Lecture 20: Cell- and Tissue-based biosensors

Last time: detection methods
Surface plasmon resonance biosensors

Today: cell- and tissue-based sensors
Primary transducers and biosensor design with living cells
microphysiometer

Reading: J.J. Pancrazio et al., 'Development and application of cell-based biosensors,' Ann. Biomed. Eng. 27, 697-711 (1999)

Cell-based biosensors[1-6]

General concepts

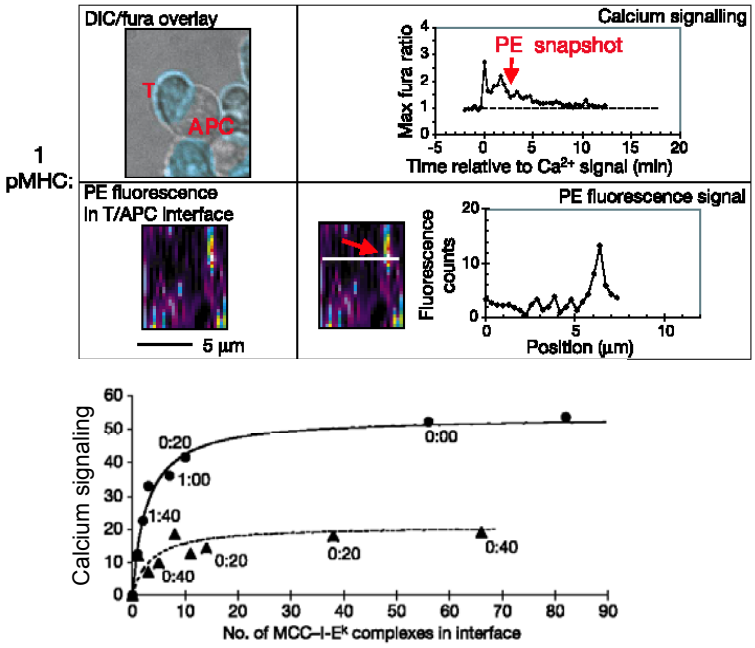
- Why cell-based biosensors?
  - **Known ultrasensitivity of cells:**
    - Olfactory neurons respond to single odorant molecules
    - Retinal neurons triggered by single photons
    - T cells triggered by single antigenic peptides[7]

¥Potential for single-molecule sensitivity

-retinal neurons triggered by single photons
-olfactory neurons detect single odorant molecules
-T cell recognition of foreign peptide (shown at right)

¥Cellular machinery maintains physiological status of receptors involved in detection

¥Complex ëvaluationÕof agents



(Irvine et al. 2002)

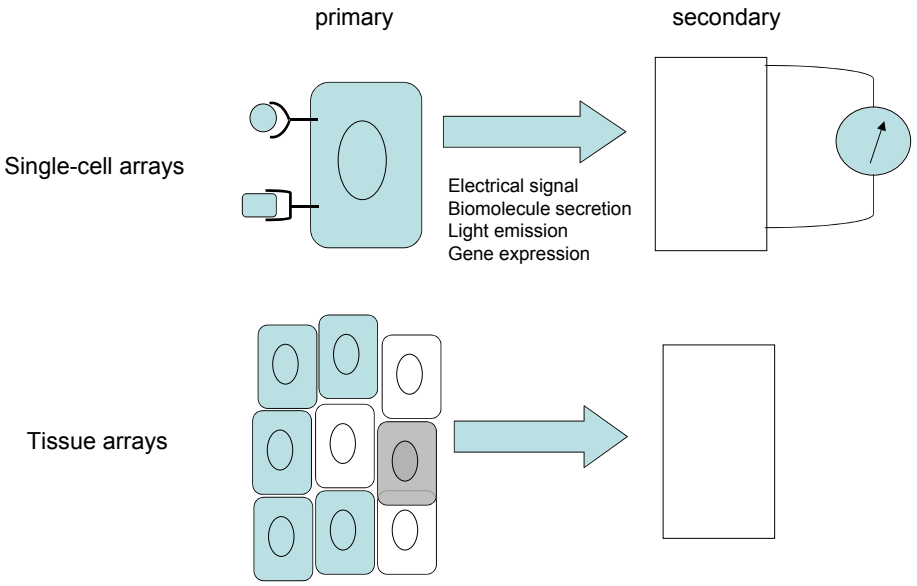
Error!

