Lecture 6: Biodegradable Polymers for Tissue Engineering

Last time: enzymatic degradation of solid polymers
Engineering biological recognition of polymers

Today: Designing polymers for tissue engineering

Reading: 'Tissue engineering- current challenges and expanding opportunities,' L.G. Griffith and G. Naughton, *Science* **295**, 1009 (2002)

Overview of Biomaterials in Tissue Engineering

Let's review the main approaches and applications in Tissue Engienering before getting into the details of materials for TE

We will review the fundamental approaches that have been taken here and return to this topic later when we discuss integration of biological molecules in synthetic biomaterials

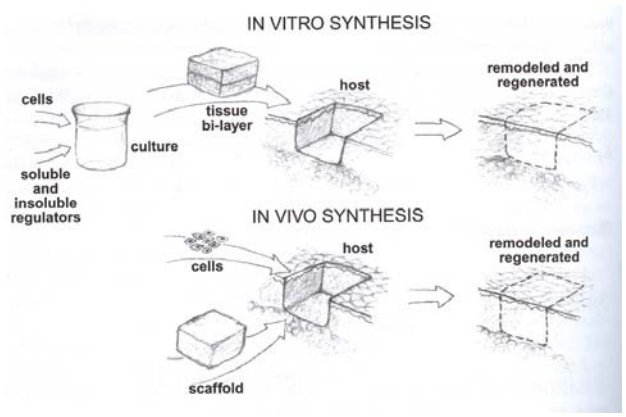
- TE scaffolds seek to provide a surrogate for natural ECM
  - Provide functions of native ECM
  - Create a space for new tissue development
  - Deliver cells to site
  - Direct macroscopic size/shape of new tissue

Tissue Engineering Approaches

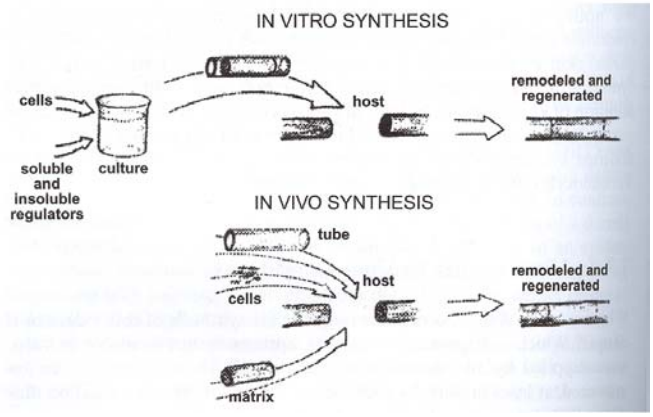
- 3 major approaches
  - *In vitro* tissue genesis → *in vivo* application
  - *In vivo* tissue genesis → *in vivo* application

Schematic comparison of *in vitro* and *in vivo* tissue engineering approaches[1]:

Skin:



bone:



- *In vitro* tissue genesis → *ex vivo* application[2]

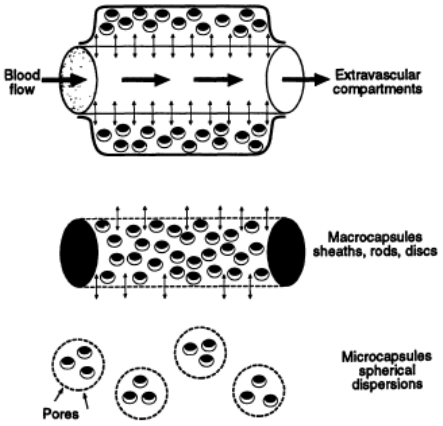


Fig. 1. There are three common closed-system configurations for cell transplant devices (*69, 70*) [figure adapted with permission from (*70*)]. In vascular devices, the cells are placed in an extracellular compartment surrounding a tubular membrane (i.d. ~1 mm) through which blood can flow. In macrocapsule systems, the cells are placed in sheaths, rods, or disks (diameter ≥0.5 to 1.0 mm). In microcapsule systems, the cells are placed in injectable spherical beads (diameter <0.5 mm). Device biocompatibility is critical because tissue reaction can block the flow of nutrients and wastes to and from the capsule. Microcapsules are commonly made of hydrogels—in particular, the polysaccharide alginate—because of the extremely mild conditions required for gel formation. The alginate can be further coated with polyanions, such as polylysine, and again with alginate if desired. Such coatings can affect the flow of nutrients and wastes, mechanical strength, and biocompatibility. Results of in vivo studies with alginate are not always consistent, possibly because of variations in alginate purity (*71*). Macrocapsules and vascular devices often consist of acrylonitrile–vinyl chloride copolymers (*69, 70*). Microcapsules have advantages over macrocapsules in that they impose fewer limitations on diffusional flow of nutrients and wastes and they can be administered by injection. Macrocapsules, on the other hand, are easier to retrieve should complications occur and are physically more stable than microcapsules.

