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Determination of optical properties of normal and adenomatous human colon tissues *in vitro* using integrating sphere techniques

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Abstract

AIM: The purpose of the present study is to compare the optical properties of normal human colon mucosa/submucosa and muscle layer/chorion, and adenomatous human colon mucosa/submucosa and muscle layer/chorion *in vitro* at 476.5, 488, 496.5, 514.5 and 532 nm. We believe these differences in optical properties should help differential diagnosis of human colon tissues by using optical methods.

METHODS: *In vitro* optical properties were investigated for four kinds of tissues: normal human colon mucosa/submucosa and muscle layer/chorion, and adenomatous human colon mucosa/submucosa and muscle layer/chorion. Tissue samples were taken from 13 human colons (13 adenomatous, 13 normal). From the normal human colons a total of 26 tissue samples, with a mean thickness of 0.40 mm, were used (13 from mucosa/submucosa and 13 from muscle layer/chorion), and from the adenomatous human bladders a total of 26 tissue samples, with a mean thickness of 0.40 mm, were used (13 from mucosa/submucosa and 13 from muscle layer/chorion). The measurements were performed using a double-integrating-sphere setup and the optical properties were assessed from these measurements using the adding-doubling method that was considered reliable.

RESULTS: The results of measurement showed that there were significant differences in the absorption coefficients and scattering coefficients between normal and adenomatous human colon mucosa/submucosa at the same wavelength, and there were also significant differences in the two optical parameters between both colon muscle layer/chorion at the same wavelength. And there were large differences in the anisotropy factors between both colon mucosa/submucosa at the same wavelength, there were also large differences in the anisotropy factors between both colon muscle layer/chorion at the same wavelength. There were large differences in the value ranges of the absorption coefficients, scattering coefficients and anisotropy factors between both colon mucosa/submucosa, and there were also large differences in these value ranges between both colon muscle layer/chorion. There are the same orders of magnitude in the absorption coefficients for four kinds of colon tissues. The scattering coefficients of these tissues exceed the absorption coefficients by at least two orders of magnitude.

CONCLUSION: There were large differences in the three optical parameters between normal and adenomatous human colon mucosa/submucosa at the same laser wavelength, and there were also large differences in these parameters between both colon muscle layer/chorion at the same laser wavelength. Large differences in optical parameters indicate that there were large differences in compositions and structures between both colon mucosa/submucosa, and between both colon muscle layer/chorion. Optical parameters for four kinds of colon tissues are wavelength dependent, and these differences would be useful and helpful in clinical applications of laser and tumors photodynamic therapy (PDT).

Key words: Optical properties; Laser; Normal and adenomatous human colon tissues; Integrating sphere

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INTRODUCTION

In the field of biomedical optics, determination of the optical properties of various biological materials is essential and important for many diagnostic and therapeutic applications of light in medicine[1-12]. Clinical protocols are usually based on clinical experience and optical properties of tissues[13-15]. For example, photodynamic therapy (PDT) is a relatively new cancer therapy, it is a superficial treatment characterized by a depth of necrosis, which depends on several parameters including delivered light dose, photosensitizer concentration and tissue optical properties[16]. The tumors of many kinds seems ideally suited to PDT, for example,

colon, bladder, *etc.* tumors are suited to PDT, as it is readily accessible using an endoscope and the entire mucosa can be easily treated simultaneously via a diffusing light fiber. Multifocal superficial disease and occult carcinoma *in situ* do not have to be localized precisely for PDT to be effective[17-19], and yet PDT treatment effectiveness is greatly influenced by photosensitizer content and light distribution in the irradiated tumor tissue[20-27], and an accurate evaluation of energy fluence in depth should allow an estimate of whether the whole tumor mass will be properly irradiated[28-31]. Consequently tissue optical properties, absorption coefficients (μ_a), scattering coefficients (μ_s) and anisotropy factors (g) of thin slabs of human colon and diseased colon tissue are important for medical applications in diagnosis and therapy[27]. The purpose of the present study is to compare the optical properties of normal human colon mucosa/submucosa and muscle layer/chorion, and adenomatous human colon mucosa/submucosa and muscle layer/chorion *in vitro* at 476.5, 488, 496.5, 514.5 and 532 nm, i.e., μ_a, μ_s and *g*. The results of the experiment were analyzed and compared, and experimental results were obtained.

