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DNA and protein change in human breast tissues by diffuse reflectance spectrum

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ABSTRACT

Breast tissues were investigated using diffuse reflectance spectroscopy to yield the absorption spectrum from Kubelka-Munk Function (KMF). A specified spectral feature measured in adipose tissue was assigned to β -carotene, which can be used to separate fat from other molecular components in breast tissues. The peaks of (KMF) at 260nm and 280nm were attributed to DNA and proteins

Keywords: *Diffuse reflectance spectroscopy, Breast Cancer Diagnose, Optical biopsy DNA, proteins,*

1. INTRODUCTION

In our past work, the fluorescence, excitation and Raman spectra from native human breast tissues have been studied as a potential clinical tool for cancer diagnostic purposes. The critical parameters of certain intensity ratios at definite wavelengths in the fluorescence and excitation spectra have been used to separate malignant from benign tissue (1-4). Due to multiple photon scattering, direct measurement of absorption spectra of tissues by conventional transmission means is not easily done. The diffuse reflectance spectrum offers a way to obtain a measure of the absorption spectrum (5).

In this paper, the diffuse reflectance spectrum was measured from breast tissues. The Kubelka-Munk function was calculated to give information about the absorption spectrum. The spectral features of the Kubelka-Munk function of breast tissue were attributed to hemoglobin (oxygenated and deoxygenated hemoglobin), collagen, and β -carotene (it exists in adipose tissue), proteins and nucleic acid components.

2. MATERIALS

Excised benign, adipose, fibroadenoma and malignant breast tissue samples were obtained from St. Vincent Hospital, Memorial Sloan Kettering Cancer Center, and National Disease Research Interchange (NDRI). Specimens were neither chemically treated nor frozen prior to spectroscopic measurements. Samples of random shapes were mounted in a 1cm $\times$ 1cm $\times$ 5cm commercial quartz cuvette and closely attached to its inner surface for measurement of spectra. Usually, the spectra were measured at up to 3 different location of the sample. The malignant breast tissue specimens were classified into carcinoma *in situ*, infiltrating or invasive carcinoma, and mixed *in situ* and invasive (part *in situ* and part invasive) according to the pathology report. In this paper, 25 invasive carcinoma, 22 mixed *in situ* and invasive, 14 fibroadenoma, 44 benign, and 31 adipose specimens have been studied.

The diffuse reflectance, fluorescence, and excitation spectra were performed from same spot of the sample, using an automated dual lamp-based spectrophotometer (Mediscience Technology Corp. CD scanner.). The measurements of diffuse reflectance spectra were selected using synchronized scan mode, in which the emission and excitation monochromators were scanning on the same wavelengths synchronously. The diffuse reflectance spectrum was scanned from 250nm to 650nm.

As a reference standard, β -carotene crystal was obtained commercially from Sigma Co. The concentration of the carotene solution was 0.8 mg/ml. The small particles of Ti_2O_3 (0.2 μ) were added into carotene solution to enhance the scattering signal during diffuse reflectance measurements.

3. MODEL

Kubelka and Munk function was obtained from diffuse reflectance R_∞ is (6,7)

$$f \equiv (1-R_\infty)^2/2R_\infty = k/s, \quad (1)$$

The function f is the ratio of absorption to scattering coefficients.

Plotting $\log f$ (r_∞) against the wavelength for a particular sample, the curve obtained corresponds to the absorption spectrum of the compound with intercept displacement given by $-\log s$ in the ordinate axis.

4. RESULTS

The K-M function of typical malignant, fibroadenoma adipose and benign breast tissue displayed in Fig. 1 Some features can be found in the K-M curves of these tissues as shown in Fig. 1(b). No carotene peak at 480nm in K-M function of benign or malignant breast tissues is observed. The feature of β -carotene peak definitely appeared in adipose tissue. The peak near 280nm appeared for all kinds of tissue, but the amplitude was different. The amplitude of malignant was higher than fibroadenoma and benign tissue. This peak is corresponding to the absorption of proteins. The another peak near 265nm only appeared for malignant tissue. This peak is corresponding to the absorption of DNA. To prove this, the absorption spectra of

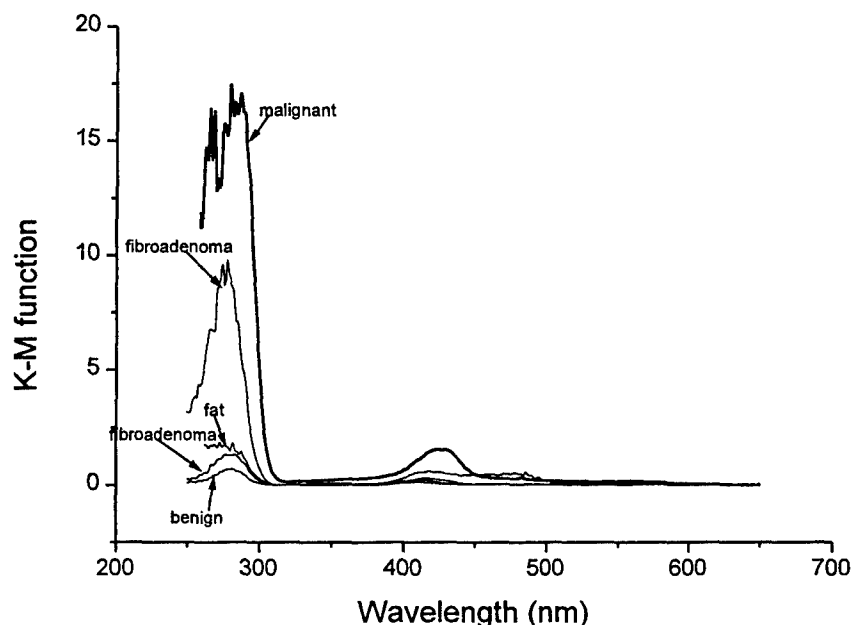


Figure 1: The Kubelka-Munk function of typical benign, malignant, fibroadenoma and adipose breast tissue.

