CIRCULATING TUMOR DNA AS A PROMISING BIOMARKER FOR BREAST CANCER DETECTION: PCR AMPLIFICATION IMPROVED BY USING GOLD NANOPARTICLES

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Abstract

Breast cancer is the most commonly diagnosed cancer in women. Circulating tumor DNA (ctDNA) is related to breast cancer development and thus, screening of tumors using ctDNA blood detection is highly sensitive and may significantly improve early-stage breast tumor diagnosis. Recently, a nanomaterial-assisted PCR using gold nanoparticles (AuNPs) has been applied to dramatically improve the specificity and sensitivity of PCR, achieving better gene detection. In this study, we aimed to optimize AuNP-assisted PCR, using a new AuNPs-ODN-ThiC3 nanocomposite. Synthesized AuNPs-ODN-ThiC3 nanocomposites, obtained after 60 min of interaction between AuNPs and ODN-ThiC3, were characterized by differential UV/VIS spectroscopy (AuNPs 35 mg/mL), AuNP peroxidase-like activity (0.5 mM TMB, 60 min, 30 cycles), PAGE 25 % gel electrophoresis with silver staining visualization (V = 120 mV, I = 150 mA, P = 20 W) and adsorptive stripping voltammetry (HMDE, from start 0 to end -1.75 V, time of accumulation 240 s). AuNP-ODN-ThiC3-assisted PCR was performed using Elizyme HS Robust Polymerase (5 U/μL), denaturation: 95 °C/15 s, annealing: 59 °C/15 s, extension: 72 °C/15 s, 35 cycles. Analysis of AuNPs modification was achieved with ODN-ThiC3 (in different concentrations) and, therefore, new AuNPs-ODN-ThiC3 nanocomposites were obtained. We observed an increased absorbance signal of AuNPs at 527 nm, with the increase of ODN-ThiC3 concentration (at 260 nm) in the AuNPs-ODN-ThiC3 mixture (y = -0.0397 + 0.0734x, r = 0.99). Mixing ODNs-ThiC3 with AuNPs increased the peroxidase-like activity of AuNPs by 17 %. LS voltammograms of cytosine and adenine (CA) reduction (the average potential at -1.34 V) showed a linear dependence (r = 0.99) between peak current and concentration of ODN-ThiC3 in the AuNPs-ODN-ThiC3. A linear dependence (r = 0.99) between bands density and ODN-ThiC3 concentration was also achieved. Analysis of PCR protocols: AuNPs-ODN-ThiC3-assisted PCR (AuNPs 814 μg/mL, ODN-ThiC3 4 μg/mL) showed a better yield (140 % of conventional PCR and 120 % of AuNP-assisted PCR). Conventional PCR can be optimized by integrating ODN-ThiC3 on the AuNP surface during PCR. This improvement represents an innovative PCR method for breast cancer detection.

Keywords: Nanomedicine, gold nanoparticles, anticancer drugs, PCR, breast cancer

1. INTRODUCTION

Breast cancer is the most commonly diagnosed cancer in women, including 28 % of all types of cancers [1]. Circulating tumor DNA (ctDNA) is related to breast cancer development and, thus, screening of tumors using

ctDNA blood detection is highly sensitive and may significantly improve early-stage breast tumor diagnosis [2]. Recently, a nanomaterial-assisted PCR using gold nanoparticles (AuNPs) has been applied to dramatically improve the specificity and sensitivity of PCR, achieving better gene detection [3]. In this study, we aim to optimize AuNP-assisted PCR, using a new AuNPs-ODN-ThiC3 nanocomposite.

2. MATERIAL AND METHODS

All used reagents were purchased in Merck-Sigma-Aldrich (USA) in analytical grade. Distilled water was prepared by the Aqual system (Tišnov, Czech Republic) and ultra-pure water was prepared by the ELGA (USA) system to the sterile 18 MΩ quality. Synthesized AuNPs-ODN-ThiC3 (5'-AGCCCTTGGGGASTTGAATTGCTG[ThiC3]-3') nanocomposites, obtained after 60 min of interaction between AuNPs and ODN-ThiC3, were characterized by differential UV/VIS spectroscopy (AuNPs 35 mg/ml), AuNP peroxidase-like activity (0.5 mM TMB, 60 min, 30 cycles), PAGE 25 % gel electrophoresis with silver staining visualization (V = 120 mV, I = 150 mA, P = 20 W) and adsorptive stripping voltammetry (HMDE, start 0 to end -1.75 V, time of accumulation 240 s). AuNP-ODN-ThiC3-assisted PCR was performed using Elizyme HS Robust Polymerase (5 U/µl), denaturation: 95 °C/15 s, annealing: 59 °C/15 s, extension: 72 °C/15 s, 35 cycles.