MATERIALS AND METHODS

Materials

Experimental materials *In vitro* optical properties were investigated for four kinds of tissues: normal human colon mucosa/submucosa and muscle layer/chorion, and adenomatous human colon mucosa/submucosa and muscle layer/chorion. Tissue samples were taken from 13 human colons (13 adenomatous, 13 normal), immediately after excision of the tissues. Each removed colon sample was immediately rinsed briefly in saline to remove surface excess blood and peeled off surface fats, and was put into bottles with saline as soon as possible, was stored in a refrigerator at 0 ℃, was sectioned by microtome before measurement. All tissue samples were respectively clamped between two glass slides of 1.03 mm thickness, and then were respectively placed between the two integrating spheres before tissue samples were measured. From the normal human colons a total of 26 tissue samples, with a mean thickness of 0.40 mm, were used (13 from mucosa/submucosa and 13 from muscle layer/chorion), and from the adenomatous human bladders a total of 26 tissue samples, with a mean thickness of 0.40 mm, were used (13 from mucosa/submucosa and 13 from muscle layer/chorion). Tissue samples were prepared and measurements were taken within at most 17 h after removal.

Methods

Measurement of tissue optical parameters An established method for measuring the optical parameters of turbid materials is the integrating-sphere technique. This technique is widely used for the determination of the optical properties of biological tissues *in vitro* and for the verification of the optical techniques intended for use *in vivo*[32-38]. The experimental set-up is schematically shown in Figure 1. The colon samples were placed between two identical integrating spheres (Anhui Institute of Optics and Fine Mechanics, Academia Sinica, China, model: F4) of 50 mm diameter with a circular sample port of 12 mm diameter and illuminated with collimated light from (1) a 2.2 mm beam diameter, 532 nm laser (LBO double-frequency, COHERENT, USA, model: Verdi-V10) operating at 0-10 W, (2) a 2.7 mm beam diameter, 476.5, 488, 496.5, 514.5 nm argon ion laser (COHERENT, USA, model: INNOVA 70) operating at 0-1.5 W. The output of all laser were collimated and attenuated (to a power at most 10 mW) by 2 mm pinhole and light attenuators, respectively. The light fluences within each sphere (the diffuse reflectance in the first sphere, the diffuse transmission in the second sphere), and the collimated transmission (at a distance of 80 cm beyond the second sphere) were measured using standard light measuring techniques[39]. The input light beams to form an intersection with light axis was approximately 1.5° were chopped mechanically at 500 Hz by using light chopper (SRS, USA, model: SR540). The collimated transmitted light signals, the diffuse reflectance R_d, and the diffuse transmittance T_d were measured by using photodiode detectors (APP, Hamamatsu, Japan, model: C5460) at 476.5, 488, 496.5, 514.5 and 532 nm. The signals were amplified using a lock-in-amplifier (SRS, USA, model: SR830) and processed by a personal computer. All measurements within the spheres were made relative to the signal when a 99% reflecting plate (Anhui Institute of Optics and Fine Mechanics, Academia Sinica, China, model: F4) was placed at the sample aperture, and the collimated transmission measurement was made relative to a measurement with no sample. For a more detailed description of the measurement method of a double-integrating-sphere set-up see Pickering *et al*[40-42], and van van Hillegersberg *et al*[43]. The optical properties, μ_a, μ_s and *g*, of thin slabs of colon tissue were determined by measuring the collimated transmission, diffuse reflectance and transmission in the aforesaid set-up. The inverse adding-doubling algorithm was used to determine the optical parameters from these measurements[44]. The algorithm accounts for the refractive index of the glass slides (n = 1.55) and of tissue (n = 1.4)[45].

Statistical analysis

Optical parameters of biological tissue samples were expressed as the mean±SD, were demonstrated by Student's

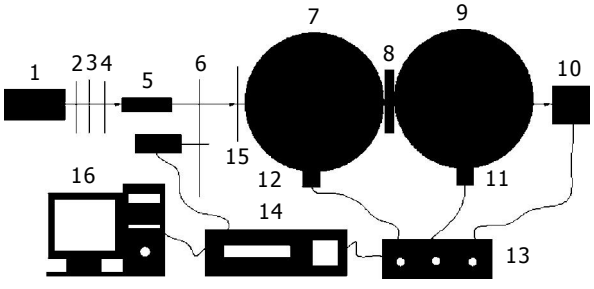

Figure 1 Experimental set-up consisting of double- integrating-sphere with an intervening sample. 1. Laser 2. Attenuator 3. Attenuator 4. 2 mm pinhole 5. Beam expander 6. 6 mm pinhole 7. Integrating sphere 8. Sample 9. Integrating sphere 10. Photodiode detector 11. Photodiode detector 12. Photodiode detector 13. Switch box 14. Lock-in-amplifier 15. Light chopper 16. PC.

t-test, and were considered significant at P values <0.01. The SPSS10 was used for the statistical analysis.

