Developing Natural Product-based Polymers for Medical Applications

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α-Methyl-D-glucose → Polymers & Co-polymers → Bulk & Nano-materials

Hydrolytic Degradation
Common Hydrolytically-degradable Polymers Prepared by Ring Opening Polymerization (ROP)

Degradation products

Use as composite materials or with non-natural co-blocks to incorporate functionality/modulate properties


Natural Product Polymers with the Functionality of Synthetic Polymers

**Synthetic Monomer**

\[
2 \text{HO} - \text{R} - \text{OH} + \text{CO}_2 \xrightarrow{\text{n H}_2\text{O}} \text{R} - \left(\begin{array}{c} \text{O} - \text{O} - \text{O} - \text{O} \end{array}\right)_{\text{n}} \quad \xrightarrow{\text{Poly(carbonate)}} \quad \text{CO}_2 - \text{R}
\]

Potentially inflammatory or toxic degradation products


**Natural products with “built in” diol for cyclic carbonate formation**


Haba, O.; Tomizuka, H.; Endo, T. *Macromolecules* 2005, 38, 3562-3563


Use as functional groups OR protect to change properties/functionality
Organobase Catalyzed Ring Opening Polymerization (ROP) Initiator/Chain-End Activation


\[ \text{benzyl alcohol} + \text{trimethylenecarbonate} \xrightarrow{1,8\text{-diaza}\text{bicyclo}[5.4.0]\text{undec-7-ene(DBU)}} \]

\[
\begin{align*}
\text{benzyl alcohol} & \quad \text{trimethylenecarbonate} \\
\end{align*}
\]

\[
\begin{align*}
\text{Initiation} & \quad \text{Propagation} \\
\text{Termination} & \quad \text{HA}
\end{align*}
\]
Degradable Polymers Derived from Natural Products: Organocatalyzed ROP of glucose-based cyclic carbonate

Commercially Available Starting Material

TBD = 1,5,7-Triazabicyclo[4.4.0]dec-5-ene

Degradable Polymers Derived from Natural Products: Organocatalyzed ROP of glucose-based cyclic carbonate

\[
\text{Repeating unit} = 248.2 \text{ Da} \\
\text{\textbullet} \text{: difference} = 248.3
\]

\[M_n \text{ laser} = 5390 \text{ g/mol (DP}_n = 21)\]

\[M_n \text{ NMR} = 7600 \text{ g/mol (DP}_n = 30)\]

\[
\text{Controlled polymerization with well-defined end groups}
\]

\[
\text{\textbullet} : \text{end group} = \begin{array}{c}
\text{K}^+ \text{ Adduct}
\end{array}
\]

\[
\begin{array}{l}
\text{DP}_n = 21, \text{ PDI} = 1.16 \\
\text{3633.6} \\
\text{5871.9} \\
\text{7315.9473} \\
\text{7381.8911} \\
\text{7860.9} \\
\text{8083.5620}
\end{array}
\]

\[
\begin{array}{l}
\text{7612.5028} \\
\text{7920.8887} \\
\text{9108} \\
\text{9274.9651}
\end{array}
\]

\[
\begin{array}{l}
\text{Voyager Spec ...} \\
\text{mass (m/z) 4101.3198 5614.7759 1101.7451 9251.3838 8591.0068 7173.1816 6359.9668 8083.5620 4620.1006 2877.5181 2345.8228}
\end{array}
\]
Controlled ROP Gives PGC with Regiorandom Propagation

Regiorandom propagation was confirmed by ESI tandem MS analysis by electron transfer dissociation (ETD)

\[ ^{13}\text{C} \text{NMR (CD}_2\text{Cl}_2, 125 \text{ MHz) } \]

\[ \text{Regiorandom propagation was confirmed by ESI tandem MS analysis by electron transfer dissociation (ETD)} \]

\[ \text{Regiorandom} \]

\[ \text{ppm} \]

\[ 155.0 \quad 154.4 \]

\[ \text{Head : Tail} \]

\[ \text{Head : Head} \]

\[ \text{Tail : Tail} \]

\[ 1 \quad 2 \quad 3 \quad 4 \quad 5 \quad 6 \quad 9 \quad 8 \quad 7 \]

\[ \text{ppm} \]

\[ 155 \quad 150 \quad 145 \quad 140 \quad 135 \quad 130 \quad 125 \quad 120 \quad 115 \quad 110 \quad 105 \quad 100 \quad 95 \quad 90 \quad 85 \quad 80 \quad 75 \quad 70 \quad 65 \quad 60 \quad 55 \]
Degradable Polymers Derived from Natural Products for Nanomedicine

Commercially Available Starting Material

- Functional Block Copolymer Assemblies?
- Robust Materials with Controlled Properties?
- Degradable Polymers?

