The application of Silver nanoparticle based SERS in diagnosing thyroid tissue

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Abstract. Surface-enhanced Raman scattering (SERS) is proved to be a powerful analytical tool for investigation of biological tissue. In this study, SERS based on Ag nanoparticles was used to investigate the normal and cancerous thyroid tissue. Preliminary results indicated that Raman peaks and the spectra profile from both normal and cancerous tissues showed a basic similarity, obvious differences are that, first, Raman peaks 563cm⁻¹, 1449cm⁻¹ and 1587cm⁻¹ in cancerous tissue decreased obviously compared with the normal thyroid tissue. Besides, Raman peaks 1004cm⁻¹ and 1128cm⁻¹ might be specific peaks for normal thyroid tissue, whereas 1294cm⁻¹ might attribute to specific peak for cancerous thyroid tissue. In addition, some peaks in normal thyroid tissue appeared to have shifted in cancerous tissue. Intensity ratio of 656cm⁻¹ vs. 725cm⁻¹ in normal tissue are significantly different from cancerous tissue (P<0.005), and it can be a reference for spectroscopic diagnostics of thyroid tissue. This study demonstrates that SERS can be used to monitor the changes at molecular level as well as a complementary tool in thyroid histopathology.

1. Introduction
Thyroid is the largest endocrine organ in human body, and there are increasing cases involved thyroid cancer, although thyroid cancer is one of the least deadly cancers, but it is mainly discovered in young adults. The pathological diagnosis of thyroid usually occurs in the following order: computerized tomography, magnetic resonance imaging and fine-needle aspiration (FNA). FNA is currently the most accepted procedure for diagnosing thyroid tissue, however, sensitivity of this procedure for the thyroid is at times poor with a high rate of false-negative results[1]. In addition, variations of the thyroid diseases present difficulties for the accurate morphological diagnosis, for example, differentiation of hyperplastic nodule, follicular adenoma or follicular carcinoma is difficult[2]. Therefore, new methods which can provide information of biochemical composition and structure, as to improve the diagnosis of thyroid diseases is quite essential.

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One of the possible alternatives to the current methods is Raman spectroscopy, which can provide a molecular fingerprint information of the sample. Thus Raman spectroscopy acts as a powerful analytical tool with many advantages for characterization of biological molecules and structures due to its unique fingerprint feature. In addition, the characteristics of low sensitivity to water, easy sample preparation, making the application of Raman spectroscopy attract more and more attention of being used in biological research and biomedical diagnosis in recent years. The application of Raman spectroscopy covers a wide range in various human organs, such as brain, breast, gastrointestinal tract, larynx, lung, nasopharynx, and skin[3-9]. However, a major limitation of conventional Raman spectroscopy is its inherently weak signals, which presents great challenges for instrumentation and restricted its clinical application.

To overcome the low efficiency of Raman spectroscopy, a called surface-enhanced Raman scattering was found with ability of greatly enhancing the scattering effect when the analyte of interest is adsorbed onto or close proximity to a rough surface of suitable metals such as gold, silver, or copper. Research results showed several orders of magnitude enhancement effect and then it was rapidly applied to the study of single molecule and tissue. Hereafter Applications of SERS at the tissue level have been reported very recently[10-11].

In this work, we report here the application of silver nanoparticles in diagnosis of thyroid tissue. Tentative assignments of major Raman peaks and possible identities of the thyroid tissue were made. Meanwhile, attempts have been made to distinguish normal thyroid tissue from cancerous thyroid tissue based on difference SERS spectrum as well as the intensity ratio of specific Raman peaks.

2. Materials and Methods

2.1. Preparation of silver nanoparticles
Colloidal silver was prepared by the aqueous reduction of silver nitrate with hydroxylamine hydrochloride using a simple and fast method reported by[12]. Briefly, Nicolae and Bernhard.12 4.5 mL sodium hydroxide (0.1 mol/L) was added to 5 ml hydroxylamine hydrochloride (6×10^{-2} mol/L) and then the mixtures were added rapidly to 90 mL silver nitrate (1.11×10^{-3} mol/L) with vigorous shaking. The maximum absorption band of the silver colloid located at 416 nm.

2.2. Sample preparation
Thyroid tissue samples excised from patients were kindly provided by Fujian Provincial Tumour Hospital. Following surgical removal of the thyroid tissues, specimens were sliced and then immediately transported to the lab and kept at 0°C, standard Ag colloidal solution was directly dropped onto the thin cross-sectioned pieces which were then dried at the room temperature, finally both normal and cancerous thyroid tissue slices were selected for spectral measurement. All samples were finished within 3h after removal from the patients, then the samples were processed for routine histological examination. Informed consent was obtained from patients who participated in the study, we strictly conformed to the institutional rules governing clinical investigation of human subjects in biomedical research.

