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Improvement Of Sensitivity Of Antigen-Antibody Detection of Semen Through Gold Nanoparticle.

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ABSTRACT

Due to their specific properties, nanoparticles have received a considerable interest in various fields including biology, medicine and, recently, inforensic sciences. Most of evidence encountered in biomedical area of forensic sciences include sexual assault, hence, the ability to accurately detect semen (with/without sperm) in such cases is an essential component in serological tests. PSA is a marker for semen detection with a concentration very higher than those of other body fluids in mature men's semen, soonly the presence of PSA in the vagina can indicate the presence of semen. Today, the success of nanoparticles in the diagnosis and regulation of various diseases and molecules can also be used to detect this social disorder. Gold nanoparticles (AuNPs, 35 nm) synthesized from gold salt by Turkevich method were conjugated to anti-PSA antibody by covalent binding and detected PSA-containing solution. This interaction was examined through color imetric change in the colloidal sample resulting from a change in absorption spectra at the time of a new structure development, an also from spectrophotometric absorption spectra. A color change from blue to red in the nanoparticle solution indicated the aggregation of anti-PSA conjugated AuNPs around the PSA. In addition, a high amount of PSA in the solution changed the absorption spectra of the sample. This colorimetric biosensor can be applied as an accessible and rapid method for PSA detection in forensic medicine, which will minimizefalse positives due to a high specificity.

Keywords: Sexualassault; Gold-nanoparticles; antibody; PSA;

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INTRODUCTION

The development and application of genetic science in the last 20 years have resulted in fundamental changes in forensic sciences. Most of evidence encountered in the biological area of forensic medicine often include sexual assault, hence, accurate detection of semen is an essential component in serological tests in such cases (1). International statistics suggest that one out of five woman around the world is raped or threatened with sexual traumas (2). This social disorder affects both physical and psychological issues. Women and girls who have been raped are emotionally depressed rather than being injured physically, for whom it takes a long time for forgetting about and consolation of this event. In this regard, it is highly important to make an accurate and reliable detection on the incidence of such a harsh event. If this incident can be proven with a high reliability, it will be possible to pursue a suspect and eventually execute a court order.

Positive detection of seminal fluid can be a very important evidence for confirmation of a claim on rape. A simple test require se valuating the amount of the enzyme acid phosphatase, which is present in high concentrations in seminal fluid. Other body fluids (e.g. saliva and vaginal secretions)contain low concentrations of this enzyme, so yielding a positive result (3). Protein P30, a prostate-specific antigen(PSA) also known as gamma-semen protein or Kallikre in 3, is another marker of seminal fluid detection. It is a 34KD glycoprotein enzyme coded by KLK3 gene on chromosome 19. PSA is secreted by the epithelial cells of the prostate gland and also produced to ejaculate the sperm and move it toward the uterus (4).

PSA physiological performance is to solubilize coagulum, i.e.a sperm covered by a gel containing semenogelin and fibronectin. PSA proteolytic activity fluidizes the coagulum resulting in an easier spermmobility. PSA was first reported by researchers seeking for identifyinga substance in the semen to detect rapecases. Concentration of PSA in the semen of adult malesis very higher than those of other body fluids, soonly the presence of PSA in the vagina can demonstrate of the presence of semen (5). PSA is now used as a marker to detect sexual assaults, which need expensive complex devices with typicallya low sensitivity.

The use of PSA for the reaction is superior than acid phosphataseas PSA is produced in association with sperm production, thus, it can be used for both spermic and azoospermic samples. A final test for semen fluid includes colorimetric preservation rendering the stained sperm visible under a high-resolution microscope. The commonly used stains are haematoxylin and eosin (H & E) and Christmas tree (6).

Techniques and diagnostic devices act more accurately, reliably and efficiently over time with scientific advances and application of such sciences as genetics, forensic, and medical biotechnology, the latter being regarded as one of the most effective areas in forensic medicine. Through application and development of advanced technology as well as identification of biomarkers, this science has been able to unveillots of molecular wonders in human. In this regard, medical biotechnology has been able to diagnose many types of cancers and viral/microbial diseases using techniques and markers (7). The latest related news announce the success of using nanoparticles in the diagnosis and regulation of various diseases and molecules. Designing a diagnostic kit using nano science and biotechnology can be very effective in the diagnosis of this social disorder.

The presence of PSA in semen can be detected by nano diagnostic kits in medical biotechnology with naked eye only without the need for expensive laboratory devices(8).

The size of nanoparticles approximates 1-100 nm in diameter, which are good markers in designing biosensors because many assessment methods such as absorption spectra, fluorescence, Raman scattering, magnetic force and electric current can be used to detect them. These particles are used in the diagnosis of DNA, protein, microorganisms, etc. (9). Gold nanoparticles (AuNPs) are among those commonly used in medical sciences.

