GOLD NANOPARTICLE AS AN ALTERNATIVE TOOL FOR A URINE PREGNANCY TEST

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SUMMARY

Objective: The urine pregnancy test is an easily available diagnostic test in the present day and is routinely performed. The test is based on an immunochromatography technique. Here, we used an advanced nanomedicine technique for modification of the urine pregnancy test.

Materials and Methods: The preparation of gold nanoparticle solution in this study followed the standard method. We performed an experiment on both pregnancy-positive and -negative urine samples. First, a mixture with an equal amount (500 μL) of gold nanoparticle solution and urine sample was prepared. Then, it was further tested for pregnancy by the urine pregnancy test strip.

Results: The pregnancy-positive mixture became pink, while the pregnancy-negative mixture became gray. The urine pregnancy test strip for a positive mixture had two lines, while the negative mixture had one line.

Conclusion: This application can help the diagnosis of pregnancy and can be an alternative method for a urine pregnancy test. To our knowledge, this is the first report on this application. [*Taiwan J Obstet Gynecol* 2008;47(3):296–299]

Key Words: gold, nanomedicine, pregnancy test, urine

Introduction

The urine pregnancy test is an easily available diagnostic test and one which, due to its ease of use and low cost per test, is widely used nowadays.

Because of the simple immunologic principle [1–3], many urine pregnancy diagnostic tests have been produced [4]. Generally, the urine pregnancy test measures human chorionic gonadotropin (hCG), which is secreted by the trophoblastic cells of the placenta soon after implantation of the fertilized egg into the uterine wall [5].

The routine urine pregnancy test is based on an immunochromatography technique. Here, we used the advanced nanomedicine technique for modification of the urine pregnancy test. It is interesting to note that gold nanoparticles can be a useful alternative method for a urine pregnancy test.

Materials and Methods

Preparation of gold nanoparticle solution

The preparation of gold nanoparticle solution in this study was according to the standard method reported by Frenkel et al [6]. The size of the gold nanoparticles was stabilized at 9 nm.

Experimental study

This study was a laboratory experiment. The leftover samples from routine urine pregnancy tests were used.

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We performed an experiment on both pregnancy-positive and -negative urine samples. In this study, the positive urine sample contained an hCG level equal to 50 mIU/mL. First, a mixture of equal amounts (500 μL) of gold nanoparticle solution and a urine sample was prepared. The urine was then further tested for pregnancy using a urine pregnancy test strip (WH Accu Test; WHPM Inc., El Monte, CA; the sensitivity of the test is 100%).

Determination of the performance characteristics

The performance characteristics of our new gold nanoparticle system were examined for accuracy, specificity, sensitivity, precision, interfering substances, expected value, calibration, and limitations according to the guideline protocol provided by the United States' Food and Drug Administration in 2001. For analysis of accuracy, specificity and sensitivity, 2,100 samples were analyzed per parameter. For precision analysis, 96 samples were analyzed at the same time. For stability, repeated analysis was performed for 1 week. For interfering substances, the effects of glucose and ketone were tested. To study the expected value, calibration and limitations of the system, the detection limit was determined by UV-visible titration between gold nanoparticle solution and hCG-added urine. First, the UV-visible absorption spectrum of gold nanoparticle solution was recorded. Synthetic urine with various concentrations of hCG was then added and the UV-visible absorption spectra of each addition were recorded consecutively. UV-visible absorption was measured using a Varian UV-visible spectrophotometer with CARY 50 probe (Varian Inc., Lincoln, NE, USA). All tests were performed under standard chemistry laboratory conditions at the Faculty of Science, Chulalongkorn University.

