Nanosponge Delivery Systems and Reconfigurable Polyglycidol Networks for Combination Therapy

Eva Harth
Department of Chemistry
Nature of Therapeutics

Small Molecule Therapeutics  Growth Factors / Proteins / Antibodies

Size

Solubility

Compatibility
Individual and Multi Component Drug Delivery Systems

How to prepare delivery systems to facilitate optimum conditions for therapeutics of different nature

Nanoponge delivery systems

Semibranched Polyglycidols

Small molecule delivery

Growth factors, proteins and antibodies
Nanosponges — Areas of Application

- Bone Healing
- Tailored release
- Cancer Therapeutics
- Diabetes
- Nanosolubilization
- Dual-Component Hydrogels
Allyl and amine functionalities — Post-modification


Ring Opening Polymerization
Controlled Linear Polymer Formation

\[ \text{O} \to \text{O} \]
\[ \text{Sn(OTf)}_2 \]
EtOH, DCM
RT, 15hr

Retention Time (Minutes)

RI

<table>
<thead>
<tr>
<th>t</th>
<th>Mw (Da)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>24h</td>
<td>3350</td>
<td>1.11</td>
</tr>
<tr>
<td>7h</td>
<td>2643</td>
<td>1.13</td>
</tr>
<tr>
<td>3.5h</td>
<td>2187</td>
<td>1.15</td>
</tr>
<tr>
<td>1h</td>
<td>84.8</td>
<td>1.19</td>
</tr>
</tbody>
</table>

One-Pot Synthesis

Polymer & diamine in solution

Linear polymer
X% epoxide incorporation

Cross-linker
(1-10 equivalence of amine to epoxide)

Graph showing diameter (nm) as a function of Amine/1 Epoxide ratio:
- 2% epoxide
- 7% epoxide
- 19% epoxide
Importance of Crosslinking Density

Various cross-linking densities while achieving similar sized nanosponges
Polycarbonate Nanoparticles

Epoxide -Amine Reaction

Thiolene – Click Reaction

Nanosolubilization – Post-Loading

1. Drop-in

2. Centrifuge

3. Multiple washes with water

4. Lyophilization yields powder of nanosolubilized drug

5. Readily dispersed in aqueous buffer for direct administration

Homogenous DMSO solution

NANOSOLUBILIZATION

Water (Vit E-TPGS)
Tailored Drug Release with Nanosponges of different Crosslinking Density

0.15mM paclitaxel
PBS pH 7.4, 37 °C, TWEEN

Paclitaxel Release

% Released vs. Time (days)

- 4% cross-linking density
- 7% cross-linking density
- 10% cross-linking density
Tailored Drug Release with Nanosponges of different Crosslinking Density

0.15mM paclitaxel  
PBS pH 7.4, 37 °C, TWEEN

control release rate by mixing particles of different crosslinking densities together, combination treatment, tailored drug amount
Hh Activation for Bone Healing

Purmorphamine

Smo Agonist → Hedgehog Activation
Promotes Osteoblast Differentiation
Water-insoluble

X. Wu et al. / Cancer Treatment Reviews 38 (2012) 580–588
Hh Activation for Bone Healing

4% crosslinking

Encapsulation of drug preserves the mechanism of action in vivo

3x higher Gli1 and Patch1 mRNA after 24 hr treatment at fracture site

Gli1/Hprt  Ptch1/Hprt

CTL  Purmo  CTL  Purmo

*
Cancer Therapeutics—Targeting Peptide Attachment

Characterization and proof of attachment via NMR

Thiol-ene Click

Tumor targeting nanosponge (αv β3 integrin receptor)

Macromolecule
Cancer Therapeutics
—Targeting Peptide Attachment

Attached Peptide

cRGD-Nanosponge

Polyester Nanosponge

cRGD

[ppm]
Paclitaxel

Camptothecin

![Chemical Structures]

---

**Graph Description:**

- **Y-axis:** Fold Change
- **X-axis:** Time (Days)
- **Legend:**
  - HV/GGSSV-NP Taxol 10 mg/kg *
  - HV/GGSSV-NP Taxol 10 mg/kg * + HV/GGSSV-NP Camptothecin 10 mg/kg *
  - NP Camptothecin 10 mg/kg *
  - RT only
  - HV/GGSSV-NP Camptothecin 10 mg/kg *
  - NP Taxol 10 mg/kg *
  - Systemic Taxol 10 mg/kg *
  - None (control)

---

*Note: Significance markers are indicated by (*) and (#) for respective comparisons.*
Biodistribution and Pharmacokinetics
Dual-Component Drug Delivery Systems: Two Drugs of synthetic and biological Nature

Semibranched polyglycidol the better “PEG” for the stabilization of growth factors

Treatment of **Neurofibromatosis (NF1)**
Bone healing impossible and amputation necessary
Dual-Component Drug Delivery Systems: Two Drugs of Different Nature

Polyglycidol protects and mediates sustained release of protein

In Collaboration with Florent Elefteriou Lab: Vanderbilt Center for Bone Biology
Dual-Component Therapy

Diffusion and degradation of Nanoparticle allow release of MEK inhibitor
Is there a better “PEG”? 

Pegylated Proteins and Therapeutics – Solvatization and Bioavailability

Linear, high molecular weight PEG
Mostly graft-on Approaches

Crystallization in the Kidney and limited clearance

Branched structures are sought

Semibranched Polyglycidol – The better “PEG”?

