestic testing, mass screening of infectious and contagious diseases, clinical diagnosis, and border chemo/bio-security programs. This is because immobilised aptamers can be engineered to develop miniaturised sensors with a high surface density as compared to immunoassay sensors [19, 20]. This will certainly be of much importance during pandemics in offering a rapid and easy-to-use detection system capable of breaking the mode of transmissions. Medical diagnostic and therapeutic applications of aptamers are receiving much attention from researchers, and this is pivotal to improve the detection of pathogenic entities rapidly and accurately from human samples [21, 25, 38]. Some of the interesting developments in the application of immobilised aptamers include immobilisation on

engineered glass nanopores, sepharose, magnetic beads, silica, and also as oligosorbents. Table 2 shows different applications of aptasensors.

5.0 Polymeric Aptasensors for Bioscreening of Targets

5.1 Immobilisation of aptamers on a single glass nanopore

Immobilisation of aptamers on a glass nanopore of a pipette for the detection of IgE and ricin, the third most toxic substance and a potential bio-threat, has been successfully performed and reported [13]. This follows the successful work done by Gao *et al.* (2009) in fabricating a synthetic nanopore at the tip of a micropipette [12]. Prior to that, protein nanopores with attached receptors were used for single molecule detection. However, this method lacked the capacity for real-time detection and possessed fragile lipid membranes. The aftermath of the development of synthetic glass nanopores led to modifications with antibodies. However, antibodies, unlike aptamers, are mostly limited to non-toxic targets, present difficulty during immobilization, and are relatively difficult to synthesise. Aptamer-embedded nanopores are very robust, specific and effective for molecular detection of pathogens with nano sizes [13]. Future applications of this immobilised aptameric system could be multiple target biosensing. Significant challenges of single glass nano-pores include prolong recognition time, low binding frequency and clogging by DNA targets [13]. These are attributed to the diffusion-collision rate and the binding activation energy [14].

5.2 Application of immobilised aptamers as oligosorbents

Another unique development of immobilised aptamers is the synthesis of oligosorbents. Oligosorbents are produced by immobilising aptamers on solid supports. They are able to detect and screen targets with high affinity and specificity from complex matrixes by employing the established biomolecular recognition mechanisms of aptamers [64]. Oligosorbents can be applied in purification processes for specific detection and screening of

target molecules from complex samples. Prior to the advent of aptamer-based oligosorbents, molecular screening and purification of target species relied on several unit steps such as ion-exchange or hydrophobic interaction liquid chromatography, and solid phase extraction with either hydrophobic supports or immunoaffinity columns. Table 3 compares the characteristics of various molecular screening and purification techniques with oligosorbents.

Efficient immobilisation of aptamers on supporting systems depends on the following factors: the type of immobilisation support, the functional groups of the support, pore framework of the matrix, the type of bonding between the supporting system and the aptamer, and the length of the spacer arm if required. By studying these characteristics of a known support system, an optimal immobilised aptameric sensor can be developed for detecting and screening of target molecules. Research reports indicate that high throughput recovery efficiencies within the range of 67% to 96% were achieved with oligosorbents for different analyses of food samples [16, 63-67]. Current aptamer applications as oligosorbents have mostly been devoted to the detection of cocaine samples and ochratoxin A in food, drinks and blood plasma. The challenge, however, now lies in the application of this approach or modified versions for enhanced bio/chemical detection and screening of other target molecules. Nonetheless, this can be achieved by generating and characterising specific aptamers for the chosen targets of interest.

Even though chemo-physical modifications have led to improvements in throughput, there are two inherent setbacks in the use of polymeric beads, such as silica, in their particulate form as supports for aptamers. In Figure 2, the pore size and transport mechanism of particulate oligosorbent system is shown. The configuration for solid phase extraction using oligosorbent packing usually results in small pore sizes. Due to the small pore size and the distribution of empty spaces between the oligosorbent particles, the mechanism of mass transfer of solutes is by diffusion through the interstitial voids. The experimental void

fraction (ε) between particles in the stationary phase of a packed column is estimated to be between 30-50% of the entire volume of the column for smooth diffusion to take place without channelling [68]. A classic expression relating the void fraction and effective diffusivity (D_{AB}) of solutes in the liquid analyte is shown by equation (i). It must be emphasized that the rate determining step of the solid phase extraction of targets with oligosorbents is the rate of diffusion. Solutes such as proteins and DNA with large hydrodynamic sizes, will take a longer time to diffuse through the matrix.