RESULTS

In this experiment, five different wavelengths of laser were respectively used for radiating the two incision areas of tissue slices, each of the measured value at each wavelength of laser in the same condition of experimentation was the mean arithmetical value that was gained by 24 measurements repeatedly at each kind of tissue sample of a total of 13 tissue samples at the same wavelength of laser, and per tissue sample was measured twice for each incision area of the tissue slice, respectively. The position of luminous spot of incident light radiation on the sample was changed after each measured datum was obtained. The two incision areas of the same kind of all samples were respectively radiated at each one wavelength of laser respectively, the measured results were reproducible for a specific sample at specific wavelength. There were no significant differences in the measured values R_d or T_d and or T_c of the two incision areas of the same kind of all samples at the same wavelength. Consequently, the collimated transmittance, diffuse reflectance and transmittance of the two incision areas of the same kind of all samples at the same wavelength were estimated with arithmetical average. By the inverse adding-doubling algorithm, we gained the wavelength dependence of the absorption coefficients, the scattering coefficients and the scattering anisotropy factors of these tissues from these measurements. Tables 1 and 2 summarize the results of our measurements for four kinds of tissues at the five wavelengths. The optical properties are expressed as the mean±SD for all measurements within one group of samples (e.g., normal human colon mucosa/submucosa at 488 nm).

DISCUSSION

In vitro optical properties of both normal and adenomatous human colon mucosa/submucosa and muscle layer/chorion were determined at 476.5, 488, 496.5, 514.5 and 532 nm, the measurements were performed using a double-integrating-sphere setup and the optical properties were assessed from these measurements using the adding-doubling method that was considered reliable[42,44,46]. In our study, it is interesting to note the differences in optical properties measured between these tissues at five different laser wavelengths. We believe these differences in optical properties should help the differential diagnosis of human colon tissues by using optical methods.

Absorption coefficients

Table 1 shows that the absorption coefficient for normal human colon mucosa/submucosa is 2.32/cm at 476.5 nm but increases to 3.27/cm at 488 nm and drops to 2.58/cm at 496.5 nm but increases to 3.12/cm at 514.5 nm and to 3.33/cm at 532 nm, and that for adenomatous human colon mucosa/submucosa is 5.27/cm at 476.5 nm but increases to 5.34/cm at 488 nm and drops to 4.87/cm at

Table 1 Anisotropy factors, absorption and scattering coefficients of normal and adenomatous human colon mucosa/submucosa at five different wavelengths of laser irradiation by the inverse adding-doubling method

Tissue	λ (nm)	μ_a (/cm)	μ_s (/cm)	g
Normal human colon mucosa/submucosa	476.5	2.32±0.09	214±5.35	0.885±0.019
	488.0	3.27±0.13	228±5.69	0.891±0.021
	496.5	2.58±0.10	212±5.27	0.897±0.024
	514.5	3.12±0.12	216±5.38	0.902±0.026
	532.0	3.33±0.14	208±5.16	0.908±0.029
Adenomatous human colon mucosa/submucosa	476.5	5.27±0.21	233±5.72	0.897±0.023
	488.0	5.34±0.22	238±5.84	0.903±0.027
	496.5	4.87±0.19	228±5.67	0.907±0.028
	514.5	4.37±0.17	231±5.69	0.917±0.033
	532.0	5.16±0.20	223±5.63	0.913±0.031

Table 2 Anisotropy factors, absorption and scattering coefficients of normal adenomatous human colon muscle layer/chorion at five different wavelengths of laser irradiation by the inverse adding-doubling method

Tissue	λ (nm)	μ_a (/cm)	μ_s (/cm)	g
Normal human colon muscle layer/chorion	476.5	1.31±0.05	221±5.61	0.923±0.037
	488.0	1.73±0.07	215±5.33	0.932±0.044
	496.5	1.27±0.05	200±5.08	0.927±0.041
	514.5	1.14±0.04	189±5.03	0.933±0.045
	532.0	1.53±0.06	193±5.05	0.941±0.048
Adenomatous human colon muscle layer/chorion	476.5	3.17±0.12	233±5.71	0.927±0.042
	488.0	3.51±0.14	223±5.62	0.935±0.046
	496.5	2.90±0.11	216±5.36	0.933±0.044
	514.5	2.57±0.09	198±5.07	0.936±0.047
	532.0	2.75±0.10	208±5.14	0.945±0.049

