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Environmentally compatible bioconjugated gold nanoparticles as efficient contrast agents for colorectal cancer cell imaging

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ABSTRACT

In this study we show, for the first time, that gold nanoparticles (AuNPs) synthesized by a simple, inexpensive, and environmentally-correct method can be easily conjugated with the antibodies anti-β-catenin and anti-E-cadherin to specifically target colorectal carcinoma cells. The antibody/AuNPs conjugates were then successfully applied for imaging cancerous cells with fluorescence confocal microscopy. The AuNPs as well as the conjugates were very stable in high-salinity medium, a pre-requisite for application in physiological-like environments. Fluorescence results suggest that conjugation was achieved by direct adsorption of antibodies on the AuNPs surface. Finally, compared with a standard method of cell staining, our method is less laborious and the preparation time (from immobilization of cells onto glass cover slips until observation by confocal microscopy) decreased from 27 h to about 1 h, which makes the method eligible for colorectal cancer diagnostic.

1. Introduction

Current cancer diagnostic methodologies include optical imaging techniques such as coherence tomography and fluorescence confocal microscopy. The image is generated by the backscattered light from the endogenous chromophores present in the tissue. The weak optical signal results in poor intracellular contrast and subtle spectral differences between malignant and benign cells hamper a proper diagnostic [1]. In order to overcome these problems, exogenous chemicals (mainly organic dyes) that function as optical contrast agent have been used to enhance the visibility of both precancerous and cancerous cells. However, organic dyes are subjected to rapid photobleaching [2], therefore becoming inefficient as the analysis proceeds. Other disadvantages of conventional dyes include poor hydrophilicity, low quantum yield, and low detection sensitivity in biological environment [2].

Recent advances of nanotechnology have allowed the development of new functional nanomaterials with enhanced specificity for molecular imaging. In particular, colloidal Au nanoparticles (AuNPs) are extremely advantageous as contrast agents in biomedicine [1,3–6] as they can be easily combined with a recognition agent. First, AuNPs present a size-tunable surface plasmon resonance (SPR) that leads to strong absorption and scattering in the visible-to-near-infrared region, a characteristic which makes them considerably superior to the conventional dyes applied in biomedical imaging. For instance, the plasmon resonance absorption of spherical gold nanoparticles has an absorption coefficient orders of magnitude larger than those of regular dyes. As an example the absorption coefficient (ε) for 40-nm gold nanoparticles [7] at a wavelength of 530 nm was calculated to be $\sim 7.7 \times 10^9\ M^{-1}\ cm^{-1}$, while the ε of rhodoamine 6G is $1.16 \times 10^5\ M^{-1}\ cm^{-1}$ [8]. Second, AuNPs do not suffer photobleaching and seem to be biocompatible [9]. Third, the chemistry of gold is quite rich. The AuNPs surface binds strongly with amines, thiols and disulfides, allowing the surface bioconjugation with a great variety of peptides, DNA and proteins, enabling selective targeting of cancer cells. Furthermore, in opposition to dyes and organic molecules, several functional

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groups can be attached onto the same nanoparticle, making them also eligible for drug delivery [10].

In this paper we synthesized AuNPs bioconjugated with β-catenin antibody (anti-β-catenin) and E-cadherin antibory (anti-E-cadherin) and applied them as novel contrast agents for the detection of colorectal cancerous cells with fluorescence confocal microscopy. E-cadherin is localized on the surface of epithelial cells in regions of cell–cell contact known as adherens junctions and is implicated in cell–cell adhesion in epithelial tissues [11–13]. β-Catenin interacts with cadherins through its cytoplasmic domain. α-catenin connects the E-cadherin and β-catenin complex to actin filaments. The dissociation of E-cadherin-catenin complex from cell membrane is important in malignant progression. In many epithelial cancers, membranous E-cadherin is lost and β-catenin dissociates in the cytoplasm and accumulates in the nucleus as a transcription factor, concomitantly with tumor progression [12]. Down-regulation of membranous E-cadherin and β-catenin, and cytoplasmic/nuclear accumulation of β-catenin have been previously reported in several cancers and hold promise as prognostic markers [14]. In humans β-catenin and E-cadherin are encoded by the adenomatous polyposis coli (APC) and CDH1 genes, respectively, and their mutation may result in colorectal cancer leading to an overexpression of β-catenin and E-cadherin by the cell [11–16]. The strategy was therefore to combine the specificities of anti-β-catenin and anti-E-cadherin with the fluorescent properties of the AuNPs to produce a selective contrast agent for targeting cancer. In this system, the AuNPs function as the signaling part and the anti-β-catenin and anti-E-cadherin act as recognition units for specific binding with the overexpressed β-catenin and E-cadherin in the cell. The confocal microscopy revealed that the antibody-conjugated AuNPs were very efficient in marking cancerous cells. On the other hand, no specific interaction between normal cells and conjugated AuNPs was observed. Another interesting feature of the present work is the use of an environmentally friendly route to produce the AuNPs based on the reduction of gold ions by glycerol in alkaline medium. Glycerol's non-toxicity and biodegradability make it an excellent alternative to commonly used reducing agents. The conjugations with anti-β-catenin and anti-E-cadherin are achieved by simple incubation after reducing the pH of the nanoparticle colloidal solution to around 7. In comparison to current methods for production and conjugation of AuNPs, our method is quite simple, low-cost and environmentally compatible, with a potential to be routinely applied in cancer diagnostics.

