Mammalian Cell Culture Technology

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Cell Culture Technology

• Introduction
• Techniques in cell culture technology
• Applications-Cell culture systems
Sources of cell cultures

- Mammalian cells/tissues
- Insect cells
- Plant cells/tissues
- Fish cells
- Hybridoma cell lines (man made)

We could generate cell culture from any life forms !!!!
<table>
<thead>
<tr>
<th>Date</th>
<th>Scientist</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1907</td>
<td>Harrison</td>
<td>Frog embryos in clots of frog lymph, survived few weeks.</td>
</tr>
<tr>
<td>1943</td>
<td>Earle</td>
<td>First rodent continuous cell line (rat)</td>
</tr>
<tr>
<td>1952</td>
<td>Gey</td>
<td>Hela – First human tumour cell line, from <em>Henrietta Lack</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cervical Cancer, most notorious cell line – contaminates other lines.</td>
</tr>
<tr>
<td>1961</td>
<td>Heyflick &amp; Moorhead</td>
<td>Normal Cells – finite lifespan</td>
</tr>
<tr>
<td>1950 to</td>
<td></td>
<td>Explosion in cell culture due to disposable plastic wear, laminar</td>
</tr>
<tr>
<td>present</td>
<td></td>
<td>flow cabinets etc..</td>
</tr>
</tbody>
</table>
Cell/tissue culture-Terminology

- The *in vitro* growth of cells (animal; including human) *Mimic internal environment outside the body*.

- Widely used technique in cell and molecular biology, genetic and toxicology.

- **Cell lines**: Cells grown in culture *Cell lines can be grown indefinitely*.

- **Static culture**: cells grown on solid surface *(mostly plastics)- anchorage dependent (tumour/haemopoietic)*

- **Suspension culture**: cells grown as suspension in culture media- *anchorage independent (normal cells)*
Mammalian cell culture -

Types of cells that may be cultured.

- Connective tissue
- fibroblast/muscle

- Epithelial Cells
- organs/skin

- Neural Cells
- neurones

- Endocrine Cells
- pancreatic islet

- Tumour Cells
- various types
# Mammalian cell culture - Types of Tissue Cultures

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary Explant Culture</strong></td>
<td>Piece of tissue cultured on grid or raft at liquid-gas interface</td>
</tr>
<tr>
<td></td>
<td>Maintains tissue architecture</td>
</tr>
<tr>
<td><strong>Organ Culture</strong></td>
<td>No new growth can occur</td>
</tr>
<tr>
<td></td>
<td>Lasts several days to weeks.</td>
</tr>
<tr>
<td></td>
<td>Each experiment requires new sample</td>
</tr>
<tr>
<td><strong>Cell Culture</strong></td>
<td>Tissue or outgrowth from primary explant</td>
</tr>
<tr>
<td></td>
<td>Broken in cell suspension by enzymatic or mechanical means</td>
</tr>
<tr>
<td></td>
<td>Primary culture is culture direct from tissue</td>
</tr>
<tr>
<td></td>
<td>Once subcultures, referred to as cell line</td>
</tr>
</tbody>
</table>
# Mammalian cell lines

<table>
<thead>
<tr>
<th>Producer cell lines</th>
<th>Medical (cancer) research</th>
<th>Hybrid cell lines</th>
<th>Primary cell lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHK-21</td>
<td>MRC-5, HEK, MDBK, LLC-MK₂, BS-C-1, CV-1, AGMK, WI-38, Vero, Hela, 3T3-L1</td>
<td>NSO NS1 SP2/0-Ag14 P3-X63 Ag8.653</td>
<td>Fibroblast cells Skin cells Liver cells Lung cells Retina cells etc</td>
</tr>
<tr>
<td>CHO-K1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Namalwa (therapeutic protein production)</td>
<td>(commonly used for viral propagation in vaccine production)</td>
<td>(protein production for therapeutic &amp; diagnostic)</td>
<td>(to make tissue equivalents- mortal cell lines recovered from tissue)</td>
</tr>
</tbody>
</table>
Hybridoma TBC3.bcl-2, producing mouse IgG1