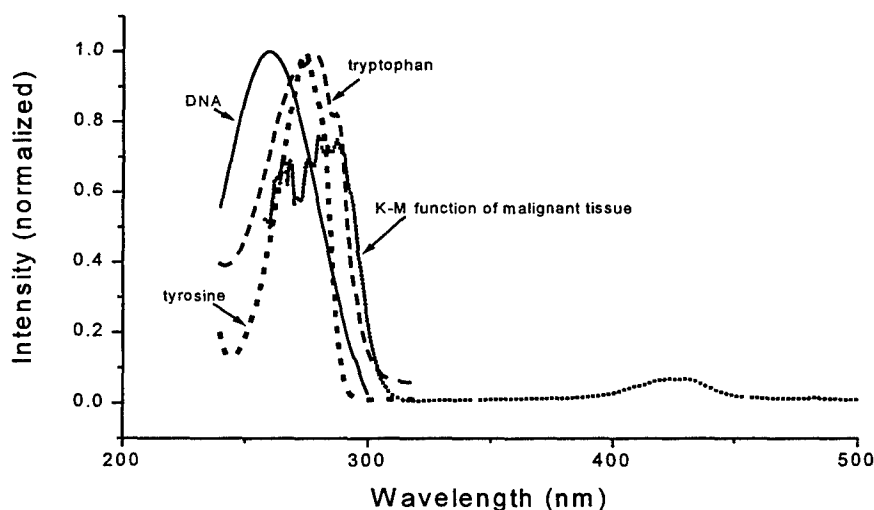


Figure 2: The absorption spectra of DNA, amino acid of tryptophan, tyrosine and K-M function of malignant breast tissue.

DNA, amino acids tryptophan and tyrosine were displayed in fig. 2. The K-M function of malignant tissue was also displayed in Fig. 2. It is clear that two peaks of malignant are overlap with the absorption peak of DNA and amino acids. For fibroadenoma, the peak of K-M curve near 280nm is uncertain. Some specimen was higher some was lower but less 265nm peak was existed as compared to malignant tissue.

Table 1: The averaged value of K-M function $f(r_{\infty})$ and logarithm K-M function ($\log f(r_{\infty})$) at 275nm–285nm and 255nm–265nm for different kinds of breast tissues.

Type of specimens	Averaged at 275nm to 285nm		Averaged at 255nm to 265nm	
	$f(r_{\infty})$	$\log f(r_{\infty})$	$f(r_{\infty})$	$\log f(r_{\infty})$
Invasive carcinoma	13.11±10.39	0.98±0.39	19.05±18.70	1.06±0.52
Mixed invasive and <i>in situ</i>	5.23±4.16	0.58±0.36	8.78±13.30	0.65±0.50
Benign	0.88±0.69	-0.15±0.29	0.66±0.70	-0.36±0.39
Fibroadenoma	5.23±5.69	0.48±0.46	3.62±4.41	0.24±0.56

Fibroadenomas are usually found in young women. It is a tumor composed of epithelial and stromal elements that originates from the terminal duct lobular unit. It is important to distinguish fibroadenoma from malignancy. From Table 1, it was found the averaged amplitude of $f(r_{\infty})_{255\text{nm}-265\text{nm}}$ was higher than $f(r_{\infty})_{275\text{nm}-285\text{nm}}$ for malignant tissue and lower for benign and fibroadenoma tissue. The another ratio parameter $A=[f(r_{\infty})_{275\text{nm}-285\text{nm}}]/[f(r_{\infty})_{255\text{nm}-265\text{nm}}]$ was selected. The statistical averaged values, standard deviations and peak position of Gaussian fit curve of parameter A were given in Table 2.

Table 2. The averaged value of parameter $A=[f(r_{\infty})_{275\text{nm}-285\text{nm}}]/[f(r_{\infty})_{255\text{nm}-265\text{nm}}]$ for different kinds breast tissues.

Type of specimens	$A=[f(r_{\infty})_{275\text{nm}-285\text{nm}}]/[f(r_{\infty})_{255\text{nm}-265\text{nm}}]$	
	Statistical mean value and standard deviation	Gauss fit center and width
Invasive carcinoma	0.93±0.52	0.85±0.35
Mixed invasive and <i>in situ</i>	0.89±0.36	0.85±0.31
Benign	1.97±1.42	1.49±0.84
Fibroadenoma	1.82±0.56	1.64±0.48

5. CONCLUSION:

KMF function in UV spectral range was used to determine the absorption peak at 265nm and 285nm. The proteins and DNA absorption peak was observed in malignant tissues. For benign tissues, the absorption of proteins was lower and there was less DNA absorption. Furthermore, for fibroadenoma tissue, while protein absorption was sometimes in the range of malignant samples, but relative weaker DNA absorption was found. The KMF function gives information of DNA and proteins content in tissues.

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