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Multimodal optical spectroscopic approach to design a complete protocol for early diagnosis of oral cancer

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ABSTRACT

Oral cancer is one of the most malignant cancers in the world. Early-stage diagnosis of oral cancer is complex process due to the multifocal unspecific development of non-malignant lesions into cancer and impossibility to take biopsy of every lesion. The aim of this study is to develop a screening method for oral cancer diagnosis at early stages using surface enhanced Raman spectroscopy (SERS) and validate the performance of a multimodal system including Raman spectroscopic (RS) and diffuse reflectance spectroscopy (DRS) oral cancer diagnosis and accurate margin detection. The study will involve the identification and integration of spectral biomarkers involved in the carcinogenesis process from different modalities.

Each modality SERS, RS and DRS is calibrated and standardized individually. Patients suffering from oral squamous cell carcinoma and other malignant diseases going through biopsy or histopathological examination are enrolled in this study. Ex vivo study involves the SERS analysis of saliva specimen and in vivo analysis will involve measurements on various tissue types, including malignant tissue and healthy contralateral site to evaluate the reproducibility and signal-to-noise ratio using fiber-optic probes for Raman and DRS systems. Feature selection methods and further machine learning tools will be used to discriminate between healthy, benign and cancer lesions based on spectral information and to identify important biomarkers. After data collection, clinician will perform a normal biopsy procedure and histopathological analysis, which will serve as gold standard to determine the sensitivity and specificity of the spectroscopy techniques.

1. INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common cancer occurring in oral cavity. Along with other head and neck tumours it is the sixth most common cancers worldwide. According to a recent report of World health organization (WHO), number of new cases of lips and oral cavity cancer in 2020 were approximately 0.38 million, which is estimated to increase to 0.47 million by 2030 [1]. In early stages of oral cancer many lesions may present as a flat white (leukoplakic) or red (erythroplakic), or exophytic (verrucous) lesions [2]. These lesions may show variable degrees of dysplasia, which could be associated with potential pre-malignancy. At later stage, lesion can present itself as a painful non-healing ulcer[3]. Early diagnosis followed by appropriate treatment can significantly improve the survival rate and post treatment quality of life by reducing the need for extensive enervating treatments.

Raman spectroscopy (RS) and surface enhanced Raman spectroscopy (SERS) could play a significant role in diagnosing oral cancer at early stage by providing chemical finger prints of the sample under consideration. Since water is a weak Raman scatterer, SERS analysis of saliva sample has a potential to diagnose oral cancer and other oral malignancies [4,5]. After cancer detection, next important step is to detect accurate tumour margin detection for complete excision of malignancy and for this DRS and RS *in vivo* analysis can play a key role. In this study, we are reporting the development of a novel non-conventional method to diagnose oral cancer in early stages by

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identifying the biochemical changes in saliva specimen and later on confirmation of lesion location with healthy margin through in vivo analysis.

1. MATERIALS AND METHODS

The study enrols the participants having oral malignancies and undergoing histopathological examination. Participants who provided the consent were asked to provide 1 ml of saliva specimen for SERS analysis. Later on, the *in vivo* tissue data was collected from the same participants with the help of a multimodal optical fiber probe for RS and DRS. Figure 1 present the SERS signal from the saliva specimen collected from one of the participant. Spectrum represents sharp protein peaks that can be further used to differentiate between a healthy participants and participant having any oral malignancy.

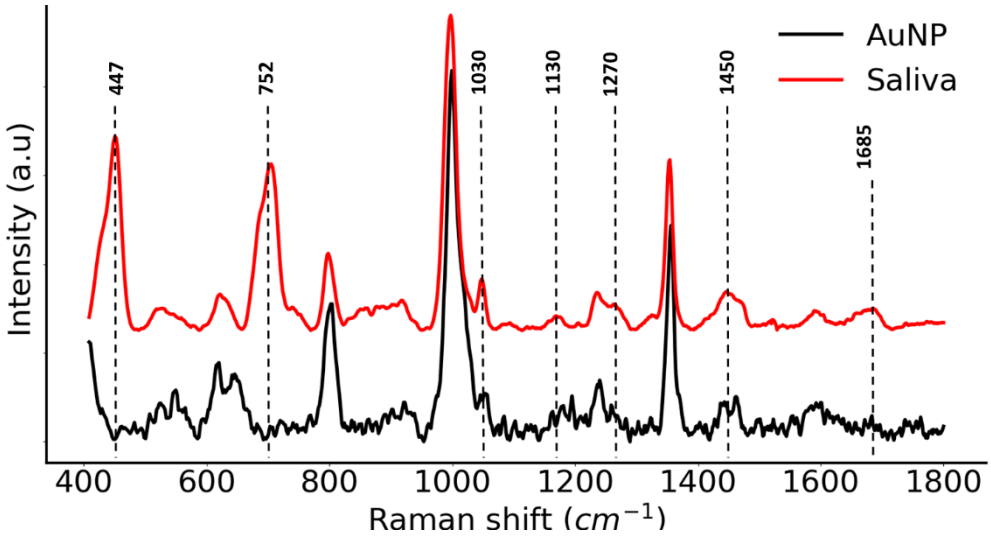

Figure1: Preliminary SERS spectrum of saliva specimen

Table 1: Spectral interpretation of dominated Raman signatures observed in Raman data of saliva samples[6, 7].