  - **Ability to 'integrate' cellular or tissue response to compounds**
    - Detect functionality of compound in addition to its chemical presence
      - i.e. tell the difference between a dead and live virus

Design of CBBs:

- Cell-based biosensors are based on a primary transducer (the cell) and secondary transducer (device which converts cellular/biochemical response into a detectable signal)
  - Secondary transducer may be electrical or optical
  - Example pathways for signal transduction:
    - Toxin -> cell stress -> changes in gene expression
    - Analyte -> cell metabolism -> changes in extracellular acidification rates

Transducers (Haruyama 2003)

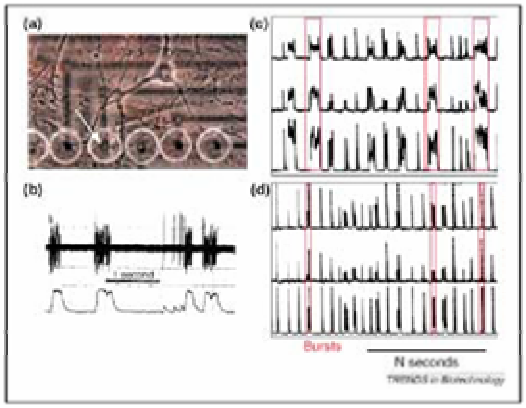


- Detection of arbitrary targets
  - Transfect cells with receptors to introduce responsiveness of e.g. neuronal cells to a chosen compound

- Basis of electrical secondary transducers
  - Electrically-excitable cells
    - Example cell types
      - Neurons[2,8]
        - Non-sensory neurons grown in culture outside of normal homeostasis and the insulation of the blood-brain barrier behave in a 'sensory' manner (Gross 1997)
        - Electrical signals play physiological role in control of secretion
      - Cardiomyocytes
        - Electrical signals play physiological role in control of contraction
    - Generate electric signals in a substance-specific and concentration-dependent manner
    - Signals generated can be monitored by microelectrodes

Cardiomyocytes
Neuronal cells

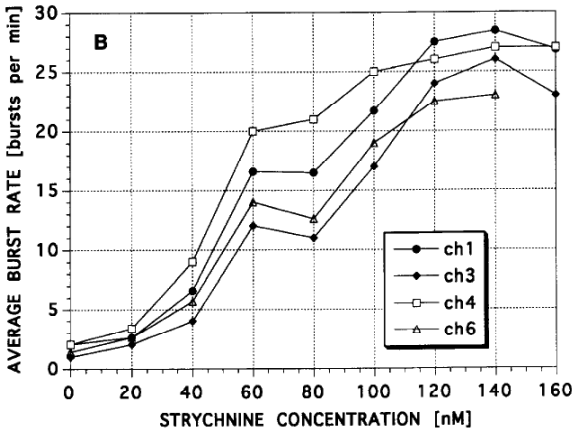
1. changes in native activity patterns
   - Synaptically active (e.g. nerve) agents
2. changes in network signalling oscillations
   - Ion channel blockers and toxins
3. paroxysmal responses due to pathological membrane currents



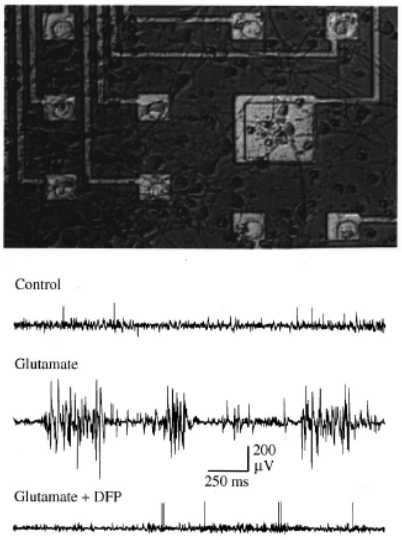
Baseline network activity

Activity following exposure of network to 2 μM trimethylolpropane phosphate (seizure-inducing compound)

(Stenger et al. 2001)



(Gross et al. 1997)

