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

Last time: principles of controlled release from solid polymers

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

Reading:

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
• Many applications would be best-served by non-monotonic and multi-cargo release profiles
  o 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>
<thead>
<tr>
<th>Hormone</th>
<th>#Pulsed/Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth hormone</td>
<td>9-16, 29</td>
</tr>
<tr>
<td>Prolactin</td>
<td>4-9, 7-22</td>
</tr>
<tr>
<td>Thyroid stimulating hormone</td>
<td>6-12, 13</td>
</tr>
<tr>
<td>Adrenocorticosterone hormone</td>
<td>15, 54</td>
</tr>
<tr>
<td>Luminizing hormone</td>
<td>7-15, 90-121</td>
</tr>
<tr>
<td>Follicle stimulating hormone</td>
<td>4-16, 19</td>
</tr>
<tr>
<td>ß-Endorphin</td>
<td>13</td>
</tr>
<tr>
<td>Melatonin</td>
<td>18-24, 12-20, 0</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>12-18</td>
</tr>
<tr>
<td>Resin</td>
<td>6, 8, 12</td>
</tr>
<tr>
<td>Parathyroid hormone</td>
<td>24-139, 23</td>
</tr>
<tr>
<td>Insulin</td>
<td>105-144, 120</td>
</tr>
<tr>
<td>Pancreatic polypeptide</td>
<td>96</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>72</td>
</tr>
<tr>
<td>Glucagon</td>
<td>103, 144</td>
</tr>
<tr>
<td>Estradiol</td>
<td>8-16, 8-19</td>
</tr>
<tr>
<td>Progesterone</td>
<td>5-12, 6-16</td>
</tr>
<tr>
<td>Testosterone</td>
<td>13, 8-12</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>6, 9-12</td>
</tr>
<tr>
<td>Control</td>
<td>15, 39</td>
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</tbody>
</table>

Table 1
Examples of pulsatile secretion of various hormones in man. Extracted from a review article by Santini et al. (Reference 7). Differences among a given hormone correspond to different primary references cited in the review article.
Example: HIV-1 DNA vaccine delivered with boosters to elevate Ab titers:

- 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**

![Diagram of multilayered delivery device]

- 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:

Water penetrates into device

Polyphosphazene only degrades quickly at neutral/basic pH:

Model drug release profiles:

Drug release from core
(Bioreactor release)

Drug release from outer drug-containing layer

Surface erosion of outer drug-containing layer

No drug release

Drug release from outer drug-containing layer

Regulated release devices: case example- drug delivery microchips

- work from M. Cima and R. Langer labs.⁴

refs for theory: J Contr Rel 20, 201 (1992); J Cont Rel 18, 159 (1992)
- Principle of a gold electrochemical cell in the presence of aqueous chloride solution:
  - ON BOARD:

```
H2O, Na+Cl-  \[\text{Au cathode}\]  \[\text{Au anode}\]
```

```
4 \text{Cl}^- + \text{Au} \rightarrow [\text{AuCl}_4]^- + 3 \text{e}^-
```

```
\[\text{Au} + 4\text{Cl}^- \rightarrow [\text{AuCl}_4]^- + 3 \text{e}^-\]
```

```
\[\text{Au} + \text{mH}_2\text{O} \rightarrow \text{Au(H}_2\text{O)}_{\text{m}}^{3^+} + 3 \text{e}^-\]
```

```
2 \text{Au} + 3\text{H}_2\text{O} \rightarrow \text{Au}_2\text{O}_3 + 6\text{H}^+ + 6\text{e}^-
```

```
2\text{Cl}^- \rightarrow \text{Cl}_2 + 2\text{e}^-
```

```
\text{Au}_2\text{O}_3 + 8\text{Cl}^- + 6\text{H}^+ \rightarrow 2[\text{AuCl}_4]^- + 3\text{H}_2\text{O}
```

- In reality, multiple reactions occur simultaneously at the anode under an applied voltage in the 'passive and transpassive' regime:
  - Au + 4Cl^- \rightarrow [AuCl4]^− + 3 e−
  - Au + mH2O \rightarrow Au(H2O)m3^+ + 3 e−
  - 2 Au + 3H2O \rightarrow Au2O3 + 6H^+ + 6e−
  - 2Cl^- \rightarrow Cl_2 + 2e−
  - Au_2O_3 + 8Cl^- + 6H^+ \rightarrow 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 \textit{in vivo}
      - many metals spontaneously form an oxide layer by reaction with water/O_2 \textit{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

![Pourbaix diagram for the gold-chloride-water system containing 0.145M chloride ion.](image)

**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.

- 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 cm²
    - microchip itself could be reduced to ~ 2 mm x 2 mm

![Cross section of a typical controlled-release microchip illustrating the principle of the electrochemical reservoir opening mechanism.](image)
Pourbaix diagram: thermodynamic stability

**WHY DOES GOLD DISSOLVE AT pH 7? POURBAIX DIAGRAM SHOWS OXIDE IS STABLE FORM**

![Pourbaix diagram](image1)

> 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)
- **Release properties:**

![Release properties](image2)

> 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. Pulselase release of multiple substances (sodium fluorescein and ⁴⁰Ca⁴⁺) from a prototype controlled-release microchip into 0.154 M sodium chloride solution. The release rate, r, of sodium fluorescein (•) is given in units of ng/min⁻¹, and that of ⁴⁰Ca⁺ (△) in units of mCi/min⁻¹. t is 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:

Skin:

<table>
<thead>
<tr>
<th>IN VITRO SYNTHESIS</th>
<th>IN VIVO SYNTHESIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>cells</td>
<td>host</td>
</tr>
<tr>
<td>culture</td>
<td>scaffold</td>
</tr>
<tr>
<td>soluble and insoluble regulators</td>
<td>remodelled and regenerated</td>
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bone:

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<td>tube</td>
</tr>
<tr>
<td>remodelled and regenerated</td>
<td>matrix</td>
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</table>

- 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

- Angiogenesis

**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 invasion with mural cells, local activation of TGF-β, and basement membrane production. The angiogenic factors 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:

![Diagram of dual growth factor delivery](image)

• Fabrication process:
  1. PDGF encapsulated in PLGA microspheres by double emulsion approach
  2. 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
  3. Disk equilibrated with CO₂ at 800 psi 48hrs
  4. Pressure rapidly dropped to ambient (14 psi)
  5. Salt leached by soaking in distilled water 48 hrs
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:

controlled release scaffolds induce formation of more blood vessels with larger diameters:
vessels formed in controlled release systems show smooth muscle actin staining indicative of mature vessel formation:

![DIAGRAM](image1.png)

References