

## 자가 형광계측술을 이용한 구강암 조기진단

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### <ABSTRACT>

#### Early Detection of Oral Cancer by an Optical Measurement System Measuring Autofluorescence

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Oral squamous cell carcinoma (OSCC) has been a focus of cancer prevention studies due to the fact that it occurs by a multistep process and that a precancerous lesion in the oral mucosa is easily accessible. The present study was aimed at developing an optical detection system using autofluorescence spectrum measurements for the early detection of oral cancer. The optical detection system was designed to use an excitation wavelength of 337 nm emanating from a Xenon lamp. Precancerous and cancerous lesions were created in the hamster buccal pouch by treatment with 7,12-dimethylbenz[a]anthracene (DMBA). Four groups of five hamsters each were used in this experiment. The right buccal pouch was treated with 0.5% DMBA to induce carcinogenesis while the left buccal pouch was treated with mineral oil as a control. The autofluorescence of both buccal pouches was measured weekly. A difference in the excitation pattern between the normal and the carcinogen-treated tissue was noticed after three weeks. Specifically, the intensity of the autofluorescence spectrum in the DMBA-treated buccal pouch was increased at wavelengths between 400 and 450 nm. The results of the autofluorescence measurements were compared to histological findings and show that the intensity of the autofluorescence increased along with the stage of epithelial dysplasia. Based on the fact that one of the autofluorophores in this tissue is NADH, we measured the fluorescence at the 450-nm NADH wavelength to conclude that the increased autofluorescence in the dysplastic areas may be caused by NADH. Based on these data, we suggest that autofluorescence optical methods are a useful tool for the early detection of oral cancer.

*Key words* : Oral squamous cell carcinoma, Precancerous lesions, Early detection, Autofluorescence, Optical detecting system, NADH

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\* This work was partly supported by priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0094028) and partly by research grant from Korea Electrotechnology Research Institute.

### I. INTRODUCTION

The World Cancer Report published by the International Agency for Research on Cancer (IARC) and the World Health Organization (WHO) predict that 15 million new cases of cancer will be diagnosed

globally in 2020 compared to the 10 million cases diagnosed in 2000. This is a dramatic increase in the population of cancer patients<sup>1</sup>. Accordingly, a globalized effort to reduce the cancer burden of the population and to achieve higher cure rates is necessary. Importantly, to overcome cancer in our society, public health actions to reduce tobacco consumption, promote a healthy lifestyle and diet, and achieve early detection through screening have been the focus<sup>2</sup>. Specifically, early detection of cancer before the tumor progresses to an invasive or to a distant metastasis can markedly reduce cancer mortality despite the increased number of cancer cases in the population.

Oral squamous cell carcinoma (OSCC) has been a focus for cancer prevention studies based on the fact that it occurs by a multistep process and that its early-stage precancerous lesion (OPL) is easily accessible by a routine clinical check-up<sup>3,4</sup>. The assessment of the risk of cancer development in OPL using histological evaluation of an epithelial dysplasia has been the most reliable method to predict the biological behavior of the lesion<sup>5</sup>. However, the presence and degree of an epithelial dysplasia, should also be shown by biopsy which is a more invasive tool. Furthermore, the degree of epithelial dysplasia does not always correspond to its biological behavior. Therefore, attempts have been made to visualize genetic lesions<sup>6</sup>. The visualization of lesions with toluidine blue has been used clinically for the early detection of oral cancer. Using this method, abnormal staining reflects histological dysplasia, loss of heterozygosity (LOH), and increased cancer risk<sup>7-9</sup>.

Fluorescence visualization and spectroscopy is a promise technology for the in vivo detection of epithelial dysplasia. A number of studies using fluorescence visualization and spectroscopy have been performed in a variety of organ sites. The development

of optical imaging systems using autofluorescence is an emerging new technology<sup>6</sup>. To determine the presence of an oral malignancy, blue excitation light (400–460 nm) has been used<sup>10,11</sup>. Poh et al. attempted to use fluorescence visualization to screen the genetically condemned area using blue excitation light at 400–460 nm. In that study, Poh et al. showed the effectiveness of a simple handheld light to guide the surgical removal of oral cancer in the operating room.<sup>12</sup> Recently, a multispectral optical imaging device has also been developed using excitation wavelengths of 365–600 nm<sup>14</sup>. In that study, Darren et al. designed a multispectral digital microscope (MDM) as a tool to improve the detection of oral neoplasia. They observed an alteration in the autofluorescence of the lesions and visibility of vasculature using orthogonal polarized reflectance imaging. These results demonstrate that this method has the potential to provide additional diagnostic information. Richard et al. studied a clinical spectroscopy system with a depth-sensitive fiber-optic probe to measure the depth response spectra and showed differences in the intensity, peak emission wavelength, and shape of the fluorescence spectra<sup>13</sup>.

