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RESEARCH PAPER

Colorimetric method based on salt-induced aggregation of gold nanoparticles and aptamer does not work for detection of tacrolimus

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ABSTRACT

Objective(s): Tacrolimus (TAC) is used in autoimmune diseases, organ transplantation, and nephrotic syndrome treatment. Therapeutic drug monitoring (TDM) of TAC is critical since it has narrow therapeutic index. Thus, the development of an easy and sensitive method is critical for detecting TAC in blood samples.

Materials and Methods: In this study, we aimed to design a fast, simple,and specific colorimetric sensor based on aptamer/AuNPs to detect. Initially, the aptamer was adsorbed on the surface of the gold nanoparticles (AuNPs).

Results: Then, it was expected that when the aptamer bound to TAC, AuNPs were aggregated by salt, and the color changed from red to blue. However, in our study, color did not change, and NaCl could not aggregate the AuNPs. In this approach, the optical properties of AuNPs and high affinity of aptamer were used for the detection of TAC.

Conclusion: However, according to our data, this colorimetric aptasensor was not appropriate for the detection of TAC.

Keywords: *Aptamer, Gold nanoparticles, Sensor, Tacrolimus*

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INTRODUCTION

Tacrolimus (TAC) is a an antibiotic extracted from *Streptomyces tsukubaensis* and has an immunosuppressive function. TAC is used in autoimmune diseases, organ transplantation, and the treatment of nephrotic syndrome [1]. Therapeutic drug monitoring (TDM) of TAC is critical since it has narrow therapeutic index. The TAC blood concentration to diminish its side effects is about 6.21–24.87 nM [1, 2]. Thus, the development of an easy and sensitive method is critical for detecting TAC in blood samples [3]. So many techniques have been reported for TDM of TAC [4] such as enzyme-linked immunosorbent assay (ELISA) [5], HPLC-MS, and HPLC-MS/MS [6, 7]. Though these methods are appropriate for TDM, they need expert person, and expensive equipment, thus, for TDM of tacrolimus, new assay with easy, sensitive, inexpensive, and fast detection time is needed [8]. Aptamers are single-stranded RNA or DNA generated by an *in vitro* selection method, called systematic evolution of ligands by exponential enrichment (SELEX) [9]. Aptamers bind to their targets tightly and specifiacally [10]. Aptamers have advantages over antibodies, including high stability, simple synthesis, inexpensiveness, low immunogenicity and toxicity, high affinity and

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sensitivity for their targets [11, 12]. Due to these attributes, aptamers are the proper selection for the assembly of biosensors [12, 13]. Colorimetry is a valuable technique for analytical applications which can detect with human eyes [14]. Using gold nanoparticles (AuNPs) in colorimetric aptasensors is possible due to their unique features including high stability and sensitivity, absorption, easy use, and lack of biological reactivity [15-17]. This paper employed aptamer having great affinity to TAC obtained from our previous study [18] to design a colorimetric sensor based on AuNPs to detect TAC.

MATERIALS AND METHODS

Materials

Tacrolimus (TAC) was purchased from Sigma-Aldrich (St. Louis, Missouri, USA). specific aptamers for TAC (CTACAGCTTGCTATGCTCCCCTTGGGGTA) was purchased from Microsynth (Switzerland).

Synthesis and charactrezation of water resuspended AuNPs

AuNP synthesis was done *via* citrate-mediated decrease of HAuCl4, according to the published protocol previously [19]. After AuNPs solution was synthesized(Au particles (Around 13 nm diameter) were primed by the citrate reduction of HAuCl4. then cleanes glassware with aqua regia , washed with Nanopure H_2O. solution of $HAuCl_4$. An aqueous solution of HAuCl4 (1 mM, 500 mL) was took to a reflux while stirring and then added to this sulotion 50 mL of a 38.8 mM trisodium citrate. after that color change), it was centrifuged at 15000 rpm for 25 min at 4°C. The supernatant was discarded and the obtaind pellet was dissolved in ultrapure water. The concentration of AuNPs was calculated by an extinction coefficient of 2.7 × 10 8 M-1 cm-1 at λ = 520 nm for AuNPs with 20 nm. The prepared AuNPs were characterized with transmission electron microscopy (TEM) (LEO 912 AB, Germany) and dynamic light scattering (DLS) (Malvern, UK).

Absorbance of AuNPs after addition of different concentration of aptamer

Various concentrations of aptamer 0, 0.05, 0.1, 0.25, 0.5, 1 , 3 µM were added to 5 nM AuNPs (constant concentration 5 nM) in ultrapure water and incubated 20 min at room temperature, then 100 mM NaCl was added and incubated for 5 min and data was read with Synergy H4 microplate reader (BioTeK, USA). the attachment of Apt to nanoparticle was perfoemed by Agarose (2.5%) gel electrophoresis. The DNA Apt, TAC-AuNPs -Apt nanocomplex were loaded on the gel . the gel run in Tris/Borate/EDTA (TBE 1%) buffer for 50 min .after that gel observed in Gel Doc System (Bio-Rad Laboratories, Hercules, CA).

