SERS Gold Nanoparticles for Cancer Cell Surface Marker Detection

C.T. Nguyen*, J.T. Nguyen*, Chen Wang** and Gilbert Walker*

* Department of Chemistry, Lash Miller Building
University of Toronto, 80 St. George Street, Toronto, ON Canada, M5S 3H6.
tcnguyen@chem.utoronto.ca, gwalker@chem.utoronto.ca
** Department of Pathology & Laboratory Medicine, Faculty of Medicine,
Mount Sinai Hospital, 600 University Avenue Toronto, ON Canada M5X 1G5.
cwang@mtsinai.on.ca

ABSTRACT

Surface Enhanced Raman Scattering (SERS) gold nanoparticles may provide novel optical tags for cellular analysis. This study describes the engineering, synthesis and testing of SERS gold nanoparticles for selective targeting and imaging of Chronic Lymphocytic Leukemia (CLL) cells. The SERS nanoparticles were conjugated to monoclonal antibodies that selectively target the CD19 antigen, a diagnostic marker of B-cell CLL [1]. The nanoparticles were protected with polyethyleneglycol to impart stability and prevent aggregation. The specific SERS signals of CLL cells were detected by using Raman spectrometry. The SERS nanoparticle specificity was examined using control nanoparticles that are conjugated to anti-CD4 antibody. A strong Raman signal was observed, providing the optical tag. The results indicate that SERS gold nanoparticles can be used to selectively target cell surface markers and Raman analysis of CLL cells.

Keywords: Immunopathology, Surface Enhanced Raman Scattering, chronic lymphocytic leukemia, cancer, nanoparticle conjugates.

INTRODUCTION

Surface Enhance Raman scattering (SERS) for molecular targeting and cellular imaging is a novel technique that has evolved rapidly within the past five years. With recent applications in biomedical research, nanostructures are revolutionizing the ways to study molecular processes during disease progression. Nanomaterials of various dimensions and compositions have been successfully employed for targeting and imaging of different cancers and molecular targets [2-4]. Gold pegylated nanoparticles imbedded with Raman reporter molecules have shown increasing usage in detection of malignant tumors [5-6]. Furthermore, colloidal gold has been shown to be safe, biocompatible and non toxic to human cells [7]. These vital characteristics of non-toxicity and biocompatibility make gold an attractive candidate for medical applications. When conjugated with biomolecular ligands such as peptides or monoclonal antibodies, these nanoparticles can be used to target specific tumors with high specificity and affinity.

Here we describe a strategic approach to construct SERS nanoparticles to detect the CD19 antigen. CD19 is a glycoprotein with a molecular weight of 95 kDa expressed on cell surface. It is commonly used as a marker for B lymphocytes and B–cell malignancies [1]. Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in the United States and Western countries, accounting for more than 30% of leukemias [8]. CLL diagnosis requires characteristic phenotype. The SERS nanoparticles may be used as optical tags for detection of CLL cell markers. In addition, pegylated particles exhibit qualities such as improved biocompatibility with cells in suspension [9]. Finally, the PEG layer allows for efficient conjugation of SERS nanoparticles with anti-CD19 antibody for vitro targeting of CLL cells. Our results demonstrate the successful application of SERS to detect surface markers of leukemia cells.

MATERIALS AND METHODS

Reagents: 60-nm citrate-stabilized gold particles were purchased from Ted Pella Inc. Malachite green isothiocyanate (MGITC) was obtained from Invitrogen. mPEG-SH (MW 5 kDa) and HS-C_2H_4-CONH-PEG-C_3H_6-COOH (MW 5 kDa) (Rapp Polymers).

Preparation and Characterization of SERS Nanoparticles

The 60 nm gold nanoparticles were tagged with a Raman reporter (MGITC). The particles were protected with mPEG and functionalized with CD19 antibodies (Fig 1). The nanoparticles were characterized by UV-vis (Fig 2a, b), Transmission Electron Microscopy (Fig 2c), Atomic force microscopy (Fig 2d) and Dynamic Light Scattering (Fig 2e). Raman measurements were performed 633 nm (Fig 2f). The spectral resolution was 3 cm^{-1}.

Conjugation of Antibodies to gold particles

To selectively target CLL cells, human anti-CD19 antibodies were covalently conjugated to the pegylated SERS nanoparticles using a heterofunctional linker HS-PEG-COOH. For covalent conjugation, the carboxylate groups on the particle surface was activated through ethyl

dimethylaminopropyl carbodiimide (EDC) and sulfo-NHS chemistry. The gold nanoparticles were then exposed to excess mPEG-SH to facilitate a well protected particle surface. The gold nanoparticles were further purified by centrifugation and resuspended in PBS. Using the same method, human anti-CD4 antibodies were functionalized to the SERS nanoparticles as one of the negative controls.

