Cell viability in TE constructs; Cartilage structure/function

Module 3, Lecture 4

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Topics for Lecture 4

• Review of Module 3 so far
• Cell viability: influence and measurement
• Overview of Module 3 week 3
• A closer look at cartilage
  – background on collagens and proteoglycans
  – how cartilage structure influences function
Module progress: week 2

- **Day 3: viability/cytotoxicity testing**
- **Practical matters**
  - focusing takes practice
  - too low cell concentration
- **Most groups found**
  - low cell recovery, especially in 3D
  - mostly live cells in 2D samples
  - 3D samples more variable
- **How can we explain these results?**
- **How can we improve the assay?**
- **Day 3: morphology observations**
  - what did you see in brightfield?

W/F Green group
Factors affecting cell viability

• What factors affect viability in a TE construct?
• Cell level
  – density: competition for nutrients, O$_2$
  – interactions (+ or -) between different cell types
• Cytokine level
  – may promote viability and/or proliferation
  – may promote apoptosis
• Materials level
  – permeability of material (to nutrients, O$_2$)
  – pore size, percent porosity
  – toxicity of material or its degradation products
Nutrient use in 3D constructs

- Parameters affecting diffusion
  - size of construct ($R$)
  - cell density ($\rho$)
  - diffusivity ($D$)
  - bulk concentration $[O_2]_{\text{bulk}}$

- In simple cases, boundary conditions can be used to get analytical solution

- Characteristic diffusion limit (nutrients, $O_2$): $\sim 100 \, \mu m$
  - diffusion profiles tend to correspond with viability profiles

- Solution in vitro: dynamic/perfusion culture
- Solution in vivo: promote angiogenesis quickly
Modeling cell viability in TE constructs

- Porous PLGA scaffolds
- Seeded cells in every other (A) or in each (B) layer
- Observed after 10 days
- Model
  - diffusion, O₂ use, and cell growth
  - quasi-steady state
  - no depletion in fluid
- Results
  - A has improved cell uniformity
  - cell growth matches O₂ tension
  - claim for predictive capability

\[ < 1 \text{M cells/cm}^3 \]
Modeling diffusion in a defined porosity


- Diffusion in colloidal crystal templated scaffolds
  - geometrically defined model
  - Brownian dynamics (time evolution)
  - Monte Carlo simulations (particle moves, Boltzmann weighting)

- Results
  - $D_{\text{eff}} = 0.3 \, D_o$ = upper-bound
  - decreases with size of inter-pores
  - with particle size ($O_2$ vs. protein)
  - with further confinement of particles by cells, or utilization by cells
Cell death: apoptosis and necrosis

• Apoptosis
  – programmed cell death
  – role in development and immunity
  – process: cell condensation and fragmentation
  – misregulated apoptosis implicated in disease

• Necrosis
  – response to trauma
  – process: cells burst and release contents
  – necrotic cells promote inflammation

• Morphology or biochemical assays can distinguish apoptotic and necrotic cells

S. Elmore *Toxicol Pathol* **35**:495 (2007)
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 4

1. Collect supernatant
   Test for collagen proteins (by ELISA)

2. Collect and lyse cells

Purify mRNA from cells  Amplify collagen cDNAs

Compare collagen I and II transcript levels
Day 4: RNA isolation

1. Collect cells
   lyse cells in buffer
   homogenize over column

2. Isolate total RNA
   on silica-gel columns that bind RNA > 200bp
   using buffers, ethanol precipitation
   enriched for mRNA due to size exclusion

www.qiagen.com

Working with RNA requires extremely clean technique. Why?

RNases are pervasive, e.g., on your hands
Day 4: RT-PCR

- RT = reverse transcriptase
  - what does this enzyme do?

- Unique primer design needs
  - how to isolate transcript but not genomic DNA?

- RT and PCR can be done in one reaction or two
  - enzyme de/activation by temperature
  - which enzymes when?

- What kinds of controls are desired?
Revisiting cartilage tissue

Avascular, highly water-swollen, heterogeneous tissue.
Collagen structure

- Collagen primary structure:
  - Gly-X-Y repeats
  - high proline, hydroxyproline content

- Collagen tertiary structure: triple helix
  - Gly contributes flexibility
  - Hyp contributes hydrogen-bonding

- Collagen quaternary structure: fibrils
  - true for many types, including I and II
  - cross-links via lysine and hydroxylysine
  - periodic banding structure observed

Image made using *Protein Explorer* (PDB ID: 1bkv)

Collagen types in cartilage

• Collagen types vary with respect to
  – location: II in cartilage, vitreous humor; I in skin, bone, vitals, etc.
  – homo- (II) or hetero- (I) trimeric helices
  – supramolecular structure formation
  – glycosylation

• Collagen composition in cartilage
  – Type II (fibrils) covalently linked to IX and XI
  – exact roles of IX and XI unknown
    ▪ IX may form inter-fibrillar cross-links
    ▪ XI may modulate collagen II fibril diameter
    ▪ mutations to IX, XI, II cause disease
  – Types III, VI, X, XII, and XIV also present

• Little collagen turnover in adult cartilage

Proteoglycan structure

• Proteins with GAG side chains
  – many negatively charged groups COO⁻ SO₃⁻
• Most common PG in cartilage is aggrecan
  – aggrecans polymerize via hyaluronin (HA)
  – GAG is primarily chondroitin sulfate (CS)
  – monomer > 1M, aggregates > 100M Da

Aggrecan monomer


Aggrecan aggregate

Cartilage structure and function

• Composition of cartilage
  – CN is 50-75% and PG is 15-30% of dry weight
  – water: 60-80%
  – cells: 5-10% by volume
• Requirements of a joint
  – load transfer (bone/bone, bone/muscle)
  – flexibility, lubrication
• Role of PG
  – high compressive strength due to osmotic swelling: water is pumped out during compression
  – low permeability, friction coefficient reduces wear and tear
• Role of CN
  – high tensile strength (~GPa)
  – contain swelling forces of PG

Lecture 4: conclusions

- Cell viability in TE constructs is affected by factors at the cell, materials, and cytokine level.
- Modeling is one useful tool to study the effects of nutrient diffusion on cell viability.
- RT-PCR is a technique for studying gene expression, with special considerations beyond PCR.
- The structure of the cartilage extracellular matrix promotes its function in joints.

Next time: gene and protein assays, *in vitro* and *in vivo* models for cartilage TE