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Analysis of serum from type II diabetes mellitus and diabetic complication using surface-enhanced Raman spectra (SERS)

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Abstract In this paper, we show surface-enhanced Raman spectra (SERS) of serums from type II diabetes mellitus and diabetic complication (coronary disease, glaucoma and cerebral infarction), and analyze the SERS through the multivariate statistical methods of principal component analysis (PCA). In particular, we find that there exist many adenines in these serums, which maybe come from DNA (RNA) damage. The relative intensity of the band at 725 ± 2 cm^{-1} assigned to adenine is higher for patients than for the healthy volunteers; therefore, it can be used as an important 'fingerprint' in order to diagnose these diseases. It is also shown that serums from type II diabetes mellitus group, diabetic complication group and healthy volunteers group can be discriminated by PCA.

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1 Introduction

In diabetes mellitus, the body fails to produce or to respond to insulin which regulates glucose fluctuations, resulting in abnormal metabolisms of carbohydrates, fats, and proteins. Failure to regulate these levels within tight limits leads to severe secondary complications. According to the World Health Organization (WHO), an estimated 0.24 billion people have diabetes mellitus (type I and II) in the world today. Therefore, there is an urgent need for rapid, dependable detection of diabetes mellitus and complication. Many spectra technologies, including the microspectrum technique, spectrophotometric analysis technique, fluorimetry method and so on, have found application in the medicine from several different aspects. Compared with these technologies, Raman spectroscopy is an important tool for chemical analysis because of its specificity for molecular group identification. Rohleder et al. found the differences between sera originating from patients with diabetes mellitus and from healthy volunteers by Raman spectra [1]. However, the weak signal of bio-specimen restricts its application in medical diagnosis.

The surface-enhanced Raman spectra (SERS) is superior for identifying the molecular composition of complex materials because the enhancement of Raman intensities of as much as 10^9–10^{15} times by molecules adsorbed onto nanostructured metal surface has generated the possibility of detecting very small amounts of the substances [2–4]. Therefore, it has become one of the best techniques to assay single molecule and bimolecular spectroscopy [5, 6]. A few studies have been made where introduced biomolecules or intracellular components have been identified independently from each other in single cells with SERS [7, 8]. Fabriciova et al. and Miskovsky et al. studied the interaction between antitumoral drug and human serum albumin [9, 10] by the SERS technique, respectively. But there are few reports on the SERS of serums from patients up to now.

Principal Components Analysis (PCA) is an exploratory multivariate statistical technique for simplifying complex data sets. Recently, principal component analysis (PCA) has been applied to Raman spectroscopy to analyze serum samples from breast cancer patients [11–13]. Here, the SERS and PCA are used to probe the serums from type II diabetes mellitus, diabetes mellitus with coronary disease, glaucoma and cerebral infarction.

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2 Materials and method

Some materials used in this experimentation such as adenine, uracil, thymine, guanine, cytosine and the corresponding nucleotides are purchased from Shanghai BioAsia Biotechnology Co. Ltd (Shanghai, China). The samples are put into the ultrapure water separately. Mix supernatant solution of each sample and the silver colloidal nanoparticles for SERS measurement, respectively. Here the silver colloidal nanoparticles are synthesized by deoxidizing method using trisodium citrate and silver nitrate [14, 15]. The experimental serums are selected from 20 healthy volunteers as the control group, from 20 patients who have been diagnosed with type II diabetes mellitus by Liaocheng People's Hospital as the second group, and from 20 people with diabetic complications (glaucoma, cerebral infarction and coronary heart disease) as the third group. The serum is mixed with the silver colloidal nanoparticles according to 1:1 proportion and put into a quartz glass capillary for Raman measurement.

Raman spectra were collected at confocal Raman microspectroscopy (British Renishaw) in the range of 400–1800 cm^{-1}, with NIR 780 nm laser whose power was maintained at 25 mW and the spectral resolution was less than 2 cm^{-1}. Spectrometer scans, data collection and processing were controlled by a personal computer.