Objective: Create natural product based polymers, with variable functionalities/properties, capable of degrading into natural by-products

Macroinitiation of Glucose Monomer Generates PPE-\(b\)-PDGC Block Copolymer

Phosphodiesters constitute the backbone of DNA, RNA

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Conv. (%)(^a)</th>
<th>PDI</th>
<th>(M_n) (NMR, Da)</th>
<th>(T_g) ((^\circ)C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGC(_{46})</td>
<td>98</td>
<td>1.13</td>
<td>11500</td>
<td>123</td>
</tr>
<tr>
<td>PPE(_{42})</td>
<td>96</td>
<td>1.12</td>
<td>7500</td>
<td>-36</td>
</tr>
<tr>
<td>PPE(<em>{42}-b)-PDGC(</em>{36})</td>
<td>85</td>
<td>1.10</td>
<td>16400</td>
<td>5</td>
</tr>
<tr>
<td>PPE(<em>{42}) + PDGC(</em>{46})</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>-37 / 95</td>
</tr>
</tbody>
</table>

Supramolecular Self-assembly: Potential temperature sensitive phase behavior?

Functionalized polymers do not undergo phase segregation

**LCST**
- Functional PPE$_{42}$-b-PDGC$_{36}$ precipitates from aqueous solution upon warming from 4 °C to room temp
Improving Materials and Degradation Characteristics

**Ethylene glycol is concerning for medical applications**

**Strategies:**
1. Develop robust materials with controlled properties via modification of the 2,3-glucose protecting groups.
2. Design fully degradable poly(saccharide)s.
3. Replace the PPE backbone linkages with those comprised of bioresorbable natural products.

**Fully degradable poly(saccharide)s**

**Hydrolytic Degradation**

LCST

Amphiphilic PPE-b-PDGC

$R, R' = $ Ionic Functional Group

$\text{CO}_2$
From Polymer Design to Applications: Degradable polymers with potential for use in medical applications

- Implantable Devices
- Therapeutics
  - Nanomaterials for Drug Deliver
- Diagnostics and Imaging
  - Improving diagnostics – contrast agents
  - Following and characterizing nanotherapeutics \textit{in vitro} and \textit{in vivo}

OsteoFab Patient Specific Cranial Device
Oxford Performace Materials (PEKK)

Absorb Bioresorbable Vascular Scaffold System
Abbott (PLLA & PDLLA + Everolimus)

Detection of angiogenesis

BIND Therapeutics
PMSA targeted (NSCLC, prostate)
Shell Crosslinked Knedel-like (SCK) Nanoparticles for Improved Therapeutics Delivery

Shell Crosslinks for:
- Structural stability
- Gating of guest release
- Addition of functionality
- Alteration of biological properties

Hydrophobic Core For:
- Packaging of therapeutics
- Determination of particle shape, rigidity, etc.

Functionalities in the Shell and/or Core:
- Radiolabeling sites
- Fluorescent probes
- Targeting ligands
- Control biodistribution
- Controlled Payload Release

Hydrophilic Shell (−, +, neutral) for Packaging of:
- Ag⁺
- DNA
- siRNA

Lung Infections

Lung Injury

Cancer Therapeutics

www.openbiosystems.com

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Advantages of Nanotherapeutics for Drug Delivery and Imaging

Rate of Extravasation and Clearance
(Also dependent on route of administration)
Polymeric Nanoparticles for Treatment of Lung Metastasis of Osteosarcoma