Hybridoma, NS0

CHO-K1

BHK-21

Hybridoma TBC3.bcl-2, producing mouse IgG1
Primary cell lines isolated from chick embryo

Chick brain cells

Chick skin cells

Chick heart cells

Chick muscle cells
Mammalian cell cultures—properties

**AVERAGE SIZE**
5 TO 5 MICRONS

**AVERAGE COMPOSITION**
(DRY WEIGHT BASIS)

- PROTEIN 40%
- CARBOHYDRATE 25%
- LIPID: 20%
- DNA: 2.5%
- RNA: 6.9%
- WATER: 80-85%

**SENSITIVITY TO ENVIRONMENT**

OSMOLARITY WINDOW: - 280-350 MILLIOSMOLE/Kg

PH RANGE: 6.5 TO 7.2 TEMPERATURE: 35 TO 370°C ± 0.20°C

LACK OF PROTECTIVE CELL WALL
(ADVERSELY AFFECTED BY LIQUID SHEAR FORCES, GAS BUBBLES)

**LOW RATE OF GROWTH**

NUMBER DOUBLING TIMES: 18 TO 36 HOURS
LOW CELL DENSITY
(TYPICALLY 1 X 10^6 CELLS/ML 0.6 grams/Liter)
FASTIDIOUS NUTRITIONAL REQUIREMENTS
EASE OF CONTAMINATION
Cell Growth Characteristics

Growth Characteristic and Productivity (IgG1) of TB/C3.pEF

- **Viability (%)**
- **Viable Cell No (x 10^5 cells/ml)**
- **IgG1 Concentration (µg/ml)**

**Time (Hour)**: 0 20 40 60 80 100 120 140

**Viability (%)**
- 0 20 40 60 80 100

**Viable Cell No (x 10^5 cells/ml)**
- 0 2 4 6 8 10 12 14

**IgG1 Concentration (µg/ml)**
- 0 10 20 30 40 50 60 70

**Legend**:
- Red dotted line: Viability (%)
- Red solid line: Viable Cell No (x 10^5 cells/ml)
- Blue dashed line: IgG1 Concentration (µg/ml)
Cell Growth Kinetics

Growth rate: \( \frac{dN}{dt} = \mu N \)

\[ \mu \text{ (typical): } 0.015 \text{ to } 0.03/\text{Hr} \]
\[ T_D = 24 \text{ to } 48 \text{ Hrs} \]

Product formation rate: \( \frac{dp}{dt} = \alpha \mu N + \beta N \)

\( \alpha = \text{ growth associated} \)
\( \beta = \text{ non growth associated} \)

Specific product synthesis rate (\( q_p \))

\[ \frac{Dp}{N dt} = q_p = \alpha \mu + \beta = \text{Pg/Cell-Day} \]

\( q_p \) (typical) = 5 to 20 Pg/Cell-Day
Mammalian cell culture—Nutritional requirements (COMPLEX MEDIUM)

**Minimal essential medium**
- ANIMAL CELLS UNABLE TO SYNTHESIZE AMINO ACIDS: (13 AMINO ACIDS: 0.05 mM TO 4 mM)
- ALL VITAMINS MUST BE SUPPLEMENTED (8 VITAMINS: 0.3 TO 11 μM)
- INORGANIC ION SUPPLEMENTATION (6 INORGANIC IONS: 1.1 TO 116 mM)
- CARBOHYDRATE: GLUCOSE MOST COMMON (5 TO 8 mM)
- GLUTAMINE (4 mM)
  - SERVES AS ENERGY SOURCE
  - INCORPORATED AS AMINO ACID
  - AMINO GROUP: TRANSAMINATION
  - NaCl (116 mM)
  - MAINTAINING PROPER OSMOLARITY
  - GLUCOSE (5 TO 8 mM)
  - PARTIAL SOURCE OF ENERGY

