Tracing Individual Cancer Cells in Blood with Artificially Intelligent Nanoarray

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Introduction

There is increasing effort being put into finding and producing new technologies that will allow personalized and evidence-based medicine, including early diagnosis and accessible follow-up tools. A promising approach is the detection and monitoring of Individual Cancer Cells (ICCs). However, currently available methods suffer from low sensitivity, complicated technical procedures, low cost-effectiveness and are not suitable for continuous monitoring.

Here we show that artificially intelligent nanoarray that is based on heterogenous set of chemisensitive nanostructured films could serve as a diagnostic tool for the detection of ICCs via Volatile Organic Compounds (VOCs) emanated to the air trapped above blood samples. The potential ability to continuously record, store and pre-process these signals suggest this nanotechnology can be used for early detection of the cancer and continuous monitoring of disease progression.

Study Design

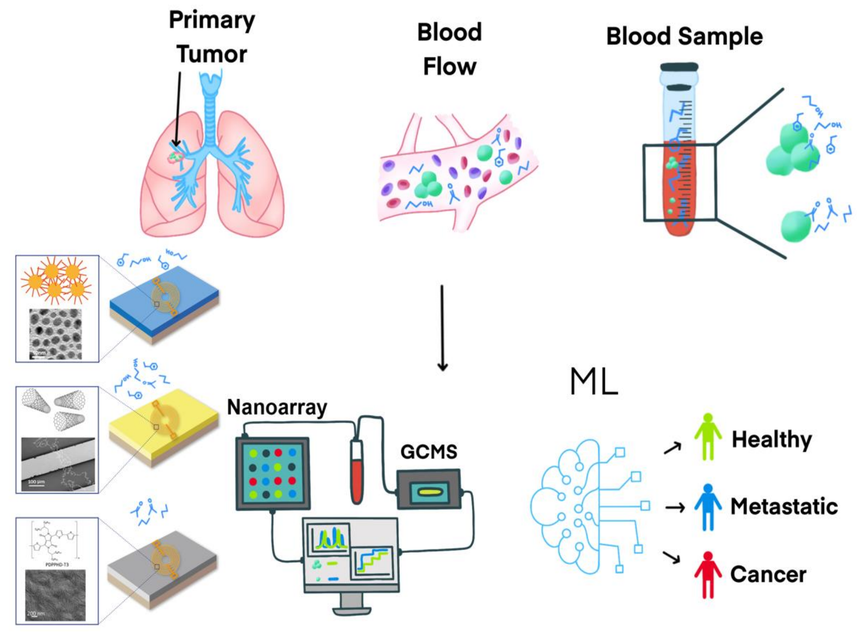

Figure 1. Illustration of the experimental approach to the hypothesis. A. Primary tumor formation or cell dissemination in the metastatic process leads to B. SCCs are releases into the blood stream and therefore to C. a change in the blood VOCs pattern. D. Exposure of the headspace to the sensors-array in combination with machine-learning methods can provide high accuracy data on the subject's health. Chemiresistors are based on organically stabilized spherical gold nanoparticles (core diameter 3-4 nm), 2D random networks of single-walled carbon nanotubes (RN-SWCNTs) capped with different organic layers, and polymeric composites. Complementary GC-MS analysis of blood headspace samples strengthens the sensor's results, and helps improve sensor sensitivity to different health states.

Results

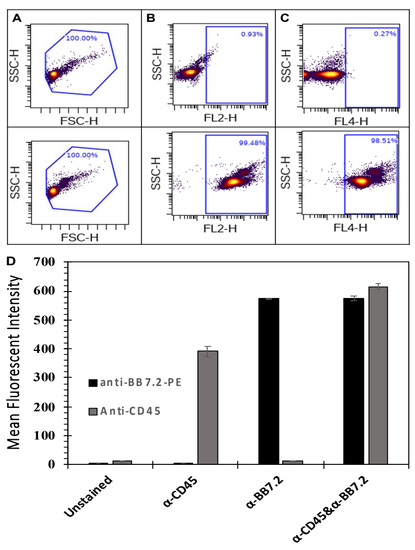

Figure 2: HLA-A2 compatibility test. Representative Dot Plots of flow cytometry data. **A.** SSC vs. FSC of PBMCs. **B.** SSC vs. FL2 signal collected from anti-BB7.2-PE stained PBMCs. **C.** SSC vs. FL4 collected from anti-CD45-Cy650 stained PBMCs. Top row, unstained cells, bottom row, antibody-stained cells. **D.** Mean fluorescent intensity of the FACS results. SSC - Side scatter; FSC - Forward scatter; FL- Fluorescent; PBMCs- Peripheral blood mononuclear cells.