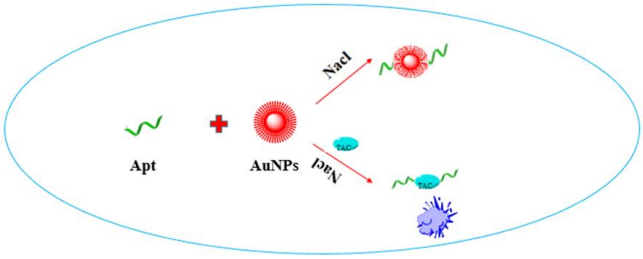
Detection of tacrolimus based on colorimetric technique

According to our previous study, specific aptamers for TAC were isolated *via* systematic evolution of ligands by the exponential enrichment method [18]. The mixture of 0.5 µM Apt and 5 nM AuNPs was incubated for 20 min at room temperature. Afterwards, different concentrations of TAC, ranging from 0-500 nM, were added to the mixture. Finally, 100 mM NaCl was added and the absorbance was recorded at 520 nm.

RESULTS AND DISCUSSION

Sensing design

Colorimetric methods based on aptasensor generally work following the interaction between aptamer and AuNPs. According to the recent studies, removing sodium citrate from the environment of AuNPs could enhance the sensitivity of the colorimetric aptasensors [17]. In the designed colorimetric aptasensor, if TAC was not in the environment, the aptamer was adsorbed on the surface of AuNPs with electrostatic interaction between the positive and negative charges of aptamer and AuNPs, respectively. Thus AuNPs were protected against salt-induced aggregation by the



Scheme 1. Schematic description of TAC detection based on colorimetric aptasensor

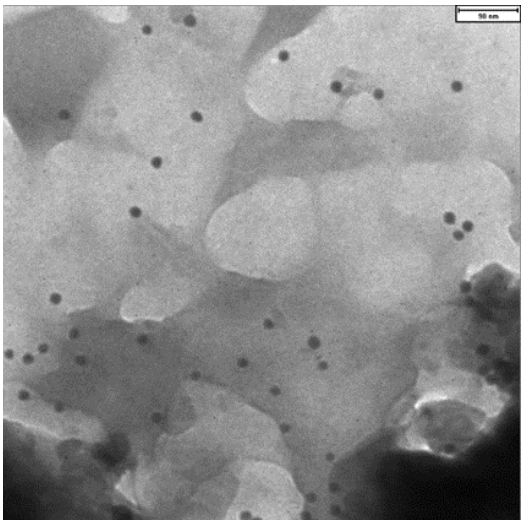


Fig 1. TEM image of water suspended AuNPs

salt. In the presence of TAC, Apt/TAC conjugate is formed thereby leading to AuNPs aggregation by NaCl, thus the color of AuNPs changed from wine-red to blue Scheme 1 [20].

Characterization of AuNPs

AuNPs were prepared and characterized with TEM. The results indicated well-dispersed nanoparticles (Fig 1). The result showed AuNPs with size of 12.7 nm (Fig 2) and zeta potential of -36.4±1.15 mv.

Optimization of aptamer concentration

In order to obtain the best concentration of TAC needed for the development of the aptasensor, different concentrations of aptamer were added to a constant concentration of AuNPs. According to the result, 0.5 µM aptamer could protect AuNPs from aggregation (Fig 3). In order to approved the conection of the Apt to AuNPs used agarose gel electrophoresis . the data showed that Apt was unable to run on the gel after incubation with the AuNPs (Fig 4).

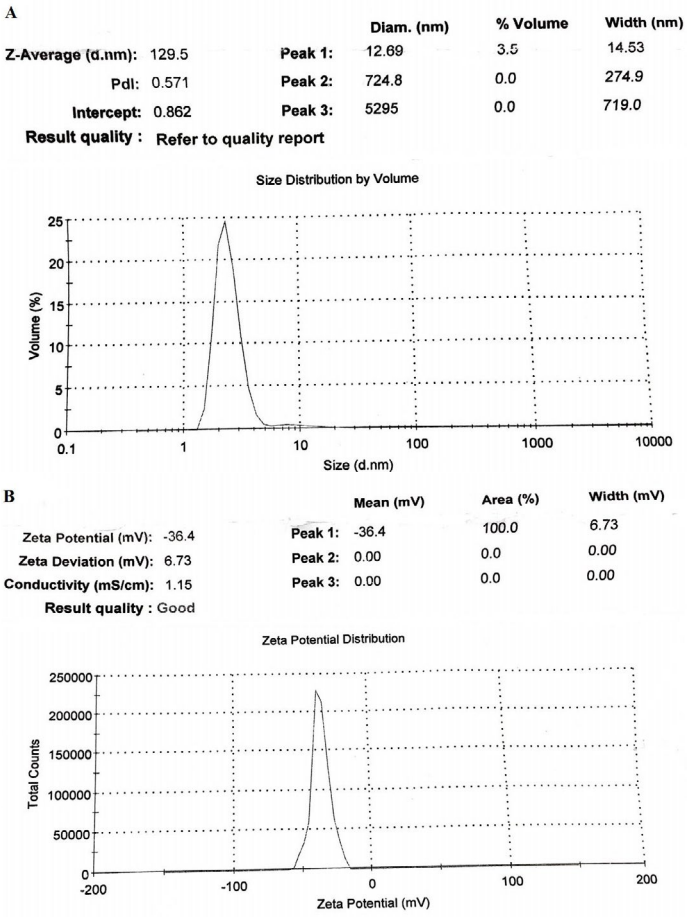


Fig 2. DLS images of water suspended AuNPs. A and B are particle size and zeta potential of AuNPs respectively

