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One-step facile synthesis of hyaluronic acid functionalized fluorescent gold nanoprobes sensitive to hyaluronidase in urine specimen from bladder cancer patients

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ABSTRACT

Gold nanoparticles (AuNPs) have been widely used to develop fluorescence resonance energy transfer (FRET) sensors to detect biological substances, environmental pollutants, and disease markers due to their superior quenching capacity to fluorescence signals. In this study, we report the one-step facile synthesis of fluorescein isothiocyanate-labeled hyaluronic acid (FITC–HA) functionalized fluorescent AuNPs based FRET nanoprobes (FITC–HA–AuNPs) *via* chemical reduction of $HAuCl_4$ by using FITC–HA as both a reducing and stabilizing agent. Then the FITC–HA–AuNPs FRET nanoprobes were used to detect hyaluronidase (HAase), a new type of disease marker, based on the specific enzymatic degradation of HAase to HA. Compared with similar work, the FITC–HA–AuNPs nanoprobes were much easier to prepare and the detection sensitivity was also high for HAase to reach a detection limit of 0.63 U mL^{-1}. More importantly, they also allowed for rapid HAase detection (within 3 h) even in complex biological specimens (urine specimens from patients with bladder cancer) with satisfactory accuracy (recovery efficiency in the range of 92.8–106.9% with RSD $\leq$ 4.85%). Our studies suggested that such a novel design of FITC–HA–AuNPs FRET nanoprobes developed for sensitive, rapid and accurate detection of HAase had exciting potentials for clinical diagnosis of HAase-related diseases, such as bladder cancer.

1. Introduction

Recent studies have showed that hyaluronic acid (HA) and hyaluronidase (HAase) had a close relationship with the proliferation, differentiation, migration, and adhesion of tumor cell and the tumor angiogenesis based on the highly efficient targeted delivery tumor cell with HA receptors such as hyaluronan receptor for endocytosis (HARE) and cluster determinant 44 (CD44) [1–3], and they have played an important role in tumor invasion, metastasis and progression [4,5]. HA is a macromolecule of linear polysaccharide, composed of repeating disaccharide units, D-glucuronic acid and N-acetyl-D-glucosamine, with good water solubility [6]. The HAase is an enzyme that degrades HA specifically by cleaving the internal β-N-acetyl-D-glucosamine linkages in the HA polymer, thereby increasing the tissue permeability [7]. As HAase had been reported to over express in certain patients with cancers (such as bladder, colon, prostate, and so on), it is becoming a new type of tumor marker [7–9]. Therefore, it will be of great significance to develop simple, rapid, and sensitive methods for detection of HAase. There are various strategies for HAase detection including turbidimetric [10,11], viscometric [12], zymography [13], immunoassay[14,15], instrument [16,17], and chemical methods such as colorimetric method [18–20], spectrophotometric [21], fluorescence detection [22–24], and chemiluminescence-assisted assay [25]. Among the strategies for HAase assay, the classical methods (turbidimetric, viscosimetric and colorimetric methods) often lack sensitivity and selectivity [26]. Zymography method is simple but not suitable for sensitive quantitative analysis. Immunoassay is sensitive and selective but needs specialized and expensive reagents (anti-HAase antibodies). And instrument-based method is sensitive and accurate but time-consuming and requires complex instruments. Thus, developing new precise and accurate methods for HAase assay is needed.

Fluorescence resonance energy transfer (FRET) is a powerful analytical technique, which has been widely used for target detection and molecular interaction study because of its high sensitivity, specificity and simplification [27]. Recently, this analysis method has been developed and improved largely due to the rapid development of new materials, especially nanomaterials such as quantum dots [28], carbon dots [29], AuNPs [30], carbon

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nanotubes [31], graphene [32,33]. The nanomaterial-based FRET analytical technique not only breaks the detection distance limitation that the traditional fluorescent dye-based FRET technique requires (the distance between donor and receptor should be within 10 nm), but also improves the sensitivity and broads its applications [34]. Among the new nanomaterials, AuNPs is a kind of efficient fluorescence quencher because of the non-radioactive electronic excitation energy transfer from the fluorophore to AuNPs [35]. They also have many other advantages such as simple synthesis, facile surface modification, tunable optical properties and good stability [36]. Therefore, AuNPs have been widely used to construct FRET probes for the detection of various ions, small molecules, biomolecules, and pathogens [37–39].

Based on the above-mentioned knowledge, we can envision that it must be a very interesting work to development a new method for the detection of HAase by combining the property of degradation of HAase to HA with AuNPs-based FRET analytical technique. Currently, there are very few reports about the detection of HAase by using AuNPs based FRET nanoprobes. Lee et al. made some attempts in fabricating AuNPs FRET nanoprobes by assembling dye-labeled HA onto the pre-prepared AuNPs surface based on gold–thiol bond covalent interaction, and applying the probes for HAase detection by the specific enzymatic degradation of HAase to HA [40]. However their work involves complicated procedure for preparation of AuNPs FRET probes, resulting time-consuming, and is lack of analysis for clinical specimens.

