Microparticles as a generic platform for vaccine delivery

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Optimized sub-unit vaccines consist of three components

- **Delivery system**
  - To target and/or deliver antigens to cells of the innate immune system

- **Immune potentiator**
  - To activate the innate immune system and provide the pro-inflammatory context for antigen recognition

- **Antigen**
  - To provide sub-unit antigens with specific pathogen epitopes to generate the adaptive (specific and long-lived) immune response

**Immune potentiators**

- LPS
- Tri-acyl and di-acyl lipopeptides
- Lipidated peptides, proteins and carbohydrates
- Flagellin
- Bacterial DNA containing CpG motives
- Double-stranded RNA
- Poly(I:C): polyinosine-polycytidylic acid

**Delivery systems**

- Emulsions
- Microparticles
- Nanoparticles
- Liposomes, Virosomes
- ISCOMS
- Mucosal delivery systems
- Jet injection devices
- Microneedles
- Dermal patches

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**Phagocytosis of PEI-coated PS microspheres by dendritic cells**

L Thiele et al. JCR 76:149-68 (2001)

by pseudopods, Pp

by sinking into cells
Fusion of lysosomes with phagocytosed PLGA microspheres in macrophages

E Walter et al. 2000

Antigen presentation: MHC I and MHC II

http://www.vetmed.wsu.edu/research_vmp/ftp/
Antigen-presenting cells bridge between innate and adaptive immune response

Toll like receptors recognize pathogen associated molecular patterns (PAMPs)
The Th1-Th2 paradigm

www.ebioscience.com
N Alaverdi & D Sehy (2006)
designed by S Lee

Effector cells

Effector cell function

Humoral immunity

Cellular immunity

PLGA microparticles for vaccine delivery:
... more than controlled release
PLGA microparticles degrade in macrophages depending on their composition

13 days incubation

L Thiele et al. JCR 76:149-68 (2001)

<table>
<thead>
<tr>
<th>Polymer</th>
<th>L:G</th>
<th>Termini</th>
<th>MW</th>
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<tr>
<td>502 H</td>
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<td>-OH, -COOH</td>
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<tr>
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<td>50:50</td>
<td>ester</td>
<td>14</td>
</tr>
<tr>
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<td>75:25</td>
<td>ester</td>
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<td>202</td>
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<td>ester</td>
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</table>

Previous work on PLGA microparticles for vaccine delivery

1995 Single administration of Tetanus toxoid in PLGA microparticles elicits similar or superior T cell and antibody response to those of Alum formulations  

1997 PLGA microparticles elicit a cytotoxic T cell response when loaded with a malaria specific CTL peptide  

1999 PLGA microparticles deliver antigens via both MHC class I and class II pathways  
Encapsulation of tetanus toxoid (TT) in PLGA microspheres to prolong antigen presentation to CD4+ T cells by human MoDC


**Graph:**

- **Y-axis:** cpm x 10^3
- **X-axis:** day 2, day 6, day 10
- **Legend:**
  - MP-TT
  - empty MP
  - soluble TT
  - DC alone
- **Data Points:**
  - 1 µg/ml TT
  - 0.01 µg/ml TT

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Encapsulation of FluM protein in PLGA microparticles to enhance antigen presentation of human DC to CD8+ CTL


**Graph:**

- **Y-axis:** IFN-γ released, pg/ml
- **X-axis:**
  - Sol. FluM 50 µg/ml
  - Sol. FluM 5 µg/ml
  - MP FluM 5 µg/ml
  - MP FluM 1 µg/ml
- **Legend:**
  - FluM soluble
  - FluM in PLGA MP
- **Data Points:**
  - Incubation of immature MoDC loaded with soluble or microparticle embedded antigen
  - Maturation to mature MoDC for 2 days
  - Incubation with FluM primed CTL
  - Assessment of IFN-γ production
Can PLGA microspheres modulate the immune response?

Plain PLGA microspheres induce tolerance

Modulation of allergic responses in mice by using biodegradable poly(lactide-co-glycolide) microspheres

Samartha Jilek, Ph.D., Elke Walker, Ph.D., Hans P. Mehl, Ph.D., and Elaine Carrbery, Ph.D.
Zurich and Lausanne, Switzerland

Background: Biodegradable poly(lactide-co-glycolide) (PLGA) microspheres are a promising carrier for vaccine delivery capable of modulating antigen-presenting cells to stimulate Th2-modulated immune responses. However, the potential of microspheres to downregulate an allergic response is less understood.

