Nucleic Acid Isolation: Fundamentals of Sample Preparation Methodologies, Current Advancements, and Future Endeavors

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Abstract

The isolation of nucleic acids (NA) is an essential component of NA analysis. Without sufficient purification, contaminants co-existing with NAs often inhibit enzymatic amplification causing poor reproducibility and sensitivity. Numerous advancements in NA sampling in recent years have led to improvements in extraction yields from complex matrices and the reduction of analysis times. Perhaps most notably is the push towards miniaturization and automation to facilitate point-of-care testing. This article reviews the advancements and current trends in NA sample preparation including cell lysis and NA purification.

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1. Introduction

Sample preparation has aided NA analysis in becoming the cornerstone of forensic, environmental, and clinical applications. Advancements in NA sampling has stemmed largely from improving the yield of NAs isolated, minimizing the amount of impurities co-extracted with NAs, decreasing sample preparation time and sample volumes, and moving towards point-of-care (POC) testing. One of the biggest challenges for NA sample preparation is the co-extraction of impurities as enzymatic detection methods, such as polymerase chain reaction (PCR) and sequencing, are highly sensitive to inhibitors often causing low sensitivities and false negatives. Therefore, ideal sample preparation methods must remove inhibitors to ensure accurate and reproducible results.

The two distinct steps involved in sample preparation are cell lysis and NA purification. Ideally, a cell lysis method will efficiently disrupt the cell membrane to free intracellular components without inhibiting downstream extraction and detection methods. Recent studies in cell lysis have focused on the development of novel lysing agents as well as the development of microfluidic chips for POC testing. Liquid-liquid extraction (LLE) and solid phase extraction (SPE) are among the most popular modes of NA extraction. These methods are highly diverse and utilize electrostatic, hydrophobic, or hydrogen bonding interactions to extract and preconcentrate NAs. Recent advances in NA extraction focus on limiting the sample volume and developing POC testing methods for analysis outside of traditional laboratory settings. One approach to furthering POC diagnostics is the development of microfluidic devices that integrate multiple sample preparation steps into a single chip. In addition, sequence-specific NA extractions are also vital to preconcentrate low abundance fragments in the presence of large amounts of background DNA.

Here, we provide a review of the most recent trends in cell lysis and NA extraction. We discuss the progress that has been made in the development of novel lysis procedures and extraction solvents/sorbents, strategies used to reduce contamination by PCR inhibitors, and the miniaturization and automation of NA sample preparation.

2. Cell Lysis

Cell lysis is often the first step in NA sample preparation. There are numerous chemical and mechanical lysis methods with each offering different advantages and disadvantages. For example, chemical lysis methods are simple and require little instrumentation. However, chemical lysis reagents can inhibit downstream bioanalytical detection methods such as PCR and membrane sensors.[1,2] In comparison, mechanical lysis methods are chemical-free but require additional instrumentation to implement. However, recent studies have reduced instrumentation by developing microfluidic devices.

2.1 Chemical Cell Lysis

Detergents such as 2-[4-(2,4,4-trimethylpentan-2-yl)phenoxy]ethanol (Triton X-100) or sodium dodecyl sulfate (SDS) solubilize the cell membrane and release intracellular components. Although detergents are effective at lysing cells, high lysis efficiencies are essential when low cell counts are available. Le et al. investigated using the non-ionic detergent octylphenoxypolyethoxyethanol (IGEPAL CA-630) in conjunction with bovine serum albumin (BSA) to lyse circulating tumor cells.[3] BSA enhanced the lysis efficiency approximately 4-fold compared to the detergent alone. A 0.3% IGEPAL CA-630 and 0.1% BSA lysis solution outperformed a commercial kit at low cell counts (10 and 100 cells) but performed worse at higher cell counts due to RNase degradation. Recently, Strijp and co-workers developed a two-step

chemical lysis method to isolate RNA and DNA from a single colorectal cancer cell.[4] This on-chip method utilized an initial 0.5x Tris/Borate/EDTA (TBE), 0.5% v/v Triton X-100 buffer to release RNA in the cytosol. The nuclear membrane was subsequently lysed to release DNA using a 0.5x TBE, 0.5% v/v Triton X-100, and protease K lysis solution.

Ionic liquids (ILs) are organic molten salts with melting temperatures under 100 °C. Altering their cation or anion can provide different physiochemical properties while promoting different interactions with analytes. Ressmann et al. reported screening 20 hydrophilic ILs for their ability to lyse animal tissue cells from meat samples.[5] The choline hexanoate ($[Chol^+][Hex^-]$) IL significantly aided the lysis process. The 1-ethyl-3-methylimidazolium acetate ($[EMIM^+][OAc^-]$) and $[Chol^+][Hex^-]$ ILs have more recently been used to lyse gram-positive and negative bacteria. Gram-positive bacteria are difficult to lyse due to a thick peptidoglycan layer and require more stringent lysis conditions, such as lysozyme treatment or sonication.[6] However, the $[EMIM^+][OAc^-]$ and $[Chol^+][Hex^-]$ ILs were remarkably capable of effectively lysing four gram-positive bacteria in 5 min with mild heating at 65 °C.

