

Review on Application of Gold Nanoparticles and Paper-based Microfluidic Analytical Device in Detection of Human Chorionic Gonadotropin

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Abstract – Human chorionic gonadotropin (HCG) has been amongst of essential part of medical diagnostics in gynaecology and oncology since its discovery. Application of gold nanoparticles in detecting HCG is due to gold nanoparticles possess extraordinary optical and physical properties, making them invaluable in various prominent aspects, including its synthesis, stabilization, and functionalization as a sensor. In addition, microfluidic analytical device, a platform for point-of-care testing and numerous applications in various field, can be applied for detection of HCG using diverse types of detection methods. From the literatures, combination of gold nanoparticles and microfluidic analytical device can be assembled to obtain a rapid, simple and user-friendly analytical site for detection of HCG.

Keywords— Human chorionic gonadotropin; Gold nanoparticles; Microfluidic analytical device; Point-of-care testing; Sensor.

I. INTRODUCTION

Home pregnancy testing dates back in 1350 BC during the era of Ancient Egypt, where wheat and barley seeds used to determine whether a woman was said to be pregnant or vice versa. However, after centuries passed and science modernized the world, home pregnancy testing focused on detection of human chorionic gonadotropin (HCG), the hormone secreted after successful implantation of zygote onto endometrium wall. In female reproductive system, HCG plays an important role in enrichment of endometrium wall for development of zygote. Apart from its role in pregnancy, HCG also known as a tumor marker for gestational trophoblastic diseases in women and testicular cancer in men, hence highlighting its functionality in oncology diagnostics and treatment plans.

In recent years, numerous researchers all over the world participated in an unsaid pursuit of developing novel sensors and its platform, highlighting gold nanoparticles (AuNP) as a novelty sensor and paper-based microfluidic analytical device (μ PAD) as the sensor platform. AuNP mentioned in various publications for being a versatile material for chemical sensing due to its exceptional optical, physical, and chemical properties. Meanwhile, μ PAD was sought out for fulfilling the basis of rapid and simple analysis; cheap, simple, adaptable and disposable. In this review, both materials will be united in order to develop an assay for a frequently mentioned hormone, HCG.

II. GOLD NANOPARTICLES (AuNP)

In order to produce nanoparticles, the suitable pathways and approaches in synthesizing the intended nanoparticles must be selected to ensure the desired characteristics of the nanoparticle achieved. Highlighting AuNPs, bottom-up approach is the most common and useful in synthesizing the AuNPs needed. Bottom-up approach is a way of tailoring the desired nanoparticles from the elemental form of the particles, brought together to form the nanoparticles in one dimension at the very least. Bottom-up approach is well suited for a method that uses reactants in liquid or gaseous state in synthesizing individually fine-sized nanoparticles, yet ill-suited to produce in bulk and hard to be scaled up [1].

Highlighting the aspect of synthesis of AuNP, a pioneering protocol that was reported back in 1951 mentioned ten different methods to synthesize AuNP [2]. However, citric acid method and sodium citrate method are the most frequently referred method to produce spherical citrate-capped colloidal gold due to its low stressing parameters and relatively lower risk than other methods [2]. Later in 1995, another follow-up report renewed the protocols and methods on the synthesis of AuNP [3]. As reported, AuNP synthesized were described to be spherical, measured at 10 – 13 nm in size, and spread onto one-dimensional monolayer [3]. Comparing both researches, the former research produced citrate-capped colloidal gold, measured in 20 nm in size with 1.5 nm deviations. A recent reported study improved the synthesis method, yielding quasi-spherical AuNPs with 1.8 nm diameter [4]. Certainly, the recent

study shown an exemplary improvement in synthesizing AuNPs using citrate reduction method with addition of reductant and fine-tuning the molar ratio used.

A. Stabilization of AuNP

The synthesized colloidal gold was described as spherical citrate-capped AuNP, having the citrate ions bonded to the gold surface in three different modes of coordination; monocarboxylate monodentate, monocarboxylate bridging, and dicarboxylate bridging [5]. The structure of the stabilized AuNP was shown using analysis of solid-state nuclear magnetic resonance spectroscopy and density function theory calculation. The configurations were dependent to the molar ratio of sodium citrate to gold ions used in synthesis process.