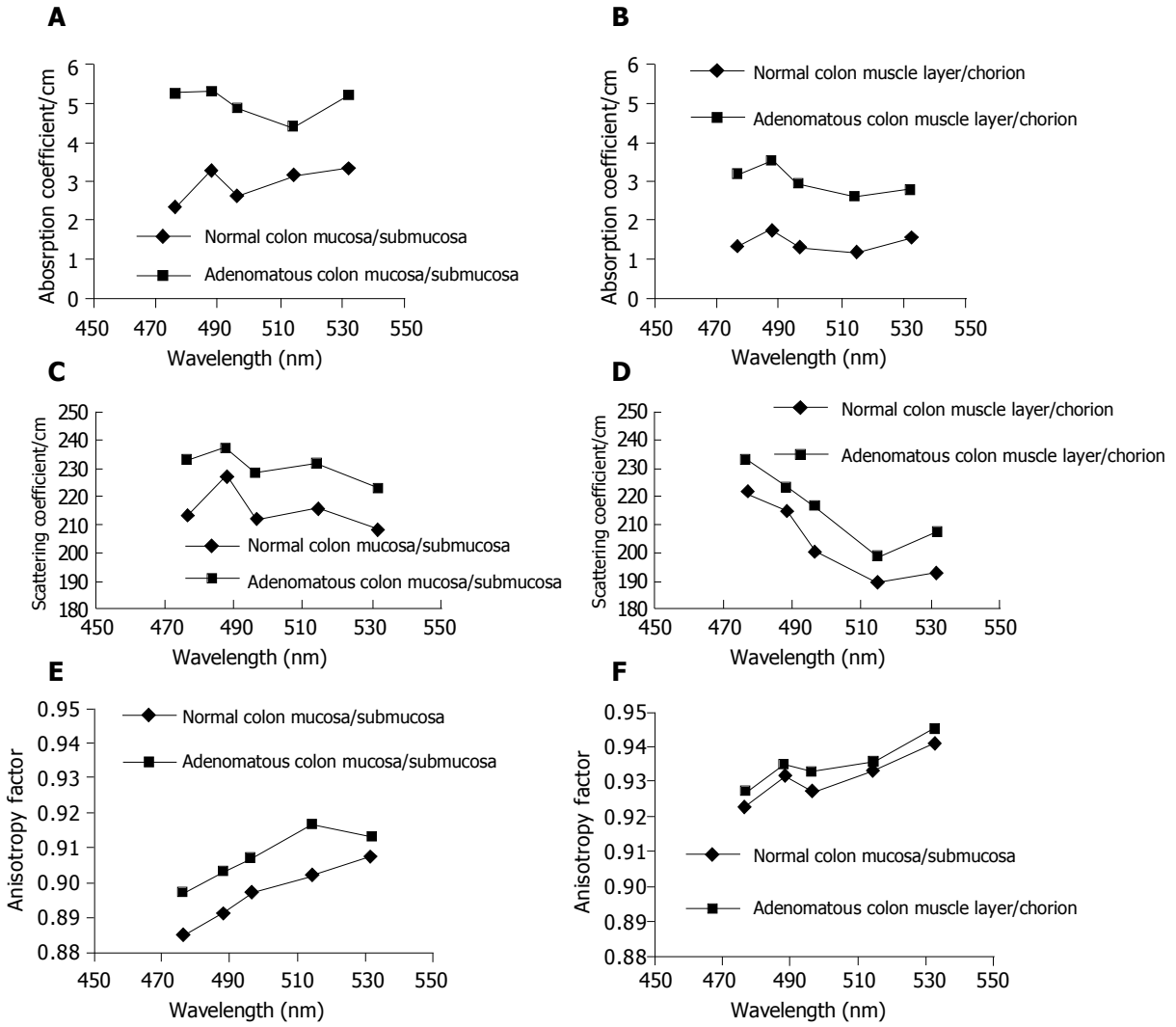

Figure 2 Optical properties of normal and adenomatous human colon mucosa/submucosa *in vitro* vary with a change of laser wavelength *in vitro*. **A**: Plots of the absorption coefficients measured *in vitro vs* wavelength of normal and adenomatous human colon mucosa/submucosa; **B**: Plots of the absorption coefficients measured *in vitro vs* wavelength of normal and adenomatous human colon muscle layer/chorion; **C**: Plots of the scattering coefficients measured *in vitro vs* wavelength of normal and adenomatous human colon mucosa/submucosa; **D**: Plots of the scattering coefficients measured *in vitro vs* wavelength of normal and adenomatous human colon muscle layer/chorion; **E**: Plots of the anisotropy factors measured *in vitro vs* wavelength of normal and adenomatous human colon mucosa/submucosa; **F**: Plots of the anisotropy factors measured *in vitro vs* wavelength of normal and adenomatous human colon muscle layer/chorion.

496.5 nm and to 4.37/cm at 514.5 nm but increases to 5.16/cm at 532 nm. It is obvious that there were significant differences in the absorption coefficients between both colon mucosa/submucosa at the same wavelength ($P<0.01$). The minimum value of the absorption coefficients for normal colon mucosa/submucosa is 2.32/cm at 476.5 nm and the maximum value is 3.33/cm at 532 nm, it is obvious that the values lie in the range between 2.32 and 3.33/cm. And the minimum value for adenomatous colon mucosa/submucosa is 4.37/cm at 514.5 nm and the maximum value is 5.34/cm at 488 nm, the values lie in the range between 4.37 and 5.34/cm, obviously there are large differences in the value range of the absorption coefficients between both tissues. Table 2 shows that the absorption coefficient for normal colon muscle layer/chorion is 1.31/cm at 476.5 nm but increases to 1.73/cm at 488 nm and drops to 1.27/cm at 496.5 nm and to 1.14/cm at 514.5 nm but increases to 1.53/cm at 532 nm, and that for adenomatous colon muscle layer/chorion is 3.17/cm at 476.5 nm but increases to 3.51/cm at 488 nm and drops to 2.90/cm at 496.5 nm and to 2.57/cm at 514.5 nm but increases to 2.75/cm at 532 nm. It is obvious that there were also significant differences in the absorption coefficients between both colon muscle layer/chorion at the same wavelength ($P<0.01$). The minimum value of the absorption coefficients for normal colon muscle layer/chorion is 1.14/cm at 514.5 nm and the maximum value is 1.73/cm at 488 nm, it is obvious that the values lie in the range between 1.14 and 1.73/cm. And the minimum value for adenomatous colon muscle layer/chorion is 2.75/cm at 532 nm and the maximum value is 3.51/cm at 488 nm, the values lie in the range between 2.75 and 3.51/cm, obviously there are large differences in the value range of the absorption coefficients between both tissues. Figure 2A,B show respectively the wavelength dependence of the absorption coefficients of both tissues obtained from the measurements, the shape of the two curves in Figure 1 is not similar, and the shape of the two curves in Figure 2A is similar, and large differences