Previous studies using fluorescence visualization systems have not been used to detect early cancer but were used to screen the extent of tumor spread in a state of malignancy<sup>6,25</sup>. At this diagnostic stage, it is very difficult to distinguish between normal tissue and neoplastic tissue because of the ambiguous boundary condition is determined by color. Previous studies of autofluorescence methods have some disadvantages, specifically, analysis is difficult due to the small difference in the signal intensity between normal and dysplastic cells<sup>23,24</sup>. Therefore, we attempted an analysis using the different pattern of the measured autofluorescence spectrum between normal and premalignant cells. To do this, we carried out our experiments in hamsters. In the present study, we

illustrate that there is a different autofluorescence spectrum pattern at wavelengths between 400 and 500 nm in DMBA-treated premalignant areas compared to control tissue. The difference in the spectrum pattern could be very useful in distinguishing between normal and dysplastic tissue.

## II. EXPERIMENTAL SETUP

We developed an optical device for the early detection of oral cancer as shown in Fig. 1. The light source was a 60 W xenon flash lamp (L7684, Hamamatsu, Japan) operated at a repetition rate of 50 Hz. The diameter of the two UV lenses was 25.4 mm and the focal distance was 12.9 mm (Korea Electro Optics, Korea). The wavelength of the bandpass filter was 337 nm (Semrock, USA). The full-width at half-maximum of the bandpass filter was 10 nm. The power of the excitation light was measured as 189 mW (Gentec Model: PSV-3103, Canada). The excitation

light was coupled into an optical fiber (1 mm in diameter), and the fluorescence signal was detected by six optical fibers (0.6 mm in diameter each). The distance from the probe tip to the tissue surface was adjusted to be 2.5 mm using an aluminum adaptor. The fluorescence of the hamster buccal pouch was delivered to a monochromator (Acton Research Spectra Pro 150, USA) and an ICCD camera (Stanford Computer Optics Inc., 4QuikE, USA) was connected to the universal serial bus (USB) port of a computer.

## III. ANIMALS, MATERIALS AND HISTOLOGICAL EXAMINATION

Six-week-old male Syrian Hamsters (Japan SLC, Inc) were used to generate premalignant lesions. Topical application of 0.5% 7,12-dimethylbenz [a] anthracene (DMBA; Sigma, St. Louis, MO) to the right buccal pouch was performed three times per week. Heavy mineral oil was applied to the left buccal pouch

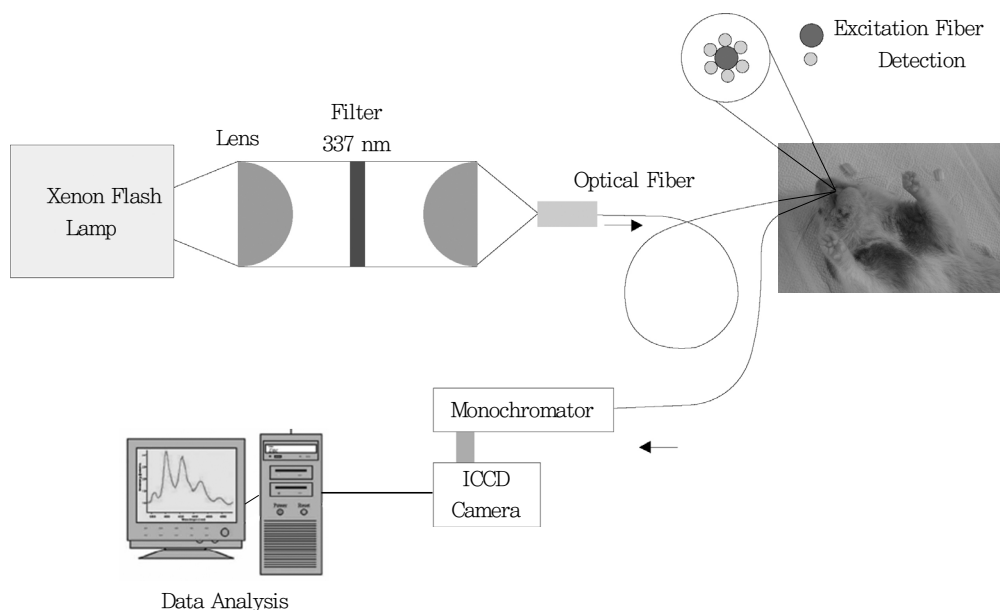


Fig. 1. Optical scheme of the oral cancer detection system.

