Original paper

Differentiation of skin biopsies by light scattering spectroscopy

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Abstract

Introduction: Spectroscopic systems are medical tools that are used for the detection of cancerous tissues *ex vivo* and *in vivo*.
Aim: To differentiate inflammatory and benign skin lesions of excised biopsy samples via a combination of multivariate statistical analysis.
Material and methods: Spectral data were obtained from a total of 22 inflammatory and ten benign skin biopsy samples from 30 patients in the visible wavelength (450–750 nm) regions. Spectral data were compared with the dermatopathology results. Spectral data analyses of biopsy samples were performed via principal component analysis (PCA), followed by linear discriminant analysis (LDA). The differentiation performance was calculated with the receiver operating characteristic (ROC) curve analysis.
Results: The classification based on the discriminant function score provided a sensitivity of 90.9% and a specificity of 80% in discriminating benign from inflammatory lesions with an accuracy of 87.5%.
Conclusions: Our study revealed that light scattering spectroscopy could discriminate between inflammatory and benign skin lesions of excised biopsy samples with high sensitivity by using multivariate statistical analysis. It can be concluded that the high diagnostic accuracy of the optical spectroscopy method has the potential to use as a supplementary system to distinguish inflammatory skin lesions from benign during the pathological examination.

Key words: spectroscopy, inflammatory, skin, principal component, linear discriminant.

Introduction

Early detection and treatment of skin cancer can significantly improve patient outcomes. In clinical practice, visual examination determines whether a skin lesion may be cancerous or normal depending on the expertise and visual skills of the clinician but diagnostic accuracy of this situation varies greatly depending on the experience. On the other hand, histopathologic examination of the excised suspicious samples still remains the "gold standard" but this investigation may last an average of 1 week for patients to receive the reports. Differentiation in the cell nuclear matrix has been associated with cell and tissue compositions that are essential features in the determination of cancer. Morphological alterations in cancer include changes in the nuclear size and shape, which are crucial characteristics used in cancer diagnosis [1, 2].

Real-time non-invasive and objective spectroscopic methods have been used to discriminate cancer from normal tissues based on either physical alteration of tissue formation or biochemical changes of tissue components [3–10]. Optical techniques present a non-invasive alternative to tissue biopsies for defining the status of the tissue, and lots of research was done in the detection of cancer by other spectroscopic techniques [11–13]. Spectroscopic systems are sensitive to the morphological, functional, and biochemical properties of tissue. As these physiological parameters vary with the progression of cancer, spectroscopic techniques allow to diagnose cancer in real-time. Additionally, these techniques provide an objective method for diagnosing cancerous tissue, and they do not require user experience as they give quantitative results. In these techniques, optical fibers placed in contact with the tissue deliver and detect light.

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The majority of the light is scattered from the nucleus, the most significant scatterer inside the cell, and also the refractive index difference between the cytoplasm and the cell membrane generates light scattering [14, 15]. The light scattered from the cell is saved as spectral data and depends on the wavelength. Evaluation of spectroscopic results does not need interpretation by an expert since acquired data give quantitative results that are objective, and provide support for the physician's decision.

Aim

In this study, our purpose was to differentiate inflammatory and benign skin lesions *ex vivo* by using the optical system in real-time and without pathologist expertise. The diagnostic algorithms are capable of discriminating between inflammatory and benign skin lesions constructed by multivariate statistical analysis in our study. The obtained spectra were assessed in combination with the principal component analysis (PCA) and linear discriminant analysis (LDA) to build a biomedical tool for decision support during the pathological examination.