On the other hand, the bonding of citrate ions to AuNPs surface is investigated using Fourier-Transform Infrared (FTIR) spectroscopy and molecular orbital (MO) calculations [6]. From the research, it was found AuNP and citrate ion interact by forming coordination bond with citrate ion acting as a monodentate ligand to AuNP. As a comparison, the characterization of bonding of citrate ion to silver nanoparticles (AgNPs) performed to determine the behaviour of citrate ion onto different types of metal nanoparticles. Differing to AuNP, citrate ion was bonded to AgNPs via ionic bond.

FTIR analysis shown that monodentate conformation of the AuNP under plasmon effect caused the stretching of $\nu_s(\text{COO}^-)$ to be equal or less than the stretching of $\nu_{as}(\text{COO}^-)$ ($\nu_s(\text{COO}^-) \leq \nu_{as}(\text{COO}^-)$) [6]. The asymmetric stretching vibration mode of citrate ion to AuNP shown a high frequent shift. This phenomenon occurred under influence of plasmon effect and varies among metallic salts and nanoparticles [6]. The monodentate bonding of citrate was proven from MO calculation as MO calculation result stated only one oxygen atom from the carboxyl group in citrate ion anchored to the gold surface.

B. AuNP as novelty sensors

In the recent years, researchers have been developing a variety of chemical sensors with high sensitivity, selectivity, and low limit of detection. Among those years, citrate-capped colloidal gold is proven useful in developing novelty sensor, especially for detection of biological compounds in medical field. In order to slave the AuNP for the detection purposes, the surface of AuNP tailored with its appropriate conjugate compound, such as antibody or proteins. **Error! Reference source not found.** states the compounds successfully detected with modified AuNP along with its working mechanism in detecting miscellaneous compounds.

Table I shows the detection of various compounds using AuNP along with its detection mechanism. From the table, AuNP can undergo several ways to give out spectral response depending on the surface modifier interaction with the target compound.

TABLE I
DETECTION OF VARIOUS COMPOUNDS USING AuNP ALONG WITH ITS DETECTION MECHANISM

| Target compound | Modification of AuNP | Detection mechanism | References |
|-----------------------------|-----------------------|--------------------------------------|------------|
| Low molecular weight thiols | Glutathione disulfide | Changes in aggregation state of AuNP | [7] |

| | | | |
|--|---|--|------|
| Cholera toxin | Monoclonal cholera antibody | Changes in aggregation state of AuNP | [8] |
| Biothiols (cysteine, glutathione, glutathione disulfide) | Cetyltrimethyl ammonium bromide, sodium borohydride | Changes in aggregation state of AuNP | [9] |
| Tumour markers (CEA, CYFRA21-1, NSE, DKK1) | Anti-NSE, anti-CEA, anti-CYFRA21-1, anti-DKK1 | Sandwich ELISA | [10] |
| α -L-fucosidase | Polyclonal antibody | Energy transfer between carbon dots and AuNP | [11] |
| Deoxyribonucleic acid | TCEP-treated thiolated DNA | Changes in aggregation state of AuNP | [12] |

One of the mechanisms of detection AuNP undergo to give out signals is using sandwich enzyme-linked immunosorbent assay (ELISA), where the target compound sandwiched between a specified antibody intended for the current immunoassay, and a marker to label the target compound via colorimetric or luminescent dyes, in this case, colour change of AuNP. The sandwich ELISA commonly used in immunoassay of proteins sourced from bodily fluids for detection or quantification of target compound in analytes. In the scope of AuNP, tumour markers of lung cancer can be detected in a colorimetric microarray [10].

Apart from sandwiching the target compound with antibody and marker, the target compound also can be sandwiched with metal nanoparticles and quantum dots with functionalized surface for high sensitivity detection and fluorescence emission signal. This concept was performed for detection of tumour marker of hepatocellular carcinoma, using antibody-functionalized AuNP and carbon dot probes. The immunoassay was based on the energy transfer between AuNP and carbon dot probes, causing the quenching of carbon dot probes and emission of photons, having wavelength at 460 nm. Since the degree of quenching of carbon dot probes is correlative to the concentration of the tumour marker, the tumour marker can be quantified in this immunoassay [11]. Quantifying tumour markers is essential for post-treatment planning as medical personnel used to determine the presence of remaining cancerous tumour in the body. Decreasing level of tumour markers can indicate successful treatment, thus highlighting the value of quantification of tumour markers.

