Biosensors and Bioelectronics 92 (2017) 349–356

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Gold aggregating gold: A novel nanoparticle biosensor approach for the direct quantification of hepatitis C virus RNA in clinical samples

Sherif M. Shawky[a,b,c], Ahmed M. Awad[a], Walaa Allam[a], Mohamed H. Alkordi[d], Sherif F. EL-Khamisy[a,b,*]

[a] Center of Genomics, Helmy institute, Zewail City of Science and Technology, Sheikh Zayed Dist., 12588 Giza, Egypt
[b] Krebs Institute, Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield S10 2TN, UK
[c] Misr University for Science and Technology, Faculty of Pharmacy, Biochemistry Department, Giza, Egypt
[d] Center for Materials Science, Zewail City of Science and Technology, Sheikh Zayed Dist., 12588 Giza, Egypt

ARTICLE INFO

Keywords:
HCV
Nucleic acid
Gold nanoparticles
RNA detection
DNA repair
Topoisomerase

ABSTRACT

The affordable and reliable detection of Hepatitis C Virus (HCV) RNA is a cornerstone in the management and control of infection, affecting approximately 3% of the global population. However, the existing technologies are expensive, labor intensive and time consuming, posing significant limitations to their wide-scale exploitation, particularly in economically deprived populations. Here, we utilized the unique optical and physicochemical properties of gold nanoparticles (AuNPs) to develop a novel assay platform shown to be rapid and robust in sensing and quantifying unamplified HCV RNA in clinical samples. The assay is based on inducing aggregation of citrate AuNPs decorated with a specific nucleic acid probe. Two types of cationic AuNPs, cysteamine and CTAB capped, were compared to achieve maximum assay performance. The technology is simple, rapid, cost effective and quantitative with 93.3% sensitivity, high specificity and detection limit of 4.57 IU/μl. Finally, our data suggest that RNA folding impact the aggregation behavior of the functionalized AuNPs, with broader applications in other nucleic acid detection technologies.

1. Introduction

Approximately 3% of the world population are infected with hepatitis C virus (HCV) with 3–4 million infections annually and at least 150 million chronic carriers at risk of developing liver cirrhosis and/or liver cancer (WHO, 2016). It was estimated that more than 15% of the Egyptian population are currently infected with HCV (Hajarizadeh et al., 2013; Mauss et al., 2013) with almost half million new cases arising annually. The infection usually progresses to fatty liver and hepatocellular carcinoma, posing significant health and economic challenges to the society (Miller and Abu-Raddad, 2010). Since an approved vaccination against HCV is yet to be established (Lee et al., 2015; WHO, 2016), the prime combating strategies rely on newly developed medications coupled to robust and affordable means of viral detection and quantification (AASLD-IDSA, 2016; Seifert et al., 2015).

HCV diagnosis is achieved by serologic and Nucleic Acid Testings (NATs) (Scott and Gretch, 2007). A major drawback of the serological approach is the inability to detect acute infections (Cloherty et al., 2016) and the associated complications of immuno-suppression patients (Atrah and Ahmed, 1996; Mauss et al., 2013). NATs are based mainly on Real-Time RT-PCR, branched- DNA (b-DNA), and transcription-mediated Amplification (TMA). NATs are relatively expensive, labor intensive, and require adequately equipped labs, posing significant limitations to their point of care testing. Thus, alternative approaches for HCV RNA detection and quantification are urgently needed.

The unique optical properties of gold nanoparticles (AuNPs), were originated from their strong Surface Plasmon Resonance (SPR) phenomena, which is responsible for their intense colors, and high extinction coefficient compared to conventional dyes (Huang et al., 2007a). Thus, AuNPs have been employed in many colorimetric assays for different biological molecules as proteins (Kim et al., 2009), and nucleic acids (Liandris et al., 2009). For example, Mirkin and co-workers were the first to develop a modified AuNPs cross-linking method for the direct detection of nucleic acids (Elghanian et al., 1997; Larguinho et al., 2015). Despite the high sensitivity and specificity of this method, it requires firm temperature control for precise target detection. To our knowledge, this method has not been used for nucleic acids detection clinically. Li and co-workers developed a method for the direct detection of nucleic acids using unmodified AuNPs (Li and

* Corresponding author at: Krebs and Sheffield Institutes for Nucleic Acids, Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield S10 2TN, UK.
E-mail address: s.el-khamisy@sheffield.ac.uk (S.F. EL-Khamisy).

http://dx.doi.org/10.1016/j.bios.2016.11.001
Received 29 August 2016; Received in revised form 30 October 2016; Accepted 1 November 2016
Available online 04 November 2016

