Lecture 6: Programmed/Pulsed Drug Delivery and Drug Delivery in Tissue Engineering

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Last time: principles of controlled release from solid polymers

Today: Pulsatile/regulated/multifactor controlled release:
3 case studies of controlled release

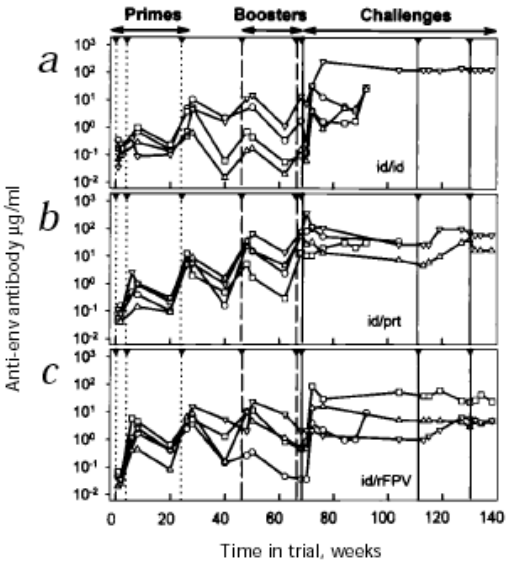
Reading: 'Polymeric system for dual growth factor delivery,' T.P. Richardson et al., *Nat. Biotech*. **19**, 1029-1034 (2001)
'Microchips as controlled drug-delivery devices,' J.T. Santini et al., *Andegwandte Chemie Intl. Ed*. **39**, 2396-2047 (2000)

Regulated controlled release

Applications of regulated and pulsatile release

- Definition: release of cargo in bursts followed by periods of little/no release in a defined temporal pattern[1]
- Many applications would be best-served by non-monotonic and multi-cargo release profiles
    - Motivation:
        - Single injection delivery of 'booster' for vaccination
        - Mimic natural secretion patterns of hormones
        - Provide optimal therapy for tolerance-inducing drugs
            - Constant drug levels cause receptor down-regulation

Vaccine boosting



hormone release patterns *in vivo*

Table 1
Examples of pulsatile secretion of various hormones in man. Extracted from a review article by Brabant et al. (Reference 7). Different ranges for a given hormone correspond to different primary references cited in the review article

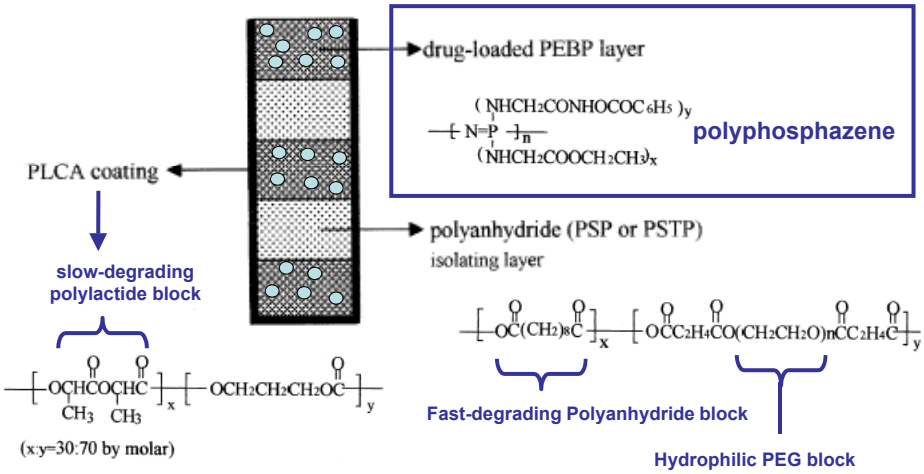
Hormone	*#Pulses/Day*
Growth hormone	9–16, 29
Prolactin	4–9, 7–22
Thyroid stimulating hormone	6–12, 13
Adrenocorticotropic hormone	15, 54
Luteinizing hormone	7–15, 90–121
Follicle stimulating hormone	4–16, 19
β-Endorphin	13
Melatonin	18–24, 12–20, 0
Vasopressin	12–18
Renin	6, 8–12
Parathyroid hormone	24–139, 23
Insulin	108–144, 120
Pancreatic polypeptide	96
Somatostatin	72
Glucagon	103, 144
Estradiol	8–16, 8–19
Progesterone	8–12, 6–16
Testosterone	13, 8–12
Aldosterone	6, 9–12
Cortisol	15, 39

        - Example: HIV-1 DNA vaccine delivered with boosters to elevate Ab titers[2]:

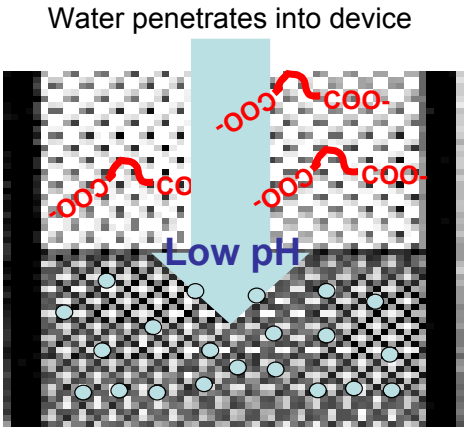
    - Mechanical and electrical devices that can provide digitized release typically require larger devices and surgical implantation (e.g. Pharm. Res. 1, 237 (1984)); also have high cost
        - Show an example
    - Degradable polymers allow submicron, injectable devices
- Two types
    - Pre-programmed
        - Release profile is encoded in structure and composition of device
    - Triggered
        - External signal drives release

Multilayer surface-eroding delivery devices

Case study: multilayered delivery devices[3]



- Polyphosphazene:
    - Base-catalyzed degradation, acid-inhibited degradation
- PEG-b-Polyanhydride:
    - Rapid bulk erosion- use hydrophilic block to make hrs-long degradation time for mm-thick caps (very fast)
    - …becomes porous during erosion, so need a means to prevent next layer from starting to degrade as water reaches drug-containing layer
    - creates acidic byproducts as it degrades
- Function of complete device:
    - First polyphosphazene layer: degrades quickly (first burst release)
    - Polyanhydride layer: degrades quickly, acidifies internal environment
        - Even though water penetrates the polyanhydride, no degradation of polyphosphazene begins and no drug is released from the polyphosphazene layer until the polyanhydride has completely eroded and acidic products are removed from microenvironment



Polyanhydride layer acidifies environment as it degrades:

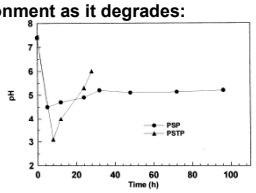


Fig. 4. pH change of 0.1 M pH 7.4 phosphate buffer solution at 37°C when polyanhydrides PSP and PSTP eroded.

Polyphosphazene only degrades quickly at neutral/basic pH :

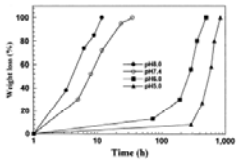
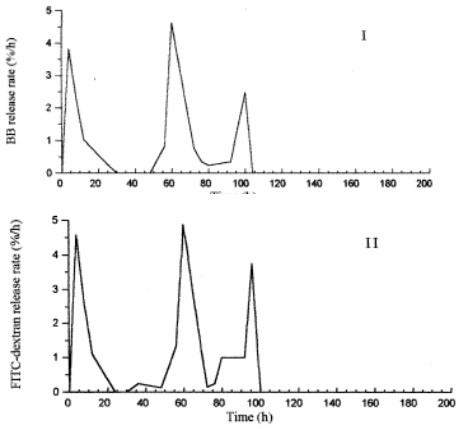


Fig. 5. Cumulative weight loss of PEBP discs (2.5 mm in diameter, 2 mm in thickness) in pH 7.4 and 8.0 boronic acid/borax buffer, and pH 5.0 and 6.0 citric acid/sodium citrate buffer at 37°C.

Model drug release profiles:



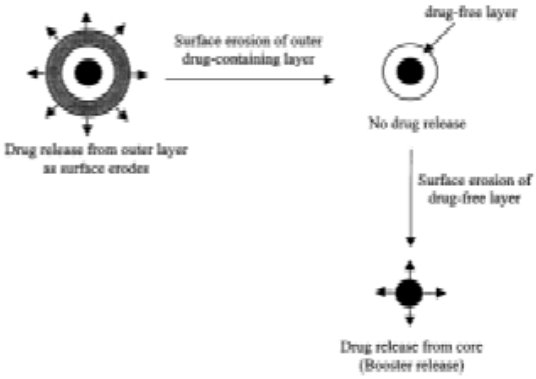


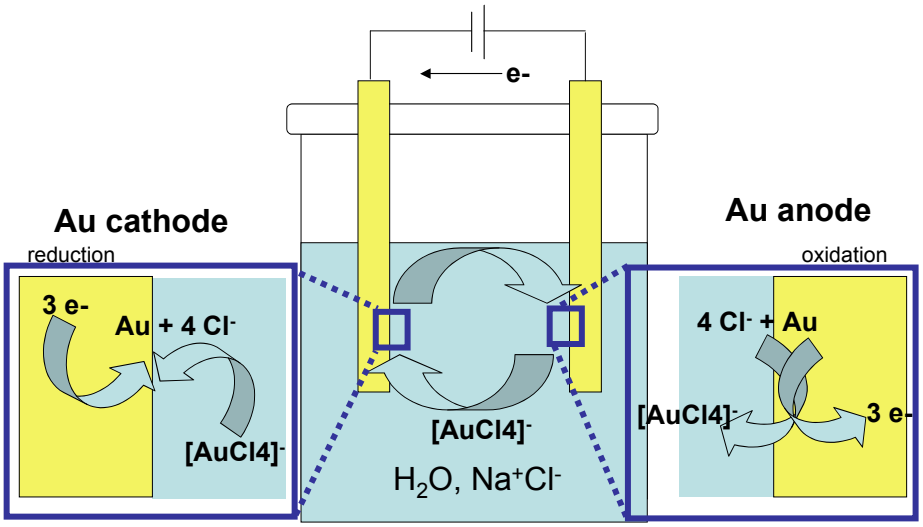
Fig. 2. Theoretical pulsatile release of a drug from a surface-eroding polymeric system. The time between initial release and booster release is determined by the erosion of the drug-free layer.

- refs for theory: J Contr Rel 20, 201 (1992); J Cont Rel 18, 159 (1992)

Regulated release devices: case example- drug delivery microchips

- work from M. Cima and R. Langer labs:[4]

- Principle of a gold electrochemical cell in the presence of aqueous chloride solution:
  - **ON BOARD:**



- In reality, multiple reactions occur simultaneously at the anode under an applied voltage in the 'passive and transpassive' regime[5]:
  - Au + 4Cl- -> [AuCl4]- + 3 e-
  - Au + mH_2O -> $Au(H2O)_m^{3+}$ + 3 e-
  - 2 Au + $3H_2O$ -> Au_2O_3 + $6H^+$ + 6e-
  - $2Cl^-$ -> Cl_2 + 2e-
  - Au_2O_3 + $8Cl^-$ + $6H^+$ -> $2[AuCl_4]^-$ + $3H_2O$
- Design of anode:
  - Need a material that is:
    - stable in the presence of chloride ions in the absence of a potential
      - many metals corrode with 0 applied potential *in vivo*
      - many metals spontaneously form an oxide layer by reaction with water/O_2 in physiological conditions
- in presence of potential, reacts to form a biocompatible soluble compound
- **Pourbaix diagram**: shows thermodynamically favored species under applied potential at varying pH
- **Evans diagram**: shows current produced due to electrochemical dissolution of the anode; the current is a measure of the rate of electrons being produced and thus measures the kinetics of the reaction

- Shows that gold membranes corrode quickly

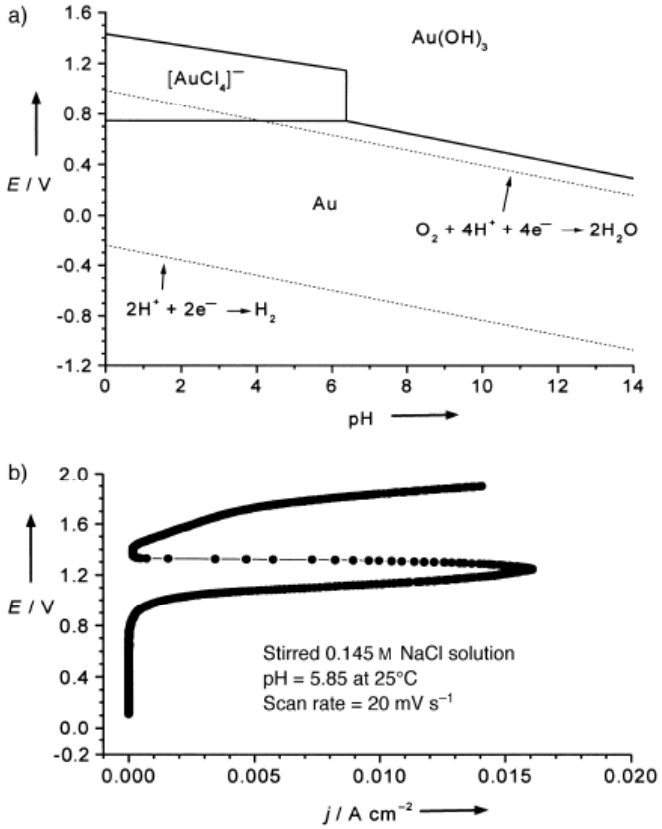


Figure 6. a) A Pourbaix diagram for the gold–chloride–water system containing 0.145M chloride ion. b) An Evans diagram for the same gold–chloride–water system obtained potentiodynamically. This diagram represents the kinetics of the gold corrosion reaction in chloride-containing solutions.

