



## Solid phase microextraction based micro-device for extraction of PCR amplifiable DNA

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### Abstract

Deoxyribonucleic acid (DNA) extraction or sample preparation from biological sample (whole blood) for downstream process on microfluidic platform has been widely studied due to its crucial role in clinical diagnostic and genetic analysis. Sample preparation can be complicated since blood comprises complex matrices and repeated blood pricking from patients can be affected their health. Thus, introduction of solid-phase microextraction (SPME) method would be suitable to isolate, fractionate and concentrate the analyte from complex sample matrices and implemented on microfluidic device with small amount of sample. SPME is an effective sample preparation method by chemical lysis followed by purification mostly by using silica-based platform developed based on direct absorption into silica resins and desorption of analyte from silica resins.

**Keywords:** DNA extraction; SPME; Whole blood; Micro-device.

### 1. Introduction

Micro total analytical system ( $\mu$ TAS) is an integration of multiple chemical analytical systems in single micro-device [1] which is breakthrough in analytical methodology and instrumentation. This micro-device is highly in demand for sample preparation in molecular diagnostics, clinical analysis, polymerase chain reaction (PCR) analysis and so on. Fundamentally, traditional bioanalytical methods involving complex matrices especially biological fluids are time consuming and difficult to perform. For example, convention DNA array consisted of DNA purification from complex sample, PCR amplification and electrophoretic size separation of the DNA fragment [2, 3]. DNA purification is necessary and most important sample preparation step for effective PCR and other genetic or clinical analysis. Basically, efficient DNA extraction from  $\mu$ l of blood requires filtering out tens of  $\mu$ g of other organic compounds as well as contaminant from sample. Previous studies reviewed that bioanalytical sample preparation method particularly solid-phase extraction (SPE) are effective for DNA extraction and purification. The insoluble particulate material in SPE with high affinity for protein and low affinity for

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nucleic acids enable the effective extraction of DNA [4]. However, this technique needed spacious extraction phase and large amount of sample volume, buffer solution or solvent plus time consuming [5]. Even some modern analytical techniques such as liquid chromatography coupled with mass spectrometry is best known for rapid plus sensitivity in quantitative and qualitative analysis but it is limited by selectivity of analytes. The biological sample such as blood and urine contains varieties of organic compounds hence sensitivity for extraction or preparation of analyte of interest might be reduced. In contrast solid-phase microextraction (SPME) needed in only small volume extraction phase to isolate, fractionate and concentrate the analyte from complex sample matrices [6, 7, 8]. This method has been widely implemented in various applications including micro-device with minimum number of handling steps and reproducible methodology. The high potential of SPME for rapid, solvent-free and simple study has continuously fascinating researchers in biochemical and clinical analysis [5, 9].

## **2. Theory of Solid Phase Microextraction (SPME)**

Analyte of interest preparation or separation from sample is vital for molecular or clinical analysis. This is difficult by traditional method as the sample consists of complex matrices and it consumes high purity and toxic solvent which hazardous to environment. Hence, SPME was introduced in the early 1990s, is a new and effective sampling and sample preparation method by chemical lysis followed by purification mostly by using silica-based resins [4]. SPME can be eco-friendly and fulfill the requirement for green analytical chemistry during the entire sample preparation stage as the utilization of solvent or buffer is reduced; the sample preparation steps are simple and fast; and cost effective analysis by removing pumps or power supply [10]. The initial idea was based on fibers and coated capillary tubes to extract analyte [11]. Rapid sample preparation can be achieved by SPME technique either in laboratory or on-site experimentation without complicated apparatus [11]. While, the latest commercial used SPME fibers are coated with liquid polymer or porous solid sorbent through immobilization of fused silica fibers. Some of these coated fibers are compatible with in-vivo SPME sampling method with gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS) device [12]. Fundamentally, SPME method was developed based on adsorption or absorption of analyte directly from sample onto micropillars, porous silica or immobilized magnetic silica beads which can be used in device directly. Thus, there are several types of methods can be used to extract analyte of interest by SPME using micropillar structure, porous solid phase or immobilized magnetic beads.

### **2.1 Microextraction by micropillar structure**

Analyte of interest in a complex sample with varieties of composition can be extracted by using SPME method in micro-device. Sample preparation using SPME is typically performed in fiber coated surface. It is based on absorption of analyte into surface of the fiber and then removal of the absorbed analyte by several washing steps. The first microchip based SPME method using pillars to extract analyte on interest was demonstrated by [13]. The method started with filtration of particle based on size-exclusion principle using microfilter silica pillars and then extraction onto silica pillar surface. Then analyte will bind to designed microbinder surface in presence of buffer solution while other interferences and other molecules will be washed out through repeated washing steps. It follows by desorption or elution stage of analyte that bound to silica pillars surface using appropriate solvent (Fig.1). According to Christel *et al.* [13], the micro-fabricated silica pillar structures

are able to eliminate some centrifugation and mixing steps which requires for conventional sample preparation method.