Hypothesis: The aim of this study was to determine whether cationic and anionic microspheres would promote Th2 predominance against allergy and to evaluate the immunomodulatory properties of microspheres alone.

Methods: Mice were treated prophylactically with ovalbumin-loaded plain PLGA microspheres before sensitization with ovalbumin (OVA) (OVA2), the major allergen of hen eggs. OVA2 or OVA2a, the minor allergen of hen eggs, in mice were measured for Th2-mediated immune polarization, and cytokine profiles were determined. Protection against anaphylaxis was evaluated after injection of an otherwise lethal dose of OVA2 a.

Results: Prophylactic PLGA2-treated groups exhibited lower levels of OVA-specific antibodies, reduced Th2 cytokine production, and improved survival in anaphylaxis challenge compared to control groups.

Abbreviations used

DC: Dendritic cell
EL: Empty vectors
PLGA: Poly(lactide-co-glycolide)
PLA2: Phosphatidylycerol
PLA2g: Phosphatidylycerol
S: Sensitivity

... cationic and anionic microspheres by themselves exert immunomodulatory properties as reflected by immune polarization.
PLGA microparticles upregulate the expression of the costimulatory signals CD83 and CD86


No impairment of cocktail induced maturation of MoDC after phagocytosis of PLGA microparticles

S Fischer et al. unpublished data
CD83 increase of dendritic cells by cationic PLL surface coatings on PS microparticles

Dendritic cell maturation (CD83) upon surface coating of poly(styrene) (PS) microspheres by conjugation of Ab

M Kempf et al. J Drug Target 2003
Assembly of surface coatings on PLGA microparticles

Surface coated PLGA microparticles as generic platform for vaccine delivery

PLGA core
- toxoid
- peptide
- protein
- pDNA, mRNA

Functionalized surface
PLL-g-PEG type block copolymer decorated with targeting ligands

Cationic coatings
- Chitosan
- Zein
- Protamine
- Poly(ethylene imine) PEI
- Poly(L-lysine) PLL
- Poly(L-arginine) Rn
- PLL-g-PEG
- PLL-g-PEG-ligand conjugate

Anionic coatings
- Plasmid DNA
- mRNA
- siRNA
- CpG rich DNA
- viral dsRNA, e.g. poly(I:C)
Layer-by-layer assembly of functional nanoscale coatings on PLGA microparticles

N Csaba et al. unpublished data

ζ-potentials of stepwise assembled coatings on PLGA microparticles

0 – PLGA microparticle
1 – Chitosan (or protamine)
2 – Plasmid DNA
3 – Chitosan (or protamine)
4 – CpG oligonucleotide
5 – Chitosan (or protamine)

One-step microparticle formation and coating through solvent extraction by static multilamination micromixer

Morphology, ζ-potential and fluorescamine reactivity of a one-step chitosan coating on PLGA microparticles


One-step manufacturing and surface coating of microparticles by cationic polyelectrolytes

S Fischer et al. unpublished data
Stable assembly of pDNA and mRNA on chitosan or protamine coated PLGA microparticles

MP loading with 0.5% pDNA, 1 d wash at 37 °C, pH 7.4; gel electrophoresis, 1% agarose, 70 V, 1 h, SYBR Gold detection

MP loading with 0.5% mRNA, 1 d wash at 37 °C, pH 7.4; RNase free; gel electrophoresis, 1% agarose, 50 V, 45 min, SYBR Gold detection

N Csaba et al. unpublished data

AFM of surface coated PLGA microparticles

Tapping mode

N Csaba et al. unpublished data
Transfection of Mph with mRNA encoding GFP with chitosan coated PLGA microparticles

N Csaba et al. unpublished data

Fluorescence microscopy Light microscopy

Phospholipase A2 immunotherapy in mice: Experimental setup

S Fischer et al. unpublished data

<table>
<thead>
<tr>
<th>Formulations and controls</th>
<th>Scheme of administration</th>
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<tbody>
<tr>
<td>Alum</td>
<td>Bleedings</td>
</tr>
<tr>
<td>PLGA</td>
<td>Time (days)</td>
</tr>
<tr>
<td>PLGA+CpG</td>
<td>0</td>
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<tr>
<td>PLGA/Prot</td>
<td>28</td>
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<tr>
<td>PLGA/Prot/CpG</td>
<td>55</td>
</tr>
<tr>
<td>ζ = -2.7± 4.6 mV</td>
<td>84</td>
</tr>
<tr>
<td>-7.6 ± 4.6 mV</td>
<td></td>
</tr>
<tr>
<td>+10.5 ± 6.6 mV</td>
<td></td>
</tr>
<tr>
<td>-12.6 ± 5.2 mV</td>
<td></td>
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</table>
Isotype profile of anti-phospholipase A2 serum antibodies analysed for IgG2a and IgG1