Material and methods

Study protocol

The clinical study was conducted at SANKO University Dermatology and Venereology Department with the approval of the SANKO University Ethics Committee (2018/08-08). Written informed consent was acquired from all patients who agreed to participate in our study. Thirty patients were selected for the study, and the spectral measurements were implemented on a total of 32 skin biopsies with the collaboration of the Dermatology and Venereology Department. Subjects with syringoma, dermatofibroma, and intradermal nevus were classified as a benign group (total $n = 10$), and chronic inflammatory lesions were classified as an inflammatory group (total $n = 22$) in our study. More than one biopsy sample was excised from some patients. During the dermatological examination, skin biopsy samples were excised via diagnostic or punch biopsy and excision biopsy from the patients. Our probe is not affected by the biopsy type since the tip is small enough to get spectral information from all different sizes of samples. We gathered a minimum of 16 spectra each, depending on its size, by touching the tip of the optical fiber randomly to their surfaces within 3–5 min. Then all samples were transported to the dermatopathology department for routine examination. It takes an average of 7 days for the final pathology report to be obtained. Spectra were obtained in the 350–1000 nm wavelength range, and each spectrum was corrected in the 450–750 nm range. Our spectra were compared with pathology results to designate correlation.

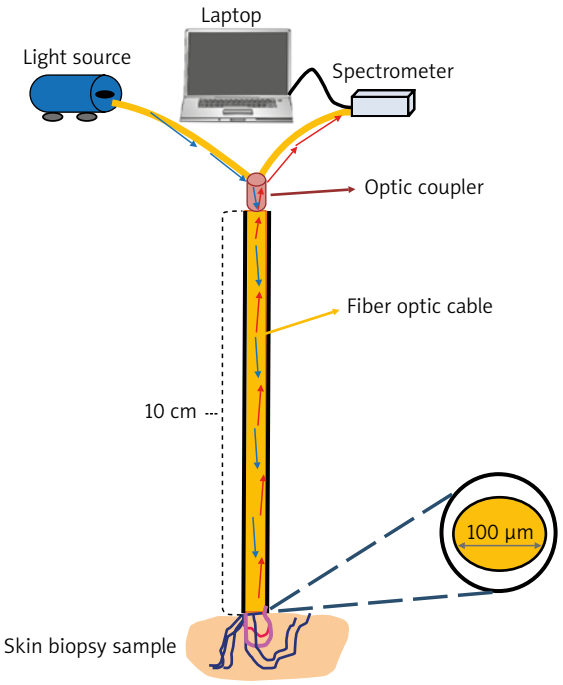

Figure 1. The optical measurement system

Instrumentation

The light scattering method (Figure 1) comprises of a single fiber optic probe (105 micron core diameter, 125 micron clad diameter, 50 : 50 coupling ratio and 0.22 numerical aperture) which is used for both transmitting and receiving light from the skin biopsy sample, a halogen-tungsten light source (HL-2000 Tungsten Halogen, Ocean Optics), a spectrometer (USB2000+VIS-NIR Spectrometer, Ocean Optics) and a laptop computer to save the spectra.

The light source was connected to one end of the fiber and the spectrometer to the other end. Fiber sent light to the skin biopsy sample, and the same fiber detected backscattered light to obtain the spectra. Each spectrum was normalized to the integration time, and three spectra were obtained for calibration. Firstly, we wanted to eliminate light as much as possible to gather a background spectrum. The fiber optic probe tip was inserted into a black container (4 cm high and 1.5 cm diameter), which contains pure water, and then we measured the back-reflection $R(\lambda)bg$. Secondly, the spectral dispersion of the light source was measured $R(\lambda)c$ from reflectance standard (Spectralon, Labsphere, Inc.). Confirmation of the calibration was achieved by observing Mie oscillation from the last spectrum, which was taken from polystyrene microspheres with a 2 ±0.02 micron diameter. After these calibration measurements, we obtained spectra from skin biopsy samples, $R(\lambda)s$ which were corrected [16] by $R(\lambda) = [R(\lambda)s - R(\lambda)bg]/[R(\lambda)c - R(\lambda)bg]$.

