

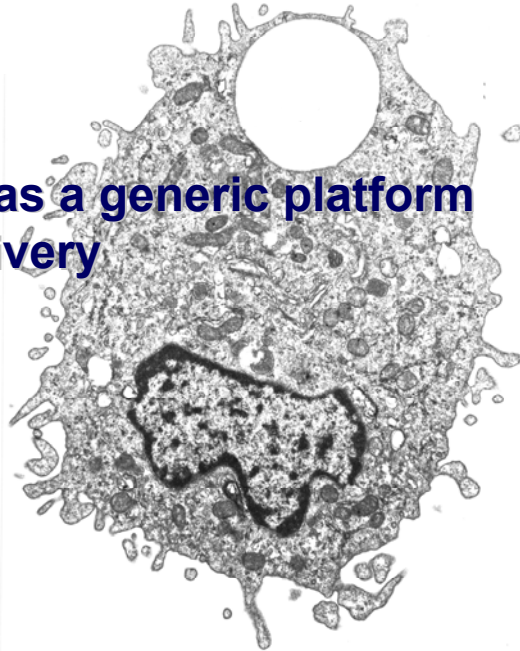
Microparticles as a generic platform for vaccine delivery

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Optimized sub-unit vaccines consist of three components

- **Delivery system**

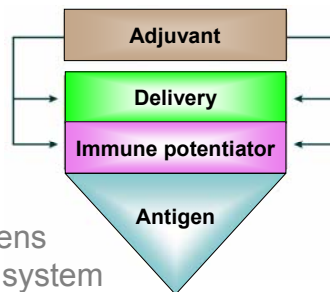
- To target and/or deliver antigens to cells of the innate immune system