## 2.2 Microextraction by porous solid phase

SPME method also can be performed using porous silica solid phase or surface as microchannel of device. This porous silica surface in micro-device can be successfully fabricated by electrochemical etching technology. The selection of porous silicon for SPME is based on its huge specific surface area that will increase the interface area significantly; the availability or obtainability of porous silicon by electrochemical etching technology; porous silicon in SPME can be controlled well due to its pore geometry, surface morphology and the absorbency by electrochemical etching conditions [14, 15]. In past few years, numerous studies have been conducted to discover the application of porous silicon in scientific fields. Nano porous structures thin films was also developed by photo-electrochemical etching technique and can be used for detection of DNA as well by using photoluminescence method [16]. Besides, chemical disruption would be suitable in microfluidic platform for porous silicon phase. The technique started with cell lysis by solvent and the targeted analyte will bind to porous silicon surface while the other impurities can be removed by several washing steps. It follows by elution of analyte that bound onto porous silica surface and collected at outlet microchamber (Fig.2).

## 2.3 Microextraction by immobilized magnetic beads

Another method of SPME is by using immobilized silica particles or beads in micro-device. It involves the typical steps of SPME method as well which comprising binding of analyte to silica surface followed by several washing steps to desorb the analyte. This method was enhanced by Wolfe et al. [17] and Breadmore et al. [18] by modifying immobilized bare-silica magnetic beads matrix in micro-device using sol-gel technology. Magnetic beads have been using in diversities of application due to their features especially in molecular biology. Magnetic beads that coated by silica resin can be used to capture particles in liquid by attaching and detaching simply by super-paramagnetic properties (controlling another magnet from the outer wall). The manipulation of magnetic beads is based on magtration technology (developed by Precision system science). Magtration was created from magnetic filtration process where particle can be separated and bound then released by magnetic field. Silica coated beads (magnetic silica microsphere) usually have large surface area thus the targeted analyte will bind onto surface and the particle will hold by assistance of magnet on the outer wall. The magnetic particles can be effectively separated and captured on the inner wall by magnet that comes into contact with outer wall of device (Fig.3). These magnetic silica beads have been used to extract DNA/RNA cell screening and others by some chemical modification [19, 20].

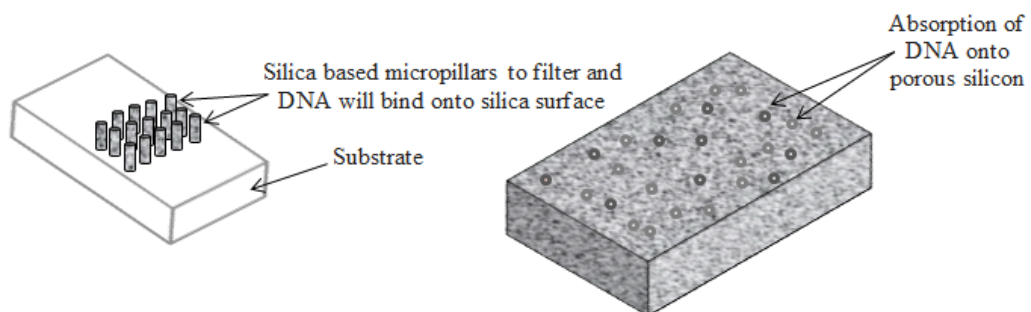


Fig. 1: Micropillars structure

Fig. 2: Porous silicon layer

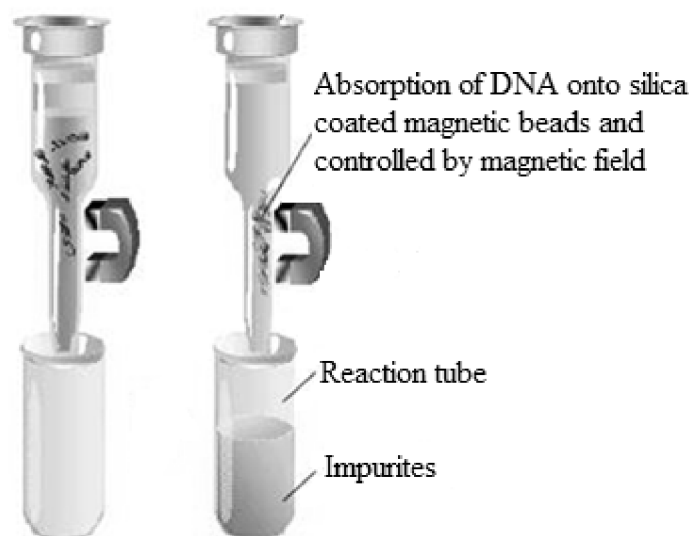


Fig. 3: Silica coated magnetic beads (modified from [www.gibthai.com](http://www.gibthai.com))