Another mechanism of detection used by AuNP nanosensor is colour changes via changes in the aggregation state. The colour changes in AuNP nanosensor occurred upon the presence of analytes that interact with AuNP via intermolecular electrostatic bonding and hydrogen bonding, influencing the distant dependent properties of AuNP that affected the aggregation state of AuNP [9], [10], [12]. In practicality, low molecular weight thiols were able to be detected via changes of aggregation state of AuNP modified with glutathione disulfide (GSSG) through disulfide bond breaking [7]. Using disulfide bond breaking to achieve change of aggregation state can be advantageous due to its low

synthesis cost, relatively low stress parameter used and able to achieve selective reaction as thiols present in hydrocarbon chain of HCG can cleave the disulfide bonds between AuNPs [13], [14].

Clustering of AuNP due to functionalization with GSSG have contrasting surface plasmon properties compared to dispersed AuNP, can provide spectral and optical signal for using UV-Vis spectroscopy analysis to observe the spectral shift of surface plasmon resonance.

III. PAPER-BASED MICROFLUIDIC ANALYTICAL DEVICE (μ PAD)

A. μ PAD as point-of-care testing kit

In medical field, μ PADs are highly sought out for its versatility in providing point-of-care testing platform due to its light weight, simple, easy to dispose, gratuitous of high cost and adaptable [15]–[17]. Point-of-care testing also commonly known as bedside testing, refers to medical diagnostics testing done at the time or location of the patient’s care for inquiry of patient’s health history. Comparing to conventional sample testing, the conventional method requires the sample to be sent to a relatively far away analytical laboratory and consumes a long time due to the long sample queue and the analysis itself. Therefore, majority of personnel involved in analysis of bodily samples prefers the usage of μ PADs for its economic value and rapid results per required [16], [17].

Numerous analysis can be done in a matter of minutes, if not seconds, to inquire the patient’s history. Due to its simplicity in its usage, various analysis has been developed, including blood sugar level, cholesterol level, and much more, described in Table II.

TABLE III
APPLICATIONS OF μ PADs AND LATERAL FLOW STRIPS (LFS) IN MEDICAL FIELD [17]

| Application | Analytes | Platform | Detection method | References |
|-------------|--|----------------|------------------|--|
| | Glucose, cholesterol, lactate | μ PADs | CD, ECD, CLD | [18]–[38] |
| | Uric acid, ascorbic acid | μ PADs | CD, ECD, CLD | [23], [27], [28], [31], [34], [39], [40] |
| | Human chorionic gonadotropin | LFS μ PADs | CD, ECD | [41]–[42] |
| | Prostate specific antigen, carcinoembryonic antigen, α -fetoprotein, circulating tumor cell, CA125, CA199 | LFS | CD, ECD, CLD | [41]–[48] |

CD: Colorimetric detection; ECD: Electrochemical detection; CLD: Chemiluminescence detection

Error! Reference source not found.II summarizes the applications of μ PADs and LFS in medical field, highlighting in general health and oncology diagnostics. The analysis done for detection of the analytes applies three detection methods; colorimetric detection, electrochemical detection, and

disulfide bond breaking. The same principle also pertain to detection of cholera toxin, using monoclonal cholera antibody functionalized onto surface of AuNP [8]. Due to high specificity of monoclonal cholera antibody to the toxin, the antibody-toxin bonding caused the antibody-conjugated AuNP amassed into a network of colloidal gold. The shifting in aggregation state can be observed visually and measurable chemiluminescence detection. All three detection methods render out signal corresponding to the concentration of the analyte in the sample. The signals given out depends on the detection method, whether in form of transmitted visible light, electron transfer from redox reaction, and photons from luminescence emission. The detection methods fitted the criteria of detection on μ PAD, having high selectivity and specificity, colored and fluorescent indicators, and requires simple to no instruments for further analysis on the μ PADs [49], [50].

B. μ PAD as point-of-care testing kit

μ PADs are one of many unique analytical platforms due to its adaptability to perform various analysis by simply tailoring the sensor in the output region of μ PAD to match the requirement of the analysis. Due to this fact, abounding number of researchers developed a diversity of novelty sensors, ranging from staple colorimetric markers to fluorometric nanosensors [43], [48], [51]. **Error! Reference source not found.**III shows various analysis done using μ PADs to show the application of different sensors for specific analysis.

