SAND2022-2375C

Transdermal Microneedle Sensors to Monitor Human Health and Performance

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Sandia National Laboratories

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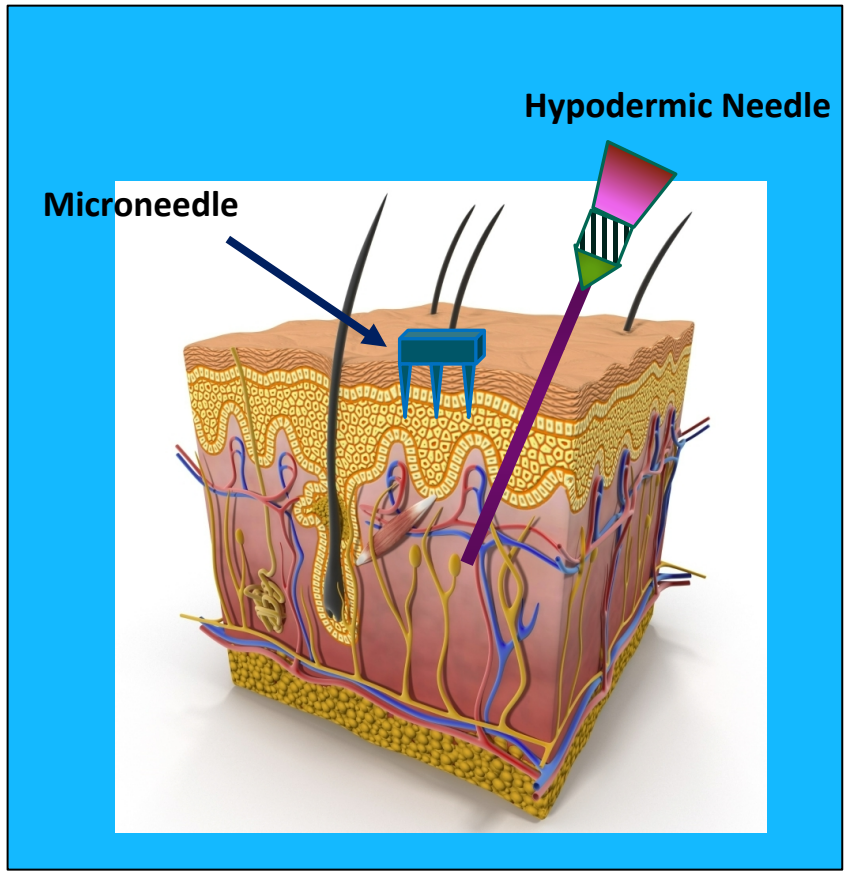
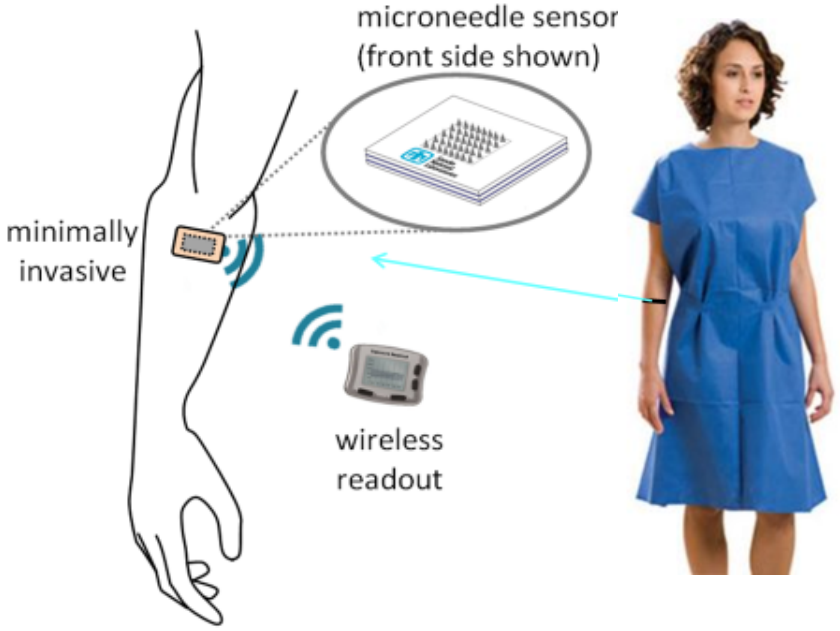
U.S. DEPARTMENT OF ENERGY

National Nuclear Security Administration

Biosciences and National Security

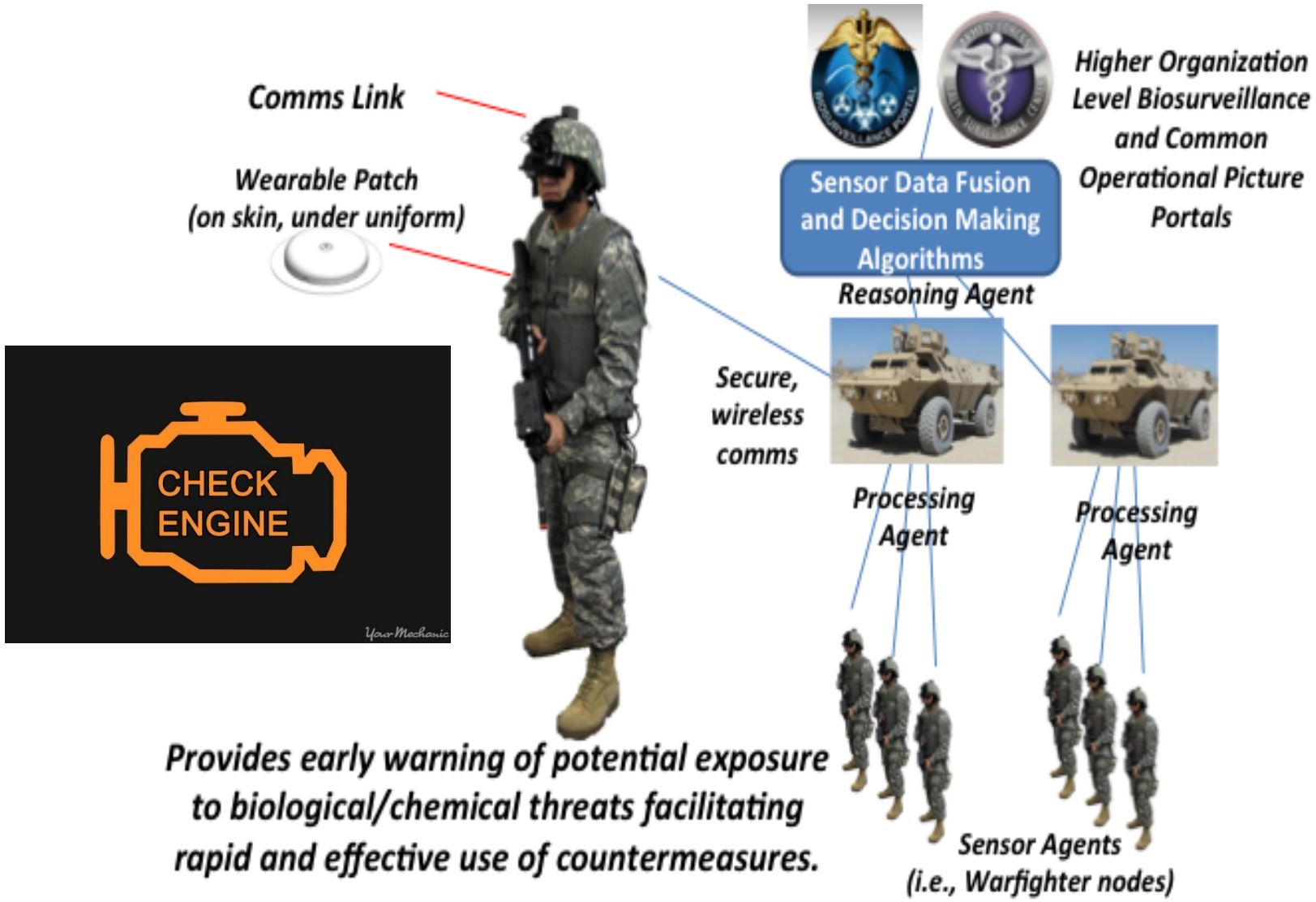
Microneedles and Health Monitoring





DTRA1002717470, Project 189920- PM Nate Adams

Bringing the diagnostic lab to the field!



