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University of Westminster, London, UK
Visiting Professor, Imperial College, London
ANTIMICROBIAL POLYMERS OF BACTERIAL ORIGIN

Polyhydroxyalkanoates

Bacterial Cellulose
Polyhydroxyalkanoates, the biodegradable and biocompatible polymers

Polyhydroxyalkanoates are water-insoluble storage polymers which are polyesters of 3-, 4-, 5- and 6-hydroxyalkanoic acids produced by a variety of bacterial species under nutrient-limiting conditions. They are biodegradable and biocompatible, exhibit thermoplastic properties and can be produced from renewable carbon sources.

Philip et al., 2007, JCTB, 82 (3):233-247
Akarayonye et al., 2010, JCTB, Volume 85 (6): 732-743
Keshavarz et al., 2010, Current Opinion in Microbiology 13 (3): pp. 321-326
The general structure of Polyhydroxyalkanoates

$R_1 / R_2 = \text{alkyl groups (C}_1-\text{C}_{13})$

$x = 1, 2, 3, 4$
SCL and MCL Polyhydroxyalkanoates

Total Carbon chain length in monomer = 4-5; **SCL PHAs**
Total Carbon chain length in monomer = 6-14; **MCL PHAs**

**SCL-PHAs** - Thermoplastics
**MCL-PHAs** - Elastomerics
## Properties of SCL and MCL Polyhydroxyalkanoates

<table>
<thead>
<tr>
<th>Type of PHA</th>
<th>Melting Temp (°C)</th>
<th>Glass Transition Temp (°C)</th>
<th>Young’s Modulus (GPa)</th>
<th>Elongation at break (%)</th>
<th>Tensile strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(3HB)</td>
<td>171</td>
<td>2.7</td>
<td>3.5</td>
<td>1</td>
<td>40</td>
</tr>
<tr>
<td>P(3HB-co-20%3HV)</td>
<td>145</td>
<td>-1</td>
<td>1.2</td>
<td>3.84</td>
<td>32</td>
</tr>
<tr>
<td>P(4HB)</td>
<td>60</td>
<td>-50</td>
<td>0.149</td>
<td>1000</td>
<td>104</td>
</tr>
<tr>
<td>P(3HB-co-16%4HB)</td>
<td>152</td>
<td>-8</td>
<td>ND</td>
<td>444</td>
<td>26</td>
</tr>
<tr>
<td>P(3HO-co-18%3HHx)</td>
<td>61</td>
<td>-35</td>
<td>0.008</td>
<td>400</td>
<td>9</td>
</tr>
<tr>
<td>P(3HB-co-3HHx)</td>
<td>120</td>
<td>-2</td>
<td>0.5</td>
<td>850</td>
<td>21</td>
</tr>
</tbody>
</table>
Metabolic Pathways involved in PHA Biosynthesis
Polyhydroxyalkanoate Synthases, the enzymes involved in PHA Biosynthesis

PHA synthases catalyse the stereo-selective conversion of \((R)-3\text{-hydroxyacyl-CoA}\) substrates to PHAs with the concomitant release of CoA
Production of Polyhydroxyalkanoates in Large Scale Fermenters
Production of SCL-Polyhydroxyalkanoates using *Bacillus cereus* SPV, a Gram positive bacteria

Valappil et al., 2007, Journal of Biotechnology, Volume 127(3), 475-487
Philip et al., 2009, Biomacromolecules 10(4): 691 – 699
Akarayonye et al., 2010, Biotechnology Journal 7(2) 293-303
Akarayonye et al., 2016, Polymer International,65 (7) 780–791
Large scale production of P(3HB) using fed batch fermentation in Kannan and Rehacek medium (Yield 38% dcw)

Valappil et al., 2007, Journal of Biotechnology, 132; 251-258
Material and Thermal Properties of the P(3HB) produced

<table>
<thead>
<tr>
<th>Type of PHA</th>
<th>Melting Temp (°C)</th>
<th>Glass Transition Temp (°C)</th>
<th>Young’s Modulus (GPa)</th>
<th>Elongation at break (%)</th>
<th>Tensile strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(3HB)</td>
<td>169</td>
<td>1.9</td>
<td>1.7</td>
<td>3.8</td>
<td>25.7</td>
</tr>
</tbody>
</table>
Production of MCL-Polyhydroxyalkanoates using *Pseudomonas mendocina*, a Gram negative bacteria

