Lecture 14:
Quantifying Cell Function

c. Cell Proliferation Assays

Importance:
- wound healing
- immune host defense
- cancer therapy
- tissue engineering
- bioprocessing/synthesis of therapeutic glycoproteins

➢ Cell number

1. count via microscopic observation or Coulter counter

2. compute specific cell proliferation rate const:
\[ k_g = \frac{1}{N} \frac{dN}{dt} = \frac{d(\ln N)}{dt} \quad \text{(units: t}^{-1}) \]

\( N = \# \text{ of cells at time } t \)

➢ Cell phase populations

In some cases, we want to know the population of cells in each phase of the cell cycle (eukaryotes):
- \( M = \text{Mitosis (~1 h)} \)
- \( G_1 = \text{gap between cell division & DNA synthesis (~18-72 h)} \)
- \( S = \text{DNA synthesis (~6-8 h)} \)
- \( G_2 = \text{gap between DNA synthesis & mitosis (~2-3 h)} \)
- \( G_0 = \text{quiescent cells} \)
tritiated-thymidine uptake

1. cells exposed to $[^3H]$thymidine pulse which labels S-phase cells only

2. % S obtained from autoradiography (Ag precipitates in an overlying emulsion film reveal S-phase cells, similar to a photograph emulsion*)

3. % M and $t_M$ obtained from optical microscopy (visually distinct)

4. by following % labeled mitoses after $[^3H]$thymidine pulse through 2 cell divisions, $t_{G2}$, $t_S$ and $t_{cycle}$ can be determined → $t_{G1}$

*autoradiography is also used with PAGE for protein identification
**flow cytometry**

1. cells’ DNA labeled with DNA-specific fluorescent dye (ex. acriflavine, ethidium bromide)

2. count cell # vs. fluorescence using laser excitation

3. fluorescence intensity ~ DNA present

\[ G_1:S:G_2:M \] DNA (& fluorescence) ratio is 1:1-2:2:2

<table>
<thead>
<tr>
<th></th>
<th>G₁</th>
<th>S</th>
<th>G₂+M</th>
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<tbody>
<tr>
<td>area %</td>
<td>52</td>
<td>41</td>
<td>7</td>
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Advantages: 
- no radiolabeling
- faster than autoradiography

Drawback: 
- M & G₂ populations lumped

*flow cytometry is also used to determine expression of specific cell receptors by labeling with fluorescent antibodies*
**d. Cell Differentiation Assays**

**Importance:**
- change in phenotype renders specialization of function
- characterized by changes in protein synthesis, genes expressed & secretions

**2D Gel Electrophoresis (SDS-PAGE):** cell proteins separated on basis of mass and charge—providing a “signature” of the cell

**Method**

1. cell is lysed (contents extracted)

2. protein solution is separated by pI (isoelectric point = pH where net charge = 0) using *isoelectric focusing*

   ![Diagram](image)

   - i. ampholyte mixture is placed under E-field to create a **stable pH gradient** in a polyacrylamide gel.
   - ii. protein solution is added and E-field reapplied
   - iii. proteins diffuse to their **isoelectric point**

3. gel strip is next placed crosswise on a second gel incorporating sodium dodecyl sulfate (SDS)

4. SDS binds & denatures proteins ⇒ native charge becomes negligible
   \[ \sim 1 \text{ SDS per 2 amino acids} \]
   \[ \text{charge/mass} \approx \text{constant} \]
5. gel is placed under E-field, separating protein chains via molecular weight

low molecular weight species move faster

\[ \mu = \mu(c_{gel}, E, MW_{protein}) \]

**SDS-PAGE:** Sodium dodecyl sulfate polyacrylamide gel electrophoresis

6. compare “before” and “after” signature to discern differentiation
**DNA microarrays**: test for gene expression  
(first commercialized by Affymetrix)

array of “probes” comprising surface-bound gene fragments  
(~20 bases/fragment)

- 2M probes/chip  
- up to 40,000 genes/chip

\[ \text{~50 } \mu\text{m}^2 \text{ probe area} = 10^6 \text{ DNA fragments} \]

**Method**

1. cells lysed to retrieve mRNA

2. mRNA transcribed to cDNA (complement), which is then transcribed to cRNA

3. cRNA is cut into 35-200 base fragments & labeled with biotin (vitamin H)—referred to as “biotinylation”

**Biotin** has strong binding affinity for streptavidin  
\[ K_a \sim 10^{15} \text{ L/mol} \]
4. cRNA “cocktail” is added to array, hybridizes with DNA fragments

5. surface-bound cRNA-biotin is labeled with fluorophore-labeled avidin

6. fluorescence spatial readout determines genes being expressed
DNA Array Fabrication

Lithographic masks used to build up A-C-T-G sequences base-by-base

e. Secretion

*Enzyme-linked immunosorbent assay (ELISA):* tests for a particular protein’s presence in cell secretions

1. Cell secretions aspirated from culture medium

2. Surface coated with soln of monoclonal antibody (MAb) for protein of interest—protein binds MAb if present

3. 2\textsuperscript{nd} antibody (enzyme-linked) is added—binds to protein on surface to create a “sandwich”

4. The enzyme (ex., alkaline phosphatase) catalyzes conversion of a compound to a colored product (amplification)

5. Color intensity is read in spectrophotometer

5. Nonspecific adsorption is determined and subtracted
Alternately, proteins directly adsorbed on a surface may be probed by ELISA in which the second (enzyme-linked) antibody is an antibody to the first antibody.