Review

A Polymer Instigated Path in the Engineering of Sensors and Biosensors for Effective Amelioration of Therapeutics

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Abstract: Nanotechnology and polymer engineering are steering towards a new development to invade deleterious mysteries. In the last few decades, polymer engineering has grabbed researchers' attention and similarly, polymeric network-engineered structures have been vastly studied. Prior to therapeutic application, early and quick detection analyses are critical. Therefore, developing nanoscale sensors to manage the acute expression of feeble states and malignancies to draw therapeutic lines demands advanced nanoengineering. In spite of nano-therapeutics have emerged as an alternative approach to tackling strenuous diseases. Also, studies have shown highly biocompatible biomedical engineering has emerged with sheer progression. Moreover, hydrogels have emerged as a three-dimensional (3D) polymeric network that consists of hydrophilic natural or synthetic polymers. The resemblance of hydrogels with tissue structure makes it more unique to study inquisitively. Preceding studies have shown a vast spectrum of synthetic and natural polymer deployment in the field of biotechnology and molecular diagnostics. This review explores recent studies on synthetic and natural polymer engineered hydrogel-based biosensors and their applications in multipurpose diagnostics and therapeutics. We reviewed the latest studies on hydrogel-engineered biosensors, exclusively DNA-based and DNA hydrogels fabricated biosensors.

Keywords: - DNA-based hydrogels; biosensors; stimuli-responsive; Progesterone detection; mRNA quantification; sensors

1. Introduction

The detection systems in therapeutics are widely studied in the earlier decade due to the necessity of rapid and robust analysis of feeble human pathology.[1] The rise in drug toxins and side effects that emerged from drug overdose are the critical elements to be analyzed.[2] Preceding studies have shown a vast and emerging need for biochemical detection methods.[3–6] The conventional methodologies in the early detection of biochemicals were studied using HPLC, PCR, GC, and GC-MS.[7–9] However, these conventional methodologies are not highly favorable since robust, affordable, and highly sensitive detecting methods are lacking.[10] Moreover, the conventional methodology does not favor working consistently under versatile humid conditions and also requires complex equipment with experienced operators.[11–17] On the other hand, researchers in early 1900 were developing polymer network nanocomplexes for biomedical applications. The architecture to draw a polymeric network confined with hydrophilic properties aims to provide a tissue-like structure for biomedical purposes. Wichterle et al in the 1960s designed a hydrophilic gel (hydrogel) for the first time with the motive of designing a 3D polymer network to utilize in the human body.[18] These immense water-rich bodies of hydrogels are easy to adapt to the microenvironment due to the similarities in tissue-like structure and are feasible to engineers having significant dynamic range.[19–22] Furthermore, hydrogels have surfaced as an excellent sensory system due to high biocompatibility and the variability that eases the tuning of gel chemistry.[23] Especially, the change in physical properties of the hydrogels such as sol-gel transitions can result in excellent target analytes.[24,25] Hydrogels are

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well known for their easy modulation of physical properties and can tend to make responsive hydrogels to the elements such as external stimuli, pH, temperature, ionic strength, light, and sound.[26] Generally, hydrogels are classified into different categories such as Natural polymers and Synthetic polymers. Hydrogels are fabricated with a polymer network using natural or synthetic materials with a high degree of flexibility owing to their large water content.[27,28] Natural polymers, such as chitosan, alginate, dextran, and hyaluronic acids.[29,30] Synthetic polymers, including polyethylene glycol (PEG), poly (N-isopropyl acrylamide) (PNIPAAm), and poly (2-hydroxyethyl methacrylate) (PHEMA).[31–33]

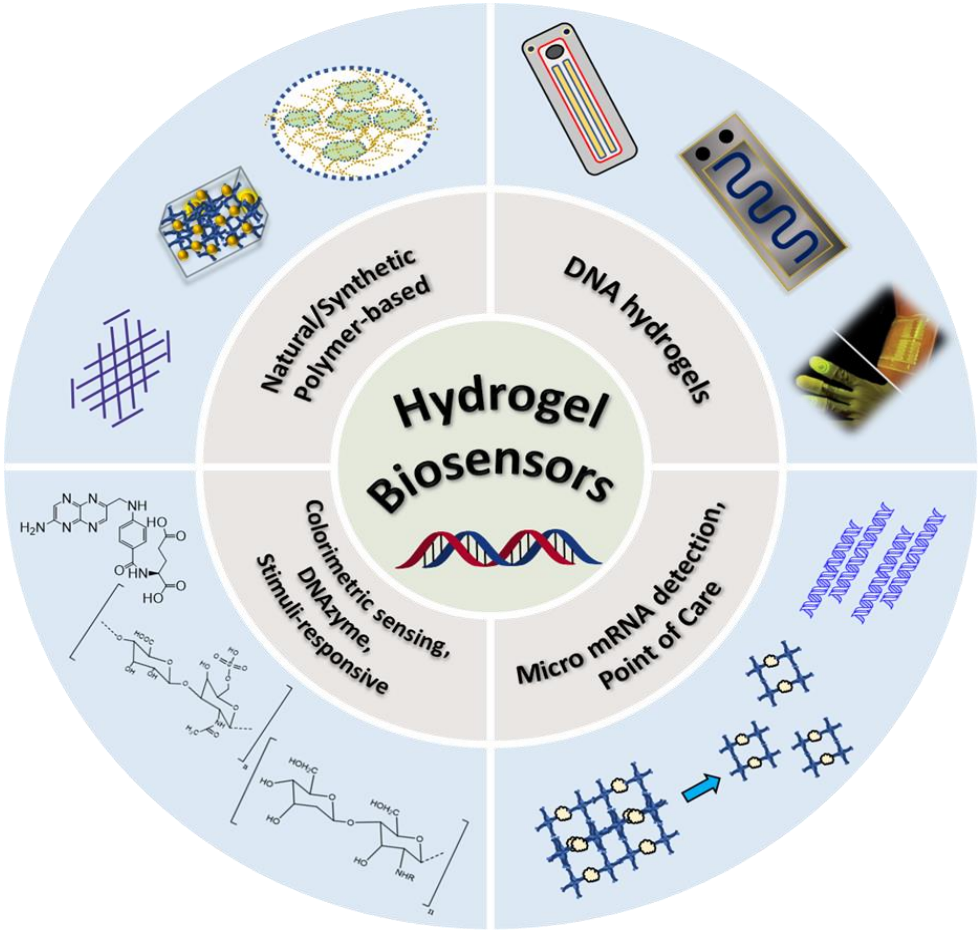

Figure 1. Schematic illustration of the designs and functional roles played by hydrogel biosensors.

The unique behavior of hydrogels in different physiological conditions can retain a large number of biological fluids resulting in them as an ideal substance for a variety of applications. Hydrogels have been vastly studied based on the classification of their type and nature.[27,34] Later on, researchers developed a hybrid hydrogel with a mixture of natural and synthetic polymers to exert and utilize the different element's unique properties[35–37]. In recent years, the global pandemic has shown the critical urgency and need for rapid, bio-susceptible sensors in detecting contagious diseases to inhibit disease transmissions.[38] Similarly, robust biosensor detecting systems have been successfully utilized to examine biochemicals, chemical drugs, or toxins and have a wide range of applications, including preventing and controlling drug abuse, preventing food contamination, and maintaining drug doses in body fluids to avoid drug overdoses.[39–41] Moreover, several studies have been well-reviewed on fabrication and application based principles and challenges in hydrogel-based biosensors.[42–47] However, our review focuses on the latest studies of hydrogel-based biosensors and explains their mechanism of action, limitations, and further future directions. We have short reviewed several first time reported hydrogel-based biosensors with impactful and significant applications. We exclusively reviewed DNA hydrogel-engineered biosensors for their diversified role and limitations.