Fuchs-Telka et al. employed hydrophobic ILs to lyse gram-negative bacteria.[7] Dissolution of the 1-butyl-1-methylpyrrolidinium bis[(trifluoromethyl)sulfonyl]imide ($[BMPyr^+][NTf_2^-]$) IL was shown to not significantly inhibit quantitative PCR (qPCR), and with mild heating could lyse over 88% of gram-negative cells in 1 min. However, the $[BMPyr^+][NTf_2^-]$ IL only lysed 5% of gram-positive cells. Further optimization of the IL structure could effectively lyse gram-positive cells in the same amount of time. Hydrophobic ILs have also been applied towards the lysis of viruses and subsequent extraction of viral RNA and DNA.[8] The IL-based method generally obtained better results with DNA viruses compared to RNA viruses. However,

92 the 1,3-dimethylimidazolium methylphosphonate IL extracted 244% more RNA compared to a
93 commercial kit.

94 Electrochemical lysis uses a localized high pH generated by a cathode to disrupt the cell
95 membrane. Compared to traditional electrical lysis methods, electrochemical lysis requires lower
96 voltages and avoids joule heating, which can lead to bubble formation. Wang et al. devised the
97 electrochemical lysis device illustrated in Figure 1 to lyse gram-negative and gram-positive
98 bacteria.[9] Cell death was noted visually in 30 s. However, a 1 min lysis step was optimum and
99 provided an 8-cycle improvement by qPCR after subsequent DNA purification.

100 *2.2 Mechanical Cell Lysis*

101 Mechanical lysing methods such as freeze/thaw, bead beating, surface acoustic waves
102 (SAW), and electrical lysis are effective chemical-free lysis methods. Recent studies have
103 integrated mechanical lysis methods into microfluidic chips, thereby reducing the amount of
104 equipment needed and providing an automated method.

105 Bead beating lysis methods utilize small (~100 μm), inert beads to induce mechanical shear
106 on cells. Berasaluce et al. developed a continuous flow microfluidic device to lyse gram-positive
107 bacteria using zirconium/silica beads.[2] The quantity and size of beads inside the lysis chamber
108 had a significant effect on the probability of the beads colliding with cells. However, too many
109 small beads were observed to clog the channel or hinder the rotation of the magnet. Yan and co-
110 workers used zirconia beads to lyse gram-negative and gram-positive bacteria followed by loop-
111 mediated isothermal amplification (LAMP) on a centrifugal chip.[10] Only 70 minutes were
112 required from the injection of the bacteria to detection with a limit of detection as low as 10 CFU
113 μL^{-1}.

Kamat et al. utilized mechanical vibrations and chitosan-coated magnetic nanoparticles to lyse six gram-negative pathogens on a microfluidic chip.[11] Frequencies above 150 Hz were capable of inducing cell lysis whereas frequencies above 240 Hz resulted in DNA damage. The nanoparticles aided in improving cell lysis by colliding with cells and imparting mechanical stress. Once lysed, the chitosan-coated nanoparticles captured DNA through electrostatic interactions.

SAWs are Rayleigh waves generated on a piezoelectric crystal surface stimulated from a radio frequency. When the SAW encounters a droplet containing a cell suspension, lysis occurs due to acoustic pressure imparting mechanical stress. Taller et al. utilized SAWs to lyse exosomes and detect microRNA (miRNA) using an ion-exchange nanomembrane.[12] The lysis efficiency was only 38%, but the lysis step did not deleteriously affect the nanomembrane. Wang et al. added polystyrene (PS) microparticles to a suspension of breast cancer cells to improve the lysis efficiency using SAW.[13] Collisions of the microparticles with the cells greatly improved the lysis efficiency from 44% (without microparticles) to 92% (with microparticles).

Electrical lysis functions by creating pores in the cell membrane and causing irreversible damage at high voltages.[9,14] Gross et al. developed a 3D-printed fluidic device to electrically lyse adhered endothelial cells.[15] The device was modified with poly(dimethylsiloxane) or PS to capture cells, and a 500 V power source allowed for lysis efficiencies as high as 96%. However, electrical cell lysis generally requires high voltages to efficiently lyse cells, which can be problematic when coupling to microfluidic chips as joule heating and bubble formation can occur. Gabardo et al. developed 3D multi-scale electrodes that lowered the required potential to only 4 V.[14] The 3D multi-scale electrodes produced over 95% lysis efficiencies by reducing inter-electrode separation and incorporating nanoscale materials, such as gold film, onto the electrode surface.

3. Liquid-Liquid Extraction

LLE utilizes two immiscible solvents to concentrate analytes in one of the phases. Early NA LLE approaches, such as phenol-chloroform LLE, utilized volatile organic solvents and required numerous sample handling steps. To reduce the use of volatile solvents, NA extractions have been miniaturized to require microliter or milliliter volumes. Recently, novel extraction solvents such as deep eutectic solvents (DES), coacervates, and magnetic ionic liquids (MILs) have been implemented to purify NAs from complex matrices.

3.1 Aqueous Biphasic Systems

Aqueous biphasic systems (ABS) form between two water-miscible solutes that are immiscible under critical conditions. Phase formation occurs when interactions between the solute molecules are more favorable than interactions with water. Zhang et al. developed an ABS containing one of 16 novel quaternary ammonium salts and polyethylene glycol (PEG)-based DESs to extract RNA.[16] DESs consist of a hydrogen bond donor and acceptor that form a homogenous mixture with a lower melting temperature than the individual components. Tryptophan was spiked into the RNA sample to assess the co-extraction of proteins with DNA. Extraction efficiencies of over 92% were achieved for both RNA and tryptophan, but only 27.02% and 86.19% of tryptophan and RNA, respectively, were recovered from the DES providing an interesting method for purifying NAs. Similarly, Xu et al. used a two-phase system comprised of ILs and DESs to extract salmon testes DNA.[17] The trimethylglycine formate IL performed the best with an extraction efficiency of 95.86%. When examining the co-extraction of cytochrome c (Cyt-c) and bovine hemoglobin (BHb), nearly 100% DNA was found in the IL phase whereas only 1.86% and 45.59% of Cyt-c and BHb, respectively, was identified in the IL phase. Differential

partitioning of DNA and two proteins was dependent on the isoelectric point of the biomolecules, suggesting that pH modification could be exploited to isolate DNA from PCR inhibitors.

