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# Surface-enhanced Raman scattering (SERS)-active gold nanochains for multiplex detection and photodynamic therapy of cancer

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ABSTRACT

Multifunctional nanomedicine holds considerable promise as the next generation of medicine that will enable early detection of diseases, as well as simultaneous monitoring and therapy with minimal toxicity. In particular, surface-enhanced Raman scattering (SERS) technology with high sensitivity and multiplexing capabilities is emerging as a powerful alternative for identifying specific biological targets in live cells. In this paper, we present the synthesis of SERS-active gold nanochains (AuNCs) as a potential theranostic system for multiplex detection and photodynamic therapy (PDT) of cancer. AuNCs were prepared by a simple physical mixing method to assemble citrate-stabilized gold nanoparticles into nanochains using hyaluronic acid and hydrocaffeic acid (HA–HCA) conjugates as templates. In addition, Raman reporters and photosensitizers (PSs) were conjugated onto the surface of the AuNCs for multiplex detection and PDT action. After mixing with HA–HCA conjugates, citrate-stabilized gold nanoparticles formed the AuNC structure, and AuNC length was controlled by the HCA conjugation ratio in the HA–HCA conjugates. AuNCs exhibited maximal absorption in the near-infrared (NIR) spectral region and effective SERS property. Confocal microscopy, flow cytometry, Raman spectroscopy and Bio-TEM measurements were used to determine cellular uptake of the Raman reporter, PS and AuNCs in HeLa cells. AuNCs conjugated with Raman reporter and PS (HA–$HCA_n$–Au–Pheo–NPT) showed more than 99% cellular uptake and exhibited excellent phototoxicity even at low PS concentrations compared with free PS after laser irradiation. This SERS-active AuNC (HA–$HCA_n$–Au–Pheo–NPT) shows promise for applications in theranostics, integrating SERS imaging and PDT.



## 1. Introduction

The development of nano-structured materials with unique optical, electronic, and magnetic properties has opened up new possibilities for molecular imaging and spectroscopic detection of specific targets in biomedical applications. In particular, surface-enhanced Raman scattering (SERS) spectroscopy is one of the most powerful analytical techniques for identification of molecular species, with the potential of reaching single-molecule detection under ambient conditions. Conventional Raman spectroscopy produces intrinsically weak scattering signals [1,2]. However, on roughened surfaces, especially on plasmonic metal nanostructures, surface plasmon resonance (SPR) leads to a strongly enhanced electric field, resulting in significantly greater efficiency of a range of surface optical processes such as Raman, fluorescence, and second-harmonic effects [3–6].

SERS is one of the most important spectroscopies inherently originated from the SPR of metal nanostructures. SERS has received intense interest since its discovery in 1974 due to various salient attributes such as ultrasensitivity (a detection sensitivity that is 10–14 orders of magnitude higher than conventional Raman spectroscopy), excellent selectivity, rapid detection capability, high signal-to-noise ratio, non-photobleaching features and its use of single photoexcitation [7–10]. Several SERS nanoprobes have been developed for biological analysis, and are capable of providing fingerprint information for probe molecules at the single-molecule level, such as *in vivo* tumor targeting and spectroscopic detection [11], and bacterial capture and culture-free analysis in human blood [12]. In addition, the wavelength used for SPR is strongly

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related to their shape and size. As compared to spherical gold nanoparticles, which exhibit SPR at wavelengths in the visible light range, anisotropically grown gold nanorods have a strong longitudinal SPR mode along the elongated direction under near-infrared (NIR) light, and their absorption wavelength can be finely tuned by controlling the aspect ratio of the gold nanorod [13,14]. Since NIR light has much longer penetration depth into biological tissue than visible or infrared light, the fabrication of various gold nanostructures has drawn more attention for biomedical applications [11,12,15–19].

With these new tools, there is growing interest in theranostic nanomedicine with integrated imaging and therapeutic modalities for simultaneous diagnosis, disease treatment, and monitoring of therapeutic efficacy. In this study we designed SERS-active AuNCs as a novel theranostic system for cancer multiplex detection and photodynamic therapy (PDT).