- **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

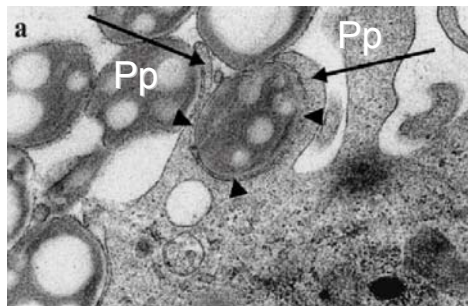


Immune potentiators

- 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



Phagocytosis of PEI-coated PS microspheres by dendritic cells

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

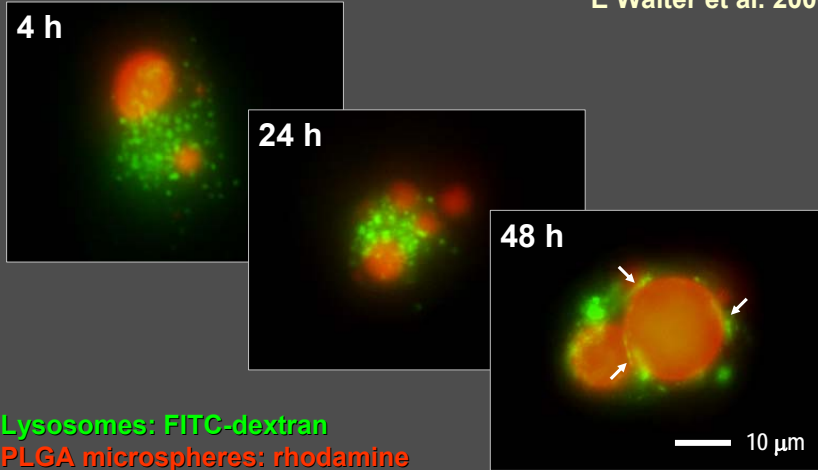
by pseudopods, Pp



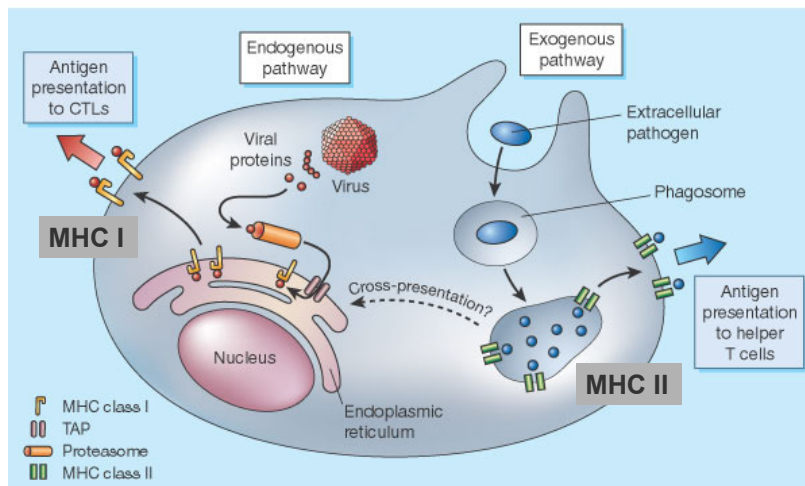
by sinking into cells

Fusion of lysosomes with phagocytosed PLGA microspheres in macrophages

E Walter et al. 2000

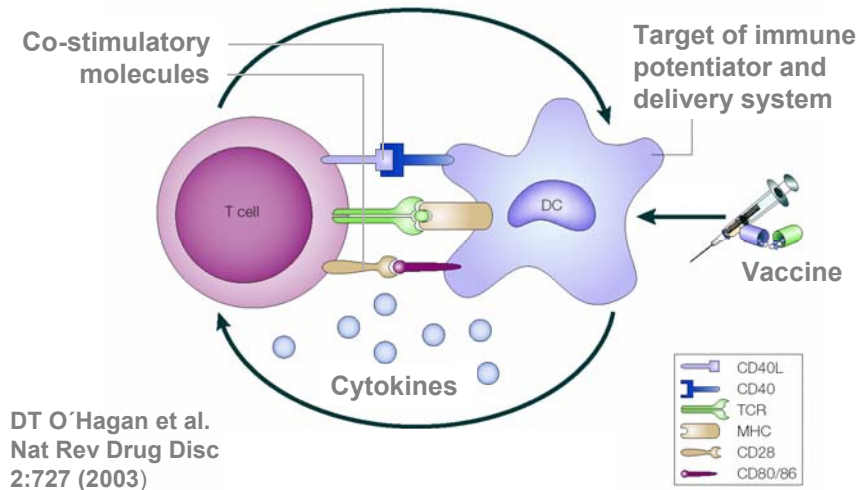


Antigen presentation: MHC I and MHC II

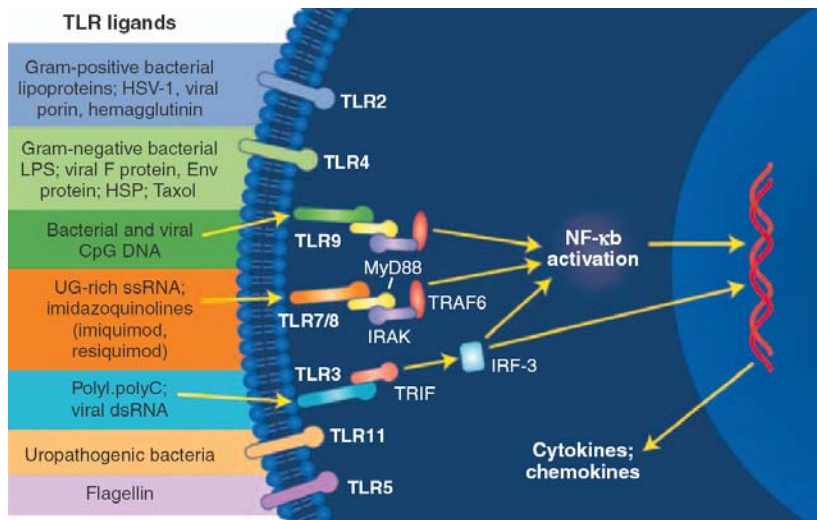


http://www.vetmed.wsu.edu/research_vmp/itp/