TABLE IIIII
APPLICATION OF DIFFERENT SENSORS FOR SPECIFIC ANALYSIS USING μ PADs

| Target compound | Source | Sensor | Reference |
|---|------------------------|--|------------|
| Transition metals (Cu, Ni, Fe, Cr) | Welding fumes | Fe: Ferriox complex Ni: Magenta complex with dimethylglyoxime Cu: Bathocuproine Cr: 1,5-diphenylcarbazide and phthalic anhydride | [51], [52] |
| Copper ions | Water sources | Hcy-DTT-AgNP | [53] |
| Metallic salts (Pb, Ba, Sb, Fe, Al, Zn, Mg) | Low explosives residue | Pb and Ba: sodium rhodizonate, sodium bitartrate, tartaric acid Sb: sodium sulfide Fe: p-aminophenol Al: ammonium acetate and aluminon Zn: dithizone Mg: xylylidil blue | [54] |
| Hypochlorite | Household bleach | Potassium iodide | [55] |
| Creatinine | Blood sample | Picric acid | [56] |
| Benzoic acid | Food | Sodium hydroxide | [57] |

As aforementioned, the sensors used on μ PADs ranges widely, from classical usage of picric acid for detection and quantification of creatinine in blood sample via Jaffe reaction, to modified silver nanoparticles (AgNP) with homocysteine and dithiothreitol for colorimetric detection of copper ions Fig. 1 [51], [54].

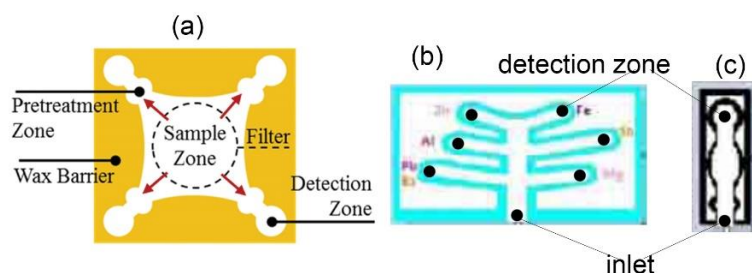


Fig. 1 Different designs of μ PADs for achieving multiplex analysis and singular analysis: (a) four-outlet with pretreatment zone and multiple simultaneous analysis [51], (b) six-outlet branched out for simultaneous analysis [54], (c) single outlet for singular target analysis [54]

Fig. 1 shows different designs used by several researchers to achieve multiplex and singular analysis. Having multiplex analysis ability on μ PAD can save a substantial amount of time on analysis by performing the analysis once to obtain various results, instead of multiple times to do analysis for each target compound in the sample.

IV. APPLYING AuNP AND μ PAD IN DETECTION OF HCG

A. Employing AuNP for detection of HCG

Recently, AuNP has been discovered to be useful in developing immunoassay of HCG through colorimetric detection and reported to have different modes of surface modification to accommodate the binding of HCG to AuNP. Differing to ELISA, most researchers reported of not employing enzymatic action, but linkage of antibodies and peptide aptamers to HCG via intermolecular bonding [7], [52], [58]–[62].

Using specific antibodies with highest affinity towards a certain protein is a staple and predominant method for detection of the said protein. HCG also has been found to be detectable using AuNP modified with anti-HCG- β mAbs for high selectivity and sensitivity of the assay [61]. The research also studied on the effects of varying AuNP sizes on the detection performances, reported that LOD values of the modified AuNP decreases along with its size, proving that medium-sized AuNP (<100 nm diameter) gave out higher detection sensitivity because of its high molar extinction coefficient and strong affinity to HCG. Meanwhile, oversized AuNP (>100 nm diameter) reported to be performed inaccurately in terms of giving out signals as larger-sized AuNPs was presumed to repress the efficiency of immunodetection reaction due to its high steric hindrance and strong light scattering [61], [63]. High steric hindrance from large AuNPs caused the absorption to become weaker due to less interaction from antibody and HCG [61], [63], [64].