CpG assembly on PLGA MP elicits enhanced and Th1 biased PLA2 immune response

S Fischer et al. unpublished data
IV Engineering surface coatings on PLGA microparticles

**PLL-g-PEG coatings:**
inhibit serum protein adsorption on flat surfaces

![Diagram showing serum protein mass on flat Nb₂O₅ surfaces for different PLL-g-PEG coatings.](image)

- PLL(20)-[18.7]-PEG(5)
- PLL(20)-[6.5]-PEG(1)
- PLL(20)-[10.1]-PEG(2)
- PLL(20)-[5.7]-PEG(2)
- PLL(20)-[3.5]-PEG(2)
- PLL(20)-[2.1]-PEG(2)

**PLL-g-PEG coatings**
inhibit IgG adsorption on coated microspheres

![Diagram showing IgG-TxRed adsorption on coated PS microspheres.](image)

- PLL(20)-[18.7]-PEG(5)
- PLL(20)-[6.5]-PEG(1)
- PLL(20)-[10.1]-PEG(2)
- PLL(20)-[5.7]-PEG(2)
- PLL(20)-[3.5]-PEG(2)
- PLL(20)-[2.1]-PEG(2)

- uncoated carboxylated PS
**DC: Adhesion vs. Phagocytosis**

PLL-g-PEGs

- Positive control
- PLL(20)-[18.7]-PEG(5)
- PLL(20)-[6.5]-PEG(1)
- PLL(20)-[10.1]-PEG(2)
- PLL(20)-[5.7]-PEG(2)
- PLL(20)-[3.5]-PEG(2)
- PLL(20)-[2.1]-PEG(2)

**Adhesion on coated glass slides**

Cells, %

**Phagocytosis of coated PS MP**

Cells, %

SEM

U Wattendorf et al. unpublished data

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**Mph: Adhesion vs. Phagocytosis**

PLL-g-PEGs

- Positive control
- PLL(20)-[18.7]-PEG(5)
- PLL(20)-[6.5]-PEG(1)
- PLL(20)-[10.1]-PEG(2)
- PLL(20)-[5.7]-PEG(2)
- PLL(20)-[3.5]-PEG(2)
- PLL(20)-[2.1]-PEG(2)

**Adhesion on coated glass slides**

Cells, %

**Phagocytosis of coated PS MP**

Cells, %

SEM

U Wattendorf et al. unpublished data
Phagocytosis vs. adhesion of DC and Mph

Phagocytosis of microparticles by DC and Mph is limited by a distinct threshold.

Mannose-mediated phagocytosis of PS microparticles by DC and Mph

Phagocytosis of mono-mannose functionalized microparticles is ligand mediated and concentration dependent.

Phagocytosis needs high density of ligand

PLL(20)-[3.5]-PEG(2)-Mannose

U Wattendorf et al. unpublished data
Embedding poly(I:C) into PLL-g-PEG coatings
access vs. protection

poly(I:C) = poly(inosine)-poly(cytidylic) acid (dsRNA)

Surface assembly of poly(I:C) on PLL-g-PEG coated carboxylated poly(styrene) (PS) microparticles

PLL[20]-g-PEG[2] polymer with/without poly(I:C)

A Hafner et al. unpublished data
Coating with PLL-g-PEG-tetrasaccharide

Leishmania Donovani lipophosphoglycan tetrasaccharide cap. Hewitt & Seeberger, 2001

- PLGA microspheres have potential to enhance and prolong antigen presentation by APC
- PLGA microspheres can be surface coated and accomodate
  - pDNA and mRNA as antigen encoding nucleic acids
  - CpG, poly(I:C) as immune potentiators
- By self-assembly, PLGA microspheres can be decorated with ligands for APC recognition
- Through surface coatings, PLGA microspheres offer chances to modulate the immune response, e.g. Th1, Th2, and Treg (?)
Collaborators

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Dr. Géraldine Coullerez

Prof. Dr. Peter Seeberger
Liu Xinyu

Dr. Pål Johansen
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The end
Thank you