Overview of Animal-Derived Cell Culture Technology

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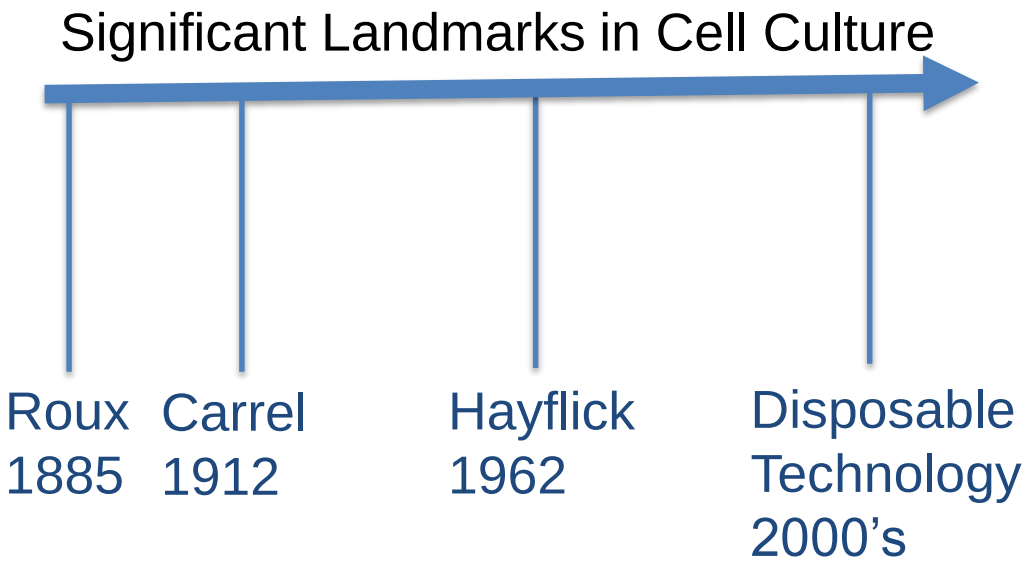
Overview of Animal Cell Culture

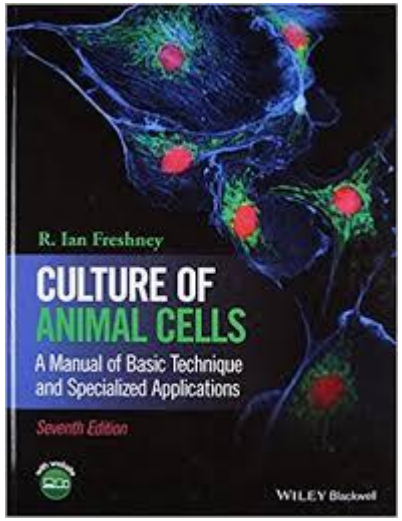
- Significant landmarks
  - Isolation
  - Aseptic technique
  - Disposable plastic-ware
  - Cell line development
  - Bioreactor
    - Disposables

Carrel Laboratory, ~1938

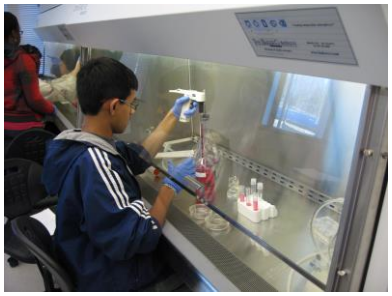


Landmarks

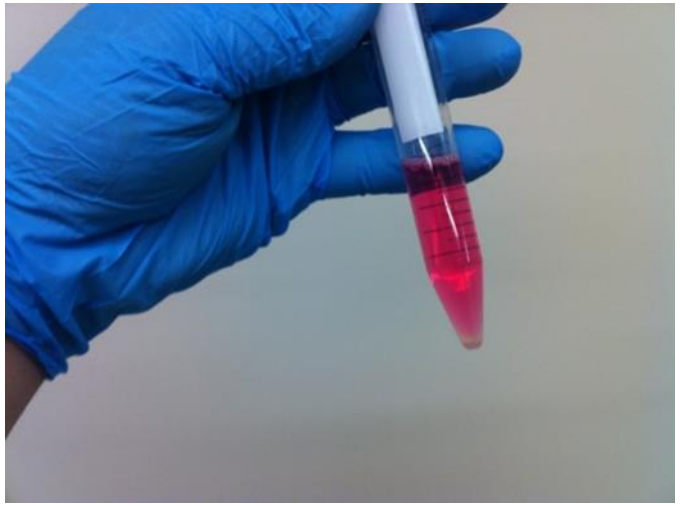
- Aseptic technique
  - Textbooks
    - 1980's
- Plasticware



State of the Art



- Animal cell culture
  - Well established
    - Techniques
    - Failure points/monitoring
- Range of products?
  - Cells
    - Muscle protein
      - Maybe?
    - Cells mixed with plant material
    - Microcarriers?
      - Edible or inedible
  - Biomanufactured muscle



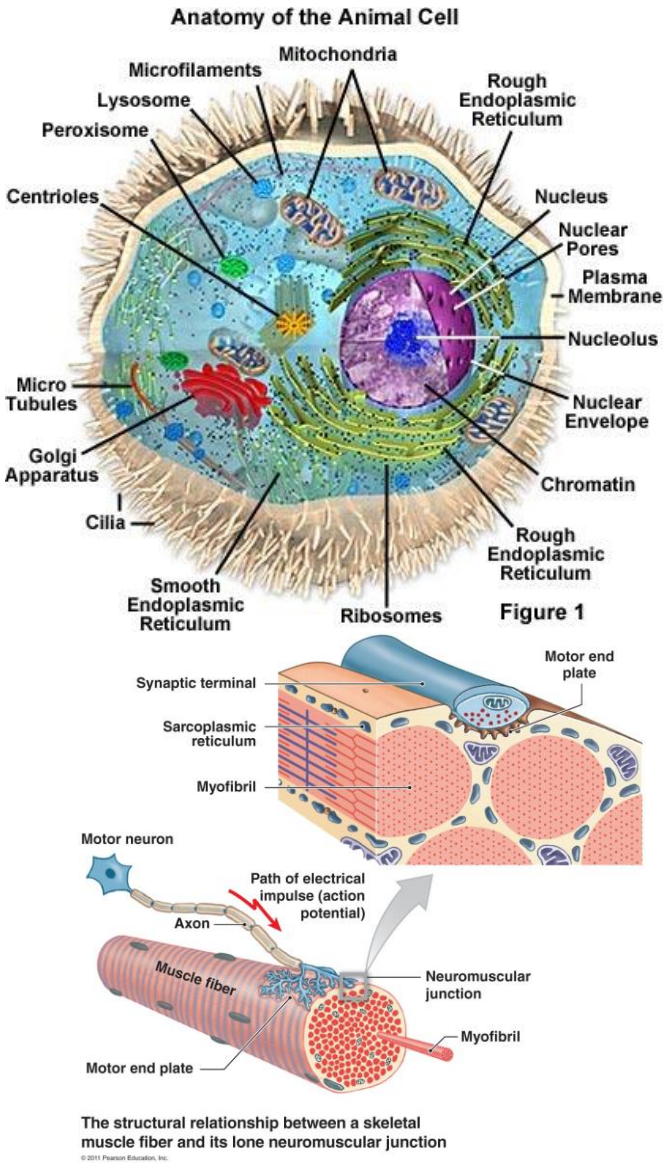
Types of Products
Central Feature For Consideration

- Formed post-harvest into an edible “meat-like” product
  - Upstream technology
    - Established
      - Avian embryonic stem cells
    - Selected cell lines adapted to suspension
    - Serum-free media
    - Qualified master cell banks
    - Guidance
      - Vaccine production-2010

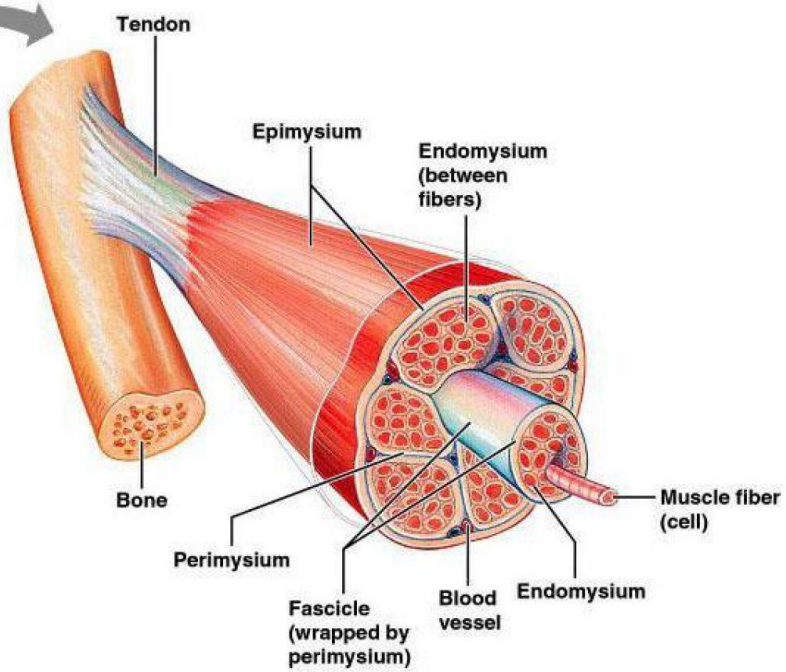
- Biomanufactured muscle
  - Specific challenges
    - Technology
    - Regulatory

