Aalto University
School of Chemical Technology

CHEM-E3225, Cell- and Tissue Engineering, 5 cr

Topic 5

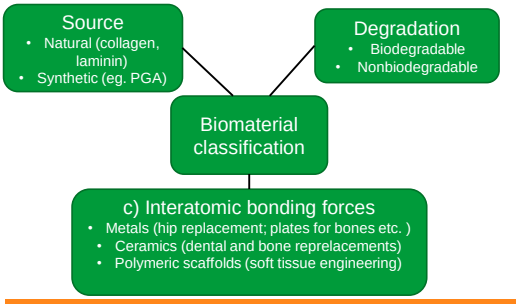
Biomaterials (and scaffolds) for tissue engineering

(Birla Chapter 3)

"A porous structure typically made out of biocompatible polymers, proteins, peptides, and inorganic materials within which cells are seeded. Scaffolds provide geometrical structure for cells to reorganize and form 3D multicellular tissue"

1) function of scaffolds
2) synthetic biomaterials
3) characterization of biomaterials in vitro and in vivo for safety and function

Classification of Biomaterials, Birla figure 3.7.



Today we will look at:

1) Function of scaffolds
2) Synthetic biomaterials
3) Properties of 3-D scaffolds
4) Characterization of biomaterials in *vitro* and in *vivo* for safety and function

Why do we need to understand these ?

- Because they are frequently needed to build an artificial tissue
- Because they are needed to help cells grow in culture and in bioreactors
- Because they must also be safe

I. Function of scaffolds

Biomaterials in Tissue engineering

- In the body cells in tissues are organized in functional units composed of different cell types arranged in a spatially defined manner.
- Engineering tissue constructs is not just putting cells into a 3D scaffold (to be discussed soon), instead it requires a spatial and temporal control of the cell arrangement to mimic the *in vivo* tissue architecture.
- Mechanical conditioning requires application of dynamic mechanical loads (compression, tension, pressure, shear etc.) to cells, also electrical stimulation, so we must understand what the materials can "take" to support normal function of cells
- Since the cells are in micrometer scale, such control of cellular environment must be in micrometer scale.

Functional tissue engineering: Goals

The key challenge is to optimize the *in vitro* culture setting in order to produce 3D implants that can meet the requirements of the *in vivo* environment with the goals of:

1. Improved definitions of functional success of tissue-engineering applications
2. Improved understanding of the *in vitro* mechanical requirements and intrinsic properties of native tissues
3. Improved understanding of the biophysical environment of cells with engineered constructs
4. Scaffold design citeria that aim to enhance cell survival and the regeneration of functional tissue-engineered constructs
5. Generate design criteria that aim to meet the metabolic and mechanical demands of specific tissue-engineered applications
6. Improve understanding of biological and mechanical responses of a engineered tissue construct following implantation

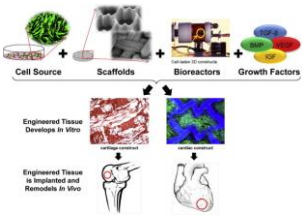
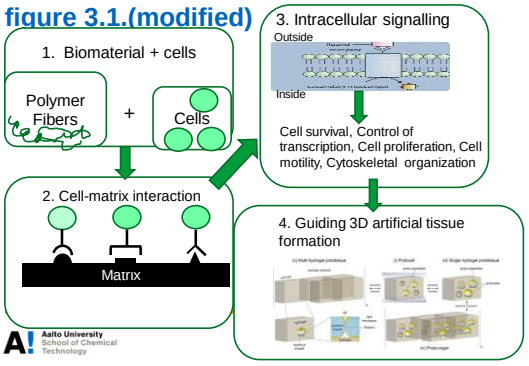


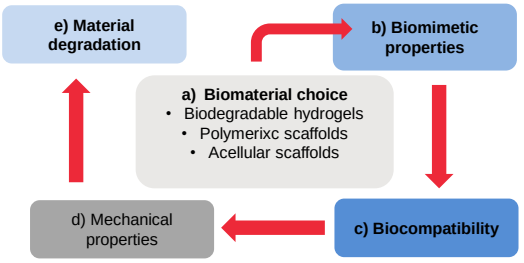
FIGURE 13.1. Strategy for engineering functional tissues *in vitro*.
Cells, scaffolds, bioreactors, and growth factors are used as tools to create functional engineered tissues. This chapter focuses on the *in vitro* culture of engineered cartilage and cardiac constructs that can be utilized for basic research and, potentially, for repair of damaged articular cartilage and myocardium.
Top Panel: scaffold images adapted from Engelmayr, Jr., G.C. et al., Nature Materials 7:1003, 2008 [67] (top) and Moutos and Guilak, Tissue Eng Part A 16:1291, 2010 [42] (bottom). Middle Panel: engineered cartilage image adapted from Valonen, P.K., et al., Biomaterials, 31(8):2193, 2010 [93]; engineered cardiac image adapted from Engelmayr, Jr., G. C. et al., Nature Materials 7:1003, 2008 [67].