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1.1. Hydrogels for progesterone detection

Progesterone plays a major role in the female body; it is a steroid hormone produced in the body to regulate the menstrual process also known as a primary biomarker for reproductive status and monitoring fatigue and irregular menstrual cycles. It has a significant role in early gestation and regulation of progesterone levels can ease the process of pregnancy of unknown location (PUL).[48] Analogously, Chen et al. have designed a PEG hydrogel with the assimilation of quantum dots (QD) and polyhistidine-tagged transcription factor (SRTF1) as a bioreceptor and Cy5 fluorophore-labeled cognate DNA sequence for the first time. The author demonstrates Förster resonance energy transfer (FRET) between a fluorophore-labeled cognate DNA and the modified QDs. The presence of progesterone leads to a decrease in fluorescence intensity (Figures 2 I) and II). This pursuit was able to demonstrate the potential of the novel genomics-to-sensor approach using allosteric transcription factors to overcome the problem of insufficient adequate bio-recognition of target elements.[49] Similarly, Velayudham et al designed a chitosan and hydroxyethyl cellulose hydrogel conjugated aptamer-based electrochemical biosensor for the detection of progesterone. Here the author utilized gold nanocubes (AuNCs) which were self-assembled with thiol-Au chemistry. The aptamers were modified with thiol and specific for progesterone (P4). The functionalities and successful applicability of the aptasensor were tested using spiked blood samples at different concentrations of progesterone P4.[50] (Figure 2 III)

Moreover, Casis and the group studied a new method based on molecularly imprinted hydrogels and demonstrated a novel module to recognize specifically progesterone. The author fabricated Hydrogel films by copolymerization of acrylic acid and ethylene glycol dimethacrylate with 2,2′-azobisisobutyronitrile as initiator. The approach includes the non-covalent imprinting method with the colloidal crystal template technique to produce membranes with pre-specified morphology. These studies suggest the engineered system can be used repetitively with high reproducibility and robust quantification of target molecules in photonic films through spectroscopic techniques.[51] (Figure 2 IV)

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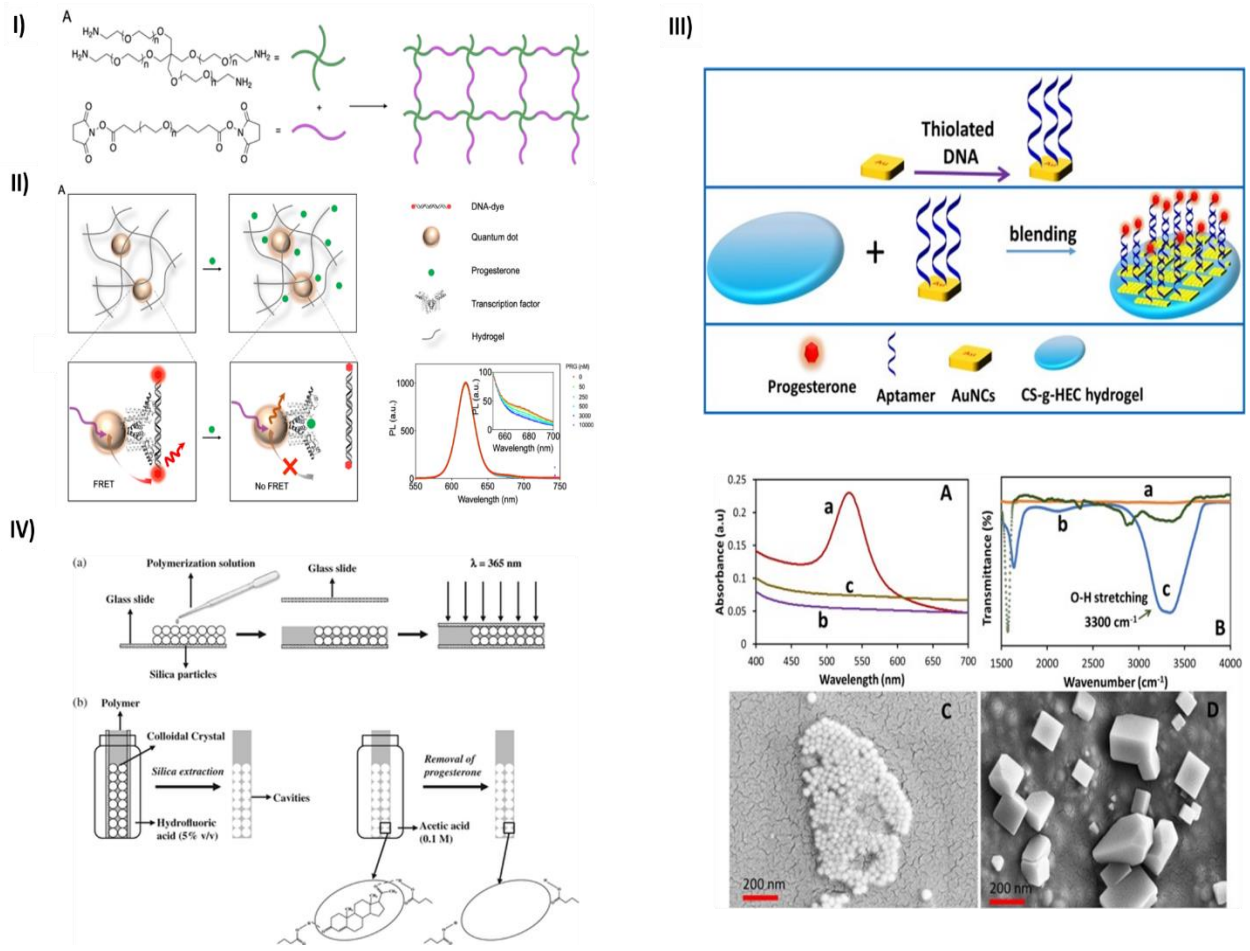

Figure 2. I) (A) Hydrogel precursors, formation, and swelling: 4-arm-PEG-NH2 and NHS-PEG-NHS. Hydrogel formation proceeds via a reaction between NHS ester (N-hydroxysuccinimide ester) and a primary amine, producing a 3D network. **II)** Schematic illustration of the progesterone diffusion into hydrogel. A FRET-based sensor utilizing TF-DNA binding mechanism and signal attenuation in graphical observations. **III)** Schematic representation of P4 aptasensor fabrication. Instrumental analysis on (A) of AuNCs (a), AuNCs-aptamer (b) and c) chitosan hydroxyethyl cellulose hybrid hydrogel CS-g-HEC conjugated AuNCs-aptamer. **IV)** a) Infiltration and polymerization process. b) Silica extraction and removal of progesterone. (Adapted from [49–51]).