The simplicity (solvent-free) and easy accessibility of this method are the main reasons for the enhancement of the SPME application in device (typically in syringe alike). Since SPME needed small space, coated fiber can be used to extract analytes in analytical chemistry application involving limited sample amount. Only a small dimension extracting phase is needed in contact with sample matrix and the extraction consider completed once analyte concentration has reached distribution equilibrium condition among sample matrix as well as fused silica fiber coating. The entire process begins with adsorption then diffusion of analyte and this can be described as Eq. (1) [11, 21]:

$$n = \frac{K_{fs}V_fV_sC_0}{K_{fs}V_f+V_s} \quad (1)$$

Where  $n$  is the extracted analyte,  $K_{fs}$  is fiber coating sample matrix distribution constant,  $V_f$  is the fiber coating volume,  $V_s$  is the sample volume and  $C_0$  is initial analyte concentration in sample. Besides that, the coated fiber enables extraction of analyte from small amount of sample such as single cell [22]. However, one of the limitations of SPME is poor sensitivity in extraction capacity. This can be solved by optimization of thicker and selective fiber coating [23]. Rapid and complete extraction is also possible for thick coating with small sample volume thus prevention of errors regarding analyte losses through decomposition or adsorption is promising. This can be applied to develop portable devices based on SPME technique. Conversely, in some cases, coating need to be modified especially when there is need for longer extraction, thus possibilities to form analyte with lower affinities are also high. Hence, modification of coating property is necessary with extraction time is less than the equilibrium time [24]. Also, analyte extraction is easier with high concentration of sample (as proven from equation 1). Additionally, there are two types of mode in SPME which includes the dynamic when the analyte pass through the tube and the static when the analyte being transferred to sorbent by diffusion process. In dynamic mode, the introduced sample transfers with linear velocity through the fused-silica capillary coated with thin film of extracting phase. The extraction is proportional to capillary length and inversely proportional to fluid flow rate [11, 25]. Whereas extraction of static mode occurs when the sample does not directly exposed to extracting phase (e.g contained

protective tubing). Thus analyte transferred to the extracting phase based on diffusion principle of Fick's first law as Eq. (2) [26, 27]:

$$dn = AD_g \frac{dc}{dz} dt = AD_g \frac{\Delta C(t)}{z} dt \quad (2)$$

Where  $D_g$  is diffusion coefficients,  $A$  is extracting phase,  $Z$  is the position of extracting phase  $C(t)$  is time dependent analyte concentration in the sample.

Based on equation 2, it can be concluded that, rate and amount of extracted analyte is proportional to sample concentration over the diffusion coefficient of analyte. A part from extracting modes, there are few other factors or parameters might be affected the extraction of distribution constant ( $K_{fs}$ ) such as temperature, pH value, salting and quantity of solvent. For instance, when temperature increases, the extraction rate increases too but  $K_{fs}$  decreases concurrently. Also, SPME only can extract neutral non-ionic from water thus pH adjustment is crucial [11]. Based on considerable amount of literature reviews, the equilibrium method of SPME seems practical way to prepare specific analyte of interest ( $K_{fs}$ ) in DNA extraction. It also provides efficient and sensitive sample clean-up (in-vitro bioanalysis) which can be applied to complex matrices such as whole blood, tissue, etc. without pretreatment of sample [8]. This analytical technique, however, also has some limitations when involving different analytes and sample matrices. Each sample matrices requires different SPME method to prepare or extract targeted analytes under specific consistent conditions. In addition, SPME method needs to be optimized with thicker and selective coating to overcome sensitivity issues caused by limited sample volume. Another limitation of SPME is matrix effect in complex sample put emphasis on usage of matrix matched standards, isotropic labeled internal standard and standard addition method for analysis or calibration [28].