Rai *et al.*, 2011, Material Science Engineering (Reviews) 72(3) 29-47  
Rai *et al.*, 2011, Biomacromolecules, 12 (6), pp 2126–2136  
Lizzaraga *et al.*, 2015, Engineering in Life Sciences 15(6) 612-621  
Material and Thermal Properties of the P(3HO) produced

<table>
<thead>
<tr>
<th>Type of PHA</th>
<th>Melting Temp (°C)</th>
<th>Glass Transition Temp (°C)</th>
<th>Young’s Modulus (MPa)</th>
<th>Elongation at break (%)</th>
<th>Tensile strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(3HO)</td>
<td>42</td>
<td>-38</td>
<td>0.8</td>
<td>1200</td>
<td>8.6</td>
</tr>
</tbody>
</table>
Large scale production of P(3HO) using batch fermentation in MSM media (Yield 31% dcw)

SODIUM OCTANOATE as the main Carbon Source
Production of a range of SCL-PHAs and MCL-PHAs

Polyhydroxyalkanoates produced using a range of different carbon sources
Production of Antimicrobial PHAs
Production of Antimicrobial PHAs by the addition of Antimicrobial agents of natural origin
Production of Antimicrobial PHAs

PHA + Trans-cinnamaldehyde
Production of Antimicrobial PHAs with Trans-cinnamaldehyde

<table>
<thead>
<tr>
<th>TC (µL)</th>
<th>Inhibition zone (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>1.5; 2.5</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>4</td>
<td>2.5</td>
</tr>
<tr>
<td>8</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Antimicrobial activity against *S. aureus* ATCC® 6538P™
Production of Antimicrobial PHAs with Trans-cinnamaldehyde

Antimicrobial activity against *S. aureus* ATCC® 6538P™

**MIC value - 5mM**
Production of Antimicrobial PHAs with Trans-cinnamaldehyde

<table>
<thead>
<tr>
<th></th>
<th>Tensile strength MPa</th>
<th>Young’s Modulus MPa</th>
<th>Extension at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(3HB)</td>
<td>21.5</td>
<td>1091.5</td>
<td>28.9</td>
</tr>
<tr>
<td>P(3HB), 8 wt%</td>
<td>16.8</td>
<td>802.3</td>
<td>53.5</td>
</tr>
<tr>
<td>P(3HB), 11 wt%</td>
<td>15.6</td>
<td>625.1</td>
<td>116.3</td>
</tr>
<tr>
<td>P(3HB), 13 wt%</td>
<td>10.7</td>
<td>444.8</td>
<td>109.1</td>
</tr>
</tbody>
</table>

P(3HB)/TC
Production of Antimicrobial PHAs with Trans-cinnamaldehyde

P(3HB)/TC
Production of Antimicrobial PHAs with Trans-cinnamaldehyde

S. aureus ATCC® 6538P™
Antimicrobial PHAs with Trans-cinnamaldehyde are effective against *S. aureus*
Production of Antimicrobial PHAs

PHA + Antimicrobial peptides
Production of Antimicrobial PHAs

- Lipophilic
- Cationic

Antimicrobial peptides:
- Cation rich (from lysine and arginine)
- Amphiphilic
- Low molecular weight

Weak, hydrophobic interaction

Strong, electrostatic interaction

Mammalian membrane
- Net neutral at surface

Bacterial membrane
- Negatively charged headgroups
Production of Antimicrobial PHAs

**AMP3**
- MIC value 50 µM

**Nut2**
- MIC value 50 µM

*S. aureus ATCC® 6538P™*
Production of Antimicrobial PHAs

**AMP3**
- MIC value 12.5 μM

**Nut2**
- MIC value 12.5 μM

E. coli ATCC 8739
Antimicrobial PHAs with Antimicrobial peptides are effective against *S. aureus* and *E. coli*
Production of Antimicrobial PHAs