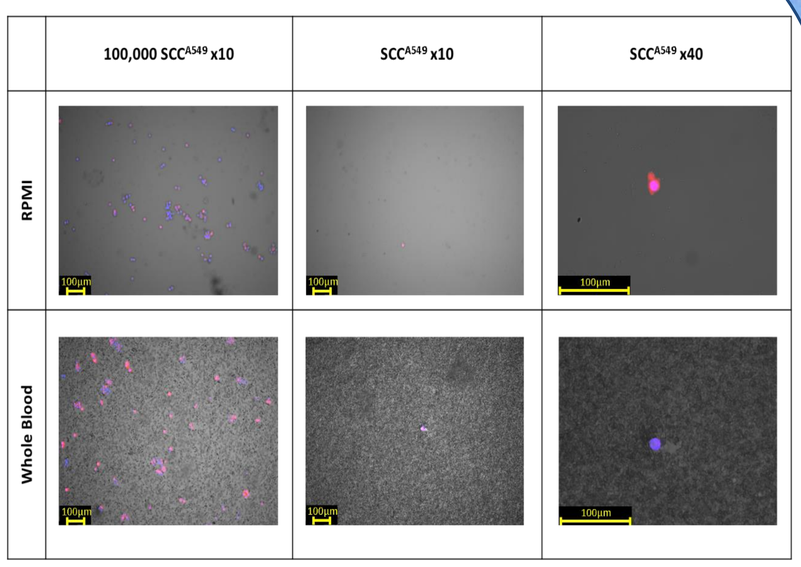

Figure 3. SCC^{A549} in blood. Bulk and SCC^{A549} were spiked in whole blood or RPMI. Samples were examined by immunofluorescence; SCC^{A549} were labeled with DiD membrane dye (red), fluorescent nuclear Hoechst staining (blue) and visualized by fluorescent microscopy. Images are from merged fluorescent channels and bright-field channel to visualize hematopoietic cells. Scale bar: 100 μm.

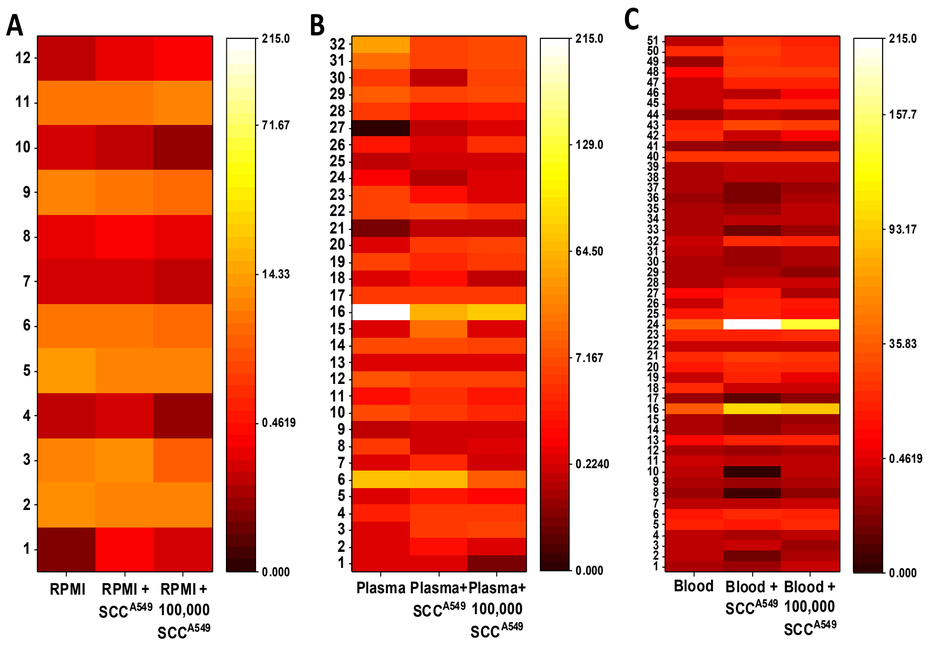

Figure 4. Colormap of SCC^{A549}-related VOC profile. Colormap shows the VOC profile (red - low, white - high, abundance of VOC as found in GC-MS) of SCC^{A549} spiked in **A.** RPMI growth medium; **B.** Human plasma; **C.** Human whole blood. Only VOCs that showed significant difference between the groups are shown.