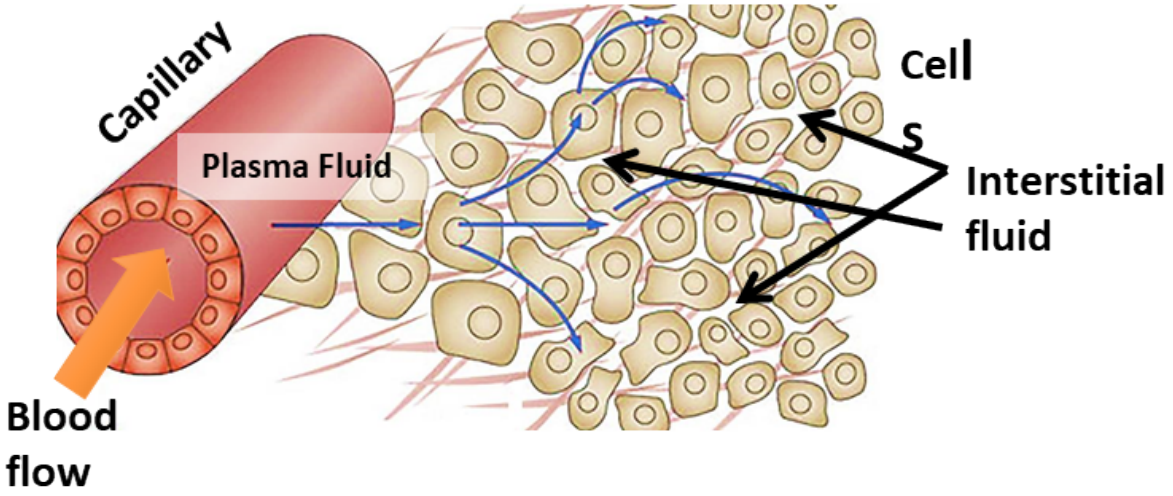
Provides early warning of potential exposure to biological/chemical threats facilitating rapid and effective use of countermeasures.

Models of Capillary Exchange of Plasma

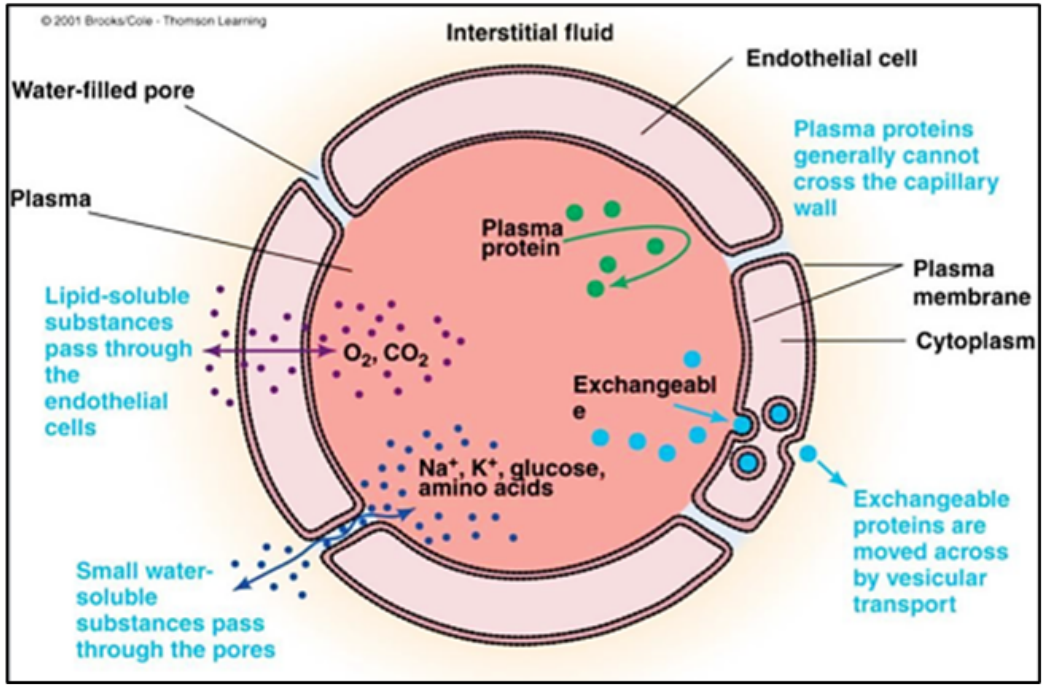
- Drinker and Field's Model 1931

Osmotic gradient and resistance changes in capillaries allow exchange of peptides and small proteins from the plasma to the interstitial fluid

- The fluid from plasma that fills the interstitial space between cells and surrounds them.
  - Continual exchange of nutrients for cells



Blood Volume = 5 L
Interstitial Volume = 12 L

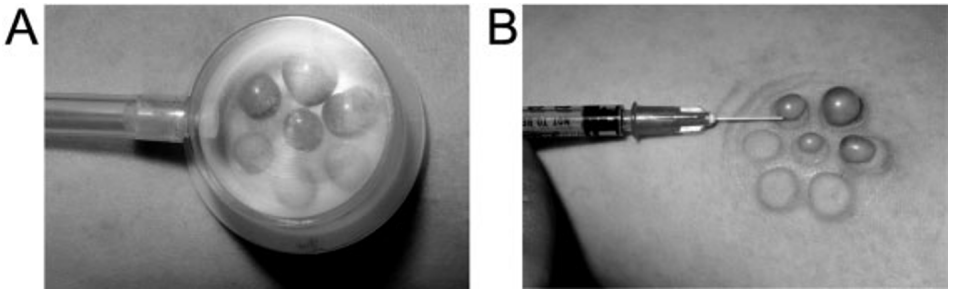


Drinker, C. K., and Field, M. E., *American Journal of Physiology*, 1931, xcvii, 32.

Interstitial Fluid Sampling Methods

Blister

Cortese TA, Sams WM, Sluzberger MB. Studies on blisters produced
by friction. II. The blister fluid. J Investig Dermatol **1968**; 50:47–53.
Vermeer BJ, Reman FC, van Gent CM. The determination of lipids and
proteins in suction blister fluid. J Investig Dermatol **1979**;73:303–5.



Microdialysis

Lonnroth P, Jansson PA, Smith U. A microdialysis method allowing
characterization of intercellular water space in humans. Am J Physiol **1987**;253:E228–31.
Jansson PA, Fowelin J, Smith U, Lonnroth P. Characterization by microdialysis of intercellular glucose level in subcutaneous tissue
in humans. Am J Physiol **1988**;255:E218–20.

Bolinder J, Hagstrom E, Ungerstedt U, Arner P. Microdialysis of subcutaneous adipose tissue in vivo for continuous glucose monitoring in man. Scand J Clin Lab Investig **1989**;49:465–71.

Effussion

Kayashima S, Tsunenori A, Kikuchi M, Nagata N, Ito N, Kuriyama T, et al. Suction effusion fluid from skin and constituent analysis: new candidate for interstitial fluid. Am J Physiol **1992**;263:H1623–7.
Janle-Swain E, van Vleet JF, Ash SR. Use of a capillary filtrate collector for monitoring glucose in diabetics. Trans Am Soc Artif Organs **1987**;33:336–40.

Wick

Aukland K, Fadnes HO. Protein concentration of interstitial fluid collected from rat skin by a wick method. Acta Physiol Scand **1973**;88:350–8.
Kramer GC, Sibley L, Aukland K, Renkin EM. Wick sampling of interstitial fluid in rat skin: further analysis and modifications of the method. Microvasc Res **1986**;32:39–49.

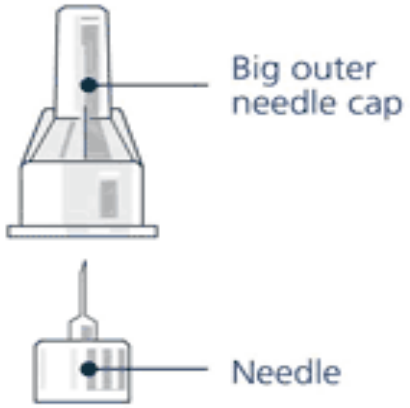
Electric Fields

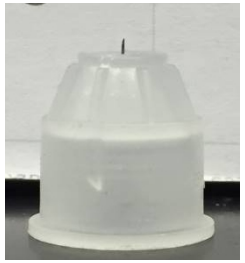
S. K. Li, A. Ghanem, K. D. Peck, and W. I. Higuchi, "Characterization
of the transport pathways induced during low to moderate voltage iontophoresis in human epidermal membrane," *J. Pharm. Sci.*, vol. 87, no. 1, pp. 40–48, Jan. **1998**.

Ultrasound

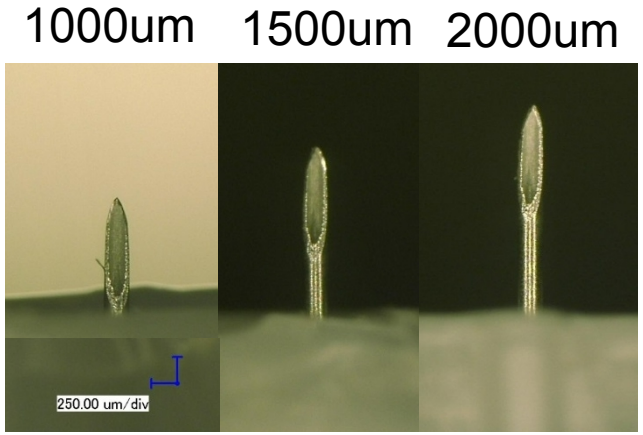
S. Mitragotri, D. Blankschtein, and R. Langer, "Transdermal drug delivery using low-frequency sonophoresis," *Pharm. Res.*, vol. 13, no. 3, pp. 411– 420, Mar. **1996**.
J. Gupta and M. R. Prausnitz, "Recovery of skin barrier properties after sonication in human subjects," *Ultrasound Med. Biol.*, vol. 35, no. 8, pp. 1405–1408, Aug. **2009**.

