Toward Mitigating High Risks for Soldiers: A Salivary Diagnostic Tool for Detection of Multi Organ Injury

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Abstract

InnoSense LLC (ISL) is developing **Nano-Mos™, a handheld rugged carbon nanotube-based point-of-care (POC) diagnostic device to detect salivary biomarkers indicative of multiorgan injury.** Currently, there are no POC devices which detect multiorgan injury due to toxic chemical exposure. The diagnostic platform is compatible with multiple sample matrices (serum, cerebrospinal fluid (CSF), urine, other bodily fluids) and can target specific biomarkers indicative of cancer, diabetes, Alzheimer's, cardiovascular and emerging infectious diseases. The envisioned Nano-Mos will:

- Produce results with minimal lag time in a POC setting or doctor's clinic.
- Quantitatively determine systemic toxicity using clinically validated biomarkers in saliva to indicate organ injury due to chemical/xenobiotic exposures.
- Detect multiple biomarkers indicative of overall stress, inflammation and brain and heart injury and xenobiotic exposure in a single assay.
- Sensitively and selectively detect target biomarkers in femtomolar (fM) levels in saliva.

To date, ISL has demonstrated successful fabrication of Nano-Mos sensors and functionalized them with antibodies (Abs) for relevant biomarkers. The sensors:

- Detected fM levels of salivary cortisol in stressed human saliva samples within 15 minutes.
- Detected fM levels of C-reactive protein (CRP-biomarker for overall inflammation) in human saliva samples demonstrating a wide dynamic range of biomarker targets, from fM to nanomolar (nM).
- Produced an unambiguous response for the target cortisol and CRP biomarkers with minimal cross-reactivity.
- Detected S100B (biomarker for brain injury) in spiked saliva samples.

The **results demonstrated the effectiveness and versatility of the nanomaterial-based detection platform**. Additional work, in collaboration with Oasis Diagnostics, will transition Nano-Mos into a field-deployable prototype assay with a fast response requiring minimal user training.

Background

Nano-Mos is being developed with DoD funding primarily for defense personnel and soldiers. They are at an unprecedented risk of exposure to chemical warfare agents and subsequent adverse health outcomes. Developing assays to predict behavior and outcome of exposure to more than 84,000 available toxic chemicals or its combinations is unrealistic. A feasible alternative is to use clinically validated biomarkers indicative of multiorgan injury due to exposure to harmful chemicals. **Salivary biomarkers are considered attractive targets since saliva sampling is a non-invasive procedure.** This is ideal for testing in military field combat scenarios.
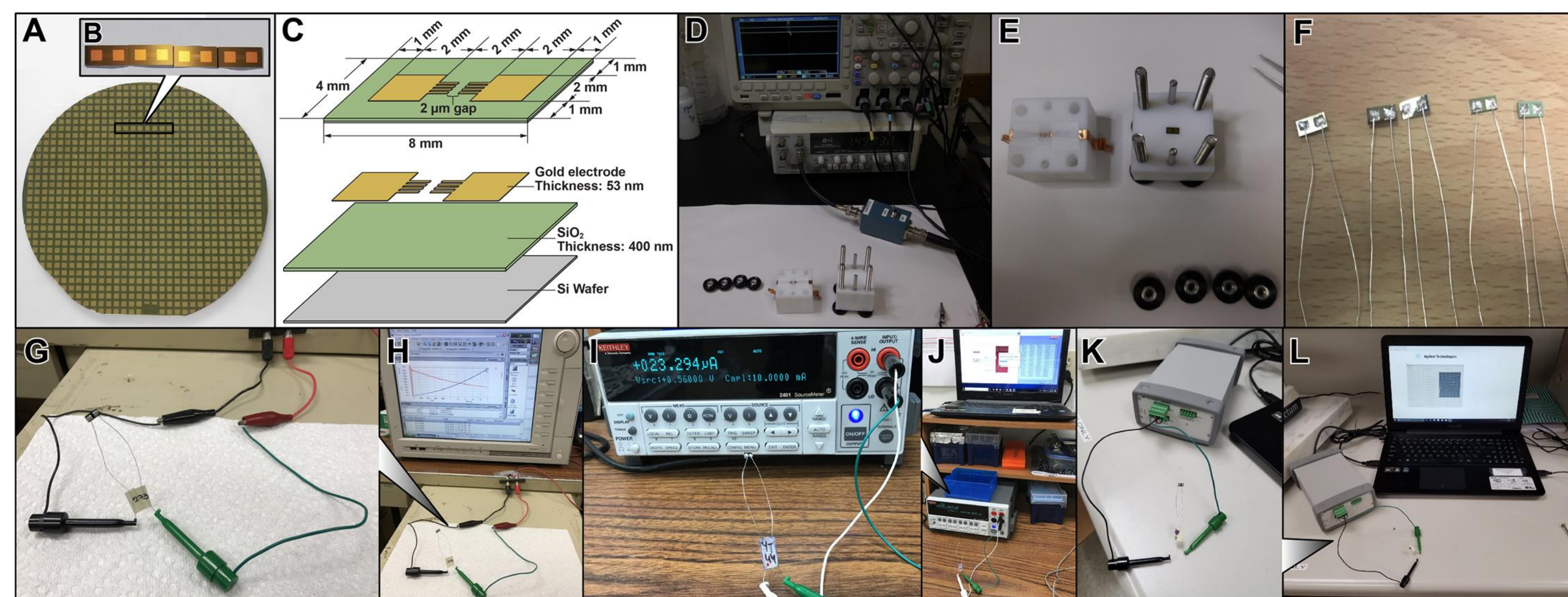
ISL's Solution

