Dual-Face and Tri-Functional Nanoparticles for Cell diagnosis: SEARs/Fluorescence Signaling, Protein Targeting, and Drug Delivering

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Abstract

We report an approach to fabricate dual-faced polystyrene beads (DFPSBs) with tri-functions of tumor cells recognition, drug delivery, and real-time Raman sensing. One-step oxygen-plasma treatment process was used to etch commercially available fluorescent polystyrene beads into a corrugated upper hemisphere and simultaneously change the entire surface with carboxylic groups. After depositing gold onto the corrugated hemisphere for surface enhanced Raman scattering (SERS) while leaving the other smooth and clean hemisphere for fluorescence detection, the DFPSBs are formed with dual-surfaces of plasmonic gold semishells on the top and fluorescent carboxylated polystyrene at the bottom. Sulfo-NHS-SS-biotin disulfide linkers and anti-CD44 antibodies can be modified and added onto the top gold surfaces and the bottom carboxyl groups through Au-S and peptide bonds, respectively. Then, the surface-modified AuFNM suspension can be employed to target overexpressive glycoproteins (CD44) on the surfaces of cancer cells and release their loads via the cleavage of disulfide bonds in the cytoplasm environment. These anti-CD44-modified DFPSBs exhibit a 12-fold cancer targeting ability on HeLa cells when compared to a normal chondrocyte cell.

Keywords: SERS, Nano particle, Bio sensing, Single molecule detection

1. Introduction

Ushering as miniature nanodevices in biological research, smart nanoparticles remarkably execute parallel multifunctions, such as combining two or more characteristics of high image contrast, fluorescent emission, thermal therapy, cancer targeting, and/or drug delivery, leading to simultaneously investigate complicated cell behaviors, visualize particle distribution, and/or cure cancer. In the past decade, label-free Raman spectroscopy incorporated with surface enhanced Raman scattering (SERS) techniques have also made a great progress for dynamic monitoring cell performances, including the differentiation of stem cells, the observation of biomolecule dynamics, and the transport pathways of endocytosed nanoparticle. Undoubtedly, new-smart-nanoparticles (NSNPs) merging of the conventional smart nanoparticle with the Raman sensing function will possess more widely applications for cell theranostics.

By employing a robust one-step process for surface roughness treatment on polystyrene NPs with self-grafted carboxyl surface functional groups, a smart particle, AuFNM, is carried out and consists of SERS/fluorescence sensing ability with dual molecule conjugation flexibility (Fig. 1 (a)-(c)). We also successfully demonstrate the multifunctions on the AuFNMs for cancer cells targeting, drug delivering into cells, and SERS Raman sensing. For live cell applications, it can perform Raman mapping of biomolecule dislocation (Fig. 1(d)) and 3-D visualization of the AuFNMs (Fig. 1(e)) through the upper SERS-active gold nanocorrugations and the lower anti-CD 44 modified fluorescent polystyrene, respectively.
Therefore, it becomes a powerful platform not only useful for tumor cells treatment but also for real-time monitoring cells metabolism.

Fig. 1. Schematic for the basic design of (a) AuFNMs that possess dual-module surfaces including gold-coated nanocorrugations and fluorescent carboxylated polystyrene. (b) On the upper gold surface, thiol SAMs can be modified through the Au–S bond. (c) On the lower surface with carboxylic groups, the –NH2 functional group of protein can be modified through a peptide bond. Moreover, (d) the upper hemisphere of the AuFNMs can provide SERS Raman sensing of the surface-modified thiol molecules or the surrounding biomolecules. (e) The lower hemisphere with long-lasting fluorescence can be used for 3D particle tracking in a confocal system.

2. Fabrication and Utilization of AuFNMs

Vigorous oxygen plasma etching on pure polystyrene beads (PSBs) without intrinsic carboxyl groups can reportedly undergo surface conversion via the cleaving of C-C-C bonds to form carboxylic functional groups. In this paper, we combined the outstanding characteristics of the oxygen-plasma-treated PSBs (surface roughness and carboxyl functional groups) with Au deposition (Fig. 2(a)-(d)), to produce the dual-faced polystyrene beads (DFPSBs) shown in Fig. 2(e). When the rough upper surface was covered with gold, a localized enhanced electric field accumulated on the inter-gaps (hotspots) between the neighboring nanopillar structures.

