Application of nanodiamonds in human body fluid analysis by matrix-assisted laser desorption/ionization mass spectrometry

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Direct mass spectrometric analysis of complex biological samples is very important and challenging. In this paper, nanodiamonds have been successfully used in matrix-assisted laser desorption/ionization mass spectrometric analysis of human serum and urine. As a practical tool and platform, it can be widely used in the field of humoral proteomics, and it plays a very promising role in clinical diagnosis, including identification of novel disease-associated biomarkers.

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Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) has served as a very important technique for analyzing peptides, proteins, oligonucleotides, and other biopolymers[1−4]. Combined with other preseparating methods, identification of new potential biomarkers in various body fluids by mass spectroscopy has been employed[5,6]. In order to fulfill direct analysis of such complex samples, improved methods, such as surface-enhanced laser desorption/ionization (SELDI), have also been developed and applied[7,8]. In our earlier works, we found that carboxylated/oxidized nanodiamonds have exceptionally high affinity to proteins and peptides through both hydrophilic and hydrophobic forces, and thus can be directly used in MALDI-MS analysis, especially for those highly diluted and contaminated samples[9−12]. The method has also been developed as a fast and general platform for proteome analysis with mass spectrometry nominated as "SPEED" (solid-phase extraction and elution on diamond)[12,13]. And recently the diamond is modified as the new bright fluorescent source with a quantum efficiency close to 1, which has been used successfully in life science research[14,15]. Here, the promise of carboxylated/oxidized nanodiamonds application in MALDI-MS for clinic proteomics research is further demonstrated with applications to human blood serum and urea.

Nanodiamonds with a nominal size of 100 nm (Kay Industrial Diamond, KDM, USA) are employed in this work. Their surfaces are prepared by strong acid pretreatment and are predominantly carboxylated and oxidized[9−16]. Stock suspensions of diamond particles were prepared with deionized water. Dihydroxybenzoic acid (DHB), 4-hydroxy-α-cyanocinnamic acid (4HCCA), and trifluoroacetic acid (TFA) were gotten from Sigma and used without further purification. All blood serum samples and urea samples were from healthy individuals (male) without obvious signs of disease. The matrix solutions consisted of 4HCCA or DHB are with concentrations of 10 and 100 mg/mL in TFA-acetonitrile-water with the volume ratio of 0.001:1:3 respectively. Mass spectra in positive ion mode were acquired using a delayed ion-extraction linear time-of-flight (TOF) mass spectrometer with a flight length of 2.2 m[10,11,17]. The acceleration voltage is 20 kV. A pulsed Nd:YAG laser operating at 355 nm with the energy of 20 μJ/pulse was used here. The desorbed/ionized molecules were detected by a triple microchannel plate assembly biased at −2.8 kV. All mass spectra were collected at 100 laser shots with a digital oscilloscope operating at its sampling rate of 100 MS/s.

Figure 1 gives the whole process of sample preparation[10,11]. The sample solution was first mixed with the diamond suspension in a centrifuge tube, followed by separation of the nanodiamonds with centrifugation. After removal of the supernatant, the diamond particles were additionally rinsed with deionized water. Then, matrix solution (4 μL) was added to the microcentrifuge tube and mixed with the precipitate. At last, an aliquot (~ 2 μL) of the mixed solution was deposited on a stainless steel probe and allowed to dry in air at room temperature for MALDI analysis.

Direct analysis of human blood serum with MALDI-MS is always unstable and can display only several distinct features in the mass-to-charge ratio (m/z) range of $2000 - 10000$[10]. When using nanodiamonds and the

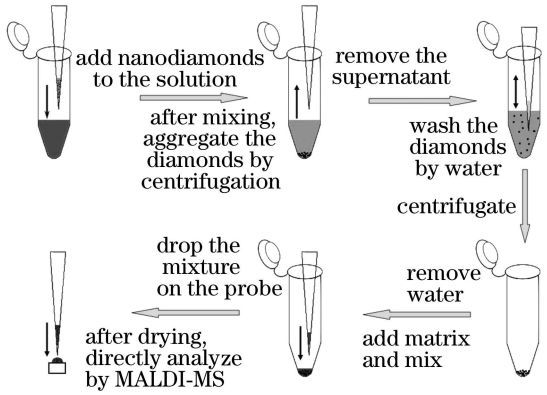

Fig. 1. Process of sample preparation using nanodiamonds to enrich proteins for MALDI-MS analysis.

procedure suggested above, the mass spectra are quite different. As shown in Fig. 2(a), with the help of nanodiamonds, it has been found that more than 80 peaks can be clearly identified in the same mass range even for just 10-μL sample (diluted in 1000-μL water) when using 4HCCA as the matrix. It is also interesting to find that different matrixes can cause difference in mass spectra for the same sample. Though the total ion signals are less when using DHB as the matrix, some new features still can be identified in its mass spectrum, as Fig. 2(b) shows. By now, it is still very hard to identify what these ions are. But the mass distributions or mass spectral patterns of those spectra and some characteristic peaks may provide very important information about different kinds of diseases by comparing the samples coming from normal persons and patients[18−21].