Principles of Tissue Engineering (Fourth Edition), 2014, 237–259
Kristen L. Moffat, Rebekah A. Neal, Lisa E. Freed, Farshid Guilak
http://dx.doi.org/10.1016/B978-0-12-398358-9.00013-6
Chapter 13 – Engineering Functional Tissues: In Vitro Culture Parameters

Definition of Biomaterials (Birla 2014, figure 3.1.(modified)



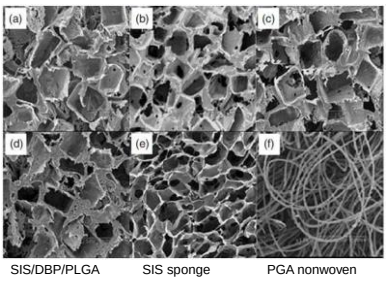
Biomaterials development for Tissue engineering (Birla 2014, figure 3.2.)



Functions of scaffolds

1. Promote cell-biomaterial interactions, cell adhesion and ECM deposition
2. Permit sufficient transport of gases, nutrients and regulatory factors to allow cell survival, proliferation and differentation
3. Biodegrade at a controllable rate
4. Provoke a minimal degree of inflammation or toxicity *in vivo*
5. *Scaffolds – word refers to a functional role, where the composition of the scaffold can be a biomaterial or also some other non-biological support*

Various types of scaffolds

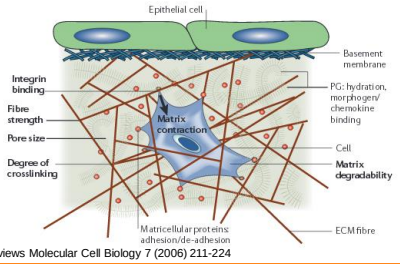


SIS/DBP/PLGA SIS sponge PGA nonwoven

Hybrid, composite, and complex biomaterials for scaffolds

- Composite materials are made from at least two or more constituent materials that have different properties and are not soluble in each other.
- There are at least two phases in a composite, a continuous phase and a dispersed phase.
- Bone and teeth are composites containing hydroxyapatite and collagen.
- They are developed to obtain better properties.
- For example, PLGA/collagen sponge
  - Collagen facilitates cell seeding
  - PLGA provides mechanical support

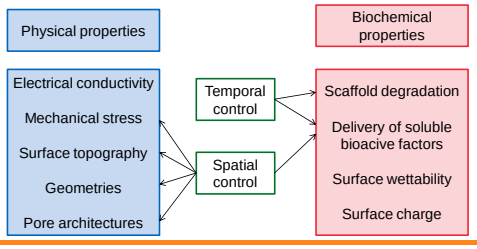
The importance of 3D environment for engineering cell function



Nature Reviews Molecular Cell Biology 7 (2006) 211-224

Properties of 3D scaffolds

- Spatiotemporal control of scaffold biochemical and physical properties



Pore architectures in 3D scaffolds

- Mass transport: how fluid, solutes, and cells move in and out of a tissue construct
- Related to: pore size, porosity, pore interconnectivity, and surface area
- In metabolically active tissues *in vivo, most cells reside within* 100 µm of a capillary.
- In engineered tissue constructs cultivated in static culture without medium perfusion, neotissue formation is generally limited to the peripheral 100-200 µm of the scaffold.
- Pore size also affects stem cell differentiation

2. Synthetic biomaterials

Synthetic biomaterials

Selection of synthetic biomaterials (metals, ceramics, and polymers)

↓

Fabrication methods

↓

Spatiotemporal* control of scaffold properties ➡ Porous 3D scaffolds

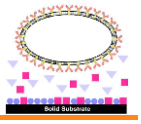
↓

Cell interaction with scaffolds

* Will we define ”spatiotemporal” later in the lecture

Non-specific cell-synthetic biomaterial interaction

- Cells "see" the adsorbed proteins, but not the synthetic biomaterial.
- Protein adsorption to synthetic biomaterials causes non-specific interactions
  - The material in cell culture medium or in the body becomes coated with proteins within seconds to minutes.
  - In general, proteins preferentially adsorb onto a hydrophobic surface, as mediated by their hydrophobic domains.
  - Many proteins have a net negative surface charge, which promotes their adsorption to a positively charged surface.
  - Adsorption is often irreversible.
  - Adsorption may lead to protein denaturation.
  - There is no control of protein orientation.
  - It is often undesired.