Extraction of ISF using novel microneedle device

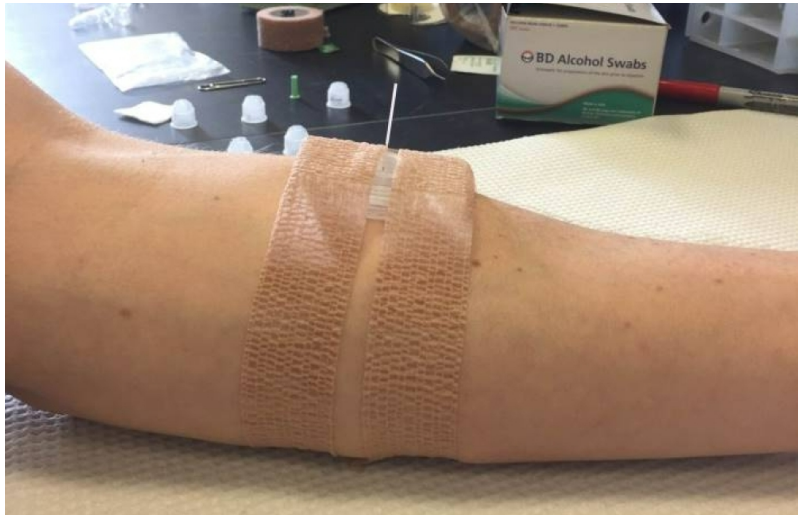


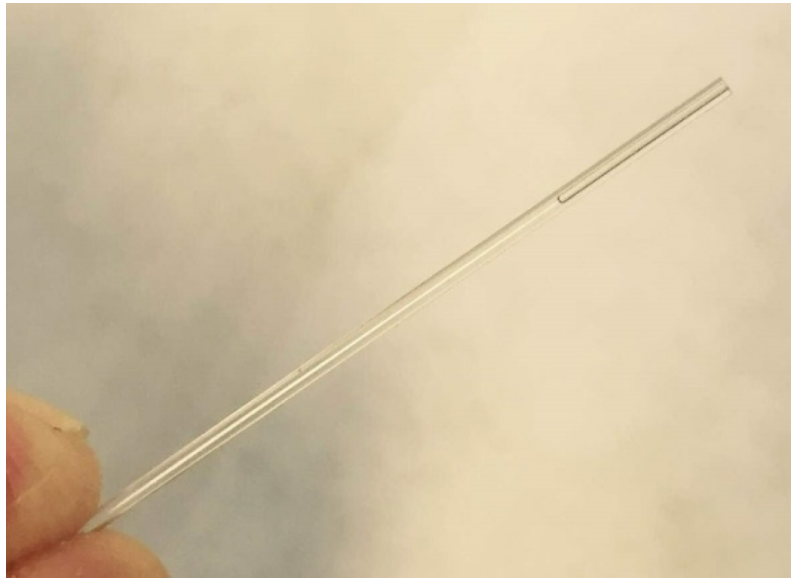


Assembled device with capillary for fluid storage



Varying PEN needle lengths cut at different lengths

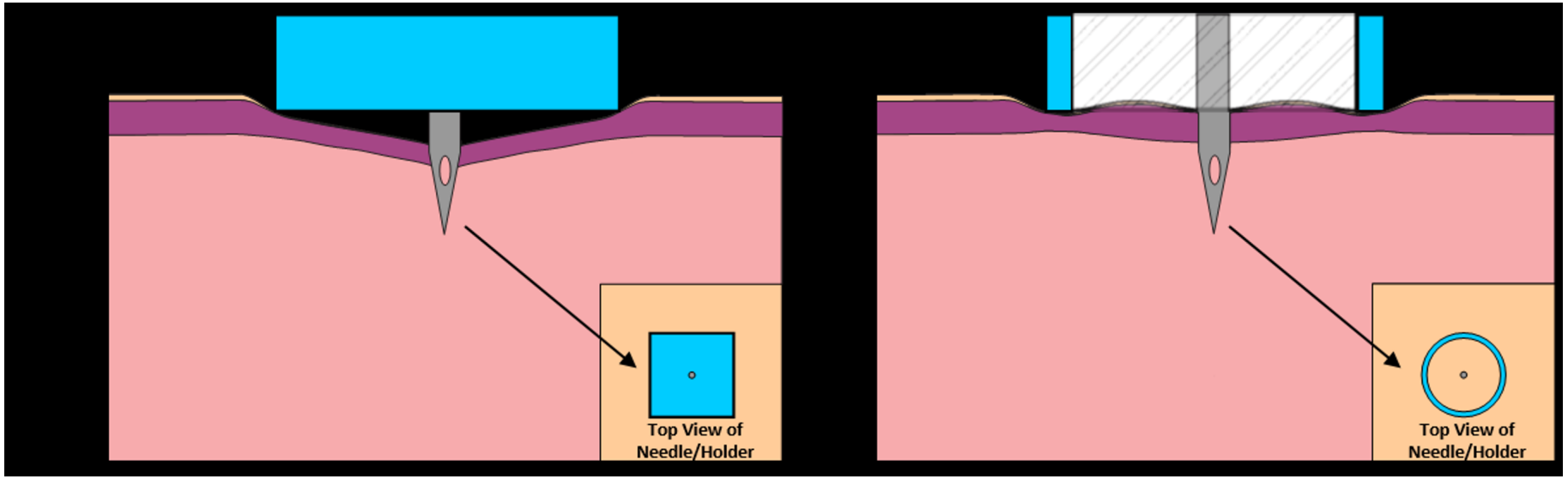




Extracted Fluid

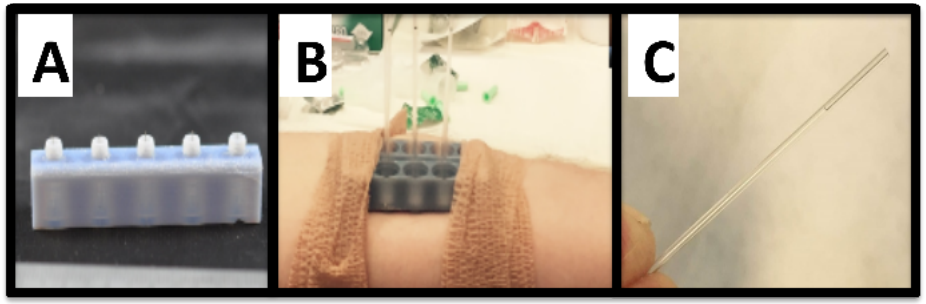
Extraction of ISF using novel microneedle device

Comm. Bio. (2018) 1:173 | DOI: 10.1038/s42003-018-0170-z



In plane Needles

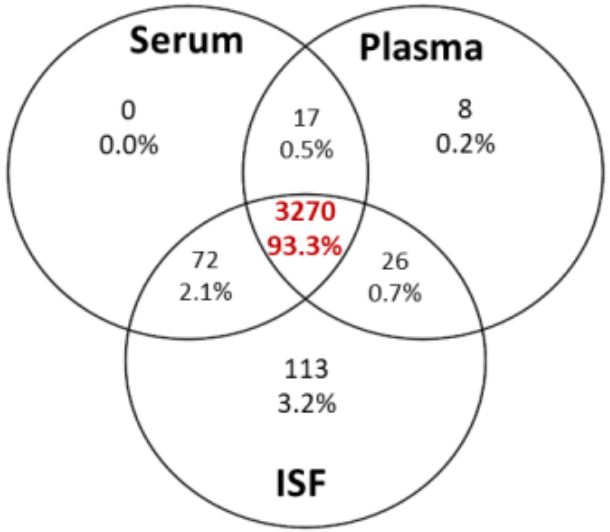
Spaced Needles



A) 3D printed microneedle holder with five needles of 1500 µm lengths. B) Two 3D microneedle holders adhered to a human subject for ISF extraction and collection in glass capillary tubes. C) Glass capillary collection tube with ISF collected from a human subject.

Proteomic Analysis of ISF

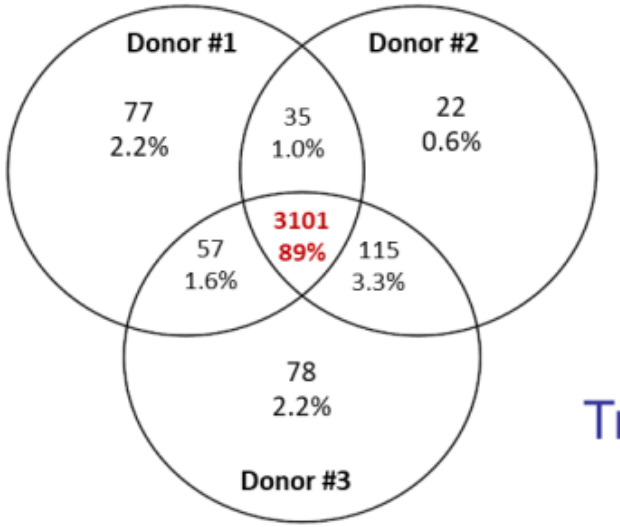
J. Proteome Res. 2018, 17, 479−485



- Of 3506 proteins identified in human subject, 3270 proteins were common between plasma, serum and ISF

- 93% match in protein content

- Indicates ISF can be used as a blood proxy!

