Small molecule analysis using MALDI-TOF MS with solid nanostructure matrices

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MALDI-TOF mass spectrometry has been widely applied for the analysis of biomolecules with high molecular weights, such as proteins, because of its advantages on easy sample preparation, sensitive detection, and so on. However, the conventional organic matrices are also ionized to produce their own peaks at low mass-to-charge (m/z) ratio range (<500) while the sample analytes are ionized by pulsed laser. Moreover, the organic matrices usually formed non-uniform co-crystals with samples while drying on the surface of target plate. Resultingly, spot-to-spot intensity deviations were too high to apply MALDI-TOF mass spectrometry to the quantitative analysis. From such reasons, the application of MALDI-TOF has difficulties in applying to the analysis of small molecular weight molecules. In this work, two kinds of solid matrices, TiO_2 nanowire arrays and functional nanoweb matrix, were synthesized and applied to MALDI-TOF mass spectrometry. Furthermore, small molecules were analyzed from human serum and milk samples qualitatively and quantitatively using solid matrices.

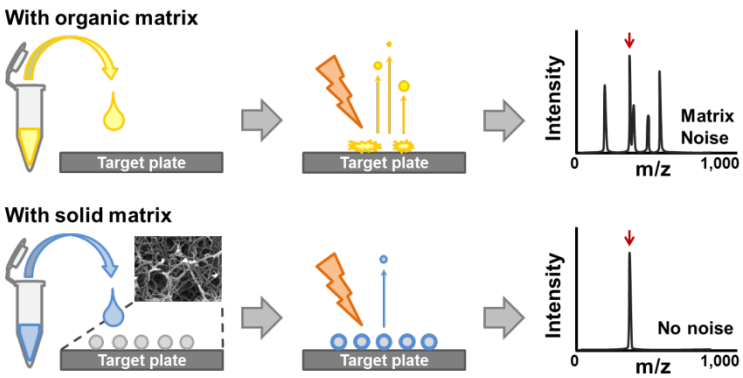

Figure 1. Scheme of applying solid matrices to MALDI-TOF MS and its effects

In order to utilize TiO_2 nanowires for MALDI-TOF mass spectrometry as matrices, TiO_2 nanowires were synthesized by top-down hydrothermal process directly on the surface of thin titanium plates using alkali solution, subsequently treated with water and heat. For the actual application as solid matrix target plates, the TiO_2 nanowire target plates were patterned with parylene thin film to have 81 nanowire zones on the surface. Synthesized TiO_2 nanowires were then characterized their morphology and structures by scanning electron microscopy (SEM), X-ray diffraction (XRD), Raman spectrometry and photo luminescence spectrometry.

In case of nanoweb matrix, functional nanoweb matrices were synthesized by simultaneous process of electrospinning of nanoweb and electrospraying of TiO_2 nanoparticles on the metal target plate. The synthesis process was monitored by a quartz crystal microbalance sensor. The characteristic of functional nanowebs were determined by SEM and a dynamic light scattering method.

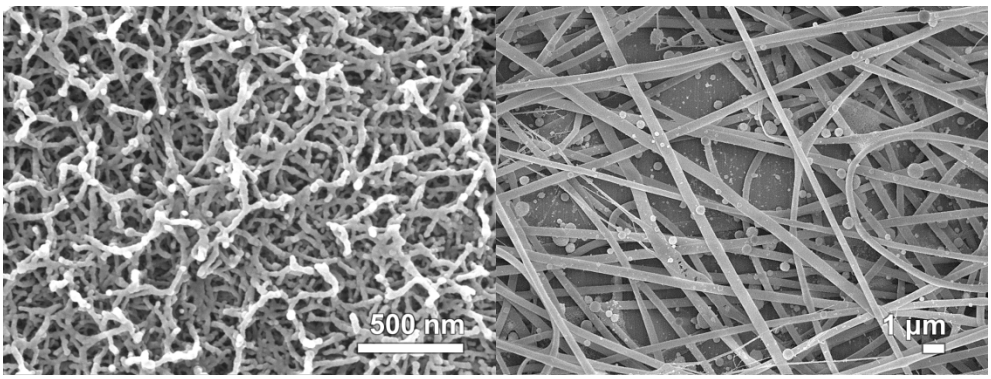

Figure 2. The morphology of TiO_2 nanowires (left) and functional nanoweb (right).

The feasibility of applying these solid matrices to MALDI-TOF mass spectrometry was demonstrated by the analysis of short peptides (leu-enkephalin) and amino acids. Amino acids are generally categorized into 4 groups; polar, non-polar, acidic and basic. Here, 2 amino acids from each group were selected as model small biomolecules. With TiO_2 nanowires and functional nanoweb matrix, the selected amino acids were clearly analyzed without matrix noise which hindered analyses of amino acids in conventional MALDI-TOF mass spectrometry. Even qualitative analyses were achieved, and the limit-of-detection (LOD) was established about 10 pM for amino acids analysis using TiO_2 nanowires and 10 pM for short peptide analysis using functional nanoweb matrix. The inter-spot relative standard deviation values were calculated to be between 10% and 30%. From the aspect of ion species,

$[M+K]^+$ ions were detected when TiO_2 nanowires were used and $[M+H]^+$ ions were detected when functional nanoweb matrices were used.

As well known, human sera include various kinds of molecules, such as albumins, globulins, antibodies and amino acids, even sugar. The presence of such proteins in a sample was known to have an influence on desorption/ionization of analytes with low molecular weights. To reduce the influence of proteins in human sera, the methanol precipitation method was used in this study. As a result, arginine could be clearly detected from methanol treated arginine-spiked sera using functional nanoweb matrices, whereas non-treated arginine-spiked sera could not.

Commercial milk also contains lot of components including fats and proteins, and showed the same phenomenon as sera. Analysis of antibiotics in milk is a big issue of public health. However, the presence of such large molecules challenges the detection of small antibiotic molecules. In this study, benzylpenicillin-spiked milk samples were analyzed by MALDI-TOF mass spectrometry using TiO_2 nanowires after simple centrifugation as a preparation step. As a result, the quantification of benzylpenicillin was achieved and LOD was established to be 0.4 ng/mL in milk samples.

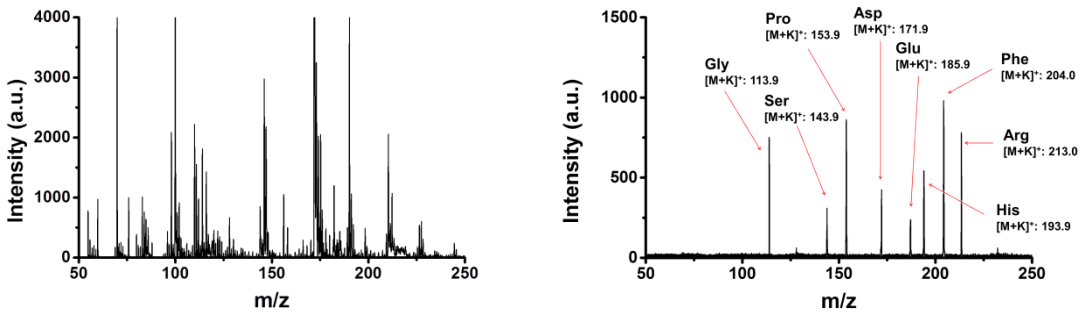

Figure 3. Amino acid analysis spectra obtained from the organic matrix (left) and solid matrix (right).