Wavenumber (cm^{-1})	Spectral Assignments
447	Ring torsion of phenyl
752	v15 (Porphyrin breathing mode)
1030	C-H in plane bending of phenylalanine
1130	C–C stretch acyl chains (trans) lipids; C-O stretch polysaccharides; v(pyrrole half-ring) asymmetric stretch (hemoglobin)
1220 – 1320	Amide III- combination of NH in-plane bending and CN stretching
1450	CH_2 bending mainly in proteins
1630 – 1690	Amide I: mainly C=O stretch in proteins

Figure 2 represents preliminary *in vivo* Raman data collected from a lesion on ventral surface of tongue and healthy contralateral site from one of the participants. Written consent was obtained from the participant.

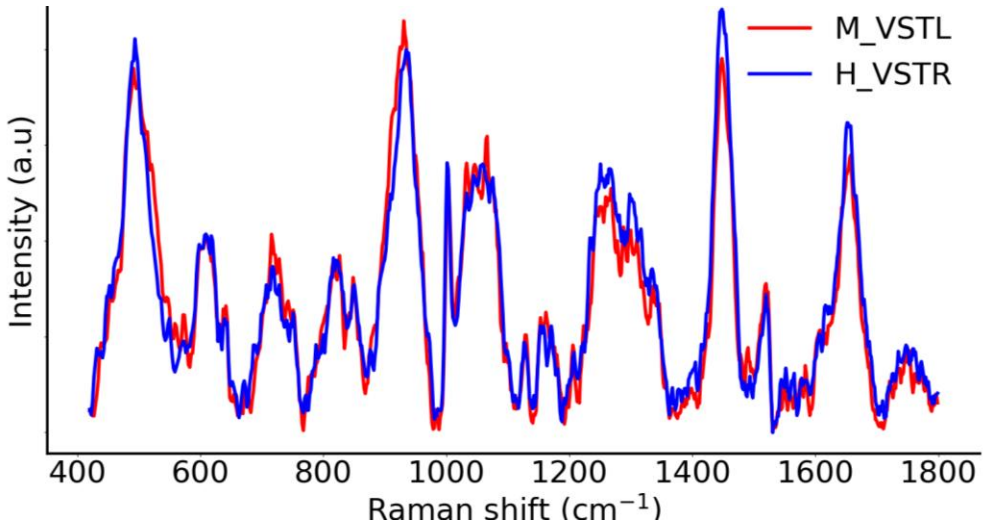

Figure 2: Preliminary *in vivo* Raman data from a lesion on ventral surface of tongue and healthy contralateral site

Figure 3 represents PCA analysis of preliminary DRS data from four patients having lesion on ventral surface of tongue and healthy contralateral site of the tongue as the healthy tissue. Which shows quite promising classification among the two tissues.

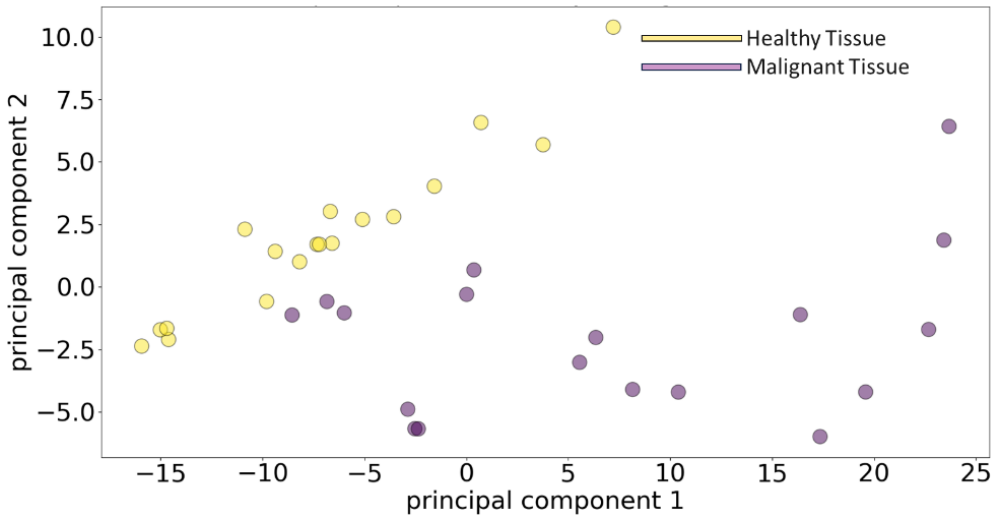

Figure 3: PCA analysis of preliminary DRS data from four patients having lesion on ventral surface of tongue and contralateral site of the tongue as the healthy tissue

2. CONCLUSION

The aim of this study is to develop a screening method for oral cancer diagnosis at early stages using surface enhanced Raman spectroscopy (SERS) and validate the performance of a multimodal system including Raman spectroscopic (RS) and diffuse reflectance spectroscopy (DRS) oral cancer diagnosis and accurate margin detection. The results obtained are quite promising to distinguish between healthy and malignant tissue. *Ex vivo*

saliva analysis also shows a potential to differentiate saliva collected from healthy participant and a participant having a lesion on the basis of proteomic biomarkers. This study aims to detect biochemical alterations in saliva and tissue for oral cancer detection through different modalities. This multimodal approach also has potential to analyse cancer margins in real-time.

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