**PHA** + **Garlic extract (allicin)**
Production of Antimicrobial PHAs

Antibacterial assay-agar well diffusion against *S. aureus* ATCC® 6538P™

<table>
<thead>
<tr>
<th>Concentration of dehydrated garlic</th>
<th>Dehydrated garlic/Inhibition zone (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mg/ml</td>
<td>1.1</td>
</tr>
<tr>
<td>5 mg/ml</td>
<td>1.5</td>
</tr>
<tr>
<td>7 mg/ml</td>
<td>1.7</td>
</tr>
</tbody>
</table>
Production of Antimicrobial PHAs
MCL-PHA

Antibacterial activity of P(3HO-co-3HD) films against *S. aureus* ATCC® 6538P™

<table>
<thead>
<tr>
<th>Concentration of agent</th>
<th>Dehydrated garlic/Inhibition zone (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.25 mg/ml</td>
<td>1.5</td>
</tr>
<tr>
<td>22.5 mg/ml</td>
<td>2.7</td>
</tr>
<tr>
<td>90 mg/ml</td>
<td>3.8</td>
</tr>
</tbody>
</table>
Production of Antimicrobial PHAs
MCL-PHA

Antibacterial activity of P(3HO-co-3HD) films against *E. coli* ATCC 8739

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</tr>
<tr>
<td>90 mg/ml</td>
<td>2.7</td>
</tr>
</tbody>
</table>
Antimicrobial PHAs with Dehydrated Garlic (Allicin) are effective against *S. aureus* and *E. coli*
Inherently Antimicrobial PHAs
Thio-PHAs
Thio-Polyhydroxyalkanoates

Sulphur containing PHAs

Sulphur in the backbone
- 3-mercaptodipropionate (3MP)
- 3-mercaptopentanoate (3MB)
- 3-mercaptopentanoate (3MV)
- copolymers with 3-hydroxybutyrate (3HB)

Sulphur in the side chains

Thioester groups

Thioether groups

Thio-Polyhydroxyalkanoates
Sulphur containing PHAs
Sulphur in the side chains: Thioester groups

Proven intrinsic antimicrobial properties
Against methicillin-resistant \textit{Staphylococcus aureus} (MRSA) both \textit{in vitro} and \textit{in vivo}
Production of Thio-Polyhydroxyalkanoates

Co-feeding experiment:
- Decanoic acid
- 6 acetylthiohexanoic acid

<table>
<thead>
<tr>
<th></th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH₂PO₄</td>
<td>13.6 g/L</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>0.2 g/L</td>
</tr>
<tr>
<td>FeSO₄ * 7H₂O</td>
<td>0.5 mg/L</td>
</tr>
<tr>
<td>Trace elements</td>
<td>1 mL/L</td>
</tr>
</tbody>
</table>
Characterisation of the Thio-PHAs
Chemical characterization: FT-IR

Thio-PHAs

Absorbance

-CH, -CH₂, -CH₃

2930 cm⁻¹

O-C=O

1735 cm⁻¹

S-C=O

1690 cm⁻¹

C-H
Thio-PHAs

Thermal characterization: DSC

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$T_m$ (°C)</th>
<th>$T_g$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(3HHx-3HO-3HD)</td>
<td>51.3</td>
<td>-41.4</td>
</tr>
<tr>
<td>P(3HHx-3HO-3HD-3H4ATB-3H6ATH)</td>
<td>-</td>
<td>-32.5</td>
</tr>
</tbody>
</table>
Thio-PHAs

Antibacterial activity (R)

- S. aureus
- E. coli

P(3HO) vs S-PHA
Scaffolds/devices/structures made using PHAs

P(3HB) and P(3HB)/Bioglass® composites

Drug Delivery

Biodegradable Drug Eluting Stents

Biodegradable Nerve Conduits
PHAs
The new emerging medical materials!

Valappil et al., 2006; Expert Review in Medical Devices 3(6): 853-868
Rai et al., 2010; Material Science Engineering (Reviews) 72(3):29-47
Dubey et al., 2014 Novel cardiac patch development using biopolymers and biocomposites; ISBN13: 9780841229907
Regulatory Body Approval of Polyhydroxyalkanoates for Medical Applications

- **Apr 2, 2007** Tepha, Inc. Receives **FDA Clearance** for TephaFLEX® Absorbable Suture product for marketing in the U.S. TephaFLEX® is the first medical device derived from PHAs developed by Tepha and the MIT.