**Notable nutrients in high concentrations**
- AMINO GROUPS
  - 13 AMINO ACIDS: 0.05 mM TO 4 mM
  - AMINO GROUP: TRANSAMINATION
- VITAMINS
  - 8 VITAMINS: 0.3 TO 11 μM
- INORGANIC IONS
  - 6 INORGANIC IONS: 1.1 TO 116 mM
- CARBOHYDRATE
  - GLUCOSE (5 TO 8 mM)
- MAINTAINING PROPER OSMOLARITY

**Antibiotics** (STREPTOMYCIN, GENTAMICIN, PENICILLIN)
- REDUCE CONTAMINATION

**Animal serum**
- DIALYZED FETAL CALF SERUM (5 TO 10%)

**Serum free medium**
- FORMULATED PRODUCTS-ADD HORMONES AND GROWTH FACTORS
- PROPRIETARY FORMULATIONS
# Mammalian cell culture-
## Application of Cell Cultures

| 1. Cells Themselves | Skin Cells – Grafting in burn treatment  
|                      | Blood Cells – Transfusions/ New Veins  
|                      | Glandular Tissue – Replacements  
| 2. Viral Vaccine Production | Industry is largest producer of animal cells  
| 3. Production of cellular macromolecules. | Pharmaceuticals: interferons, monoclonal antibodies, hormones and enzymes  
| 4. In vitro toxicology | Testing drugs on cell cultures instead of animals  
| 5. Research tool | Cancer research-compare normal and tumour cells  
|                  | Predict chemosensitivity to cancer cells  

New Biotech Drug and Vaccine Approvals/New Indication Approvals by Year

![Bar chart showing the number of approvals by year from 1982 to 2002. The chart indicates a general increase in approvals over time, with a peak in 2000 with 32 approvals, followed by 24 in 2002.]
List of biopharmaceutical products
(some examples)

**Herceptin®** (Trastuzumab)
Anti-HER2 antibody
For metastatic breast cancer in HER2 overexpressed tumors

**Rituxan®** (Rituximab)
Anti-CD20 antibody
For relapsed or refractory low-grade or follicular, CD20 positive, B-cell non-Hodgkin's lymphoma

**Thrombolytics Activase®** (Alteplase, recombinant)
A tissue-plasminogen activator
For acute myocardial infarction, acute ischemic stroke and acute massive pulmonary embolism

**Cathflo® Activase®** (Alteplase)
Thrombolytic agent
For the restoration of function to central venous access devices as assessed by the ability to withdraw blood

**TNKase™** (Tenecteplase)
Single-bolus thrombolytic agent
For the treatment of acute myocardial infarction (AMI)

**Nutropin AQ®** [somatropin (rDNA origin) injection]
Liquid formulation growth hormone
For GHD in children and adults; growth failure associated with chronic renal insufficiency (CRI) prior to kidney transplantation; short stature associated with Turner syndrome

**Nutropin®** [somatropin (rDNA origin) for injection]
Growth hormone
For GHD in children and adults; growth failure associated with CRI prior to kidney transplantation; short stature associated with Turner syndrome

**Protropin®** (somatrem for injection)
Growth hormone
For growth hormone deficiency (GHD) in children

**Pulmozyme®** (dornase alfa)
Inhalation Solution
For management of cystic fibrosis (including patients under age 5)
## Rationale for using mammalian cell cultures