Bio-based ILs often contain an amino acid or carbohydrate-based anion. Therefore, bio-based ILs are considered more environmentally-friendly compared to traditional classes of ILs. Quental et al. demonstrated that amino acid-based ILs can form ABSs with polypropylene glycol (PPG) and quantitatively extract RNA.[18] The [$Chol^+$] L-glutamate and [$Chol^+$] L-aspartate ILs preserved RNA for up to 15 days, with the [$Chol^+$] L-aspartate IL also preventing RNase degradation. However, in a bacterial lysate, residual RNA was found in the PEG along with co-extracted DNA. Additional optimization of the mixture composition or operational conditions has the potential to reduce matrix effects and co-extraction of DNA.

To reduce the number of sample handling steps, Cheung et al. combined Triton X-100 ABS with thermophilic helicase dependent amplification (tHDA) to isolate and amplify DNA from *E. coli* in a one-step procedure.[19] After lysing the cells in the detergent phase, DNA migrated to the tHDA buffer phase where it was amplified. This method has the potential for POC diagnostics if visual detection is utilized as only a heat block would be required for analysis.

3.2 Coacervate Separation

Polyelectrolytes can form liquid droplets known as coacervates permitting phase separation between the aqueous and solute-rich phase. Frankel et al. generated the coacervates, shown in Figure 2, to capture RNA via ion-exchange.[20] Coacervates used were comprised of poly(allylamine hydrochloride, three adenine nucleotides (5'-AMP, 5'-ADP, and 5'-ATP), and Mg^{2+}. RNA extractions from complex matrices using coacervates were not examined, and future studies should examine extraction from different matrices to assess the method's practicality.

3.3 Membrane Separation

Membrane separations have been applied for the preconcentration of DNA and proteins using DESs as draw solutions.[21] Using forward osmosis, the feed solution is passed through the membrane into the draw solution leaving larger analytes to collect within the feed solution. A thin-film composite polyamide membrane hindered the flux of DNA and proteins into the choline chloride and ethylene glycol DES draw solution. Enrichment factors greater than 3 and 6 for DNA and BSA, respectively, were achieved while maintaining the integrity of biomolecules.

3.4 Liquid-Liquid Microextraction

Liquid-liquid microextractions revolve around using only microliter volumes of extraction solvents to efficiently isolate analytes and minimize solvent waste. Clark et al. demonstrated the first DNA extraction with three hydrophobic MILs.[22] MILs are a subclass of ILs containing a paramagnetic component allowing droplets to respond to an external magnetic field. Extraction efficiencies over 90% were obtained by dispersing the trihexy(tetradecyl)phosphonium tetrachloroferrate(III) ($[P_{66614}^{+}][FeCl_4^{-}]$) MIL. However, the benzyltrioctylammonium bromotrichloroferrate(III) ($[(C_8)_3BnN^{+}][FeBrCl_3^{-}]$) MIL extracted significantly less albumin compared to the other Fe(III)-based MILs demonstrating that altering the MIL structure can have a significant effect on the extraction of impurities. Emaus et al. investigated the effect of the paramagnetic metal center of acetylacetonate-based MILs on DNA extraction.[23] The nickel-based MIL produced 4-6 times higher enrichment factors compared to the cobalt and dysprosium-based MILs. In addition, the manganese-based MIL poorly extracted DNA compared to the Ni-, Co-, or Dy-based MILs. Desorbing DNA from either a liquid solvent or solid sorbent can be time-consuming and is often incomplete. Therefore, Emaus et al. integrated DNA-enriched MILs into

custom-designed qPCR buffers to thermally desorb DNA during qPCR without impacting the amplification efficiency.

4. Solid Phase Nucleic Acid Extraction

The extraction of NAs using solid sorbents is a promising area of research capable of isolating NAs with limited use of organic solvents. Silica-based techniques comprise most commercially-available SPE kits. These kits use chaotropic salts to disrupt the solvation of the biopolymer and facilitate the adsorption of NAs. Adsorbed NAs are then washed with alcohol and eluted with a low ionic strength solvent. Recent work by Günal et al. compared the DNA extraction behavior of silica microspheres of varying pore sizes and polymer coatings.[24] Monodisperse silica particles extracted more DNA when silica gel possessed a bimodal distribution of pore sizes due to an increased surface area and rate of mass transport.

Juang et al. developed a method called centrifugation-assisted immiscible fluid filtration (CIFF) to extract DNA and messenger RNA (mRNA).[25] As shown in Figure 3, dehydrated NAs were adsorbed to the microbeads, followed by pelleting of the beads in a fluorinated oil to isolate NAs from impurities. This method allows for the high-throughput analysis of NAs without manual wash steps, thereby reducing the risk of contamination. CIFF was successfully able to isolate enough DNA for qPCR detection from 10 cancer cells with only 0.5% carryover from the sample solution.

4.1 Magnetic Nanoparticles

Traditional silica-based extraction platforms require specialized equipment and significant user intervention. In comparison, magnetic nanoparticles only require an external

magnet to recover DNA-enriched particles. Magnetic nanoparticles are also attractive due to their large surface area and ease of modification.

