Neuron Based Biosensors

- Definition: a biosensor that uses living neural cells to detect substance of interest
- Why neuron based biosensors?
- Key advantage: a single neuron-based sensor can potentially detect a vast number of chemical and biological agents
  - A healthy neuron generates voltage pulses ("action potentials") spontaneously on the membrane of the axon.
  - Changes in environment (presence of chemicals or biological agents) modulate the neuron’s electrical activity.
  - Neuron exhibits a unique electrical response to particular agents

Review: Detection

• Favored method of detection is Microelectrode Arrays (MEAs).
  – Electrodes fabricated on surface of device
  – Monitor signal externally; doesn’t damage cells
• Neural signals typically in the range of 100s of uVpp
• Many working neuron-based sensors utilize MEAs
• Much research focused on improving control of neural growth on MEAs.

Analysis of Neural Response

• Time domain analysis
  – Characterize response for various substances
    • Amplitude
    • Duration of burst
    • Time interval between bursts

MEA recordings of neural activity
  a) Spontaneous activity
  b) Cyclothiazide
  c) MK-801
  d) NBQX

Source: Chiappalone et al. “Networks of neurons coupled to microelectrode arrays: a neuronal sensory system for pharmacological applications” Biosensor and Bioelectronics, 18:5-6 (2003), 627-634
Analysis of Neural Response

• Frequency domain analysis
  – Example: Prasad et al. 2004: Examine and characterize frequency components of neural response for particular substances

Neural response to hydrogen peroxide

Source: Prasad et al. "Neurons as sensors: individual and cascaded chemical sensing" Biosensor and Bioelectronics, 19:12 (2004), 1599-1610
Challenges

- Controlling interaction of living neuron to device.
  - Ideal of 1:1 association of neurons to electrodes is difficult to achieve
  - Affects signal-to-noise ratio
  - Affects reproducibility and repeatability of response
- Long term maintenance of cells in vitro
- Stability of device (corrosion, biofouling, etc)
Review: Cell Patterning Techniques

*Goal is to enhance detectibility of action potentials by patterning neurons over electrodes*

**Topographical Patterning**


**Dielectrophoresis**


**Physical Immobilization**


**Chemical Patterning**

Source: James, et al., 2004.
Cell Patterning Using SAMs

• SAMs form a single layer of molecules on a substrate.
• Advantages:
  – Creates a biocompatible membrane like microenvironment
    • Supporting structure for growth
    • Directs growth
  – Relatively easy to create
  – Long term stability
  – Customizable
• Many Types of SAMs
• Recent research has focused on using thiols on gold substrates
Early work

- Potember (1995)
  - SAM: n-octa-decyltrichlorosilane
  - Selective UV irradiation to remove/pattern SAM
  - Surface made bioactive using synthetic peptide, covalently attached using a cross-linker
  - 5um line widths
  - Optical microscopy to evaluate neural attachment and growth
  - Cells remained restricted to pattern for over 15 days in culture

Thiol-based SAMs

- Structure:
  - Alkane chain, typically with 10-20 methylene units
  - Head group with a strong preferential adsorption to the substrate used. Eg: Thiol (-SH) head groups and Au(111) substrates
  - Tail group gives the SAM its functionality

Source: “Self Assembled Monolayers”
http://www.ifm.liu.se/applphys/ftir/sams.html
Thiols on Au(111)

- Thiol head group bonds to the threefold hollow site on gold surface.
- Van der Waals forces between alkane chain causes them to lie at 30 degree angle

Commonly used SAMs:
- MUA: 11-mercaptopoundecanoic acid
- 11-AUT: 11-amino-1-undecanethiol

Nam et al. 2004

- Contribution: Coated microelectrode arrays with gold in order to use alkanthiol-based SAM techniques
- Technique:
  - Coat MEAs with 50-80A of gold
  - Immerse in MUA solution for 2 hours to create SAM
  - Expose SAM to other compounds to produce layer of NHS esters
  - Use uCP to apply poly-D-lysine. Stable PDL layer created by covalent linking to SAM layer
  - Unstamped areas covered with chemical that inhibits cell growth