The optical and thermal properties of separate and complex AuNPs probes are used as a diagnostic method. A specific interaction between oligonucleotides(DNA probe) stabilized on AuNPs and target DNA causes aggregation of interconnected network of AuNPs resulting in a color change. Such a color change results from the scattering characteristics, interaction between particle surface plasmons, and a change in the spacing of AuNPs, which indicates the presence of target molecule in the sample, whichis also visible optically (10).

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Today, an advantage of AuNPs compared to other nano particlesis their easy binding to biological molecules. AuNPs are capable of covalent binding to proteins forming a solid bond (11). In this report, PSA was detected using size-specific AuNPs coupled with anti-PSA.

The use of AuNPs-anti-PSA construct will have a great potential for detecting the presence of PSA and can play a significant role in many sexual offenses.

MATERIALS AND METHODS

In this study, AuNPs were first synthesized by Turkevich method (12) using gold salt HAuCl4 (Sigma-Aldrich) and sodium citrate(Sigma-Aldrich). Next, the synthesized nanoparticles reached the desired concentration. Then, anti-PSA (Sigma-Aldrich) was also diluted to 14mg/ml. The conjugation of anti PSA-AuNPs was always carried out under the same conditions, temperature and pH, making a covalent binding. The antibody (100 µL) was added to the nanoparticle solution (1 ml) and placed on a rotator at room temperature for 20 min, followed by centrifugation (140000 rpm, 4° C, 20 min) to remove additional antibodies. The precipitate was dissolved in a preservation buffer. Conjugated AuNPs are stable and can be stored in the refrigerator.

A prepared PSA solution (1mg/ml, Sigma-Aldrich)was used as a PSA-containing sample. There after, the prepared PSA solution containing antibody-conjugated AuNPs was added to the above solution to examine the interaction between antibody-conjugated AuNPs and PSA.PSA bindsits specific antibody via hydrogen bonds, the specificity of which increases by other weaker attractions such as van derWaals, electrostatic, and hydrophobic (13).

Findings

In the present research, AuNPs in three sizes of 20, 10 and 35 nm were prepared in aqueous medium using Turkevich method. The final solution color and absorption spectra were the most important factors determining the size of AuNPs (Fig. 1). The color of AuNPs varies with different sizes, and the absorption of nanoparticles also rises with increasing size. As shown in Figure 1, AuNPs with a size of 35 nm showed the best absorption, which were used in the rest of the experimental procedure.

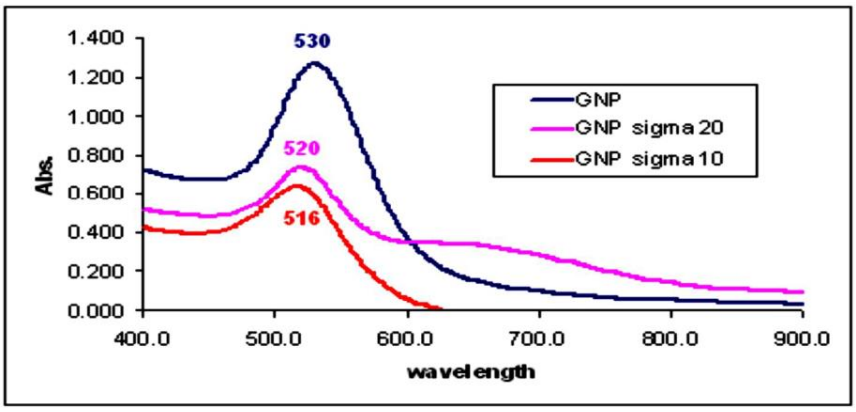

Figure 1: Absorption of different sized nanoparticles. The blue, pink, and red curves represent nanoparticles sized 35, 20 and 10 nm, respectively.

Examination of appropriate nanoparticle-anti body coupling: A shift of < 3 nm is expected if the antibody is appropriately coupled to the nanoparticles; a higher shift indicates the aggregation of nanoparticles.