Antigen-presenting cells bridge between innate and adaptive immune response



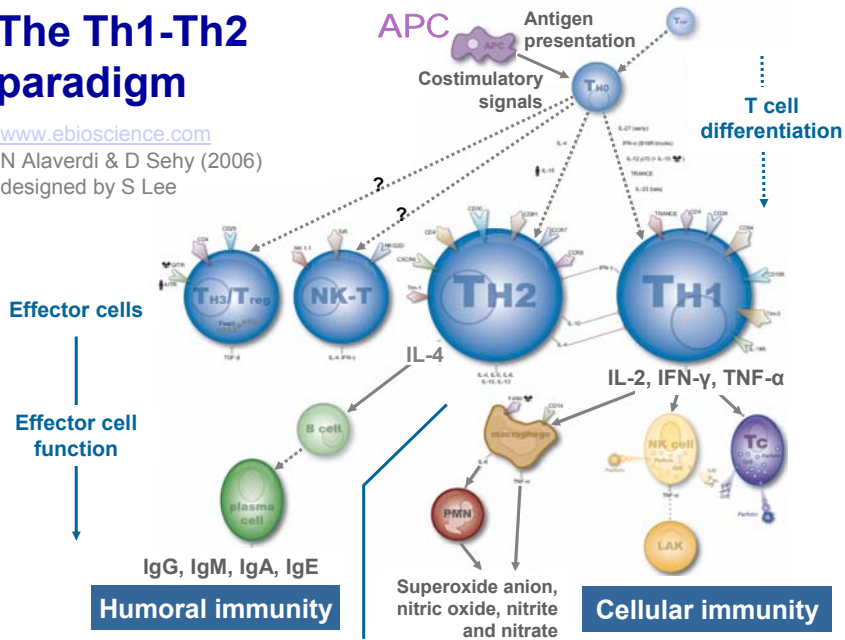
Toll like receptors recognize pathogen associated molecular patterns (PAMPs)



The Th1-Th2 paradigm

www.ebioscience.com

N Alaverdi & D Sehy (2006)
designed by S Lee



I

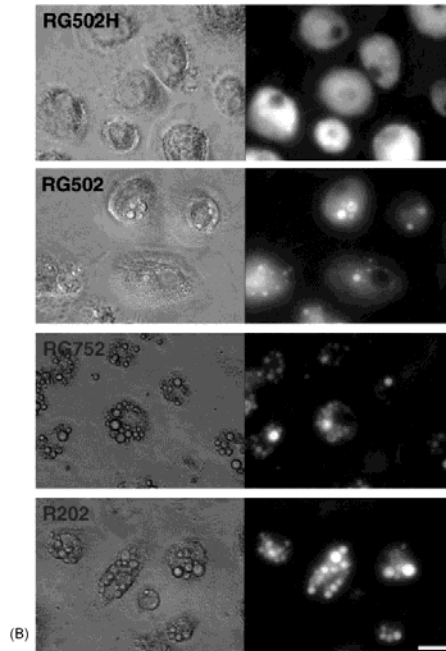
**PLGA microparticles for vaccine delivery:
... more than controlled release**

PLGA microparticles degrade in macrophages depending on their composition

13 days incubation

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

Polymer	L:G	Termini	MW
502 H	50:50	-OH, -COOH	14
502	50:50	ester	14
752	75:25	ester	17
202	100:0	ester	14