Previously, we had successfully developed N-acetylglucosamine-functionalized AuNPs by using polysaccharide as a ligand [41]. Herein, as a further step, by employing fluorescein isothiocyanate-labeled hyaluronic acid (FITC–HA) as both a reducer and stabilizer to deoxidize chloroauric acid ($HAuCl_4$), we have prepared FITC–HA functionalized fluorescent AuNPs (FITC–HA–AuNPs) as FRET nanoprobes in one step in a facile manner. The resultant FITC–HA–AuNPs FRET nanoprobes are of uniform size and good stability, which are subsequently used to develop a simple, rapid, and sensitive strategy for detection of HAase in urine specimens from patients with bladder cancer (Scheme 1). Compared with similar work [40], our work not only simplifies the preparation procedure of AuNPs FRET probes, but also improves the detection limit (as low as 0.625 U mL^{-1}) for HAase analysis. Moreover, the AuNPs FRET nanoprobes could detect HAase in clinical specimens (urine specimens from patients with bladder carcinoma) directly with good accuracy within 3 h, which is the first report to the best of our knowledge. Such FITC–HA–AuNPs FRET nanoprobes show promising potential for clinical diagnosis of HAase-related diseases.

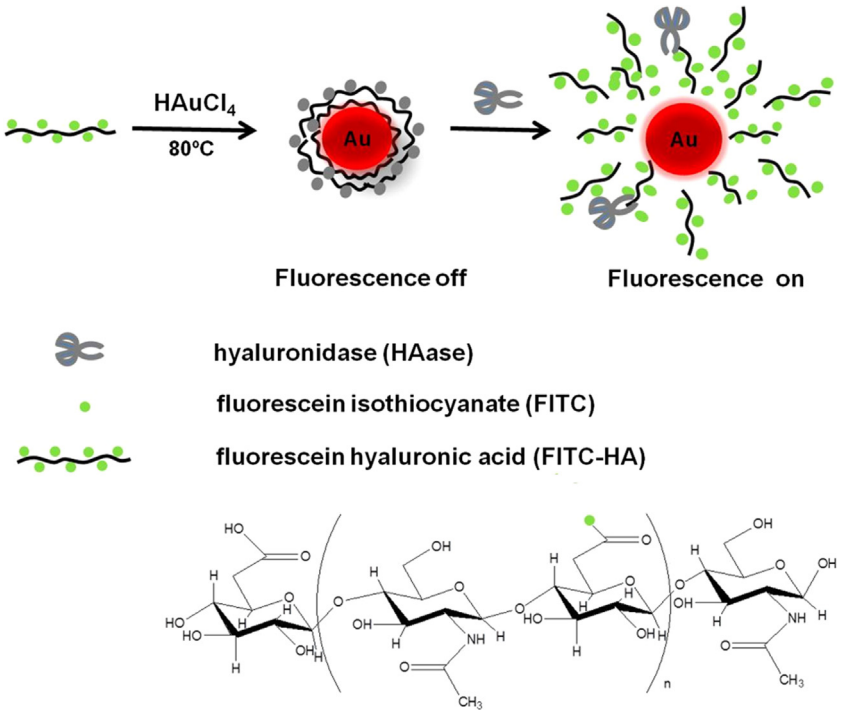
2. Experimental

2.1. Instrumentations

The fluorescence spectra were obtained using a fluorescence spectrophotometer (F-7000, Hitachi). UV–vis absorption spectra were recorded by a UV–vis spectrophotometer (UV-2450, Shimadzu). Morphology and microscopic structure were characterized using a transmission electron microscope (TEM) (LIBRA 200PE, German Carl Zeiss Company). Size distribution was recorded by Zetasizer Nano ZS (Malvern Instruments Ltd.).

2.2. Standard solutions and reagents

Fluorescein hyaluronic acid (FITC–HA) and hyaluronidase (HAase) were purchased from Sigma-Aldrich Co. Ltd.; sodium citrate was obtained from Chongqing Chuandong Chemical Co. Ltd.(China); chloroauric acid ($HAuCl_4$) was provided by Tianjin Guangfu Chemical Research Institute; human serum albumin (HSA) was purchased from Beijing Dingguo biotechnology Co. Ltd.; carbamide, uric acid, creatinine, benzoylglycine were obtained from Sinopharm Chemical Reagent Co., Ltd.; human serum (HS) and human urine specimens were supported by Department of Oncology, the Ninth People's Hospital of Chongqing (China); all the buffers were prepared with double-distilled water which was purified with a water purification system (ELGA, British) to a specific resistance of 18 MΩ cm.



Scheme 1. Schematic illustration of the one-step synthesis of FITC–HA–AuNPs nanoprobes and their application as optical nanoprobes for HAase detection.