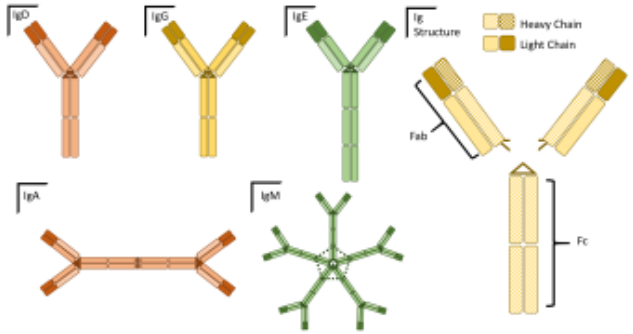


89% of these proteins were consistently detected in three examined human individuals.

Trevor Glaros, Bao Tran, Nicole Rosenzweig
ECBC

Immunoglobins

The five classes of immunoglobulins and generalized structure



We found similar profiles in IgG, IgA and IgMs in ISF to serum and plasma using our extraction method

J. Prot. Res. 2019 (just accepted)
Collaboration with ECBC

Identified Immunoglobulins from Extracted ISF vs Paired Plasma and Serum

Immunoglobulin	Description	Accession	% Coverage	# PSMs	# Unique Peptides	Serum/ISF	Plasma/ISF
IgA1	heavy constant alpha 1	P01876	63	369	8	1.3	1.3
IgA2	alpha-2 heavy chain	P0DOX2	57	230	8	1.0	1.0
IgA2	heavy constant alpha 2 (Fragment)	A0A0G2JMB2	70	256	1	1.1	1.2
IgD	delta heavy chain	P0DOX3	40	32	3	1.0	0.9
IgE	epsilon heavy chain	P0DOX4	4	2	2	1.0	1.3
IgG1	gamma-1 heavy chain	P0DOX5	63	1026	15	0.8	0.9
IgG2	heavy constant gamma 2	P01859	60	828	7	0.8	0.8
IgG3	heavy constant gamma 3	P01860	66	696	10	0.9	0.9
IgG4	heavy constant gamma 4 (Fragment)	A0A286YFJ8	43	593	5	1.0	1.0
IgM	heavy constant mu	P01871	72	357	9	1.5	1.5
IgM	mu heavy chain	P0DOX6	44	251	2	1.6	1.5

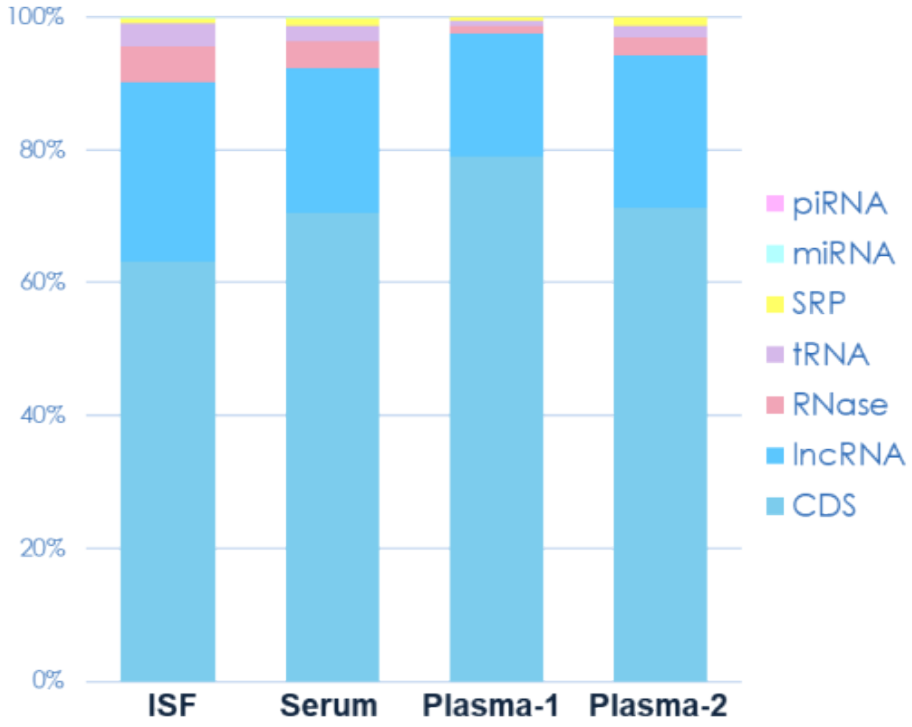
Transcriptomics

Overview of RNA species detected in ISF, serum, and plasma samples

A.

	ISF		Serum		Plasma-1		Plasma-2	
Mapped Reads (M)	1.9	(1.7 - 4.8)	2.2	(1.3 - 5.6)	4.9	(2.3 - 6.8)	3.7	(2.5 - 4.8)
CDS (%)	63.2	(22.3 - 84.2)	70.6	(41.1 - 87.9)	78.9	(71.5 - 88.8)	71.4	(62.1 - 76.9)
lncRNA (%)	26.9	(7.4 - 74.4)	21.8	(7.1 - 52.3)	18.6	(7.2 - 26.6)	22.9	(18.8 - 28.0)
RNase (%)	5.4	(1.4 - 11.3)	3.9	(0.4 - 5.3)	1.2	(0.4 - 2.6)	2.6	(0.5 - 5.1)
tRNA (%)	3.5	(1.3 - 5.7)	2.4	(1.4 - 4.2)	0.7	(0.4 - 1.4)	1.9	(0.7 - 3.0)
SRP (%)	0.9	(0.2 - 2.6)	1.2	(0.2 - 3.2)	0.5	(0.3 - 0.7)	1.2	(0.6 - 1.7)
miRNA (%)	0.05	(0.01 - 0.09)	0.08	(0.04 - 0.13)	0.03	(0.01 - 0.05)	0.05	(0.01 - 0.10)
piRNA (%)	0.04	(0.00 - 0.07)	0.03	(0.01 - 0.06)	0.01	(0.00 - 0.01)	0.01	(0.00 - 0.02)

B.



In agreement with proteomic study, RNA content was similar between Plasma, Serum, and ISF

Comm. Bio. (2018) 1:173 | DOI: 10.1038/s42003-018-0170-z

Dynamic Response of RNA in ISF

Acute hypoxia can cause physiological alterations affecting pulmonary, cardiovascular, renal, neurological, and hematologic systems, as well as declines in overall performance and health conditions such as heart disease and tumor microenvironment.

- Three CD Hairless rats were exposed to normoxia (21% O_2) and three exposed to acute hypoxia (10% O_2) conditions
- Serum, Plasma, and ISF was collected and RNA-Seq was used
- Changes in miRNA abundance versus normoxia and hypoxia were compared

Clinical Significance

Similar to previous studies of plasma content, annotated functions of genes encoding transcripts are differentially expressed in ISF and can be monitored to diagnose hypoxia

Function	Genes
Matrix Metalloproteinases (MMPs)	Mmp8, Mt1
Chemokines	Cxcl13, Ccr1
Leukocyte Migration	Stk10
Innate Immunity	Hmgb1-ps3, Lacc1, Bnip1
Transcription Regulator	Creg1, Brwd3, Mecp2, Sertad2, Tada2b, Tle1
Mitochondrial Processes	Pcca, Tomm40, Mtx1
Heat Shock Protein Regulation	Dnajb9
Cytoskeleton and Cell Adhesion	Mpzl2, Actn3
DNA Repair	Tdg

Transcripts that are enriched (red) or depleted (blue) in ISF in rats experiencing hypoxia (10% oxygen), as compared to rats experiencing normoxia (21% oxygen).

Comm. Bio. (2018) 1:173 | DOI: 10.1038/s42003-018-0170-z

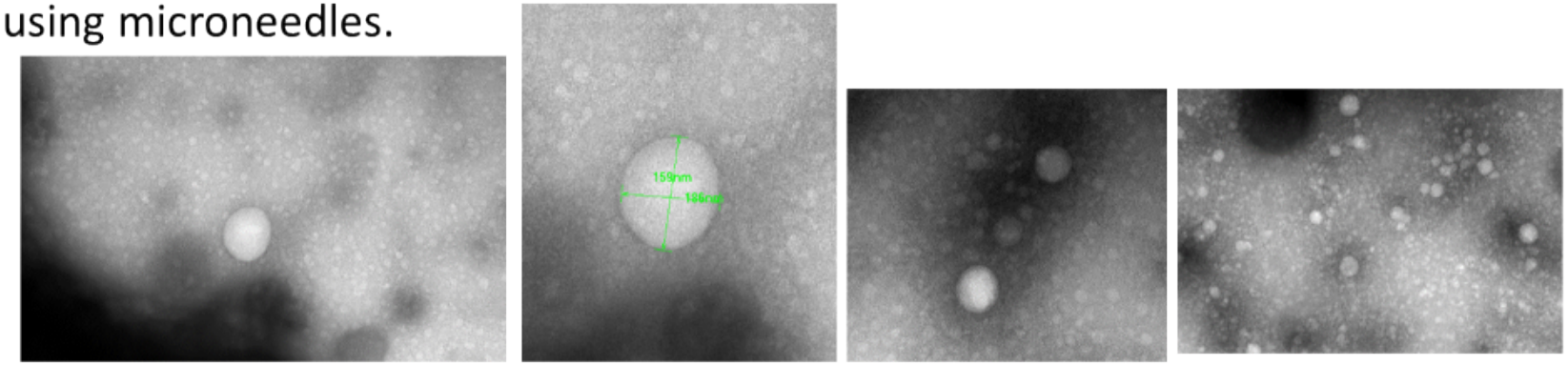
Presence Of Exosomes in Interstitial Fluid

First Demonstration!