3. RESULTS

3.1. UV-visible spectrophotometry

Analysis of AuNPs' modification: was achieved with ODN-ThiC3 (different concentrations) and, therefore, new AuNPs-ODN-ThiC3 nanocomposites were obtained. We observed an increased absorbance signal of AuNPs at 527 nm, with the increase of ODN-ThiC3 concentration (at 260 nm) in the AuNPs-ODN-ThiC3 mixture (y = -0.0397 + 0.0734x, r = 0.99) (**Figure 1**), suggesting that ODN-ThiC3 interaction with AuNPs surface may change its absorption properties. It should be noted that coating of AuNPs with ODNs impact the surface density, hybridization with the target sequences, and the non-specific interactions. We hypothesize that chemisorption of ODN-ThiC3 on AuNPs surface increases AuNPs solubility, which may explain the increase of AuNPs'absorbance peaks when binding ODN-ThiC3.

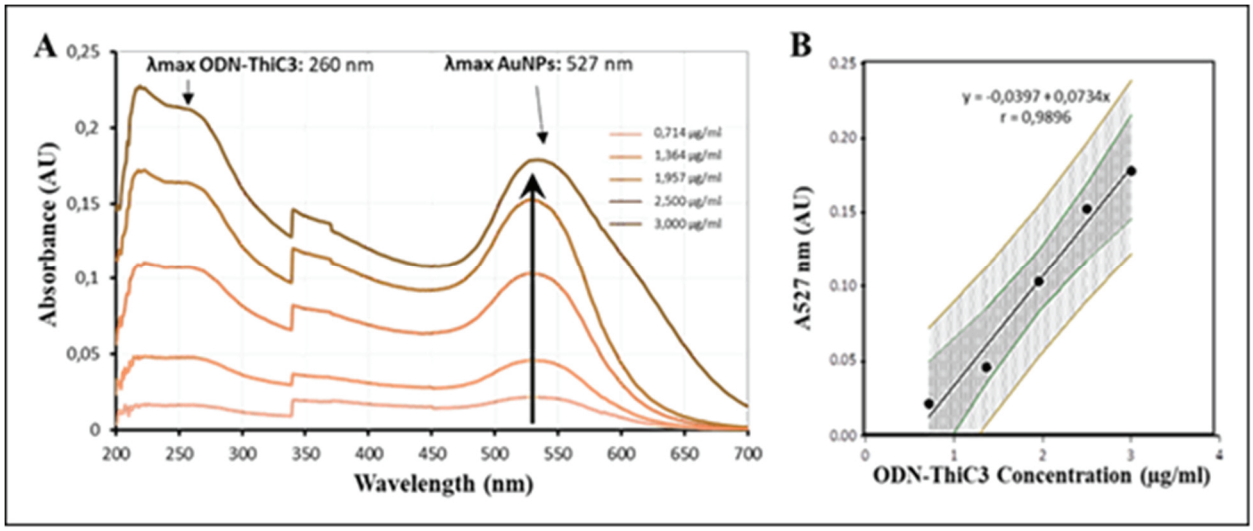

Figure 1 Effect of functionalization of AuNPs surface with increasing concentrations (0.714, 1.364, 1.957, 2.500 and 3.000 µg/mL) of ODN-ThiC3 on the AuNPs absorbance at 527 nm. (A) Increasing concentrations of ODN-ThiC3 leads to absorbance increase at 527 nm. (B) The occurrence of this effect was linear between 0.714 and 3.000 µg/mL.