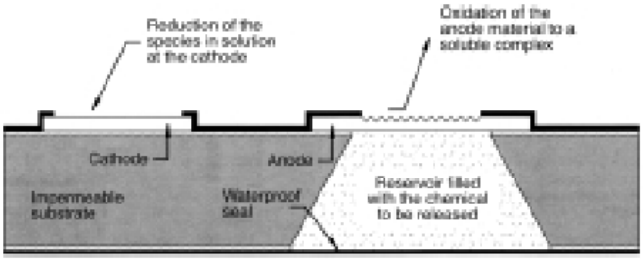
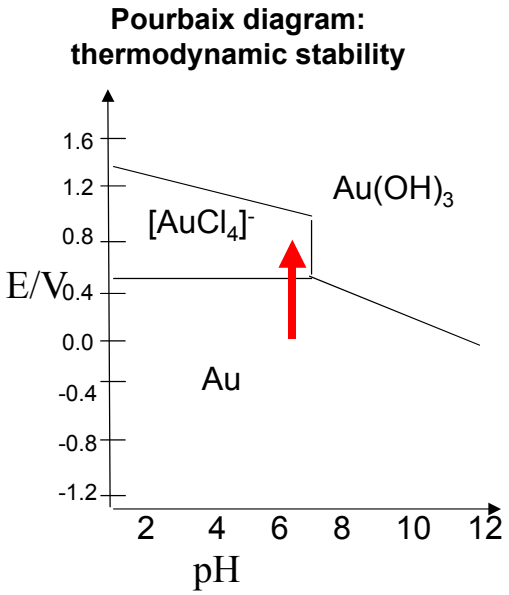
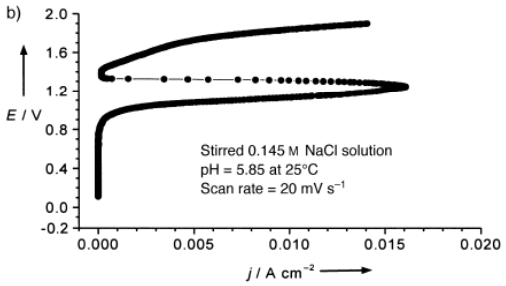


Figure 3. A cross section of a typical controlled-release microchip illustrating the principle of the electrochemical reservoir opening mechanism. The materials used in prototype microchips are described in Section 4.2.

- Structure of the controlled release microchip:
  - anode is a gold membrane 0.3 μm thick
  - current limitation in design is size of battery needed to operate the device: ~ 1 cm^2
    - microchip itself could be reduced to ~ 2 mmx 2mm





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- WHY DOES GOLD DISSOLVE AT PH 7? POURBAIX DIAGRAM SHOWS OXIDE IS STABLE FORM

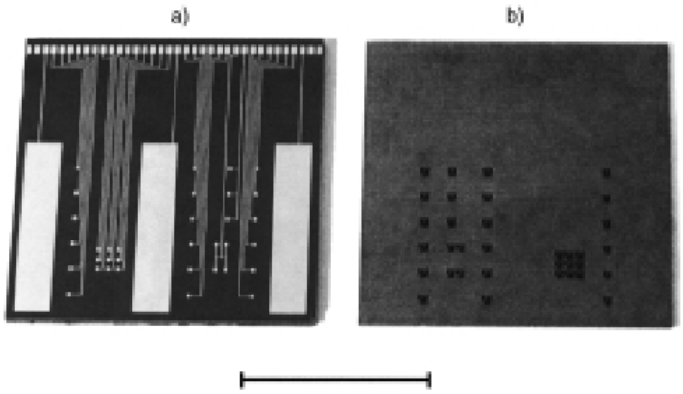


Figure 4. Photographs of a prototype microchip. a) The electrode-containing front side, b) the back side with the openings for filling the reservoirs. (Scale bar: 10 mm; photographs by Paul Horwitz.)

(Cima work)[6]

- Release properties:

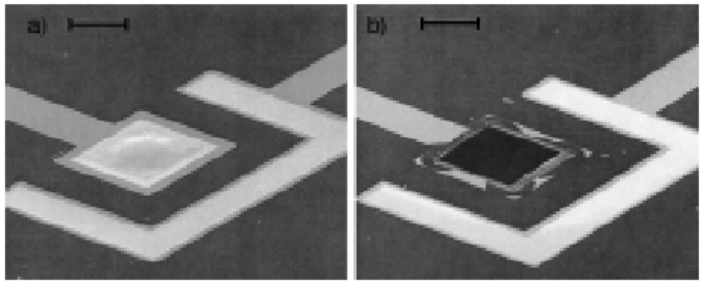


Figure 7. Scanning electron micrographs of a gold anode membrane covering a reservoir a) before and b) after the application of +1.04 V vs. SCE for several seconds in phosphate-buffered saline (PBS). (Scale bar: 50 μm.)