$$\varepsilon = \frac{void\ volume\ (V)}{total\ volume\ of\ the\ column(V_T)} \qquad (i)$$

Considering the configuration of an oligosorbent system and molecular diffusion as the means of mass transfer, Fick's law can be used to estimate the effective diffusivity as

$$D_{AB,eff} = \frac{\varepsilon D_{AB}}{\tau} \qquad (ii)$$

Diffusivity of a solute A in a liquid solvent B can be theoretically estimated from Stokes-Einstein equation. This expression is given as:

$$D_{AB} = \frac{kT}{6\pi r_A \mu_B} \qquad (iii)$$

Where τ is the tortuosity of the pore-path, r_A is the radius of the solute in the solvent, T is the operating temperature in K, k is the boltzmans constant and μ_B is the viscosity of the liquid solvent B. From the above equations, it becomes clear that the effective diffusion of particles is a function of the void fraction, the radius (size) of the solute, which in this sense is the target and in other terms the viscosity of the solvent as well. This can be expressed as

$$D_{AB} = f(\varepsilon, r_A, \mu_B) \qquad (iv)$$

The first parameter of the function, ε, is a design parameter which can be controlled and improved by considering other adsorbents such as engineered macroporous continuous polymers.

5.3 High throughput immobilised systems

Considering the drawbacks of oligosorbent systems as discussed earlier, a more effective approach will be the use of a continuous adsorbent system as the stationary support. Such supports have been demonstrated to be effective for high throughput results. Zhao *et al.*, (2008a) were the first group to successfully host aptamers on the monolithic polymer (glycidyl methacrylate-co-trimethylolpropanetrimethacrylate) [69]. They used 61mer G-quartet DNA aptamer-monolith system for the detection and separation of thrombin and cytochrome c in diluted serum samples. The density of immobilised aptamers on the column was ~164pmol/μL. The estimated K_d ~150μM after aptamer immobilisation was about 30 times more than the reported SELEX value of ~4.6μM [69]. The reduction in binding affinity could be because of some of the factors explained earlier for K_d.

In another experiment by Zhao *et al.*, (2008b), they used a similar polymeric monolith to host 15mer and 29mer DNA aptamers and obtained K_d values of ~100nM and ~0.5nM respectively [70]. The detection limit of thrombin were observed to decrease from 4nM to 0.1nM for both aptamers [70]. The improvement was attributed to the increase in pre-concentration duration of thrombin from 0.5min to 5min, and the increase in the immobilisation density of aptamer to 250pmol/μL [70]. Han *et al.*, (2012) demonstrated an online screening of lysozyme from a protein mixture by covalently immobilising an anti-Lys DNA aptamer on a poly (glycidyl methacrylate-co-ethylene dimethacrylate) monolithic rod [71]. They attained a low back pressure of 1.0MPa at a volumetric flow rate of 0.8mL/min, an

aptamer immobilisation density of 290pmol/μL compared to 204pmol/μL for microbeads, and a high precision, functionality, stability and reproducibility for 20 experimental runs using the monolithic rod [71]. Hybrid silica monolithic rod with immobilised amino-modified apt-29 was used to screen thrombin at a detection limit of 3.4nM [72]. They achieved an immobilisation density of 568 pmol/μL and a binding capacity of $1.95x10^{-24}$ mol/nm^2 compared to $1.4x10^{-25}$ mol/nm^{2}for open tubular capillaries [72]. Brothier *et al.*, (2014) demonstrated the design and application of a miniaturised hybrid silica monolith for the extraction of ochratoxin A and cocaine [73]. The monolith was characterised in terms of its stability, reproducibility, permeability and morphology. Back pressures of ~4.8bars and ~12.1bars at flow rates of 200nL/min and 500nL/min were observed for aqueous buffer and acetonitrile/water respectively. Also, the density of aptamer immobilisation was ~6.27nmol/μL and ~5.14nmol/μL for ochratoxin A and cocaine extraction [22]. Figure 3 shows an illustrative scheme for the immobilisation of an aptamer on a macroporous polymeric support and Table 4 reports a comparison between aptamer immobilised continuous polymer systems and particulate oligosorbent systems.