3.2. Adsorptive Stripping Voltammetry (ASV) measurements

Mixing ODNs-ThiC3 with AuNPs increased by 17 % the peroxidase-like activity of AuNPs. Electrochemistry of DNA at electrodes provides valuable insights into the mechanisms involved in DNA-mediated charge transport at electrodes. Moreover, electrochemistry provides the opportunity for highly sensitive DNA detection. ASV voltammograms of cytosine and adenine (CA) reduction (average potential at -1.34 V) showed a linear dependence (r = 0.99) between peak current and concentration of ODN-ThiC3 in the AuNPs-ODN-ThiC3. A linear dependence (r = 0.99) between bands' density and ODN-ThiC3 concentration was also achieved. Analysis of PCR protocols: AuNPs-ODN-ThiC3-assisted PCR (AuNPs 814 µg/ml, ODN-ThiC3 4 µg/ml) showed a better yield (140 % of conventional PCR and 120 % of AuNP-assisted PCR). This data clearly demonstrates that interaction with AuNPs minimizes the reduction of ODN-ThiC3 on the electrode's surface, interfering with ODN-ThiC3 detection using electrochemical-based methodology (**Figure 2**).

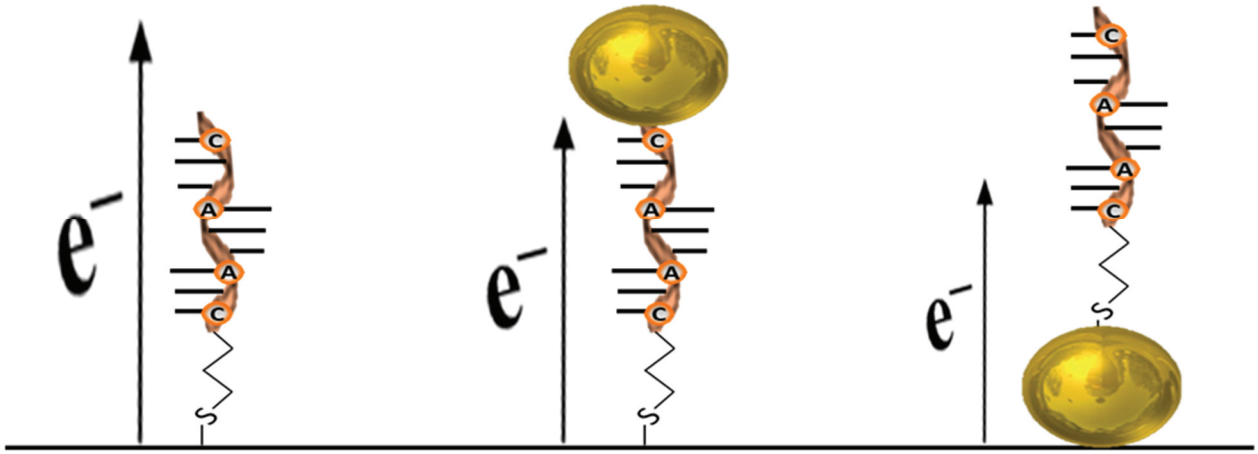

Figure 2 Schematic illustration of attenuation of ODN-ThiC3- mediated electrochemistry by gold nanoparticles (AuNPs)

3.3. AuNP-assisted PCR

The specificity of the primer pairs for the desired DNA region was analyzed first by single conventional PCR. The target gene was amplified specifically using its designed primers. The size of the amplified products was identical with the expected target fragments (237 bp). To perform AuNP-assisted PCR, different reaction mixtures were made by adding a different volume (0.55, 1.10, 2.20, 3.30, 4.40, 5.50 and 8.75 µL) of synthesized AuNPs (18.50 mg/mL) to conventional PCR mixtures, obtaining different AuNPs' final concentrations (407, 814, 1628, 2442, 3256, 4070 and 6475 µg/mL) in each mixture. The resulting PCR products were migrated on 15 % SDS-PAGE gel (**Figure 3A**). After executing silver staining protocol, the gel was scanned and processed by programmed ColorTest, which assigns intensity to the individual pixels of the studied image in the color area and allows densitometry quantifications of the bands as well as the drawing of its electrophoretic graphs. The enhancement of amplification yield (%) of the desired DNA sequence was defined as a ratio of the density value of the target DNA band obtained with AuNP-assisted PCR to the density value of the target DNA. band observed with conventional PCR, which is assigned to a value of 0 %. In AuNP-assisted PCR, the concentration of AuNPs that enabled the PCR to produce the large amount of DNA target band (maximum efficiency) on the gel was identified to be the optimum concentration. This study revealed that AuNP-assisted PCR using AuNP concentrations of 407 µg/m and 814 µg/mL showed improved yield of gene amplification when compared with conventional PCR (**Figure 3B**). Taken together, these data confirms that, at an appropriate concentration, presence of AuNPs may significantly improve the efficiency of PCR, which can be explained by three general effects of AuNPs in PCR already reported (**Figure 3C**)