2. DNA-based hydrogels as biosensors

DNA-based nanostructures have become adaptable building blocks for the manufacturing of soft materials at the nanoscale.[52] The flexibility of conjugation with other biomolecules, and the structural and functional properties of DNA made it favorable for the utilization of a variety of biomedical applications.[52–54] The hydrogels formed by DNA are biocompatible, stable, tunable, and biologically versatile, thus, these have a wide range of promising applications in bioanalysis and biomedicine.[55] Due to their characteristics and use in biosensing, bioimaging, and therapeutics, DNA-based hydrogels have attracted a lot of attention in recent years.[56–59] Nano-engineered structures and devices built on DNA nanotechnology have shown potential in a wide range of soft materials. Ned Seeman proposed modulating the structure of DNA on the nanoscale based on its physical properties, DNA long recognized for its role as genetic material emerged as an ideal building material on the nanoscale.[60] Next, the renowned Watson-Crick base pairing makes it the deterministic and controllable, sequence-specific hybridization between DNA strands.[61] In particular, DNA nanotechnology has evolved in a transition from one-dimensional structures to 3D dimensional structures. The frequent studies of structure building in dimensions from DNA wires to nanotubes, lattice-crystal structures to diverse DNA polyhedra origamis with 3D structures.[62,63] To our knowledge and findings DNA hydrogel's role in biosensors is highly limited and rare to observe. However, in this

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section, we reviewed several reported studies on DNA hydrogel-based biosensors in the sensing of microRNA, colorimetric sensing, point-of-care devices, and label-free detection-based applications. Additionally, we have overviewed the recent and well-approached unique terms such as "wireless infection detection on wounds" (WINDOW) and other significant usages.

2.1. Micro RNA (miRNA) quantification and detection

In recent times, miRNAs have gained recognition as promising molecular biomarkers.[64] Earlier reported studies demonstrate, several methods have been established to detect miRNA expressions, such as stem-loop reverse transcription PCR, rolling circle amplification, ligase chain reaction, and many other enzyme-catalyzed reaction-based techniques.[65–68] Moreover, abnormal miRNA expression has been used to categorize and diagnose and predict the prognosis of cancers. miRNA expression levels that are abnormally high are linked to several human diseases.[69–72] Therefore researchers amend their ideas to construct hydrogel-engineered sensors for miRNA detection and quantifications. Similarly, Hui Wang et.al developed a simply prepared capillarity self-driven DNA hydrogel sensor by fixing DNA hydrogel film at a capillary end. The direct hybridization between miRNAs and the DNA probes from the hydrogel sense and detect miRNA targets. The DNA hydrogel sensor applies miR-21 as a proof-of-concept target (Figure 3 I).[73] This visually quantitative detection of miRNAs stands, cheap and self-driven by DNA hydrogel sensor, along with the little volume of DNA hydrogel can detect target successfully. Similarly in another study, Li and colleagues designed a versatile fluorescence strategy based on DNA hydrogel to detect microRNA-141s. A polydirectional hybridization chain reaction based DNA hydrogel on SiO_2 microspheres was designed. By coupling with DNA walking amplification, it served as a flexible fluorescence signal amplifier for the ultrasensitive detection of miRNA-141. The author claims based on the findings this strategy can be applied to miRNA-141 detection in human prostate cancer with favorable precision, suggesting promising applications of the sensing strategy in disease diagnosis and biomedical analysis. Designing and use of functional nanomaterials properties in sensing, detection, and imaging are widely known.[74] Li and group studies the selectivity of the DNA hydrogel-based fluorescence strategy for miRNA assay with several other miRNAs including miRNA-21, miRNA-155, miRNA-182, and mismatched miRNA-141.[75] Based on miRNA responsive/targeting strategies Si, et al designed a novel surfaced-enhanced Raman scattering sensor array (SERS) with a Raman signal "ON" and "OFF" strategy. The design includes nine sensor units that can detect multiple cancer-responsive miRNAs in one sample. The researcher first synthesized DNA hydrogel followed by AuAg nanoparticles as SERS tags and incorporated certain MNAzymes (Multi-component nucleic acid enzymes) with the SERS array (Figure 3 II). [76] The design is based on the mechanism of miRNA interactions and the AuAg alloy nanoparticles penetration to the hydrogels which allow and interact with the SERS array to produce the Raman intense signal. The miRNA interaction proceeds to configuration and restores the activities of MNAzymes which lead to the breakage of the substrate linker and followed by penetration of alloy nanoparticles to the hydrogel.

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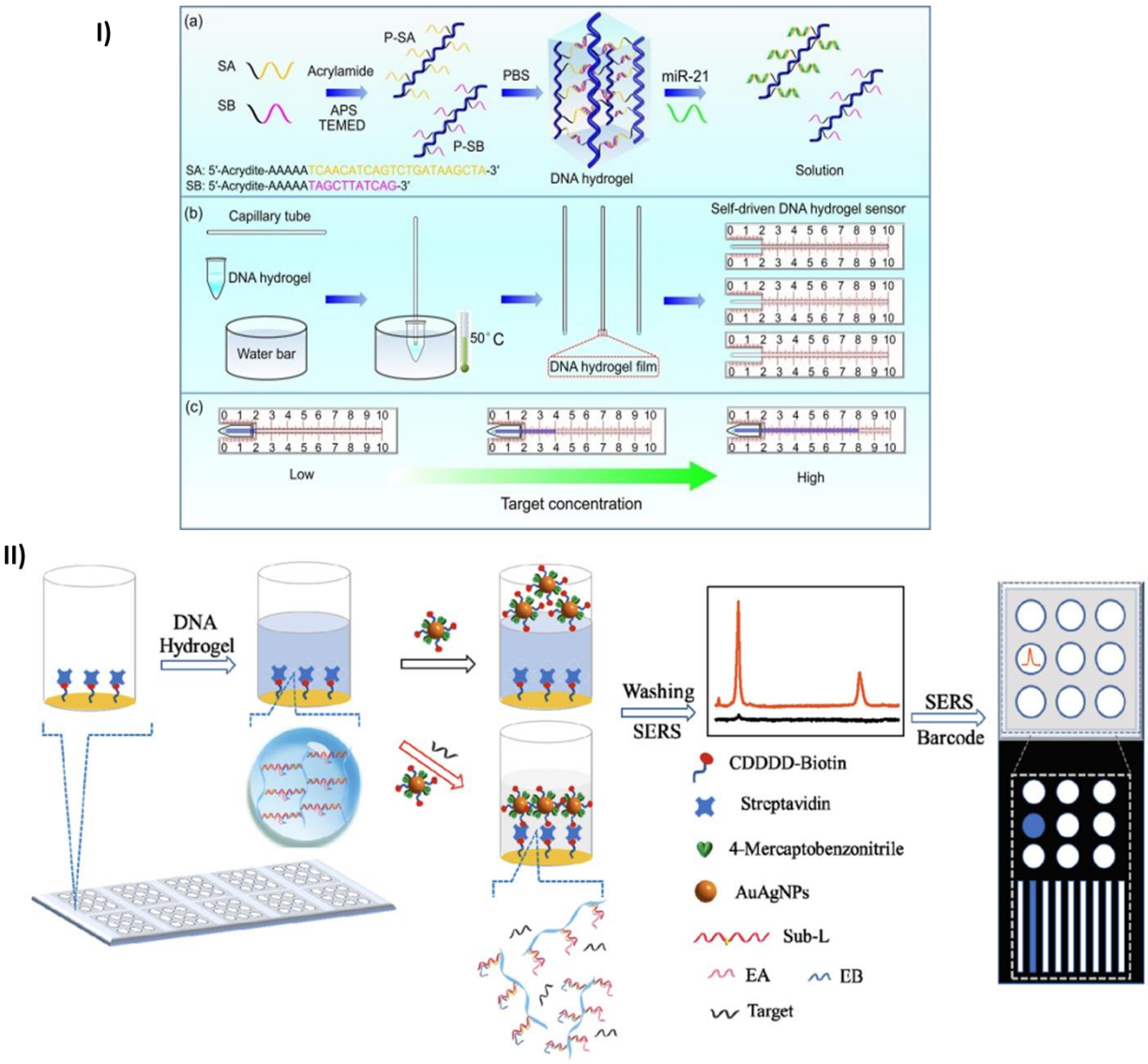