6.0 Challenges to aptameric binding and application

Challenges relating to aptameric binding and application can be classified as follows: (i) Technological gap between aptamer research and practical applications (ii) Limitations to aptamer generation by SELEX and, (iii) Limitation to molecular recognition of some specific targets. With all the aforementioned benefits of aptameric binding and applications, only the Macugen aptamer has been accepted for clinical application. So what is hindering the progress of aptameric binding applications through the clinical phases? First and foremost, Baird (2010) highlighted that since aptamers are aimed at replacing antibody-systems for molecular binding, it is natural for practitioners to resist the change [74]. There still exists a knowledge gap between researchers and practitioners concerning the benefit of aptamers over

antibodies in all application spheres [74]. Moreover, it generally takes about fifteen to twenty years for new scientific discoveries to be implemented and accepted as a whole. As a result, applications of aptamers for diagnosis, therapeutic, and environmental analysis will take some time.

Radom *et al*. (2013) also discussed three different challenges that the SELEX approach brings to bear despite its' simplicity and significance in aptamer selection [1]. One possibility is the hybridization between the primers and random regions. This leads to a destruction of the secondary and tertiary structures of the aptamer responsible for binding the target. As a result, significant attention is required in designing the primers in order to circumvent the problem of sequence overlapping during SELEX. The second challenge SELEX poses are the post-amplification steps in each round of aptamer selection [1]. This step involves separation of strands to ensure that only the appropriate strands are left for the next cycle. The techniques for the separation of nucleic acid aptamers have their own advantages and disadvantages. For RNA aptamers, separation is achieved by transcripting ssDNA templates into ssRNA and later digesting the DNA template. However, the separation process for DNA aptamers is achieved by eliminating the negative strands by employing either lambda-exonuclease digestion of the negative dephosphorylated strand, asymmetric PCR, denaturing polyacrylamide gel electrophoresis, or with streptavidin-coated magnetic beads. The third challenge identified by Radom *et al.* (2013) is the susceptible formation of incomplete complementary dimers [1]. This can lead to the formation of concatamers, which are aptamers of undesired sizes. This problem can be circumvented by carefully monitoring the number of cycles of amplification for selected nucleic acids.

Another peculiar problem facing aptameric binding is the selection of aptamers against hydrophobic targets such as steroids and alkaloids, as well as negatively charged molecules

[23, 24]. Efforts to resolve this challenge include the attachment of non-polar functional groups and the exploitation of non-stacked base pairs [23].

7.0 Conclusion

Aptamers have a wide range of applications and this has triggered significant research interests. Immobilising aptamers on surfaces for target detection has the potential to achieve high throughput screening with high accuracy, sensitivity and specificity at a relatively low cost. Immobilised aptamers have been used in solid phase extraction units/columns, in the development of biosensors and in the fabrication of glass nanopore sensors. Potential applications exist in the health sector for mass screening during disease outbreaks, and also for the food and beverage industry, pharmaceutical industry, defence and domestic use. Immobilisation of aptamers in glass nanopore and solid phase extraction media have some challenges relating to low pore sizes, high back pressure and the limited capacity for high throughput screening. This can be resolved by applying continuous adsorbent systems. Such adsorbents, unlike the oligosorbents, are macroporous and possess significant longitudinal permeability with aptamers functionalised in their poresurfaces. These can be engineered to have minimal void fractions and a convective mass transfer mechanism. Application of continuous adsorbents will further strengthen the development of immobilised aptameric binding for full-scale application in a chromatographic format. However, it must be noted that the binding characteristics of aptamers under immobilised conditions can be significantly affected by controlled spatial mobility at the point of attachment and its' performance. Also, research into various polymeric supports with the capacity to enhance convective fluid transport for high throughput screening is essential.

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Table

Table 1. List of demonstrated advantages of aptamers for biomedical applications.

