SERS measurement of the bladder cancer cells with the nanoparticles

Di Jin, Haige Chen, Ming Cao, Guoliang Yang, Wei Xue and Yiran Huang*
Department of Urology, Ren Ji Hospital School of Medicine, Shanghai Jiao Tong University, Dongfang Road, Shanghai, PR China

Abstract: The surfaced enhanced Raman spectroscopy (SERS) of bladder cancer cells and tissues were measured in this paper. Both depth SERS and map SERS of SCABER bladder cancer cells were measured with confocal Raman microscope using gold nanoparticles as the enhance substrate. We also measured SERS of normal bladder tissue and bladder cancer tissue, and analyzed the difference of two different tissues. The SERS spectra of more samples need to be measured and analyzed for bladder cancer tissue and the normal bladder tissue in the future and the spectra will be helpful for bladder cancer diagnosis.

Keywords: Raman spectra; depth spectrum; mapping spectrum; bladder cancer cell.

INTRODUCTION

Bladder cancer is the ninth most frequently diagnosed cancer and accounts for 5-10% of all malignancies in males worldwide (Draga *et al*, 2010). Bladder cancer ranks first of genitourinary cancer incidence in our country, while ranking No. 2in the West, after the incidence of prostate cancer. Therefore, early detection of bladder cancer is essential for preventing early recurrence and provides the chance for patient survival (Grimbergen *et al*, 2009).

The bladder cancer diagnosis methods were mainly included routine urianlysis, urine cytology, urinary tumor markers, pelvic B ultrasound, pelvic CT, pelvicMR. And the other diagnosis methods were also studied using protein or gene analysis or fluorescence. Fluorescence-guided cystoscopy using 5-aminolevulinic acid could improve the detection of bladder tumors, particularly carcinoma in situ (Jichlinski *et al*, 2003; Blanco*et al*, 2010). The elastic scatter measurements as a tool for diagnosing bladder cancer was applied using optical fiber probe through one of the lumens of a urological cystoscope with an algorithm for distinguishing malignant from nonmalignant tissue (Judith *et al*, 1995). The gene assays for the diagnosis of the bladder cancer were also studied (Eissa *et al*, 2014, Mengual *et al*, 2014). In general, these methods need the label or high costly.

Surface-enhanced Raman Spectroscopy (SERS) appears particularly promising because it can provide the molecular vibration spectrum information with label-free. It has been applied in many fields such as analytical chemistry (Fan *et al*, 2011), and life science (McNay *et al*, 2011). Raman Spectroscopy also plays an important role in the aspect of cancer treatment, several researchers have been reported in breast cancer (Krishna *et al*, 2008), lung cancer (Li *et al*, 2012), esophageal cancer (Bergholt *et al*, 2011), skin cancer (Lui *et al*, 2012) and bladder cancer (Barman *et al*, 2012). In this paper, the SERS of bladder cancer cells was detected using the gold nanoparticles as the substrate with the confocal Raman micro-spectroscopy. Depth spectra measurement and mapping spectra measurement were both used for determining the compositions of the bladder cancer. We also measured the SERS of bladder cancer tissue and normal bladder tissue in this work.

Methods and the measurements setup

Gold nanoparticles preparation

The gold nanoparticles were prepared by tri-sodium citrate reduction method with microwave heating. 1mL 1% $HAuCl_4$ solution was dipped into 100mL deionized water, and heated to boil in microwave, then quickly dropped 2mL 1% tri-sodium citrate solution into the above mixtures solute ion, so that the gold nanoparticles were synthesized.

Cell preparation

SCABER cells were cultured in dulbecco's modified eagle medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS). Cells were maintained under standard cell culture conditions at 37°C with 5% CO_2. The cell dissociation was processed using 0.25% Trypsin-EDTA, and the cells were transferred to different Silicon wafer placed in 6-well plate and incubated with the SERS substrate gold nanoparticles at the ratio of 2:1 (medium: gold colloids) for approximately 24 hours in the incubator. After 24h in exponential growth conditions, cell samples on the Silicon wafer were picked up from culture medium and washed with Phosphate Buffered Saline (PBS) for two times in order to remove free gold nanoparticles on the surface of the cells before measuring on the silicone slide. The bladder tissue cancer cells were placed on the silicone slide for the measurement.