<table>
<thead>
<tr>
<th><strong>Rationale</strong></th>
<th><strong>Details</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HYBRIDOMA FOR ANTIBODY PRODUCTION</strong></td>
<td><strong>RAPID ISOLATION AND PURIFICATION FOR ANALYTICAL USAGE</strong></td>
</tr>
<tr>
<td><strong>RAPID METHOD FOR EXPRESSION OF NEW PROTEINS</strong></td>
<td><strong>STABILITY OF EXPRESSION FOR MANUFACTURING FDA ACCEPTANCE FOR THERAPEUTIC PROTEINS</strong></td>
</tr>
<tr>
<td><strong>POST-TRANSLATIONAL MODIFICATIONS</strong></td>
<td><strong>BIOSYNTHESIS OF CARBOHYDRATE MOITYES (SIALIC ACID, GLUCOSAMINES, GALACTOSE)</strong></td>
</tr>
<tr>
<td><strong>SULFATION, PHOSPHORYLATION, ETC. GLYCOSYLATION ONTO PROPER SITES</strong></td>
<td><strong>CORRECT FOLDING OF ACTIVE MOLECULE</strong></td>
</tr>
<tr>
<td><strong>PROPER DISULFIDE FORMATION</strong></td>
<td><strong>SECRETION OF ACTIVE MOLECULE</strong></td>
</tr>
<tr>
<td><strong>OTHER PROTEIN PROCESSING CAPABILITIES</strong></td>
<td><strong>BIOSYNTHESIS AND SECRETION OF COMPLEX POLYPEPTIDES</strong></td>
</tr>
<tr>
<td>(NO OTHER CHOICE: e.g. MONOCLONAL ANTIBODIES)</td>
<td>(NO OTHER CHOICE: e.g. MONOCLONAL ANTIBODIES)</td>
</tr>
<tr>
<td><strong>BIOSYNTHESIS OF CARBOHYDRATE MOITYES (SIALIC ACID, GLUCOSAMINES, GALACTOSE)</strong></td>
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</tr>
<tr>
<td><strong>SECRETION OF ACTIVE MOLECULE</strong></td>
<td><strong>PROPER DISULFIDE FORMATION</strong></td>
</tr>
<tr>
<td><strong>GENETIC SYSTEM FOR HETEROLOGOUS PROTEIN</strong></td>
<td><strong>Rapid method for expression of new proteins</strong></td>
</tr>
<tr>
<td><strong>STABILITY OF EXPRESSION FOR MANUFACTURING FDA ACCEPTANCE FOR THERAPEUTIC PROTEINS</strong></td>
<td><strong>STABILITY OF EXPRESSION FOR MANUFACTURING FDA ACCEPTANCE FOR THERAPEUTIC PROTEINS</strong></td>
</tr>
<tr>
<td><strong>AI DI NG ANALYTICAL AND PRODUCT PURIFICATION.</strong></td>
<td><strong>HYBRIDOMA FOR ANTIBODY PRODUCTION</strong></td>
</tr>
<tr>
<td><strong>RAPID ISOLATION AND PURIFICATION FOR ANALYTICAL USAGE</strong></td>
<td><strong>RAPID ISOLATION AND PURIFICATION FOR ANALYTICAL USAGE</strong></td>
</tr>
</tbody>
</table>
Figure 1.3: (a) 3-D structure of human IgG1 molecule (Clark, M: http://www.path.cam.ac.uk/~mrc7/lectures/models.html) and (b) The schematic model of an IgG1 (κ) antibody molecule showing the basic 4-chain structure and domains (VH, CH1, Vk, Ck, CH2 and CH3). The sites for enzymatic cleavage (papain and pepsin) are shown.
Mammalian cell culture - Advantages of tissue culture

Environment Control
- Defined physiochemical environment (growth media) - pH, temp, O₂ etc

Sample Homogeneity
- Tissue has mixture of cell types
- Homogenous sample identical over many generations
- Indefinite if stored in liquid nitrogen (cryo-preserved -196°C)

Economy
- Less cell culture required as directly exposed to reagents
- Avoid moral, legal and ethical issues with animal testing
Mammalian cell culture
Disadvantages of tissue cultures

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**Expertise**

- Strict aseptic conditions required
- Operator requires level of skill and knowledge
- Require specialised equipment – laminar flow cabinets, CO2 incubators etc

**Facilities**

- Some continuous cell lines loose differentiated function
- Result in unstable chromosome number

**Differentiation/instability**

- Major consideration in toxicology and research
- *In vivo* cells have 3D geometry, *in vitro* have 2D
- *In vitro* lose regulation of nervous & endocrine systems

**Difference *in vivo*-in *vitro**
Working with cell culture -

**Safety**

- Safe practice need to be established due to number of possible risks
- Biohazards need to be categorised correctly
  - Risk assessment
    - How the materials or equipment is used
    - Who uses it
    - The frequency of use
    - Training
    - General environmental conditions
- Handling hazardous substances, equipment and condition should have SOP (standard operating procedure)
Safety regulations