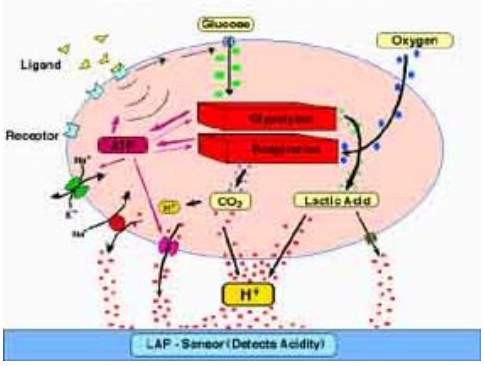


(Pancrazio et al. 1999)

FIGURE 7. Culture of spinal cord neurons for toxicological evaluation. (Upper panel) Phase contrast image of embryonic day 15 rat spinal cord neurons cultured for 18 days on a microelectrode array described in detail previously (Ref. 115). Cells were cultured under serum-free defined media conditions on artificial self-assembled monolayer substrates of aminosilanes. Microelectrode recordings shown were all from the same site. As shown from a microelectrode contact typical of this experiment, addition of glutamate (50 μM) greatly augmented spike activity. Administration of an organophosphate, diisopropylfluorophosphate (DFP; 25 μM), revealed a marked ablation of spontaneous firing, illustrating the utility of neurons cultured on microelectrode arrays for detection of toxic compounds.

Microphysiometer[9-11]

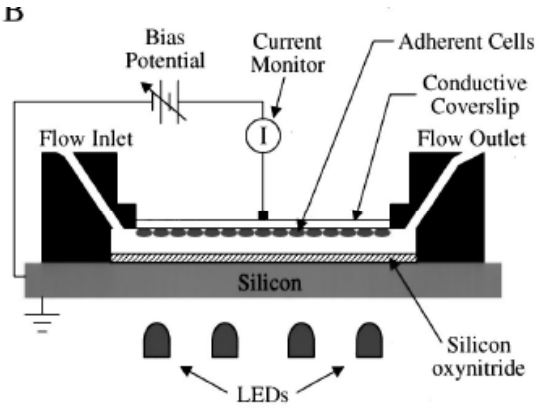
- Measures changes in extracellular acidification rate: pH changes associated with alterations in ATP consumption by cells (metabolism)
- Extremely sensitive readout of changes in cell metabolism



Effects on proton release rate:

- Receptor-ligand binding
- Metabolic drugs/poisons
- General cell stress

(McConnell et al. 1992)



(Pancrazio et al. 1999)

Detecting antigens using T cells and a microphysiometer:

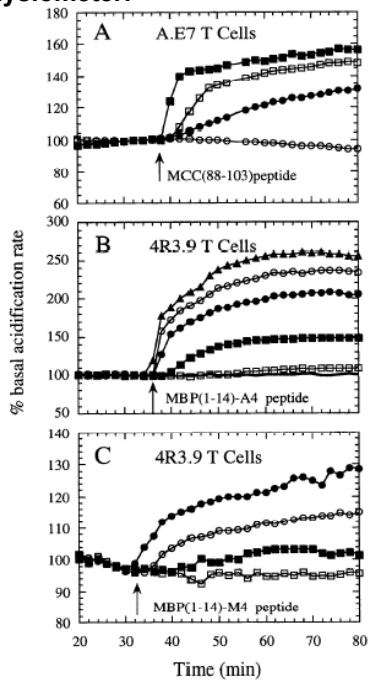


FIG. 3. Effect of peptide concentration on the acidification response of T cells. Resting T cells were prepared and loaded in Cytosensor chambers with APCs as described in Fig. 1. At the time indicated by the arrows, various doses of peptide were pumped onto the cells for 15 min, and the cells were monitored for acidification rate changes. The acidification rates were normalized to 100% prior to exposure to peptides. (*A*) A.E7 T cells were exposed to MCC-(81–104) at 0.01 μg/ml (5.5 nM) (●), MCC at 0.1 μg/ml (□), MCC at 1 μg/ml (■), or medium alone (○). (*B*) 4R3.9 T cells were exposed to AcMBP-(1–14)-A^4 at 0.01 μg/ml (6.2 nM) (□), AcMBP-A^4 at 0.1 μg/ml (■), AcMBP-A^4 at 1 μg/ml (●), AcMBP-A^4 at 10 μg/ml (○), AcMBP-A^4 at 100 μg/ml (▲), or medium alone (—). (*C*) 4R3.9 T cells were exposed to AcMBP-(1–14)-M^4 at 0.01 μg/ml (6 nM) (■), AcMBP-M^4 at 0.1 μg/ml (○), AcMBP-M^4 at 1 μg/ml (●), or medium alone (□).