To develop a nanomaterial-based multiplexed POC diagnostic to detect multiorgan injury from saliva.
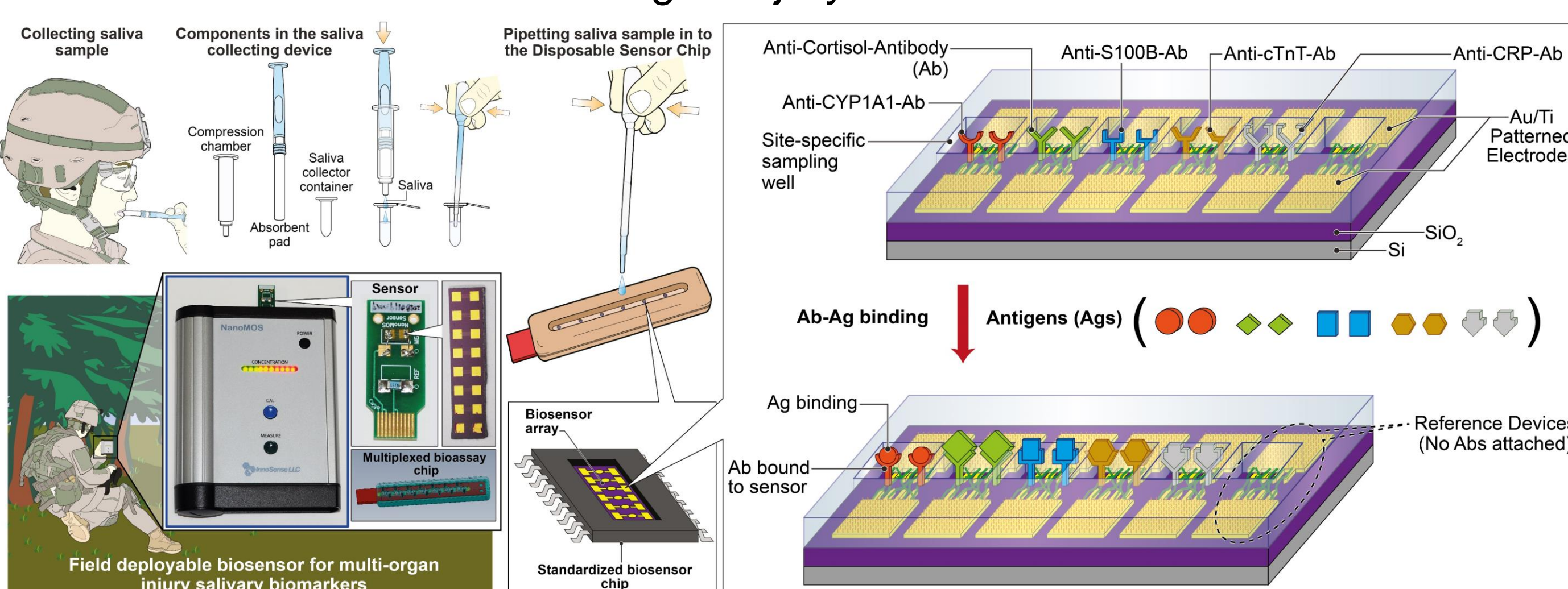
**Envisioned Nano-Mos for rapid detection of multiorgan injury in a single assay from saliva.
(Inset) Nano-Mos prototype (ver1)**