Figure 3. I) (a) The designing/working principle of the self-driven DNA hydrogel sensor. **(b)** The preparation process of the self-driven DNA hydrogel sensor. **(c)** The principle of the visual and quantitative detection of miRNA by using the proposed sensor. **II)** Schematic illustration of the preparation and application of the target miRNA-responsive DNA hydrogel-based SERS sensor array for measuring multiple miRNAs in one sample. (Adapted from ref.[73] & [76]).

2.2. Colorimetric sensing

In the development of DNA hydrogel-based biosensors, cost-effectiveness, fast response time, and ease of fabrication along with handling are the major concerns.[77] In addressing these concerns, colorimetric sensing has played a major role. Similarly, DNA hydrogel's contributions toward similar issues make it inquisitive to study, hence several reports demonstrate the functionalities of DNA hydrogel in colorimetric sensing. Here in this section, we have short reviewed the recent studies on DNA-based hydrogel for their application and impactful role in colorimetric sensing. Earlier Baeissa and the group studied and engineered DNA-functionalized monolithic hydrogels.[78] The author utilized the well-known thiol-Au chemistry to establish DNA-modified AuNPs and load them to the DNA-modified polyacrylamide hydrogels. In the presence of the target, DNAs AuNPs adherence makes it feasible to detect visually regardless of instrumental analysis. The favorable point of this study is at even ~0.1 nM target DNA can be visually detected with the DNA-functionalized AuNPs. Similarly, this approach can easily handle in a way similar to other homogeneous assays.[79,80] Moreover, recently, Zhao et al reported a new type of DNAzyme-crosslinked hydrogel that

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contributes highly to rapid colorimetric sensing of H_2O_2 with the signal accumulation strategy.[81] here author used G-quadruplex/hemin complex to catalyze the H_2O_2-mediated oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) to form blue oxidized 3,3′,5,5′-tetramethylbenzidine (oxTMB). The DNAzymes were assembled within the hydrogel network as cross-linkers, which play a major role inside the gel to reduce H_2O_2 (Figure 4 I). These peroxidase activities resulted in a color change of TMB. Similarly, the Amine group influences the electrostatic force to interact with the DNAzyme backbones to accumulate. The author claims this novel hybrid DNA hydrogel utilization in colorimetric sensing could be the first study. Also, the DNAzyme-crosslinked hydrogels are regenerable and can be employed in the detection of H_2O_2 in environmental and food-related works.

Nanomaterial's intrinsic properties are the basic element that has drawn major attention and played a significant role in designing biosensors.[5,82,83] Similarly, nanomaterials extinction coefficients, plasmonic properties, and the surface-to-volume ratio has facilitated new designs to develop biosensors.[84,85] Recently, Liu et al have designed a simple layer-by-layer assembly strategy and developed a hybrid DNA-AuNP hydrogel film as a colorimetric biosensing system. The authors worked on DNA-AuNP hybrid hydrogel system to design a portable and storable biosensing system. The simple layer-based assembly structure is composed of densely packed AuNPs and crosslinked by DNA structures based 3D hydrophilic network which was well designed on a glass substrate (Figure 4 II). [36]

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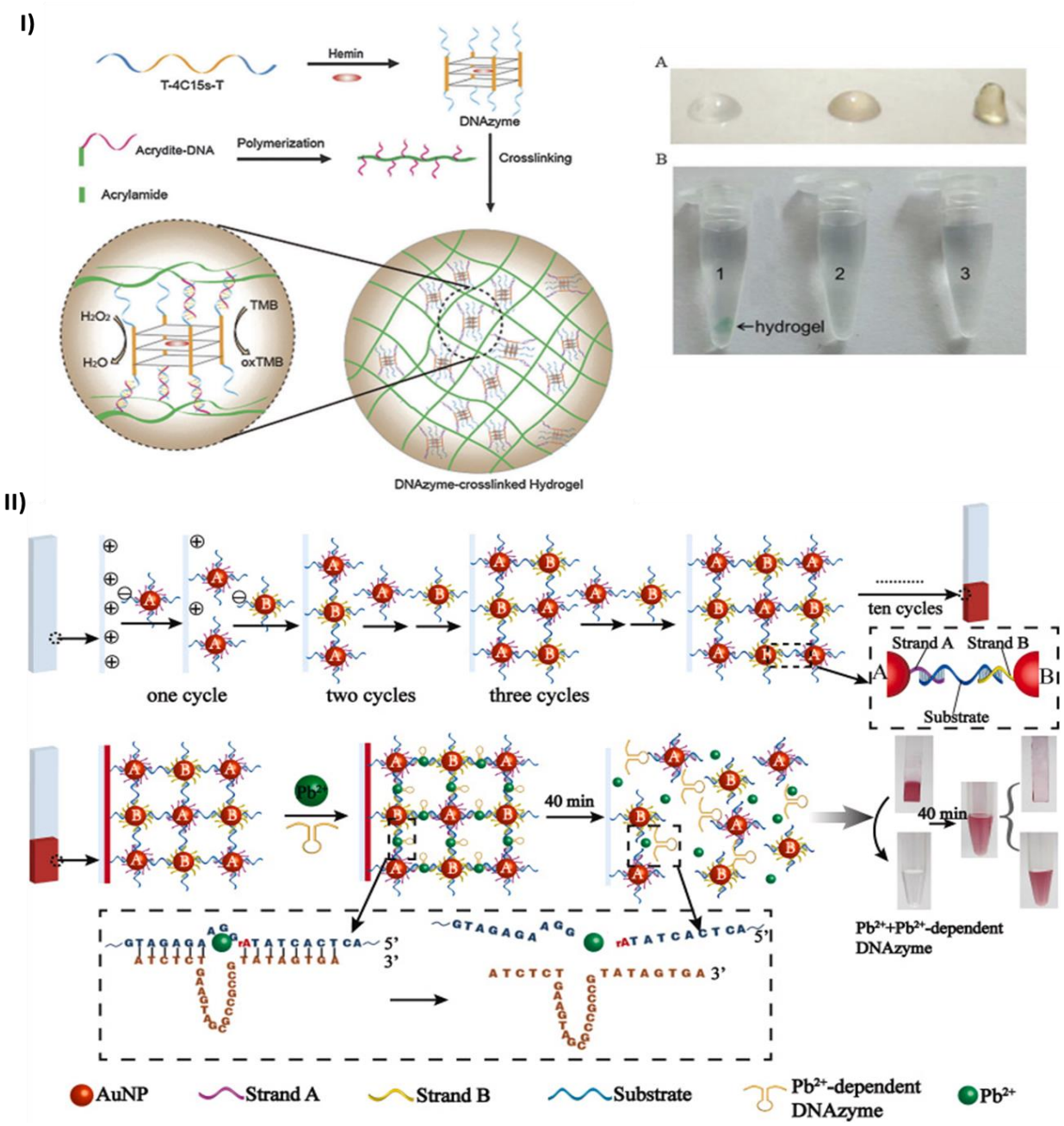