of the absolute values of two curves in Figures 2A and B, respectively indicate that there were large differences in compositions and structures between two tissues, and just large differences in compositions and structures induce large differences in absorption characteristics between both tissues.

The scattering coefficients

Table 1 shows that the scattering coefficient for normal human colon mucosa/submucosa is 214/cm at 476.5 nm but increases to 228/cm at 488 nm and drops to 212/cm at 496.5 nm but increases to 216/cm at 514.5 nm and drops to 208/cm at 532 nm, and that for adenomatous human colon mucosa/submucosa is 233/cm at 476.5 nm but increases to 238/cm at 488 nm and drops to 228/cm at 496.5 nm but increases to 231/cm at 514.5 nm drops to 223/cm at 532 nm. It is obvious that there were also significant differences in the scattering coefficients between both colon mucosa/submucosa at the same wavelength ($P<0.01$). The minimum value of the scattering coefficients for normal colon mucosa/submucosa is 208/cm at 532 nm and the maximum value is 228/cm at 488 nm, it is obvious that the values lie in the range between 208 and 228/cm. And the minimum value for adenomatous colon mucosa/submucosa is 223/cm at 532 nm and the maximum value is 238/cm at 488 nm, the values lie in the range between 223 and 238/cm, obviously there are large differences in the value range of the scattering coefficients between both tissues. Table 2 shows that the scattering coefficient for normal colon muscle layer/chorion is 221/cm at 476.5 nm and drops to 215/cm at 488 nm and to 200/cm at 496.5 nm and to 189/cm at 514.5 nm but increases to 193/cm at 532 nm, and that for adenomatous colon muscle layer/chorion is 233/cm at 476.5 nm and drops to 223/cm at 488 nm and to 216/cm at 496.5 nm and to 198/cm at 514.5 nm but increases to 208/cm at 532 nm. It is obvious that there were also significant differences in the scattering coefficients between both colon muscle layer/chorion at the same wavelength ($P<0.01$). The minimum value of the scattering coefficients for normal colon muscle layer/chorion is 189/cm at 514.5 nm and the maximum value is 221/cm at 476.5 nm, obviously the values lie in the range between 189 and 221/cm. And the minimum value for adenomatous colon muscle layer/chorion is 198/cm at 514.5 nm and the maximum value is 233/cm at 476.5 nm, the values lie in the range between 198 and 233/cm, obviously there are also large differences in the value range of the scattering coefficients between both tissues. Figures 2C and D show respectively the wavelength dependence of the scattering coefficients of both tissues obtained from the measurements, the shape of the two curves in Figure 2B is similar, and the shape of the two curves in Figure 2C is also similar, and large differences of the absolute values of two curves in Figures 2C and D respectively indicate that there were also large differences in compositions and structures between two tissues, and just large differences in compositions and structures induce large differences in scattering characteristics between both tissues.