Parameter	Advantage of aptamers	Reference(s)
Cost of manufacture	Less expensive and readily produced scalable methods compared to the production of monoclonal antibodies.	[11, 34, 46]
Product variation	Largely consistent performance. Batch-to-batch variations exist with antibodies.	[11, 47]
Duration	Short duration to biochemically synthesis aptamers. Takes weeks instead of the usual months for antibodies.	[34, 35]
Medium/suitable environments	Aptamers show a wider range of stability towards different residing media of the target.	[11, 37, 47]
Biopurity	The selection process of aptamers is not prone to viral or bacterial contamination.	[34]
Immunogenicity or toxicity	Aptamers are synthesised *in vitro* hence are mostly void of biotoxins.	[11, 29]
Target space	Aptamers virtually have an infinite array of targets.	[1, 29, 34]
Shelf life	Aptamers are stable and have a long shelf life. They can be regenerated even after denaturation.	[11, 14, 46]

Table 2. Identified immobilised aptameric supports and applications.

Type of aptamer	Type of support	Application	Reference
IgE aptamer; Ricin aptamer	Glass	To detect and screen IgE, Ricin toxin.	[13]
AptC.1	Silica	To immobilise Chymotrypsin in the construction of an enzymatic reactor for protein digestion.	[61]
Amino modified cocaine aptamer	Cyanogen Bromide - activated sepharose	Selective detection and extraction of cocaine	[16]
Amino modified cocaine aptamer	Streptavidin-activated agarose	Selective detection and extraction of cocaine	[16]
Ochratoxin A aptamer	Magnetic nanospheres	Detection and extraction of ochratoxin A from food samples	[62]

Ochratoxin A aptamer	Cyanogen Bromide - activated Sepharose	Detection and extraction of ochratoxin A	[35]
A10 RNA aptamer	Quantum Dot	Targeting of cells and sensing delivery of drug for cancer treatment	[63]
β-conglutin aptamer	Microtiter plate	Detecting of β-conglutin food allergen	[45]

Table 3. Characteristic features of various purification techniques in juxtaposition with oligosorbents.

Detection and Screening Method	Characteristic feature(s) for binding and screening			Reference(s)
Liquid chromatography	Pre-treatment of sample is required	Time-consuming	Problem of matrix effect	[16, 62, 65]
Conventional solid phase extraction	Large quantity of organic solvent required	Time-consuming	Not specific	[16, 62]
Immunoaffinity columns	Expensive to operate due to the use of antibodies	Exhibit variations from different batches of antibodies	Have a short life cycle and not suitable for high temperature and harsh solvents	[16, 62, 65]
Aptamer-based biomolecular recognition mechanism	Less expensive relative to the above methods	Specific and have a high affinity for target	Have a long life span	[16, 62, 65]

Table 4. Comparison between suggested aptameric based continuous polymer systems and oligosorbent systems.

Parameters	Oligosorbent systems	Aptameric based continuous polymer systems
Mechanism of transfer	Transfer of solute in assay is by diffusion	Transfer of solute in assay is by convective transport
Format of arrangement and number of particles	Several polymeric-like particles arranged in a packed bed format	Single medium
Duration	Relatively slow due to diffusive mass transfer	Rapid due to convective mass transfer
Rate of permeability	Low longitudinal permeability	Significant longitudinal permeability
Pore surface area	Small	Large
Presence of void fraction	30-50% void fraction present in column	Absence of void fraction
Back Pressure	High back pressure due to small pores size	Low back pressure
Throughput	Low throughput	High throughput

