Editorial

Point-of-Care Testing Blood Coagulation Detectors Using a Bio-Microfluidic Device Accompanied by Raman Spectroscopy

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An efficient technique was developed for the detection of human blood coagulation using a bio-microfluidic device based on Raman spectra. A Raman spectrometer mounted with a He-Ne laser with a maximum power of 200 mW and an excitation wavelength of 632.8 nm was used together with a Peltier-cooled charge-coupled device (CCD) detector with a measurement range of 200–3000 cm^{-1} [1]. The characteristic contents of human whole blood and the prothrombin time (PT) were monitored to prevent the interference of fluorescent signals [2]. To avoid damage to the blood samples by the laser light, the measurement time was set to 3 s. The samples were measured three to five times, and the Raman spectrum was smoothed three times using filters. The obtained spectrum was compared with previously reported Raman spectral results. The results thus obtained in combination with the bio-microfluidic device show more significant Raman spectral signals. The amino acid components in the blood could be detected in the Raman spectra as well as hormones, enzymes, antibodies, the protein composition, and other biological information which can be found in blood samples. This biological information can be determined using the characteristic Raman peaks. Such a method is an improvement over the microscopic techniques once extensively used for blood test observation, with their long analysis times and imperfect data accuracy [3].

In recent years, of all the various laser detection methods, Raman spectroscopy has become the only nonlinear scattering technique widely used for blood testing and analysis. This approach offers the benefits of requiring only a small sample amount, quick analysis, and precise interpretation [4–6]. The general bio-microfluidic device for biomedical applications is composed of two layers (upper and lower) with a flow channel and a mixing region. The Raman spectra of the blood in the upper layer exhibit several peaks corresponding to blood components, such as for isoprotein amino acids and Allo-D-isoleucine at 991.3 and 1290 cm^{-1}, respectively [7], hemoglobin (Hb) at 1614 cm^{-1}, and protein and lipids in platelets at 1446 cm^{-1} [8]. The peaks at 804, 820–920, 1726, and 494 cm^{-1} correspond to D-Mannose [9]. However, since the white blood cells (WBC) and platelets in the blood may be relatively few in number or change after coagulation, the Raman intensity is extremely low, making interpretation difficult. There are many secondary characteristic peaks ranging from 600 to 1000 cm^{-1}, which correspond to other biological compounds in the blood, such as hormones, antibodies, and enzymes. The peak at 1454 cm^{-1} most likely corresponds to platelets, with a secondary peak detected to the left of the Raman spectrum [10]. The alteration of the platelets from their usual disc shape to a spherical form occurred as a result of blood cryopreservation, and some platelet lipids were lost, leading to platelet loss.

The peaks at 964 to 1444 cm^{-1} disappeared over time. The Raman peak at 1290 cm^{-1} is related to the band value of hemoglobin, which is more pronounced than other peaks. This suggests that hemoglobin is responsible for most of the Raman peaks in the blood. Blood transmission is primarily dependent on lipoproteins in the blood; lipids will join with lipoproteins, and platelet lipids have no relationship with the protein content [11].

Therefore, blood samples were placed in a bio-microfluidic device [12], and measurement was performed inside the microchannels (the blood mixing channel is at the center). At this point, the blood clotted, and the absence of peaks in the spectrum proved that the primary component of this blood sample was platelets rather than hemoglobin [13].

The PT is a method for detecting the extrinsic coagulation pathway. The current PT detection standard is based on the international normalized ratio (INR), also known as the ProTime INR or PT/INR [14,15]. This method can be applied to evaluate the coagulation ability of the blood. The PT can detect the function of coagulation factors, and it is the time required for the conversion of prothrombin into thrombin after adding the excess tissue factor to platelet-deficient plasma, resulting in plasma coagulation. Since the average PT is 10 to 12 s, measurements were completed within 12 s after uniform mixing. The PT and partial prothrombin time (aPTT) indicate whether the subject's intrinsic coagulation pathway and common coagulation pathway are normal or not [16]. Blood coagulation or the coagulation process refers to changes in the blood from a liquid state to a non-flowing gel state, and it is an essential link in physiological hemostasis. During the blood coagulation process, the soluble fibrinogen in plasma is converted into insoluble fibrin. Here, the "prothrombin-to-thrombin conversion mechanism" is the benchmark measurement. Normally, the coagulation process must be quick, to minimize bleeding or meet the requirements of rapid treatment. For this reason, the cascade mechanism was used in the coagulation mechanism to expand the coagulation signal step by step [17].

Before measurement, the blood was injected into the microfluidic device and mixed for about 2–3 s by the automatic circulatory system [4,5]. The Raman detection system can measure minute changes in the characteristic peaks by increasing the integration time. Therefore, the intensity of the characteristic peaks at this time will be higher than before mixing. In addition, the interaction of the coagulation mechanism with other hemostatic mechanisms (especially the mechanism of platelet thrombosis) is indicted by changes in the intensity of the characteristic peaks related to the platelets. Hence, it is essential to study the difference in the characteristic peaks before and after mixing. The Raman spectra of the blood samples are often influenced by fluorescence or other ambient background light. Thus, background subtraction is necessary. Generally, polynomial fitting is used to eliminate the background noise. Here, the background was removed to obtain the processed spectrogram. The built-in Labspec software was used to smooth the spectrum with the average filter approach. Four spectra were measured in total, with each scan taking 3 s on average. The time was short to avoid damage caused by the laser light source during measurement and to measure the damage or destruction of red blood cells caused by laser light energy irradiation. Continuous use of laser irradiation to irradiate blood will cause hemolysis of red blood cells [18]. The hemolysis status signal was monitored to avoid errors caused during blood analysis, controlling the measurement time within 12 s. The influence of the environment on the red blood cells themselves was analyzed as well as the oxygen-carrying mechanism of red blood cells in the oxidized blood. The resulting spectrum was composed of five average spectra of various spectral effects taken under identical conditions.

