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Lecture 23
Tissue Engineering II

Case Examples

1. Diabetes Treatment by Cell Encapsulation

Diabetes Mellitus (Type I or insulin-dependent): pancreatic disorder in which pancreas ceases to produce insulin

- 100M cases worldwide
- 15M cases in U.S. ⇒ $94B in treatment annually
- Symptoms: tiredness, weight loss, extreme thirst

Note: In Type II diabetes, insulin is manufactured but not used.

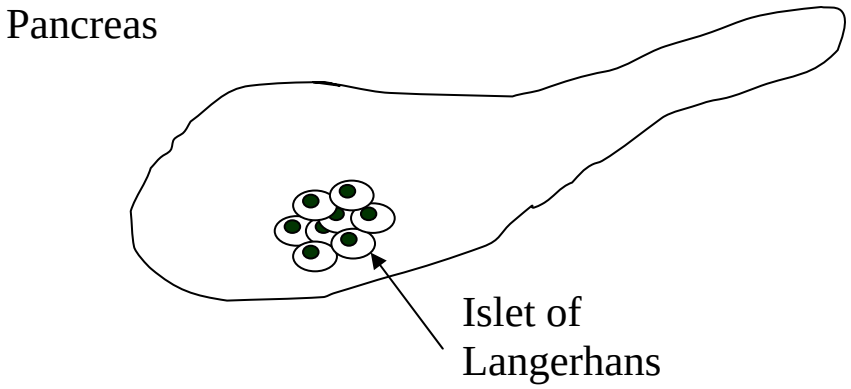
Traditional Therapy: Daily insulin injections

Problem: abnormal release pattern generates long-term complications

- Blindness
- Loss of circulation in limbs (frequently requires amputation)
- Kidney failure

What is Insulin?

- hormone (MW ~6 kD) released in response to blood glucose levels
- assists cells in glucose absorption



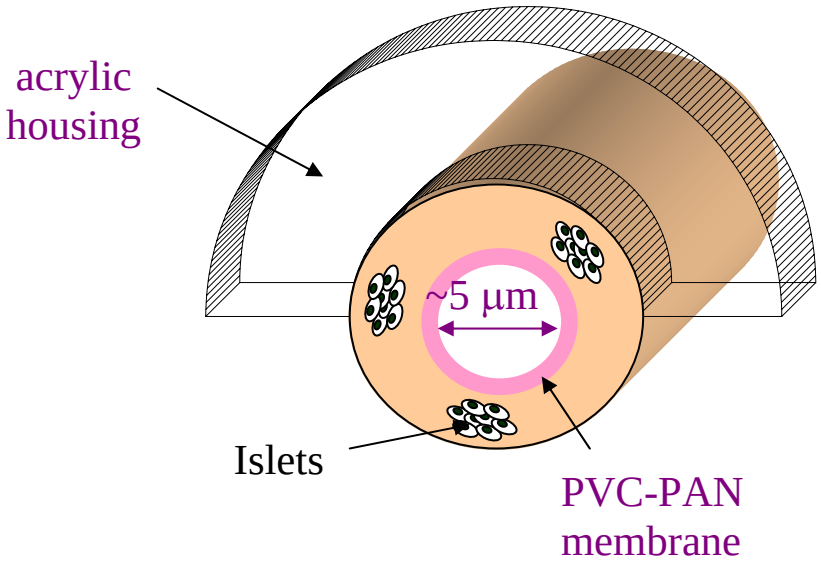
Pancreas: an endocrine gland, incorporating:

- Pancreatic Acini: release digestive enzymes to the duodenum
- Islets of Langerhans (1-2% of pancreas vol.): release insulin to bloodstream

Cell Encapsulation Therapy

- 1st reported in mid-1970's (W.L. Chick, Joslin Res. Lab., Boston)
  $\Rightarrow$ glucose homeostasis achieved in rats

- Tubular implants in pancreatectomized dogs, early 1990's (R.P. Lanza)



- Implanted in peritoneal (abdominal) cavity
- Membrane connected to vasculature by PTFE grafts
- No exogenous insulin required for > 10 weeks
- Little fibrosis up to 30 weeks (biocompatible acrylic)
- Mechanisms of failure:
  a) membrane rupture (80-90% of devices by 5-7 mo.)
  b) thrombosis
  c) infection
  d) loss of islet function, islet necrosis