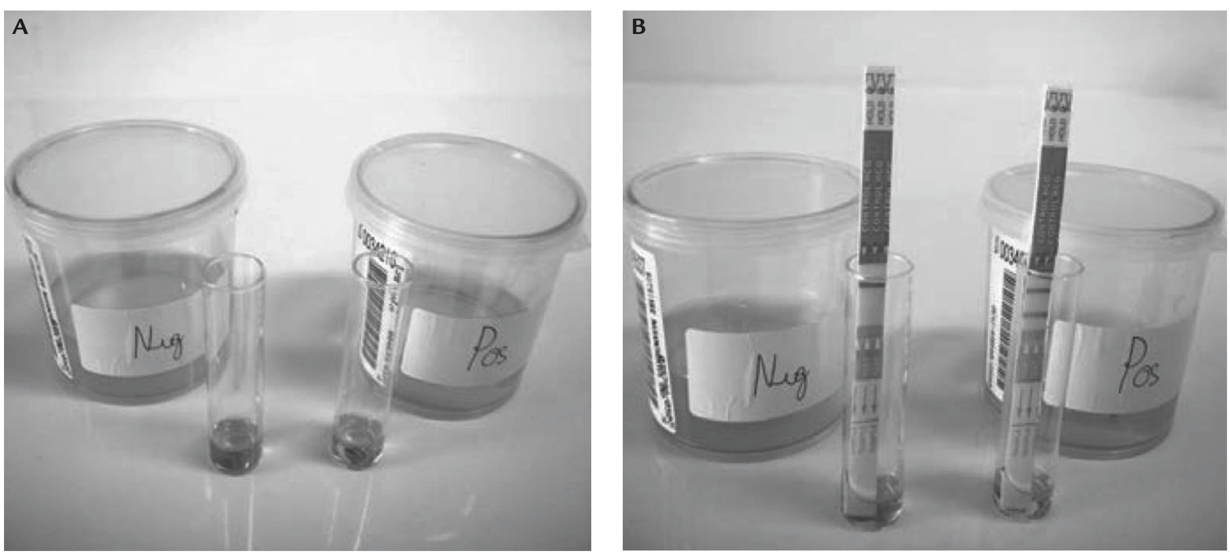

Figure 1. The mixtures of gold nanoparticle solution and positive and negative urine samples. (A) Color difference between positive and negative urine samples. (B) Confirmation by urine pregnancy strip test.

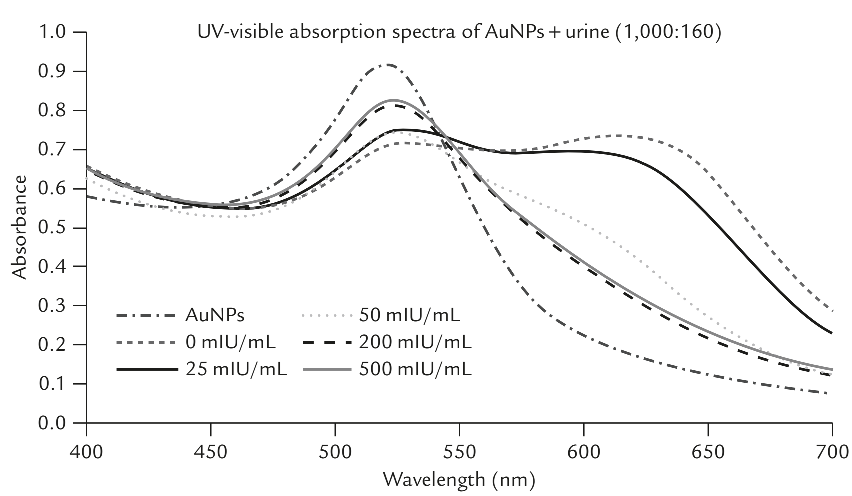

Figure 2. Results from UV-visible absorption spectroscopy. AuNPs = gold nanoparticles.

Results

The positive and negative urine samples were confirmed by urine pregnancy strips (two lines for positive and one line for negative). The mixtures of gold nanoparticle solution and positive and negative urine samples were prepared. The pregnancy-positive mixture became pink, while the pregnancy-negative mixture became gray (Figure 1). The urine pregnancy test strip for the positive mixture had two lines, while the negative mixture had one line.

The results of the examination of performance characteristics, accuracy, specificity and sensitivity were 100%, 100%, 100% and 100%, respectively. For stability, the test was stable over the 1-week test period. For interfering substances, there was no effect of glucose and ketone on the system. According to the study on expected value, calibration, and limitations, from 50 mIU/mL of hCG in urine and higher, the color of the gold nanoparticle solution did not change to gray, as shown in Figure 2. Hence, the detection limit of hCG in urine by this method is 50 mIU/mL. With respect to precision analysis, precision was equal to 100%. To study the precision of the test, urine containing 50 IU of hCG was mixed with gold nanoparticle solution at the same ratio as described in the experimental study in a 96-sample well plate for determination of color change, and no color change could be seen. In addition, at the studied level ranging from 0 to 500 mIU/mL, the linearity of the test could be observed at 25 to 200 IU/mL (Figure 3). Considering the minimal amount of urine and gold nanoparticle solution necessary for the test, at least 100 μL of samples and gold nanoparticles solution are required for formation of a visible color dot in the smallest well plate (Figure 4).