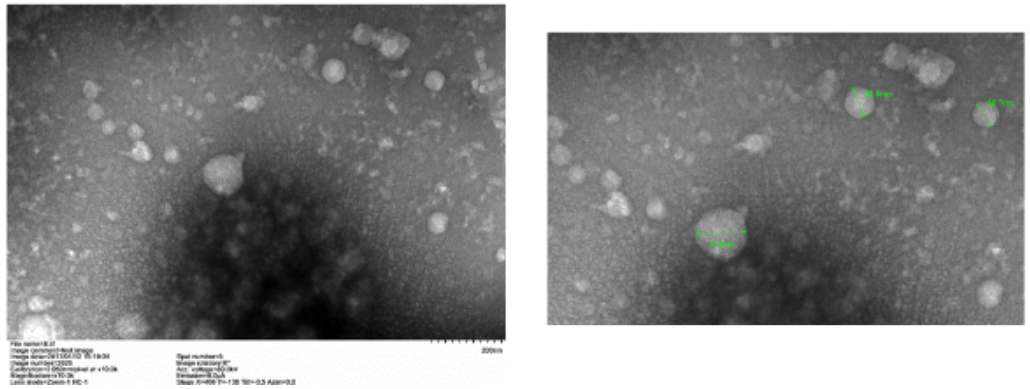
Microneedle Extraction of Interstitial Fluid (ISF)

- Have successfully purified exosomes from ISF extracted using microneedles.

ISF



Serum



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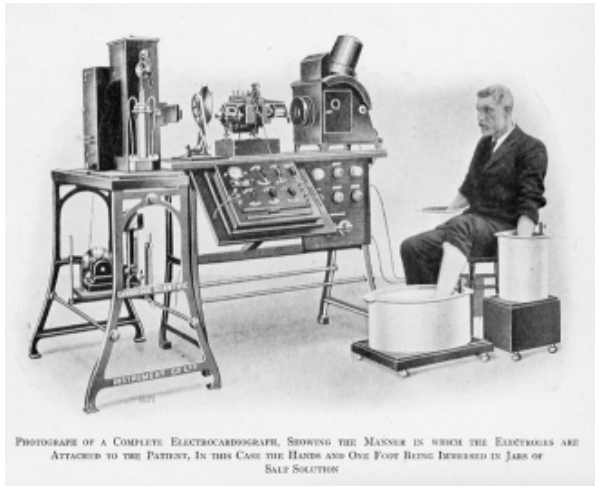
Part 2. Microneedle Sensors

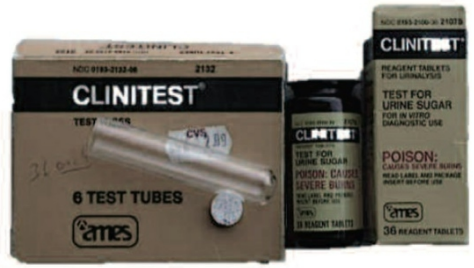
Evolution of the “Wearable”

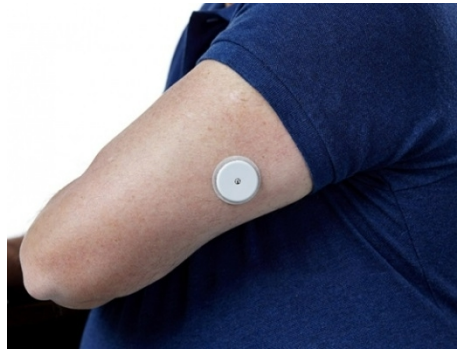
GPS 1980 → 2015



PHOTOGRAPH OF A COMPLETE ELECTROCARDIOGRAPH, SHOWING THE MANNER IN WHICH THE ELECTRODES ARE ATTACHED TO THE PATIENT, IN THIS CASE THE HANDS AND ONE FOOT BEING IMMERSED IN JARS OF SALT SOLUTION

ECG 1911 → 2015

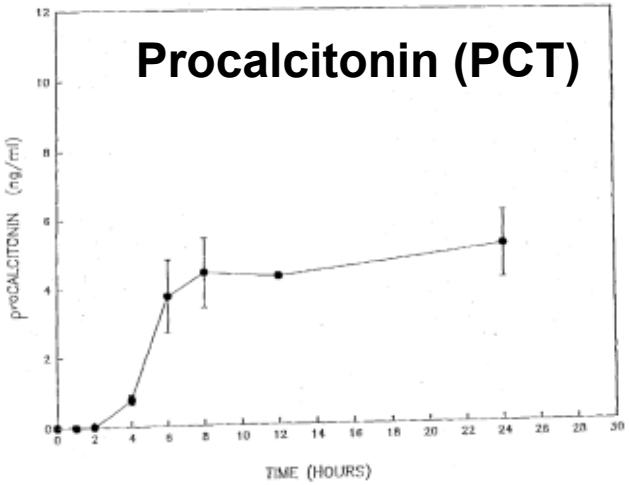


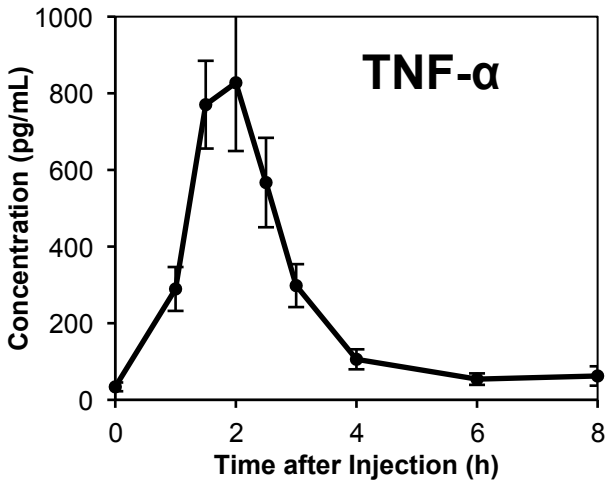


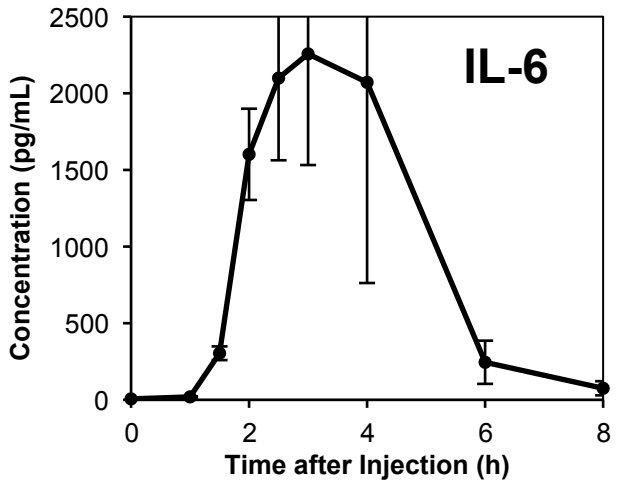
Glucose 1945 → 2015

Peptide Markers of Exposure to Biothreats

7 healthy patients, IV injected with *E. Coli* endotoxin. Serum levels of PCT, IL-6, and TNF-α measured over time.





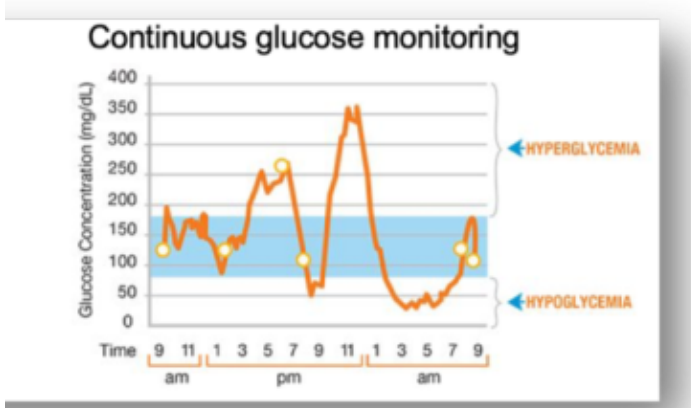


Demonstrates the importance of “kinetic” data. Unfortunately, most studies only have one data point, taken after subject is already infected and has symptoms.

*TNF-α and IL-6 plots made from tabulated data in paper.

(40) Procalcitonin increase after endotoxin injection in normal subjects. The Journal of Clinical Endocrinology and Metabolism. 1994.