Figure

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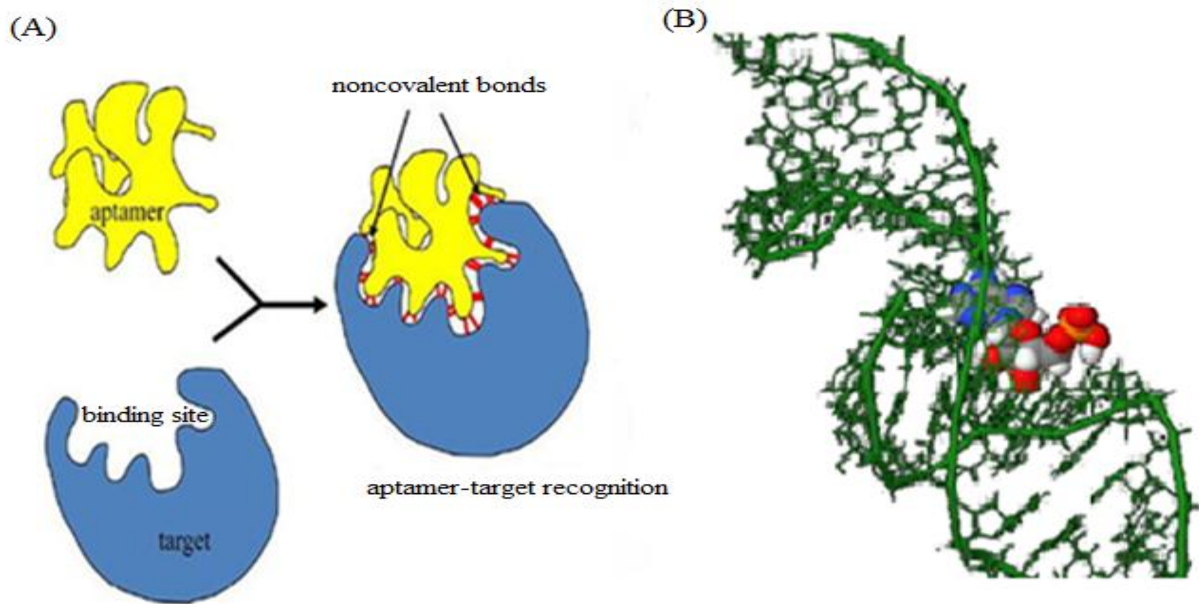
Figures



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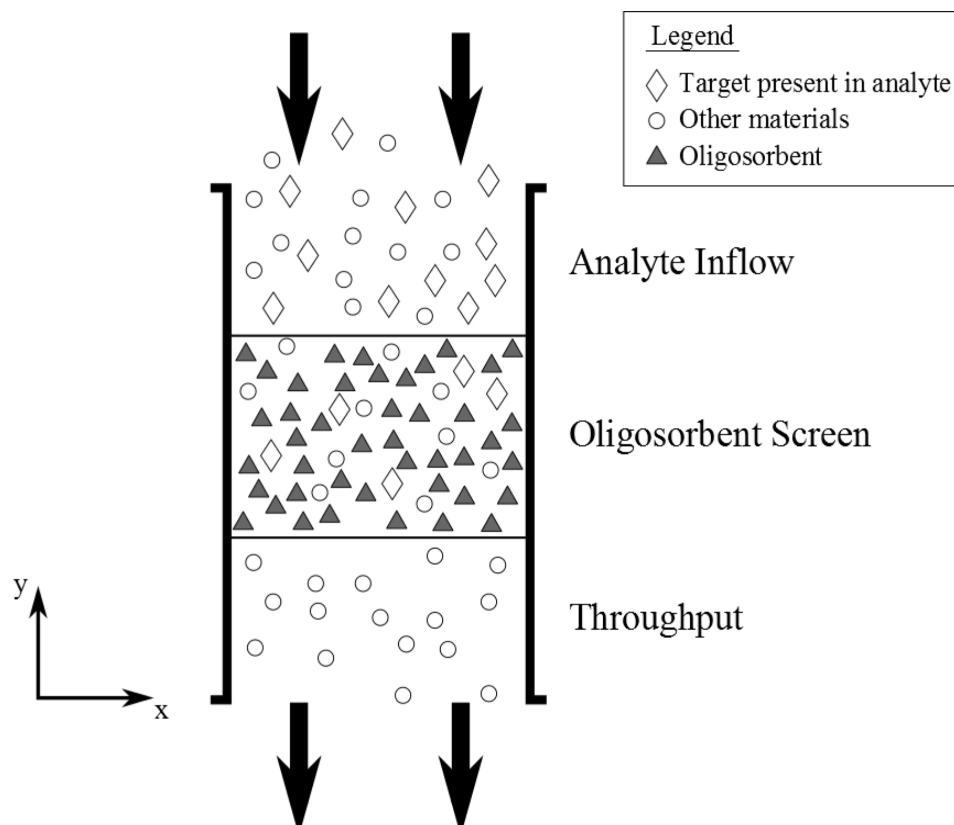

Figure 2. Process configuration of an oligosorbent chromatographic column for screening analytes.