Bare iron magnetic nanoparticles are easily oxidized and have low affinity for NAs. Therefore, studies have evaluated coating magnetic nanoparticles to improve their affinity for NAs. Nanayakkara et al. implemented chitosan-coated iron nanoparticles to extract genomic DNA from whole blood.[26] The particles lysed white blood cells through bead beating while simultaneously adsorbing DNA. The DNA-enriched nanoparticles were added to a qPCR buffer to bypass a time-consuming desorption step. Hu et al. coated iron phosphate nanoparticles with polyethyleneimine and demonstrated its ability to extract 4.6 μg of genomic DNA from whole blood via electrostatic interactions at pH 4.[27] Nearly 80% of extracted DNA could be recovered by increasing to a pH of 10. Haddad et al. examined the physical characteristics of different coatings through the measurement of particle size and zeta potential before and after DNA binding.[28] Silica and tripolyphosphate functionalized particles exhibited zeta potentials of +5.55±2.10 and +1.78±1.73 mV, respectively. The tripolyphosphate magnetic particles possessed a lower zeta potential but extracted 100-fold more DNA than the silica particles. Bai et al. developed a method using amine-rich silica-coated iron oxide nanoparticles for NA isolation.[29] The amine-rich coating greatly increased the surface area of the particles and was directly added to the PCR buffer for successful amplification.

Magnetic nanoparticles have also been widely used as composite extraction phases to recover the extraction phase. Xu et al. utilized chitosan-modified iron-magnetized multi-walled carbon nanotubes coated with a PEG-based DES to extract up to 178 mg of DNA per gram of sorbent.[30]. In comparison, the composite could only load up to 15 mg g^{-1} of BHb. Liu et al. developed a DNA extraction phase consisting of reduced graphene oxide, chitosan-

functionalized magnetic nanoparticles, and a guanidinium-based IL.[31] This phase extracted 233 mg of salmon testes DNA per gram of sorbent while extracting less than 5% of BHb. Meng et al. utilized magnetic nanoparticles functionalized with a metal-organic framework dispersed in a lactic acid-based DES to extract RNA.[32] The composite was able to selectively extract RNA over DNA, Cyto-c, tryptophan, and tyrosine through chelation, electrostatic, hydrophobic, and hydrogen bonding interactions. Yan et al. employed a reduced graphene oxide phase combined with magnetic nanoparticles to isolate miRNAs.[33] The material preferentially extracted miRNA over mRNA due to the larger diffusivity of miRNA.

4.2 Polymeric Ionic Liquid Sorbents

Solid-phase microextraction (SPME) utilizes a fiber coated with a sorbent for solventless, non-exhaustive preconcentation of analytes. SPME is a unique extraction method as it does not require significant instrumentation to extract or recover analytes, making it ideal for POC testing. Nacham et al. used a polymeric ionic liquid (PIL) sorbent coating for DNA and yeast RNA extractions.[34,35] PILs are polymers produced from the polymerization of IL monomers that retain many properties of ILs (i.e., thermal stability and structural tunability). NAs were extracted by the coating through anion exchange. Varona et al. expanded the use of the PIL fibers to isolate DNA from lysed *Mycobacteria smegatis* in an artificial sputum matrix.[36] To avoid diluting recovered DNA, a custom LAMP buffer was developed to allow direct addition of the desorption solution (1 M NaCl). This buffer was used along with hydroxy-naphthol blue [36] and sequence-specific molecular beacons for sequence-specific DNA detection.[37] With isothermal DNA amplification, the PIL-SPME procedure can be implemented in non-traditional laboratory settings for rapid and specific diagnostics.

5. Microfluidic-based Nucleic Acid Extraction

POC testing is capable of rapidly obtaining results at, or near, the sampling location. Traditional NA extraction techniques are not applicable for POC diagnostics due to numerous sample handling steps and the required instrumentation. Microfluidic systems rectify the limitations of conventional extraction methods by reducing sample volumes, improving portability, and allowing for automation by integrating multiple sample preparation steps into a single device.

5.1 Magnetic Separation in Microfluidics

The use of a magnetic field to transport and isolate NAs has been widely explored due to its potential for automation. Deraney and co-workers devised a synergistic method that integrated magnetic and electroosmotic forces in their microfluidic system to transport DNA-enriched silica-based magnetic beads within the chip.[38] Utilizing electroosmotic flow improved the DNA extraction yield 15% while the carryover volume was minimized to only 0.22%. Zhang et al. used a magnetic field to adhere silica-based magnetic beads to a coaxial channel. [39] Compared to traditional hollow channels, the coaxial channels allowed for higher DNA extraction efficiencies (~99%) potentially due to a greater surface area. Perez-Toralla et al. used magnetic microparticles to electrostatically extract DNA followed by digital droplet PCR from serum samples of cancer patients.[40] Extraction efficiencies were found to be significantly reduced when serum samples were used.

Strohmeier and co-workers integrated the lysis and extraction steps on a single centrifugal microfluidic LabDisk.[41] The chip contained silica-based magnetic beads to capture NAs from lysed white blood cells, *B. subtilis*, *E. coli*, and Rift Valley fever viruses. The beads were transferred between chambers using a magnetic field. The LabDisk extracted approximately 3-fold less DNA from whole blood compared to Qiagen spin columns. In addition, on-disk DNA

extraction suffered from up to 5.5% (w/v) ethanol co-extraction due to the limited ability of drying the beads within the chip. Ethanol can precipitate DNA as well as enzymes used in PCR. Therefore, further work should explore approaches that reduce ethanol co-elution.