Figure 4. I) Illustration of the preparation of the DNAzyme-crosslinked hydrogel. **(A)** Photograph of DNA-branched polyacrylamide solution (left), DNA-branched polyacrylamide solution containing 4c15s-DNAzyme without poly-T sequence (middle), and the T-4c15s-T-DNAzyme-crosslinked hydrogel (right) on parafilm. **(B)** Colorimetric sensing of H_2O_2 in the presence of DNAzyme-crosslinked hydrogel (1), free T-4c15s-T-DNAzyme (1.0 mM) (2), and hemin alone (1.2 mM) (3). Reaction conditions: H_2O_2 = 60 mM, MB = 0.2 mM, 20 1C, 50 min. **II)** Schematic illustration of the preparation of DNA-AuNPs hybrid hydrogel film and the biosensing system for colorimetric detection of Pb^{2+}. (Adapted from[81] & [36]).

The selective ion address causes DNAzyme activation and leads to the degradation of DNA-AuNP hydrogel film. The ion-selective active degradation causes the release of AuNPs which further acts as the sensitive colorimetric signal read with a detection limit of 2.6 nM (Pb^{2+}) and 10.3 nM (UO_2^{2+}). The designed biosensing system was significantly used in the colorimetric detection of Pb^{2+} or UO_2^{2+}. The DNA-AuNPs hydrogel film biosensing system is highly promising for future rapid on-site detection applications.

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2.4. point-of-care

In recent years, researchers highly studied certain elements to discover, ameliorate, and evolve the term "point of care".[42]The evolution of point-of-care testing involves the requirements of stability, portability, ease of storage, and cost-effective availability. Moreover, in recent times DNA hydrogels have evolved as ideal signal transduction strategies for point-of-care functional roles. Recently, Jiang et.al proposed a facile DNA-based hydrogel capillary sensor (DHCS) for the sensitive detection of Pb^{2+}. In this study, authors have proposed a strategy that inculcates a miniature, portable, sensitive, and highly selective, visual platform for the detection of Pb^{2+} in situ measurement. The biosensor work of action is demonstrated as in the presence of Pb^{2+} the crosslinker substrate strands were parted which led the hydrogel to partially fracture. This strategy results in the behavior of the solution flowing through the capillary controlled by the hydrogel film being affected by the concentration of Pb^{2+}. Hence, quantitative detection of Pb^{2+} was achieved regardless of instrumental analysis and observed with the naked eye owing to the distance and time delay in the process. The author claims the sensor can be utilized to detect Pb^{2+} in tap water with significant reliability (Figure 5. I).[86] Earlier Zhu and the group demonstrate an aptamer-based target-responsive gel to detect non-glucose targets such as cocaine. The author designed a linear polyacrylamide with complementary aptamers and grafted two short DNAs. The study was directly proportional to the target concentration and engineered hydrogel. The work of action explains that glucoamylase was trapped inside of the gel and separated its substrate on the outside of the gel (amylose) which further resulted in the gel's collapse due to the target's adherence to the aptamers. Later, Zhu et al discover the limitations of-engineered hydrogel incorporation of gel into their earlier hypothesis and demonstrates a novel quantitative assay to address the limitations by engineering an Au core/Pt shell nanoparticle (Au@PtNPs). The mechanism briefs as by enforcing negative pressure the gel fractures and releases the (Au@PtNPs) and due to the force, the supernatant comes in contact with H_2O_2, which further decomposes into O_2. The O_2 bubbles propelled red ink into the top channel, and the ink's migration distance was proportional to the target concentration. A hydrogel-entrapped Au core/Pt shell nanoparticle (Au@PtNP) and a volumetric bar-chart chip (HV-Chip) were used to visualize and quantify the data.[87,88]

2.5. Label-free detection

Optical biosensors are used in label-free technology known as label-free detection to measure the alterations that take place when an analyte binds to a ligand immobilized on a biosensor surface.[89] The increase in global water pollution, industrial toxic waste/sewage discharge, and toxic heavy metal ions have become the main micropollutants globally. These methodologies raise concerns worldwide to address the global pollution rise and engineer the countermeasure worldwide. Analogously label-free detection of such toxic metal ions studies has become appealing. [90–93] A strategical counter based on the new label-free method for Pb^{2+} biosensing was developed by Chu and colleagues, using a one-step preparation of Pb^{2+}-responsive pure DNA hydrogel material. The substrate strand and Pb^{2+}-dependent DNAzyme strand were added to fabricate DNA hydrogel. The work of action was DNA hydrogel structure is destroyed when Pb^{2+} is present in the sample because it activates the enzyme strand in the hydrogel skeleton and causes the substrate to be cleaved. With a minimum detection limit of 7.7 nM, DNA fragments released by the hydrogel's collapse were easily measured as a signal output for quantifying Pb^{2+} concentrations. The author claims that this strategy can be applied to the field detection of toxic metal ions by altering the DNAzyme and substrate sequences. (Figure 6. I & II).[94]

The use of SPR-based techniques in biosensors are well known[95] and a similar approach with an enzyme-free and label-free surface plasmon resonance (SPR) biosensing strategy was designed by Guo et al. The authors engineered DNA self-assembly aptamer-based hydrogel with streptavidin (SA) encapsulation. Followed by ML/RARα (promyelocytic leukemia, retinoic acid receptor alpha) targeting capture probes (Cp) immobilized on the chip surface engineered Cp-PML/RARα duplex. The hydrogel nanostructure was established on the gold surface by X-shaped polymer self-assemblies through target-triggered. Following Streptavidin aptamer selective binding facilitate hydrogel

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encapsulations and designed a high molecular weight complex. The high molecular weight structures then bind to the gold surface which increases the SPR signals and provides ultrasensitive detection. Based on the studies several other studies have reported similar findings, this approach can detect targets up to the range from 100 fM to 10nM with high efficiency.[96] The study demonstrates the potential of utilization in clinical diagnosis for gene fusions with high detection capability.