Device Fabrication

(A) A pre-diced silicon wafer from which the microelectrodes are cut, **(B)** Magnified to show the microelectronic devices (MEDs), **(C)** Dimensions of the MED, **(D)** Nanomaterial deposition station **(E)** Device in mold for deposition, **(F)** Soldered devices, **(G-H)** Agilent Semiconductor Device Analyzer for testing devices, **(I-J)** Keithley sourcemeter setup for testing devices, and **(K-L)** Keysight sourcemeter setup for testing devices.

Nanomaterials Characterization
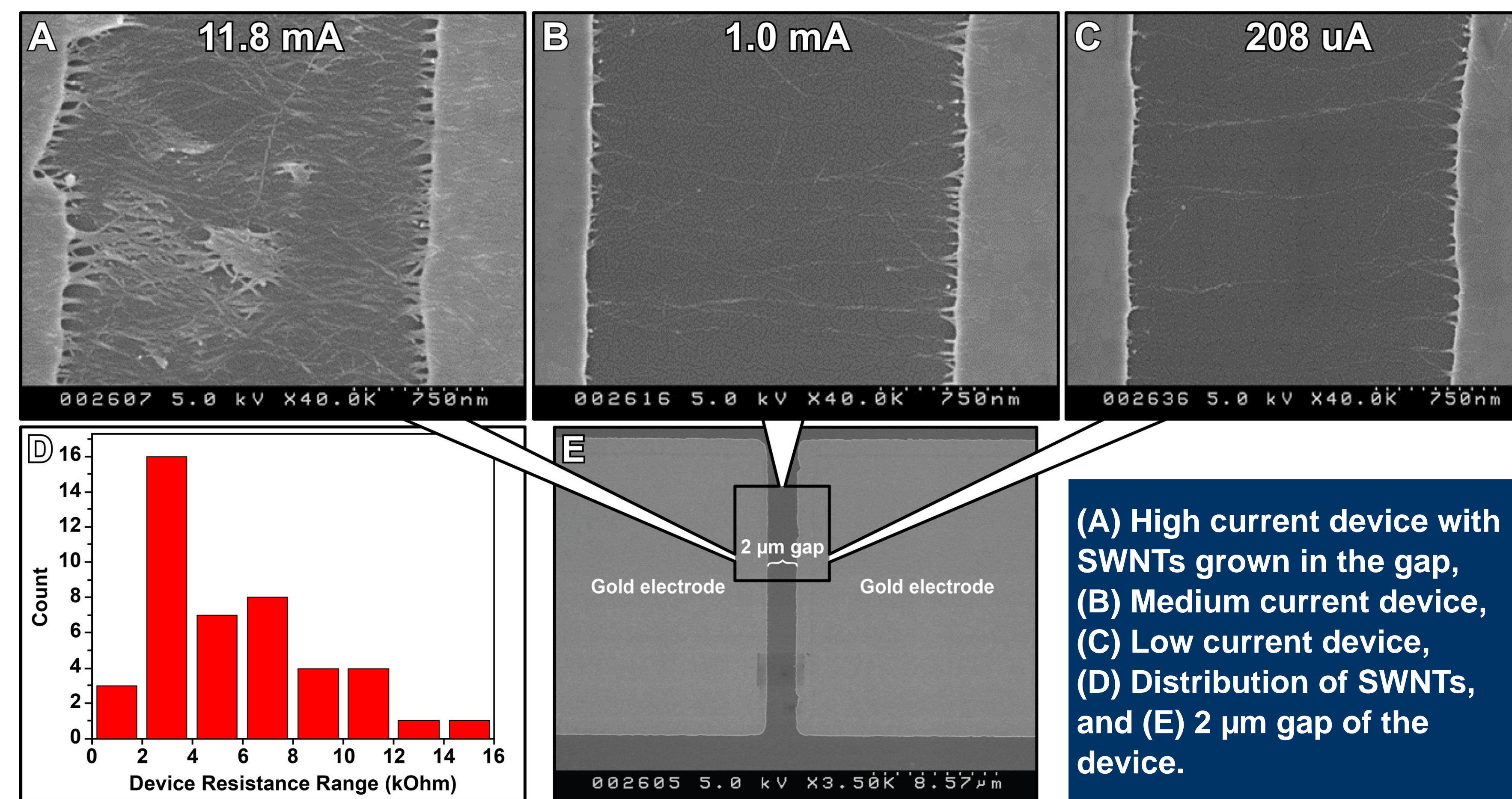
(A) High current device with SWNTs grown in the gap, **(B)** Medium current device, **(C)** Low current device, **(D)** Distribution of SWNTs, and **(E)** 2 µm gap of the device.