### 3. Development of SPME: in-tube device to microfluidic device

SPME for sample preparation is mostly conducted using fiber coated and capillary tubes coated with an applicable stationary phase. In-tube SPME devices were developed in order to make it automated instrumentation performed using fused-silica capillary with stationary phase coating as miniaturization and automation system. The development of this technique by absorption then desorption of analytes initially designed in microsyringe (in-needle SMPE) and pipette tip (in-tip SPME). These were few examples which cost effective, pollution free and automated SPME in-tube devices [29, 30]. The fiber coated needle or fiber-packed needle successfully used for microextraction in a packed syringe. This microsyringe technique for sample preparation has been applied in analysis of anesthetics in human plasma [31]. A part from that, micropipette technique has been used in isolation and purification of proteins and peptides in genomic studies. In needle SPME method provides faster and easier plus uses disposable material which makes the entire development comparable to automated sample preparation machines. This was modified to fiber packed capillary for SPME-HPLC for continuous sampling process. However this method requires pure samples due to easy blockage of capillary plus extraction is low due to higher capacity of capillary. The in-tube method has been applied in multiplicities of field such as food, pharmaceutical, biomedical analysis, forensic, and clinical analysis for sample preparation plus extraction of analytes [8, 32]. Continuously, in-vivo SPME device discovered easy way especially to solve biomedical analysis process [8, 24]. As presented by Lord et al. [33] and Zhang et al. [34] in-vivo sampling for biogenic volatile organic compounds (human skin and breath) were successfully extracted to diagnose several diseases. In detail, direct SPME

approach was done in sealed glass globe for skin emissions whereas collected human breath in Tedlar bag undergone direct exposure of SPME fiber into the bag. Different types of substances such as ketones, amines, aldehydes and others are synthesized by metabolism and release from human breath and skin. These organic compounds can be used to identify diseases, monitoring drug concentration and screening for toxicological exposure [35]. In-vivo SPME used to analyze concentration of aldehydes from collected breath exhalation (from patients who smoke and healthy control) and provides information regarding lung cancer and tumor specific composition [36, 37]. Direct SPME approach to extract organic compound from skin were used to study the features about skin cancer, wound healing and infection. Besides, in a recent study SPME sampling method were used to detect biomarkers named dimethylsulfide and dimethyltrisulfide for breast cancer in breath exhalation and skin emission correspondingly [38, 39]. A part from that, amount of protein binding of drugs in blood sample also can be extracted by SPME [8, 29]. Followed by this, the automated fiber SPME method was implemented for commercial usage. For instance, Combi-PAL auto sample (CTC analytics, Zwingen, Switzerland and other suppliers) was designed to perform various sample preparation stages such as dilution, agitation and extraction. Following by this, TriPlus (Thermo Fisher Scientific, Milan, Italy) auto sample was introduced with temperature control and stirring process [8]. Another advanced finding was the concept of multi-fiber SPME configuration with 96 robotic systems for automated high-throughput analysis (Professional Analytical System Technology, Magdala, Germany). Based on in-tip SPME micropipette method, automated sample preparation analysis of 96-tip arrays was developed researchers. Few extraction tips commercially available in market are including ZipTip (Milipore, Bedford, MA), Omix (Varian, Palo Alto, CA), NuTip and MonoTip C18 (GLScience, Tokyo, Japan). The tip also can be coupled with LC-MS/MS especially for drug analysis. This device can be connected to HPLC for further analysis and utilized mostly in drug protein binding studies [39, 40]. Based on this idea, 96-well plate technology was developed which able to hold 96 SPME fibers for multiple extraction with high precision [41]. The high efficient multi-well plate system then well modified with orbital shakers, arm robotic system and LC-MS/MS analysis for simultaneous high output usage. This has been validated by the accurate and sensitive analysis of benzodiazepines in human blood with preparation of 96 samples simultaneously within 100 minutes [42]. Accordingly, the developing technology caused progression of micro-device due to the ability to integrate multiple analyses in single  $\mu$ TAS and reduce time, cost and consumptions of reagents. Integrated microfluidic system for total analysis promises easy automation plus high throughput analysis for purification of biological molecules including DNA, protein to define genetic transformation and others. The silica based used for microfluidic device can be fabricated by electrochemical etching technology since it is compatible with latest microelectronic technology. The process in micro-device started with DNA absorption into silica surface in the presence of chaotropic solution and then removal of PCR inhibitors with appropriate solvent. Essentially, DNA carries negative charge per base pair over a wide range of pH condition. Likewise, silica surface able to exist as negatively charged at basic or neutral condition. The intermolecular forces of DNA in silica surface enable electrostatic repulsion of DNA and dissolved out from silica adsorption. Several previous studies emphasized that SPME micro-device system for nucleic acid purification, PCR amplification, and electrophoretic separation has been developed as automated and high sensitive device [35]. The latest commercial used SPME fibers are coated with liquid polymer or porous solid sorbent through immobilization of fused silica fibers in micro-device. For example polar polydimethylsiloxane (PDMS) which has high affinity extraction of non-polar compounds and blended coating with porous solid particle such as divinylbenzene (DVB) and template resin (TPR) which has larger surface area. Some of

these coated fibers are compatible with in-vivo SPME sampling method with gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS) device [30]. Some papers stated PDMS fibers can be used to extract licit drugs (cocaine) opioids (morphine and acetyl morphine) and cannabinoids from plasma, hair also urine. Subsequently analyze them in GC-MS and this method was widely used in forensic, clinical toxicology particularly for detection of drug abuse [43, 44, 45]. The entire development of SPME from in-tube to micro-device is summarized as visual in Fig. 4.