- Human trials 1994 (Sharp & Lacy)

  - Implanted subcutaneously
  - PAN-PVC hollow fiber (1.5 cm length)
  - Human islets in alginate matrix
  - 90-95% islet viability after 2 weeks

Limitation: Insulin quantity—several meters of fiber for therapeutic # of cells

Possible solutions:

a) "super" cell lines—high insulin output, low nutrient needs
b) advances in angiogenesis

References

R.H. Li, "Materials for cell encapsulation", Adv. Drug Delivery Rev. 33 (1998) 87-109.

R.P. Lanza, R. Langer, W.L. Chick, Principles of Tissue Engineering, R.G. Landes Co.: Austin, TX, 1997

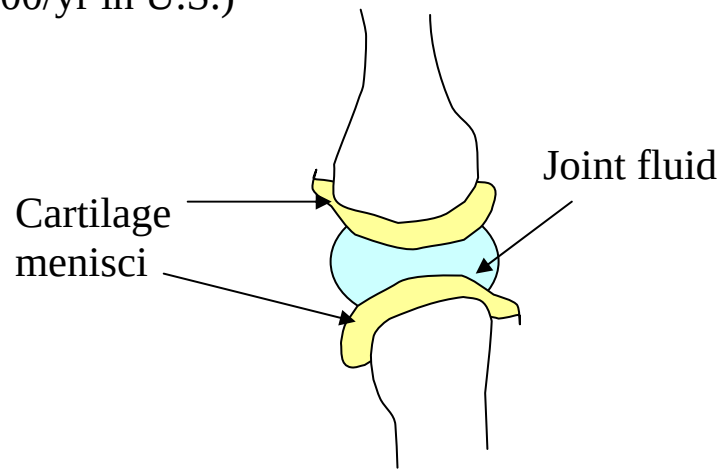
2. *In Vitro* Cartilage Regeneration

Basics of Cartilage:

- incorporates a single cell type (chondrocytes)
- low vascularization (limits *in vivo* regeneration capability)
- provides joint lubrication (knee)
- basis of soft structural members (nose, ears)

Conditions:

- torn cartilage (athletic injury) (~0.5M/yr in U.S.)
- rheumatoid arthritis (enzymes from phagocytes degrade cartilage)
- birth defects/cosmetic (28,000/yr in U.S.)

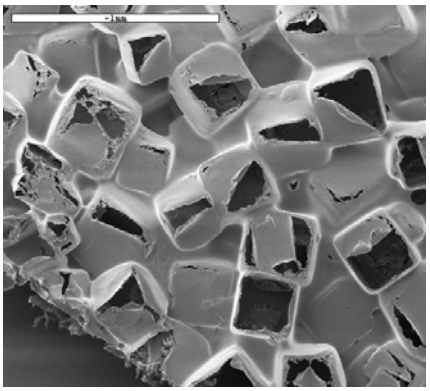


In vitro Chondrogenesis

- Materials: nonwoven PGA fiber mesh or salt-leached; ~95% porous
- Procedure: scaffold prewet with culture medium & seeded
- PGA matrix replaced over time by cells, collagen & GAGs

Salt-leached scaffold
made by 3.082 students

10% polylactide

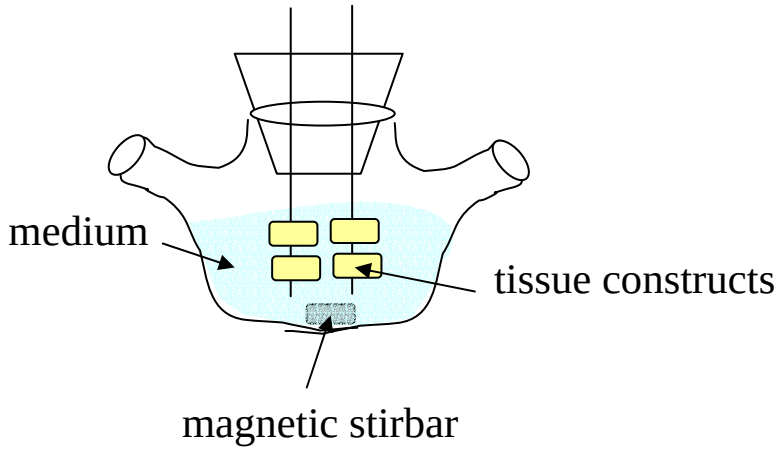


Limitations:

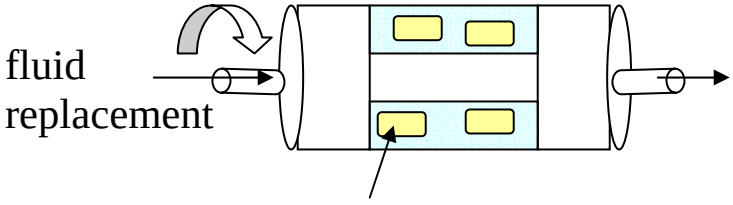
a) Low solids content: poor mass transfer in culture
b) Irregular tissue morphology: lack of flow field *in vitro*

Revised Approach: Bioreactors (R. Langer, MIT)

Spinner Flask



Rotating Vessel

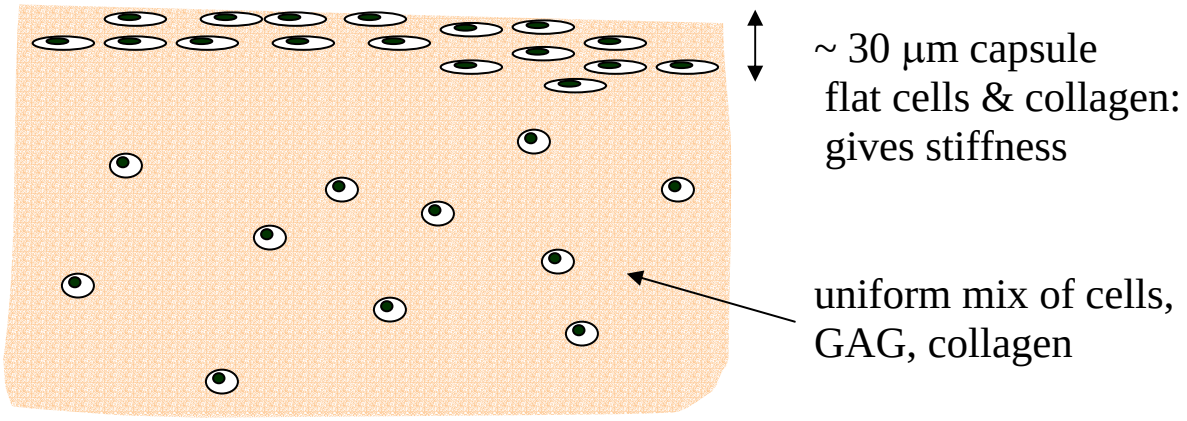


tissue constructs
(free-floating)

	Cells	GAG	Collagen	H_2O
Static	4	10	15	
Rotating	7	30	19	88
Natural	4	38	42	75

Bioreactor-Grown Tissues:

- tissue dimensions close to original scaffold
- higher solids content than static
- histology mimics natural cartilage



Commercial Status: Genzyme (Cambridge, MA)
FDA-approved for autologous knee cartilage repairs

Remaining Challenges:

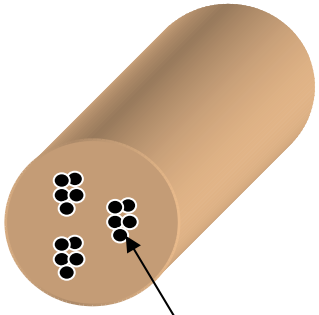
a) scale-up of tissue dimensions
b) improved mechanical properties