For each study, five periodic scans were taken with a cumulative exposure time of 3 s each to examine the red blood cell oxygenation capacity. The oxygen-carrying capacity was investigated by examining changes in the Raman spectra of the red blood cells by controlling the ambient oxygen concentration. The spectral range between 1500 and 1650 cm^{-1}, related to the oxygen-carrying capacity of red blood cells [19], was used to evaluate the oxygen-carrying capacity of these cells. The obtained data revealed that the environmental oxygen level affects the distinctive peak at 1638 cm^{-1}, resulting in changes in intensity. After coagulation, the peak intensity is reduced, which can be used to evaluate the oxygen-carrying capability. Comparison with the Spectral Database for Organic Compounds (SDBS) [20] showed that many detected signals correspond to blood amino acids. The peaks between 1200 and 1300 cm^{-1} are mainly due to C–H bond changes; those between 1300 and 1400 cm^{-1} mainly indicate pyrrole ring expansion and deformation

characteristics; and those between 1500 and 1650 cm^{-1} are due to changes in the spin state and the oxygen-carrying capacity of the red blood cells.

Diluting blood samples leads to a 1.41 times higher peak than the maximum value. However, higher dilution produces weaker peaks, and some characteristic peaks are not easy to detect. If the dilution is greater than 1:10, a higher laser power or integration time is required for measurement, but this may damage the sample. The limit is found experimentally and can be identified with a significantly smaller volume of blood. Studies have shown that a 632.8 nm Raman device can assess blood dilutions of at least 1:5 in this scenario. The characteristic peaks at 755, 1212, 1270, 1365, 1446, 1575, and 1608 cm^{-1} can be clearly detected. Hemolysis signals for erythrocyte measurement might easily impact the vibration modes [21], and they can reduce the error caused by hemolysis. Raman analysis can also show the albumin, hemoglobin, and glucose found in the blood.

Hemoglobin occupies 95% of the weight of dry red blood cells. The peak at 1001 cm^{-1} is due to the presence of phenylalanine in albumin, similar to the phenylalanine-containing proteins in saliva, and lipase and starch enzymes. Amide II vibrational modes, including C–N stretching, were detected with several subtle peaks at 1146 cm^{-1} for N–H and C–C bonds [22]. Clinical studies using Raman spectrometer detection have confirmed the diversity of biological information in the blood, with the same biological information displayed in similar blood samples. The bio-microfluidic device was used to study the behavior of the blood and calcium ions (Ca^{2+}) with an initial blood flow rate of 0.2 m/s. The coagulation reaction with calcium ions did not occur in the initial inlet area, because the prothrombin activator began to mix in this area. The blood coagulated swiftly, with the coagulation response completed in 15 s.

There was a significant reduction in the lipid Raman signals at 1223, 1247, 1446, and 1619 cm^{-1}, indicating that coagulation has a significant influence on the ester composition, and that the lipids have undergone serious changes. Heparin, a mucopolysaccharide with high polarity and a potent anticoagulant for negative tuberculosis, was examined using Raman spectroscopy [23]. Heparin has been used in clinical practice to treat thrombosis. It has a good inhibitory effect on the blood, preventing the generation of thromboplastin, the conversion of prothrombin into thrombin, and the inhibition of thrombin. It is injected into the body, and the body's new metabolism will eventually replace it. The Raman spectra show prominent features in the 700 to 1200 cm^{-1} range. These vibrational modes are associated with small changes in the dipoles and give rise to strong changes in the peaks at 1039 and 1055 cm^{-1} caused by symmetric S–O stretching to produce N–O-sulfate residues [24]. The heparin and blood mixture coagulated quickly, within 12 s. The difference before and after the mixing reaction was examined. Changes in measurement conditions can have a significant impact on platelet and coagulation performance.

Bio-microfluidic devices were used for the measurement of the PT [4,5]. They were fabricated using low-temperature plasma, and annealing processes were carried out for the bonding of heterogeneous materials and to eliminate internal stress on the silicon-based surface implantation of the components [12]. The advantages of this process are that it can prevent cracking or blood leakage. This new process successfully produced an improved bio-microfluidic device, suitable to be used for blood coagulation detection by Raman spectroscopy [2,14]. The bonding interface of the bio-microfluidic device was examined by scanning electron microscopy (SEM) and automated optical inspection (AOI) systems. The results show the interface of the bio-microfluidic device produced by the low-temperature bonding process to be very flat [12]. Raman technology can be used to accurately collect and analyze information of the components in the blood [25]. After the experiments, the comparison showed that the conventional Raman system had a good signal acquisition effect. Compared with the high-precision confocal Raman microscope system, it has the advantages of being low-priced and having a simple operating structure [26]. A blood coagulation detector using a bio-microfluidic device accompanied by Raman spectroscopy is an effective method for measuring the signals of human blood. Comparison of the above

results with previous reports showed the blood coagulation detectors to be more accurate and rapid.

Conflicts of Interest: The authors declare no conflict of interest.

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