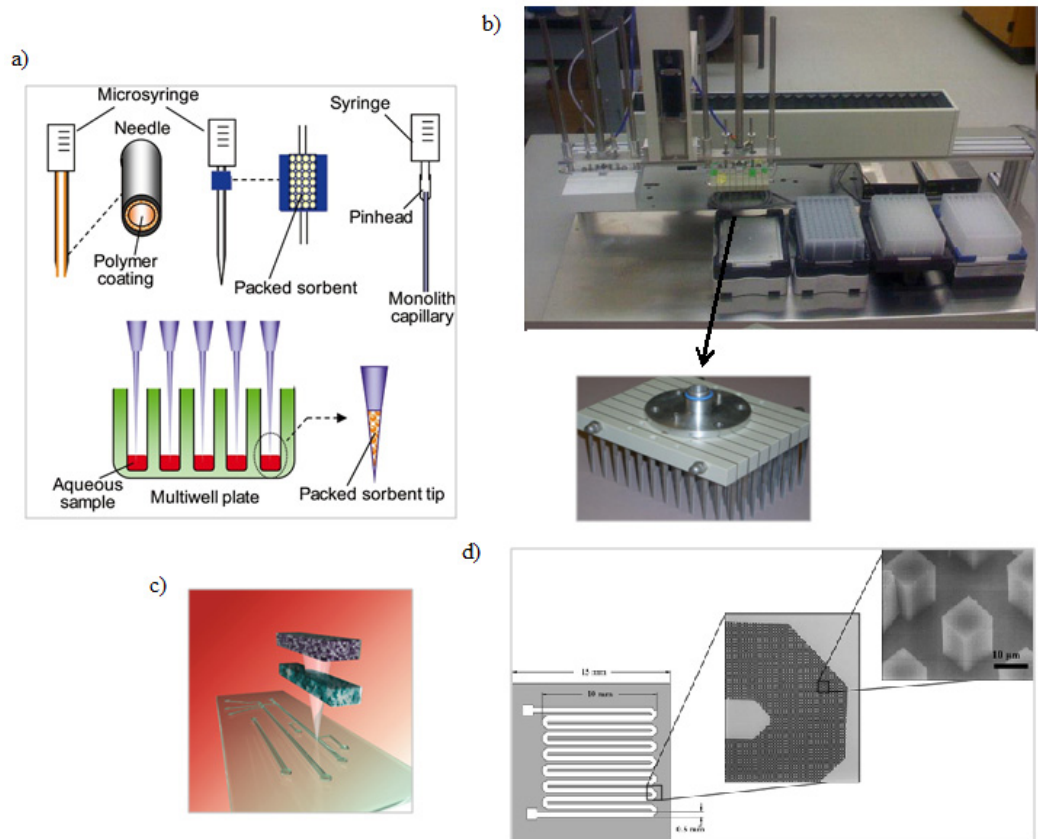


Fig. 4: Development of SPME method from in-tube to microfluidic device to extract biological compounds; a) microsyringe and micropipette in silica packed sorbent; b) auto-sampler station based on in-tip method for 96 simultaneous extractions from PAS technology, Maglada Germany; c) microfabricated device with porous silicon layer to extract and bind DNA; d) microfabricated silica pillars to filter cells based on size-exclusion and the remaining biological compound will trap into silica micropillar structure [Adapted from 12, 35, 46, 47].

#### 4. Solid Phase Microextraction for DNA extraction in Micro-device

There is a consensus among researchers that DNA-PCR microchips have wide applications in clinical diagnostics and genetic analysis. However, only limited amount of paper has been published regarding this parallel DNA-PCR microchip. In other word, integration of sample (analyte of interest) preparation step from biological compound follows by PCR and detection on single micro-device is very limited [48, 49, 50, 51]. Human blood is a superb source of DNA. Fundamentally DNA is present in white blood cell which has nuclei and approximately 51  $\mu\text{l}$  is sufficient to extract enough DNA for PCR analysis. Thus far, a number of studies highlighted factors that are associated with features of the integrated device such as sample preparing and handling method in microfluidic, selection material plus design and fabrication of device, temperature control system for