Continuous Monitoring



Long Term

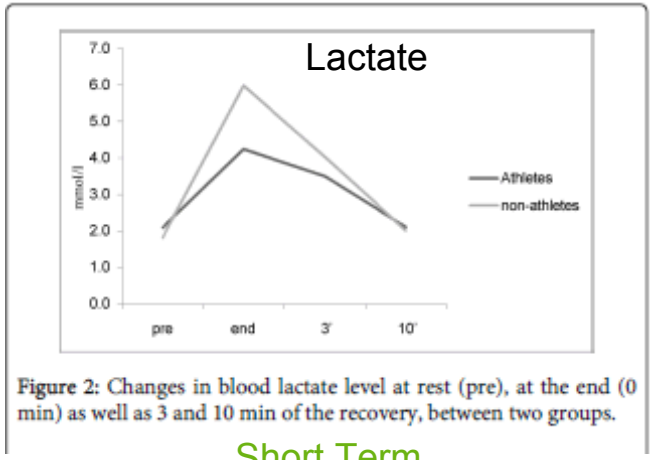
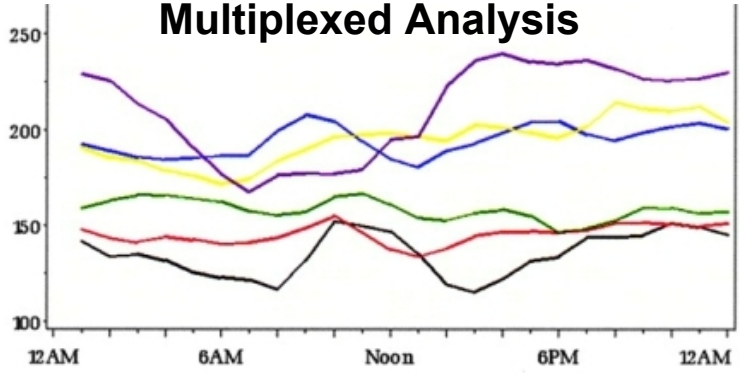


Figure 2: Changes in blood lactate level at rest (pre), at the end (0 min) as well as 3 and 10 min of the recovery, between two groups.

Short Term

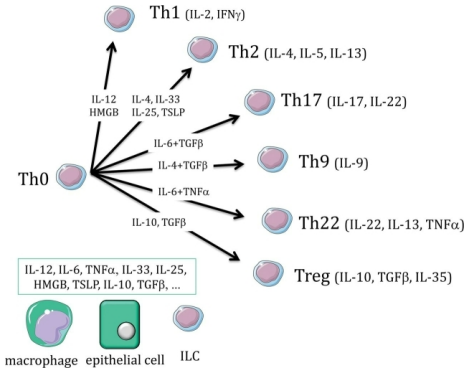
Multiplexed Analysis



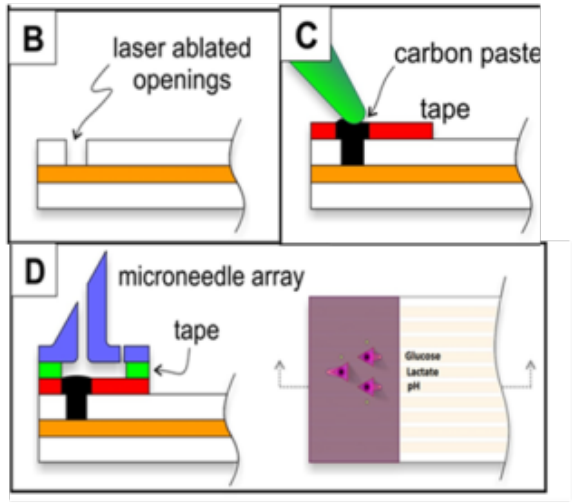
Sodium
Calcium
Potassium
Chloride
Bicarbonate
BUN
Creatine
Glucose

Chem 7 Panel

Additional Biomarkers



Multiplexed Microneedle Array



- Single MN on an array and schematic of multiplexed layout

Needle 1:

$$Lactate + H_2O + O_2 \xrightarrow{LOX} Pyruvate + H_2O_2$$

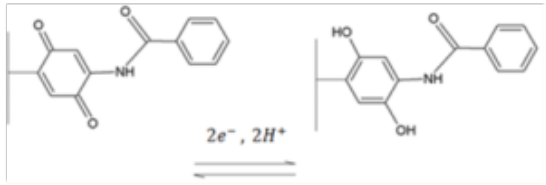
$$H_2O_2 \xrightarrow{RhonC\ @-0.15V} 2H + O_2 + 2e^-$$

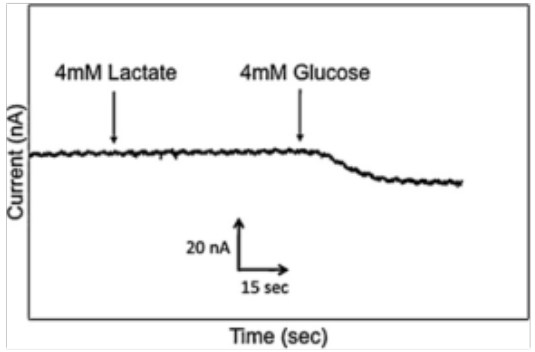
Needle 2:

$$Glucose + H_2O + O_2 \xrightarrow{GOX} Gluconicacid + H_2O_2$$

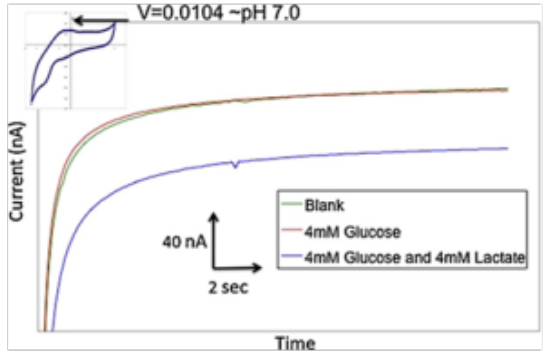
$$H_2O_2 \xrightarrow{RhonC\ @-0.05V} 2H + O_2 + 2e^-$$

Needle 3:





Spike of 4mM lactate followed by 4mM Glucose spike with detection at glucose modified carbon paste in 0.1M phosphate buffer.



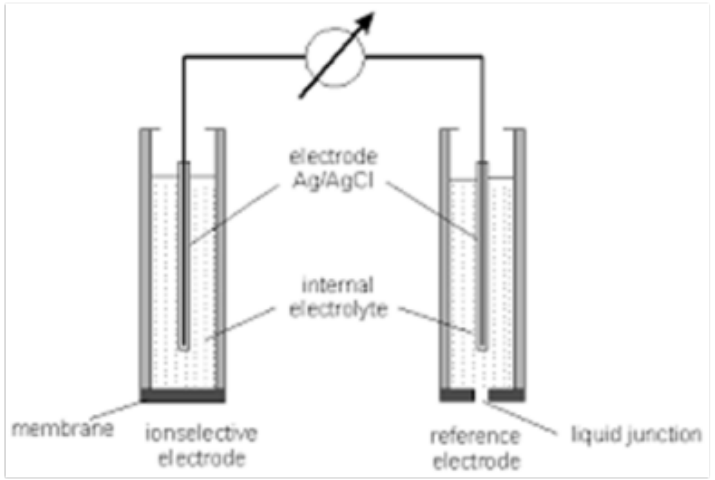
Spike of 4mM glucose followed by 4mM lactate spike with detection at lactate modified carbon paste in 0.1M phosphate buffer. CV at diazonium modified carbon paste electrode (inset).

P.R. Miller et al. ,Talanta 88 (2012)

Microneedle Electrolyte Sensor

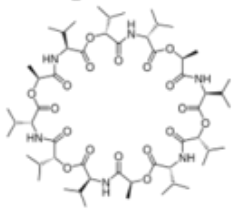
Detection Mechanism: Potential difference across an ISE membrane due to selective binding between ion and ionophore within polymer ISE membrane, measured against a reference electrode of constant potential

Traditional Liquid ISE



- Uses selective membrane to bind to ion and create potential difference
- Valinomycin used for selective potassium binding
- Difficult to miniaturize

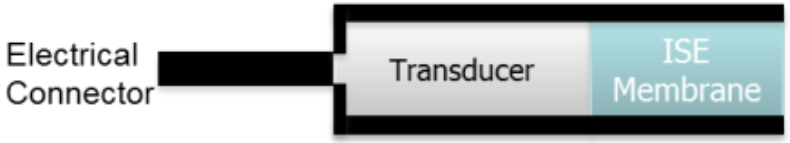
Ionophore used: Valinomycin →



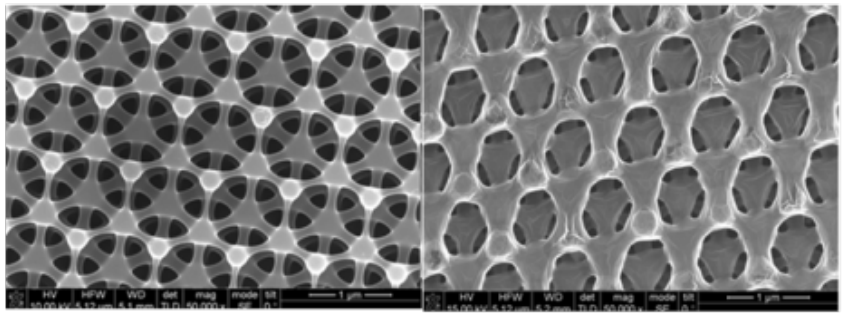
http://web.nmsu.edu/~kburke/Instrumentation/IS_Electrod.html

Solid State Polymer Membrane ISE

- Removes internal solution by coating electrode directly with polymer ISE membrane
- Facile to make and test
- Capable of being miniaturized