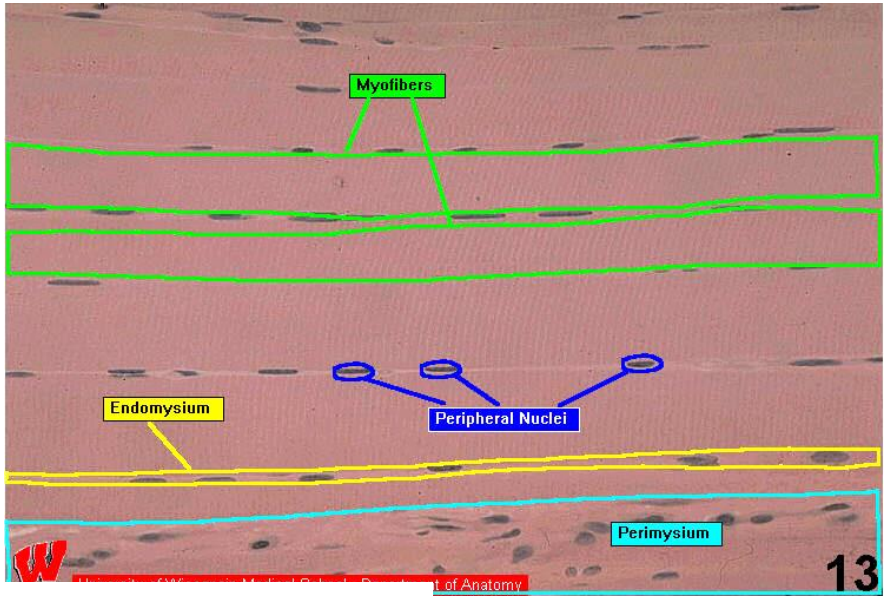
Biomanufactured Animal Cell Protein

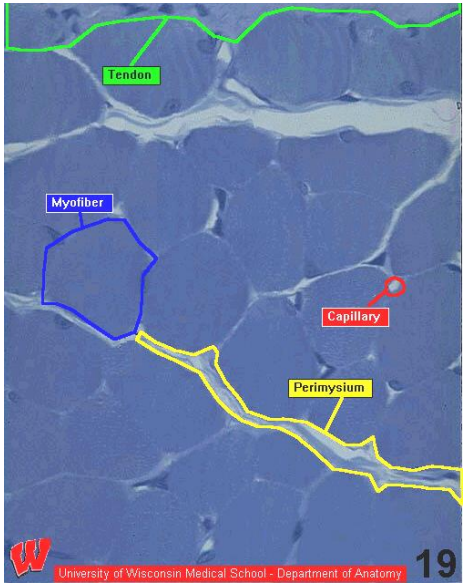
- General animal cell
  - Single nucleus
  - Red blood cell
    - 6 to 8 microns
  - White blood cell
    - 12 to 15 microns
- Muscle fiber/muscle cell
  - Multinucleate
  - 1 to 40 millimeters in length
  - 10 to 100 microns in diameter
  - Muscle architecture
    - Connective tissue/scaffolds
  - Adipose
  - Vasculature

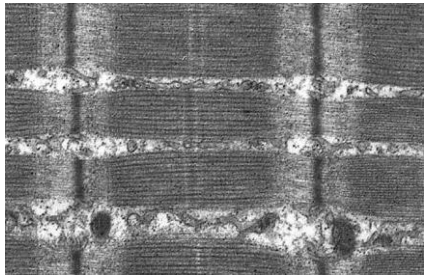


Structure of Muscle

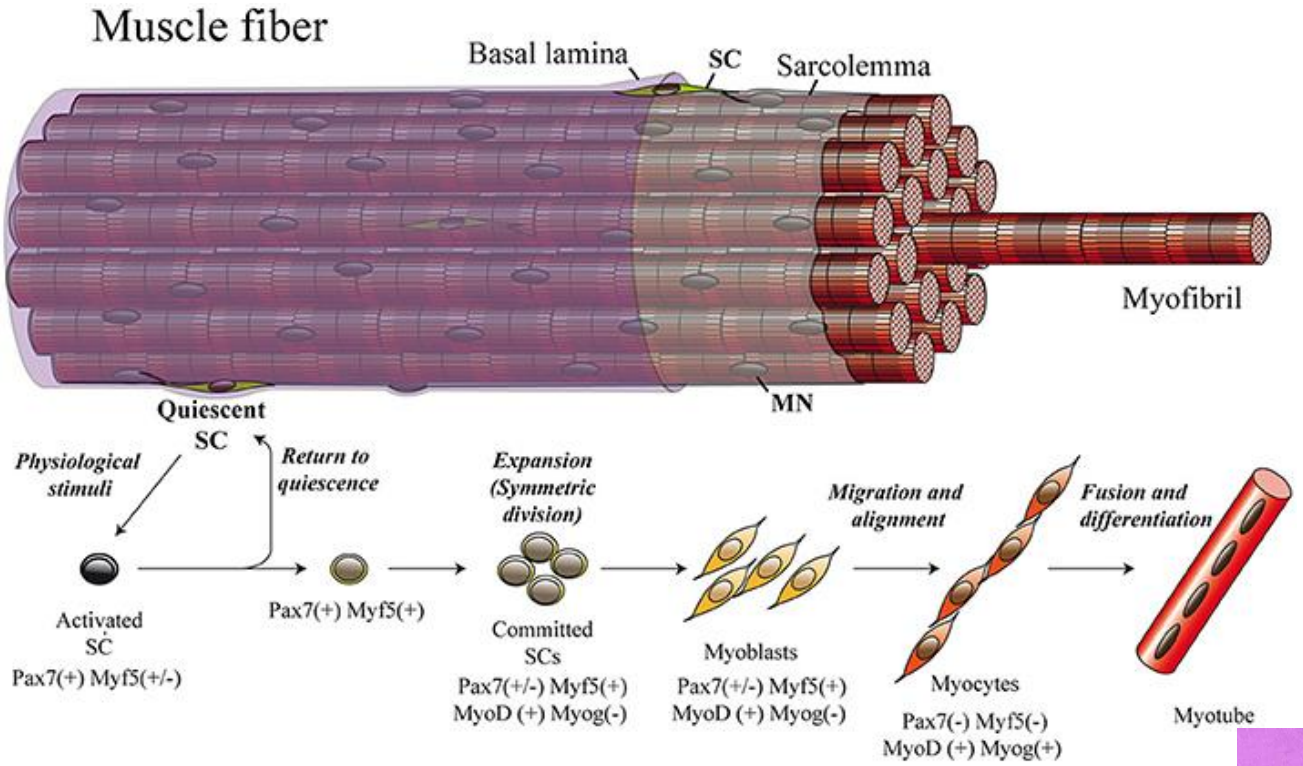








Review of Myogenesis

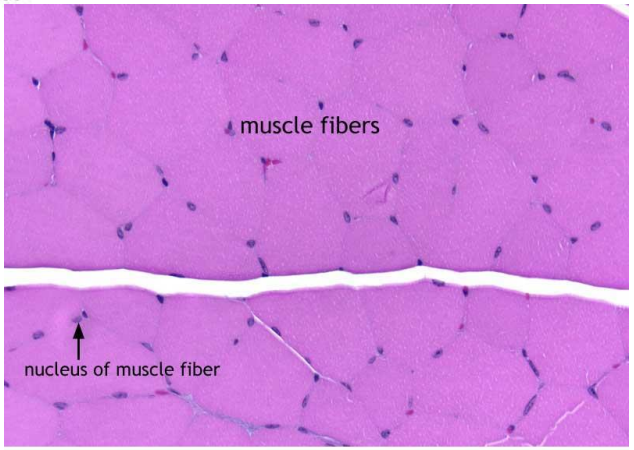


Hernendez-Torres et al. 2017

Satellite Cells (SC) lie in close proximity to the muscle fiber.

