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Gold-coated Black Silicon: An Efficient Substrate for Laser Desorption Ionization Mass Spectrometry Applications

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Abstract:
Mass spectrometry imaging is a label-free analytical technique capable of molecularly characterizing biological samples, including tissues and cell lines. The organic matrices used in matrix-assisted laser desorption/ionization mass spectrometry experiments are not suitable for low weight compounds (i.e. metabolites) due to the big ions interference generated by the organic matrix. The use of solid-state substrates instead of the classical matrices is very appropriate to overcome this problem. In this study we focus on developing a novel nanostructured substrate based on black silicon fabricated by reactive ion etching and coated with sputtered gold. The hydrophobic/hydrophilic properties of this surface have been tailored in order to detect both polar and nonpolar compounds. This surface demonstrated to be very effective for analyzing imprinted mouse brain tissues and fingerprints.

Key words: black silicon, gold nanolayer, selective detection, metabolites, imprinting.

Background

Matrix- assisted laser desorption/ionization mass spectrometry (MALDI-MS) is a popular technique that has been used extensively in mass spectrometry imaging (MSI) for protein and peptide mapping over a surface like an animal or vegetal tissue. However, the main problems of this technique are the limited spatial resolution, the lack of spatial homogeneity and the difficulty to detect low weight chemical compounds due to the high quantity of matrix interference compound detected [1].

Nanostructured functional surfaces have been developed to eliminate the use of matrix and to overcome the limitations of MALDI. These matrix-free LDI-MS methods demonstrated their great value by their versatility; both liquid analysis and tissue metabolite mapping can be achieved at low concentrations and with little or no ion fragmentation [2].

We investigated the fabrication and use of novel gold-coated black silicon substrates for LDI metabolomic analysis of imprinted fingerprint and mouse brain tissue. Characterisation of structure, reflectance, and hydrophilic/hydrophobic behaviour revealed that the gold-coated black silicon substrate possesses optimal properties for LDI analysis.

Fabrication

The black silicon substrates have been prepared using reactive ion etching (RIE) processes as described in [3]. A mixture of oxygen (O_2) and sulf hexafluoride (SF_6) plasma (1:1 ratio) was used to create the needle-like structures as presented in Fig. 1. Afterwards, a gold layer of 10 nm thickness was deposited onto the black silicon by sputtering. Finally, the hydrophilic (hydrophobic) regions were obtained using O_2 (CHF_3) plasma etching with the help of a shielding mask (Fig. 2.).

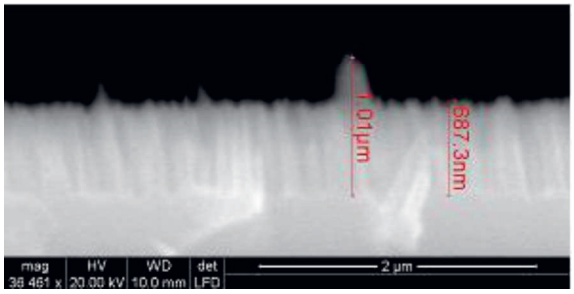

Fig. 1. Scanning Electron Microscopy cross-section image of black silicon substrate.

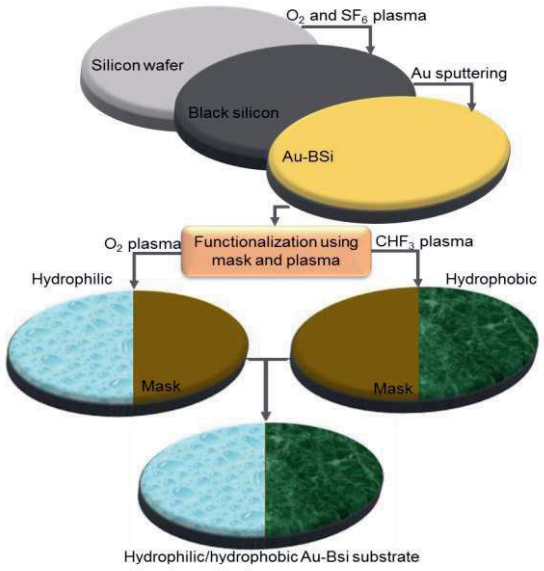

Fig. 2. Fabrication steps of selective Au-BSi substrate.

Results and Discussion

The physicochemical properties of selective black silicon substrates have been characterized. The black silicon surface presents a needle-like array structure (Fig. 1.), that absorbs visible light and gives the black aspect of silicon. Remarkably, at 337 nm (the wavelength of LDI-MS laser) the reflectance value is very low (<2%). The hydrophobic or hydrophilic property of the gold-coated black silicon surface was characterized with a contact angle (CA) measurement equipment. We fabricated the superhydrophobic surface with a mean CA of ~165º, due to CH_x terminal groups and the hydrophilic surface with a mean CA of ~45º, due to hydroxyl terminations (Fig. 3.).

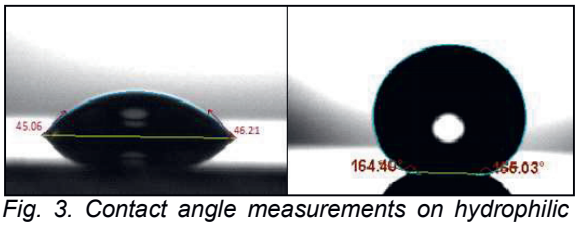

Fig. 3. Contact angle measurements on hydrophilic (left) and hydrophobic (right) Au-BSi substrates.

The gold nanolayer plays two important roles: from one part it helps the ionization and desorption processes of the analytes and from the other part, the Au ions that appear in the spectra are very helpful for the ion spectra calibration. It has been previously demonstrated that nano-sized gold layer interacts with the laser energy and with the molecules to be ionized, guiding the desorption and ionization processes.

The MS images of imprinted fingerprint and mouse brain tissue are represented in Fig.4. The gold-coated black silicon substrate has successfully distinguished between ions that have adhered to the selective areas. In the case of the fingerprint, the top half region was functionalized with hydrophilic terminations and the bottom half with hydrophobic terminations. In this case, as an example, we represented the concentration of the hydrophilic ion 284.34 Da, which is distributed unevenly throughout the selective surface, as expected. In the case of the brain tissue, we mapped the concentration of the 274.87 Da ion, which has adhered to the hydrophilic area of the surface (left side), suggesting that the ion is a hydrophilic metabolite.

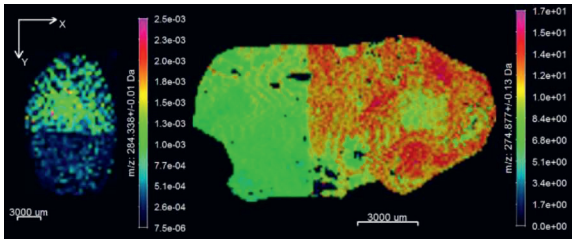

Fig. 4. Mass spectrometry imaging of fingerprint (left) and mouse brain tissue imprint (right) on the gold-coated black silicon substrate, with the color intensity scale.

Conclusion

Selective detection of hydrophobic or hydrophilic metabolite ions is possible with the help of nanostructured gold-coated black silicon functional surfaces. We differentiated between hydrophilic and hydrophobic ions of imprinted fingerprints and mouse brain tissues. The nanostructured gold-coated black silicon substrates assisted in the ionization/desorption processes, facilitating detection in the low molecular mass range without interfering background signal.

Acknowledgments

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