- Advice that might help in complying safety regulations
  - USA Dept of Health and Human Services, 1993
    - E.g: Biosafety in Microbiological and Biomedical Laboratories
    - E.g: General Laboratory Health and Safety (26 pages)
  - NIOSH, CDC, OhASIS
## Working with cell culture

### Equipment

<table>
<thead>
<tr>
<th>Essential</th>
<th>Beneficial/ additional</th>
<th>consumable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laminar flow cabinet</td>
<td>pH meter</td>
<td>Pipettes (glass or disposable)</td>
</tr>
<tr>
<td>Incubator/Incubator (CO2)</td>
<td>Cell counter</td>
<td>Culture vessels (T-flasks)</td>
</tr>
<tr>
<td>Autoclave/steriliser</td>
<td>Magnetic stirrer</td>
<td>Pipette tips</td>
</tr>
<tr>
<td>Refrigerator/freezer</td>
<td>Fluid aspirator</td>
<td>Eppendorf tubes</td>
</tr>
<tr>
<td>Inverted microscope</td>
<td>Fluid dispenser</td>
<td>etc</td>
</tr>
<tr>
<td>Upright microscope</td>
<td>Glassware washing unit</td>
<td></td>
</tr>
<tr>
<td>Purified water</td>
<td>Video camera and monitor</td>
<td></td>
</tr>
<tr>
<td>(deionised double distilled)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centrifuge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquid N2 tank</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Balance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemocytometer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Media filter unit</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 3: Flowchart showing methods for obtaining each type of cell culture.

How to obtain cell/tissue culture
Origin of Hybridoma cell lines

Myeloma cell types (tumour cells) × Spleen cells (B-lymphocytes-antibody producing cells)

Cell fusions

Hybridoma cells

<table>
<thead>
<tr>
<th>Hybridoma</th>
<th>NSO</th>
<th>NS1/1</th>
<th>SP2/0-Ag14</th>
<th>P3-X63 Ag8.653</th>
<th>P3-X63 Ag Ul</th>
</tr>
</thead>
<tbody>
<tr>
<td>properties</td>
<td>No IgG</td>
<td>Light chain</td>
<td>No IgG</td>
<td>No IgG</td>
<td>Light chain</td>
</tr>
</tbody>
</table>
Cell banking-
Liquid Nitrogen Tank (-196°C)

Cell culture (10⁶⁻⁷ cell/ml)
→
Cryo-preserved liquid
(DMSO or Glycerol, serum)
→
Deep freezing
(LN₂)
World Cell Culture Collection Centers

American Type Cell Collection
http://www.atcc.org/
The European Collection of Cell Cultures
http://www.ecacc.org.uk/
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (German Collection of Microorganisms and Cell Cultures)
http://www.dsmz.de/dsmzhome.htm
Japanese Collection of Research Bioresources (JCRB)
http://cellbank.nihs.go.jp/
Mammalian Cell culture systems
Applications

- Production of various proteins
  - Widely used producer cell lines and its products (handout 1)
- Tissue equivalents
- Organ repair/transplant
- Alternative toxicity testing
- Cloned babies!!!!!!!
Mammalian Cell cultivation systems

1. Batch cultures
2. High density cultures
3. Continuous cultures
4. Type of bioreactors
   1. stirred tank bioreactor
   2. Airlift bioreactor
   3. Hollow fibre bioreactor
5. Microcarrier systems
6. microencapsulation, packed bed etc.
<table>
<thead>
<tr>
<th>System</th>
<th>Maximum Concentration mg/l</th>
<th>Productivity mg/week</th>
<th>Required for the production of 1 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vivo</td>
<td>2,200</td>
<td>2</td>
<td>180 mice</td>
</tr>
<tr>
<td>In vitro</td>
<td>42</td>
<td>7</td>
<td>400 T-flasks</td>
</tr>
<tr>
<td>T-flask</td>
<td>47</td>
<td>180</td>
<td>39 days</td>
</tr>
<tr>
<td>Stirred-tank bioreactor, batch</td>
<td>120</td>
<td>250&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28 days</td>
</tr>
<tr>
<td>Stirred-tank bioreactor, fed-batch</td>
<td>1,600</td>
<td>1,400&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26; 5 days&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Table 1. Antibody production in different bioreactor systems**

Eg: CelliGen Plus, an all-purpose stirred-tank bioreactor
CSTR (continuous stirred tank reactor)

http://www.accessexcellence.org/LC/SS/ferm_graphics/reactor.html
A 5 litre stirred tank fermenter, equipped with 3 Rushton turbines or with 3 Intermig impellers (B.Braun).