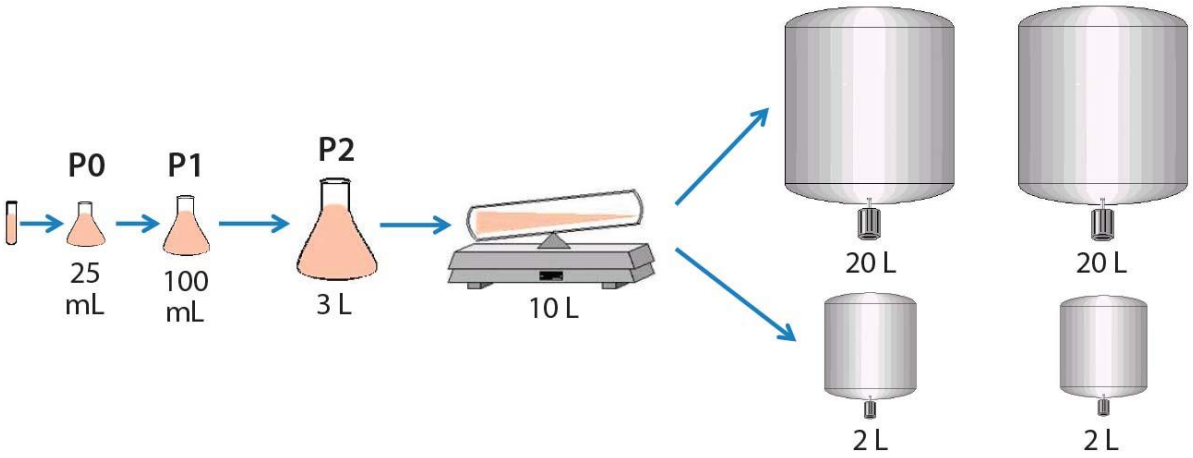
Rebuild Muscle

Donate nuclei



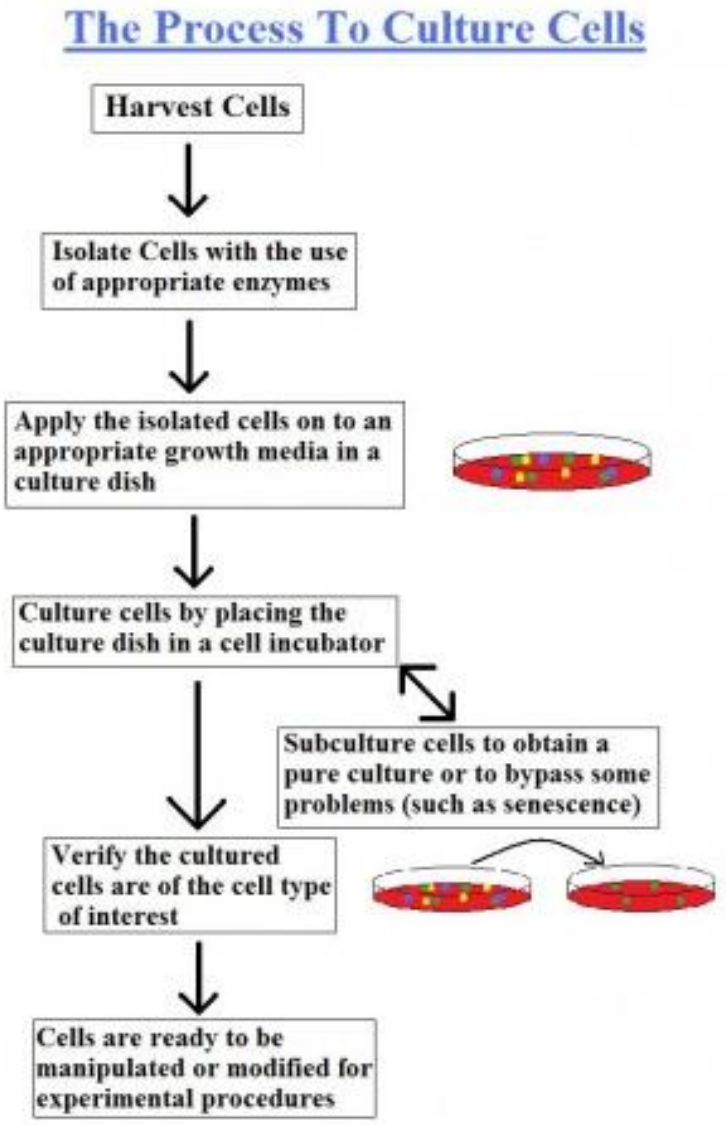
Myogenesis In Vitro

- Biomanufacturing challenge
  - Myoblasts
  - Differentiation
  - Connective tissue



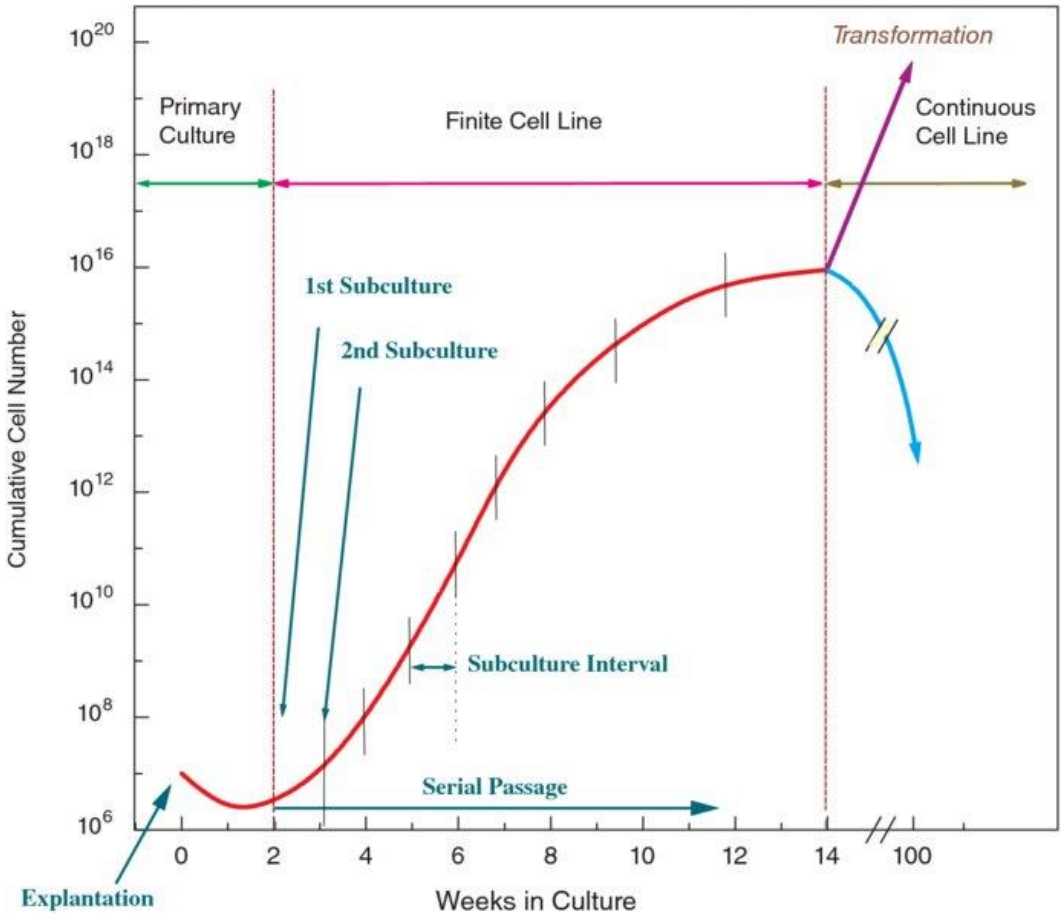
Animal Cell Culture General Procedure

- Isolate cells
- Primary isolates
- Establish cell lines
  - Potentially optional step
  - Finite cell lines
  - Immortalized
    - Naturally occurring
      - Select
      - Stem cells/primordial germ cells
    - Modified
- Media
  - Classical
  - Serum free



Concept Of Immortalization

- Finite cell lines
  - Not Immortal
- Immortalize cell lines
  - Selected
  - Naturally occurring
    - Stem cells
  - Genetic modifications



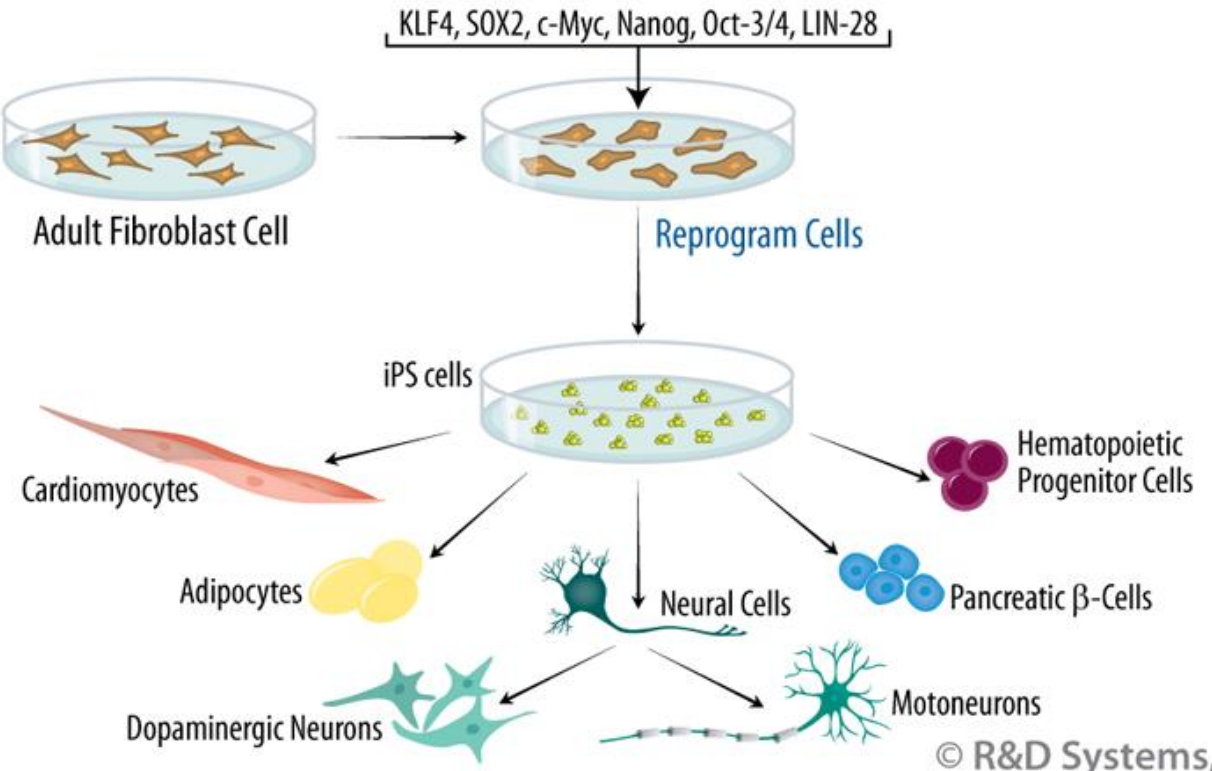
Cell Line Development

- Well established
  - Selection
    - Not genetically modified
      - C2C12 and L6
      - 1977 and 1970
  - Stem cells
    - Not genetically modified
  - Genetic modifications