5.2 Filter-based Isolation using Microfluidics

Filters present a simple yet customizable NA extraction method as the pore size, filter length, and coating can be easily modified for use in microfluidic devices. Brassard and co-workers utilized glass microfiber filters to extract DNA on a centrifugal chip.[42] Pneumatic pumping flow, which utilizes pressure ports to compress air and facilitate airflow through the channels, was integrated into the chip to reduce ethanol co-elution. A 10 min pneumatic pumping flow step reduced the amount of ethanol co-elution 10-fold, which is a significant improvement from Strohmeier et al.[41] The centrifugal microfluidic device extracted similar amounts of DNA compared to commercial spin columns.

Gan and co-workers inserted a chitosan-modified filter paper into the microchip shown in Figure 4.[43] The filter was able to extract 95% of DNA in a sample through electrostatic interactions and entanglement of DNA with the fiber matrix. An "*in situ"* PCR method was employed permitting DNA amplification within the extraction chamber without a desorption step. "*In situ"* PCR reduced the analysis time by removing a lengthy desorption step while simplifying the design of the microfluidic device.

5.3 Channel Modifications

Channels within a microfluidic chip provide a large surface area to extract NAs. Therefore, ample research has focused on modifying the channel for NA extractions. Jin and co-workers modified the channel wall with primary amines to capture dimethyl pimelimidate (DMP)-bound

NAs through the formation of amidine bonds.[44] DMP captured NAs through electrostatic interactions with DNA were released at high pH (pH>10). Compared to commercial spin columns, the microfluidic method extracted 8-fold less DNA. However, the major advantage of the DMP extraction method is its ability to remove 99% of proteins from the sample; further modification to the chip's design could help to improve the extraction efficiency. Kastania et al. utilized carboxylic acid modifications to the channel wall to extract NAs precipitated using PEG, ethanol, and sodium chloride.[45] A recovery efficiency of 96% was achieved.

5.4 Paper-based Extraction Methods

Paper-based microfluidic devices have garnered significant attention due to their low cost, portability, and disposability. Rodriguez et al. designed a paper-based platform for the isolation, isothermal amplification, and lateral flow detection of DNA.[46] DNA was precipitated in the sample port while aqueous waste was wicked through a membrane. Once purified, LAMP was performed in the sample port to amplify DNA within 30 min. The paper-based method was prone to false positives attributed to the self-priming of LAMP primers. To reduce the occurrence of false positives, sequence-specific probes can be employed.

6. Sequence-Specific Nucleic Acid Extractions

Target NA sequences often comprise a small fraction of the total NA population. To enable accurate detection and quantification of low abundance sequences, it is often necessary to include an enrichment step. NAs possess the ability to recognize complementary sequences through Watson-Crick base-pairing interactions. These specific interactions are often exploited to isolate specific sequences from complex samples. Short oligonucleotide probes are commonly used to

hybridize with target sequences. These probes are modified with functional groups (e.g., biotin) allowing them to be captured by a support possessing an affinity for the tag.

6.1 Solid Supports for DNA Analysis

Magnetic nanospheres were synthesized in a layer-by-layer fashion by Wen et al. to capture hepatitis B DNA.[47] The carboxylated nanospheres were functionalized with a streptavidin coating and modified with a biotin-labeled oligonucleotide complementary to a sequence in the HBV genome. Capture efficiencies as high as 87±1.7% were achieved using this approach. Daggumati et al. developed a method for the sequential detection and purification of specific NA sequences from complex biological samples using modified nanoporous gold electrodes.[48] The electrodes were modified with thiolated 26-mer DNA probes. After the hybridization and wash step, the newly formed duplex could be desorbed using cyclic voltammetry. The applied voltage (from 0 to -1.5 V) was successfully able to cleave the gold-thiol bond and prevent re-adsorption of the duplex.

An interesting approach was developed by Leslie et al. that allowed for the label-free, visual detection of DNA using paramagnetic bead aggregation.[49] In the presence of DNA and a rotating magnetic field, silica-coated magnetic particles underwent visual aggregation. This approach was developed further to enable the sequence-specific detection of a clinically-relevant *KRAS* mutation.[50] Single-nucleotide polymorphism (SNP) detection was achieved by functionalizing a portion of the beads with a stabilizing probe complementary to a conserved region, while the remaining beads were modified with a shorter discriminating probe complementary to the wild-type, as illustrated in Figure 5a. In the presence of the wild-type sequence, the discriminating probe was able to bind resulting in visual aggregation (Figure 5b). Conversely, when the mutant sequence was present, the discriminating probe was unable to bind

which prevented aggregation (Figure 5c). The wild-type sequence was detected in 2 min when placed in a 1:1 ratio with the mutant sequence.

6.2 Selective DNA analysis using Liquid Supports

While magnetic beads are a popular platform for sequence-specific enrichment, they suffer from aggregation and sedimentation which can lower extraction efficiencies. Recently, Clark et al. developed a method relying on the [$P_{6,6,6,14}^{+}$] tris(hexafluoroacetylaceto)manganate(II) ([$Mn(hfacac)_3^{-}$]) MIL as a support for the sequence-selective capture of DNA.[51] Ion-tagged oligonucleotides (ITOs) possessing an affinity for the MIL were synthesized using thiol-ene "click" chemistry between a thiol-modified oligonucleotide and an allylimidazolium IL. A 1000-fold greater amount of DNA was extracted from a 16.9 fmol solution using a modified oligo compared to using an unmodified oligo.

