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Gelatin-modified gold nanoparticles for direct detection of urinary total gelatinase activity: Diagnostic value in bladder cancer

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ABSTRACT

Matrix metalloproteinases (MMPs), in particularly gelatinases (MMP-2 and MMP-9) were reported as urinary markers of bladder cancer. In this work, we developed a simple colorimetric gold nanoparticle (AuNP) assay for rapid and sensitive detection of urinary total gelatinase activity based on the surface plasmon resonance (SPR) property of AuNPs. Gelatin-modified AuNPs were stably suspended in solution even upon addition of an aggregation inducer as 6-mercaptohexan-1-ol (6-MCH). Gelatinases digest gelatin capping. Subsequently, addition of 6-MCH leads to AuNPs aggregation with red to blue color shift. In a pilot study, results of the developed AuNP assay were consistent with zymography for qualitative detection of urinary total gelatinase activity. The sensitivity and specificity of both assays were 80% and 90.9% respectively. The absorption ratios, A_{625}/A_{530} of the reacted AuNP solutions were used to quantify the total gelatinase concentration. The best cut off value was 0.01895 ng/μg protein, at which the sensitivity was 87.5% and the specificity was 86.4%. The developed AuNP assay is simple, low-cost and can aid non-invasive diagnosis of bladder cancer.

1. Introduction

Matrix metalloproteinases (MMPs) are a family of zinc-containing endopeptidases that degrade components of the extracellular matrix (ECM). This cleavage is crucial in many physiological processes such as tissues remodeling during embryonic development, cell migration and wound healing [1–3]. Due to their proteolytic activities; MMPs have been implicated in many pathological conditions such as arthritis, cardiovascular diseases and cancer progression [4–6]. Among the members of MMPs family, the gelatinases MMP-2 (gelatinase A) and MMP-9 (gelatinase B) have been extensively studied owing to their consistent association with tumor invasion and metastasis [7,8]. Previous studies have reported that the expression and activity of these gelatinases are elevated in many human tumors such as bladder cancers [9–11], prostate cancers [12], endometrial cancers [13] and colorectal cancer [14].

Bladder cancer is a common cancer worldwide and the second most common malignancy of the genitourinary tract [15]. Cystoscopy in adjunction with urine cytology is the gold standard for diagnosis and follows up of bladder cancer [16]. Lower sensitivity of urine cytology especially in detecting low grade-tumor makes the recurrent use of invasive uncomfortable cystoscopy is essential. The development of a sensitive non-invasive diagnostic test that allows early detection of bladder carcinoma could help in early treatment and improve the clinical outcomes [17].

Measurement of MMP activity is a disputable matter because we need to develop sensitive, selective and rapid method. Generally for in vitro measurement of MMP activity, enzymatic, immunochemical and fluorimetric methods are commonly used techniques. Enzymatic approach depends on the cleavage of the enzyme substrate that can be either labeled or none using the popular substrate zymography technique [18]. Zymography is the most common technique used to measure extracellular MMPs from cell cultures, tissue extracts, serum and urine [19]. This technique is quite sensitive as we use a natural substrate that reflects MMP activity; however, as being multistep technique, it is not reliable and is difficult to reproduce especially for clinical diagnosis. Immunochemical assays like enzyme-linked immunosorbent assay (ELISA) precede enzymatic methods, but they are expensive and unable to distinguish between active and inactive MMPs in zymogen form [20]. Fluorimetric methods

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measure the increase in florescence associated with the cleavage of quenched florogenic substrate [21]. Although this method allows quantitative determination of MMP activity, but it shows low limits of detection. Recently, a new approach measuring the cleavage of a synthetic triple helical peptide substrate has been developed, but it is not easily synthesized [22]. In-vivo imaging of MMP activity using techniques like fluorescence molecular tomography (FMT) is a recent approach that allows determining tumor invasiveness with high sensitivity using MMP activity as imaging biomarker. However these techniques are of particular interest in cancer research, but it is not suitable for routine clinical diagnosis [23,24]. Therefore the need for a simple, sensitive and clinically applicable method for measurement of MMPs is important.

Gold nanoparticles (AuNPs) posses strong surface plasmon resonance (SPR) depending on their sizes and the relative distances between particles [25,26]. In colloidal solution, spherical AuNPs appear red when the interparticle distance is larger than the average particle diameter and blue when the interparticle distance is smaller than the average particle diameter [27]. Based on this distinctive phenomenon, many AuNP-based colorimetric assays have been developed for rapid and sensitive detection of enzymes [28], proteins [29], nucleic acids [30] and cancerous cells [31]. Change in signal takes only few seconds to occur.