Osteosarcoma:
- Peak incidence in adolescence (<5 to ~40 years)
- Most commonly in the *distal femur* or the *proximal tibia*
- High fatality rate
  - 70% survival at 5 years
  - < 30% with metastasis
- Most common site of metastasis is the lung
  - 40% present with overt metastasis
  - 90% estimated to have micrometastatic disease at diagnosis

Treatment:
- Combination chemotherapy and surgical removal of the tumor (primary tumor)
- Chemotherapy to eliminate micrometastatic disease
- Unresectible recurrent disease is uniformly fatal

“...drugs delivered to the respiratory tract in liposomal formulation resulted in high pulmonary drug concentration, reduced systemic toxicity, and reduced dosage requirements compared with parenteral and oral administration.”

Fluorescent Labels: Why are they needed and what are we observing?

1. Light absorption (excitation)
   Photon absorption
2. Relaxation & internal conversion
3. Non-radiative decay
   No observed emission
4. Radiative decay (emission)
   Photon emission observed

$h_n$  

$S_0$ (Ground State)  

$S_1$ (Excited Singlet State)
Effects of degradation on evaluation of dye conjugation?

Electrophoretic Analysis (SDS-PAGE): Effects of polymer degradation when characterizing fluorophore conjugation

A488 cPPE cSCK
Degradable
30% crosslinking

A488 PAEA cSCK
Non-degradable
5% crosslinking

Band profiles due to electrophoresis
Triggered degradation

Band profile due to polydispersity

UV/Vis Absorption Suffers from Scattering, Limiting Utility for Quantitative Assessment

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Sample Type</th>
<th>Sample</th>
<th>$\Phi_F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanopure H$_2$O</td>
<td>Dye</td>
<td>A488</td>
<td>0.92*</td>
</tr>
<tr>
<td></td>
<td>Degradable</td>
<td>A488 cPPE</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>Non-degradable</td>
<td>A488 PAEA</td>
<td>0.28</td>
</tr>
<tr>
<td>PBS pH 7.4</td>
<td>Dye</td>
<td>A488</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Degradable</td>
<td>A488 cPPE</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Non-degradable</td>
<td>A488 PAEA</td>
<td>-</td>
</tr>
<tr>
<td>5% FBS in H$_2$O</td>
<td>Dye</td>
<td>A488</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Degradable</td>
<td>A488 cPPE</td>
<td>1.93</td>
</tr>
<tr>
<td></td>
<td>Non-degradable</td>
<td>A488 PAEA</td>
<td>-</td>
</tr>
</tbody>
</table>

*The Molecular Probes Handbook, Fluorescence quantum yields

![Diagram showing absorbance spectra of Alexa 488 in various conditions]
Steady State Spectroscopy for Assessing Dye-cSCK Conjugation

**Emission Anisotropy, \( r \)**

- **Tumbling** or state change & size determination is provided thru Polarized ex./em.
- Provide information on dynamic behavior: molecular orientation & rotational diffusion
- Measure the avg. angular displacement during the time between excitation and emission.

\[
0.4 = \text{high anisotropy} \quad 0.0 = \text{low anisotropy}
\]

\[
r = r_0 \frac{\theta}{\theta + \tau}
\]

Rotational correlation time

\[
\theta = \frac{\eta V}{RT}
\]

Lifetime

\[
r \uparrow = \text{Slower } \theta
\]

\[
r \downarrow = \text{Faster } \theta
\]
Steady State Spectroscopy: Some methods offer more reliability than others

**Steady State Anisotropy**

\[ r = r_0 \frac{\theta}{\theta + \tau} \]

*Rotational correlation time*  
*Lifetime*  
\[ \theta = \frac{\eta V}{RT} \]

\[ r \uparrow = \text{Slower } \theta \]  
\[ r \downarrow = \text{Faster } \theta \]