- *In vitro* tissue genesis → *in vitro* application
  e.g. tissue on a chip approaches[3]:

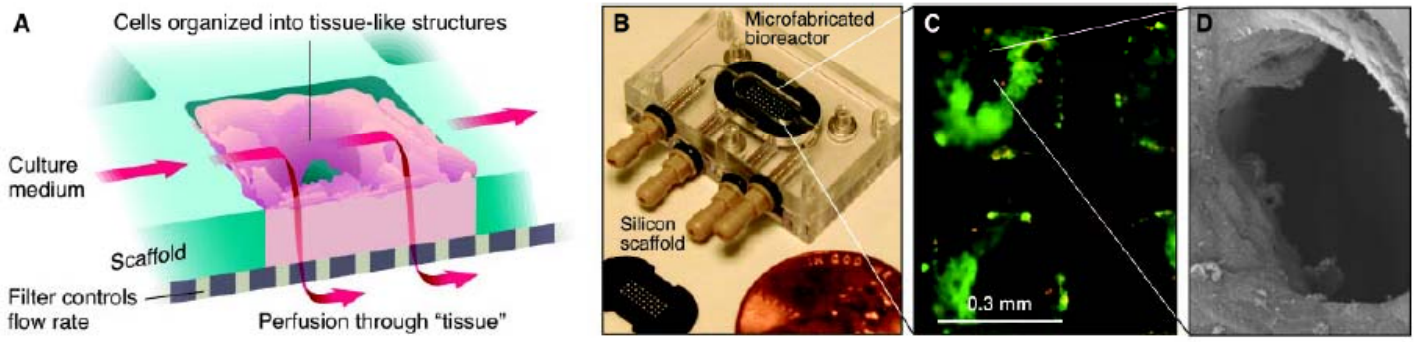


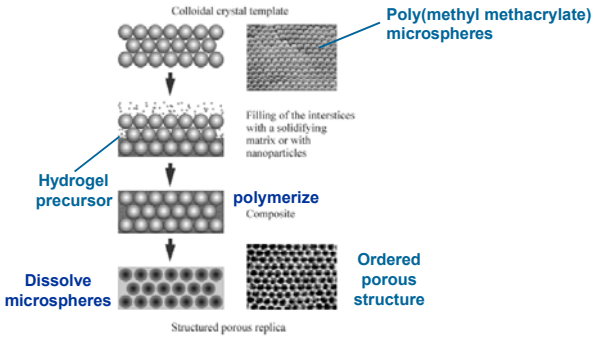
Fig. 2. A microfabricated bioreactor for perfusing 3D liver tissue engineered in vitro (*54, 55*). (**A**) A cross section showing tissue aggregates growing attached to the inside walls of the narrow channels of the silicon-chip scaffold. Culture medium flows across the top of the scaffold as well as through the narrow channels, enabling tissue aggregates to extract oxygen and nutrients. The design of the scaffold promotes self-assembly of the cells into tissues. (**B**) A bioreactor containing a 0.2-mm-thick silicon-chip scaffold etched with 0.3-mm-diameter channels. (**C**) Hepatocytes seeded onto the scaffold of the bioreactor attach to the walls of the channels (four channels are shown) and reorganize to form 3D structures that are reminiscent of liver cords. Bile canaliculi and tight junctions can be seen with high-power microscopy (*54, 55*). Live cells are green and dead cells are red as visualized with the calcein AM/ethidium homodimer stain. (**D**) Scanning electron micrograph showing vessel-like structures assembled from endothelial cells at the fluid-tissue interface in the bioreactor channels. [Illustration: Preston Morrighan]

Macroscopic TE Scaffold Structure

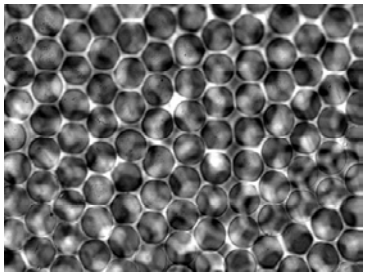
- Early attempts at designing scaffold for tissue engineering simply used forms of processed polymers:
  - PGA mesh fibers

