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Gold nanorods reflectance discriminate benign from malignant oral lesions

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Abstract

Nanoparticle-based contrast agents have been used as an imaging tool for selectively detecting cancerous processes. We aimed to evaluate the detection sensitivity of reflection measurements of gold nanorods (GNRs) bio-conjugated to anti-epidermal growth factor receptor (GNRs-EGFR) monoclonal antibodies in discriminating benign from premalignant and malignant human oral lesions. Tissue sections incubated with GNRs-EGFR and the reflectance spectrum was measured using hyperspectral microscopy. Reflectance intensity increased with the progression of the disease, lowest in the control group and increasing as the dysplastic changes increase ($P < 0.001$ for linear trend of grade). Intensity was significantly higher in the moderate and severe dysplasias and cancer patients than in the controls and mild dysplasia (t test $P = 0.0003$, Mann–Whitney $P < 0.0001$). The GNRs reflection measurements can discriminate benign and mild dysplastic lesions from the more severe dysplasia and invasive cancer, suggesting an objective, not dependent on the qualification of a technician and with less interpretation errors.

Key words: Nanoconjugates; Nanospheres; Gold nanorods; Mouth neoplasms; Oral cancer; Oral potentially malignant disorders

Oral squamous cell carcinomas (OSCC) are among the most common cancers worldwide and are of great economic and clinical burden for the health care in developing countries.[1–3] Late diagnosis and loco-regional recurrences results in poor prognosis despite advancement in treatment modalities.[4,5] Most cases of OSCC are preceded by precancerous lesions which have an increased potential for malignant transformation assumed to be between 2 and 4% annually.[6] Histopathology is the gold standard for predicting the extent of the disease, however, controversy exist in reporting dysplastic conditions which make interpretation difficult. A different diagnostic approach is therefore mandatory for early in vivo detection of oral cancer and precancerous lesions, a method that will be sensitive, and easy to perform in clinical setting.

Gold nanoparticles (GNPs) have been studied as contrast agents for imaging of cancer, mainly due to their ability to scatter and enhancing the reflection of the irradiated light in their surface plasmon resonance frequency. Epidermal growth factor receptor (EGFR) has been found to be dysregulated in OSCC and dysplastic oral lesions,[7–11] and can be served as an ideal target for nanoparticle-based contrast agents using gold GNPs bio-conjugated to anti-EGFR monoclonal antibodies. We have recently introduced a new method for cancer detection based on diffusion reflection (DR) measurements of gold nanorods (GNRs) bio-conjugated to anti-EGFR monoclonal antibody (GNR-EGFR),[12–14] successfully tested in a rat model of OSCC.[15] Using air-scanning electron microscopy, we have been able to visualize the GNRs-EGFR in tissue sections of OSCC showing a gradient of the GNRs from the tumor to its surrounding normal epithelium.[16] The absorption and scattering spectrum of the GNRs is tunable by adjustment of their aspect ratio (AR), easily controlled during the synthesis of the GNRs. It enables to adjust the GNRs optical properties to the wavelength in which the sample has the highest penetration depth.

In the present study we aimed to evaluate the detection sensitivity of the DR method in human oral tissues, discriminating benign and mild dysplastic lesions from more advanced

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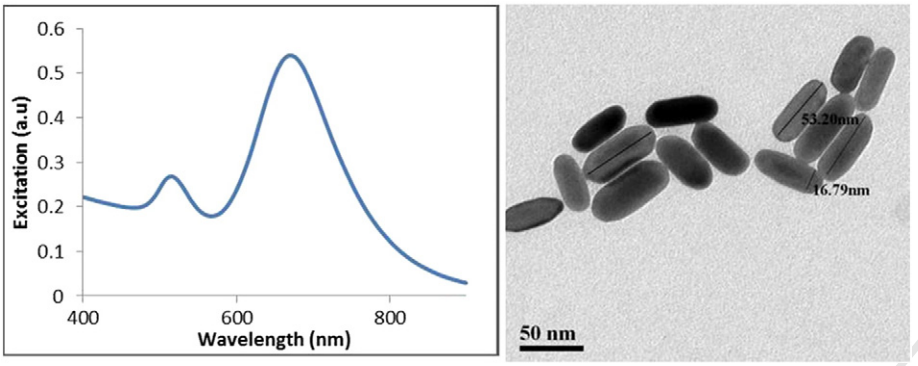

Figure 1. Excitation spectrum and TEM image of the GNRs used in the reflectance experiments. The GNRs peak is at 660 nm and their average dimension was 55×16nm.

dysplasia and invasive cancer with histopathology as a gold standard. The reflectance spectra were captured using the hyperspectral imaging microscopy, enabling the measurements of the GNRs reflectance intensity.