Fig. 2. Schematic illustration of the fabrication processes and the surface characteristics of the dual-faced polystyrene beads (DFPSBs). Dual-Faced PSB Fabrication Processes: (a) the 4” glass substrate is pre-cleaned, and (b) three-step spin-coating process is employed to obtain a monolayer of PSB array on the glass surface. (c) Oxygen plasma treatment converts pure polystyrene to carboxyl-modified polystyrene and results in a hemisphere-corrugated surface on the PSBs. (d) Following a vertically Au deposition on the oxygen-plasma-etched PSB array, the DFPSB is achieved. Surface Characteristics of DFPSBs: The upper and lower surfaces of (e) the DFPSB can be self-assembled with thiol (~SH) molecules and proteins (~NH2) via (f) Au–S bond and (g) peptide bond, respectively.
By exploiting the dual-module surface, highly selective modifications can be made to the Au-S bond using thiols\textsuperscript{[2, 3]} (Fig. 2(f)) and/or the peptide bond using a dehydration reaction between –COOH and –NH\textsubscript{2} (Fig. 1(g)).\textsuperscript{[4]} For applications in cancer therapy (cancer targeting\textsuperscript{[5]} and drug delivery\textsuperscript{[6-7]}), the DFPSBs were modified by attaching anti-CD44 antibody (on the carboxylated polystyrene) and a sulfo-NHS-SS-biotin disulfide linker (onto the amine\textsuperscript{[6, 7]} or gold surface\textsuperscript{[8-10]}). Because CD44 is overexpressed in most cancer cells, including HeLa and MCF-7, the anti-CD44-modified DFPSBs can be utilized to target cancer.\textsuperscript{[11]} For drug delivery, a relatively weak covalent bond in the disulfide linker has the advantage of being capable of cleavage via reduction. The disulfide cleavage, dividing one R-SS-R into two R-SH molecules, occurs with cell cytoplasm environment.\textsuperscript{[6, 7]} In the intracellular space, the cell regulatory mechanism can retain the redox equilibrium;\textsuperscript{[6, 7]} consequently, the disulfide linker-modified DFPSBs act as vehicles releasing their load inside the cell membrane. Therefore, surface-modified DFPSBs can integrate three functions (Fig. 3) on the nanoparticles for biomedical applications.

![Fig. 3. Tri-functions of SERS Raman sensor, cancer marker, and drug delivery carrier can be achieved by the corrugated Au surface, anti-CD44-modified lower hemisphere, and sulfo-NHS-SS-biotin linker, respectively.](image)

3. Experimental Results

In Fig. 4 the C1s spectrum of X-ray photoelectric spectroscopy (XPS) shows that the surface chemistry is changed from pure polystyrene to carbonyl groups (C=O or O-C-O) while the PSBs are treated by oxygen plasma etching. The particle diameters and the surface enhanced Raman intensity are shown in Fig. 5(a) and Fig. 5(b), respectively. The R500, G400, and G250 represent their original particle diameters of 500, 400, and 250 nm, respectively, and R and G indicate the fluorescence emission of red and green light, respectively. In Fig. 4(b) plasma etching time of about 120-160 sec on R500 and G400 can have about 10-fold improved Raman intensity. The SEM and TEM images of the surface-modified DFPSBs are shown in Fig. 6(a) and Fig. 6(b), respectively. Those DFPSBs are detached and resuspended in DI water using a ultrasonicator. In Fig. 7, the anti-CD44-modified DFPSBs (Au-O2-R400-anti CD44 and Au-O2-R400-anti CD44/biotin-strep-QDs) show up to 6-fold cancer cell targeting abilities on HeLa cell compared to that on normal cell (chondrocyte). To demonstrate the molecule delivery strategy, the gold film is modified with QDs through sulfo-NHS-SS-biotin (disulfide linker) and immersed in lysed HeLa cell cytoplasm solution. QDs were also successfully released after about 30-min immersion time inside cells.\textsuperscript{[12]}
4. Conclusion
Multifunctional nanoparticles AuFNMs have been successfully developed with capabilities such as fluorescence emission, cancer targeting, and drug delivery, which are potential tools for particle tracking, cancer therapy, and/or the investigations of particle–cell interactions.

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References