3 Results

The mean SERS of serums from type II diabetes mellitus group and diabetic complications group are presented in Fig. 1. It can be found that the characteristic SERS of serums from coronary disease, glaucoma and cerebral infarction are very similar to that from type II diabetes mellitus, indicating that these diseases associate with each other.

The mean SERS of serums from the type II diabetes mellitus group and the control group are presented in Fig. 2. The major contribution to these bands is from protein, lipids, carbohydrate, and so on. The ratio of relative intensity can be used to analyze the characteristic of biomacromolecule after the group and chemical bond changed [16] and this ratio can be defined as:

$$\Delta I_n = \frac{I'_n - I_n}{I_n} \times 100\%.$$

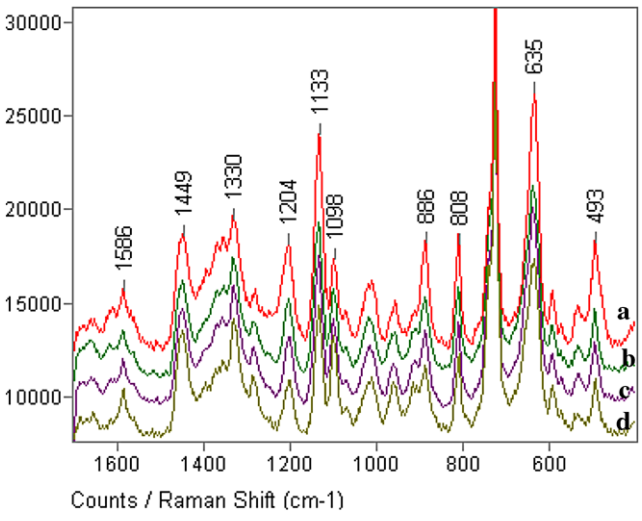

Fig. 1 The mean SERS of serum from different diseases. The characteristic bands of serums from the type II diabetes mellitus (*a*), coronary disease (*b*), glaucoma (*c*), and cerebral infarction (*d*) are very similar