Anisotropy factors

Table 1 shows that the anisotropy factors for normal human colon mucosa/submucosa is 0.885 at 476.5 nm but increases to 0.891 at 488 nm and to 0.897 at 496.5 nm and to 0.902 at 514.5 nm and to 0.908 at 532 nm, and that for adenomatous human colon mucosa/submucosa is 0.897 at 476.5 nm but increases to 0.903 at 488 nm and to 0.907 at 496.5 nm and to 0.917 at 514.5 nm and drops to 0.913 at 532 nm. It is obvious that there were large differences in the anisotropy factors between both colon mucosa/submucosa at the same wavelength. The minimum value of the anisotropy factors for normal colon mucosa/submucosa is 0.885 at 476.5 nm and the maximum value is 0.908 at 532 nm, obviously the values lie in the range between 0.885 and 0.908. And the minimum value for adenomatous colon mucosa/submucosa is 0.897 at 476.5 nm and the maximum value is 0.917 at 514.5 nm, the values lie in the range between 0.897 and 0.917, obviously there are large differences in the value range of the anisotropy factors between both tissues. Table 2 shows that the anisotropy factors for normal colon muscle layer/chorion is 0.923 at 476.5 nm but increases to 0.932 at 488 nm and drops to 0.927 at 496.5 nm but increases to 0.933 at 514.5 nm and to 0.941 at 532 nm, and that for adenomatous colon muscle layer/chorion is 0.927 at 476.5 nm but increases to 0.935 at 488 nm and drops to 0.933 at 496.5 nm but increases to 0.936 at 514.5 nm and to 0.945 at 532 nm. It is obvious that there were large differences in the anisotropy factors between both colon muscle layer/chorion at the same wavelength. The minimum value of the anisotropy factors for normal colon muscle layer/chorion is 0.923 at 476.5 nm and the maximum value is 0.941 at 532 nm, it is obvious that the values lie in the range between 0.923 and 0.941. And the minimum value for adenomatous colon muscle layer/chorion is 0.927 at 476.5 nm and the maximum value is 0.945 at 532 nm, the values lie in the range between 0.927 and 0.945, it is obvious that there are obvious differences in the value range of the anisotropy factors between both tissues. Figures 2E and F show respectively the wavelength dependence of the anisotropy factors of both tissues obtained from the measurements, the shape of the two curves in Figure 2E is not similar, and the shape of the two curves in Figure 2F is similar, and obvious differences of the absolute values of two curves in Figures 2E andF, respectively indicate also that there were obvious differences in compositions and structures between two tissues, and just obvious differences in compositions and structures induce obvious differences in anisotropy characteristics between both tissues.

Researching the results indicate that there were obvious differences in tissue optical properties between both colon mucosa/submucosa and between both colon muscle layer/chorion at the same laser wavelength. The same orders of magnitude exist in the absorption coefficients for four kinds of colon tissues. The scattering coefficients of these tissues exceed the absorption coefficients by at least two orders of magnitude. The absorption coefficients are strongly affected by the presence of blood, particularly at wavelengths below 600 nm. Tissues of various pathologies have differing absorption coefficients and scattering properties and these differences are wavelength dependent, and these differences would be useful and helpful in clinical applications of laser and tumors PDT.

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