thermal cycling and also detection module for real time analysis [51, 52]. Generally, DNA preparation or extraction from blood sample in micro-device can be effectively done in the silica based solid phase microextraction (SPME) either in the form of micropillars, porous silicon surface or immobilized silica beads [9, 46, 51]. Over the past decade, most research in chemical and molecular analysis has emphasized the use of silica surface for successful DNA extraction. Silica solid surface could be implemented in microchannel either as micro-filters, porous silicon surface or silica coated magnetic particles. The basic steps in SPME for DNA begins with injection of sample into silica based microchannel in presence of high concentration of chaotropic salts plus organic solvents and follows by injection of buffer solution for cell lysis process. Then, double-stranded linear DNA will bind or absorb to silica surface and other molecules will wash out to the outlet by several washing steps. Finally the elution buffer with high pH and low ionic strength will remove the absorbed DNA and collect at the outlet. The DNA absorption and desorption from silica surface is generally based on few driving forces such as electrostatic screening effect, dehydration effect and the presence of intermolecular hydrogen bonds [53]. Under most experimental conditions, silica surface and DNA are negatively charged particles. Silica surface can be hydrolyzed and added with silanol groups by acid treatment and silica's negative surface charge density can be decreased by lowering the solution pH. As a consequence, electrostatic repulsion between DNA and silica can be reduced. Another driving force for DNA absorption is hydrogen bond between silica surfaces and DNA molecules. When DNA absorbed to silica surface, it increases in entropy indirectly then water molecules will solvate and release the DNA. Basically, the amount of DNA bind onto silica surface depends on solution pH, ionic strength, electrolyte type and this can be studied using quartz crystal microbalance with dissipation monitoring (QCM-D). For thin, rigid, and uniform films, the adsorbed mass can be calculated using the Sauerbrey equation Eq. (3):

$$\Delta m = \frac{-C\Delta F_n}{n} \quad (3)$$

Where  $\Delta m$  the change in mass is,  $\Delta F_n$  is the change in the frequency of oscillation (Hz) at an overtone  $n$ , and  $C$  is a constant. QCM-D experiments suggest that increasing the ionic strength of monovalent electrolytes results in DNA adsorbing to silica in a more rigid and compact conformation [54, 55, 56].

Integration of multiple analysis steps is the major advantages of development of SPME DNA extraction micro-device. In molecular diagnostic, DNA isolation micro-device was initially studied by Tian *et al.* [57, 58] based on electrophoretic separation and then chip-based PCR. Consequently, considerable amount of research has been published on SPE for DNA purification. Fundamentally, DNA purification involves DNA adsorption onto a solid surface through hydrogen bond or electrostatic interactions. This technique was demonstrated first by Tian *et al.* [57, 58] by capillary packed silica-based resin SPME and then developed the real DNA purification from whole blood cell for PCR ready analysis. The demand for this method was increased as the achievable efficiency of extraction was about 70 % with 80% of intracellular protein removal. Besides, this method only used small amount of solvents as well as sample and reduces time consuming of entire analysis. However this method required pretreatment of sample before the purification step. In the following study, Christel *et al.* [13] reported first suitable approach for micro-chip based DNA purification using pillars to increase the silica surface area for adsorption. The prospective of this method was limited by the complex fabrication, cost required for each microchip and sample clogging problem could not be completely solved. Several studies have attempted to integrate rapid DNA extraction and PCR amplification in single micro-device [59, 60]. Nevertheless, this study required pure DNA with free of contamination.