Lonza Biologics, Slough, UK. The hall containing three airlift bioreactor with a total fermentation capacity of 6,500 liters in a range of process scales from 100 to 2,000 liters.
Air lift fermenter

-The usual height to diameter ration is 10/ 1 to 40/ 1

-no mechanical means of agitation.
(turbulence within the culture is brought about by injection of pressurized air mainly from the bottom. To enable efficient circulation the design operates with loops either as external loop or as internal loop (so called 'draught tube').

-also known as the 'pressure cycle fermenter'.
(since the growth medium undergoes successive cycles of high and low pressure ) main disadvantage lies in the ascending air bubbles will increase in size in the top area of the fermenter (due to the decrease in hydrostatic pressure). Since large bubbles transfer oxygen less efficient than small bubbles this results in the generation of a sub-optimal oxygen supply in the top area.
TECNOMOUSE - perfusion system for the long-term cultivation of cells

The heart of TECNOMOUSE is the exchangeable CultureCassette - a miniature bioreactor. Ideal cultivation conditions are guaranteed by optimal gas supply and media utilisation.

TECNOMOUSE is a modular design and comprises:

- A removable, programmable control unit for ease of operation.
- A modular portable rack for up to 5 Culture Cassettes.
- A media pump.
- An integrated gas supply unit that guarantees adequate gas flow to the membranes.

SEM graph of the cross-sectional structure of annular PSf/PESf hollow fiber.
NASA Bioreactor

- For growing cells in zero gravity
- A rotating chamber cells grow in suspension
- Simulated zero gravity achieved by slowly rotating the chamber, altering the sedimentation vector continuously.
- The cells remain stationary, subject to zero shear force, and tend to form three-dimensional aggregates, which, reputedly, enhances product formation.
- When the rotation stops, the aggregates sediment, and the medium can be replaced.
Example of an actual Home-made Bioreactor Run
Cell line:
YTS 177.9.6.1 rat IgG2a anti-CD4 (DA spleen x Y3/Ag1.2.3 hybridoma)
NB. This is a reliably good producer by all methods (eg 100 micrograms/ml in roller culture)!
Cartridge:
Initial seeding with 2x10**8 cells only - this took 2 weeks to reach optimal production and we would normally use more cells.
Medium:
Total usage over 6 weeks: 40L (cost 80 pounds)
Harvest:
Total harvest over last 4 weeks: 2.15L (average of 77ml/day)
Production:
Total mAb after 50% SAS purification: 3.2 g (avg. of 114mg/day at greater than 1.5mg/ml)
N.B. final mAb greater than 90% pure [native gel].
Other costs:
Estimate 40 pounds per litre of harvest for cost of nutrition bags, FCS, filters, syringes etc.
Total cost:
80 pounds; consumables + 80 pounds; medium + 12 pounds; cartridge (AltraNova 140) = 172 pounds total (for 3.2 g)
ie. approx. 50 pounds per gm (excluding labour/purification/hidden and indirect costs).

http://www.molbiol.ox.ac.uk/pathology/tig/mprod.html
Tissue cultures-organ replacement

- Skin
- Artificial organs
- Biomaterials
- Cartilage
- Blood substitutes
- Organs
- Vascular grafts

Research projects on the listed tissues. Refer handouts
Tissue Equivalents-Skin cells

- Dermal equivalents
  - Skin repair
  - To study efficacy, metabolism and toxicity of drugs
Structure of the Skin

Structure of the epidermis. Cells stratify into corresponding layers according to differential stages of keratinocytes.
Autografting Technique

Classical
Removal of patient's own skin from donor sites for grafting. Accompanies morbidity, pain, scarring and sometimes insufficient. Skin from donor site has to be at least the same size as burn site.