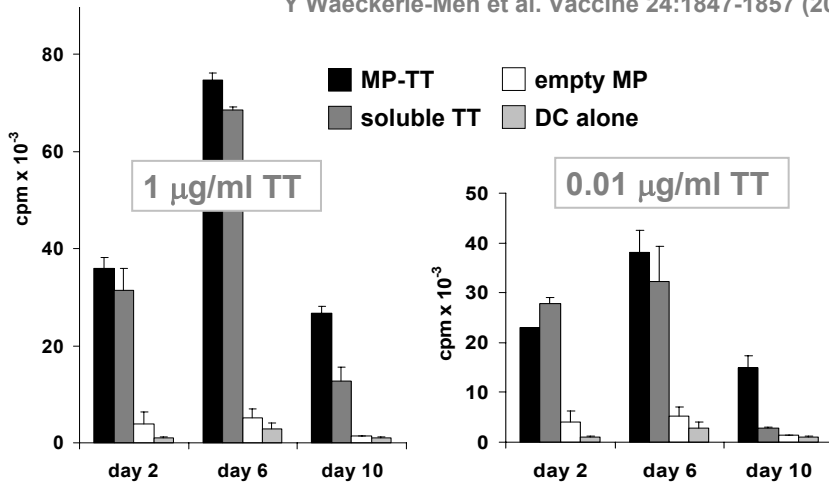


Previous work on PLGA microparticles for vaccine delivery

- 1995** Single administration of Tetanus toxoid in PLGA microparticles elicits similar or superior T cell and antibody response to those of Alum formulations [Y Men et al. Vaccine 13:683-689 \(1995\)](#)
- 1997** PLGA microparticles elicit a cytotoxic T cell response when loaded with a malaria specific CTL peptide [Y Men et al. Vaccine 15:1405-1412 \(1997\)](#)
- 1999** PLGA microparticles deliver antigens via both MHC class I and class II pathways [Y Men et al. Vaccine 17:1047-1056 \(1999\)](#)

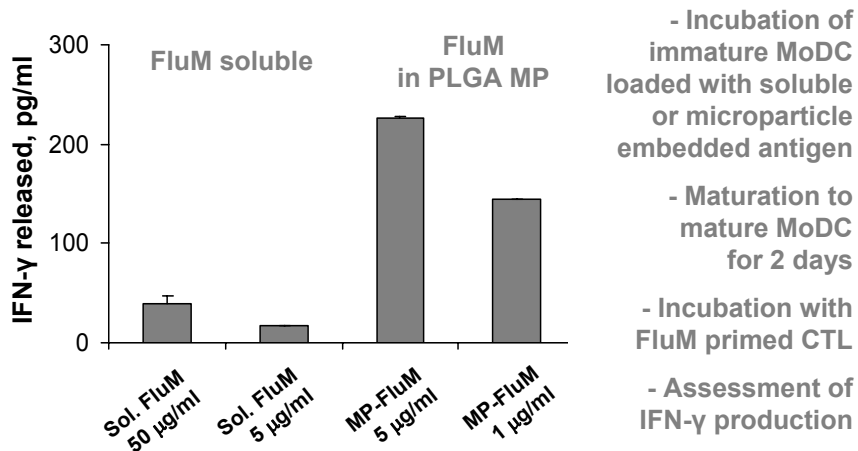
Encapsulation of tetanus toxoid (TT) in PLGA microspheres to prolong antigen presentation to CD4+ T cells by human MoDC

Y Waeckerle-Men et al. Vaccine 24:1847-1857 (2006)



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

Y Waeckerle-Men et al. Vaccine 24:1847-1857 (2006)



II

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

Samantha Jilek, PhD,^a Elke Walter, PhD,^a Hans P. Merkle, PhD,^a and Blaise Corthésy, PhD^b
Zurich and Lausanne, Switzerland

Background: Biodegradable poly(lactide-co-glycolide) (PLGA) microspheres are a promising carrier for vaccine delivery capable of maturing antigen-presenting cells to stimulate T-cell-mediated immune responses. However, the potential of microspheres to downregulate an allergic response *in vivo* is unknown.

Objective: The aim of this study was to determine whether microspheres could potentiate DNA vaccination against allergy and to evaluate the immunomodulatory properties of microspheres alone.

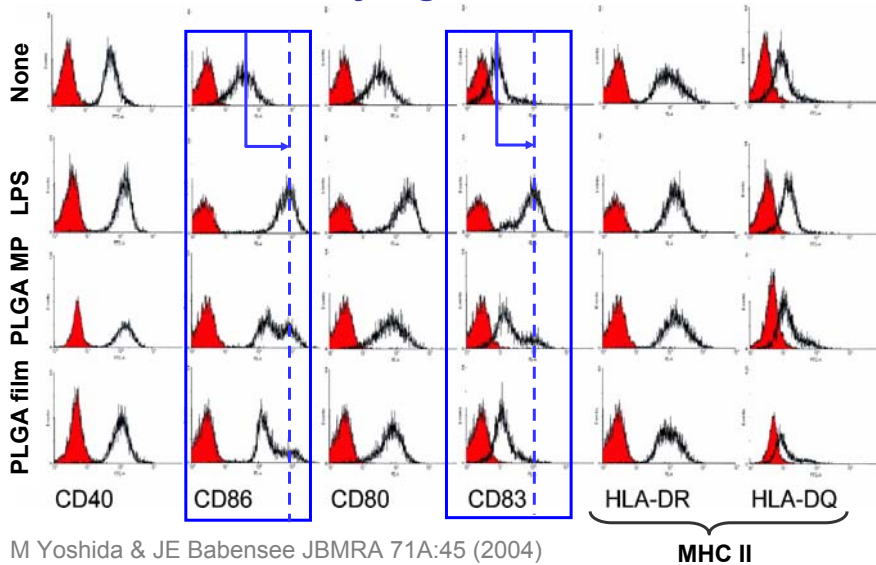
Methods: Mice were treated prophylactically with DNA-loaded plain PLGA microspheres before sensitization with phospholipase A2 (PLA2), the major allergen of bee venom. PLA2-specific IgG1, IgG2a, IgE in serum were measured for 8.5 months, and splenocyte proliferative responses and cytokine profiles were determined. Protection against anaphylaxis was evaluated after injection of an otherwise lethal dose of 10⁶ Results: Phospholipase A2