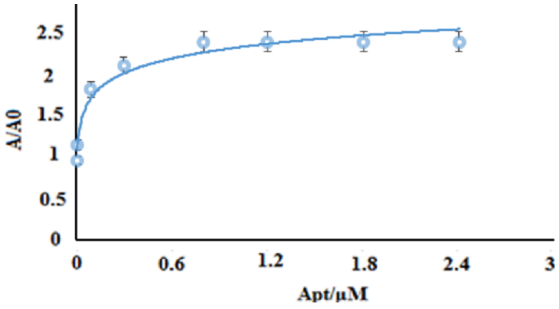


Fig 3. Absorbance of various concentrations of aptamer with AuNPs at 520 nm

Fig 4. Gel electrophoresis assay analysis of aptamer conjugated to AuNPs. Lane1: aptamer, lane2: aptamer conjugated to AuNPs

Tacrolimus analysis

In this method, we expected that the color of sample changed from wine-red to blue by increasing of TAC concentrations. However, according to our result, blue color was not observed. It shows that with increasing TAC concentration, the aptamer did not leave the surface of AuNPs and thus, AuNPs did not aggregate by NaCl (Fig 5). Fig 6 indicates the absorbance of AuNPs in which no difference in absorbtion at different TAC concentrations was observed.various methods use for detection of TAC including enzyme-linked immunosorbentassay

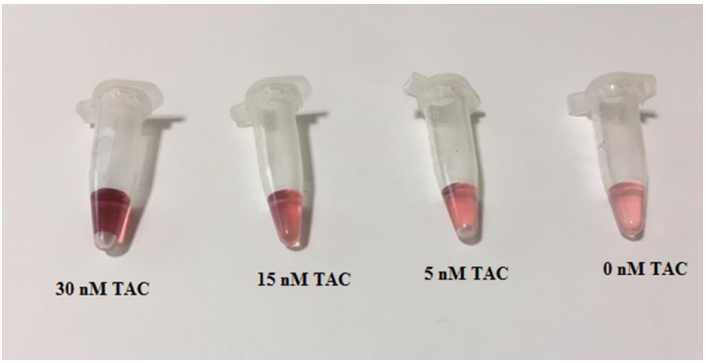


Fig 5. Optical color change following the treatment of Apt/AuNPs complex with different concentrations of TAC (0, 5, 15 and 30 nM)

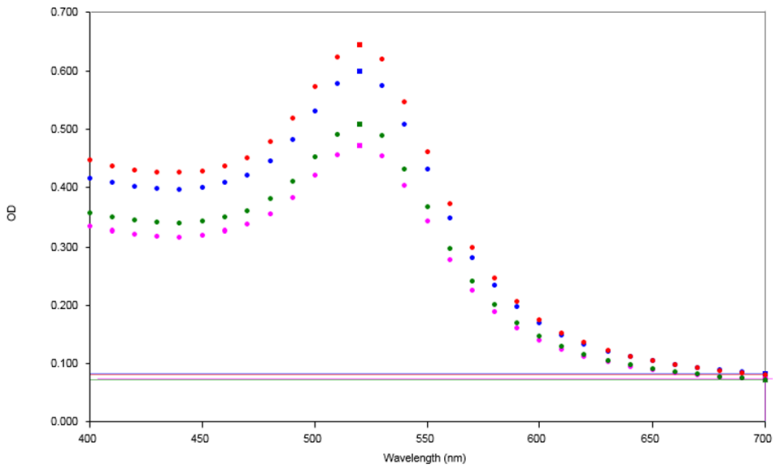


Fig 6. The absorbance of AuNPs at various concentrations of TAC (from top to bottom 0, 5, 15, and 30 nM)

(ELISA) but in this method , detect TAC based on the metabolites of TAC not real detection [21]. anather methods are HPLC-MS andHPLC-MS/MS [6]. Unlike this methods , aptasensors are simple, not expensive and need simple instruments.

CONCLUSION

In this work, we aimed to design a fast, simple,and specific colorimetric sensor based on aptamer/AuNPs to detect TAC. In this approach, the optical properties of AuNPs and high affinity of aptamer were used for the detection of TAC. However, according to our data, this colorimetric aptasensor was not appropriate for the detection of TAC.

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CONFLICT OF INTEREST

There is no conflict of interest in this article.

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