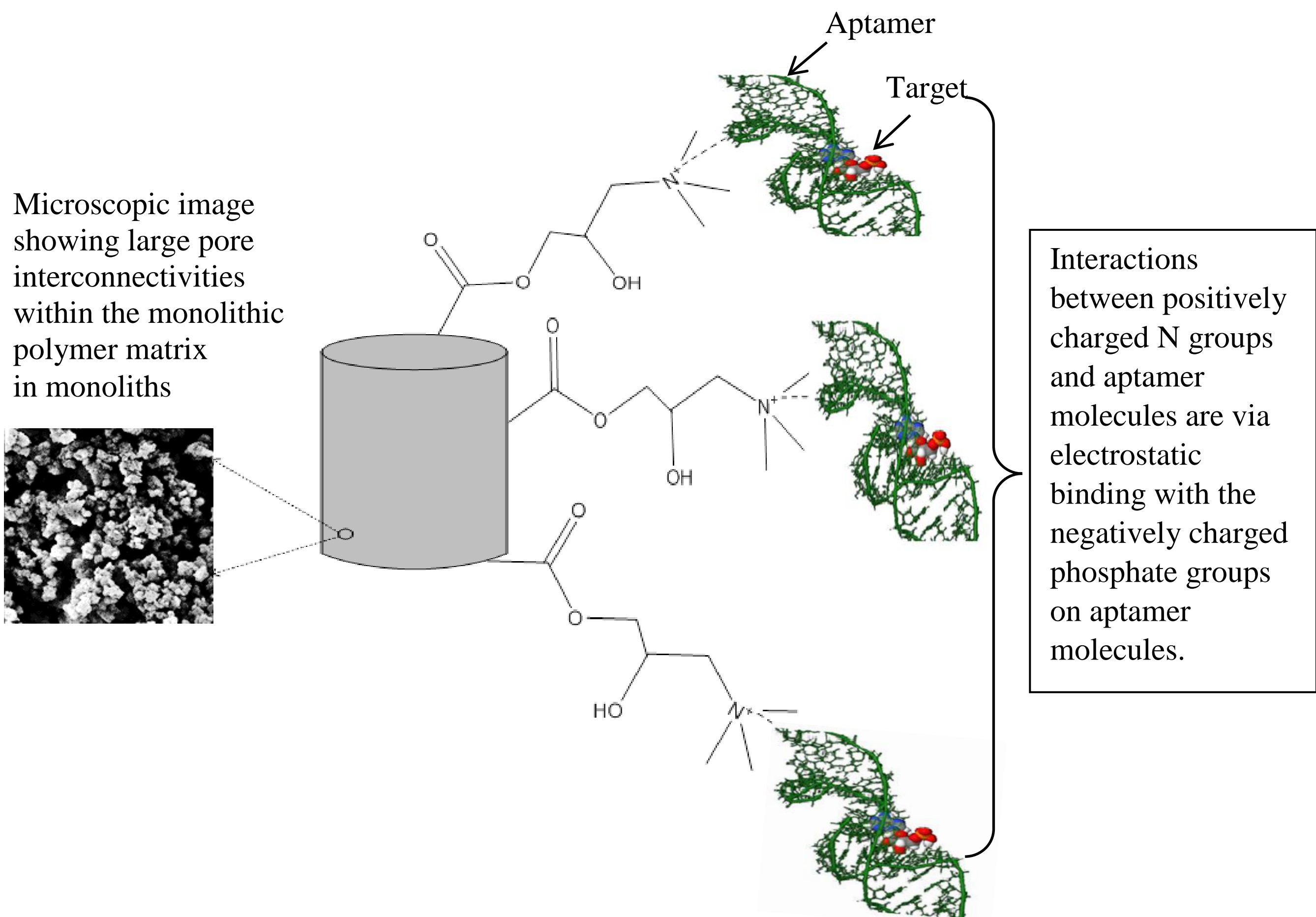

Figure 3. Aptameric immobilisation of a continuous polymeric support with non-uniform large-pore interconnectivities for molecular probing of a target molecule.

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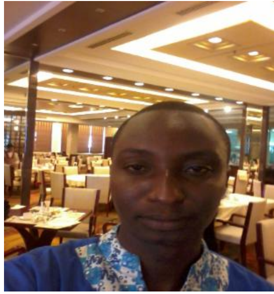

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