Name	Species and tissue	Morphology	Author and year of origin
L929	Mouse connective tissue	Fibroblast	Earle, 1948
HeLa	Human cervix	Epithelial	Gay, 1951
CHO	Chinese Hamster ovary	Epithelial-like	Puck, 1957
MDCK	Canine kidney	Epithelial	Madin and Darby, 1958
WI-38	Human lung	Fibroblast	Hayflick, 1961
BHK-21	Syrian Hamster kidney	Fibroblast	Macpherson and Stoker, 1961
Vero	African Green Monkey kidney	Epithelial	Yasumura and Kawakita, 1962
NIH 3T3	Mouse embryo	Fibroblast	Todaro and Green, 1962
MCR-5	Human lung	Fibroblast	Jacobs, 1966
SH-SY5Y	Human neuroblastoma	Neuroblast	Biedler, 1970

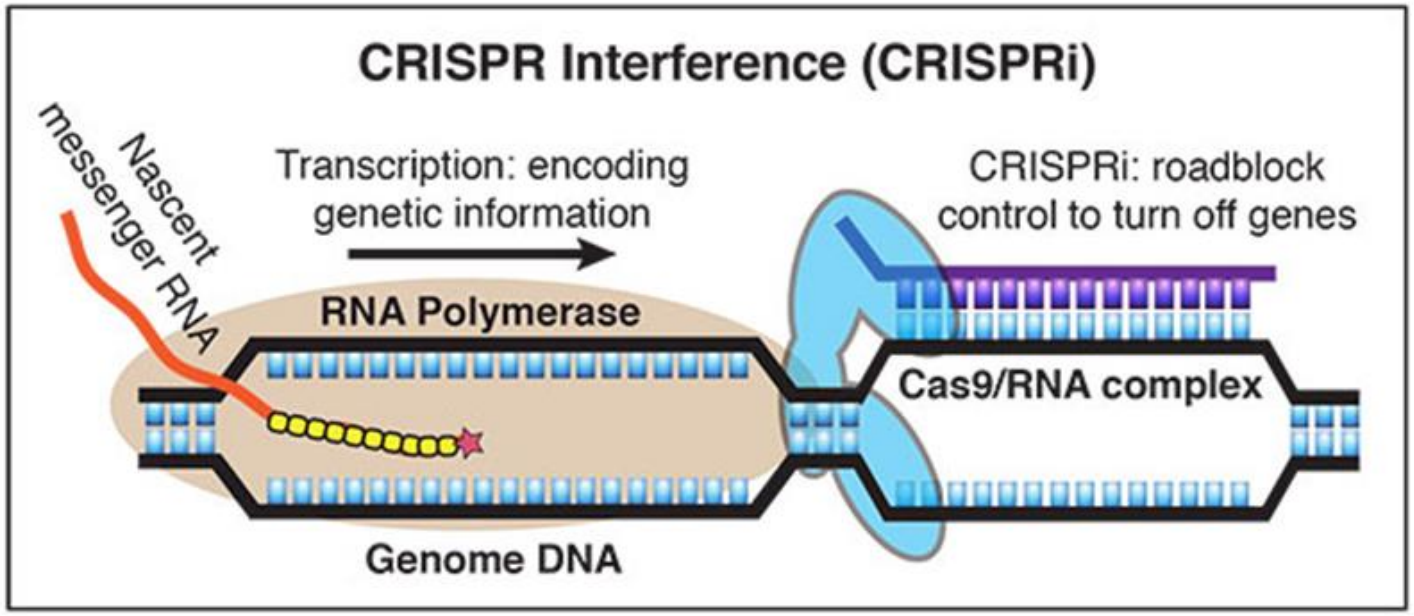
Cell Line Development

- Induced Pluripotent Stem Cells (IPSC)
  - Stem cell characteristics
    - Immortalization
  - Introduce genes
    - Transfection reagents
    - DNA



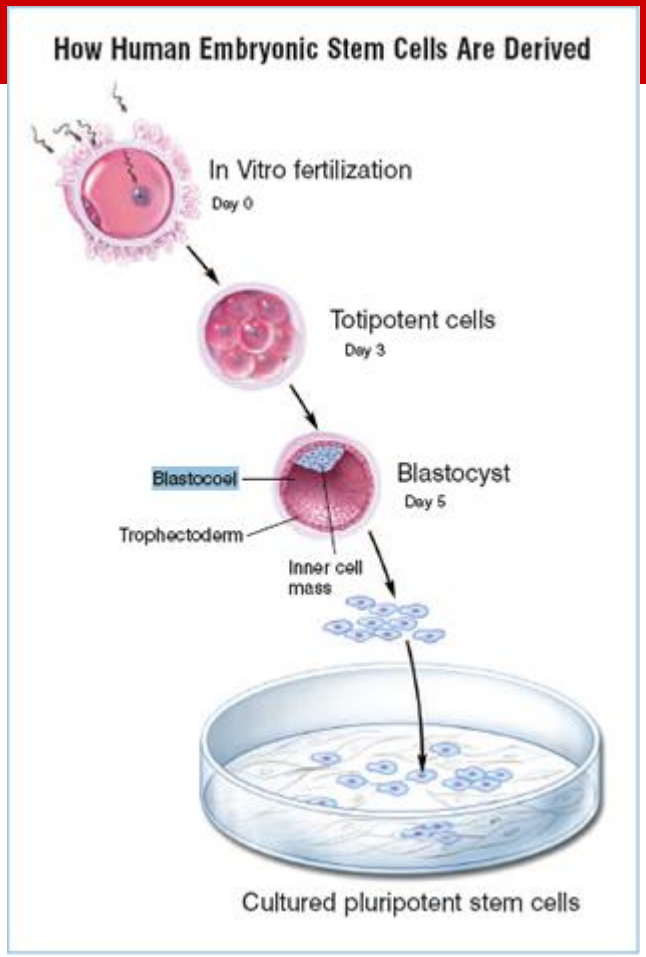
Cell Line Development

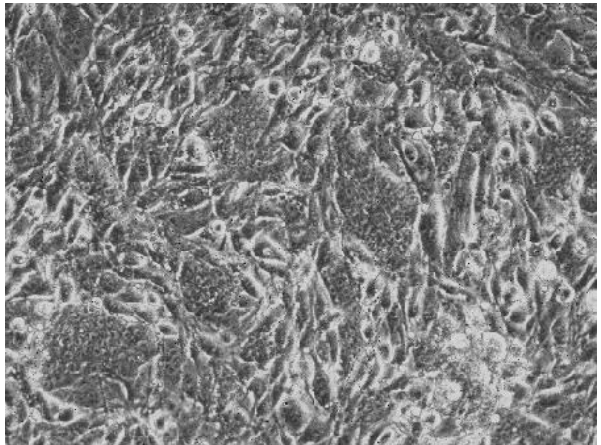
- Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)
  - Transfection reagents
    - Liposome, polymeric
- Genetically modified animals
- Remove specific DNA segments
  - Add genes?



Embryonic Stem Cells

- Avian embryo
  - Simple isolation
  - Feeder layer's
    - STO
      - Inactivated
- Sterile environment
- Immortalized
  - Not genetically modified
  - Established technology
    - Petitte et al. 2004 and Petitte et al., 1997 USPTO 5,656,470

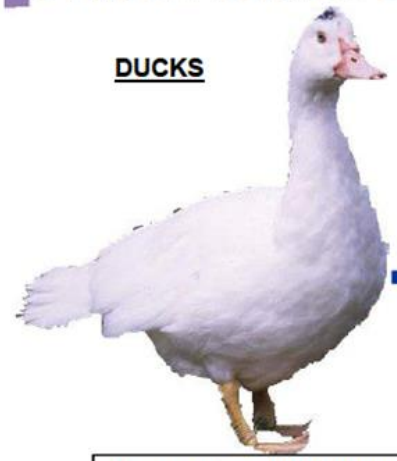




Derivation of avian EB66 cell lines

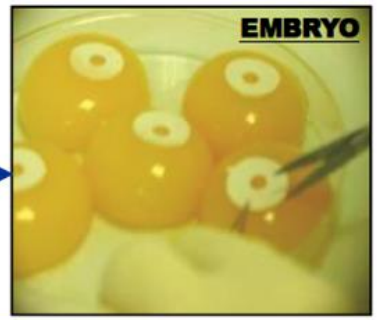
Phase I: isolation of avian ES cell

From cells to therapeutics Vivalis



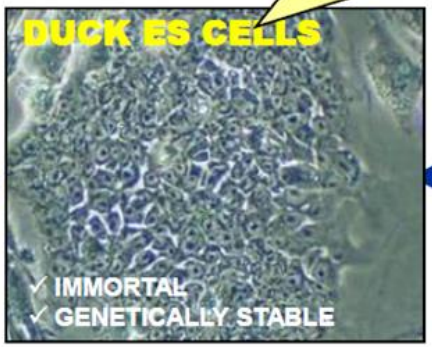
No reports on endogenous retroviral particles