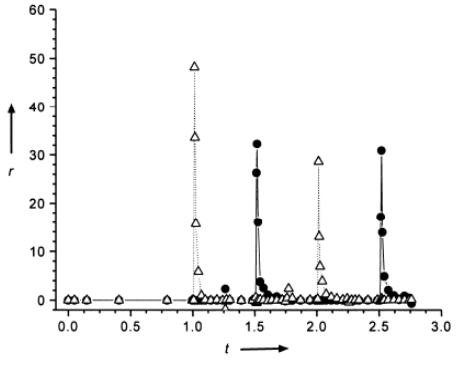


Figure 9. Pulsatile release of multiple substances (sodium fluorescein and $^{45}Ca^{2+}$) from a prototype controlled-release microchip into 0.145M sodium chloride solution. The release rate r of sodium fluorescein (●) is given in units of ng min^{-1}, and that of $^{45}Ca^{2+}$ (△) in units of 5 nCi min^{-1}. t in days.

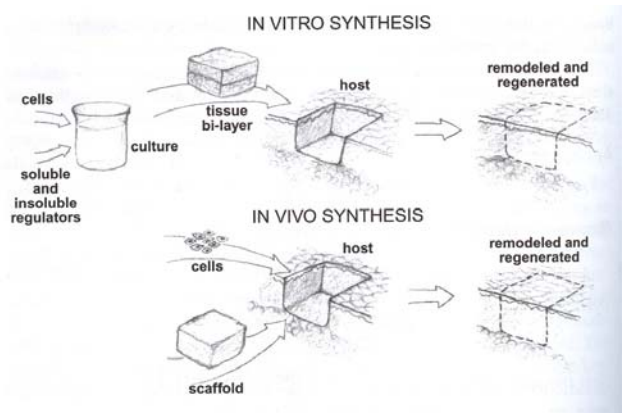
Controlled Release in Tissue Engineering

Tissue Engineering/Regenerative Medicine

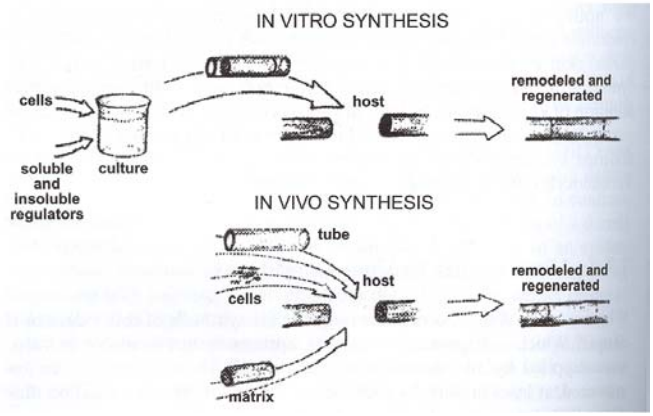
- 2 major approaches for regenerative medicine
  - *In vitro* tissue genesis → *in vivo* application
  - *In vivo* tissue genesis → *in vivo* application

Schematic comparison of *in vitro* and *in vivo* tissue engineering approaches[7]:

Skin:



bone:



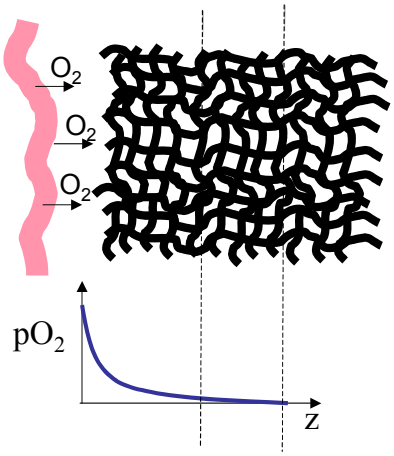
- Role of scaffold:
  - Provide functions of native ECM
  - Create a space for new tissue development
  - Deliver cells to site
  - Direct macroscopic size/shape of new tissue
- roles for soluble factor delivery in TE:
  - chemoattractant gradients used to draw desired cell types into structure
  - growth factors provided to induce cell proliferation to regenerate tissue
  - cytokines to induce tissue-specific cell functions