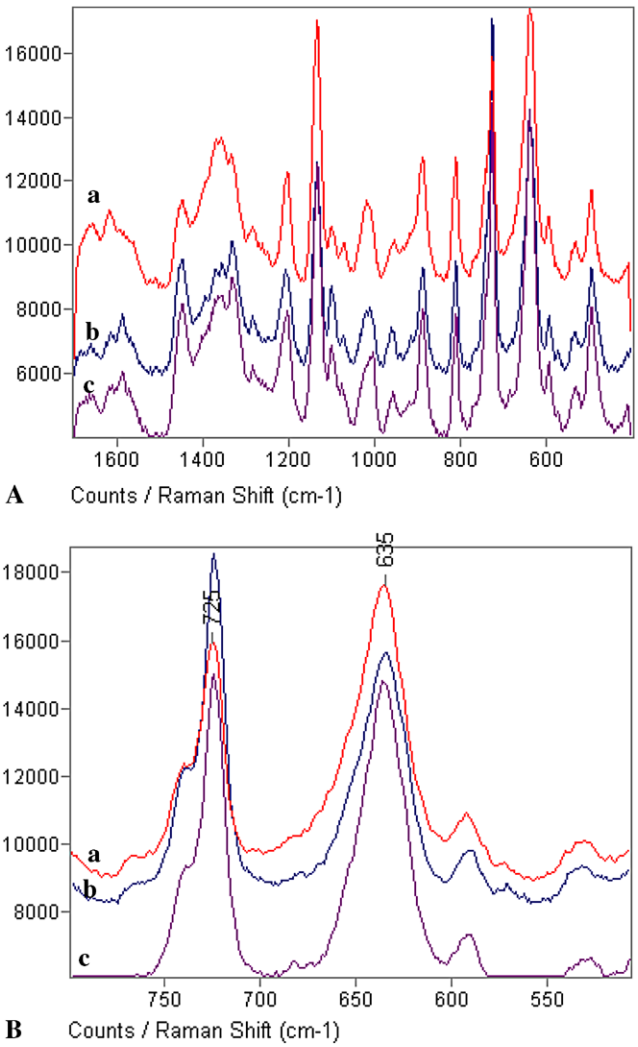

Fig. 2 The mean SERS of serum from the type II diabetes mellitus group and the healthy volunteers group. (**B**) shows the part of (**A**) between 790 and 460 cm^{-1}: (*a*) the mean SERS of serum from the healthy volunteers group with the relative intensity $\beta = I_{725\pm2}/I_{635\pm2}$ being 0.54; (*b*) β is 1.3 for the type II diabetes mellitus group; (*c*) β decreases to 1.01 for the mixture solution of DNA and the serum from the type II diabetes mellitus group, but the value of β for the mixture solution of DNA and the serum from the healthy volunteers group stays invariant

Table 1 The assignment and the ratio of the main Raman peaks of serums from the type II diabetes mellitus group, diabetic complication group and the healthy volunteers group

Control Raman shift/cm^{-1}	Type II diabetes mellitus and complication Raman shift/cm^{-1} (cm^{-1})	ΔI_n	Assignment
1620 ± 2	1586 ± 1	−1.13–1.20%	Trp, Tyr, Phe
1448 ± 3	1449 ± 2	43.1–56.5%	$\delta(CH_2, CH_3)$
1367 ± 1	1365 ± 2	−41.9–−32.1%	Trp
1331 ± 3	1330 ± 2	33.4–50.0%	C (6)-H_2, CH_3COH
1202 ± 3	1204 ± 2	−7.66–1.81%	Trp, Phe
1133 ± 1	1133 ± 1	−8.60–12.3%	Man, γ(C–N)
1097 ± 1	1098 ± 2	42.7–51.8%	Man, γ(C–N)
1071 ± 2	1073 ± 1	−12.2–−26.2%	γ(C–N)
1014 ± 2	1013 ± 2	−16.7–1.04%	Trp
887 ± 1	886 ± 1	−5.24–1.55%	Trp
810 ± 2	808 ± 3	1.18–5.49%	Asn
725 ± 2	725 ± 2	87–135%	γ(C–S)
635 ± 1	635 ± 2	0	γ(C–S)
592 ± 1	593 ± 1	−2.08–8.51%	Amide-VI
494 ± 1	493 ± 1	5.69–14.5%	Man

γ—Stretching Vibration; Phe—Phenylalanine; δ—Bending Vibration; Tyr—Tyrosine; Trp—Tryptophan; Man—D-Mannos; Asn—Asparaginase

Here $I_n'$and I_n are representatives for the relative intensity of the peaks from the type II diabetes mellitus group and the control group, respectively. The relative intensity of the peak is defined as $I_n = \mathrm{I}_n/\mathrm{I}_{635\pm2}$, by selecting the intensity of 635 ± 2 cm^{-1} as the main criterion. Table 1 presents the assignment and the ratio of the main Raman peaks of serum from the type II diabetes mellitus group, diabetic complication group and the control group.