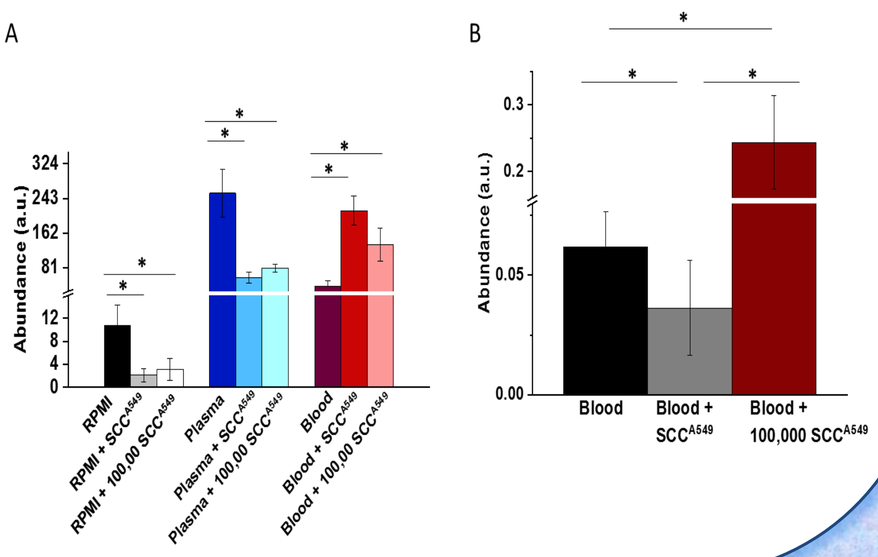

Figure 5. A. Statistically significant 2-ethyl-1-hexanol in the headspace of medium, plasma and blood. B. Statistically significant 2-methyl-2-propanol in blood spiked with A549 lung cancer cell lines, detected by the GC-MS.*p<0.05. **NOTE:** a.u. stands for arbitrary units of GC-MS peaks AUC.

Nannoarray Discrimination

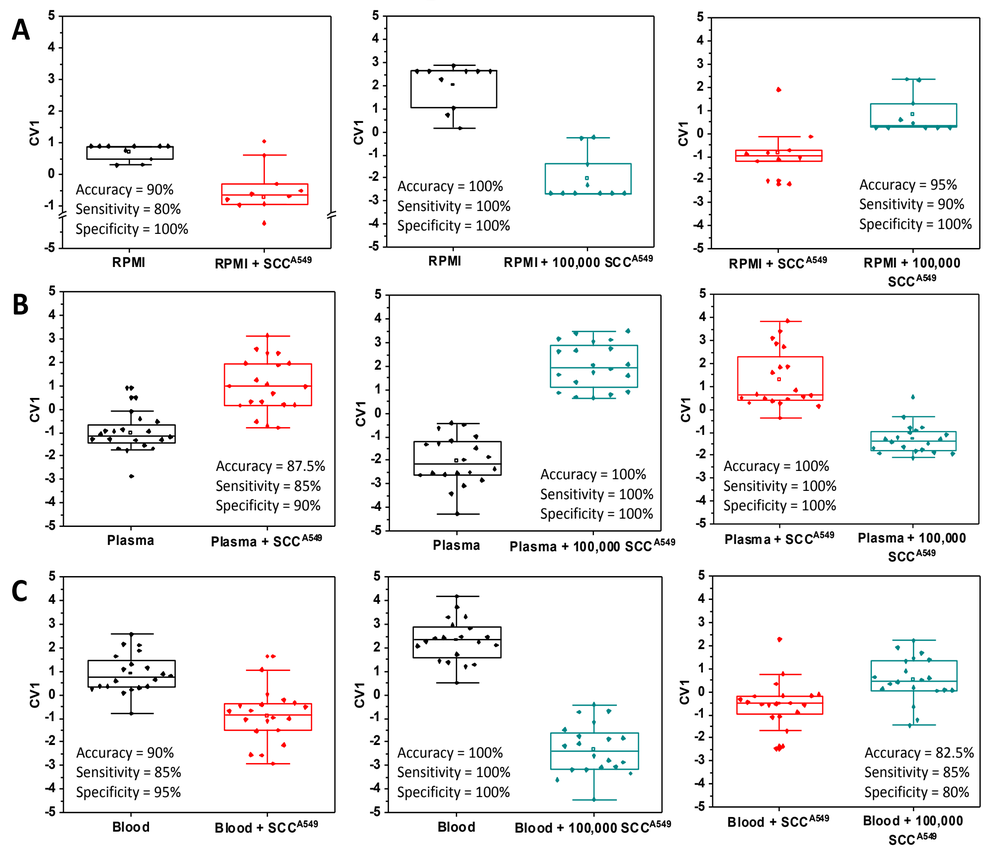

Figure 6. DFA plots of the first canonical variable (CV1) calculated from the response of GNP sensors to the headspace of (A) Medium, (B) Plasma and (C) Blood spiked with SCC^{A549} or 100,000 SCC^{A549} cells.

Discussion & conclusion

We have shown that an interaction between SCC^{A549}-specific VOCs in the blood samples and a miniaturized artificially intelligent nanoarray of cross-reactive, highly sensitive nanomaterial-based chemical sensors can serve as a diagnostics tool. We were able to detect SCC^{A549} in blood samples with >90% accuracy, >85% sensitivity and >95% specificity with a 2 features-based DFA model. Discrimination accuracy reached 100% when comparing between naïve blood samples and 100,000 SCC^{A549} and were high in comparing blood samples spiked with SCC^{A549} and with 100,000 SCC^{A549}, indicating a potential amount and/or cell communication effect. The GC-MS evidence on the cross-talk between the SCC and the blood gave an indirect indication of the existence of SCC^{A549} in the blood, even before actual SCC^{A549} capturing. GC-MS displayed a unique pattern of VOCs, depending on the presence and number of SCC^{A549} . The ability to record, store and pre-process the signals by integrated miniature on-chip electronics, and to transfer them via the internet to an external server that holds previous measurements and other clinical data of the same patient could increase the chances that this nanotechnology can be used for continuous monitoring of cancer progression.