Surface functionalization of AuNPs with macromolecules (nucleic acids, polymers and proteins) has been reported previously for different applications in bionanotechnology [32,33]. Several ways have been used for conjugation of proteins to AuNPs. One of these is the using of physical adsorption between the AuNPs and protein molecules, either by electrostatic interactions or gold-thiol bonds (from cysteine residues) [34]. In this work, gelatin molecules were grafted onto the surfaces of citrate-capped AuNPs based on electrostatic attraction between the positively charged amino groups of gelatin molecules and negatively charged citrate of the AuNPs.

In this study, AuNP-based colorimetric assay was developed to directly detect urinary total gelatinase activity to detect bladder carcinoma. Functionalization of AuNPs with gelatin as a substrate not allowed only the detection of gelatinases, but also provided a steric repulsion effect preventing the aggregation of AuNPs.

The principle of our assay as represented in Fig. 1 is based on

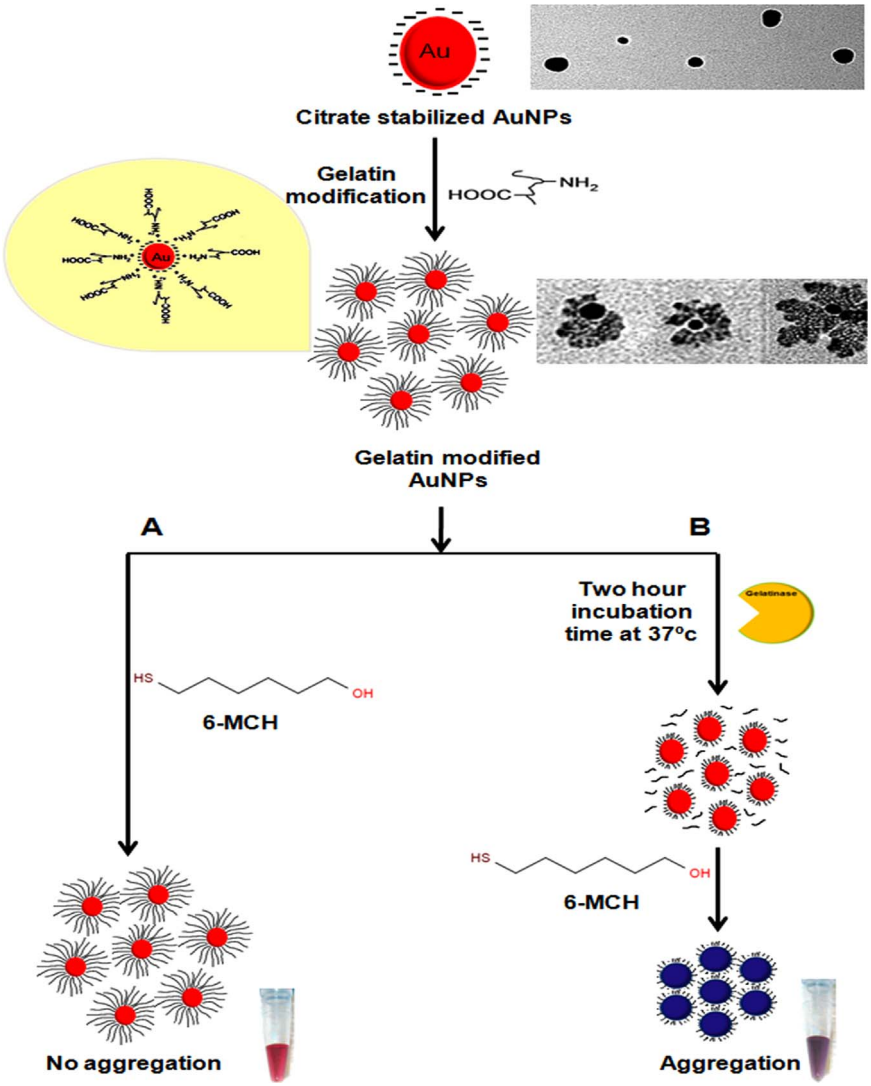

Fig. 1. The principle of gelatin-modified AuNP based colorimetric assay for direct detection of total gelatinase activity. (A) In the absence of gelatinase activity (negative sample), gelatin-modified AuNPs are stably suspended in solution (red color) even upon addition of 6-MCH. Gelatin provides a steric repulsion effect preventing the aggregation of AuNPs. (B) In the presence of gelatinase activity (positive sample), after enzymatic digestion, the gelatin capping is reduced, this allows the binding of 6-MCH molecules to the surface of AuNPs through –SH substitution with exposed –OH on the surface of the AuNPs. Exposed –OH enhances the attraction force among AuNPs leading to aggregation (blue color). The insets in the figure show TEM images for AuNPs before and after gelatin modification. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)