2. *In Vivo* Nerve Regeneration

Central Nervous System (CNS): Brain & Spinal cord

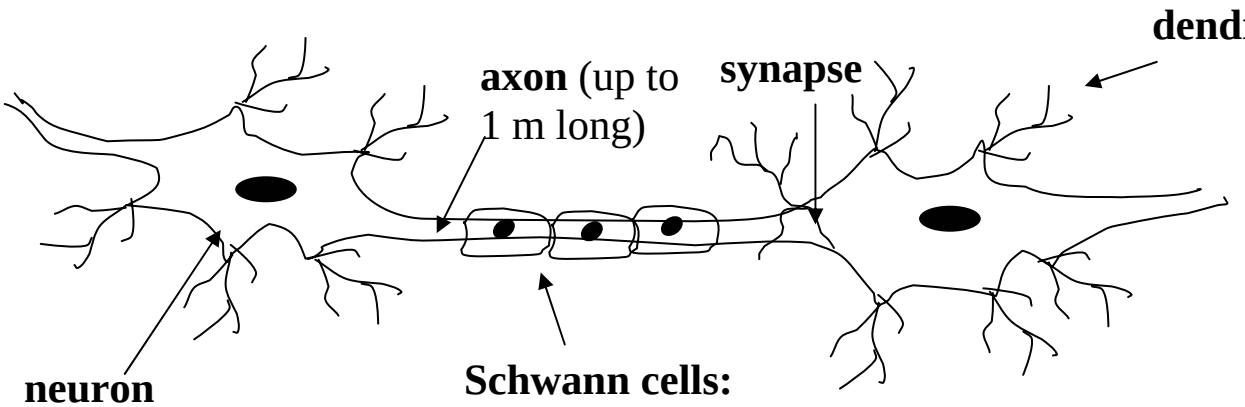
Spinal cord: neurons coated by oligodendrocytes—secretions inhibit regeneration

Peripheral Nervous System (PNS): nerve branches that process information from the environment; some regenerative capacity



Nerve: several fasicles encased in epinurium

fasicles: axon bundles surrounded by connective tissue



Condition: Severed nerve

- loss of support (neurotrophic factors)
- scar ingrowth
- displacement of resprouting axons

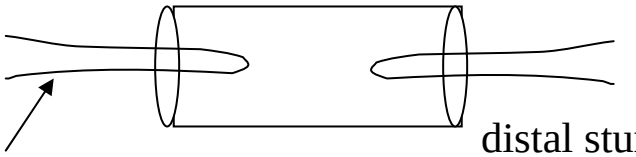
Nerve Guidance Channels: use regenerative capacity of PNS

History

WWI
- Rubber tubes used to guide axon growth
- low biocompatibility

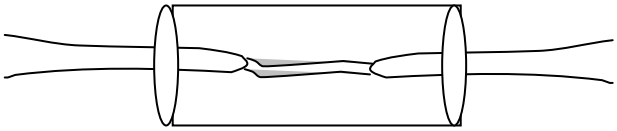
1960's
- Silastic trials (crosslinked silicone rubber)

Today
- Limited clinical use
- Silicone & PGA devices
- largest bridgeable gap ~ 1 cm



distal stump (to organ)

proximal stump (to spine)



Case Study: Severed rat sciatic nerve in silicone guide

Time elapsed	**Morphology**
hours	influx of serum, fibrin, neurotrophic factors
one week	longitudinally oriented fibrin coalesces to bridge stumps cells invade: macrophages, fibroblasts, Schwann, endothelial axons sprout from proximal stump
four weeks	axon sprouts reach distal stump (~ 1cm)

Regenerated Nerves

- less axons & thinner sheath
- slower signal conduction
- lower amplitude signal

Channel Design Considerations

Resorbable vs. Nonresorbable
resorbable—larger inflammatory response
nonresorbable—susceptible to compression injury

Impermeable vs. Porous
impermeable—poor nutrient, waste and O_2 transport
porous—interference by wound healing mechanisms, poor orientation
semipermeable—MW cutoff 50-100 kD enables transport & sequesters GF

Schwann cell-seeded gels
- secrete neurite-promoting basal lamina, NGFs
- can be genetically engineered to secrete neurotrophins
- evidence of CNS regeneration support (optic nerve regen. demonstrated)

Remaining Challenges:

a) large-gap repair
b) CNS regeneration (spinal cord work at MIT)

Important Remaining Challenges in Tissue Engineering

1. angiogenesis/mass transport limitations
2. delivering appropriate signals to cells
3. providing appropriate mechanical stimulus for growth