To form AuNCs composed of gold nanoparticles, we used the biocompatible polymer conjugate hyaluronic acid–hydrocaffeic acid (HA–HCA). HA, which consists of repeating disaccharide units of β-1,4-D-glucuronic acid and β-1,3-N-acetyl-D-glucosamine, is a linear, nonsulfated glycosaminoglycan found ubiquitously in the extracellular matrix (ECM) of virtually all mammalian connective tissues. The use of HCA, 3,4-dihydroxyhydrocinnamic acid was inspired by mussel adhesion phenomena in nature. Mussels secrete specialized adhesive proteins containing a high content of the catecholic amino acid, proteins and their derivatives have demonstrated strong interfacial adhesion strength [20,21]. We utilized HCA as a high-affinity anchor for gold nanoparticle stabilization and AuNC formation. Our SERS-active AuNCs are not only useful for cancer detection, but also for the delivery of a PS for PDT action.

PDT, a noninvasive treatment modality for a range of diseases including cancers [22], combines three components (light, PS, and oxygen) to treat malignant diseases. Upon irradiation at the appropriate wavelength, the PS can be excited and transfers energy to surrounding tissue oxygen, generating highly reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$). The ROS can react with biomolecules such as unsaturated lipids, amino acid residues in proteins, and nucleic acid bases in DNA, initiating an apoptotic or necrotic response and eventually leading to oxidative damage and cell death [23–27].

Despite its advantage over traditional treatments, PDT has not been accepted for general treatment in clinics because of technical difficulties in application. Conventional PSs often randomly distribute *in vivo* and lack selectivity to tumors [28]. A significant challenge that needs to be overcome for most treatments is the hydrophobic nature of the PS, which severely hampers intravenous administration through the bloodstream. Because of their low water solubility, hydrophobic PSs cannot be directly injected intravenously. Here, to improve solubility and enhance cellular uptake and PDT efficiency, we conjugated a hydrophobic PS, pheophorbide a (Pheo), onto our AuNCs.

Although various conventional SERS nanoprobes have been developed for biomarker assays [5,6,29], a SERS-active AuNC for cancer multiplex detection and therapy has not been reported. The main objective of this study was to describe a novel multifunctional SERS-active AuNC prepared by self-assembling of gold nanoparticles into AuNCs for the simultaneous cancer detection and PDT. Raman reporter (2-naphthalenethiol (NPT)) and thiol group-functionalized PS (Pheo-SH) were conjugated onto AuNCs through thiol groups. The chemical composition of intermediates and the final product were determined by $^1$H NMR. The photophysical properties of AuNCs in aqueous solution and morphology were determined by UV–visible spectroscopy and TEM. *In vitro* cellular localization in HeLa cells was investigated by confocal microscopy, flow cytometry, SERS and Bio-TEM. The phototoxicity of free Pheo and SERS-active AuNCs was also investigated.

## 2. Materials and methods

### 2.1. Materials and characterization

HA (molecular weight: about $1.65 \times 10^6$ Da), sodium citrate tribasic dihydrate, cysteamine hydrochloride, triethylamine (TEA), and N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Chloroauric acid, HCA, 2-naphthalenethiol (NPT), and 4-(dimethylamino) pyridine (DMAP) were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). N-hydroxysuccinimide (NHS) was purchased from Fluka (Buchs, Switzerland). Dimethyl sulfoxide (DMSO) and acetone were obtained from Samchun Pure Chemical Co., Ltd. (Gyeonggi-do, Korea). Pheo was purchased from Frontier Scientific, Inc. (Logan, UT, USA). Spectra/Por membranes were purchased from Spectrum Laboratories, Inc. (Rancho Dominguez, CA, USA). RPMI 1640 medium, Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin, and Dulbecco's phosphate buffered saline (DPBS) were obtained from Gibco BRL (Invitrogen Corp., CA, USA). All chemicals were of analytical grade and used as received without further purification.