The average spectra look similar to one another, but some peaks show differences in intensity and Raman shift (see Fig. 2 and Table 1 for more details). The relative intensity of 725 ± 2 cm^{-1} assigned to adenine and the trans-side conformation of proteins [17, 18] from the type II diabetes mellitus group increases by 87–135% ($\Delta I_{725\pm2}$), but it is not related to the level of blood sugar. The band at 1620 ± 2 cm^{-1} assigned to tryptophan, tyrosine and phenylalanine of protein [19, 20] from the control group shifts to 1586 ± 1 cm^{-1}. The relative intensity of the band at 1449 ± 2 cm^{-1} attributed to the bending stretching of CH_2- and CH_3- [21] increases by 43.1–56.5% ($\Delta I_{1449\pm2}$) in the type II diabetes mellitus group, indicating that the contents of lipids from these diseases rises. The relative intensity of the band at 1330 ± 2 cm^{-1} assigned to adenine and the stretching vibration of C–H–O of carbohydrates [22] increases by 33.4–50.0% ($\Delta I_{1330\pm2}$). In addition, the lines at 1098 ± 2 cm^{-1} belonging to D-Mannose [23, 24] also increase by 42.7–51.8% ($\Delta I_{1098\pm2}$), suggesting that the contents of glycoprotein and sugariness rise. But the relative intensity of band at 1365 ± 2 cm^{-1} assigned to the indole-ring of tryptophane (Trp) decreases by 41.9–32.1% ($\Delta I_{1365\pm2}$) in the type II diabetes mellitus group.

4 Discussion

In order to analyze the factors resulting in the change of relative intensity of 725 ± 2 cm^{-1}, the relative intensity of 725 ± 2 cm^{-1} is defined as $\beta = \mathrm{I}_{725\pm2}/\mathrm{I}_{635\pm2}$. Figure 2 shows $\beta \approx 0.54$ (the average value) for the control group and $\beta \approx 1.3$ (the average value) for the type II diabetes mellitus group. Then we put a small amount of DNA into the mixture solution of silver colloidal nanoparticles and the serum. The mean SERS of the new mixture from the type II diabetes mellitus solution is shown in Fig. 2(c). It can be found that the relative intensity of 725 cm^{-1} decreases to $\beta \approx 1.01$ (the average value), but for the healthy volunteers it stays invariant, which can be attributed to the fact that the competitive adsorption phenomenon exists between DNA and adenine on silver colloidal nanoparticles. At the same time, the relative intensity of the other bands does not change significantly, indicating that the SERS of proteins, lipids and carbohydrates are not affected by DNA. In addition, no line has been found in the mixture solution of DNA and silver colloidal nanoparticles. These results show that the change of the band at 725 ± 2 cm^{-1} has a close relationship with adenine.

We next rule out the effects of protein on the change of relative intensity of 725 ± 2 cm^{-1} and confirm it by the results from adenine. Adenine powder is put into the ultrapure