Meanwhile, in another study, Wolfe et al. [17] stated silica-based solid phase extraction system would be suitable for integrate with  $\mu$ TAS. Wolfe et al. [17] and Breadmore et al. [18] modified immobilized bare-silica beads matrix in microchannel by sol-gel technology for DNA purification in less than 30 minutes. One of the limitations with this micro-device is back pressure and clogging of crude sample due to larger surface area of packing density. Although the method was a success, but it required complex design of packing matrix plus hard to control in microchip. Schilling et al. [61] reported continuous lysis of bacteria cells were possible using  $\beta$ -galactosidase in simple T-type microfluidic device. Likewise Sethu et al., [62] demonstrated continuous flow microfluidic device based on SPME for erythrocyte lysis. Accordingly, solid phase matrix made of porous silicon has developed based on advanced technology to extract DNA due to better performance and the fabrication steps were compatible to the modern microelectronic and MEMS technology for micro-device [63, 64]. There were numerous studies concerning application of porous silicon in scientific fields. Usage of porous silicon recognized in solar cells, biochemical, sensor technology, enzyme micro reactor to absorb enzyme and protein, antibody micro-assay and just to list a few [65, 66, 67, 68, 69, 70]. Previous studies revealed the usage of porous silicon in SPME device for DNA purification has great advantages compared to conventional technique. Typically DNA extraction can be done by either cells disrupting and cell lysis step by enzymes, chemical lytic agents and mechanical forces in SPE technique. However chemical disruption is more suitable than cell lysis method in microfluidic platform. Furthermore, the fabrication process for chemical disruption in SPME device is simpler and compatible. In 2006, Chen et al. [63] designed SPE microfluidic chip with silicon-PDMS glass structure for DNA extraction. Porous oxidized silicon was used as solid phase matrix (coiled channel) for DNA purification while PDMS glass cover (withstand of continuous fluxion of buffers) was fabricated on substrate by optimal pressing method. The chip was extracted 23.5ng of PCR amplifiable DNA from 1 $\mu$ l of hole blood sample within 40-50 min. typically, the process of SPE microfluidic based on adsorption of DNA onto solid surface, then washing and elution steps. Theoretically, the solid phase matrix surface reduces electrostatic repulsion among negative DNA, and then both the matrix surface and DNA was dehydrated by high concentrated binding salt by forming hydrogen bond. Then breaking of hydrogen bonds happens in high pH (6.4~6.7) and low (4~6 M) concentrated binding salt condition. DNA desorbs from matrix when repulsion force among surface and DNA increases. The pure DNA can be achieved by continuous fluxion of the binding, washing and elution buffers [64]. As silicon based microfluidic chip to extract DNA from white bold cell needed complex structure with multiple layers of silicon, Ji et al. [71] demonstrated micro-machined silicon chip for DNA purification from bold sample. The micro-device which consisted of micromixer, microfilter, microbinder and microvalve in single layer, functioned based on high chaotropic salt agent to isolate DNA from the sample [72]. The method started with mixing of solvent to sample then filtration of white blood cell (WBC) by pillar structure filter. As the size of WBC larger than red blood cell (RBC) and RBC was able to align by its own to pass through narrow spacing, WBC was trapped based on size-exclusion principle using microfilter pillars [73]. Besides, microvalve with solid plug paraffin for one time used has been designed to isolate waste from extracted DNA. The solid plug of paraffin will activate thermally (change from solid to liquid) and block off the normal flow to redirect the flow of waste or sample to another channel. Finally the extracted DNA will bound to designed microbinder surface in high salt concentration solution [71, 74]. In another study, silicon or glass microfluidic chip integrated with mixer, paraffin valves, filter and binder was fabricated and designed with automation system for DNA extraction from white blood cell (WBC) by Hui et al. [76]. This method started with separation of WBC using filter of 3 $\mu$ m gap and lyse using high salt solution to release DNA. Then DNA was

captured reversibly by binder and purified with solvent. Based on this experiment, average 1ng of DNA was extracted from 1 $\mu$ l of sample. In order to justify the obtained results, PCR amplification was performed through gel electrophoresis and proven the method was a success as positive PCR results were shown [76]. After few attempt, Zhang and his group successfully overcome the sample clogging problem for extraction of DNA using magnetic beads by modifying the design of microfluidic platform with control of microvalves plus micropump. They introduced DNA extraction by superparamagnetic particles based SPE to perform cell lysis, DNA binding, washing and PCR on single microfluidic platform [51, 77]. Moreover, the PCR analysis in executed in droplet oil manner where the oil injected will encapsulate PCR reagent and prevent from evaporating plus contamination. Thus there is no need for chamber sealing for the PCR analysis. This research group has effectively demonstrated the capability of the main application of this microfluidic platform to analyze Rsf-1 gene. More recently, MEMS based micro-device based online cell lysis by continuous chemical disruption and genomic DNA purification by SPME was presented by Chen et al [5]. Porous silicon matrix was fabricated by electrochemical etching technology and average 39.7ng of DNA was extracted from 1 $\mu$ l whole blood (small sample volume) within less than 20 minutes [5]. Besides, Cao et al. [78] has developed microfluidic chip integrates with SPE for sample preparation which is nuclei acid extraction followed by reverse transcription polymerase chain reaction (RT-PCR) for molecular amplification. The aim of this chip was mainly to detect influenza virus in human nasopharyngeal aspirate (NPA) and nasopharyngeal swab (NPS) specimens. The entire process from sample extraction and amplification of influenza specimen was done within 3 hours or less and then product was sent for detection (off chip) through capillary electrophoresis. SPE based filtration using monolithic aluminum oxide membrane (AOM) for DNA extraction from saliva and real time polymerase chain reaction for amplification was developed by Oblath et al. [79]. They have reported effective identification *Streptococcus mutans* in a saliva sample with amplification of 300 fg (100–125 copies) of both methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) genomic DNA (gDNA) in less than 2.5 hours. On the other hand, instead of using chaotropic-based sample loading and washing, Nakagawa et al. [80] proved another method where DNA can be purified by ion exchange capture phase using amine groups in microchannel. Sample of blood could be loaded at pH 6 and eluted at pH 7.5 for microchannel coated by amine groups (e.g. 3-aminopropyltriethoxysilane) thus usage of chaotropic salts and organic solution can be avoided. Although the analysis steps were towards greener technology, yet it only extracted 27-40% of efficient DNA [81]. Following by this, a novel method where DNA captured by pH-induced and bind by chitosan coated was proposed by Cao et al. [75] Chitosan is categorized in amino group, linear polysaccharide produced from deacetylation of chitin (crab and lobster shells). Chitosan coated channels responsible to bind DNA at pH 5 and release them at pH 9. Besides, protein binding can be reduced by the hydrophilic surface of chitosan as well [81]. This method has reported high efficiency of DNA extraction (~75%) with low protein binding to microchannel using small elution volume of 1.5 $\mu$ l. the summary of DNA extraction based on SPME was listed in Table 1.