2. Experimental

2.1. Chemicals and reagents

Gold trichloride (30 wt% in HCl), polyvinylpyrrolidone (PVP, M_W = 10.000), sodium hydroxide, dialysis tubing cellulose membrane, and glycerol were products of Sigma-Aldrich Chemical Co. Sulphuric acid and hydrogen peroxide were purchased from Vetec. Phosphate-buffered saline (PBS) solution and bovine serum albumine (BSA, 5%) were purchased from Life Technologies Corporation©. β-Catenin antibodies (anti-β-catenin) and E-cadherin antibodies (anti-E-cadherin) were acquired from ABCAM® at the concentration of 200 μg L^{-1} in PBS buffer. Dulbecco's modified Eagle's medium (DMEM) and heat-inactivated bovine serum were purchased from Life Technologies Corporation© and Cultilab Ltda/Brasil, respectively.

2.2. Production and characterization of AuNPs

AuNPs were produced using a previously reported method [17]. Briefly, all glassware was kept overnight in $KMnO_4$ + NaOH solution, rinsed with deionized water, kept for 10 min in H_2O_2 + H_2SO_4 solution (1:1 v/v), again rinsed with deionized water and dried prior to use. Known amounts of PVP (M_W = 10.000) and gold chloride were dissolved in 10 mL of water. In a separate beaker, determined quantities of glycerol and NaOH were dissolved in 10 mL of water. The glycerol–NaOH solution was then added to the $AuCl_3$-PVP solution to yield the following final concentrations: 1.0 mM Au^{3+}, 0.10 M NaOH, 0.10 M glycerol and 10 g L^{-1} PVP. The final mixture had a deep-red color due to the formed AuNPs. The AuNPs colloidal

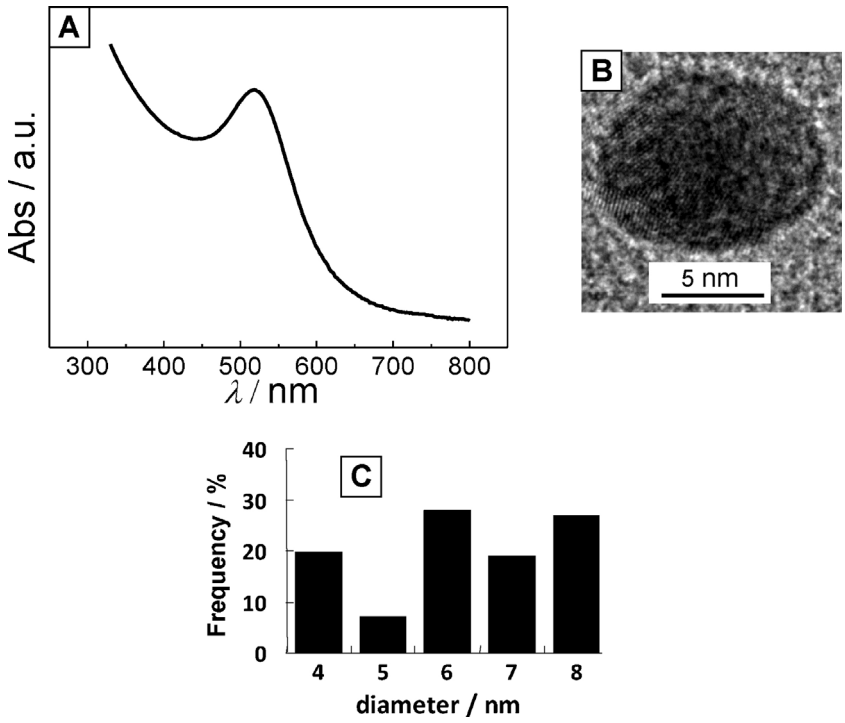

Fig. 1. (A) UV–vis spectra of the colloidal AuNps, (B) high-resolution TEM images of a AuNp and (C) size distribution of the AuNps.