Multivariate statistical analysis

PCA is a simple numerical method of data reduction and which extracts relevant information from the large dataset. LDA is used for pathological feature extraction [17, 18]. The spectra within 450–750 nm were used for the multivariate statistical analysis. PCA [19, 20] followed by LDA [21] was performed to find specific patterns in these wavelength regions. PCA was used to identify and extract major trends within a given spectral data set. Statistically significant components were utilized in LDA to achieve differentiation of skin biopsy samples. For confirmation and validation of the analysis, leave-one-out cross-validation (LOOCV) was performed [17, 18, 22]. We used the software package R-Studio Open Source Statistical Language for PCA, LDA, and receiver-operating-characteristic (ROC) analysis [23]. The results of LDA were compared to determine the sensitivity and specificity values.

Results

In the present study, the histopathologic examination was accepted as the "gold standard" for skin biopsy sample differentiation. The detailed anatomic location, histopathology results, and biopsy type of excised samples are shown in Table 1. The spectral pattern related to scattering of the light from the nucleus and organelles is used as a diagnostic parameter. A total of 32 skin biopsy samples were removed from 30 patients, and a total of 1104 spectra (368 spectra from benign and 736 spectra from inflammatory skin biopsies) were analysed. Figure 2 displays the corrected spectra of the fiber optic system for benign and inflammatory skin biopsies. Multivariate statistical analysis, i.e., PCA followed by LDA, was applied to discriminate the spectral patterns in our study.

Firstly, spectra were centred and scaled (using a mean of 0 and a standard deviation of 1) before statistical comparison. Then PCA was performed to reduce the number of predictor variables for the differentiation of skin biopsy samples. PCA was achieved by first finding the direction having the most significant variance (PC1: 48%), and after that finding the following directions (PC2: 36%, PC3: 2%, PC4: 2%, PC5-36 collectively provides 1% or less of variance however still contributed significantly). After that, an independent-sample *t*-test on all the PC scores comparing benign and inflammatory skin biop-

Table 1. Anatomic location, histopathology results and biopsy type of excised inflammatory and benign samples

Benign			Inflammatory		
Histopathology	**Location**	**Biopsy type**	**Histopathology**	**Location**	**Biopsy type**
Syringoma	Breast	Punch	Chronic inflammation	Knee	Punch
Dermatofibroma	Arm	Excision	Chronic inflammation	Face	Punch
Intradermal nevus	Armpit	Excision	Chronic inflammation	Face	Punch
Syringoma	Neck	Punch	Chronic inflammation	Arm	Punch
Intradermal nevus	Head	Punch	Chronic inflammation	Ear	Punch
Syringoma	Breast	Punch	Chronic inflammation	Arm	Punch
Syringoma	Breast	Punch	Chronic inflammation	Arm	Punch
Syringoma	Breast	Punch	Inflammation	Head	Punch
Intradermal nevus	Face	Punch	Chronic inflammation	Sole	Punch
Intradermal nevus	Face	Punch	Impetigo	Head	Punch
			Chronic inflammation	Ear	Punch
			Chronic inflammation	Leg	Punch
			Chronic inflammation	Nose	Punch
			Chronic inflammation	Leg	Punch
			Chronic inflammation	Abdomen	Punch
			Chronic inflammation	Head	Punch
			Chronic inflammation	Nape	Punch
			Chronic inflammation	Dorsum	Punch
			Chronic inflammation	Abdomen	Punch
			Granulomatous dermatitis	Face	Punch
			Chronic inflammation	Hand	Punch
			Chronic inflammation	Face	Punch