Maruyama et al. developed a different approach to enable sequence-specific DNA extraction of short sequences using oleoyl-modified DNA probes.[52] These probes hybridized to their target and formed reverse micelles with dilauroylphosphatidylcholine and 1-hexanol to enable partitioning into an organic phase. A subsequent study demonstrated the sequence-selective extraction of an aptamer using this strategy.[53] It was shown that the aptamer was capable of recognizing its target following the extraction and back-extraction processes, revealing that the method maintains the structural integrity of the target DNA. The method was also expanded for the extraction of short RNA sequences.[54] However, this technique is limited to short sequences and required long analysis times, reducing its practicality.

6.3 mRNA Analysis

The analysis of mRNA is of significant interest due to its role in gene expression. mRNA possesses a poly-adenosine tail on its 3' end that prevents it from being enzymatically degraded in the cytoplasm. This portion of the mRNA molecule can serve as a molecular handle for sequence-specific extractions with oligonucleotide probes containing consecutive thymine bases. Nacham et al. developed a SPME sorbent using commercially-available polyacrylate (PA) fibers.[35] The PA fibers were modified with oligo deoxythymine 20 (dT_{20}) for the selective extraction of mRNA. Successful analysis of mRNA using RT-qPCR was performed from a suspension of 3.5×10^7 *S. cerevisiae* cells mL^{-1}.

Scherp et al. developed a similar method called solid phase gene extraction (SPGE).[55] Pulled borosilicate glass rods were used to create needles that were subsequently modified with oligo-dT_{10} nucleotides for selective mRNA extraction. Extractions were performed from Drosophila eggs and embryonic flax roots and provided unique spatial resolution. One drawback to the SPGE method was the brittle glass needles used as the support. Park et al. improved the method by generating SPGE probes using stainless steel acupuncture needles to generate a more robust support. These probes were used to analyze *Brassica* roots in two different studies.[56,57] More recently, Nestorova et al. interfaced SPGE with a lab-on-a-chip device for the seamless extraction and desorption of mRNA for RT-qPCR analysis.[58] The method analyzed mRNA from rat glioblastoma cell spheroids in 7 min.

Drop-seq is a popular technique for gene expression analysis permitting the capture, amplification, and barcoding of the transcriptome for individual cells. Next-generation sequencing is subsequently used to reveal the gene expression profile of each cell. However, these techniques are limited to information near the poly-A tail. Hanson et al. developed DNA barcoded beads capable of capturing the T-cell receptor α and β chain mRNAs using a reversible chain blocking

group.[59] This allowed for the development of individual beads capable of simultaneously capturing two different sequences. The ability to obtain paired information for two mRNA sequences can be potentially useful in understanding various disease states.

6.4 Sequence-Specific Nucleic Acid Capture for Proteomic Analysis of Bound Protein

The analysis of proteins associated with chromatin and RNA is of significant interest in developing an in-depth understanding of gene expression and regulation. One method that has been developed to understand these proteins is called hybridization capture of chromatin-associated proteins for proteomics (HyCCAPP).[60] This approach uses DNA oligonucleotides bound to magnetic beads to capture specific chromosomal loci for LC-MS/MS analysis of the bound protein. This method relies on formaldehyde crosslinking of chromatin and subsequent capture of specific loci using magnetic beads. DNA oligonucleotides modified with desthiobiotin were used as capture probes to enable the detection of low-abundance proteins, as biotin-labeled probes produced a higher background. In a separate study, a multiplexed approach was developed to capture the 25S rDNA, ARX1, CTT1, and RPL30 genes from yeast and subsequently analyze the bound protein.[61] This was done using strand-displacement probes that enabled the selective displacement of each gene-protein pair for mass spectrometric analysis. This allowed for the capture of all sequences on individual beads, which significantly decreased the analysis time. A similar approach was utilized by Spiniello et al. for the study of proteins associated with specific long noncoding RNA sequences.[62]

7. Conclusion and Future Outlook

Recent advancements in NA sample preparation have improved the recovery of pure NAs while reducing the time required for sample preparation. The co-extraction of potential PCR

inhibitors has been minimized through the use of new NA extraction phases such as DESs, MILs, and magnetic composites. A move towards automation has also occurred by integrating sample preparation steps in microfluidic devices.

While continual efforts towards ensuring NA purity from increasingly more complex samples are needed, future endeavors should also focus on improving detection limits. Sequence-specific extractions have aided this goal by enriching low abundance mutations that would otherwise be masked during PCR. Enhanced NA preconcentration has the potential to be achieved by using lower desorption volumes or by integrating NA-enriched sorbents/solvents into a PCR assay to circumvent diluting recovered DNA. In addition, modifying the design of microfluidic chips has the potential to improve NA enrichment while still permitting POC testing. Lower detection limits will aid clinical and forensic applications by reducing the amount of sample required for analysis or permitting earlier detection of diseases through the analysis of viral NA or circulating tumor DNA.