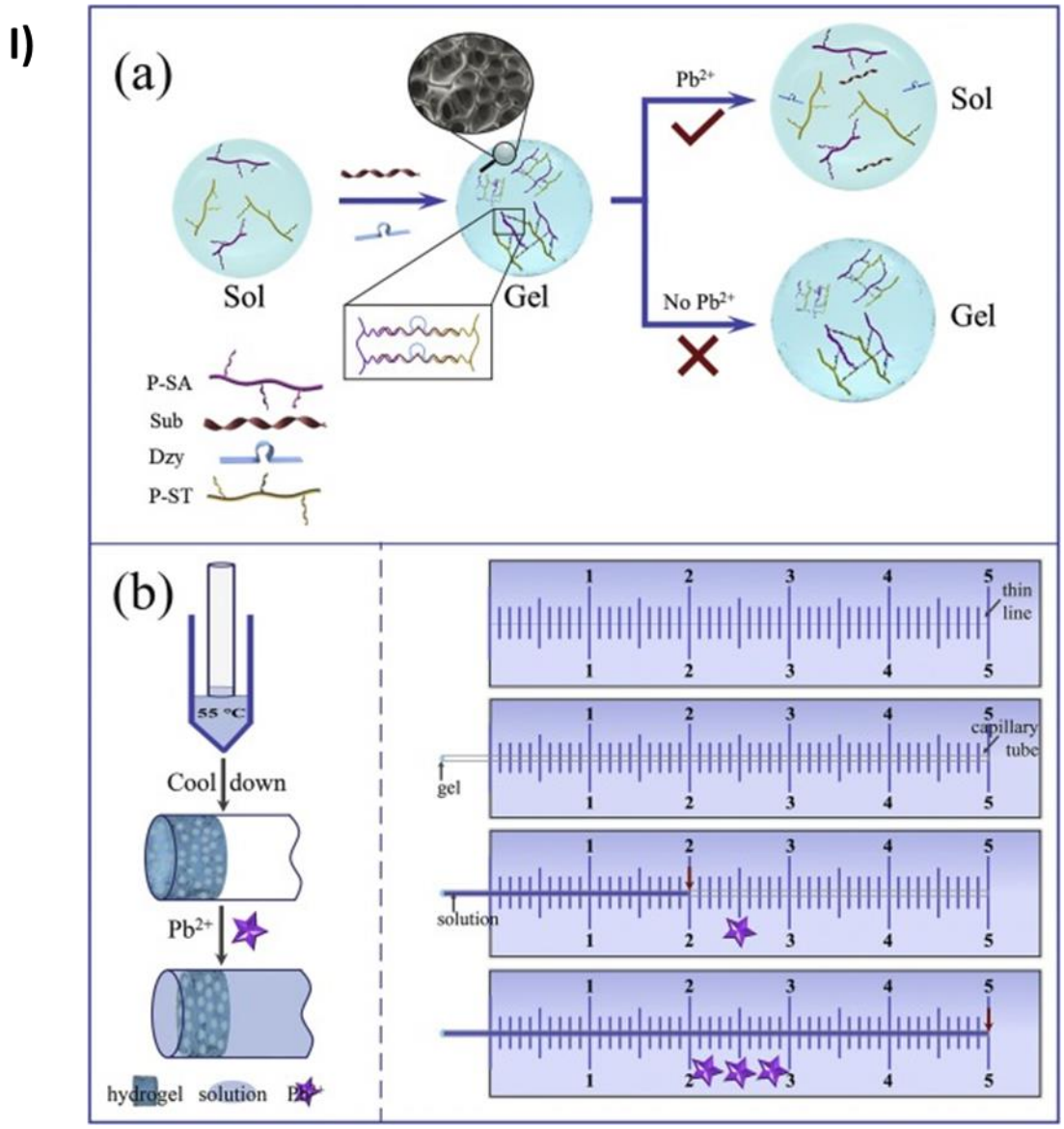

Figure 5. I) (a) Schematic illustration of the DNA-based hydrogel for the detection of Pb^{2+}. (b) Fabrication and response processes are monitored by the naked eye of the DNA hydrogel capillary sensor (DHCS). (Adapted from [86]).

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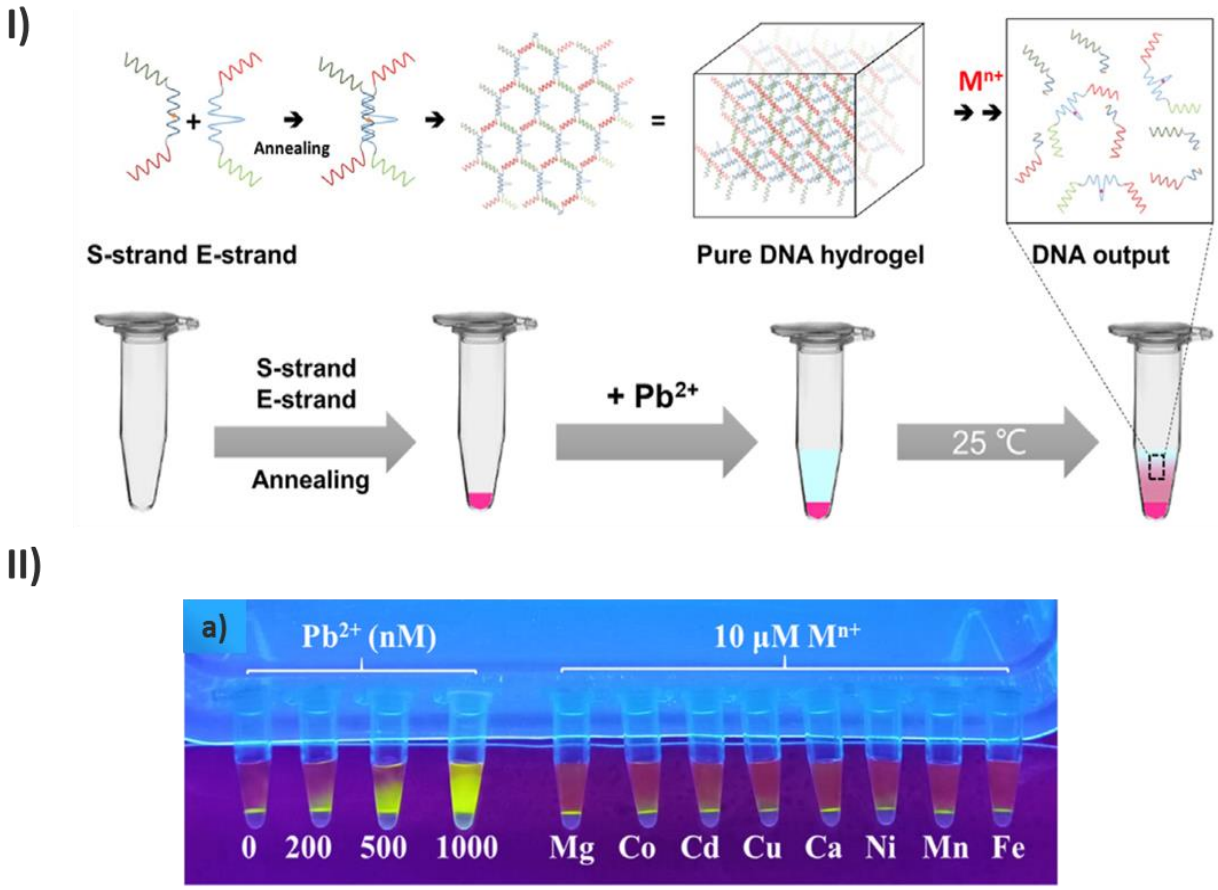

Figure 6. I) Schematic of DNA hydrogel construction and metal ion response. (A) Preparation of responsive pure DNA hydrogel and principle of metal ion detection. The E-strand and S-strand are mainly composed of complementary domains, from 5′ to 3′, respectively. DNAzyme sequence highly specific for metal ions such as Pb^{2+} was designed in the middle domain of E-strand. After the target is added to the hydrogel, it can gradually diffuse into the hydrogel and activate the DNAzyme to cleave the substrate, causing the breaking of the hydrogel. **II)** a) the visual verification and selectivity of the responsive DNA hydrogel for Pb^{2+} (Adapted from [94]).