- From here, the need for a higher surface area and more 'enclosed' structure were recognized and polymer foams were developed:
  - Freeze-dried scaffolds
  - particulate-leached scaffolds (Mikos 1994, Lu 2000)
  - Supercritical CO_2-based scaffolds (Hile et al 2000, J Contrl Rel 66, 177)
  - Effervescent salt leaching (Yoon et al 2001, JBMR 42, 396)

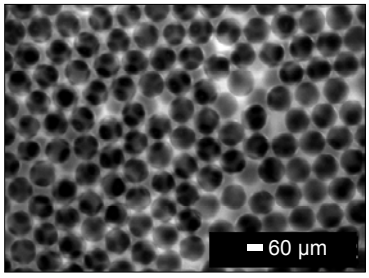
- More elegant approaches are now being considered:
  - Colloidal crystal templating



Optical micrograph/20 μm pores



Fluorescence micrograph/60 μm pores



  - Nanofiber-based structures (P. Ma)
  -
- Researchers have also investigated natural materials as scaffolds for tissue engineering- using processed ECM for tissue engineering (we won't pursue here, covered in Biomaterials-Tissue Interactions)
  - Example materials
    - Decellularized tissues (Badylak 1998, Hilbert 1989)
    - Collagen-based gels (Ellis et al 1996)
  - Advantages:
    - Native cues present
    - Can preserve natural tissue microstructure
  - Disadvantages:
    - Poor mechanical properties in some cases
    - Difficult to process
    - Poor reproducibility
    - High cost

Cellular Interactions with Synthetic Degradable Solids Used as Scaffolds?

Review older literature looking directly at cells on PLGA, PLA, etc.

Molecularly-Designed Surfaces for TE

Reconstruction at Scaffold Surfaces

Controlled Release in Tissue Engineering

Cytokine delivery from scaffolds

Case Study: Induction of vascularization in TE scaffolds

- Structure of vasculature
- Dual growth factor delivery from degradable scaffolds for *de novo* blood vessel synthesis[4]:

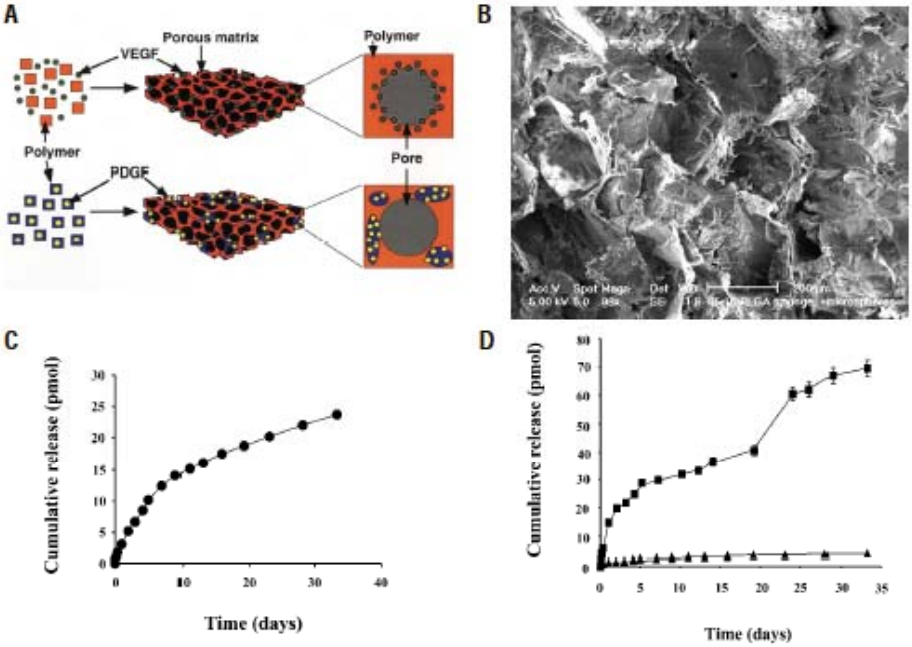


Figure 1. Schematic of scaffold fabrication process and growth factor release kinetics. (A) Growth factors were incorporated into polymer scaffolds by either mixing with polymer particles before processing into scaffolds (VEGF), or pre-encapsulating the factor (PDGF) into polymer microspheres used to form scaffolds. The VEGF incorporation approach results in the factor being largely associated with the surface of the polymer, and subject to rapid release. In contrast, the PDGF incorporation approach is predicted to result in a more even distribution of factor throughout the polymer, with release regulated by the degradation of the polymer used to form microspheres. The two growth factors were incorporated together into the same scaffolds by mixing polymer microspheres containing pre-encapsulated PDGF with lyophilized VEGF before processing into scaffolds. (B) Scanning electron micrograph of a typical scaffold utilized for dual growth factor release. (C) *In vitro* release kinetics of VEGF from scaffolds fabricated from PLG (85:15, lactide:glycolide), measured using ^{125}I-labeled tracers. (D) *In vitro* release kinetics of PDGF pre-encapsulated in PLG microspheres (▲ 75:25, intrinsic viscosity = 0.69 dl/g; ■ 75:25, intrinsic viscosity = 0.2 dl/g), before scaffold fabrication. Data represent the mean (n = 5), and error bars represent standard deviation (error bars not visible are smaller than the symbol).

controlled release scaffolds induce formation of more blood vessels with larger diameters:

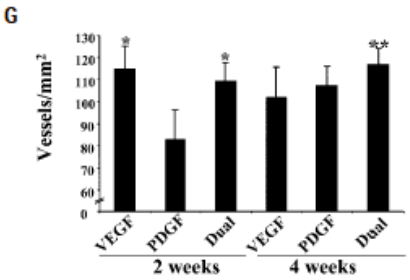


Figure 3. Sustained, dual delivery of VEGF and PDGF rapidly forms dense vasculature. Scaffolds incorporating VEGF alone, PDGF alone, and both VEGF/PDGF were implanted as described earlier. Scaffolds that rapidly release VEGF (Fig. 1C) with a slower release of PDGF (Fig. 1D, lower curve) were utilized in these experiments. Hematoxylin and eosin staining of tissue sections from subcutaneous implants ($n = 4$) of scaffolds containing VEGF only, after two weeks (A) and four weeks (B); scaffolds containing PDGF only, after two weeks (C) and four weeks (D); and scaffolds releasing both VEGF and PDGF, at two weeks (E) and four weeks (F). The vascular density within tissue sections was quantified in each condition (G). * indicates statistical significance relative to blank at the same time point ($P < 0.05$); ** indicates statistical significance relative to VEGF and PDGF ($P < 0.05$). Magnification for all photomicrographs was 400×.

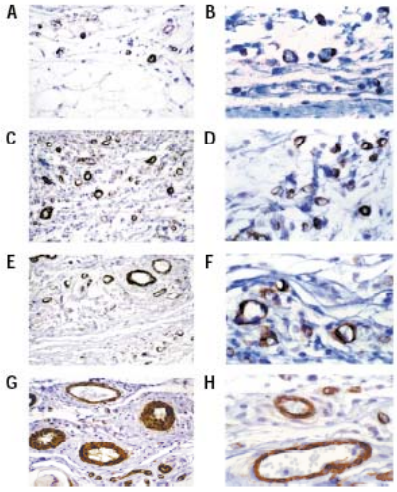


Figure 4. Dual delivery of VEGF and PDGF induces mural cell association. α-Smooth muscle actin staining of tissue sections of subcutaneous implants of blank scaffolds after two weeks (A, B); scaffolds containing VEGF only (C, D), PDGF only (E, F), and dual release of VEGF and PDGF (G, H). Magnification for photomicrographs (A, C, E, G) 400×; (B, D, F, H) 1,000×.

DNA delivery from scaffolds

Objective – *in situ* gene therapy

Microenvironments for Stem Cells

Application Focus: Engineering Vasculature

References

1. Yannas, I. V. *Tissue and Organ Regeneration in Adults* (Springer, New York, 2001).
2. Langer, R. & Vacanti, J. P. Tissue engineering. *Science* **260**, 920-6 (1993).
3. Griffith, L. G. & Naughton, G. Tissue engineering--current challenges and expanding opportunities. *Science* **295**, 1009-14 (2002).
4. Richardson, T. P., Peters, M. C., Ennett, A. B. & Mooney, D. J. Polymeric system for dual growth factor delivery. *Nat Biotechnol* **19**, 1029-34 (2001).