Methods

The archives of the Department of Oral Pathology and Medicine School of Dental Medicine, Tel-Aviv University were searched for cases diagnosed as OSCC and various dysplastic lesions. The study has been reviewed and approved by the Tel-Aviv University Ethics Committee; all cases were anonymous and informed consent was not required. The cases were divided according to histopathologic diagnosis based on the WHO diagnostic criteria as followed: mild dysplasia, moderate dysplasia, severe dysplasia, and invasive squamous cell carcinoma.[17] The original slides were reviewed and the diagnosis was confirmed by two of the researches, both certified oral pathologists (Abraham Hirshberg, Irit Allon). Only cases with definitive diagnosis based upon histopathology were included. Data concerning age, gender and oral site were recorded.

From each paraffin-embedded block, two consecutive 5 μm sections were cut on a glass slide. One slide was stained with hematoxylin and eosin (H&E) to confirm the diagnosis and an unstained slide was submitted for the hyper-spectral imaging and the DR experiments. The epithelium, which was the area of interest (AOI) was marked on the H&E stained slide and copied exactly on the unstained consecutive slide; the DR measurements were performed only within the AOI.

GNRs fabrication and targeting

GNRs present the highest scattering and absorption properties compared to gold nanoshells or gold nanospheres so they were utilized as targeted contrast agents. The GNRs were synthesized using seed mediated growth method,[18] and their size, shape and uniformity were characterized using transmission electron microscopy (TEM, Figure 1). Seed solution: A freshly prepared, ice-cold aqueous $NaBH_4$ solution (0.01 M, 0.6 mL) into an aqueous mixture solution composed of $HAuCl_4$ (0.01 M, 0.25 mL) and CTAB (0.1 M, 7.5 mL), followed by a rapid mixing for 2 min. The seed solution was kept for 1 h at 25 °C. The growth solution was composed of CTAB (0.1 M, 200 mL), $HAuCl_4$ (0.01 M, 10 mL), $AgNO_3$ (0.01 M, 1.5 mL) and ascorbic acid (0.1 M, 1.6 mL). 480 μL seed solution was added into the growth solution, which was kept under the same condition with seed solution. The resultant average shape was 55×16nm, with a narrow size distribution. The GNRs extinction coefficient spectrum was determined using a spectrophotometer, and the resultant extinction peak was 660 nm (Figure 1).

For the bioconjugation process, a protective layer of polyethylene-glycol (PEG) was adsorbed on the surface of the GNRs in order to prevent aggregation.[19] For cancer cell targeting, the heterofunctional PEG was covalently conjugated to an anti-EGFR monoclonal antibody.[12,13] Bioconjugation of the GNRs to the anti-EGFR antibody was achieved according to the method described by Lvov using polystyrene sulfonate.[20] Each slide was scanned before and after adding the GNRs for negative control.

All slides were viewed under hyper-spectral microscopy, and the reflectance intensity was captured. The measurements were performed blinded to the tissue origin.

Hyper spectral imaging system

Reflectance measurements of GNRs on tissues were captured using the hyper-spectral imaging system (Nuance, CRi, MA). By using this method one can easily prove that the GNRs are really location in the tissue. A Halogen illumination (UN2-PSE100, Nikon, Japan), along with a 32-bit ultrasensitive CCD camera detector (N-MSI-EX) and X40 objective (0.75NA) were used for imaging in RGB mode. Microscopy was performed with an 80i Nikon Microscope (Nikon Instruments). Images were acquired using the Nuance software version 2.1. The value units of the images are arbitrary intensity units (IU). The reflectance intensity from each sample was collected and analyses were performed at 660 nm, according to the GNRs excitation peak. A schematic diagram of our method is illustrated in Figure 2.

Statistics

The main goal of the analysis was to investigate the power of GNRs reflectance measurements in discriminating patients that do not need invasive treatment (controls or mild dysplasia) from