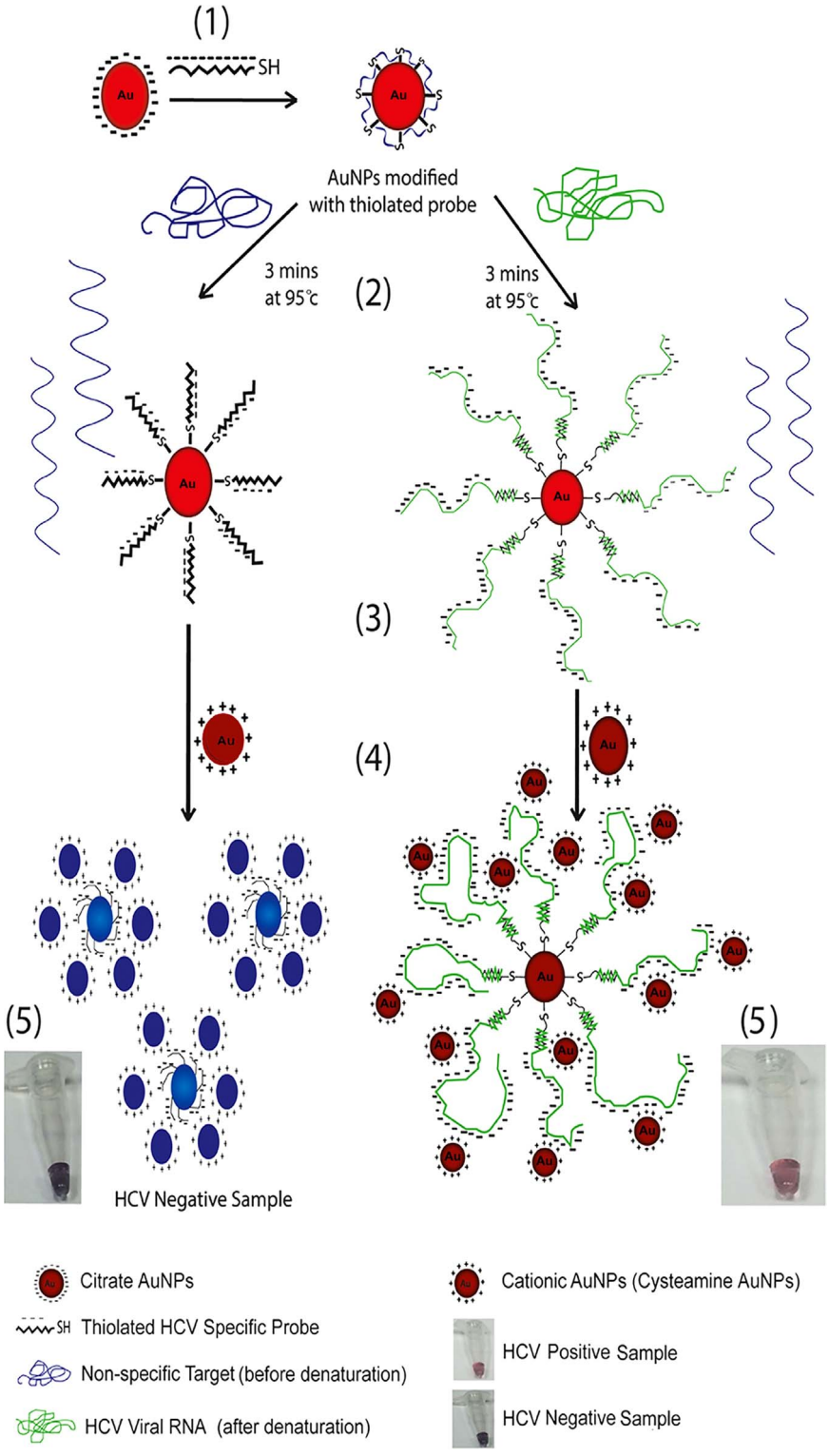

Fig. 1. A scheme depicting the nano-assay principle and procedures. (1) Citrate capped AuNPs, was functionalized with thiolated HCV specific probe forming nanoprobes. (2) The nanoprobe is then mixed with the RNA sample and heated at 95 °C for 3 min. (3) *Right panel*; the HCV viral RNA was hybridized to the HCV specific nanoprobe by sequence complimentarily. *Left panel*; no HCV RNA is present (non-specific RNA target), thus no hybridization takes place. The mixture incubated at room temperature. (4) The cationic AuNPs was added. (5) *Right panel;* in the presence of HCV RNA, the mixture solution remains red, reflecting the dispersion of the AuNPs onto the folded HCV RNA, thereby protecting the nanoprobes from aggregation by cationic AuNPs. *Left panel;* in the absence of a complementary target, the cationic AuNPs will bind to the probe phosphate backbone electrostatically, thereby reducing the inter-particle distance between the nanoprobes and the cationic AuNPs, resulting in aggregation and color change from red to blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Rothberg 2004a, 2005). The technique is based on the adsorption behavior of single and double stranded nucleic acid onto AuNPs surface and the medium ionic strength. Despite its sensitivity and specificity, it requires precise control of the probe, salt, and AuNPs concentrations.

The non-cross linking method was first introduced by Sato and co-workers (Sato et al., 2003), which is based on functionalizing AuNPs with single stranded thiol-modified probe. AuNPs aggregation is based also on the ionic strength of the medium. However, the desired results