2.6. Target responsive

Engineering of biosensors modulated with the advanced approach and followed by selective target responses are the need of urgencies and trending. Similarly, advancing such issues Tan et al designed a graphene oxide (GO) hydrogel as a fluorescent biosensor for the first time based on a target-responsive strategy to detect antibiotics presence. The author constructed a 3D macrostructure by co-crosslinking the GO sheets with adenosine and aptamer. The adenosine and aptamer were employed as the co-crosslinkers to rope the GO sheets. The method includes a simple gelation, immersion, and fluorescence determination process which led to the development of a fluorescent sensing platform via GO hydrogel. The functional role of engineered hydrogel includes high mechanical and thermal stability. The promising role of the hydrogel had the lowest detection limit i.e., limit to quantitation (LOQ) of 25 g/L. The first fluorescent GO based target responsive study showcases significant sensing for oxytetracycline antibiotics and similarly, authors hypothesize the studies could be broadened towards the other biomolecules through slight modifications in hydrogels.[97]

Next, Ma and the group studied and designed a target-responsive aptamer cross-linked hydrogel for the visual detection of glucose. The target aptamer and two short complementary DNA sequences were used as cross-linkers which were attached to a linear polyacrylamide chain to design the glucose-responsive hydrogel. The thiol-PEG-modified AuNPs were used as the output signal for obvious detection which were encapsulated in the hydrogel. Ma et al demonstrate using glucose complex one can detect glucose easily with the naked eye and along with sensor detection limits of

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0.44 mM with Uv Visible spectrophotometric analysis. The boronic acid derivatives (Shinkai's receptor) play a major role in the complex which ropes with the aptamer to deform the hydrogel and cause to release of AuNPs with an evident red color in the supernatant (Figure 7).[98] A similar study based on target-responsive DNA hydrogel with a chemiluminescent biosensor was constructed to sense adenosine. The designed DNA hydrogel carries prominent selectivity with high loading capacity. The designed AuNPs coating on HKUST-1 (Au@HKUST-1) showcases stronger peroxidase-like activity than the original HKUST-1 (HKUST-1 Cu-based MOFs having peroxidase activity). The Au@HKUST-1 was employed as a regulated chemiluminescent signal amplifier. A blend of partially complementary, acrydite-modified single-strand DNA, acrydite-modified adenosine aptamer and hemin aptamer designed the DNA hydrogel. The DNA hydrogel completely disintegrated as a result of the interaction between adenosine and an adenosine aptamer, whereas in the presence of only hemin G-quadruplex/hemin was formed and as a result, DNA hydrogel remained integrated. Followed by DNA hydrogel disintegration, Lin et al studied the release of Au@HKUST-1 and G-quadruplex/hemin in the chemiluminescent system, and dual signal amplification of chemiluminescent biosensor was enabled. The author demonstrates the adenosine target responsive detection in urine.[99]

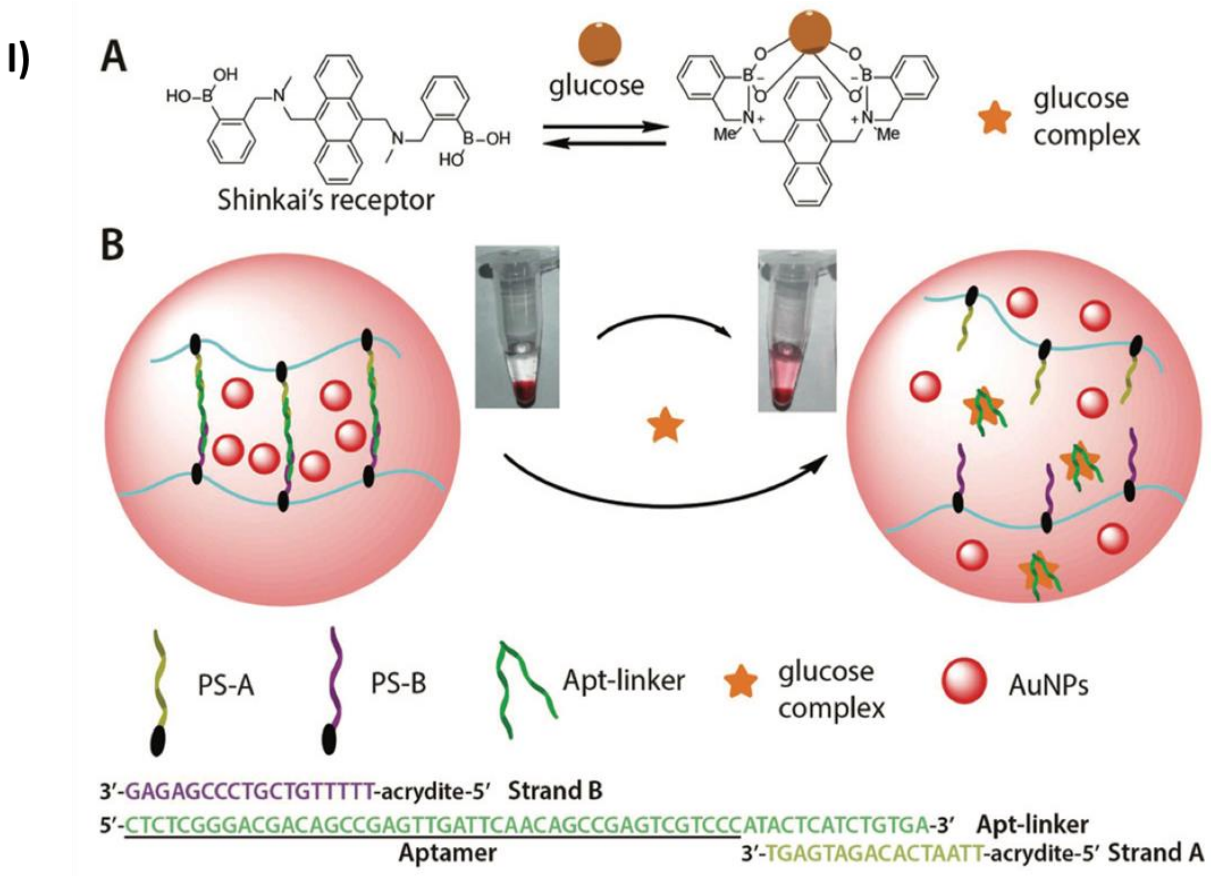

Figure 7. I) (A) Schematic illustration of the fabrication of glucose complex. **(B)** Work of action of the DNA hydrogel embedded AuNPs for the visual detection of glucose. In the presence of the glucose complex, the DNA hydrogel is disrupted, and encapsulated AuNPs are released into the supernatant. The supernatant's red color can be observed with the naked eye. (Adapted from [98]).