Linear

Polyglycidol derivatives
semibranched

Hyperbranched

Semibranched Polyglycidol – Control of the Abundance of the Dendritic Carbon - Branching

Sn(OTf)$_2$ reacts with epoxides and acts as catalyst – Kinetic Control

Preferred branching
More linearity

non-preferred branching
Leads to more dendritic carbons
Degree of Branching

\[ DB = \frac{2D}{2D + L_{1,3} + L_{1,4}} \]

# DB in Glycidol Homopolymers

<table>
<thead>
<tr>
<th>Region</th>
<th>Shift (ppm)</th>
<th>40°C</th>
<th>20°C</th>
<th>0°C</th>
<th>-20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>L_{1,3}</td>
<td>81.0-82.0</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>D</td>
<td>79.5-80.5</td>
<td>0.79</td>
<td>0.62</td>
<td>0.60</td>
<td>0.48</td>
</tr>
<tr>
<td>2 L_{1,4}</td>
<td>73.5-74.5</td>
<td>3.69</td>
<td>3.65</td>
<td>3.98</td>
<td>4.80</td>
</tr>
<tr>
<td>2D, 2T</td>
<td>72.0-73.5</td>
<td>7.05</td>
<td>7.52</td>
<td>7.62</td>
<td>8.44</td>
</tr>
<tr>
<td>L_{1,3}, L_{1,4}</td>
<td>70.5-72.0</td>
<td>3.14</td>
<td>3.01</td>
<td>3.17</td>
<td>2.93</td>
</tr>
<tr>
<td>T</td>
<td>64.0-65.0</td>
<td>1.74</td>
<td>2.13</td>
<td>2.17</td>
<td>3.53</td>
</tr>
<tr>
<td>L_{1,3}</td>
<td>62.0-63.5</td>
<td>3.15</td>
<td>2.97</td>
<td>2.76</td>
<td>2.87</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Degree of Branching</th>
<th>0.24</th>
<th>0.21</th>
<th>0.20</th>
<th>0.15</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Relative Abundance of Dendritic Carbons</th>
<th>10.5%</th>
<th>8.2%</th>
<th>8.0%</th>
<th>5.2%</th>
</tr>
</thead>
</table>

**Temperature**

**Degree of Branching**
Formation of BSA-Polyglycidol Conjugates

BSA-OH Macroinitiator Formation

Phosphate Buffer pH=7.2

Polymer Growth: Graft-from

1. DMF (Sn)
2. DMSO (Sn)
3. PB pH=6

Polyacrylamide Gel Electrophoresis and MALDI-ToF

<table>
<thead>
<tr>
<th>Lane</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Protein Ladder</td>
</tr>
<tr>
<td>2</td>
<td>BSA</td>
</tr>
<tr>
<td>3</td>
<td>BSA-OH</td>
</tr>
<tr>
<td>4</td>
<td>BSA-ExB-PB</td>
</tr>
<tr>
<td>5</td>
<td>BSA-ExB-DMF</td>
</tr>
<tr>
<td>6</td>
<td>BSA-ExB-DMSO</td>
</tr>
</tbody>
</table>

BSA

DMF

DMSO

PB

78K

Polymerization of Polyglycidol in PBS Buffer

PB Reactions

PB pH 6.0

DB slightly than metal catalyzed higher but with 15%, just between hyperbranched and purely linear polyglycidols
Semibranched Polyglycidols with incorporated Aminooxy Functionalities

Varied degrees of aminooxy – content
Primary amino groups 50% or fully modified
Bring it all together - Reconfigurable and Responsive Network Systems

Semibranched Functionalization Polyglycidol + Polyglycidol

Amino-oxy

Functionalization Polyglycidol

Allyl -ester

Functionalized polyesters or polycarbonates

Network Formation

Azide

Functionalized Nanosponges and/or

Thiol
Non-Functionalized Polyglycidol as Network component

0.2 eq DMPA
DMF
UV 5 min

Equilibrium Water Content (%)

Water (25 °C)  water (37 °C)  SGF (37 °C)

PC
PC-PG
PC-1.5k
Non-Functionalized Polyglycidol as Network component

Vitrimer?

Transesterification – Network responsive to stimuli

Other properties – UMASS collaboration V. Muthukumar

Silica-Like Malleable Materials from Permanent Organic Networks Damien Montarnal, Mathieu Capelot, François Tournilhac, Ludwik Leibler* Science 18 November 2011: Vol. 334 no. 6058 pp. 965-968
Polycarbonate 2-D crosslinked Materials

Degradation
In PBS buffer and with and without lipase

With incorporated polyglycidol faster degradation and drug release

Drug Release: Paclitaxel release in buffer and SGF
Functionalized Polyglycidols and the Network Formations

Amino-Oxy polyglycidol with polyester (OPD)

In PBS buffer (pH 6) or organics

With or without the presence of
2. Reaction: Transesterification with zincacetate under heat. These reactions are reconfigurable and are not “set”.

Zinc Acetate 120°C

Functionalized Polyglycidols and the Network Formations
Functionalized Polyglycidols and the Network Formations

Development of Library of Hydrogels with functionalized polyglycidols

Crosslinking reaction to hydrogel

Network rearrangement with Zn(ac)2- Vitrimer
Conclusions

Cancer Therapeutics

Chemotherapeutic

Bone Healing

Smo Agonist

Combination Therapy

MEK inhibitor

Protein

Protein Therapeutics

Multifunctional Combination Treatments - Particle and Polyglycidol Hydrogels
Acknowledgements

Collaborators:
- Florent Elefteriou, VU Center for Bone Biology
- James Crowe, VU Pediatrics Department
- Fatih Uckun, University of Southern California
- Jamie Grunlan, Texas A&M
- Charles Manning, VU Imaging Institute

Present Members
- Dr. GuangZhao Li
- Benjamin Spears
- Artez Sims
- Ghazal Hariri
- David Stevens
- Dain Beezer
- Kelly Gillmore

Undergraduates
- Sicheng Ma
- Josh Greenbaum
- Tyler Merill
- Harry Watson
- Julian Waksal