Principles and Practice of Tissue Engineering

Module 3, Lecture 1

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Topics for Lecture 1

• Introduction to tissue engineering
  – basic principles
  – examples

• Introduction to Module 3
  – background: cartilage
  – module structure
  – focus on week 1
What is tissue engineering?

“TE... applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function.”


• By what means?
  – natural or synthetic materials and cells

• Which functions?
  – the replacement is not usually identical to native tissue
Why tissue engineering?

• Severe trauma (acute or disease-state) challenges natural repair
  – e.g., shallow vs. deep cuts or burns
  – scar tissue formation
• Donor tissue problematic
  – scarcity of available tissue
  – immune response (graft or host)
• Autologous tissue can be problematic
  – no available site with excess tissue
  – permanent damage at donor site

[Public domain image, Wikimedia Commons]
Components of a TE construct

**scaffold/matrix**
- usually degradable, porous

**soluble factors**
- made by cells or synthetic
- various release profiles

**cells**
- precursors and/or differentiated
- usually autologous

**integrated implantable or injectable device**
Principles of TE: scaffolds

• Why a degradable, porous scaffold?
  – scaffold initially provides mechanical support for cells
  – degradability: scaffold may block new tissue growth, and/or overstimulate inflammation
  – porosity: promotes nutrient+oxygen diffusion

• How is the scaffold made degradable?
  – cross-links susceptible to chemical cleavage
  – cross-links susceptible to enzymatic cleavage

• Example: collagen or collagen-mimetic scaffolds
Principles of TE: soluble factors

- Types of soluble factors (cytokines)
  - growth factors for proliferation or differentiation (TGF, BMP)
  - factors promoting angiogenesis (VEGF)
  - chemokines that attract the cell type(s) of interest

- Delivery of soluble factors:
  - release from transplanted cells or scaffold itself

- Example: CCL21 promotes T cell migration
Principles of TE: cells

• Progenitors vs. differentiated cells
  – progenitors: hard to obtain large numbers
  – differentiated: may have lost functions

• Transplanted vs. in situ cells
  – cell expansion in vitro: can transplant large numbers

• Example: tumor-infiltrating lymphocytes (TIL)
  – T cells lose function in tumors
  – expand TIL ex vivo, treat with cytokines, and transplant: regression in some patients

Putting it all together: TE construct

Successful TE example

- Skin regeneration after severe burns
    - top layer protects wound, prevents fluid loss
    - bottom provides scaffold for growth
  - results in neotissue comparable to native skin
    - *not* contracted scar tissue
    - however, lacks sweat glands and follicles
  - sold as Integra Dermal Regeneration template

1. silicone
2. collagen-GAG

www.integra-ls.com/products/?product=46
Our focus: cartilage tissue

Avascular, highly water-swollen, heterogeneous tissue.
Cartilage TE basics

• Progenitor cells: mesenchymal stem cells
  – require growth factors for differentiation
  – may be difficult to obtain or work with

• Differentiated cells: chondrocytes
  – require special environment to maintain phenotype
  – otherwise, may de-differentiate to fibroblasts

• Our goal: *in vitro* culture of chondrocytes to preserve or destroy phenotype
  – observe collagen content, morphology, viability
  – collagen II:collagen I ratio reflects cell state
  – ultimately, knowledge of key environmental effects can help with design of cartilage TE constructs
Module overview: lab

Day 1: design
Day 2: seed cultures
Day 3: viability assay
Day 4: prep RNA+cDNA
Day 5: transcript assay
Day 6: protein assay
Day 7: remaining analysis
Day 8: your research ideas!
Module overview: week 1

Days 1+2: design and seed cultures

- 2D culture: plastic surface
  - prepare in duplicate
  - design maintenance plan

- 3D culture: alginate beads
  - prepare in duplicate wells
  - vary one parameter
Alginate: material for 3D culture

- Water-swollen gel
- Seaweed-derived polysaccharide
- Co-polymer of M and G acids
  - mannuronic and glucuronic
- G-block polymer chains cross-linked by cations (e.g., Ca\(^{2+}\))
- G/M content and MW influence
  - degradability
  - swelling
  - mechanical properties
  - viscosity of solution

**Method preview:**

liquid droplets into Ca\(^{2+}\) solution semi-solid gel beads
Lecture 1: conclusions

• Tissue engineering is an emerging field at the interface of engineering, science and medicine
• Maintaining cell function is a key part of TE
• Monolayer and alginate cultures provide a system for testing microenvironmental effects on chondrocytes

Next time… types of biomaterials, their properties, and cell-biomaterial interactions.
Ideas for varying culture conditions

**scaffold/matrix**
→ usually degradable, porous

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Other targets for change? Most realistic options?