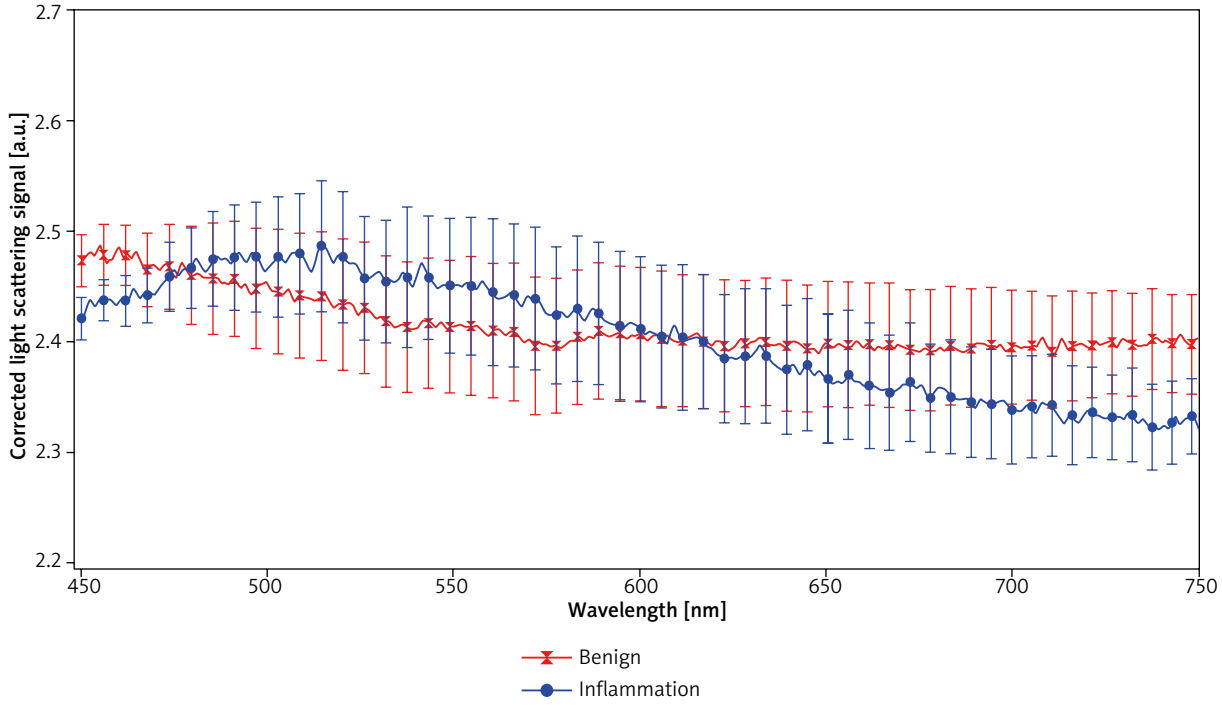

Figure 2. Corrected light spectra for benign and inflammatory skin biopsy samples

Table 2. Distribution of the inflammatory and benign samples based on the measurement by using the pathology results as a gold standard

		Fiber optic spectroscopy		
Pathology	**Sample type**	**Inflammatory**	**Benign**	**Total**
	Inflammatory	20 (TN)	2 (FP)	22
	Benign	2 (FN)	8 (TP)	10

TP – true positive, TN – true negative, FP – false positive, FN – false negative.

sies showed that there was only one most diagnostically significant ($p < 0.05$) PC (PC2) for discriminating benign and inflammatory skin biopsy samples. The vital PC2 ($p < 0.05$) component was used as the input variable of LDA. The cross-validation was performed by using the leave-one-out technique to prevent over-fitting [22]. The areas under the ROC curve (AUC) and its 95% confidence interval (CI) were computed. AUC as well as the specificities and sensitivities for the optimal cut-points were calculated using the discriminant function scores which was acquired by LDA [24].

In a group of 22 inflammatory lesions, 2 cases were misclassified as benign (False positive: FP), and in a group of 10 benign, 2 cases were misclassified as inflammatory (False negative: FN) samples (Table 2). It was determined that two FN tissues were nevus samples having a dense nucleus structure. Besides, one of the two FP tissues was found to have dense proliferation, and the other had dense nuclei. ROC analysis with a discriminant function score produced an AUC of 0.795 for separating benign from inflammatory samples, as seen in Figure 3. The population of the benign group was smaller than that of the inflammatory group, and we had 10 benign samples during the study. The minimal required sample size is calculated as 10 per group by using AUC value (0.795), type I error (Alpha, Significance) value 0.05 (5%), type II error (Beta,1-Power) value 0.20 (power is 80%) and null hypothesis value 0.5 [25]. This calculation shows that the number of the minimum sample size used in our study is sufficient.