2.3. Raman Instrumentation
All SERS spectra were recorded with Renishaw InVia Raman microscope system (Renishaw Plc., New Mills, Wotton-under- Edge, UK)which equipped with 785 nm diode laser and 514nm argon-ion laser. The SERS spectra were recorded using 785 nm excitation. The laser power on the sample was about 3 mW, and the exposure time was 10s. A 20x (N.A.=0.75) objective was used for focusing the excitation beam and collecting the backscattered signals. The detection of Raman signal was accomplished with a Peltier cooled charge-coupled device (CCD) camera and the Raman instrument was calibrated using a silicon wafer by centering the band at 520cm^{-1}. The WIRE 2.0 software was used for spectra acquisition and analysis.
3. Results and discussion

Figure 1 shows the mean spectra from normal and papillary thyroid tissue and the corresponding difference spectrum in the region of 400–800 cm\(^{-1}\). Prior to other treatment the spectra were preprocessed by sequential 5-point smoothing method to reduce the noise[13]. It can be noted that most Raman peaks as well as the spectra profile from both tissues show a rough similarity, which means that the biochemical compositions are somewhat similar in this two type. Relatively strong bands of 570 cm\(^{-1}\), 656 cm\(^{-1}\), 725 cm\(^{-1}\), 895-912 cm\(^{-1}\), 963 cm\(^{-1}\), 1094 cm\(^{-1}\), 1331 cm\(^{-1}\), 1374 cm\(^{-1}\), 1449 cm\(^{-1}\), 1587 cm\(^{-1}\) were observed in both tissue. Obvious differences are that, first, in cancer thyroid tissue Raman peaks 563 cm\(^{-1}\), 1449 cm\(^{-1}\), 1587 cm\(^{-1}\) which can be attributed to nucleic acid, collagen and lipids decreased obviously compared with the normal thyroid tissue, besides, Raman peaks 1004 cm\(^{-1}\) and 1128 cm\(^{-1}\) might be specific peaks for normal thyroid tissue, whereas 1294 cm\(^{-1}\) might attribute to specific peak for cancer thyroid tissue. In addition, some peaks such as 1374 cm\(^{-1}\), 1449 cm\(^{-1}\), 1587 cm\(^{-1}\) in the normal thyroid tissue appeared to have shifted in comparison with cancer thyroid tissue. The tentative assignment for the observed SERS bands were listed in table 1.

The preliminary results indicated that SERS spectra can characterize the normal and cancerous thyroid tissue due to their intrinsic differences of biochemical components and molecular structure. Raman peaks at 563 cm\(^{-1}\) and 1449 cm\(^{-1}\) which can be assigned to DNA were easily found to be different in their intensity between normal and cancerous thyroid tissue, and this coincided with the basic concept that DNA content variates during the carcinogenesis process. Meanwhile, we found a peak at 1587 cm\(^{-1}\) which attributed to lipids decreased significantly in cancer tissue compared with the normal tissue. Recent research results showed that obvious difference was found in lipid content between normal tissue and malignant tissue, and they ascribed this phenomenon to the change in energy distribution of lipids[14].

Figure 2 shows a scatter plot for the intensity ratio of I\(_{656}\) vs. I\(_{725}\) for each tissue sample, previous studies have shown a simple but effective algorithm of intensity ratio based on Raman peaks can be used to classify tumor vs. normal tissue in brain breast, colon and lung[15-17]. In our preliminary study, the mean ratio of I\(_{656}\) vs. I\(_{725}\) (mean ± SD) for normal tissue (0.409±0.0.082) was significantly different from the mean ratio for cancer tissue (0.900±0.362), (unpaired Student’s t-test, P<0.005), and this intensity ratio can be a potential diagnostic parameter for detecting and/or classifying different type of thyroid malignancies, and the next work is to increase the number of sample measurements.

4. Conclusion

In this preliminary study, we have analyzed and compared the spectra obtained from normal and cancerous thyroid tissue. Although some peaks appear in both two tissue type, but significant spectral differences for cancerous thyroid compared to normal thyroid tissue were found at specific peaks, further calculation based on intensity ratio of I\(_{656}\) vs. I\(_{725}\) for each tissue sample gives a potential and valuable spectroscopic reference for discriminating normal thyroid tissue from cancerous thyroid tissue. Furthermore, we are working on the development of new statistical methods based on SERS spectra which reflects the changes at molecular level for diagnosis and pathologic analysis of thyroid tissue.
**Figure 1.** Comparison of Mean Raman spectra between normal (a, n=11) and cancerous (b, n=8) thyroid tissue. Shaded area represents the standard deviations (SD). Also shown at the bottom is the difference spectrum (c).

**Figure 2.** Scatter plot of the intensity ratio of Raman peak at 656cm⁻¹ vs. 725cm⁻¹. The mean ratio (0.409±0.0.082) for normal tissue is significantly different (P<0.005) from the mean value (0.900±0.362) for cancer tissue.
Table 1 The peak positions and tentative band assignment of Raman spectra

| Normal thyroid tissue | Cancerous thyroid tissue | components               |
|-----------------------|-------------------------|--------------------------|
| 563                   | 570                     | Nucleic acids, Nucleotide|
| 656                   | 658                     | Tryptophan               |
| 725                   | 725                     | Adenine, Coenzyme A      |
| 803                   | 803                     | RNA                      |
| 895-912               | 897                     | DNA backbone             |
| 963                   | 960                     | Citric acid              |
| 1004                  | -                       | Phenylalanine, Tyrosine  |
| 1050                  | 1050                    | Tryptophan               |
| 1094                  | 1094                    | Lipid, Nucleic acid      |
| 1128                  | -                       | Protein                  |
|                       | 1294                    | Lipids                   |
| 1331                  | 1333                    | Nucleic acid bases       |
| 1374                  | 1376                    | Nucleic acids            |
| 1449                  | 1458                    | Collagen, Phospholipid   |
| 1587                  | 1591                    | Lipids                   |

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