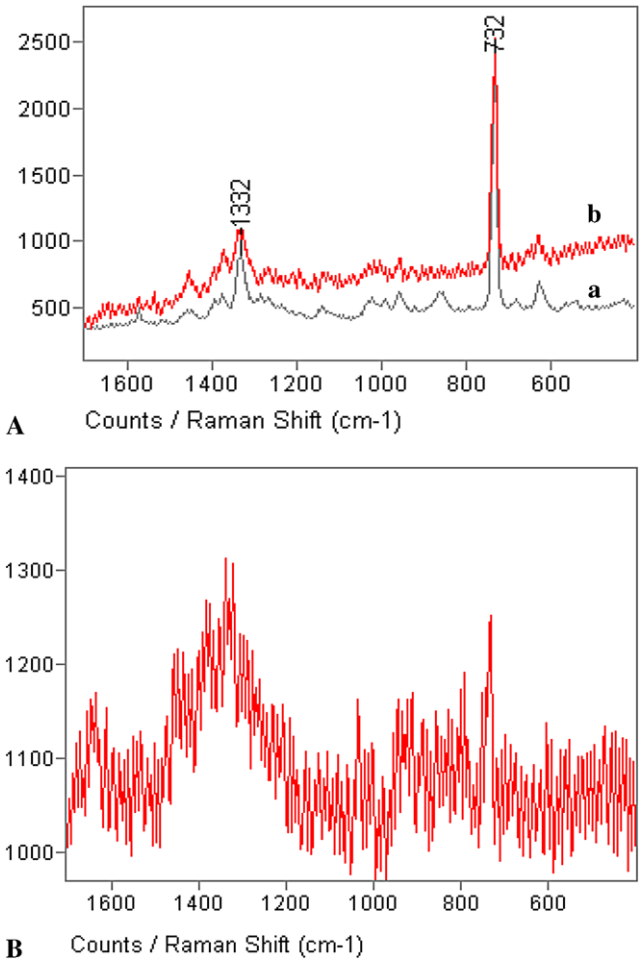

Fig. 3 (**A**) A comparison of SERS of adenine with the serum without protein: (*a*) the SERS taken for adenine solution; (*b*) the mean SERS of the residual solution after the serums of the type II diabetes mellitus group and diabetic complication group are incubated at 100°C until solidified. It appears that the SERS of the residual solution have the same characteristics as that of adenine. (**B**) The characteristics of adenine become indistinguishable in the residual solution of serum from the healthy volunteers group

water, and the supernatant solution of the sample is mixed with the silver colloidal nanoparticles. The SERS of adenine solution is given in Fig. 3(a). We select 16 serums from the control group, 16 serums from the type II diabetes mellitus group and 16 serums from the diabetic complication group randomly, and then incubate those serums at 100°C until solidified. After being extracted by quantitative filter paper, the mean SERS of the residual solution from the type II diabetes mellitus group (13 samples) and the diabetic complication group (12 samples) is shown in Fig. 3(b). It is apparent for the SERS of the residual solution that the peaks belonging to protein disappear, but two peaks at 732 and 1332 cm^{-1} have the same characteristics as that of adenine. However, the characteristics of adenine become indistinguishable in the residual solution of serum from the control group (14 samples) in Fig. 3(B), showing that there are small amounts

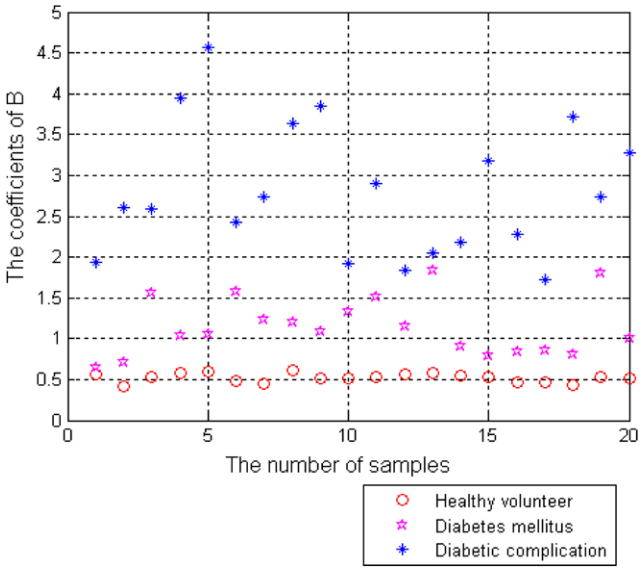

Fig. 4 The coefficients of variation of $\beta = I_{725\pm2}/I_{635\pm2}$ from the type II diabetes mellitus group (☆), diabetic complication group (∗), and the healthy volunteers group (○). The range of β in the serums from the healthy volunteers group is 0.4–0.6 with the mean of 0.54, whereas it is 0.6–1.7 with the mean of 1.3 in the type II diabetes mellitus group and 1.7–5.0 with the mean of 2.8 in the diabetic complications group

of adenines in the blood of healthy volunteers. In addition, we also measure the SERS of the other bases and their nucleotides, and find that these spectra are not consistent with Fig. 3(b). This may mean that many adenines exists in the serum of patients with type II diabetes mellitus and diabetic complication and that adenines combine with protein so that the band at 732 cm^{-1} moves to 725 cm^{-1}.