**Table 1**
Composition of HA–HCA conjugates.

| Samples | Feed ratio HA:HCA[a] | Conjugation ratio (%)[b] | Molecular weight (Da)[c] |
|---|---|---|---|
| HA–$HCA_{0.0}$ | 1: 0.0 | 0.00 | $1.65 \times 10^6$ |
| HA–$HCA_{0.1}$ | 1: 0.1 | 8.96 | $1.72 \times 10^6$ |
| HA–$HCA_{0.3}$ | 1: 0.3 | 28.17 | $1.85 \times 10^6$ |
| HA–$HCA_{0.5}$ | 1: 0.5 | 38.47 | $1.95 \times 10^6$ |
| HA–$HCA_{1.0}$ | 1: 1.0 | 40.70 | $1.97 \times 10^6$ |
| HA–$HCA_{2.0}$ | 1: 2.0 | 48.60 | $2.03 \times 10^6$ |

[a] Molar feed ratio of HCA to HA repeat unit.
[b] Percent of HA repeat units were functionalized by HCA, determined by UV–Vis spectra.
[c] Molecular weight of HA–$HCA_n$ was calculated based on the average molecular weight of HA ($1.65 \times 10^6$ Da) and the conjugation ratio of HCA.

**Table 2**
Properties of SERS-active AuNCs with different HCA conjugation ratios.

| Samples | Feed ratio[a] (HA:HCA) | Absorption wavelength (nm)[b] | EF[c] | DCC (%)[d] |
|---|---|---|---|---|
| HA–$HCA_{0.0}$–Au | 1: 0.0 | 519, – | | |
| HA–$HCA_{0.1}$–Au | 1: 0.1 | 519, 605 | | |
| HA–$HCA_{0.3}$–Au | 1: 0.3 | 519, 612 | | |
| HA–$HCA_{0.5}$–Au | 1: 0.5 | 519, 627 | | |
| HA–$HCA_{1.0}$–Au | 1: 1.0 | 519, 646 | | |
| HA–$HCA_{2.0}$–Au | 1: 2.0 | 519, 658 | | |
| HA–$HCA_{0.0}$–Au–NPT | 1: 0.0 | | – | |
| HA–$HCA_{0.1}$–Au–NPT | 1: 0.1 | | – | |
| HA–$HCA_{0.3}$–Au–NPT | 1: 0.3 | | $2.7 \times 10^5$ | |
| HA–$HCA_{0.5}$–Au–NPT | 1: 0.5 | | $2.2 \times 10^6$ | |
| HA–$HCA_{1.0}$–Au–NPT | 1: 1.0 | | $5.7 \times 10^7$ | |
| HA–$HCA_{2.0}$–Au–NPT | 1: 2.0 | | $1.8 \times 10^8$ | |
| HA–$HCA_{2.0}$–Au–Pheo–NPT | 1: 0.3 | | | 9.0 |
| HA–$HCA_{2.0}$–Au–Pheo–NPT | 1: 2.0 | | | 9.0 |

[a] Molar feed ratio of HCA to HA repeat unit.
[b] UV absorption wavelength of AuNCs (HA–$HCA_n$–Au) in aqueous solution, where n is the molar feed ratio of HCA to HA repeat unit.
[c] Raman Enhancement Factors (EF) (HA–$HCA_n$–Au–NPT), $EF = (I_{sig}/C_{sig})/(I_{ref}/C_{ref})$, where $I_{sig}$ and $I_{ref}$ represent the intensities of the 1375 $cm^{-1}$ band for the NPT adsorbed on the quartz substrate with and without AuNCs, respectively, whereas $C_{sig}$ and $C_{ref}$ represent the corresponding concentrations of NPT on these substrates.
[d] Drug conjugation content (DCC), determined by $^1$H NMR. $DCC\ (\%) = \frac{\text{Amount of Pheo in AuNCs}}{\text{Amount of Pheo-loaded SERS-active AuNCs}} \times 100\% = \frac{\text{Pheo}}{\text{HA–HCAn–Au–Pheo–NPT}} \times 100\%$.

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