as a control. The autofluorescence excitation pattern was observed every week starting at the second week to compare the DMBA-treated areas with the control areas using an excitation light source of 337 nm emanating from a Xenon lamp. Animals were sacrificed at 8, 10, 12, and 14 weeks of treatment to examine the histological changes and to correlate them with the excitation pattern. The excitation light of 337 nm was also emitted by nicotinamide adenine dinucleotide (NADH, Sigma, Japan) and collagen(Collagen, Sigma, Japan) to evaluate the source causing the unique spectrum pattern in DMBA-treated areas and the control. Buccal pouch tissue was fixed with 10% neutral formalin and embedded in paraffin. For histological examination, routine hematoxylin-eosin

staining was done. We observed epithelial hyperplasia, dysplasia, papilloma formation and invasive carcinoma,

## IV. RESULTS

### 1. Autofluorescence pattern and its correlation to histological findings

Differences in the fluorescence spectra were found after three weeks between the DMBA-treated and control-treated areas. Fluorescence intensity was enhanced at wavelengths between 400 and 450 nm in the DMBA-treated group as shown in Fig. 2. This same pattern was consistently found from week 3 to

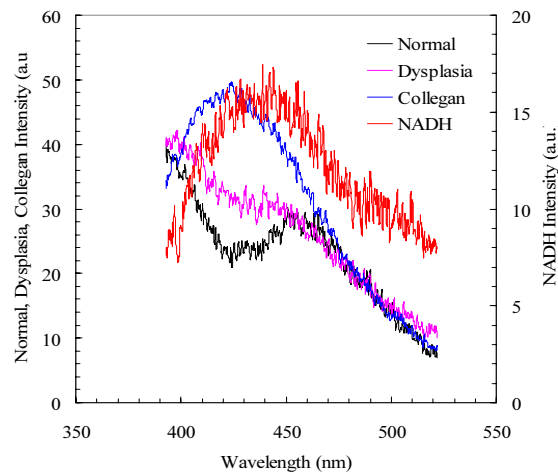


Fig. 2. Fluorescence measurement results in normal (left buccal pouch), epithelial dysplasia (right buccal pouch), collagen and NADH.

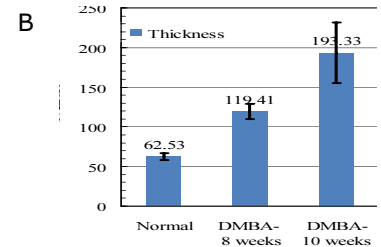
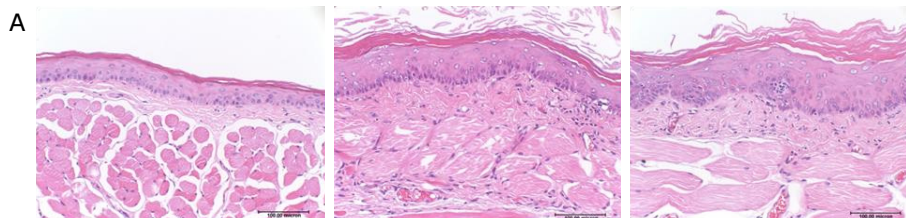


Fig. 3. A: Hamster oral mucosa biopsy results (From the top: normal, DMBA-treated week 8, DMBA-treated week 10) B: Measurement of the thickness of the epithelium, DMBA-treated buccal pouch at week 10 averaged  $193.32 \pm 38.02$  mm while the epithelium of the control measured  $62.52 \pm 4.47$  mm

week 10. At week 10, the histological findings revealed hyperkeratosis and epithelial hyperplasia with dysplasia in the DMBA-treated hamster buccal pouches. At week 12 and week 14, papillomas and invasive carcinomas had developed and the corresponding fluorescence showed an irregular variable pattern. When we measured the thickness of the epithelium, DMBA-treated buccal pouches at week 10 averaged  $193 \pm 38$   $\mu\text{m}$ , while the epithelium of the control measured  $62 \pm 4$   $\mu\text{m}$ , as shown in Fig. 3 (a) and Fig. 3 (b). Assuming that excitation light penetrates 200  $\mu\text{m}$ , the control may be influenced by the underlying muscle layer, including connective tissue.