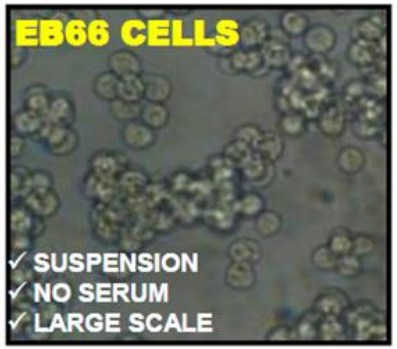
22,000 eggs



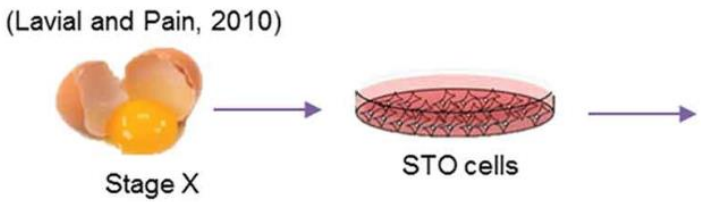
CRITICAL PARAMETERS

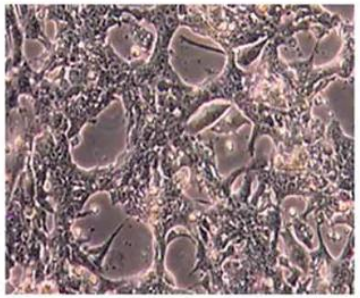
- Cell Culture Medium
- Cell Growth factors
- Feeder Cells
- Temperature
- Cell Dissociation Agents…



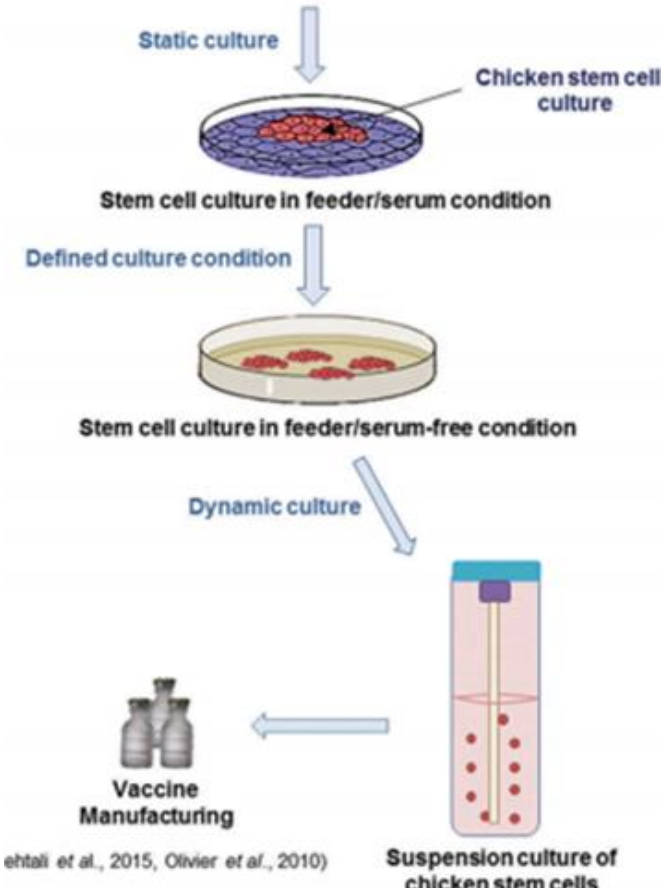


Suspension Embryonic Stem Cells



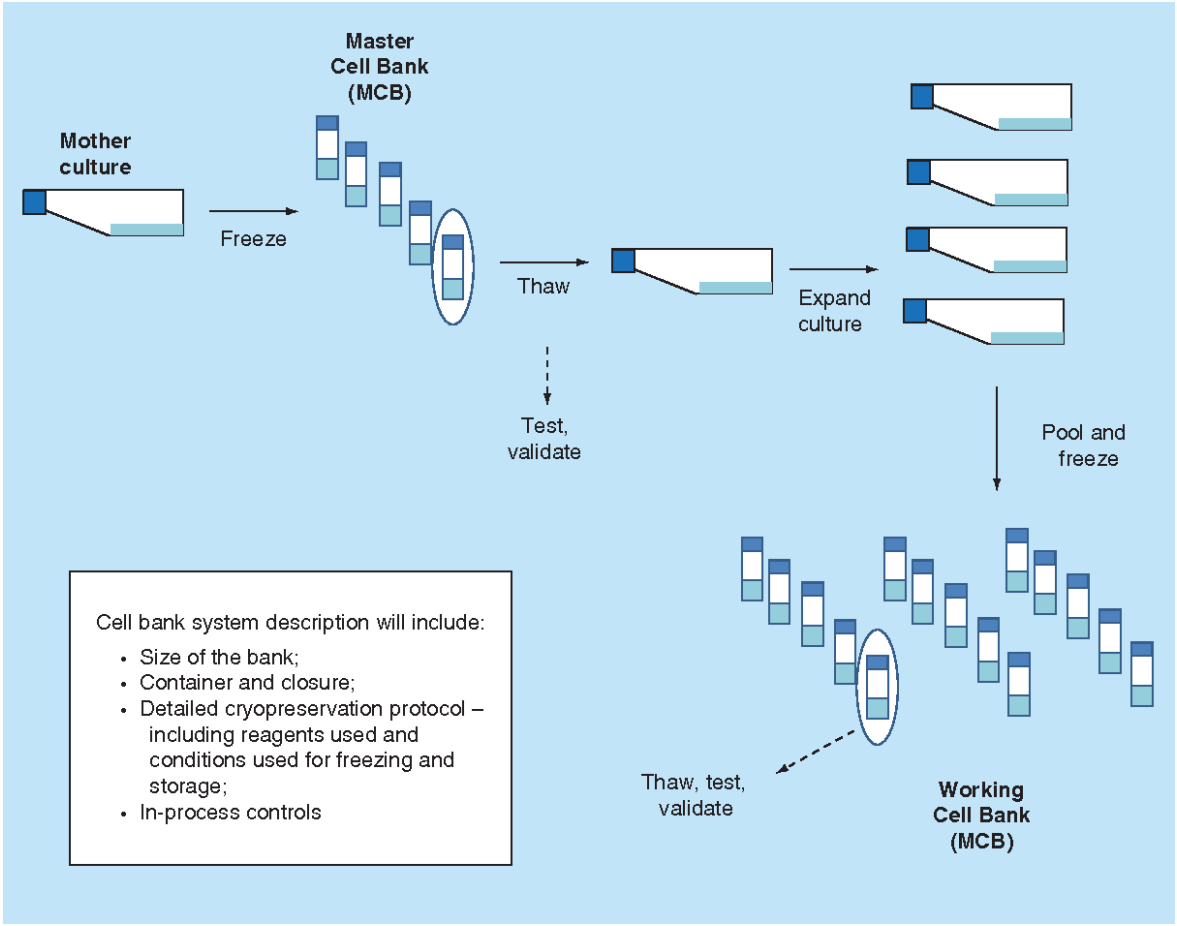

Chicken ESCs

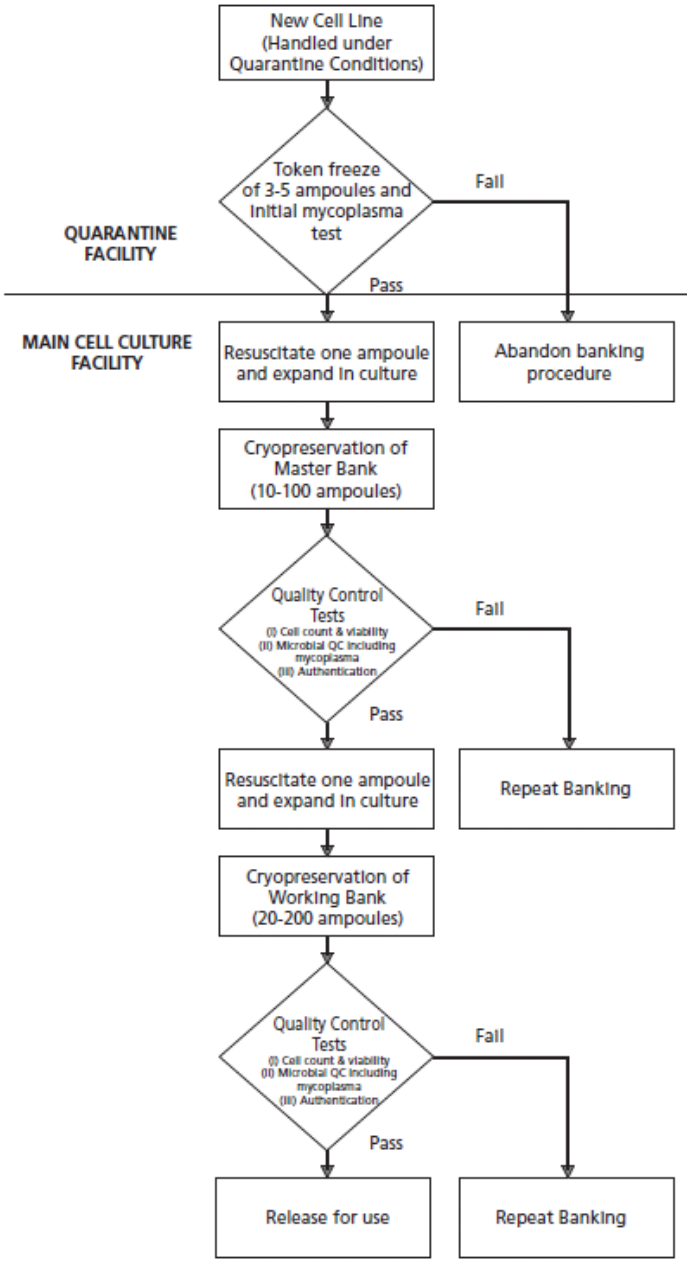
- Adapt to suspension
  - Grow under agitation
    - No modifications
    - Bioreactors
- Serum free media
  - Buffers
  - Amino acids
  - Vitamins
  - Recombinant growth factors



