Tissue Engineering: culture systems, bioreactors, biological signals

In vitro tissue engineering vs In vivo tissue engineering

Types of tissue culture:
Organ culture (tissue explant retains architecture and 3D shape)
Primary explant (fragment of tissue at liquid-solid interface and cells can migrate in 2D)
Cell culture (tissue or outgrowth mechanically and enzymatically digested and cultured as adherent monolayer or non-adherent suspension)

Medium
– liquid in which cells grow. Individually tailored.

Dissolved gases: CO_2, 5-10%
Sodium BiCarb in medium

$H_2O + CO_2 <—> H_2CO_3 <——> H^+ + HCO_3$
pH = 6.0966 + log ($[HCO_3^-]/[CO_2]$)

Medium components.

NaCl	control osmotic pressure
Inorganic salts	electrolyte balance (similar to blood)
Sodium Bicarb	buffer, pH
d-glucose	energy and carbon source
amino acids	nitrogen source for protein synthesis
vitamins	cofactors for biochem processes
phenol red	pH indicator
serum	cell growth, attachment factors, hormones (insulin, steroids), carrier proteins (albumin), minerals
antibiotics	
growth factors	

Biological Signals Relevant to Tissue Engineering:

- Growth Factors
- Cytokines
- Osmolarity
- pH
- Oxygen
- Mechanical Signals
- ECM molecules

Growth factor effects.

- Cell state, gene expression, migration, polarity, proliferation
- Cell-cell contacts (adhesion)
- ECM environment (cofactors)
- Mechanical Stimulation (cofactors)

Significant impact on embryo development and adult biology. Many different functions in different tissues. Concentration (biphasic response often)

Transforming Growth Factor Superfamily

- Control growth, proliferation, and lineage specificity of many tissues
- Activate Smad family of transcription factors
- TGFb important in cartilage and bone development
- BMPs are largest group in this family
- A number of related extracellular signaling molecules that play widespread roles in regulating development in both invertebrates and vertebrates
- These are derived from inactive precursor proteins through proteolytic processing

BMP.

- Derived from bone (inducing activity)
- Differentiation factor
- Also found in gut and heart during development, involved in limb development (with FGF)
- Stem Cell Differentiation

Ones to recognize the name:
PDGF (first one isolated), similar to VEGF – large developmental role and proliferation (serum), VEGF, NGF, HGF.

FGF.
1974, 22 distinct proteins.
Requires heparin or heparin sulfate essential for binding and activation.
All tissues express FGF at some time – different forms of FGF: intracellular, secreted, ECM bound. During development and postnatal.

FGFRs play important role – defect mice – many craniofacial problems
Significant role in skeletal and bone and chondrocytes regulation, limb induction
Also effects in epidermal (wound healing), lung dev., CNS, mammary, ear, hepatic

IGF-1.
Made in liver and in specific tissues. Found in serum or **stored in matrix (relevance to tissue engineering)**

Development, growth, CNS, bone, skeletal muscle, mammary, pancreas, reproductive, life span and aging (DAF gene encoding IGF receptor)

Has proliferative and differentiation effects
Can control glycogen synthesis and protein synthesis

6 binding proteins inhibit or potentiate activity

Example in muscle.
First stimulates increase in cell cycle markers, proliferation, via MAP kinase pathway
Second, induces myogenic regulatory factors, through phosphatidylinositol (PI)-3-kinase pathway.

IGF hyperexpressed in muscle → myofiber hypertrophy, high muscle mass

Importance of drug delivery in tissue engineering.
Review by Saltzman.
Provides continuous release of a biological agent and the potential for multiple drug release at different times (ie, important for angiogenesis). How to incorporate into tissue engineering system….

Bioreactors in tissue engineering.
Cardiac: perfusion improves engineering of cardiac tissue (Carrier et al).
Electrical stimulation improves cardiac TE (Vunjak novakovic, also relevant for nerve)
Balloon mechanical stimulation (Seliktar)

Vascular: vessels cultured in a flow bioreactor mimicked better normal vessels (strength, morphology, biochemical content, Niklason).

Cartilage: mechanical stimulation significant for cartilage (too much will damage cartilage, right amount improves matrix properties (Sah et al, Grodzinsky). Static compression will inhibit proteoglycan production, dynamic improves it).

Static, mixed, or rotating bioreactors for engineering cartilage – static cultures made fragile small constructs, mixed (turbulent flow) cultures had larger fibrous capsule, both with weak mechanics, and rotating cultures produced strong cartilage (freed).

Bone: indentor bioreactor significant increased bone production (matrix) and MSC differentiation (Kaplan).

Tendon: Pull on MSCs on fibers and they differentiate towards tendon (Types I and II collagen, Tenascin, Altman et al.)-