(McConnell et al. 1995)

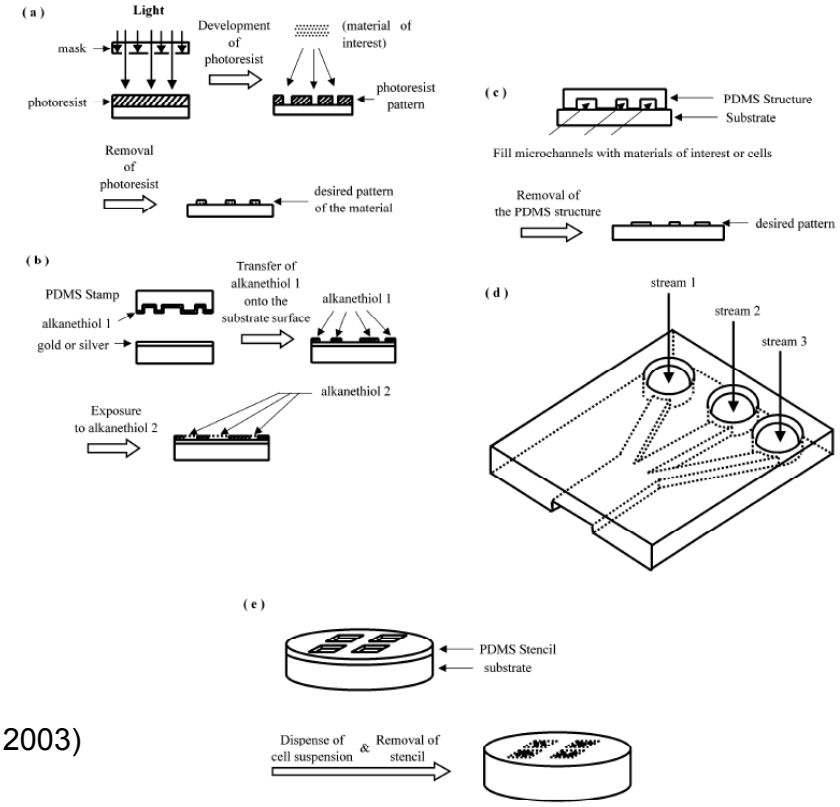
Relative advantages and disadvantages of cell-based sensors

- Pros
  - Cell-based sensors may utilize the ability of cells to respond to complex mixtures of signals in a unique way
  - Receptors, channels, and enzymes maintained in a physiologically-relevant state by the machinery of the cell
  - May provide alternatives to animal testing in the future

- Cons
  - Issues of maintaining cell viability and reproducibility in measurements
  - Issues of cell sources
    - Often require primary cells in current systems

Patterning cells for sensing[12]

- Techniques used:
  - Photolithography
  - Microcontact printing (soft lithography)
  - Microfluidic patterning
  - Membrane lift-off



(Park and Shuler, 2003)

Figure 1. Schematics of the processes of micropatterning: (a) photolithography, (b) microcontact printing, (c) microfluidic patterning using microchannels, (d) laminar flow patterning, (e) stencil patterning.

soft lithography and self-assembled monolayers

- Techniques based on the formation of gold (or other metal)-thiol bonds and spontaneous assembly of close-packed alkyl chain structures on a surface

Tissue-based biosensors

- Any papers out on the liver chip? GRIFFITH LAB

In vitro toxicology studies: tissue biosensors

- Shown below is a model of the pharmacology of naphthalene[13]
  - Tissue distribution and toxic chemistry outlined is a multi-organ, multi-compartment phenomenon
- Potential methodology: Animal-on-a-chip
  - 2 cm x 2 cm Si chip
  - designed to have ratio of organ compartment size and liquid residence times physiologically realistic
  - minimum 10K cells per compartment to facilitate analysis of chemicals and enzyme activity
  - physiologic hydrodynamic shear stress values