One of the most characteristic features of carboxylated/oxidized nanodiamond is that its surface has maximum absorption ability for the protein with the isoelectric point (pI) value closest to the surrounding pH value[9]. This is quite useful for capturing proteins selectively from mixed and complicated solutions. Here the serum samples were diluted into phosphate buffers with different pH values, and then analyzed by nanodiamonds and MALDI-MS respectively. Figure 3 shows the results for solutions with pH values of 2, 5, 7, and 10. It can be seen many different peaks can be identified respectively. And the mass spectra gotten under extreme pH values are very different with those gotten at neutral surroundings. The characteristic is very valuable for the detection of low concentration peptides or proteins in serum or plasma, such as a majority of biomarkers or potential biomarkers, because by selecting suitable pH value to those ions, the abundant but non-interested ions (with different pI values) can be suppressed simultaneously.

Another very valuable example is the direct analysis of

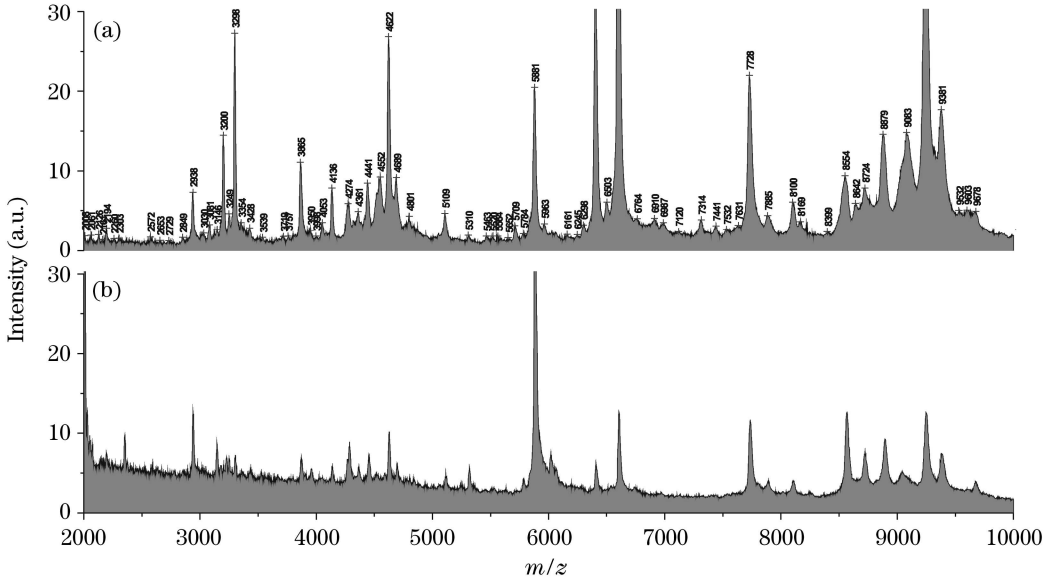

Fig. 2. MALDI-TOF mass spectra of 10-μL human serum (diluted in 1000-μL water) pretreated with nanodiamonds, while the matrixes are (a) 4HCCA and (b) DHB respectively.