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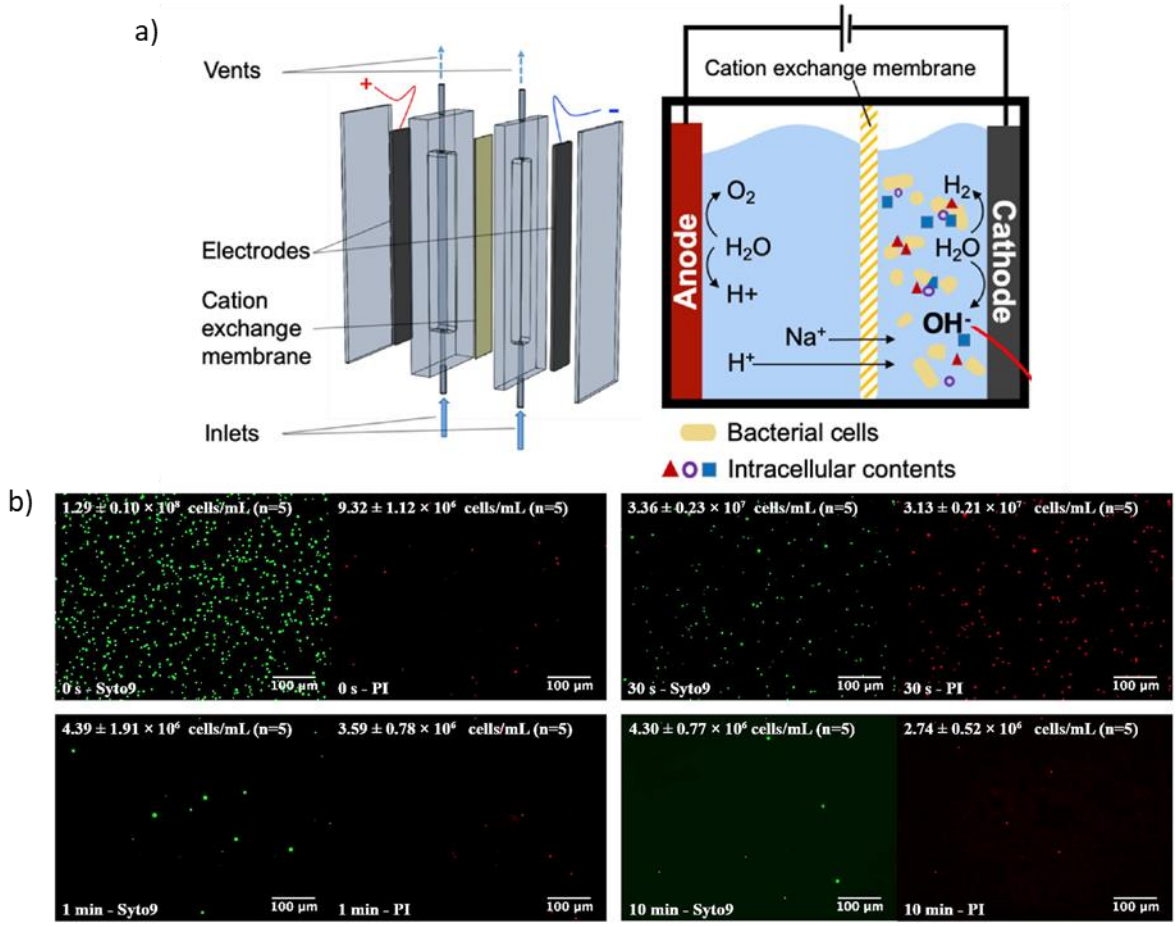

Figure 1 (a) Schematic of the electrochemical device used to lyse waterborne pathogens. (b) Fluorescent monitoring of viable (green) and recently dead (red) *E. coli* cells during electrochemical lysis. Reproduced with permission form Ref. [9]

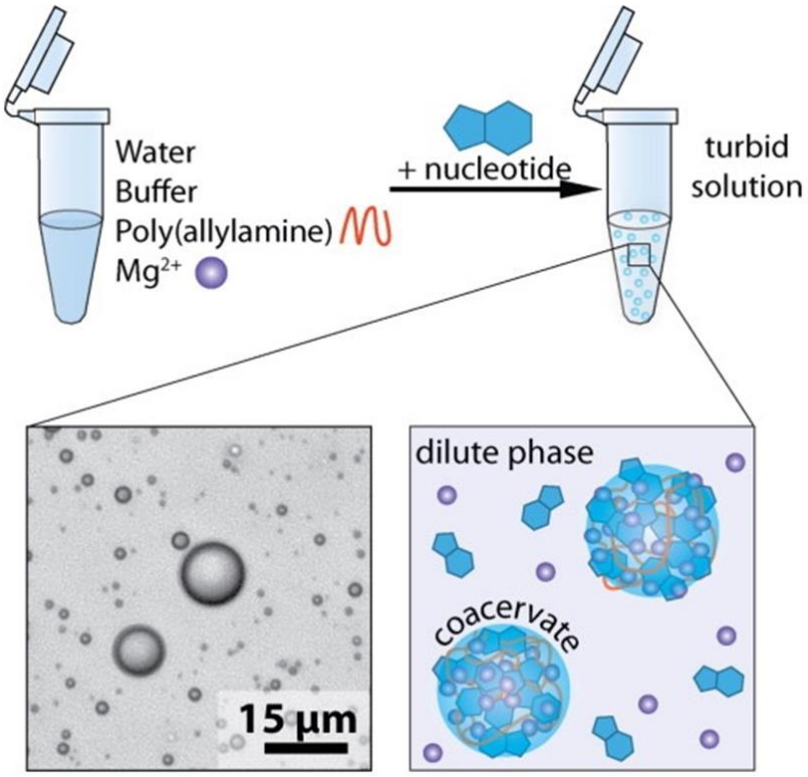

Figure 2 Experimental approach for the formation of coacervates from electrolytes. Reproduced with permission from Ref. [20]