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Sample Type</th>
<th>Sample</th>
<th>( r )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanopure H(_2)O</td>
<td>Dye</td>
<td>A488</td>
<td>0.015 ± 0.003</td>
</tr>
<tr>
<td></td>
<td>Degradable</td>
<td>A488 cPPE</td>
<td>0.114 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>Non-degradable</td>
<td>A488 PAEA</td>
<td>0.129 ± 0.006</td>
</tr>
<tr>
<td>PBS pH 7.4</td>
<td>Dye</td>
<td>A488</td>
<td>0.013 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>Degradable</td>
<td>A488 cPPE</td>
<td>0.089 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>Non-degradable</td>
<td>A488 PAEA</td>
<td>0.132 ± 0.008</td>
</tr>
<tr>
<td>5% FBS in H(_2)O</td>
<td>Dye</td>
<td>A488</td>
<td>0.259 ± 0.006</td>
</tr>
<tr>
<td></td>
<td>Degradable</td>
<td>A488 cPPE</td>
<td>0.185 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>Non-degradable</td>
<td>A488 PAEA</td>
<td>0.217 ± 0.012</td>
</tr>
</tbody>
</table>

\( r \) = steady state anisotropy \( \lambda_{\text{ex}} = 430 \text{ nm} \), \( \lambda_{\text{em}} = 580 \text{ nm} \)

It's not only about confirming conjugation: How to obtain information on environmental interactions?
Excited State Lifetime

**Radiative processes:**
- a) Intrinsic building blocks (dye)
- b) Macromolecular (dye-polymer or dye-protein conjugates)
- c) Supramolecular (dye-nanoparticle conjugates)

**Non-radiative processes (environmental interactions):**
1) Solvent
2) Collisions
3) Aggregation
Possible explanations for divergence:

1) Size/volume:

2) Shell Rigidity: PAEA-\(b\)-PLA \(T_g = 48 \, ^\circ C\) vs. cPPE-\(b\)-PLLA \(T_g = -7.49 \, ^\circ C\)

3) Different shell character:
   1) Average intrinsic dipole moment
   2) Ammonium density
   3) Charge density

Steady state anisotropy trends rule out size and rigidity
Fluorescence Lifetime Changes Indicates Increased Polymer-solvent Interactions for Degradable cPPE

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Sample Type</th>
<th>Sample</th>
<th>$\tau_1$ (ns)</th>
<th>$\tau_i$ (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{H}_2\text{O}$</td>
<td>Dye</td>
<td>A488</td>
<td>4.22 (100 %)</td>
<td>4.22</td>
</tr>
<tr>
<td>PBS pH 7.4</td>
<td>Dye</td>
<td>A488</td>
<td>4.08 (100 %)</td>
<td>4.08</td>
</tr>
<tr>
<td>$\text{H}_2\text{O}$</td>
<td>Degradable</td>
<td>A488 cPPE</td>
<td>1.15 (27 %)</td>
<td>3.04</td>
</tr>
<tr>
<td>PBS pH 7.4</td>
<td>Degradable</td>
<td>A488 cPPE</td>
<td>1.42 (28 %)</td>
<td>3.26</td>
</tr>
<tr>
<td>$\text{H}_2\text{O}$</td>
<td>Non-degradable</td>
<td>A488 PAEA</td>
<td>1.05 (30 %)</td>
<td>3.86</td>
</tr>
<tr>
<td>PBS pH 7.4</td>
<td>Non-degradable</td>
<td>A488 PAEA</td>
<td>0.79 (21 %)</td>
<td>4.06</td>
</tr>
</tbody>
</table>

Lifetime Trends in PBS:
1) $\uparrow$ in $\tau_1$ for A488 cPPE
2) $\downarrow$ in $\tau_1$ for A488 PAEA
3) Rotational correlation time ($\theta$):
   a. A488 cPPE $\uparrow$ 46%
   b. A488 PAEA $\uparrow$ 4%

$\theta = \frac{\eta V}{RT}$

An understanding the spectral signature-structure-function relationship provides a fundamental understanding of nanomaterials before the complexity of drug delivery and biology become a factors.
Polymer-based Nanotherapeutics

Intelligent synthetic design offers unique opportunities to improve upon traditional synthetic materials; natural product-based materials may offer increased biocompatibility, control of degradation products, etc.

Importance of evaluating polymeric nanomaterials as therapeutics and imaging agents:

What is the goal?

How to evaluate polymeric materials to ensure they work in a consistent and reproducible manner

How can polymer/nanoparticle design impact observations during in vivo studies?
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