Tissue preparation

Bladder tissues were obtained from patient who was undergoing bladdertomy, the samples were snap-frozen in

*Corresponding author: e-mail: hyrrenji2@aliyun.com

liquid nitrogen for storage and then passively thawed at room temperature. We cut tissues in pieces and cultured them in DMEM, tissues were maintained under standard cell culture conditions at 37°C with 5% CO_2, then added some gold nanoparticles in the DMEM. After approximately 24h in exponential growth conditions, tissues were washed with PBS (Phosphate Buffered Saline) for three times, then were transferred to 1.5mL centrifuge tubes. Then centrifuge the suspensions at 1000 rpm for 5 min. The tissues cluster on the bottom of the tubes was re-suspended in PBS. And then centrifuge at 1000 rpm for 5 min. Repeated more than three times, the tissue sample was prepared.

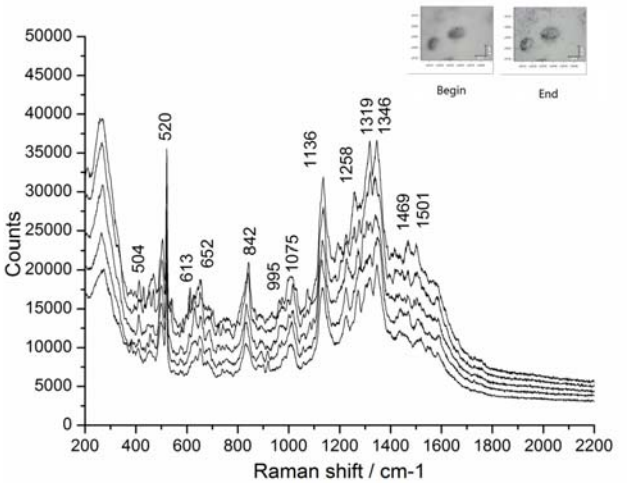

Fig. 1: The depth spectra of the bladder cancer cells.

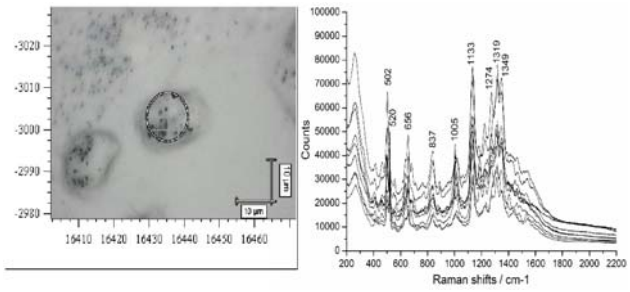

Fig. 2: The mapping spectra of the bladder cancer cells.

Raman measurements setup

Raman measurements were performed on the Renishaw Invia Confocal Raman Microscope system. 785 nm laser light was used as the excitation source and the laser beam was focused on the single cell through an objective lens (×50L magnification), and then measured with the depth spectra measurements mode and mapping spectra measurements mode. Finally SERS of bladder cancer and normal bladder tissues were measured. The output laser power was about 10mW and the wavelength scan range was between 200cm^{-1} and 2200cm^{-1}.

RESULTS

Depth spectra measurements

The 50× objective lens and the depth profiling measurements of the bladder cancer cells were performed in high confocal mode with the 785nm laser, as shown in fig. 1. In this experiment, the step was setto 2um and the number of acquisitions was 5. The obvious peaks appeared at the positions of 652, 842, 995, 1075, 1136, 1258, 1319, 1346, 1469 and 1501cm^{-1}. The peaks could be assigned to chemical constituents of proteins, lips, DNA and others. The tentative bands assignments were listed in table 1.