As earlier described the pollutant and drawbacks happening through metal ion-induced, sewage discharge, and toxic side effects in water bodies fascinate to draw researcher's attention in develop constructive actions. Huang et al engineered a DNAzyme-based responsive smart hydrogel system for the portable and quick detection of Uranium (UO_2^{2+}). Huang and the group studied the hydrogel-entrapped AuNP's role in the visual detection UO_2^{2+}. Huang et al used DNA-grafted polyacrylamide chains to crosslink enzymes and substrate strands of a DNAzyme complex to design a

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DNA hydrogel.[100] The encasing of gold nanoparticles (AuNPs) in the DNAzyme-crosslinked hydrogel and colorimetric analysis of the concentration of UO22+ was accomplished. The enzyme strand is inactive without UO_2^{2+}. The UO_2^{2+} in the sample activates the enzyme strand and causes the substrate strand to separate from the enzyme strand, which resulted in lowering the density of crosslinkers and weakening of hydrogel. The weakening of hydrogel allows the encapsulated AuNPs to be released. The author demonstrates through this strategy it is possible to visually detect UO_2^{2+} as low as 100 nM. Also, the target-responsive hydrogel worked in naturally occurring water that had been injected with UO_2^{2+}. Moreover, the authors studied and quantitively detected UO_2^{2+} through the earlier reported "volumetric bar-chart chip (V-Chip)" to avoid errors.[100–102]

2.7. DNA Hydrogels induced miscellaneous application in sensing

As earlier discussed, hydrogels oftentimes appeared as a tremendous and biocompatible element in designing biosensor systems.[103–108] Correspondingly, the conjoint approach towards a combination of biosensors and wireless technology could possibly open up the ease in diagnosing and treating medical conditions away from clinical settings.[109–113] Referring to that Xiong et al proposed the term "wireless infection detection on wounds" (WINDOW). The study demonstrates a sensing technology, which significantly detects bacteria virulence using DNA hydrogel-based, wireless, and battery-free sensor (Figure 8 I). Here, the author designed a strategy to detect an enzyme secreted by Staphylococcus aureus and Pseudomonas aeruginosa, and Streptococcus pyogenes. The enzyme, known as deoxyribonuclease (DNase) was detected by a customized DNA hydrogel base sensor that provides radio frequency followed by enzyme detections. The study incorporates animal model-based demonstration with the near-field communications that are detectable by dielectric changes through the selective detections of deoxyribonuclease in pathogenic bacteria.[114]

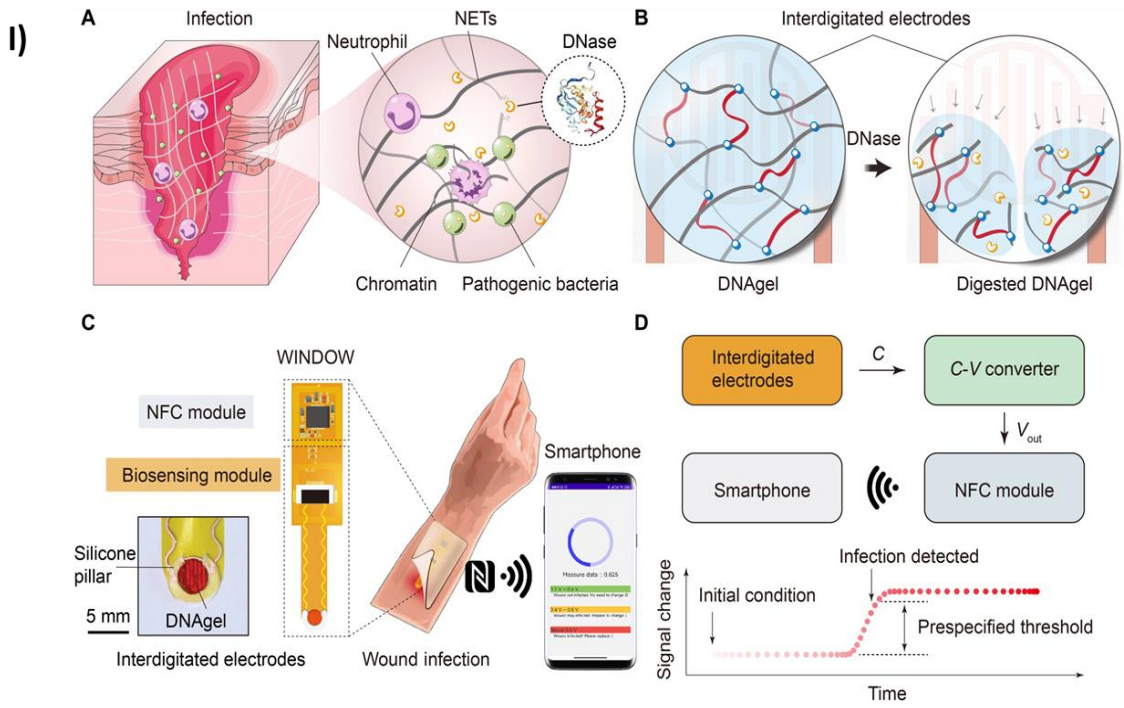

Figure 8. I) (A) DNase is a virulence factor in wound infections. Pathogenic bacteria secrete DNase to evade neutrophil extracellular traps (NETs), which are integral to the host's immune response. (B) Schematic of the infection sensing mechanism. DNA gel is degraded upon exposure to DNase, resulting in a change in the capacitance of the sensor. **(C)** Schematic of the wireless wound infection sensor. WINDOW integrates the bio-responsive DNA gel, a half-wave–rectified LC biosensing module, and an NFC module to enable smartphone readout of the wound status. Inset image: Sensor-integrated DNA gel stained with rhodamine B. **(D)** System block diagram showing signal transduction from the DNA gel-based biosensor to the NFC module and to a smartphone for wireless readout and display. (Adapted from [114]).

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3. Outlook and future directions.

In this review, we presented detailed and recent studies on the development of natural and synthetic hydrogel evolution and procurement as biosensors. We aim to provide a simplified study to reach the basic goals of 3D hydrophilic, water-rich bodies' amplified role in biomedical, label-free biosensing, target responsive, and point-of-care detection roles. We specifically demonstrate DNA hydrogels and DNA-based hydrogel's role in a diversified field that showcases DNA as a bio-susceptible, eco-friendly, easy-to-produce, and cost-effective element to construct biosensors. DNA hydrogels could result in enormous applications due to their easy adaptability and unique features, which don't limit themselves to genetic materials. DNA hydrogel studies are widely known, although specific target detection, enzyme-mediated stability, and easy conjugations with metal ions are still lacking. DNA hydrogels could evolve as potential biosensor building elements to exert multidimensional roles and functionalities. In this review, earlier we discussed several strategies and DNA hydrogel-based studies which clearly demonstrate stringent methods, lack of regenerations, and off-target oriented stringencies. The major outcomes throughout the reports were the reproducibility and lack of ease in handling the methodologies. Numerous studies on the fabrication and utilization of biosensors have been studied with spectral signal readout in *in vitro* and *in vivo*. These techniques have high sensitivity and good selectivity; however, the stability and reproducibility of biosensors are suboptimal. Similarly, reproducibility, and reuse with significant detection limits were absent. We believe our review article could ease the understanding of biosensors, their designs, and their limitations. The DNA hydrogels and also hybrid hydrogels could be engineered with the incorporation of selective DNA oligomers with ligands, carbon/QD, or aptamers which can play an advantageous role in selective and sensitive detection and quantification of targeted analytes. Also, we believe to design and construct the perfect biosensors DNA hydrogels could be utilized as a potential element. The DNA nanotechnology fields are adapting and evolving. Therefore, future outcomes can focus on robust and selective target-based biosensors with great stabilities which can be conceived with DNA hydrogels at significant potential scales.

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