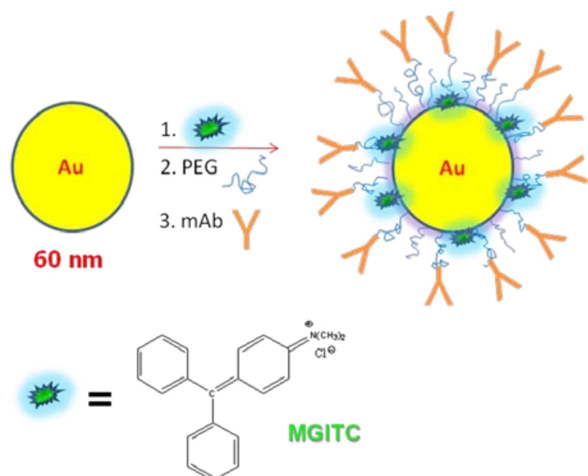

Figure 1. Schematic illustration of pegylated SERS gold nanoparticles functionalized with CD19 antibodies for SERS imaging of Leukemia cells.

Preparation of Leukemia cells

CLL cells were prepared from routine blood samples at Mount Sinai Hospital. CLL diagnosis was established by hematology, morphology and flow cytometry phenotype analysis. Mononuclear cell fraction was separated by using Ficoll-hypaque gradient method. Clonal CLL cells accounted for over 95% of the separated cell population as determined by flow cytometry phenotype analysis. The cells were suspended in PBS (1% FCS) for antibody staining.

Cell marker labeling

CLL cells were incubated with the functionalized CD19 conjugated SERS nanoparticles. The cells were then washed several times and resuspended in PBS before SERS measurement. To control for non-specific bindings, CLL cells were also incubated with control SERS nanoparticles (with no antibodies attached), and with SERS nanoparticles functionalized with CD4 antibodies. The cells were resuspended in PBS at a cell density of 10^6 cells per ml and aliquots of cells were placed slides in suspension. The attachment of the gold nanoparticles to the cell surface was examined under a dark-field microscopy; a technique that detects only scattered light. Images were captured with an upright microscope equipped with a camera.

RESULTS AND DISCUSSION

The SERS nanoparticle-antibody conjugates were tested for the detection of CLL cells. Cell imaging and spectroscopic detection are illustrated in Figure 3. CLL cells prepared on glass slides were examined using dark-field microscopy. Gold particle attachment to CLL cells were detected using dark field microscopy (both Raman and Rayleigh scattered light is seen in the first 2 columns of Fig 3). In contrast, the light scattering spectra show the presence or absence of Raman scattering and are collected in such a way that Rayleigh scattering by the gold core is excluded. A long-pass emission filter (λ>655 nm) was used to pass Raman scattered light and exclude 633nm Rayleigh scattered light.