Cytokine delivery from scaffolds

Case Study: Induction of vascularization in TE scaffolds

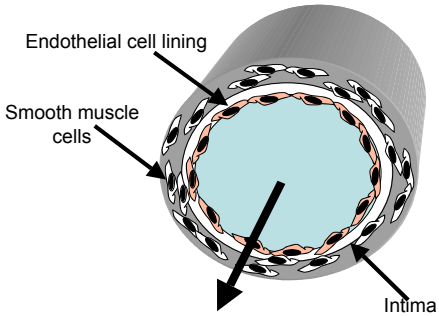
- Challenge of providing nutrients and oxygen to large tissue constructs
  - Constructs ~500 μm thick or greater cannot be supported by diffusive transport- need vascularization

- Structure of vasculature



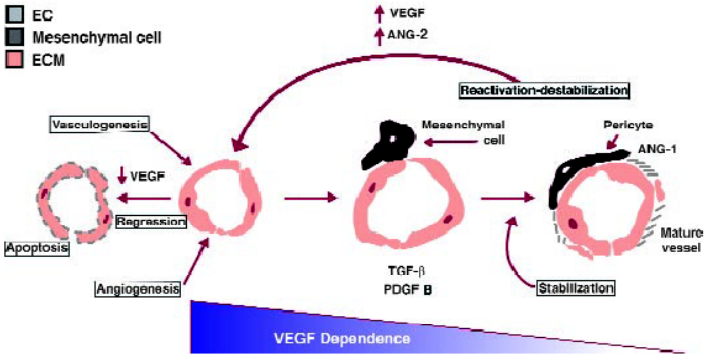
Extensive cell death
in center of construct

blood vessel structure:



- Angiogenesis[8]

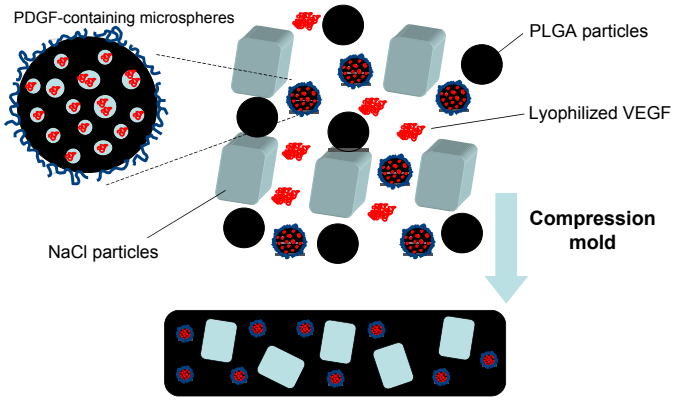
Figure 1
Vessel maturation: vessel development proceeds from a stage of growth-factor dependence where loss of a survival factor leads to apoptosis. Vessel stabilization is marked by investment with mural cells, local activation of TGF-β, and basement membrane production. The angiopoietins are clearly implicated, though their precise roles are yet to be determined.



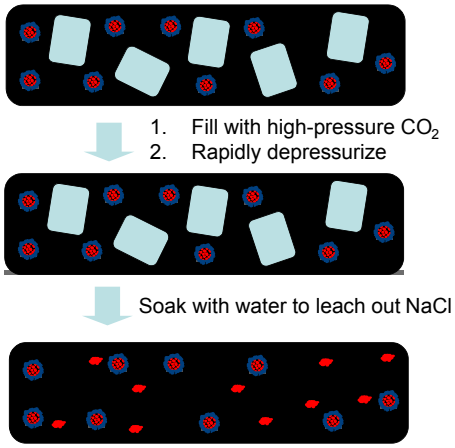
Steps in angiogenesis:

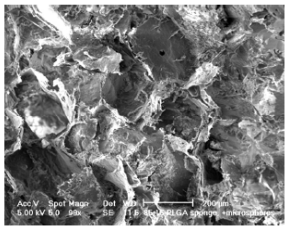
1. VEGF (vascular endothelial growth factor)
   -attracts endothelial cells, induces proliferation
   -induces tube formation
2. PDGF (platelet-derived growth factor)
   -attracts smooth muscle cells, stabilizes new vessels

- Dual growth factor delivery from degradable scaffolds for *de novo* blood vessel synthesis[9]:



- Fabrication process:[10]
  - PDGF encapsulated in PLGA microspheres by double emulsion approach
  - Microspheres (5-50 μm) mixed with PLGA particles (150-250 μm), NaCl particles (250-500 μm), and lyophilized VEGF particles (5-50 μm) in mold and compression molded to form a solid disk
  - Disk equilibrated with CO_2 at 800 psi 48hrs
  - Pressure rapidly dropped to ambient (14 psi)
  - Salt leached by soaking in distilled water 48 hrs