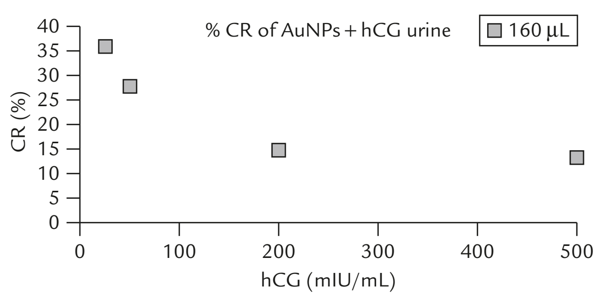

Figure 3. Linearity of the test. AuNPs = gold nanoparticles; CR = colorimetric response; hCG = human chorionic gonadotropin.

Discussion

In this study, we report success in application of gold nanoparticle solution in a pregnancy test. The significant difference in the color of the mixture between pregnancy-positive and -negative urine samples is a new finding in obstetrics and gynecology. The gold nanoparticle has many interesting properties. An important property is the change of color due to the aggregation of the particles [7]. This can be applied to diagnostic tests in medicine. In this situation, the specific binding between the positive charge within the particle and negative charge within hCG in pregnancy-positive urine samples can be expected, while this reaction will not occur in negative urine samples. This is believed to be the explanation for the difference in color (Figure 5). The proposed chemical reaction is shown in Figure 6.

This study had also confirmed the theory that metallic nanoparticles can offer control over the size and protection against aggregation with relation to color change [8]. According to this work, at least 100 μL of sample and gold nanoparticle solution are required for formation of a visible color dot in the smallest well plate. This is the reasonable level for visualization by human beings, and this technique requires a significantly

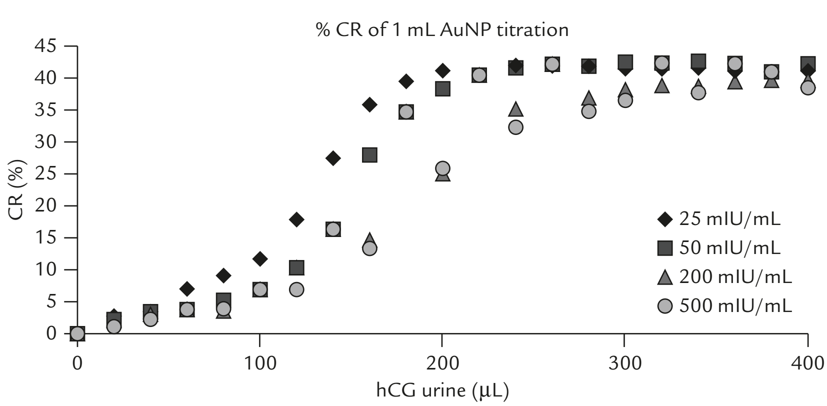

Figure 4. Titration for the minimal amount of urine and gold nanoparticle (AuNP) solution necessary for the test. CR = colorimetric response; hCG = human chorionic gonadotropin.

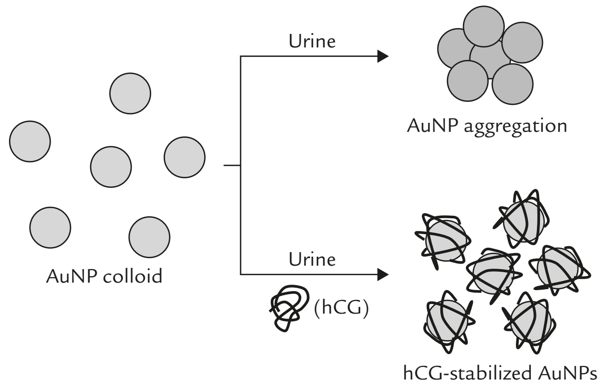

Figure 5. Diagram showing the reaction between human gonadotropin (hCG) and gold nanoparticle (AuNP).

$$HAuCl_{4(aq)} + Na_3(citrate)_{(aq)} \rightarrow AuNPs$$

Figure 6. Proposed chemical reaction. AuNPs = gold nanoparticles.

lower amount of urine than routine urine pregnancy tests (2 mL). In addition, if a microscope is used, less than 100 μL of sample can also be applied. A similar colorimetric system for blood determinants was proposed in February 2007 [9].

This application can help the diagnosis of pregnancy and can be an alternative method for a urine pregnancy test. It should also be noted that the gold nanoparticle is cheaper than the urine strip test. The cost of the gold nanoparticle (about US$0.01 per test in Thailand) is 150 times cheaper than the urine strip test (about US$1.5 per test in Thailand).

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