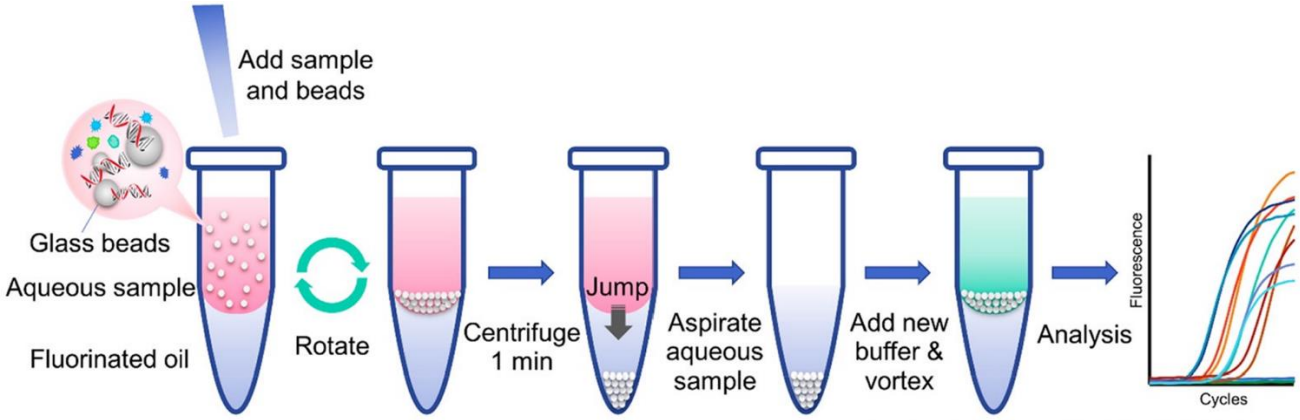

Figure 3 NA extraction procedure using the CIFF method where NAs are adsorbed onto glass beads then isolated from the sample matrix by being pelleted in a fluorinated oil. Reproduced with permission from Ref. [25]

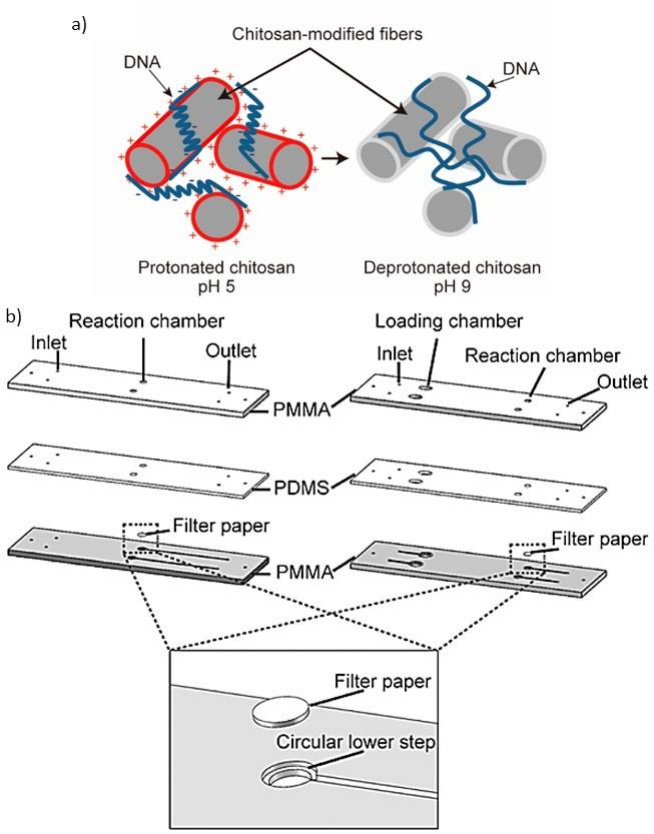

Figure 4 a) DNA capture mechanisms using the chitosan-modified filter paper. b) Exploded view of the microchips that contain a (left) single reaction chamber and (right) a sample loading and reaction chamber used for DNA capture with the chitosan-modified filter paper. Reproduced with permission from Ref. [43]

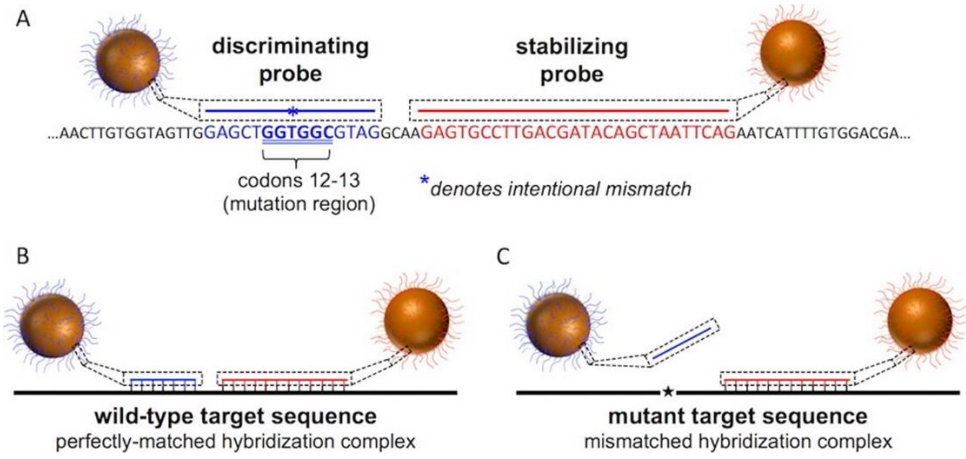

Figure 5 (a) Design of the discriminating and stabilizing probes. (b) Both the stabilizing and hybridization probe bind to the wild-type target causing visual aggregation. (c) Only the stabilizing probe binds to the mutant target preventing aggregation. Reproduced with permission from Ref. [50]