Aptamer-based method also recently adapted for detection of HCG due to its specificity and affinity to the target compound, and high stability in diverse media [60], [65], [66]. Although aptamer-based methods scarcely reported on being a novelty sensor, aptamer is proven to have several advantages

[53], [56]. The ability of μ PADs to perform multiplex analysis also shown in the analysis of welding fumes and low explosives residue to determine the quantity of metal content within the analytes, which depends on the design of μ PAD and the number of sensor placement on the μ PAD as shown in

over antibodies, including low cost and uncomplicated manufacturing [65], [66]. Conjugating with polymeric dendrimer also possesses the similar edge to aptamer as it shown high linearity and sensitivity on HCG detection [58]. Several studies using AuNPs reported HCG detection using antibody-free both electrochemical and colorimetric detection assay using functionalized AuNPs [59], [60], [67]. Adapting non-antibody sensing moiety on AuNPs reduces the cost of producing the sensor and able to achieve high selectivity for HCG detection as antibody-based detection. Hence, usage of antibody-free detection assay for HCG is recommended as it holds competitive advantages compared to antibody-based methods.

B. Utilization of μ PADs

Detection of HCG using paper-based analytical device has been recorded ever since the development of commercially available pregnancy test strip kits. The test strips also known as lateral flow strips (LFS), utilizing immunochromatographic assay, using monoclonal anti-HCG antibody for highly selective detection [68]. The earliest LFS for detection of HCG fabricated and commercialized in 1978 due to discovery of highly selective monoclonal antibody and development of facile and low-cost immunoassay for domestic pregnancy testing [69]. Developed LFS for domestic purpose works via sandwich ELISA of binding of mobile anti-HCG antibody (Ab1) and immobile anti-HCG antibody (Ab2) while having HCG in between. The binding of both antibodies results in production of a distinct dye from enzymatic reaction occurred. Figure 2 describes the indications of test results from commercial LFS.

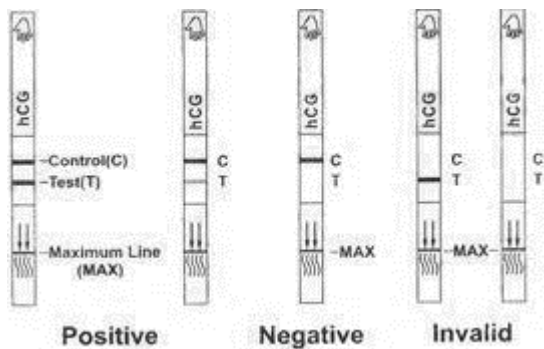


Fig. 2 Types of results from commercial LFS for detection of HCG in urine samples [70]

Positive results were achieved when both control and test lines were present, albeit the test line were less visible or faint looking. Usage of LFS was limited to its sensitivity on HCG level present in the used sample, causing false negatives as the sensing component has higher detection level and unable to detect HCG below the detection level [71].

μ PADs also have its fair share in detection of HCG as a platform for various types of sensor, as numerous biosensor has been developed, including AuNP-based nanosensor for detection of HCG exclusively on μ PADs. AuNP-based nanosensor used on the μ PADs works on either sandwich ELISA or electrochemical detection, depending on the surface modification of colloidal gold and fabrication of μ PAD [68], [72].

On another report, μ PAD used for electrochemical detection utilized differential pulse voltammetry to study the electrochemical responses generated from catalytic oxidation of p-nitrophenyl phosphate via alkaline phosphatase conjugated to immunocomplex of HCG. As a result, the microfluidic paper-based electrochemical immunosensor was reported to detect HCG as low as 0.36 mIU/ml (0.0146 ppm) with linear range of 1.0 mIU/ml to 100 IU/ml (0.0404 – 4043 ppm) [18]. This shows microfluidic sensing device can achieve high sensitivity HCG detection, as lowest detected level of HCG in healthy human body is 0.2021 ppm. Thus, the future project to integrate the application of AuNP and μ PAD can enhance and assist in pregnancy testing and diagnostics in cancer and tumor.

V. CONCLUSIONS

AuNPs have been truly valuable in detecting various compound, including HCG. In order to augment AuNPs for HCG detection, using improved citrate reduction method to produce AuNPs with suitable characteristics is indeed paramount. Various types of detecting moiety can be functionalized onto AuNPs for application of AuNP-based nanosensor on μ PAD. Furthermore, the assay requires further analysis for quantification of HCG in sample. Simplicity and convenience of usage of μ PAD can provide the point-of-care testing site needed by both medical and non-medical personnel for easy and fast pregnancy test kit. The fusion of AuNP-based nanosensor and μ PAD is notable, that it may achieve the need of simple, rapid, user-friendly analytical device for detection of HCG, both for pregnancy testing and tumour marker detection.

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