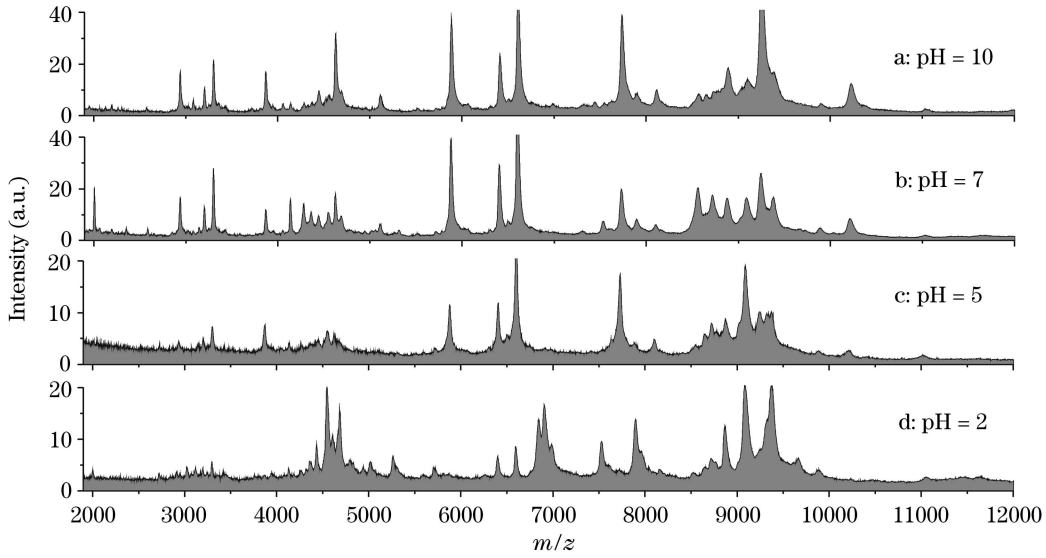

Fig. 3. MALDI-TOF mass spectra of human serum after 100-fold dilution in phosphate buffers with different pH values and then pretreated with nanodiamonds. The matrix is 4HCCA.

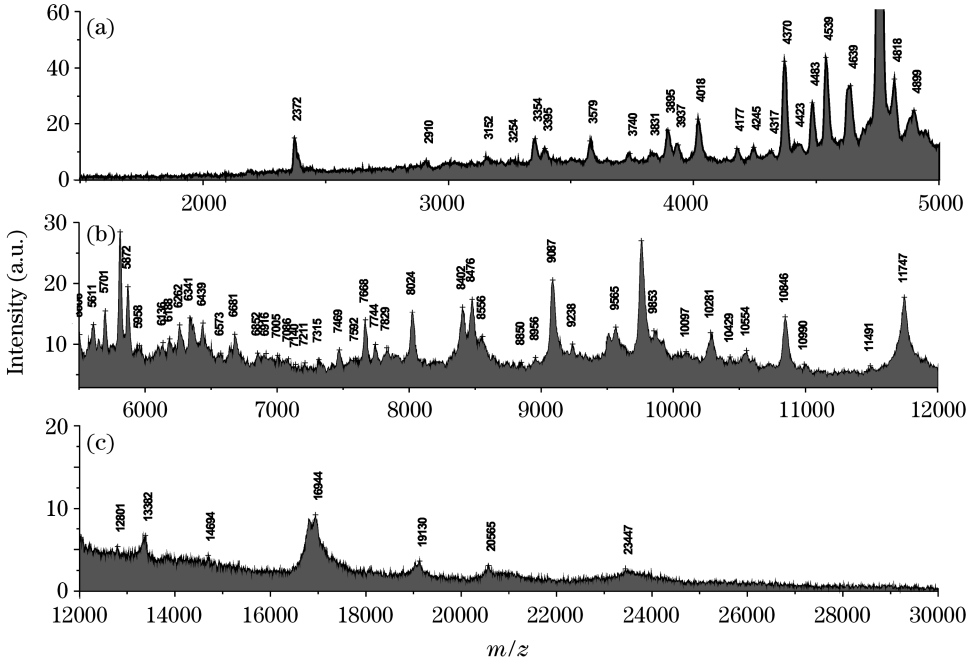

Fig. 4. MALDI-TOF mass spectra of human urine pretreated with nanodiamonds, while (a), (b), and (c) come from one mass spectrum, which are displayed separately here just for clarity. The matrix is 4HCCA.

human urine, which is a readily obtainable biological fluid[22]. For example, diseases which adversely affect the function of kidneys will cause excessive losses of proteins in the urine, thus the pattern of urinary protein excretion can be used to identify the cause of the disease[23]. Protein separation methods are always needed when using mass spectrometry, because the existence of urea and other salts is always causing the loss of signals[10,22−24]. Because of its high affinity for proteins and peptides, nanodiamonds play very important roles in the urinary proteomics. It can concentrate proteins in large volumes and can be separated readily, and the negative effects of urea to mass spectrometry can be easily removed by just washing with water, as we proved early[10]. The whole process is faster, easier and more effective compared with other existing methods[5,6,23,24]. Figure 4 shows the direct analysis of 4-μg nanodiamonds in 100-μL urea of normal person with 4HCCA as the matrix, in which more than 70 peaks can be clearly identified in the m/z range of 2000−25000, while the direct analysis of the urea sample gives no stable peaks.

In brief summary, it has been demonstrated that the carboxylated/oxidized nanodiamonds can be used in the direct analysis of human serum and urine with MALDI-MS. By adjusting pH values of the solutions and selecting suit matrix, the analysis of peptides and proteins with low abundance becomes feasible, which makes it as a promising way for searching disease-related biomarkers. Considering the various preanalytical variations of human body fluids, automatization should be further combined with the method and thus huge deals of samples can be analyzed systematically.

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