Sensor Response

Bar graphs summarize the average response of multiple Nano-Mos devices to different concentrations of Cortisol, CRP and S100B in phosphate buffered saline (PBS) and human saliva samples.
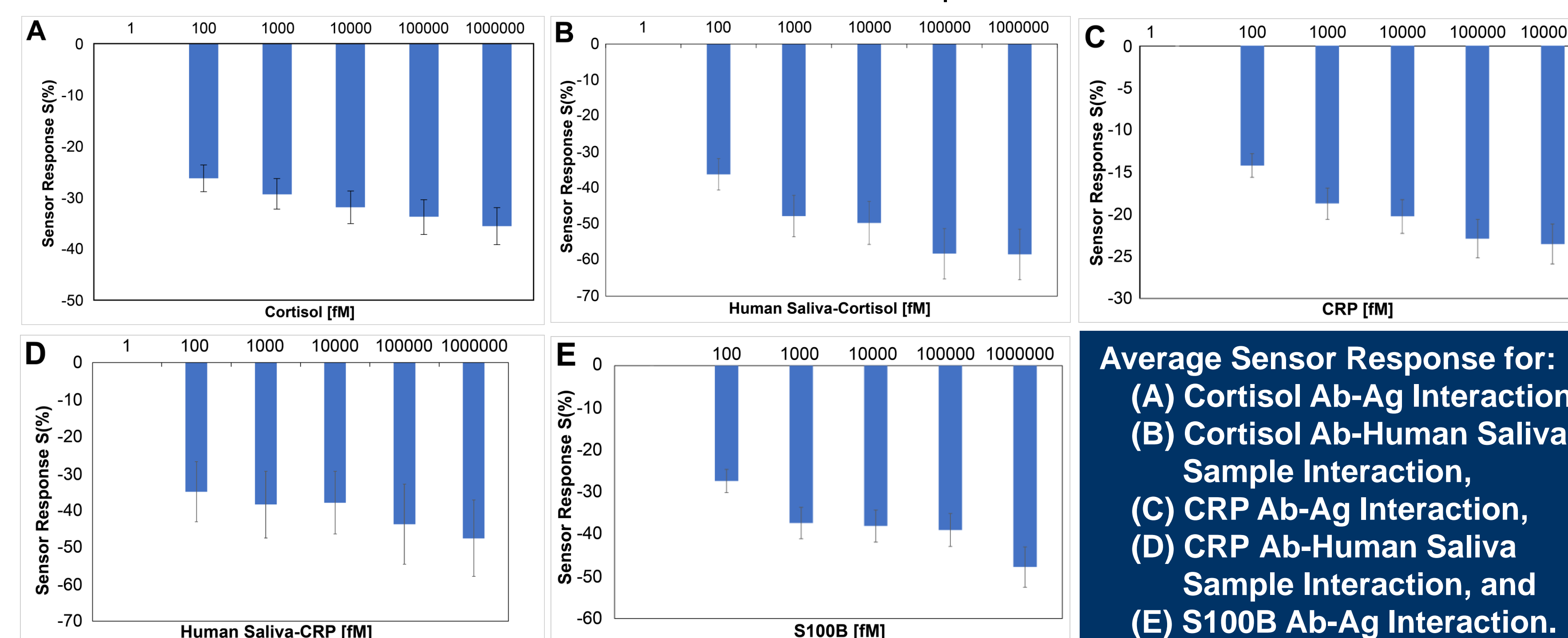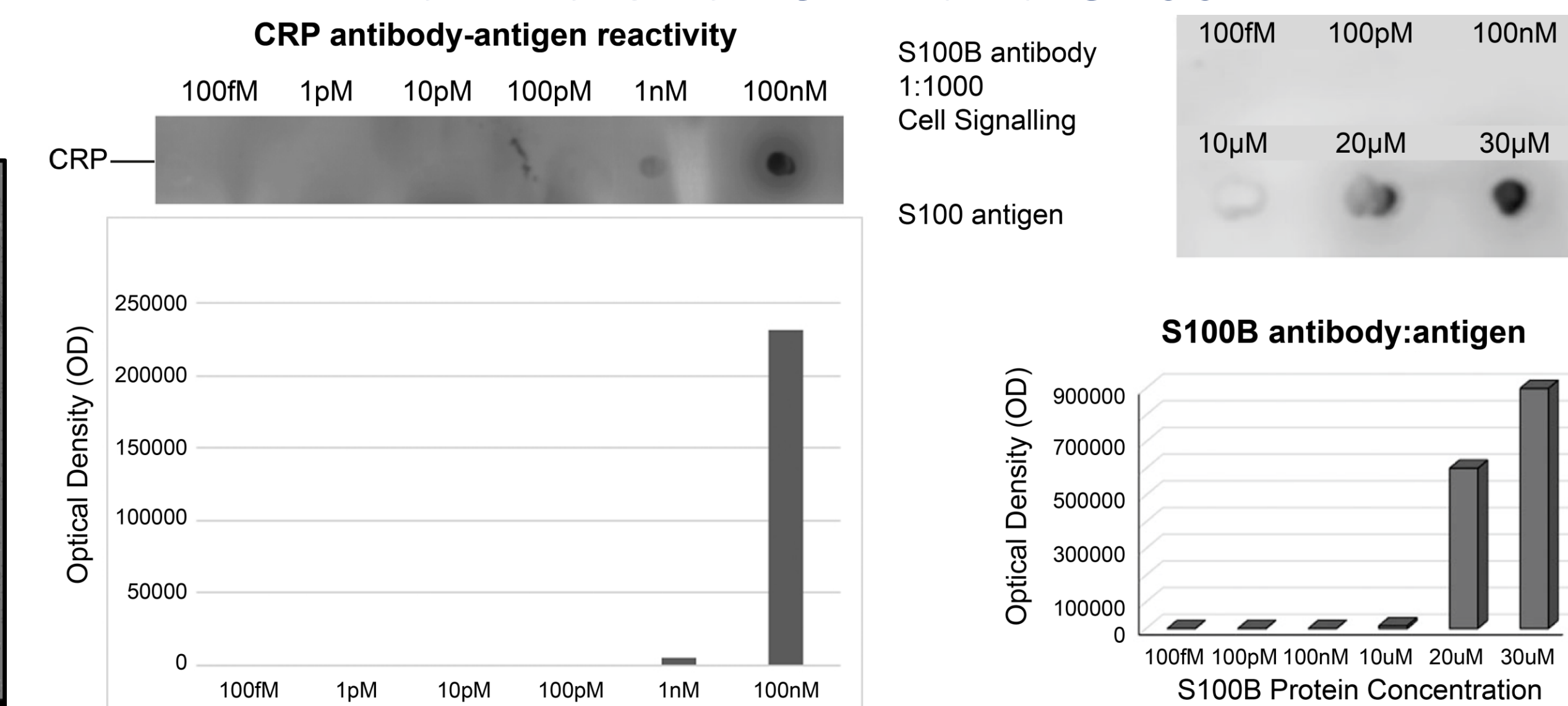
Average Sensor Response for:
(A) Cortisol Ab-Ag Interaction
(B) Cortisol Ab-Human Saliva Sample Interaction,
(C) CRP Ab-Ag Interaction,
(D) CRP Ab-Human Saliva Sample Interaction, and
(E) S100B Ab-Ag Interaction.