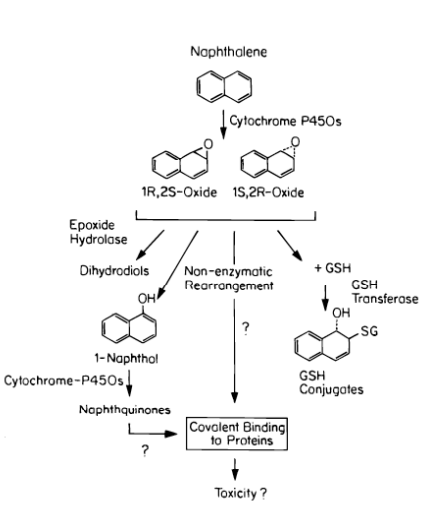
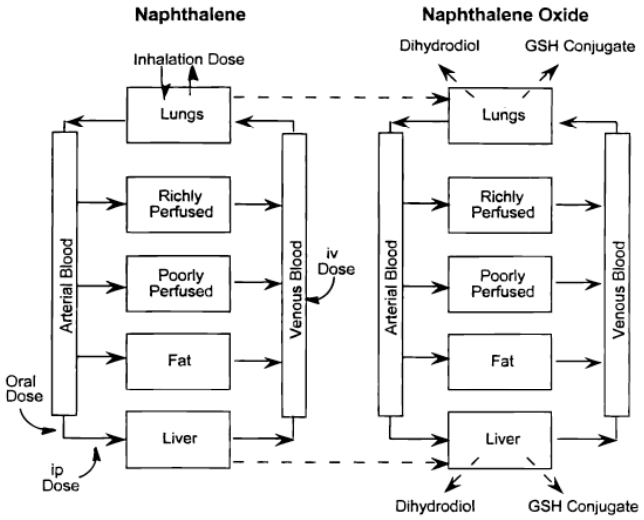
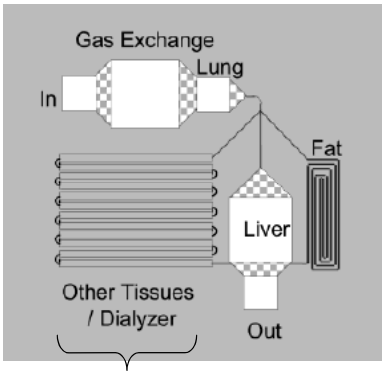


Figure 1. Reaction scheme for naphthalene and its products.



(Quick and Shuler 1999)



Models retention of chemical in blood and interstitial fluid

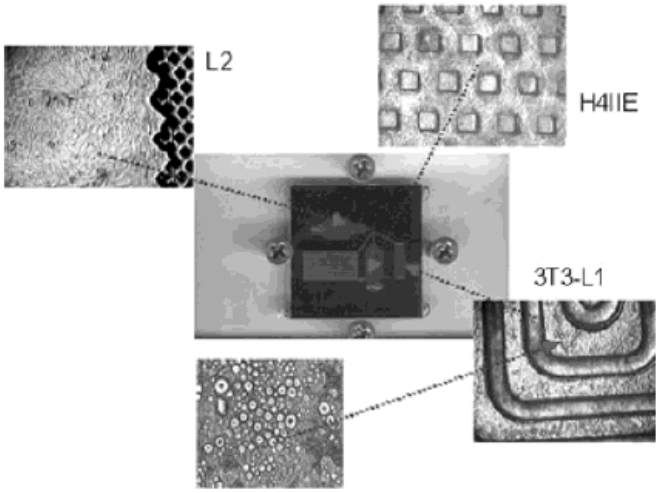


Figure 4. (a) Microscopic CCA system with four chambers. The dimensions (w × l × d) of the chambers are: lung 2 mm × 2 mm × 20 μm; liver 3.5 mm × 4.6 mm × 20 μm; other tissue 0.4 mm × 109 mm × 100 μm; fat 0.42 mm × 50.6 mm × 100 μm. Cells are cultured as monolayers on the silicon surfaces modified by adsorption of polylysine and collagen (b).

(Park and Shuler 2003)

In vivo detection

- Biofouling typically limits lifetime of *in vivo* measurements to 1-2 days
  - Inflammation
  - Fibrosis
  - Loss of vasculature

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