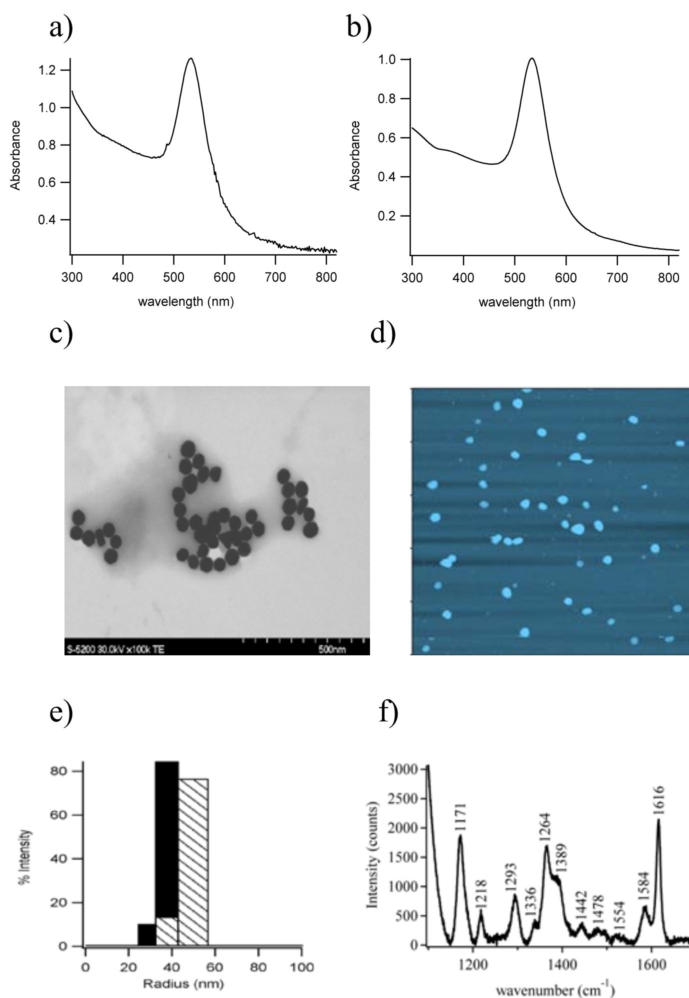

Figure 2. (a) Optical absorption before and after pegylation (b), (c) transmission electron microscopy (TEM), (d) Atomic Force Microscopy (AFM) image 5 x 5 µm, and (e) dynamic light scattering (DLS) size data obtained from before (black) and after (hash) pegylation of gold nanoparticles. Raman spectra of SERS nanoparticles (f).

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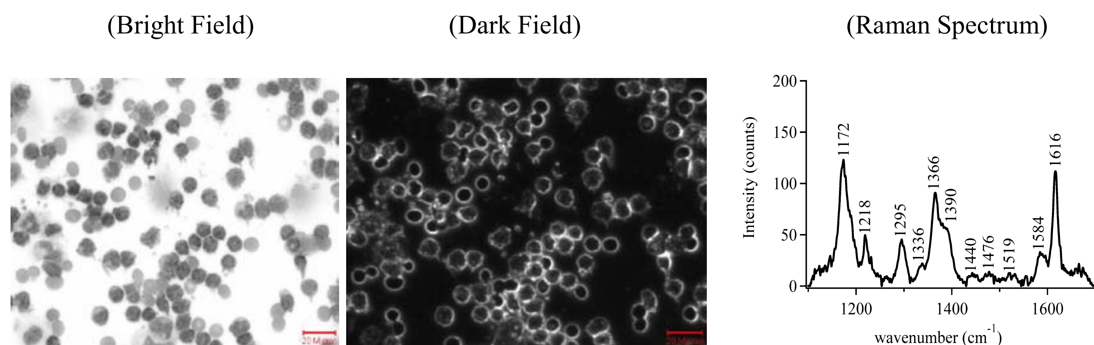
Nanoparticles conjugated to CD19 antibodies showed specific targeting CLL cells (Figure 3a), while unconjugated nanoparticles or the control conjugates with anti-CD4 antibody showed minimal particles bound to cell surface (Figure 3b, c). The MGITC origin of the Raman signal is confirmed by the frequencies of the Stokes shifted bands [10]. Spectroscopic analysis of the negative controls, nanoparticles without antibody conjugation or nanoparticles functionalized with CD4 antibodies, showed no Raman signals.

a) CLL cells labeled with anti-CD19 antibody-SERS-nanoparticles



b) CLL cells incubated with anti-CD4 antibody-SERS conjugates

c) CLL cells incubated with control SERS-nanoparticles (unconjugated)

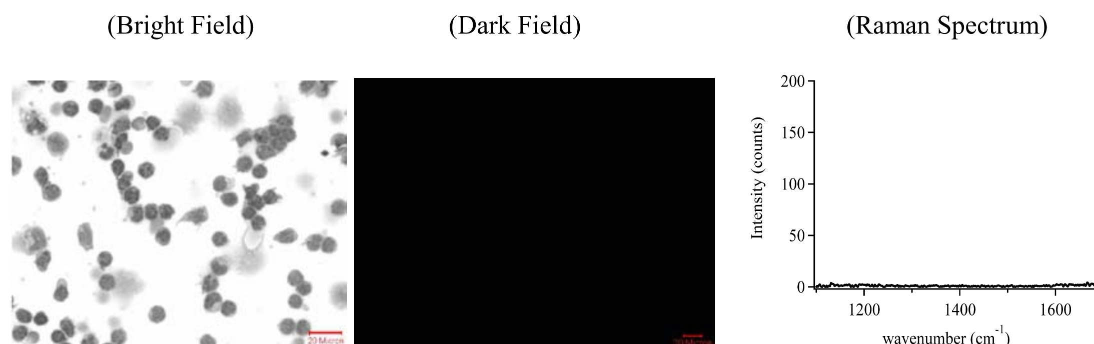


Figure 3. Bright and Dark-field images and accompanying Raman spectra of CLL cells stained with anti-CD19 and nanoparticle conjugates (a); anti-CD4 antibody and nanoparticle conjugates (b) and Unconjugated nanoparticles (c). Original magnifications, 20X.

CONCLUSION

SERS nanoparticle conjugates demonstrated selective targeting and significant strong Raman signal was detected. Our long term goal is to utilize these particles to detect multiple cell markers simultaneously. Thus by passing the limitation associated with conventional methods such as fluoresences.

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