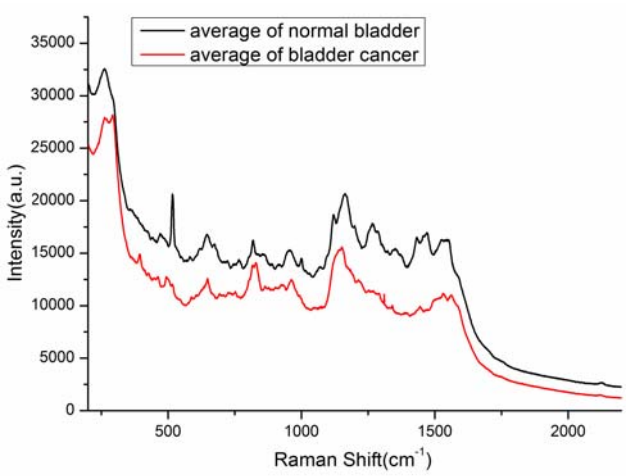

Fig. 3: SERS of bladder cancer and normal bladder tissue

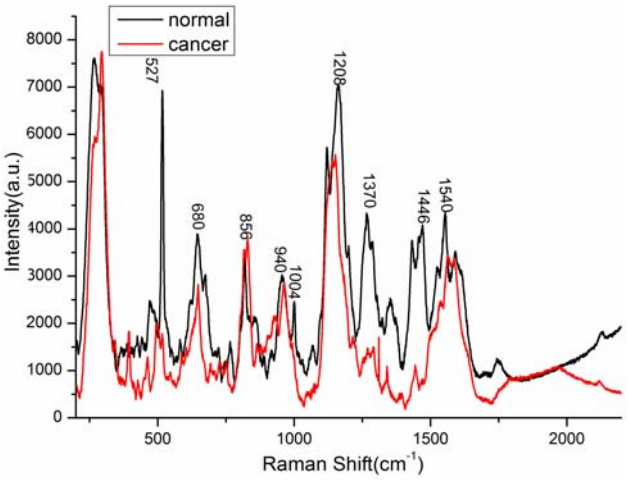

Fig. 4: SERS of bladder cancer and normal bladder tissue after background subtracted

Mapping spectra measurements

Fig. 2 showed the mapping spectra of the bladder cancer cells. Some peaks were different among the cell spectra, but several peaks were same to each other.

Table 1: Peak position and major assignment (Draga *et al*, 2010)

Raman shift (cm^{-1})	Tentative assignment
652	DNA (guanine)
995	Phenylalanine
1075	C-N stretching of proteins (and lipids mode to lesser degree)
1136	Undefined
1258	Amide III (C-N stretching mode of proteins), collagen
1319	CH3, CH2, lipids, DNA (adenine, cytosine)
1346	DNA (adenine, thymine, guanine), lipids
1469	CH2 deformation lipids and proteins

Table 2: Peak position and major assignment

Raman Shift (cm^{-1})	Tentative assignment	Raman Shift (cm^{-1})	Tentative assignment
680	DNA (guanine)	1206	Tryptophan, phenylalanine
856	Tyrosine	1370	DNA (adenine, thymine, guanine), lipids
940	C-C stretching of proteins	1446	CH2 deformation lipids and proteins
1004	Protein: ring breathing mode (phenylalanine)	1540	Tryptophan, DNA (guanine, adenine), phenylalanine

Spectra measurements of the normal bladder and bladder cancer tissues

The SERS of the normal bladder and bladder cancer cells with direct detection method was measured, as shown in fig. 3. fig. 4 showed the spectra after background fluorescence subtracted. In fig. 3 we can find that intensity of normal tissues was stronger than bladder cancer tissues. In fig.4 we can obviously see the different parts of two different tissues, table 2 showed the major assignment of Raman peaks. Raman peaks were obvious in SERS of normal tissues, but disappeared in SERS of bladder cancer tissues. The SERS spectra of the tissues were different with the spectra of the SCABER bladder cancer cells strain. These represented the specific chemical component changes in the process of cancer, for example, Raman peak position of normal bladder tissue in 1370cm^{-1} was much stronger than cancer tissue, it demonstrated that tryptophan, DNA (guanine, adenine) and phenylalanine may be degraded in cancer tissues.

CONCLUSIONS

Raman spectra were studied for the diagnosis of bladder cancer with high specificity and sensitivity (Lopez *et al*, 2014; Barman *et al*, 2012; Praveen *et al*, 2013). The depth spectra and mapping spectra of the SCABER bladder cancer cells were detected for determining the chemical compositions. The Raman spectra of the bladder tissue cancer tissues and normal tissues were measured to analyze the difference of two tissues, which may be helpful for Raman measurements of cells and tissues used in clinical diagnosis in the future.

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