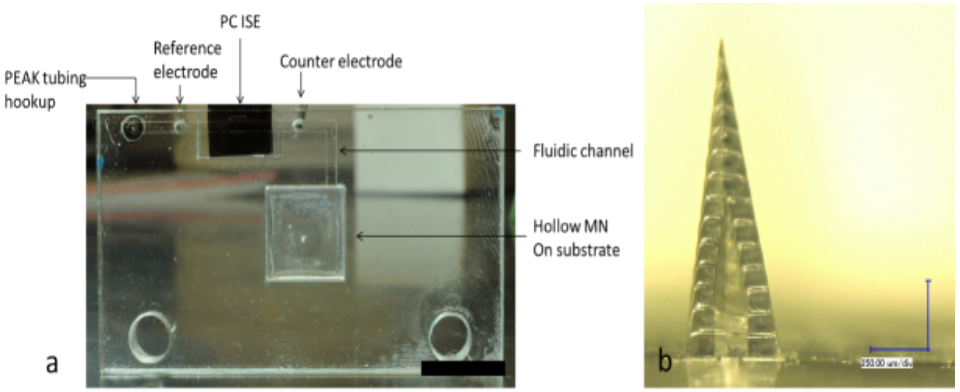


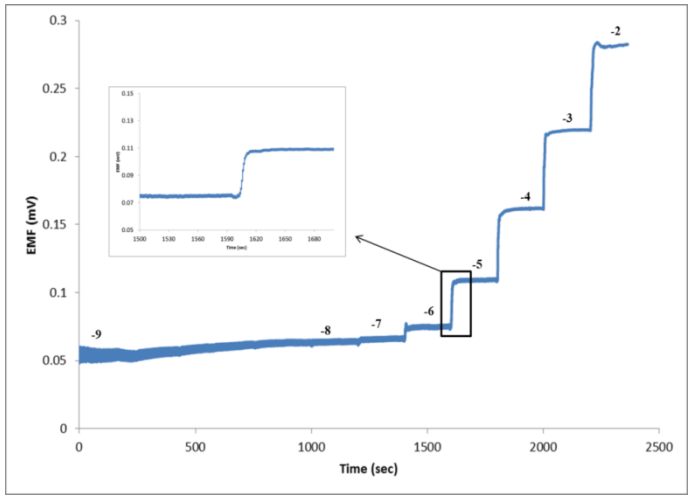
Porous Electrodes used as Transducers

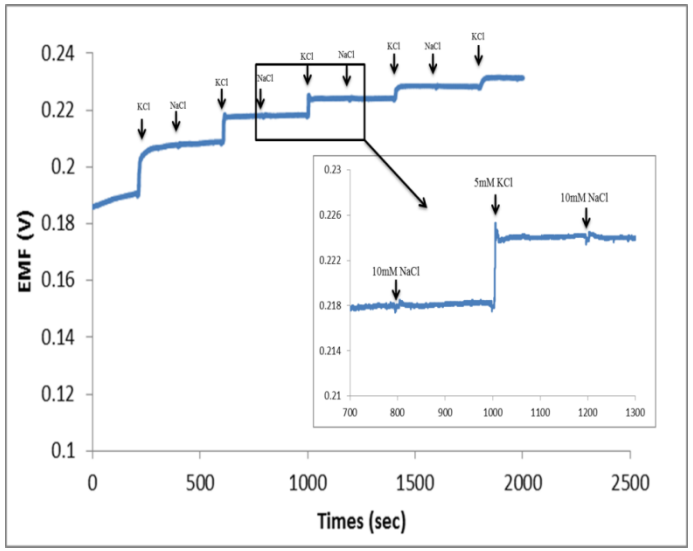


-Porous carbon (left) and porous graphene (right) electrodes used in this study

Microneedle Electrolyte Sensor



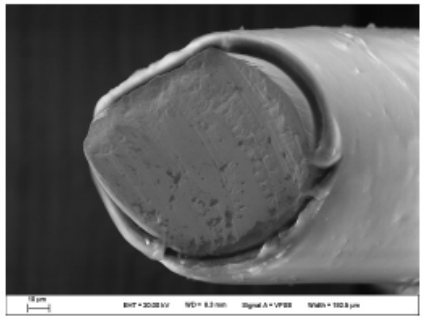


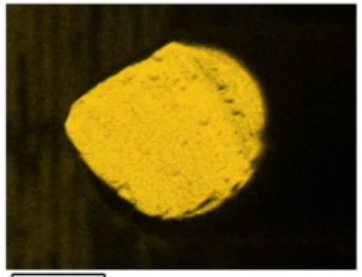


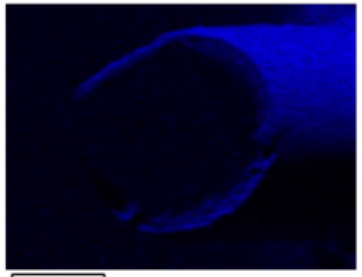
Caoxial Microneedle Electrode

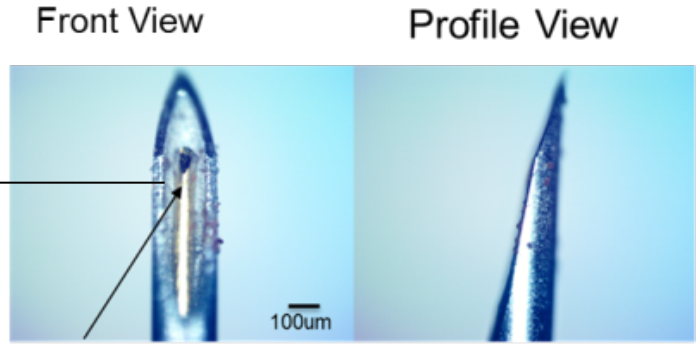
Top-Down View of Sensor

- Biosensor
- Dielectric
- PEN Needle
- Reference Electro



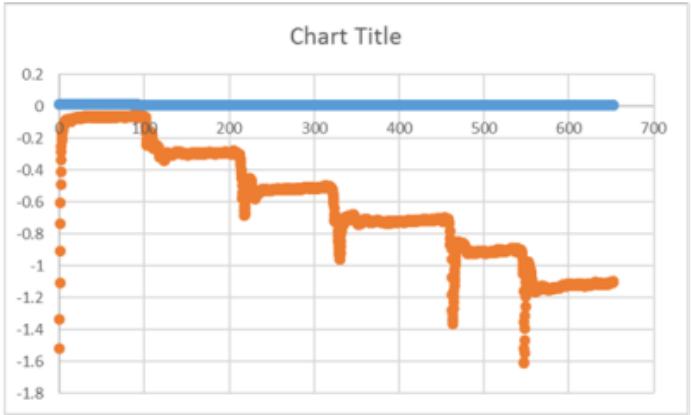






- Created method for protecting electrode within needle
- Can be used for reference and/or working electrode
- Wire electrically isolated from needle
- Each needle is a sensor and large arrays of individually addressable sensor possible

50 µm Au insulated enzymatic electrode with only tip exposed can be inserted into microneedle without causing electrical short. Thus, microneedle serves as only housing, protecting against biofouling along shaft while sensor provide very precise probing of tissue metabolites.

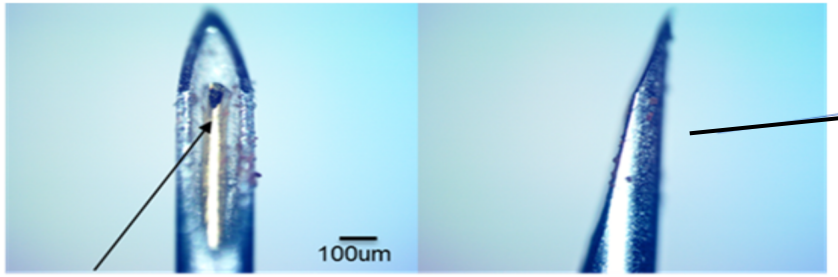


Coaxial Microneedles

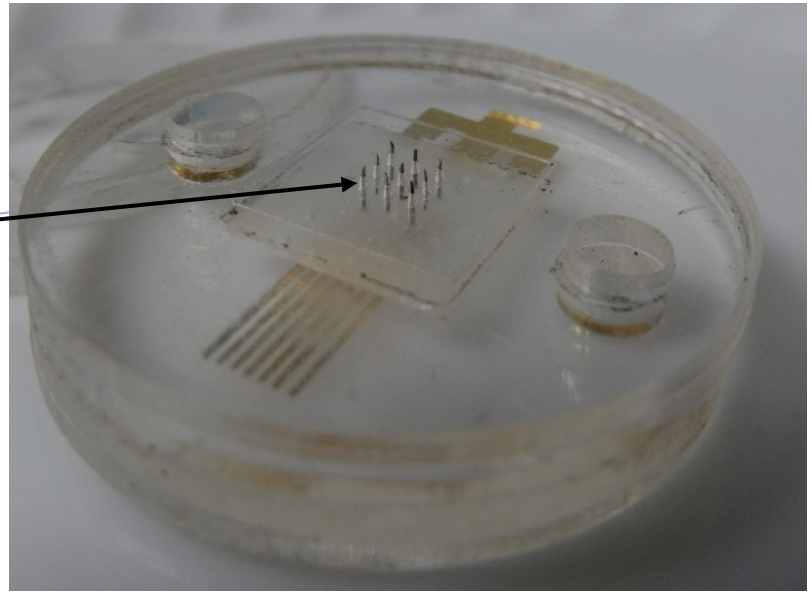
Top-Down View of Sensor

- Biosensor
- Dielectric
- PEN Needle
- Reference Electrode

Individual Microneedle Sensor

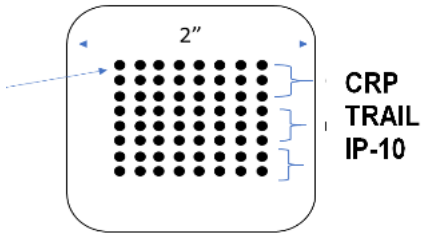


Front View Profile View



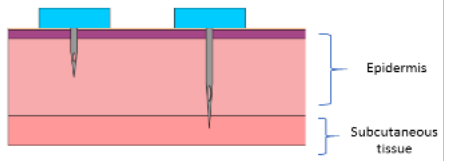
-Each coaxial sensor can self-reference
-Potential for multiple sensors per needle