5 Statistical methods

The band at 725 ± 2 cm^{-1} can be regarded as an important 'fingerprint' to diagnose these diseases, Fig. 4 shows the coefficients of variation of β ($\beta = I_{725\pm2}/I_{635\pm2}$) from the serum of the type II diabetes mellitus group (☆), diabetic complication group (∗) and the healthy volunteers group (○). The range of β in 20 cases of serums from the healthy volunteers group is 0.4–0.6 with the mean of 0.54, and the range of β is 0.6–1.7 with the mean of 1.3 in 20 cases of serums from the type II diabetes mellitus group, except for one case which belongs to the range of the healthy volunteers group. So the sensitivity of β is 95% if we discriminate between the type II diabetes mellitus group and the healthy volunteers group by $\beta \approx 0.6$. Nevertheless, the range of β is 1.7–5.0 with the mean of 2.8 in the diabetic complication group, where there are two cases that belong to the range of the type II diabetes mellitus group, so the sensitivity of β is 90% if we discriminate between the type II diabetes mellitus group and the diabetic complication group by $\beta \approx 1.7$.

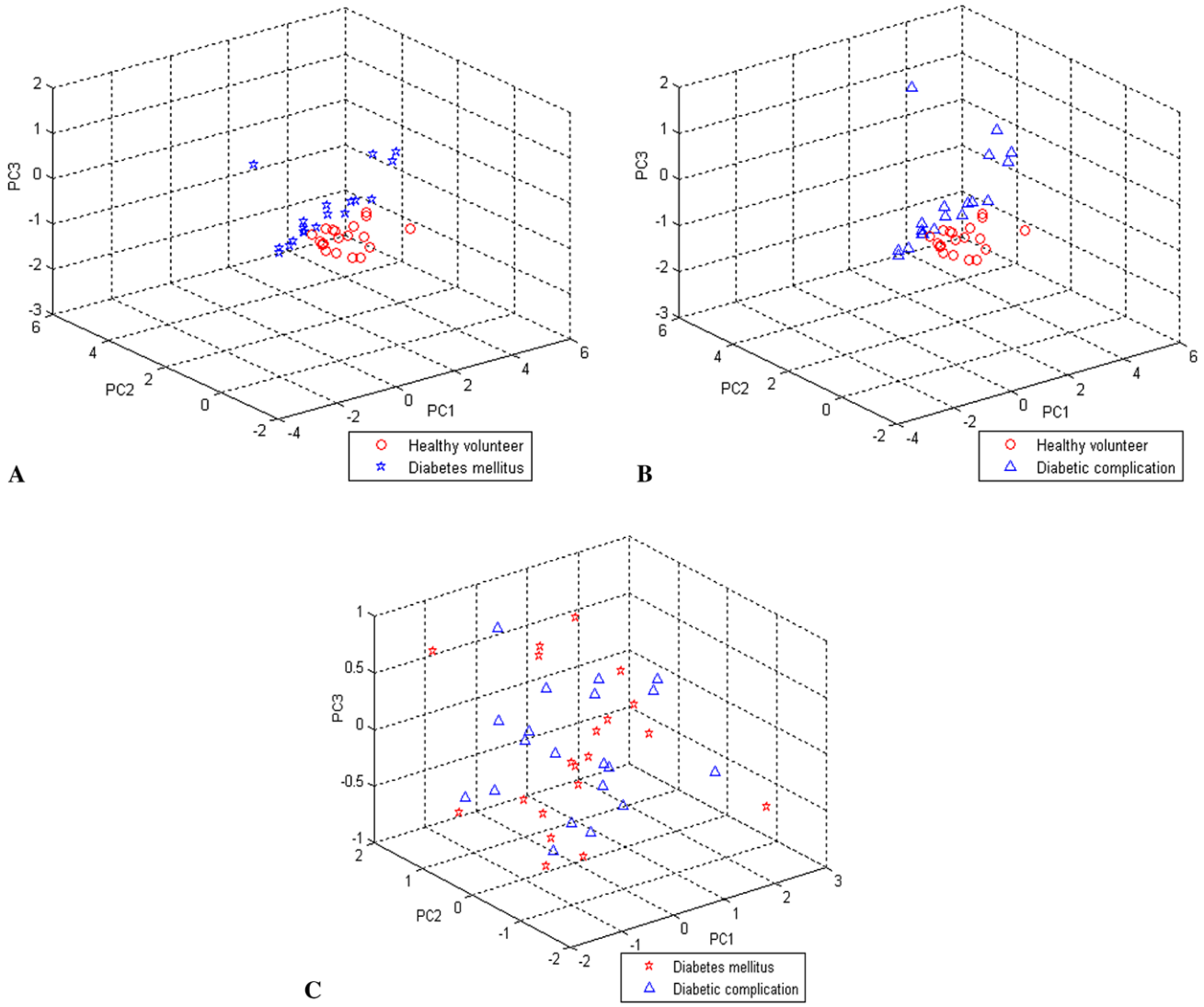

Fig. 5 A three-dimensional mapping of PCA from the type II diabetes mellitus group, diabetic complication group and the healthy volunteers group. (**A**) The results of PCA from healthy volunteers group (○) and the type II diabetes mellitus group (☆). Sample points are distributed in separate areas. (**B**) The results of PCA from the healthy volunteers group (○) and the diabetic complication group (△). The points can be separated completely. (**C**) The results of PCA from the type II diabetes mellitus group (☆) and the diabetic complication group (△). The points fall into similar regions, indicating that they are undistinguishable

The images are analyzed with multivariate statistics in order to improve the sensitivity of discernment and to extract the remarkable results. Principal components analysis (PCA) is a statistical technique for determining the key variables in a multidimensional data set that explain the differences in the observations, and can be used to simplify the analysis and visualization of multidimensional data sets [25, 26]. Firstly, the raw images are processed by baseline correction, second order-derivative and area normalization, and then