Nam et al. 2004

- Results:
  - Demonstrated cell viability on PDL linked gold surface
  - Good resolution stamped 100 x 100um grid pattern of 10um line width
  - Cells complied to pattern for > 2 weeks
  - Recording of *spontaneous* neural activity to verify cell activity.
  - Enhanced amplitudes up to 500uVpp (100-200uVpp typical)
  - Gold MEAs were not reusable

Nam et al. 2006

• Updated process
  – different SAM 3-glycidoxypropyl trimethoxysilane (3-GPS)
  – uCP for protein pattern stamping

• Results
  – Neurons complied to patterns for 2-3 weeks
  – Spontaneous neural activity recorded:
    • Note SAM increased impedance by factor of 2-3
    • Mean SNR of 6.5 at 2 weeks
    • Mean amplitude of extracellular spikes was $25 \text{uV}_{\text{pp}}$ at 7 DIV and $50 \text{uV}_{\text{pp}}$ at 20-24 DIV.
    • Background noise $2.9 \text{uV}_{\text{pp}}$

Source: Nam et al. “Epoxy-silane linking of biomolecules is simple and effective for patterning neuronal cultures.” Biosensors and Bioelectronics 22 (2006) 589–597
**Palyvoda et al. 2007**

- **Technique:**
  - Create gold electrodes
  - Immerse in 11-AUT solution to create SAM
  - Studied effect of pad size on neural guidance

- **Contribution:** used SAM to support and guide neural growth directly
  - No intermediate protein layer, e.g. polylysine (which is difficult to pattern, nonphysiological, toxic under some conditions)


Image of neurons on 50x50um SAM coated gold electrode
• Results
  – Effects of pad size on cell counts:
    • 350x350um: 57 +/- 10
    • 200x200um: 16 +/- 5
    • 100x100um: 4.14 +/- 2
    • 50x50um: 1 +/- 0.87
  – 50x50um pad size comes close to single neuron immobilization, with error.
  – No measurement of electrical activity was performed

Other Characterization Studies

• Naka et al. (2002)
  – Investigated effects of different functional tail groups of thiol based SAMs on neuron growth: Amino groups (NH2), carboxyl (COOH), methyl (CH3).
  – Concludes 11-amino-1-undecanethiol is best
  – Neurons detach after about 2 weeks.

• Slaughter et al. (2004)
  – Characterized protein attachment to two thiol SAMs on gold electrodes using florescence microscopy, atomic force microscopy, and ac impedance.

• Faucheux et al. (2004)
  – Characterized SAM with various functional groups by examining wettability, layer thickness, and roughness
  – Examined protein adsorption to these SAMs
Other Recent Studies

- **Romanova et al. (2006)**
  - Studied how chemical modifications to SAM affected neuron growth and neurite extension.

- **Widge et al. (2007)**
  - Studied how mixed SAMs of thiol and conductive polymers affected electrical impedance and phase of gold coated MEAs.
  - Adding conductive polymers reduces impedance by adding surface area (roughness).

- **Lin et al. (2008)**
  - Characterized physical structure (thickness, bonding characteristics) of gold electrodes modified MUA-SAMs coated with poly-D-lysine using infrared reflection and AFM.
  - Demonstrated that neurons adhere better to MUA-SAM modified gold electrodes better than to bare gold.
Conclusions

• Neuron-based biosensors appear poised to become an effective biosensor technology.

• Challenges:
  – Stability of culture and micro device
  – Reproducibility of results
  – Maintaining health of cells over long term
Conclusions

• SAMs are attractive as a possible solution to these challenges
  – Relatively simple compared to other options
  – Highly biocompatible
  – Customizable
  – Increases stability of neuron-device interface

• But…
  – 10-15 years or more of characterization studies of SAMs
  – No fully functional SAM-based biosensor for specific application to date
Questions?
References


References