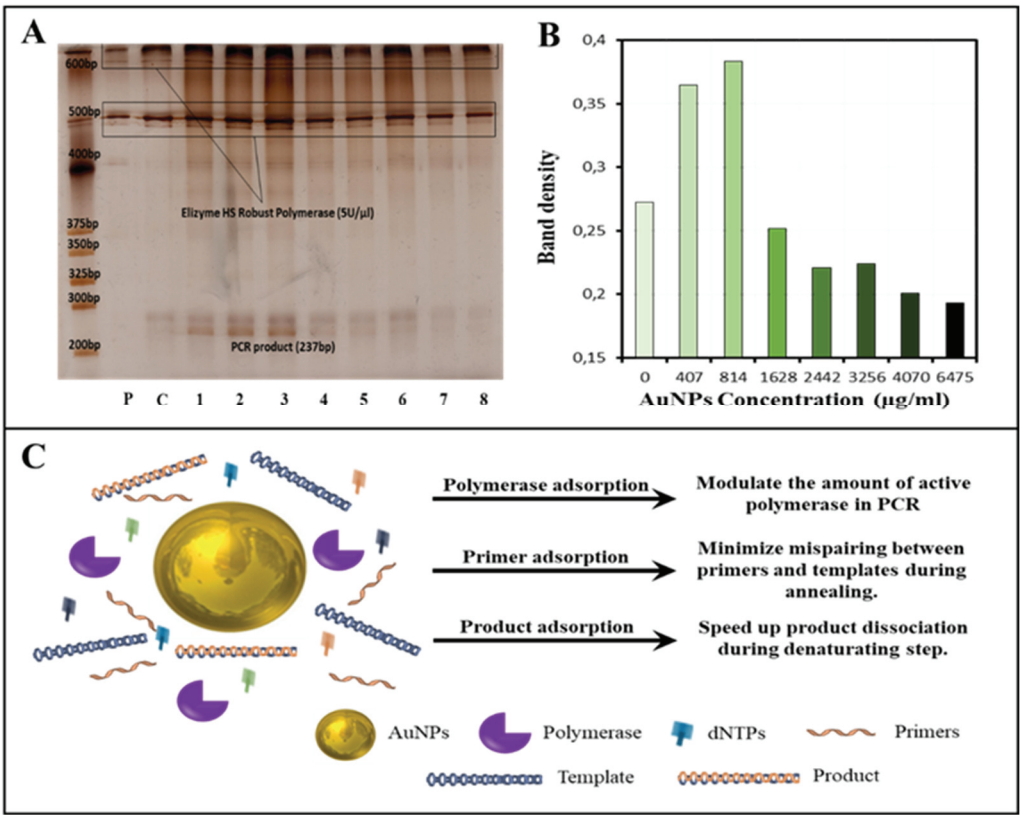

Figure 3 (A, B) The effect of the concentration (µg/mL) of gold nanoparticles on the yield of PCR: lane P (polymerase): 0; lane C (control): 0; lane 1: 0; lane 2: 407; lane 3: 814; lane 4: 1628; lane 5: 2442; lane 6: 3256; lane 7: 4070; lane 8: 6475. (C) Schematic illustration of potential effects of gold nanoparticles (AuNPs) in PCR

4. CONCLUSION

The AuNPs did indeed improve the yield of PCR. Conventional PCR can be optimized by integrating ODN-ThiC3 on the AuNP surface during PCR. Thus the use of AuNPs open new opportunities for improving PCR-based techniques, among which the improvement of PCR-based ctDNA detection may optimize breast cancer diagnosis, allowing earlier tumor detection and improved breast cancer management.

ACKNOWLEDGEMENTS

This work was supported by the H2020 ERASMUS+ and NANO LPR 2017 Liga proti rakovině Praha and international collaboration project of The European Technology Platform for Nanomedicine.

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