Cultured Keratinocyte Sheet
Large number of sheets can be grown from a small piece of patient's normal skin.
1. **Type:** Normal human epidermal keratinocytes (NHEK).
2. **Genetic make-up:** Single donor.
3. **Derived from:** Neonatal-foreskin tissue.
4. **Alternatives:** NHEK from adult breast tissue.
From Whole Skin to Cultured Skin: Possible Methods

whole skin → epidermis + dermis

Irradiated 3T3 → keratinocytes

fibroblast

collagen gel
Processing of Initial Skin Biopsy into Single Cells

1. Separation of epidermis from dermis

3. Filtering to exclude crude tissues, followed by centrifugation and cell count.

4. About $1 \times 10^6$ cells from $1\text{cm}^2$ skin biopsy $\Rightarrow$ 4 to 5 T-75 tissue culture flasks (3000-5000 times total area expansion).
Fully grown keratinocytes will form a transparent skin sheet in the culture dish. Using the enzyme dispase, the graft sheet is detached from plastic within 30-45 minutes. At this stage, a petrolatum guaze is used as backing dressing to support the graft and preserve the orientation. The graft is detached and then transferred to the operating room under sterile conditions.
Procedures for In Vitro Production of Bovine Embryos

R.M. Rivera, J.L. Edwards, A.D. Ealy, V.M. Monterroso, A.C. Majewski, and P.J. Hansen
Dept. of Animal Sciences, University of Florida

The procedures for in vitro production [IVP; i.e. in vitro maturation (IVM), in vitro fertilization (IVF), and in vitro culture (IVC)] of embryos described here are based on procedures developed in other laboratories at the University of Wisconsin (Parrish et al., 1986), University of Guelph (Xu et al., 1992), and University of Missouri (Hernandez-Ledezma et al., 1993). These procedures as used by our group have been published previously (Edwards et al., 1997; Paula-Lopes et al., 1998). Keep in mind that the protocols described here are not fixed but rather constantly evolve as new developments take place. Therefore, practitioners of IVP will be well advised to experiment with the procedures used, especially after reading of improvements made by other laboratories. This protocol is organized by day of the protocol with d 0 being the day of fertilization.

DAY-BY-DAY PROCEDURE
Day -2: Getting Prepared
Day -1: Collection of Ovaries | Oocyte Collection | Preparation of IVF Medium
Day 0: In Vitro Fertilization
Day 1-9: Culture of Embryos
Biomaterial-
Artery, knee cartilage replacement up for FDA approval (Salubria)

A unique biomaterial developed for patients needing artery or knee cartilage replacement. It may also be used to speed repair of damaged nerves in patients with spinal cord injuries and as the basis for an implantable drug delivery system. It is biocompatible with body tissue because of

- its attraction to water,
- mechanical strength can be adjusted as needed,
- it is compliant like normal body tissue
- it is made from an organic polymer (Hydrogel), rather than silicone.
Left image: fabrication of various geometric shapes of sapeptide scaffolds. (A) The tape is approximately 8cm long, 0.5cm wide and 0.3mm thick. (B) The rope is about 2mm in diameter. (C) Membrane form.

Right image: molecular models of the ionic self-complementary peptides that form distinct polar and nonpolar sides. These peptides undergo molecular self-assembly to form the nanofibers that further assemble into scaffold.

Images courtesy Nature

This peptide-based scaffold, on which neurons grow fibers to communicate with each other and establish functional synapses, ideal medium for growing replacement nerve cells

CAMBRIDGE, Mass. -- Researchers at the Massachusetts Institute of Technology and New York University report in the June 6 issue of the Proceedings of the National Academy of Sciences (PNAS) that they have made a biomaterial that supports living nerve cells.
Cell culture—Phytochemical testing
(Dosage effects of MHCP extract on growth of TB/C3.bcl2 cell culture)
Thank You For Your Kind Attention