- **May 1, 2009** Tepha, Inc. announced that its corporate partner, Aesculap AG, has received a **CE Mark** and is launching its MonoMax monofilament absorbable suture for general surgical indications in Europe. The product is made with TephaFLEX® fibre.
Medical applications of PHAs being explored in my Group

**Bone tissue engineering**
- P(3HB) and P(3HB)/Bioglass® composites

**Cartilage Tissue Engineering**
- P(3HB)/MFC composites

**Semiartificial Pancreas**
- P(3HO)/P(3HB) Blends

**Skin Tissue Engineering/Wound Healing**
- P(3HO)/NanoBioglass Composites

**Cardiac Tissue Engineering**
- P(3HO) and P(3HN-co-3HP)

**Drug Delivery**
- P(3HB)/P(3HB-co3HV)

**Medical Device Development:**
- **Biodegradable Drug Eluting Stents**
- SCL/MCL PHAs

- **Biodegradable Nerve Conduits**
- SCL/MCL PHAs
Bacterial cellulose based antimicrobial materials
Bacterial cellulose

Bacterial cellulose (BC) produced by bacteria from different genera (for example *Gluconacetobacter*). Bacterial cellulose shows a peculiar tridimensional structure. It is produced as nanosized fibrils with high degree of purity and crystallinity, giving it unique physical and mechanical properties like strength and water retention. Moreover, it is much purer than plant cellulose which is normally in the form of lignocellulose and is known to be highly biocompatible, so it is very well suited for applications in the biomedical field.

Production of Bacterial cellulose

*Gluconacetobacter xylinus*
5-7 days at 30 °C

Bacterial cellulose pellicle

Bacterial cellulose pellicle after washing
Characterisation of Bacterial cellulose

OH
C-O-C
H₂O
C-CH₂
CH₂

3344.67 cm⁻¹, 0.04 A
2896.36 cm⁻¹, 0.01 A
1645.59 cm⁻¹, 0.03 A
1428.02 cm⁻¹, 0.02 A
1543.91 cm⁻¹, 0.02 A
1161.70 cm⁻¹, 0.04 A
1315.07 cm⁻¹, 0.03 A
1032.08 cm⁻¹, 0.09 A
1055.60 cm⁻¹, 0.10 A
557.99 cm⁻¹, 0.09 A
435.29 cm⁻¹, 0.08 A
665.43 cm⁻¹, 0.07 A
1108.43 cm⁻¹, 0.06 A

Cellulose
Name
Sample 041 By Students Date Monday, March 21 2016
Description
Characterisation of Bacterial cellulose

SEM
Antibacterial activity of additive ‘a’

10 μL, ZOI = 1.7 cm
30 μL, ZOI = 2.3 cm

20 μL, ZOI = 2.1 cm
40 μL, ZOI = 2.5 cm

30 μL, ZOI = 2.3 cm
50 μL, ZOI = 2.8 cm

50 μL, ZOI = 2.8 cm
70 μL, ZOI = 3.1 cm

S. aureus ATCC® 6538P™
Surface modification of Bacterial cellulose

1) NaOH;
2) a
65 °C, oil bath
Bacterial Cellulose
Surface Antibacterial testing

S. aureus ATCC® 6538P™
Conclusions

• Polyhydroxyalkanoates (PHAs) are an emerging class of biodegradable and biocompatible polymers of natural origin with huge potential in biomedical applications.

• *Bacillus* sp. and *Psuedomonas* sp. have been used in the Roy lab to produce SCL-PHAs and MCL-PHAs respectively.

• The PHAs produced have been used successfully in development of antimicrobial polymers using additives-TC, AMP, Allicin.

• Thio-PHAs are another emerging class of antibacterial polymers

• Bacterial Cellulose is another natural polymer with potential in biomedical applications including wound healing.
Key Scientists

Sheila Piarali
(TC, AMP and PHA)

Isabel Orlando
(Bacterial Cellulose)

Elena Marcello
(Thio-PHA)

Alexandra Paxinou
(Allicin and PHA)

Dr Pooja Basnett
(All aspects)
Funding for this work was provided by the European Commission’s “Horizon 2020 Programme” under Grant agreement No. 643050 (HyMedPoly).
My Group
Thanks for your attention!