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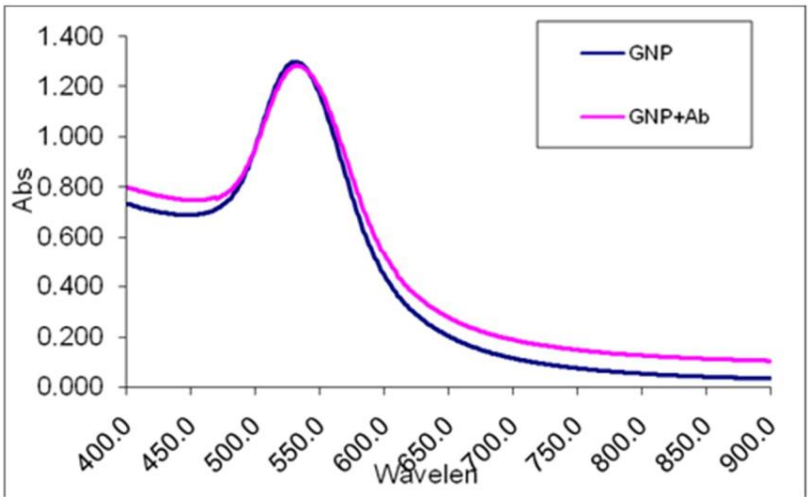

Figure 2: Appropriate anti PSAAuNPs coupling. Blue curve belongs to the absorption of nanoparticles alone, and the pink curve shows absorption of anti-PSA conjugated nanoparticles.

Examination of the interaction between anti-PSA conjugated nanoparticles: Appropriate nanoparticle-antibody coupling with PSA solution led to a color change from red to violet (Fig. 1) indicating the accuracy of the experiment.

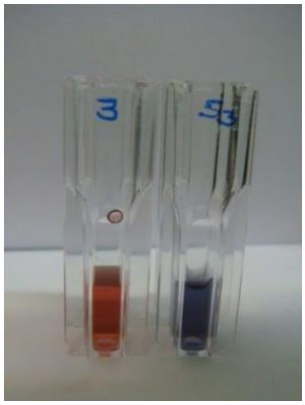

Figure3: A color change observed due to PSA coupling of anti-PSA conjugated nanoparticles. The cuvette with a red solution contains anti-PSA conjugated nanoparticles, and that with a blue solution contains anti-PSA conjugated nanoparticles coupled to PSA.

DISCUSSION AND CONCLUSION

Forensic is a completely new branch in legal medicine, which concerns the development of nano-sensors for real-time monitoring of crime scenes and terrorist activities using remains of biological factors. Nanotechnology plays a role inforensic medicine from two aspects: 1) Nanotechnology can examine and detect samples in nano scale, which was impossible to detect in the past due to the limitation of tools, and 2) Nanomaterials have properties that can be used to collect samples and evidence that were not accessible in the past. Applications of nanotechnology in forensic medicine include fingerprinting, heavy metals, explosives, DNA in fingerprints, and so on.

Most of the well-known applications of nanotechnology in forensic medicine are associated with the improvement and development of DNA microchips and DNA microarray. Information on its other applications, however, is not available. Lin et al. found that gold nanoparticles could significantly improve the efficiency of polymerase chain reaction (PCR). They showed that addition of gold nanoparticles to PCR reactors reduced the reaction time through increasing the speed of heat/cold cycle (14).

Other evidence encountered in the biological area of forensic medicine is rape or sexual assault, for which precise detection of semen is a necessity in such cases. PSA is a prostate-specific antigen commonly used as a marker of prostate cancer diagnosis. Most of studies have focused on the use of gold nanoparticles to detect PSA for early diagnosis in patients with prostate cancer.

A number of studies have investigated PSA detection using immune sensors with different markers such as enzymes, DNA, and nano particles. Lind and Kubitsa applied antibody-conjugated DNA to increase immuno-PCR sensitivity in PSA detection, which showed a higher sensitivity over ELISA (15). Thaxton et al. reported that the efficiency of nanotechnology-made biobarcodes for identification of serum PSA presented a strength about six times the conventional methods (16).Choi et al. observed a 10^3-fold increase in the signal emitted from a nanoparticle-made biosensor for PSA detection (17). They used 35 nm anti-PSA conjugated AuNPsto detect PSA. PSA binding to nanoparticle-conjugated antibody causes a change in the optical properties, a fact that allows optical detection of the molecule througha color change only. Application of these techniques enables an easy and fast detection without the need for complex laboratory equipment with very low cost of detection (18).

This study developed a new method for PSA detection using nanotechnology, which is of a rather good sensitivity and also a simple and fast procedure compared to conventional techniques. Yet, further studies including specificity examination and its quantification using such available techniques as spectro photometry are required in this area. Besides, it is recommended to analyze the efficiency of these antibody-conjugated nanoparticles in clinical specimens to confirm their sensitivity and specificity. Some of problems with the use of this method in clinical samples include false positive and negative cases, which require designing calibration curves and cut-off point definition in forensic medicine samples.

There are limitations in this project, such as maintaining a fixed nanoscale of exactly 35 nm at all stages of the project. Another challenge was preservation of the AuNPs-anti PSA construct at room temperature owing to the presence of antibody.

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