Comparison Showing Interaction and Controls for Cortisol Ab-Ag Pair
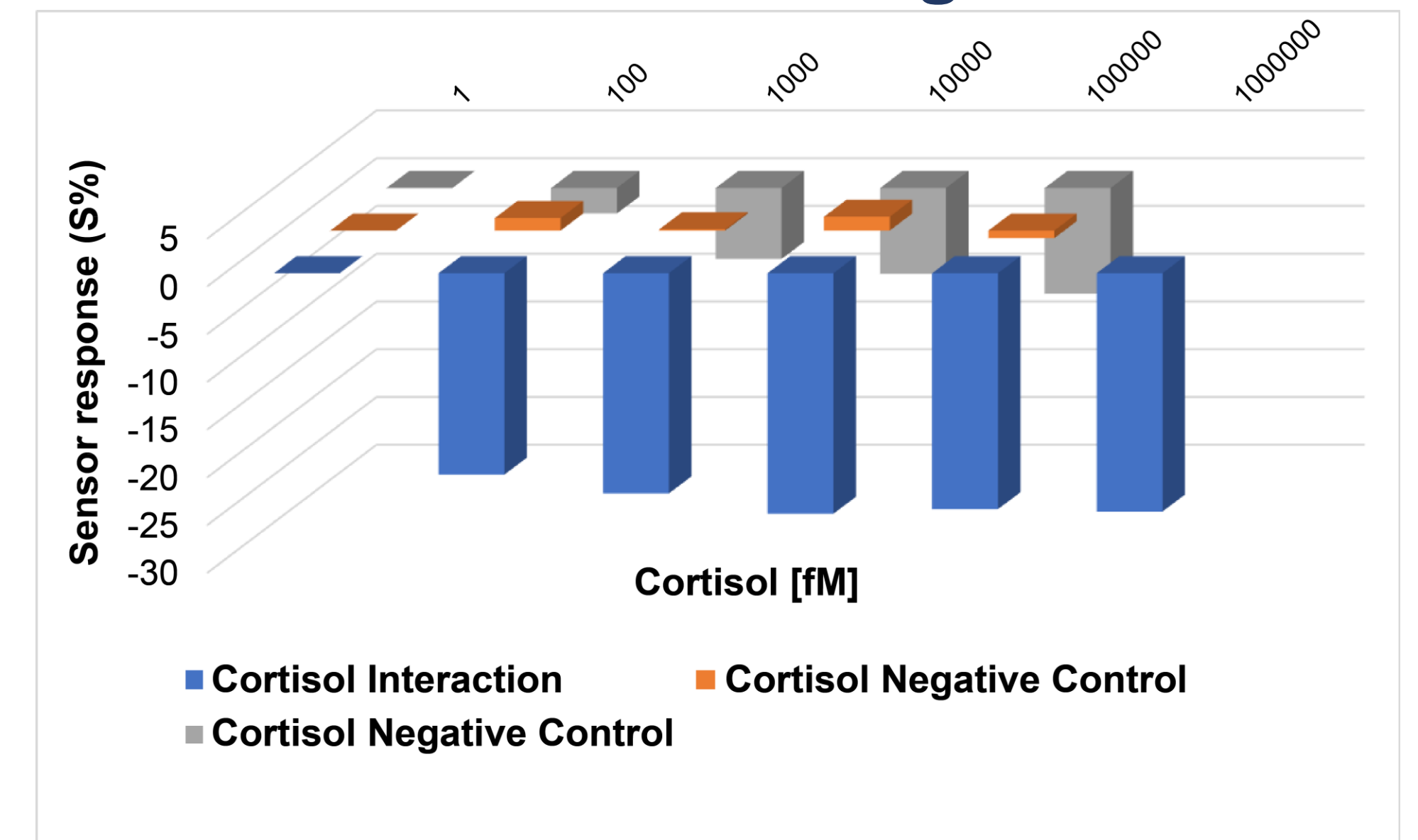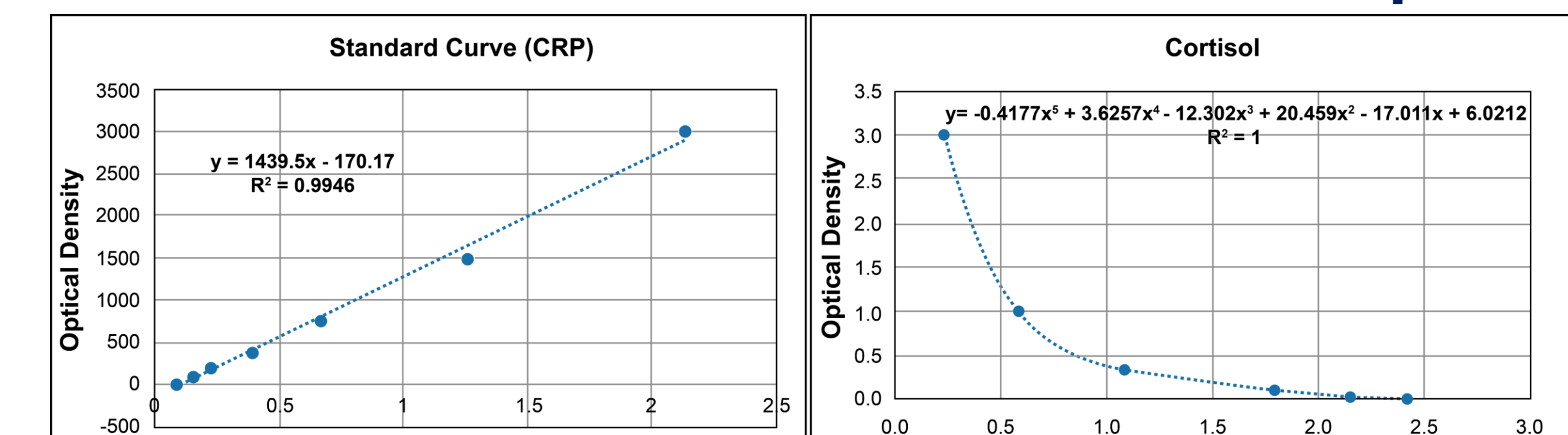
Bar graph summarizes the average response of multiple Nano-Mos devices (n = 5-6) to varying concentrations of Cortisol-Ag compared to minimal response for positive control (No Ag) and negative control (No Ab).

Cortisol Interaction — Cortisol Negative Control — Cortisol Negative Control

Dot Blots for CRP and S100B

CRP antibody-antigen reactivity

S100B antibody 1:1000 Cell Signalling

S100B antibody:antigen

ELISA for CRP and Cortisol Saliva Samples

Standard Curve (CRP)
$y = 1439.5x - 170.17$
$R^2 = 0.9946$

Cortisol
$y = -0.4177x^4 + 3.6257x^3 - 12.302x^2 + 20.459x^2 - 17.011x + 6.0212$
$R^2 = 1$

Conclusions

- Nano-Mos fabrication with ~85% reproducibility.
- Quantitative detection of fM levels of salivary cortisol and CRP.
- Dynamic range of fM to nM established for cortisol and CRP.
- Specific Ab-Ag interaction confirmed by dot blots.
- Saliva samples validated by ELISA.
- Nano-Mos prototype (ver1) constructed.

About ISL

- Founded in 2002
- Located in Torrance, CA
- 28 full-time employees
- About 11,400 square ft. of fully equipped R&D space