Array of Sensor on Wearable Patch



-Each row of sensors can be customizable to the analyte of interest
-Suitable sample sizes can help determine System flux
-Compatible with suite of sensors and ISF fluid extraction microneedles

Sensor Placement Depth Control

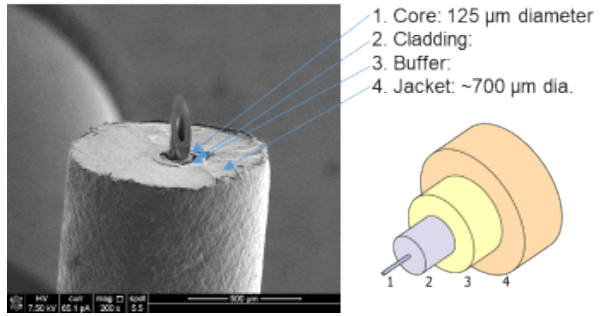


-Microneedle sensor depth control allows monitoring different skin layers
-May help distinguish physiological responses that are driven by external factor or via internal ones

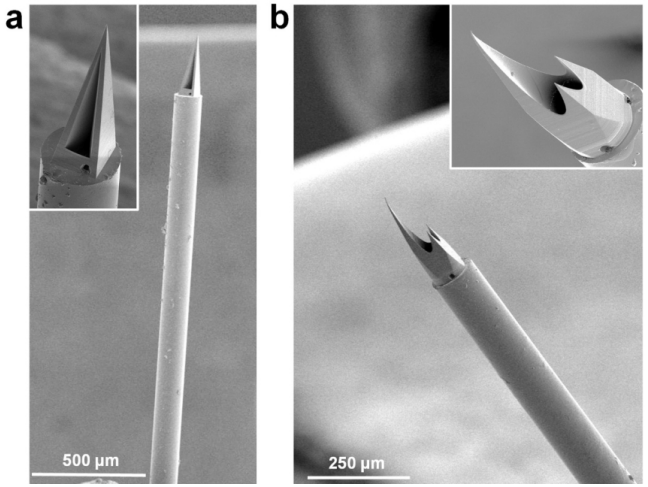
Fiber Optic Microneedle

Rapid prototyping of microneedles via direct laser writing onto fiberoptic using Nanoscribe.

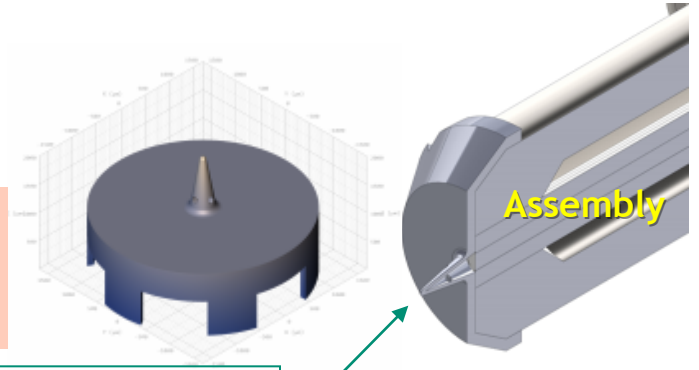
Method 1: Direct fabrication on clad fiber…



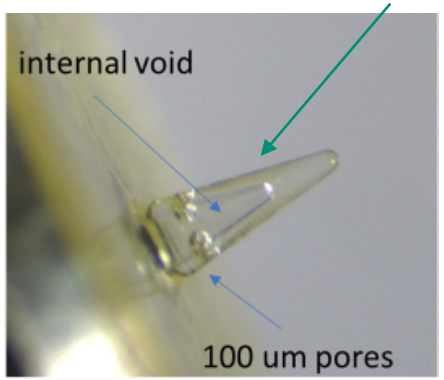
Diverse MN geometries are possible.
However MNs do not adhere well onto cladding

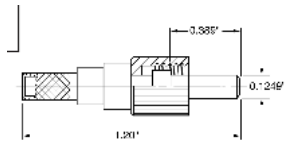


Method 2: Needle-cap printed separately to fit/align onto standard SMA905 fiber terminal.



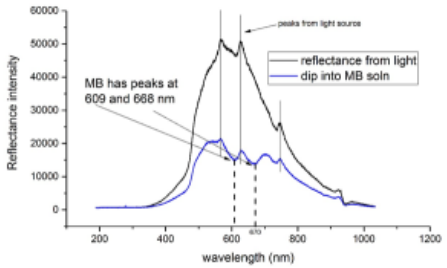
Hollow core needle



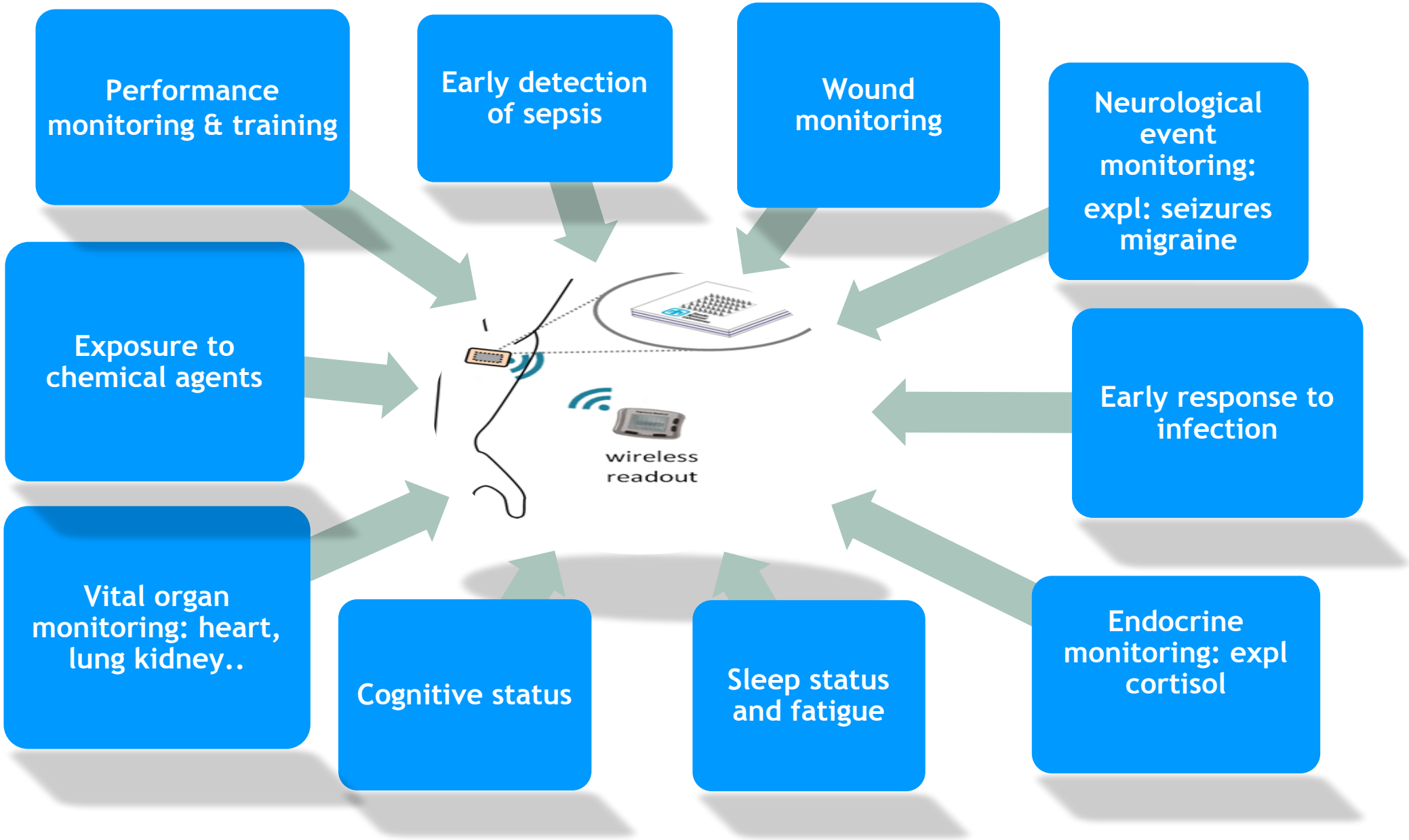


For **IR-biosensing**, Au will be plated in the interior of the needle (~20nm = 100% reflectance at λ 2.0-2.5µm) to avoid interference from the photocured polymer.

UV-Vis: No signal detected unless the 'reflection' tip is attached Methylene blue spectra detected indicating sampling from needle core.



Application Areas for Microneedle Array Wearable Biosensors



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Thank you