we can obtain K-components after the new data K–L transformation of Matlab 7.0 (The Mathworks Inc.). The contribution rate to total variation of spectra from the first to the third of principal component (PC1, PC2 and PC3) reached over 89.68% so that the first three principal components can account for over 89.68% of the total variability. Figure 5 shows the results of these calculations: three-dimensional mapping of PCA from the type II diabetes mellitus group, diabetic complication group, and the healthy volunteers group.

Figure 5(A) and 5(B) show the results of PCA from the type II diabetes mellitus group with healthy volunteers group, and diabetic complication group with healthy volunteers group, respectively. We can clearly see that they are distributed in separate areas, which means that we are able to discriminate between the spectra of the type II diabetes mellitus group and the healthy volunteers group, or between the diabetic complication group and the healthy volunteers group. Figure 5(A) and 5(B) also show that the regional dis-

tribution of the type II diabetes mellitus group and the diabetic complication group is wider than for the healthy volunteers group, and even some distribution close to the healthy volunteers group. But the results from the type II diabetes mellitus group and diabetic complication group fall into similar regions in Fig. 5(C), indicating that they are undistinguishable.

6 Conclusion

Surface-enhanced Raman scattering technique and multivariate analysis are employed to study serums from type II diabetes mellitus and diabetic complication patients. It is demonstrated that these spectra have similar characteristics, indicating these diseases associate with each other. It is also shown that the relative intensity of the band at $725 \pm 2\ \mathrm{cm}^{-1}$ (β) assigned to adenine becomes higher for these patients than for the healthy volunteers, revealing that these serums contain many adenines, so this parameter (β) can be regarded as an important 'fingerprint' to diagnose type II diabetes mellitus and diabetic complication, but the sensitivity of β is not 100%. PCA gives a clear discrimination between the spectra of type II diabetes mellitus and healthy volunteers, or diabetic complication patients and healthy volunteers.

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