Cell Banking General Procedure

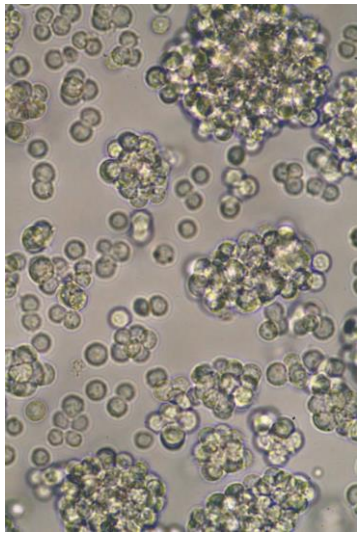
- Master cell bank
- Working cell bank

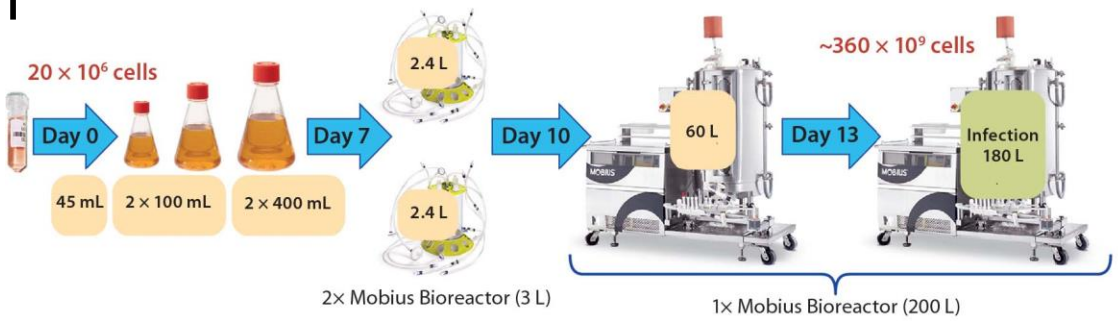


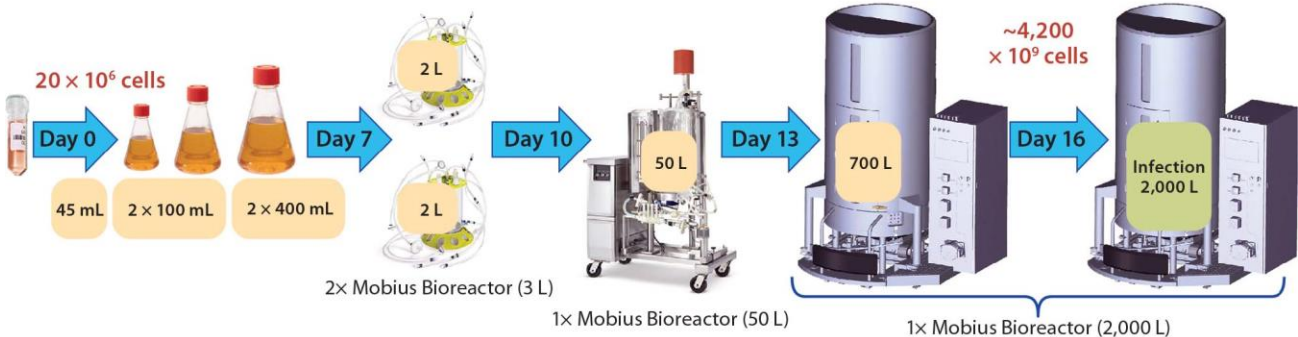


Process at Scale

- Grow cells
  - Suspension efficient for scale-up
    - Seed on scaffolds
    - Use directly
- EB66 duck embryonic stem cells
  - Bioreactor
  - Contamination
    - Personnel

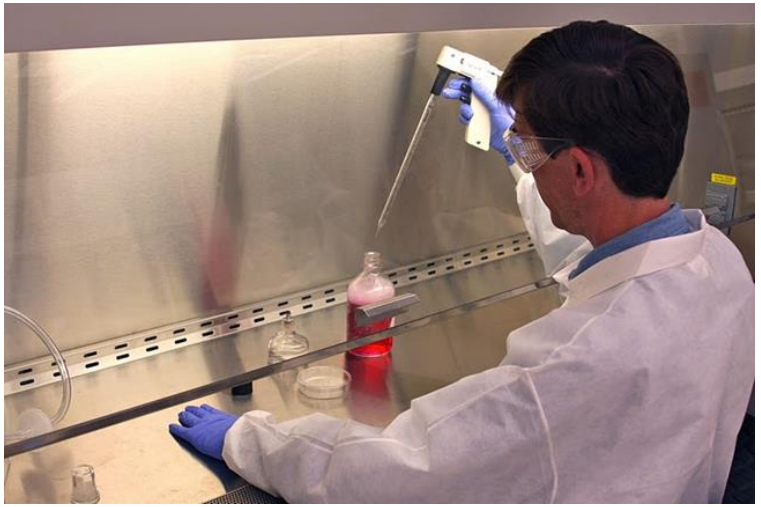






Future

- Embryonic stem cells
  - Regulatory framework exists
  - Serum-free media exists
    - Avian
    - Animal component free
    - EX-CELL® EBx™
- Other production platforms
  - Technological issues
  - Regulatory issues
    - Cell source
    - Serum
    - Process
      - Contaminants



Biomanufactured Muscle

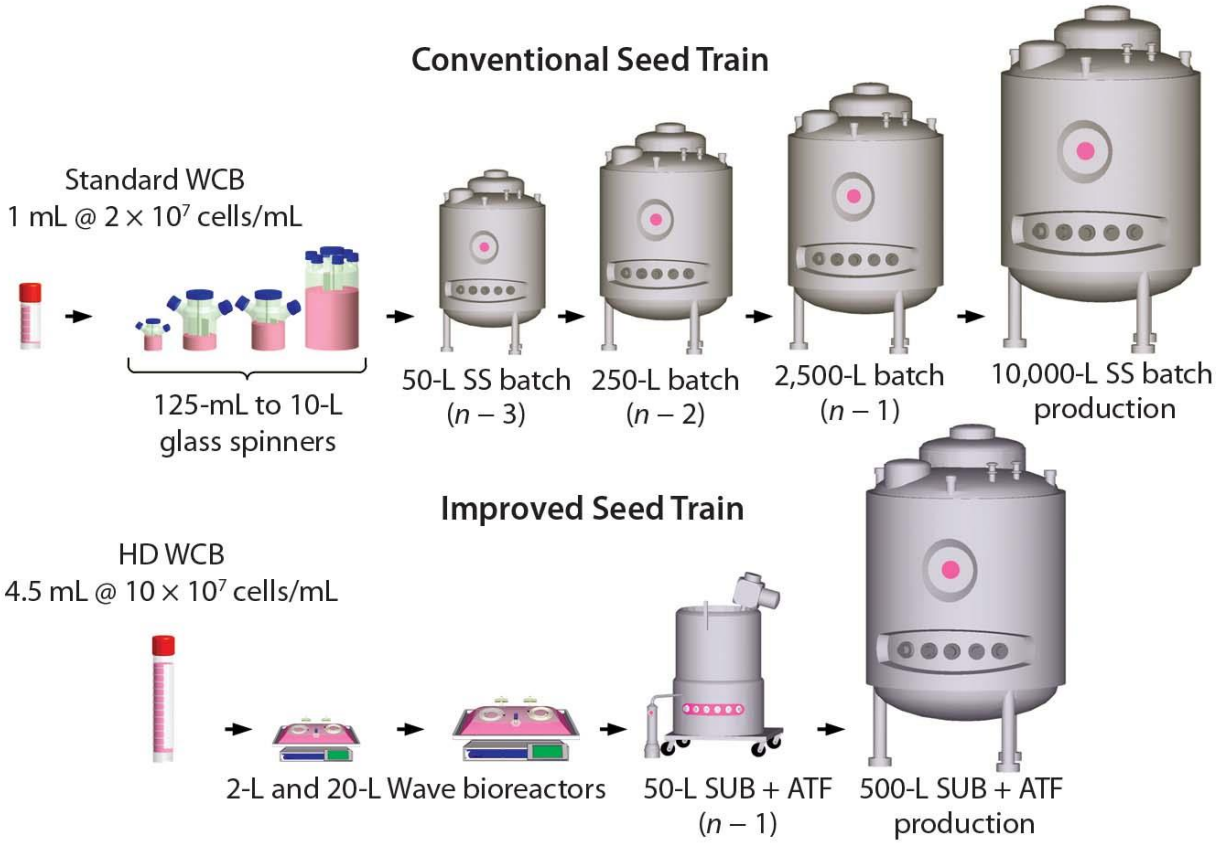
- Cells committed to forming muscle
- Mammalian species are not immortalized
- Issues
  - Animal
  - Harvest
    - Collection fluid
  - Isolation of cells
    - Enzymes
      - Trypsin
      - Collagenase
      - Pronase
      - Dispase
      - Recombinant or classical
      - Routine cell culture
  - Validated cell lines
    - Master cell banks