## 2. Measurement of NADH and collagen

To explore whether the source of the increased fluorescence intensity at 400–450 nm in the tissue was NADH, we measured the fluorescence spectrum of NADH as shown in Fig. 2. The fluorescence spectrum of NADH had a peak fluorescence level at 440 nm. Overlaying the spectra of the DMBA-treated tissue, control tissue and NADH, suggests that the enhanced fluorescence in the DMBA-treated area is at least partially due to increased NADH. Additionally, we measured the spectrum of collagen. Collagen had a peak fluorescence level at 425 nm as shown in Fig. 2.

## V. DISCUSSION

Many endogenous fluorophores have been identified in human tissue<sup>15)</sup>. Among these various optical signatures, NADH has a peak emission at 440 nm and the fluorescence quantum yield of NADH increases approximately four-fold when bound to proteins. Additional, flavin adenine dinucleotide (FAD), another major electron acceptor, and FAD, its oxidized form,

are both fluorescent. When excited in the near UV region, the endogenous fluorescence at 500 nm is reduced in the tumor tissue compared to normal tissue. The reason behind this difference may be due to a decrease in the oxidized form of FAD and the relative amount of NADH<sup>15)</sup>.

An increase in the reduced form of NADH has been reported with cancer progression<sup>16)</sup>. In the present study, the fluorescence intensity was enhanced at wavelengths of 400–450 nm. The 337 nm excitation source can penetrate the level of 150–200  $\mu\text{m}$  of tissue depth<sup>21)</sup>. Therefore, the spectrum pattern of a normal cell reflects autofluorescence emitted from collagen fibers, as well as the epithelial layer. As a result, we measured the fluorescence spectra of both collagen and NADH. In case of normal tissue, the spectrum pattern showed a combination of the fluorescence of both collagen and NADH at wavelengths between 400–450 nm, which is where we identified a unique pattern between normal tissue and dysplasia. On the other hand, the depth of the epithelial layer of DMBA-treated dysplastic and hyperplastic area was measured as  $193 \pm 38$   $\mu\text{m}$ . Therefore, the excitation light mainly penetrates the epithelial layer of DMBA-treated dysplastic and hyperplastic areas. Therefore, the spectrum pattern of the DMBA-treated dysplastic and hyperplastic area likely depends on NADH fluorescence. Using an excitation wavelength of 337 nm from a Xenon lamp, NADH displayed fluorescence between 400–450 nm with a peak at 440 nm. These data support the hypothesis that the increased fluorescence intensity at 400–450 nm in DMBA-treated tissues may be due to increased NADH activity derived from a thickened epithelial layer with an increase in dysplastic cells. Based on these data, the increased fluorescence intensity of the dysplastic cells at the 400–450 nm wavelengths can be attributed to the influence of NADH.

Studies of visual diagnostic tools for the early detection of intraepithelial neoplasias have focused on several organs, including the lung, uterine cervix, and oral cavity<sup>17-19</sup>. Different fluorescence spectra were found between normal cervical tissue and intraepithelial cervical neoplasia using a laser-induced excitation source at 337 nm. Ingrams et al. showed that differences in the spectra between normal and dysplastic oral mucosa were most pronounced at an excitation wavelength of 410 nm<sup>20</sup>. Many endogenous fluorophores that are present in human tissue have previously been reported<sup>22</sup>. Among the various optical signatures present, we focused on 440 nm, as the NADH peak, given that the optical properties at this wavelength were clearly different between normal and cancerous tissues in the oral cavity.

Our results suggest that an optical detection system using a Xenon lamp excitation source could be useful for the early detection of OSCC. Importantly, early detection and treatment are key factors for cancer patients to overcome cancer. The successful development of an optical detection system would be a highly useful tool for cancer screening.

## VI. CONCLUSIONS

An optical method for the early detection of oral cancer was demonstrated in a carcinogen-induced hamster buccal model. The present study showed that a different pattern of autofluorescence was seen between normal tissue and dysplastic at wavelengths between 400-450 nm. The reason for the different spectra pattern was likely due to be the influence of NADH, based on optical and biopsy data. We are presently furthering this study in a clinical setting, applying the scheme described above.

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