Scaffold degradation

- Ideally when the regeneration of a fully functional tissue is complete, the scaffold is degraded and absorbed.
- Polymer molecular weight and copolymerization ratio can be adjusted to control the degradation rate.
- Some degraded products may cause side effects
  - PGA, PLA, and PLGA produce glycolic/lactic acid byproducts that are very acidic
- Degradation mechanisms:
  - Passive degradation
  - Cell-triggered degradation

Hydrogels

- 3D networks composed of cross-linked hydrophilic polymer chains
- Can absorb up to thousands of times their dry weight in water
- Can be cast into practically any shape, size or form
- Polymeric structures
  - Primary covalent cross-links
  - Ionic forces
  - Hydrogen bonds
  - Affinity or bio-recognition interactions
  - Hydrophobic interactions
  - Polymer crystallites
  - Physical entanglements of individual polymer chains
  - A combination of two or more of the above interactions

Hydrogels as tissue engineering matrices

- Advantages
  - Aqueous environment can protect cells and fragile drugs (peptides,proteins, oligonucleotides, DNA)
  - Good transport of nutrient to cells and products from cells
  - May be easily modified with cell adhesion ligands
  - Can be injected in vivo as a liquid that gels at body temperature
  - Usually biocompatible
- Disadvantages
  - Can be hard to handle
  - Usually mechanically weak
  - May be difficult to load drugs and cells and then crosslink in vitro as a prefabricated matrix
  - May be difficult to sterilize

Biomedical applications of hydrogels

- Contact lenses
- Blood-contacting hydrogels
- Drug delivery from hydrogels
- Targeted drug delivery from hydrogels
- Tissue engineering scaffolds from hydrogels

- **3. Characterization of biomaterials in tissue engineering *in vitro* and *in vivo***

  a) physicochemical characterization
  b) investigation of cell-biomaterial interactions
  c) setting up animal models
  d) Biocompatibility testing

Fundamental criteria for engineering functional tissues

1. At the time of implantation, an engineered tissue should posess sufficient size and mechanical integrity to allow for handling and permit survival under physiological conditions
2. Immediately following implantation, an engineered tissue should provide some minimal level of biomechanical function that should improve progressively until normal tissue function has been restored
3. After implantation, an engineered tissue should mature and integrate with surrounding host tissue

a) Physicochemical characterization of biomaterials

- Mechanical properties
  - Stress-strain
  - Viscoelastic properties
- Morphological characteristics (especially pore characterization)
  - Light microscopy
  - Scanning electron microscopy
  - Atomic force microscopy
  - Permeation of aqueous fluids
- Chemical characteristics (especially surface characterization)
  - Contact angle
  - Infrared surface studies
  - Electron spectroscopy for chemical analysis
  - NMR
- Chemical stability (biodegradation)
  - In aqueous media
  - In enzyme solutions
  - In oxidant solutions

b) Investigation of cell-biomaterial interactions

- *In vivo studies*
  - Biocompatibility:
    - Inflammation
    - Fibrosis
    - Coagulation
    - Immune response
    - Angiogenesis
  - Functionality:
    - Intended function depending on the applications

Cells used in cytocompatibility test of biomaterials *in vitro*

Primary cells: most similar to the *in vivo cells,* limited availability of human primary cells

Cell line: subcultured cells from primary cells

- Finite cell line: it dies after a set number of polulation doublings (generations).
- Continuous cell line: it survives indefinitely, either spontaneously (particularly cells from rodents) or induced by transformation (tumorigenic) or immortalization, e.g.
  - Cancer cell lines: unlimited life span, abnormal phenotype and genotype
  - Immortalized non-transformed cell lines: minimal chromosome abnormalities, unlimited life span

Cells used in functionality test of tissue engineered products *in vitro*

- Primary, differentiated cells
- Stem cells
- Stem cell-derived cells

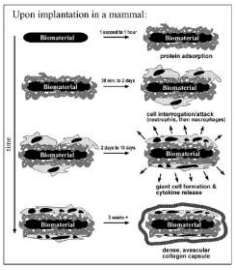
c) Establishment of animal models

- To evaluate the biocompatibility and functionality of biomaterials, both *in vitro and in vivo studies are* required.
- Results from *in vitro studies can be difficult to* extrapolate to the *in vivo situation.*
- For this reason the use of animal models is an essential step prior to clinical use in human.
- However, animal models differ significantly from humans and several promising products fail in clinical testing

d) Biocompatibility testing: four factors

1. Toxicology
   - Unreacted monomer, oligomers, cross-linker, stabilizer, additives, ions released from biomaterials, degraded products...
2. Extrinsic organisms
   - Bacterial and fungi can cause inflammation
   - Bacterial endotoxin contamination
3. Mechanical effects
   - Mechanical mismatch: a hard biomaterial and a soft tissue
   - Biomaterial implant has sharp corners, is moving, rubbing in contact with tissue
4. Cell-biomaterial interactions
   - Less understood, complex
   - Foreign-body reaction
   - For example: macrophages adhere to polystyrene but not polyHEMA in vitro; but both biomaterials generate the same foreign-body capsule in vivo.

Foreign body reaction (FBR)



When the capsule is thin and the reaction site is quiescent after one month, this is an acceptable FBR and the implant is biocompatible.

Summary

- Studies are performed to characterize tissue engineered materials to understand its performance *in vivo* and to make sure that the materials are safe to use.
- The levels of cell culture in tissue engineering
  - 1. Static level (traditional cell culture)
  - 2. 3D culture (scaffolds)
  - 3. Dynamic cell culture
  - 4. Bioreactors (to follow in topic 6)