Classical Media

- Basal medium
  - Empirical
    - Dulbecco's, Ham's, Eagle's
  - Defined
    - Vitamins, amino acids, lipids, nucleosides, inorganic salts
  - Bicarbonate buffer
    - Carbon dioxide
- Serum undefined
  - Growth factors
  - Lot to lot variation
  - Viral contamination
  - Commercial interest?
  - Mycoplasma
- Antibiotics
  - Penicillin-Streptomycin
  - Gentamicin
  - Zero

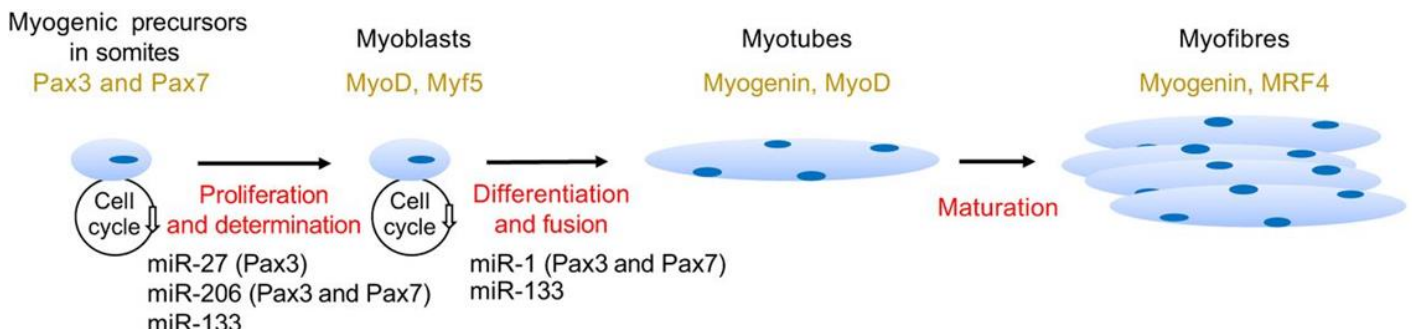
Serum-Free Medium

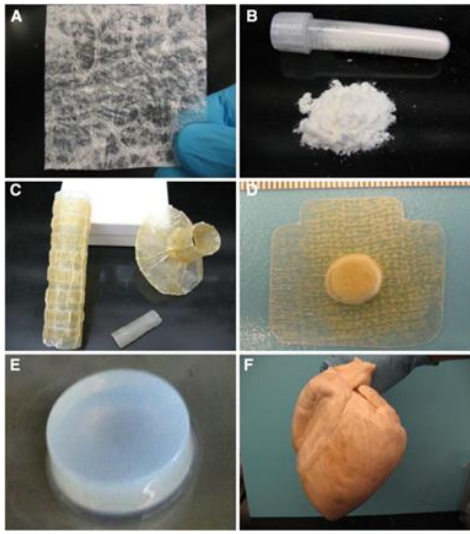
- Components of classical media
  - Additionally
    - albumin, transferrin, fibronectin,
    - recombinant derived growth factors
  - Defined
  - Proliferation
    - Serum-based
    - Serum-free



Scaling-Up

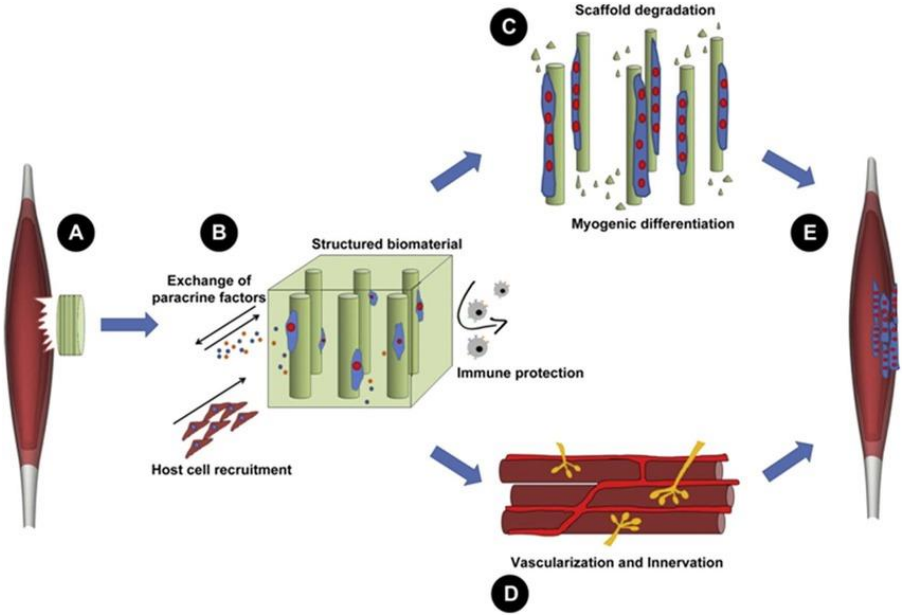
- Bioreactor
- Roller bottle
- Scaffold
- Differentiate
  - Reduce serum/growth factors
- Coatings
  - Roller bottle or scaffold
    - Collagen
    - Fibronectin
    - Laminin
    - ECM components

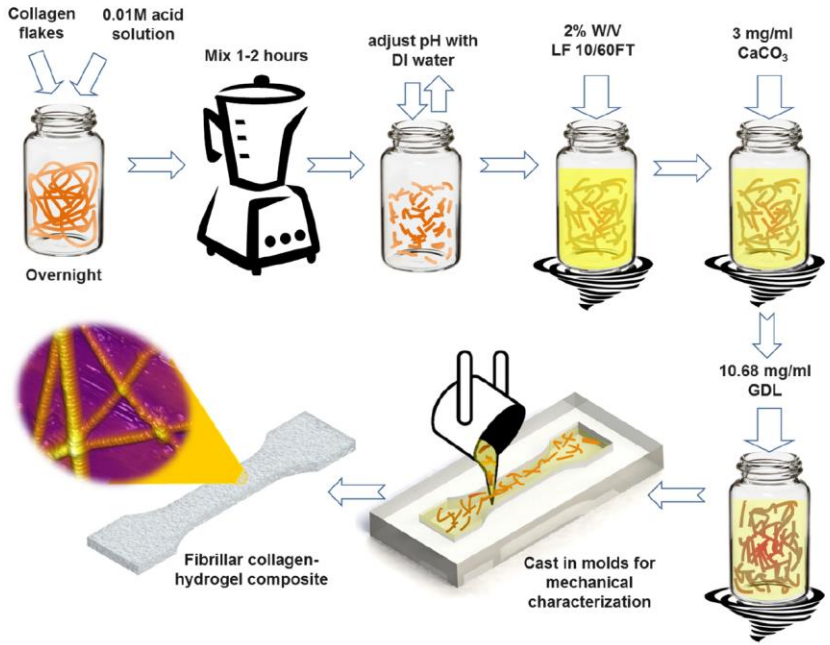


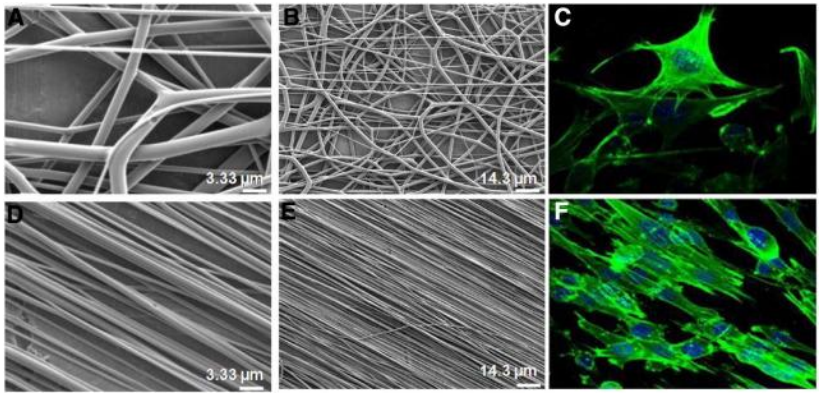


Myogenic Cell Lines

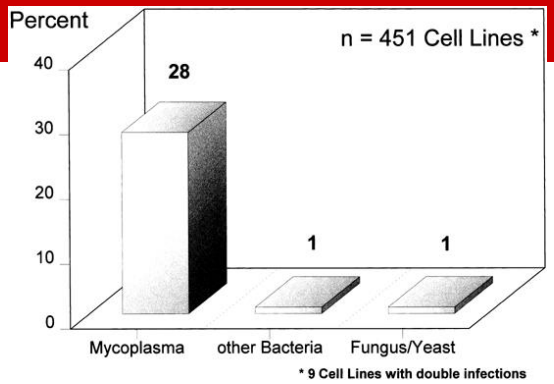
- Scaffold
  - Interest in plant materials
  - Hydrogels
    - Edible materials
    - Alginate, agarose, collagen, fibrin