Table 1: Summary of DNA extraction method based on SPME

<b>Phase</b>	<b>Sample</b>	<b>Remark</b>	<b>Ref.</b>
SPME chip based on micro-pillars	Blood cells	Complex fabrication, costly, sample clogging problem	[13]
Capillary packed silica-based resin	Blood cells	Achieved 70 %efficiency of extraction but required pretreatment of sample before the purification step	[57, 58]
Immobilized bare-silica beads matrix in microchannel by sol-gel technology	Blood cells	DNA purification in less than 30 minutes but back pressure and clogging of crude sample problem	[17, 18]
Lysis of bacteria cells using $\beta$ -galactosidase in simple T-type microfluidic device.	Bacteria cells		[61]
Purification by ion exchange capture phase using amine groups coated microchannel.	Blood cells	Avoiding chaotropic salts and organic solution, towards greener technology, yet it only extracted 27-40% of efficient DNA	[80]
Microfluidic chip with silicon-PDMS glass	1 $\mu$ l of whole blood cells	Extracted 23.5ng of DNA within 40-50 min.	[64]
Micro-machined silicon chip with micromixer, microfilter, microbinder and microvalve in single layer	Blood cells		[71]
Silicon microfluidic chip with mixer, paraffin valves, filter and binder	1 $\mu$ l white blood cell	1ng of DNA was extracted	[76]
DNA captured by pH-induced and bind by chitosan coated microchannel	Blood cells	Achieved ~75% efficiency of DNA extraction using small elution volume of 1.5 $\mu$ l.	[78]
20-30 nm Porous microfluidic channels in silicon via anodization in an HF electrolyte.	Blood cells	Achieved ~80% efficiency of DNA extraction	[63]

DNA extraction by super paramagnetic particles based SPE and PCR on single microfluidic platform. PCR analysis is executed in droplet oil manner where the oil injected will encapsulate PCR reagent and prevent from evaporating plus contamination.	Blood cells	Successfully overcome the sample clogging problem for extraction of DNA using magnetic beads and analyze Rsf-1 gene	[19]
SPE for sample preparation (nuclei acid extraction) followed by reverse transcription polymerase chain reaction (RT-PCR). The aim of this chip was mainly to detect influenza virus.	Human nasopharyngeal aspirate (NPA) and nasopharyngeal swab (NPS) specimens	The entire process from sample extraction and amplification of influenza specimen was done within 3 hours or less and then product was sent for detection (off chip) through capillary electrophoresis.	[78]
Micro-device based online cell lysis by continuous chemical disruption	1µl whole blood	39.7ng of DNA was extracted within less than 20 minutes	[5]
SPE based filtration using monolithic aluminum oxide membrane (AOM) for DNA extraction from saliva and real time polymerase chain reaction for amplification.	Saliva	Effective identification <i>Streptococcus mutans</i> in a saliva sample with amplification of 300 fg (100–125 copies) of both methicillin-susceptible <i>Staphylococcus aureus</i> (MSSA) and methicillin-resistant <i>S. aureus</i> (MRSA) genomic DNA (gDNA) in less than 2.5 hours.	[79]

## 5. Conclusion

Selecting an appropriate method for sample preparation is essential for further analysis and it's greatly influences the accuracy of yield. Extraction of pure sample is the key factor for success of further downstream process especially in clinical analysis to diagnosis diseases. Separation of analyte from most of biological sample is complicated thus, specific analyte extraction and purification method or micro-device would be suitable for rapid analysis. SPME can be used for effective sample preparation from whole blood with minimal error and high accuracy.

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