Discussion

The fiber optic probe has a core diameter of 100 microns, which is used for both transmitting and receiving light from the skin biopsy sample. In our study, due to the geometry of the probe, it is sensitive to sub-cellular morphology and structural alterations associated with the characteristics used by the pathologists in the evaluation. Spectroscopic methods are quantitative approaches that give more unbiased results than the conventional method, which is subjected to pathologists' interpretation. In our study, spectra obtained from skin biopsy samples were examined based on multivariate statistical analysis. Spectra were used to determine the most significant spectral patterns and may improve the

diagnostic execution of skin biopsy sample analysis and classification. For this purpose, ROC analysis further confirms PCA-LDA based distinguishing algorithms using the whole spectral characteristics of fiber optic system could separate benign and inflammatory skin biopsy samples with high sensitivity under *ex-vivo* conditions as seen in Figure 3.

In recent years, real-time and non-invasive diagnostic studies have been carried out using optical methods. Molecular and morphological changes in the tissue during the progression of cancer more depend on the scatterers of tissue, including nucleus size, nucleus-cytoplasm ratio, and mitochondrial size [26, 27]. These parameters can be related to characteristics that are applied by pathologists as histological evaluation. The scattering of light from the tissue is more sensitive to variations in the cellular morphology than biochemical alterations. The progression of cancer is correlated with necessary changes in tissue organization, composition, and cellular morphology, which can be detected by the fiber-optic reflectance spectroscopy technique. Therefore, it is specified in the literature that this optical technique could be used as a biomedical tool in discriminating between normal and cancerous tissue [28]. When examining the discrimination accuracy in the published articles which are involving the fiber optic reflectance spectroscopy with different probe geometry, malign versus benign or normal human skin tissues, sensitivity, and specificity values ranged from 77.8 to 95.2% and from 80.1 to 93.3%, respectively [28–30]. In these studies, all inflammatory samples had been combined with benign lesions, and there was no discrimination between inflammatory and benign ones. The distinction between these two structures is clinically important because the benign structure is the beginning of the tumoral process that requires follow-up, whereas patients with inflammatory lesions could be treated with suppressive therapy.

To our knowledge, this is the first study that differentiates inflammatory skin biopsy samples from benign samples with high sensitivity by using an optical method. This technique could aid the pathologist in accurately identifying inflammatory skin samples and could reduce the false positive of a simple naked eye examination.

Conclusions

This study revealed that the fiber optic method is time-saving, gives unbiased results and has the potential to be used in the differentiation of inflammatory and benign skin biopsy samples. In addition, the high sensitivity of the method increases the potential to be applied in examination *in vivo* as a new medical instrument for differentiating inflammatory lesions. Also, this system has the potential to be used not only in dermatology but also in other clinical branches that require biopsy evaluation.

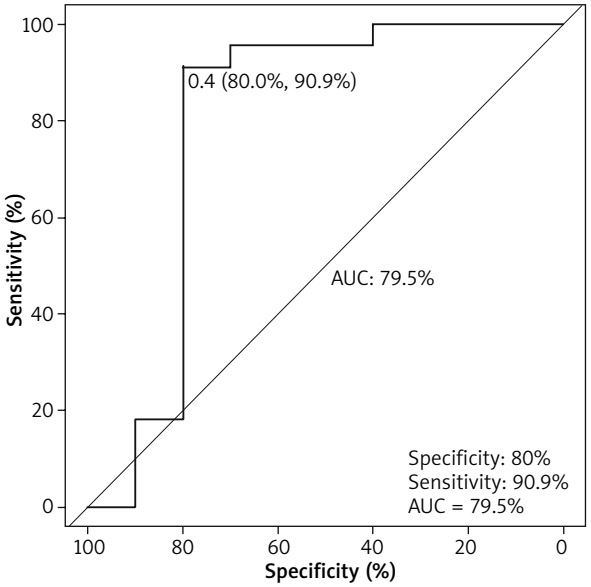

Figure 3. ROC curve, comparing benign and inflammatory skin biopsy samples

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Conflict of interest

The authors declare no conflict of interest.

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