Lung Cancer Detection: Analysis of Trace Volatile Organic Compounds in Exhaled Breath Using Silicon Microreactor Technology

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Introduction

Studies[1-2] have shown that certain volatile organic compounds present in the breath can serve as metabolic biomarkers for the detection of lung cancer. This implies that by accurately identifying and quantifying biomarkers in exhaled breath that are specific to lung cancer, a minimally invasive, safer, and more cost-effective screening and diagnostic tool for the detection of lung cancer can be developed that has the potential to improve long-term survival rates.

Methods

Tedlar bags were spiked with known amounts of acrolein, 4-hydroxyhexenal and 4-hydroxynonenal. Exhaled breath samples from healthy controls and patients with indeterminate pulmonary nodules were collected at the Brown Cancer Center. The silicon microreactors were loaded with one of two types of ammonium salt coatings: 2-(aminooxy)-*N*,*N*,*N*-trimethylethanammonium (ATM) iodide or 2-hydrazinyl-*N*, *N*, *N*-trimethylethan-1-ammonium (HTM) iodide for reaction with aldehydes. The tedlar bags were evacuated through the microreactors and samples were eluted from the microreactors using methanol. Internal references were added to the samples and were analyzed under UHPLC-MS.

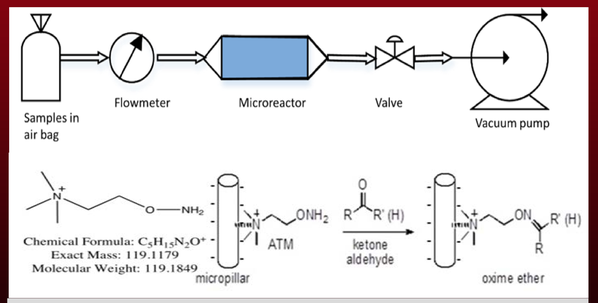

Figure 1: Schematic of standard experimental setup for capture of volatile compounds

Methods (cont.)

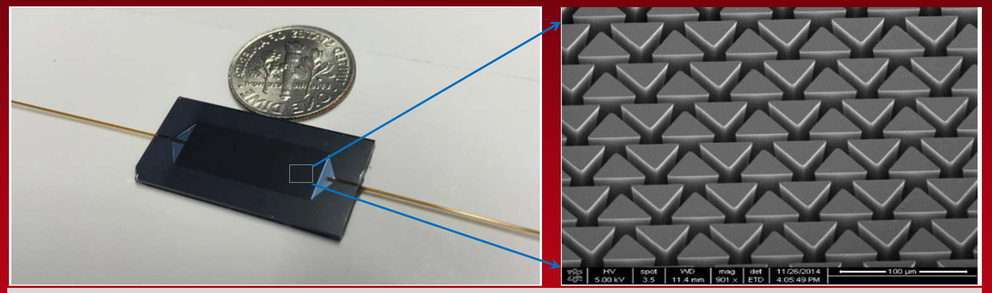

Figure 2: Silicon microreactor (left) and SEM images of triangular micropillar array (right)

Results

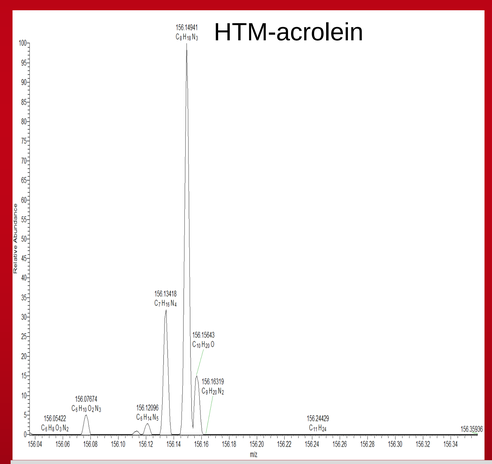

Figure 3: High resolution MS spectra of adducts of HTM reacted with unsaturated aldehydes such as acrolein and 4-HHE in exhaled breath.

Table 1: Comparative analysis of HTM (top; N=4) vs. ATM (bottom; N=34) coated silicon microreactors for capture of unsaturated aldehydes in exhaled breath of patients with pulmonary nodules

HTM	4-HHE	4-HNE	C_3H_4O	C_4H_6O	C_5H_8O
Range	0.0005-0.3681	0	0.0353-0.3413	0.0162-1.1866	0.0001-0.0052
Mean	0.12425	0	0.12695	0.3576	0.00215
Median	0.0642	0	0.0656	0.1138	0.00165

ATM	4-HHE	4-HNE	C_3H_4O	C_4H_6O	C_5H_8O
Range	0-0.012825	0-0.0111	0.0084-0.1433	0	0.00224-0.1452
Mean	0.002756	0.00329	0.04598	0	0.04288
Median	0.000983	0.00148	0.031325	0	0.02846

Results (cont.)

Table 2: Comparison of concentration of unsaturated aldehydes in patients with pulmonary nodules (top; N=2) vs. healthy controls (bottom; N=3) using HTM-coated silicon microreactors.

HTM	4-HHE	4-HNE	C_3H_4O	C_4H_6O	C_5H_8O
Range	0.1215-0.3681	0	0.0886-0.3413	0.1984-1.1866	0.0031-0.0052
Mean	0.2448	0	0.21495	0.6925	0.00415
Median	0.2448	0	0.21495	0.6925	0.00415

HTM	4-HHE	4-HNE	C_3H_4O	C_4H_6O	C_5H_8O
Range	0.182-0.214	0	0.123-0.307	0.636-1.288	0.006-0.014
Mean	0.204	0	0.199	0.860	0.014
Median	0.214	0	0.167	0.656	0.014

HTM coated microreactors show much higher capture efficiencies than ATM. As a result, HTM coated microreactors for breath analysis provided higher concentration ranges of acrolein (0.09-0.34 nmol/L), crotonaldehyde (0.20-1.19 nmol/L), pentenal (0.003-0.005 nmol/L), 4-HHE (0.12-0.36 nmol/L) in the breath of patients with pulmonary nodules. The preliminary results indicate that 4-HHE in exhaled breath samples of a small number (n=2) of patients with indeterminate pulmonary nodules show higher concentrations than that in exhaled breath samples of healthy controls (0.18-0.21 nmol/L). Also, pentenal in healthy controls is in the range of 0.006-0.014 nmol/L higher than the range of patients with pulmonary nodules.

Conclusion

The preliminary results support the hypothesis that quantitative analysis of unsaturated aldehydes in exhaled breath is promising for developing a noninvasive diagnostic tool for lung cancer screening. More patients for breath analysis are required for statistical analysis to validate specific unsaturated aldehydes as lung cancer biomarkers.

References

1. X. A. Fu, M. Li, R. J. Knipp, M. H. Nantz, M. Bousamra, Noninvasive Detection of Lung Cancer Using Exhaled Breath". *Cancer Medicine* 3, (2014) 174-181.
2. E. Schumer, J.R. Trivedi, V. van Berkel, M.C. Black, M. Li, X.A. Fu, M. Bousamra, "High sensitivity for lung cancer detection using analysis of exhaled carbonyl compounds" *J. Thoracic Cardiovascular Surgery* 150 (2015) 1517-1523.

Acknowledgements

Research supported by a grant from R25-CA-CA134283 and the School of Medicine Summer Research Scholar Program.