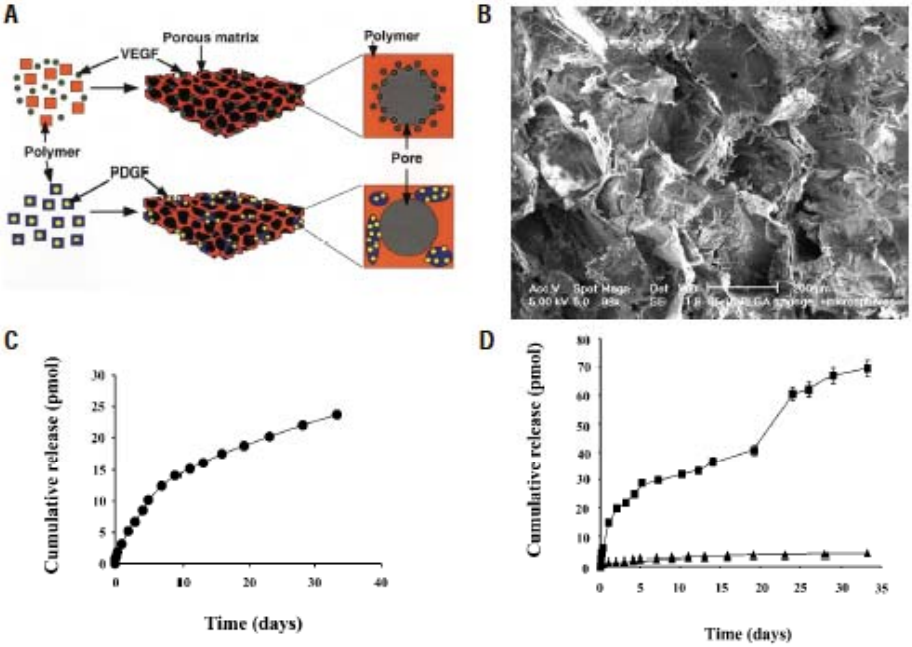


Figure 1. Schematic of scaffold fabrication process and growth factor release kinetics. (A) Growth factors were incorporated into polymer scaffolds by either mixing with polymer particles before processing into scaffolds (VEGF), or pre-encapsulating the factor (PDGF) into polymer microspheres used to 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).

- In vivo experiments:
  - Scaffolds implanted subcutaneously in Lewis rats, examined histologically at 2 weeks and 4 weeks
  - Comparisons:
    - Free cytokine injections with scaffolds vs. controlled release scaffolds
    - Dual vs. single factor controlled release scaffolds

- Bolus injection of free cytokines is ineffective:

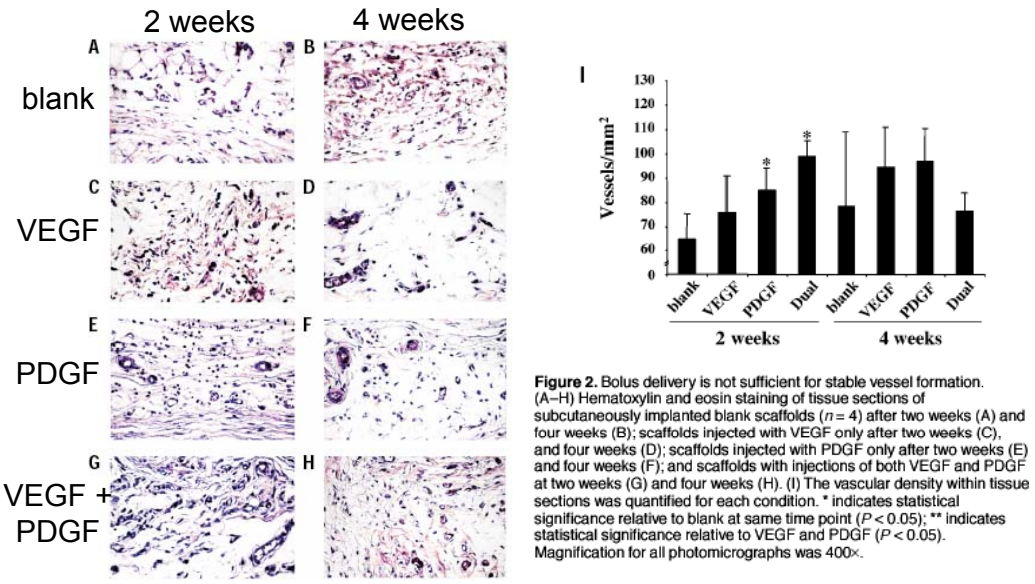


Figure 2. Bolus delivery is not sufficient for stable vessel formation. (A–H) Hematoxylin and eosin staining of tissue sections of subcutaneously implanted blank scaffolds ($n = 4$) after two weeks (A) and four weeks (B); scaffolds injected with VEGF only after two weeks (C), and four weeks (D); scaffolds injected with PDGF only after two weeks (E) and four weeks (F); and scaffolds with injections of both VEGF and PDGF at two weeks (G) and four weeks (H). (I) The vascular density within tissue sections was quantified for each condition. * indicates statistical significance relative to blank at same time point ($P < 0.05$); ** indicates statistical significance relative to VEGF and PDGF ($P < 0.05$). Magnification for all photomicrographs was 400×.