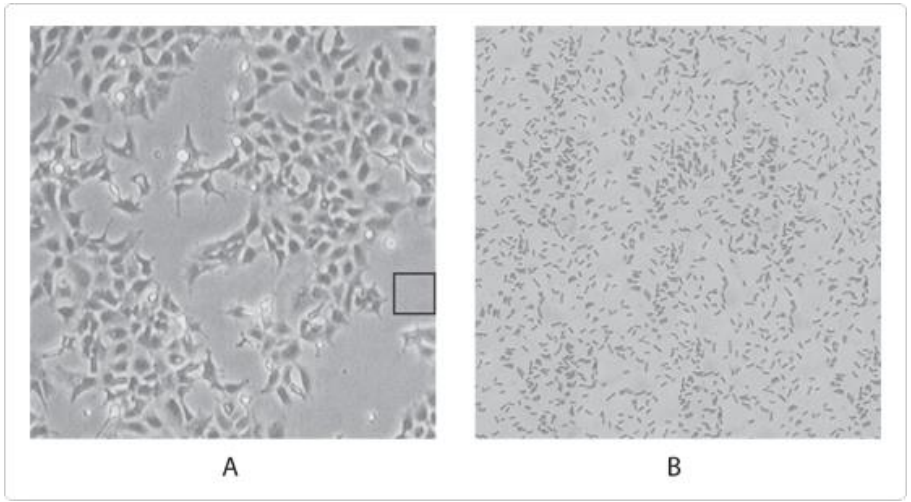


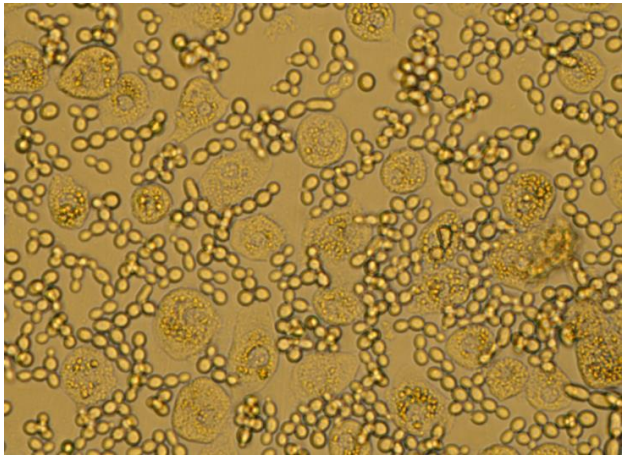


Contamination



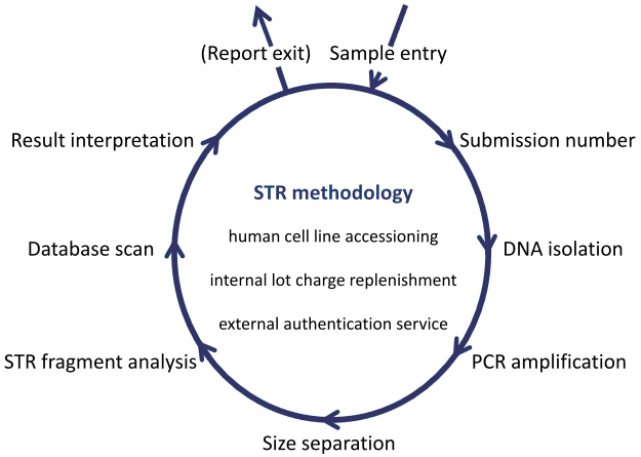
- Biological
  - Personnel
  - Bacteria
    - Yeast
  - Mycoplasma
  - Viral
- Validated cell banks
- Post-production media test
- Chemical
  - Uncommon
    - Plasticizers
    - Metals

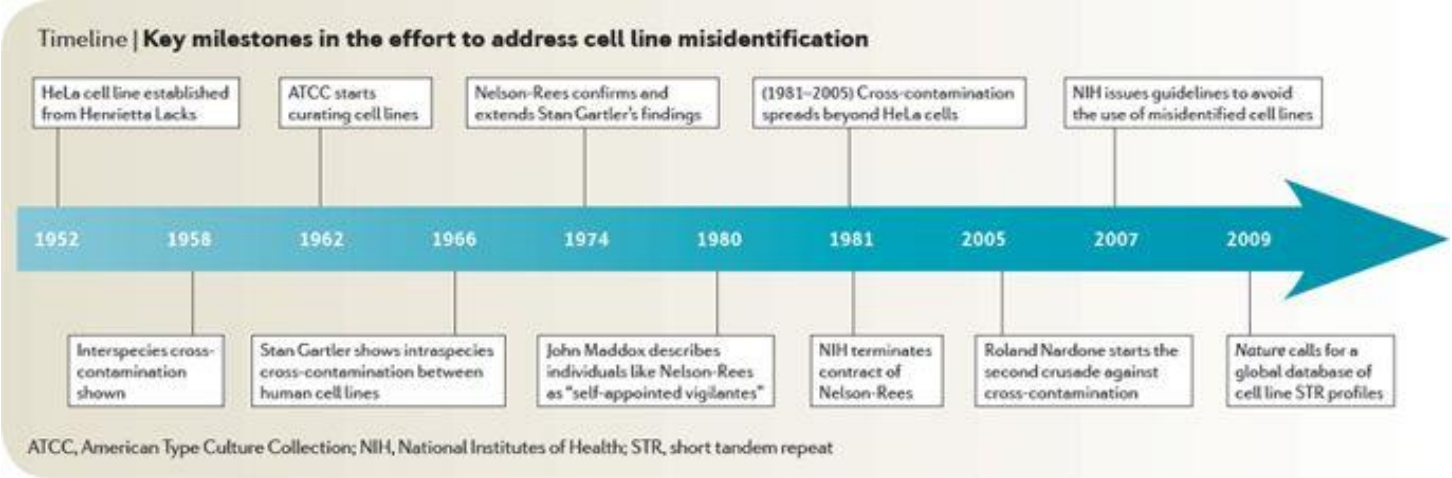




Contamination

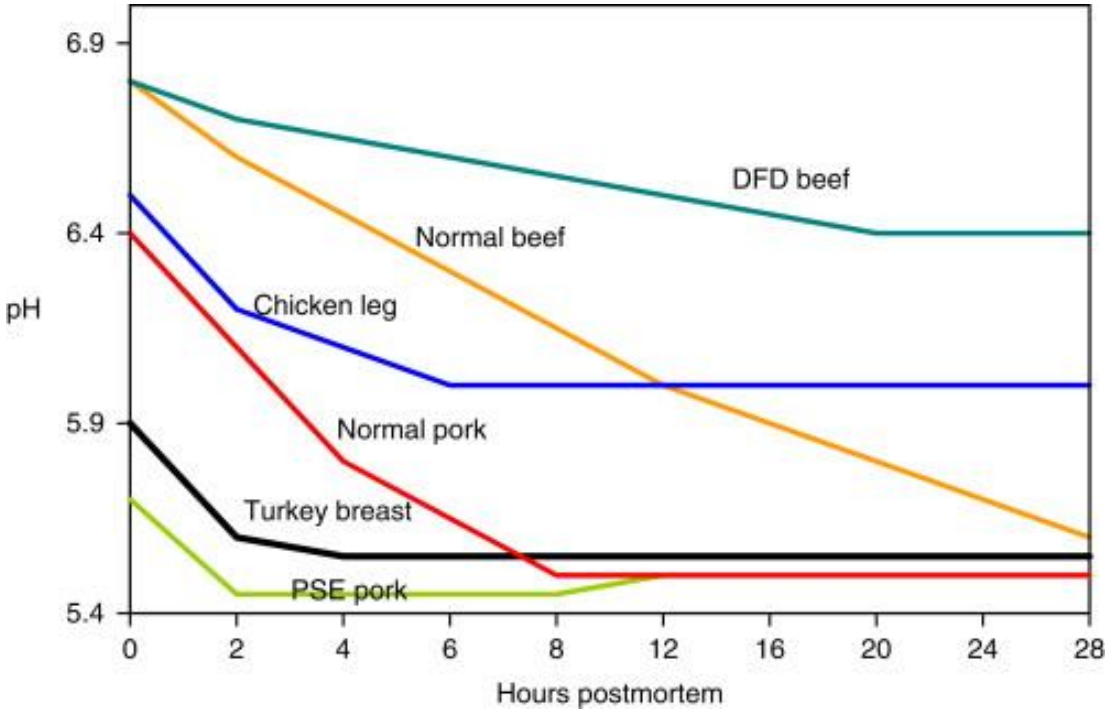
- Cell-line authentication
  - Cross-contamination
    - HELA catastrophe
  - Short-tandem repeat analysis
  - Next-gen Sequencing





Harvest and Downstream Processing

- Harvest
  - Enzymes
- Post-harvest
  - Tenderness
  - Enzymes?
- Microbial contamination
  - Critical control points
  - Other materials
- Packaging



Some Final Thoughts

- Established technology
- Production platforms
  - Cell lines
- Food intersections with biomanufacturing

Everything was going along fine until they discovered their HeLa cell line expressed Y chromosome markers.