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

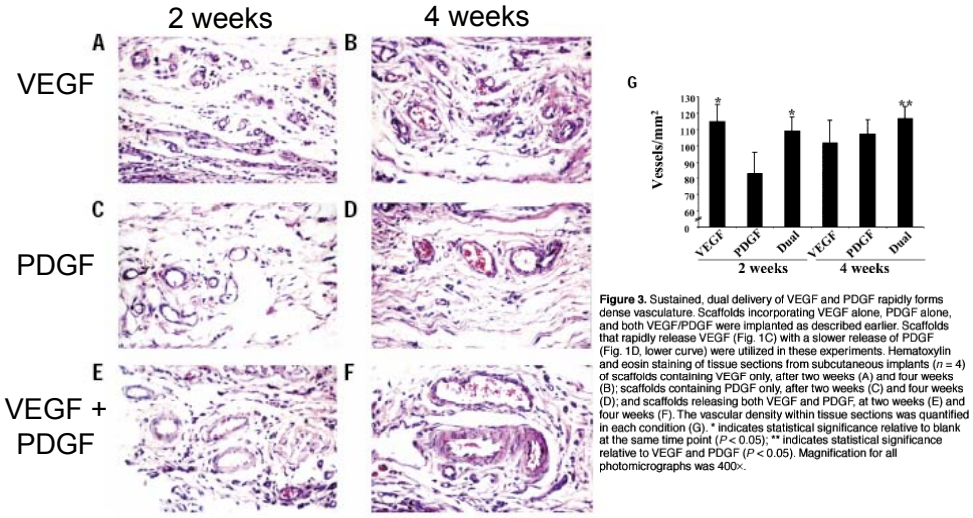


Figure 3. Sustained, dual delivery of VEGF and PDGF rapidly forms dense vasculature. Scaffolds incorporating VEGF alone, PDGF alone, and both VEGF/PDGF were implanted as described earlier. Scaffolds that rapidly release VEGF (Fig. 1C) with a slower release of PDGF (Fig. 1D, lower curve) were utilized 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×.

- vessels formed in controlled release systems show smooth muscle actin staining indicative of mature vessel formation:

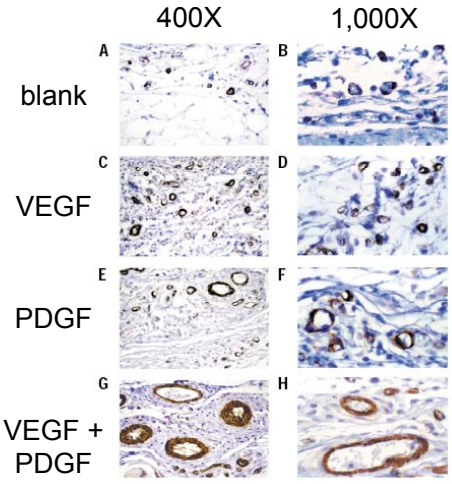


Figure 4. Dual delivery of VEGF and PDGF induces mural cell association. α-Smooth muscle actin staining of tissue sections of subcutaneous implants of blank scaffolds after two weeks (A, B); scaffolds containing VEGF only (C, D), PDGF only (E, F), and dual release of VEGF and PDGF (G, H). Magnification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

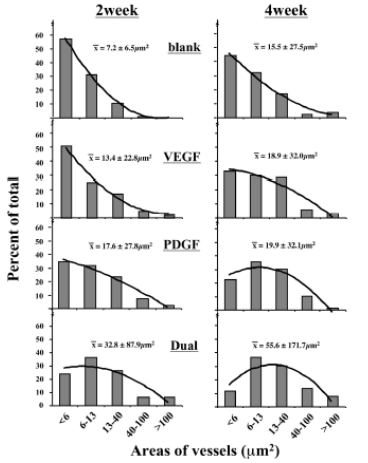


Figure 5. Dual delivery of VEGF and PDGF induces formation of larger vessels. Cross-sectional areas of blood vessels at two weeks and four weeks were measured from hematoxylin and eosin–stained tissue sections. For each condition, at least 150 blood vessels from 10 tissue sections were counted.

References

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