Abbreviations used
DC: Dendritic cell
EV: Empty vector
PLGA: Poly(lactide-co-glycolide)
PLA2: Phospholi
PLA2V: Phospholi
SI: Stimulato

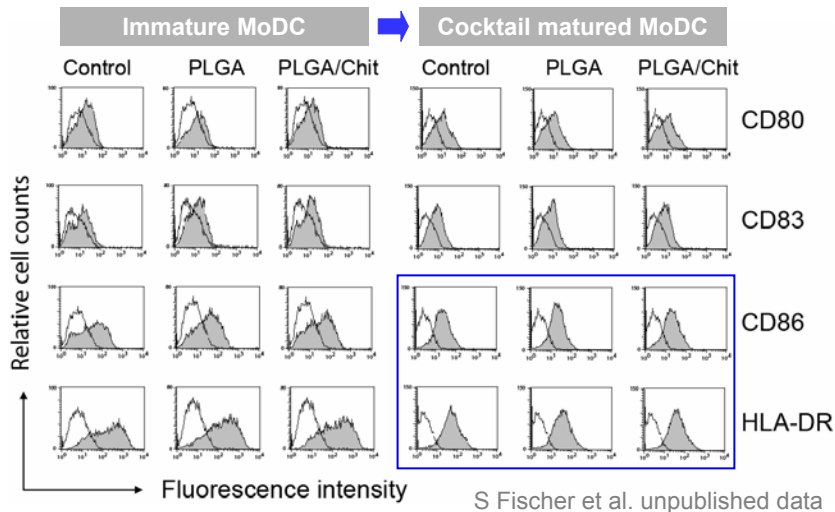
... cationic and anionic microspheres by themselves exert immunomodulatory properties as reflected by immune polarization.

S Jilek et al. J Allergy Clin Immunol. 114:943-50 (2004)

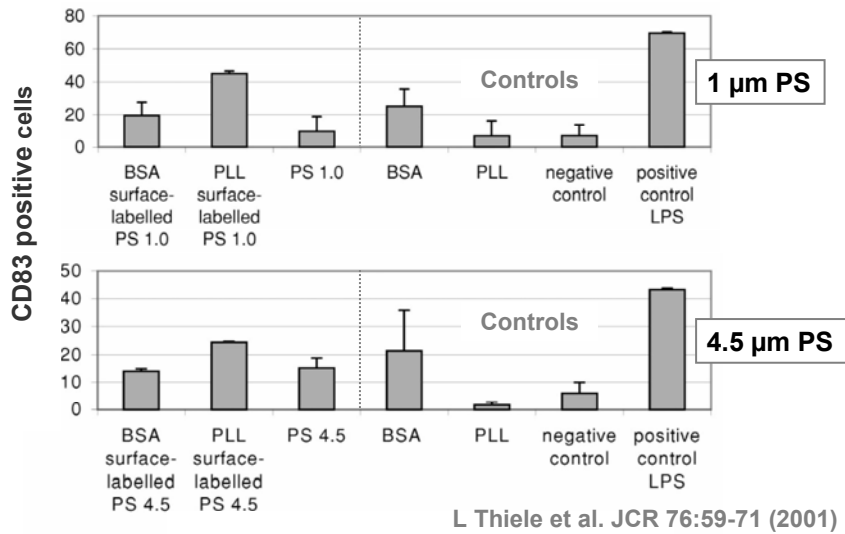
PLGA microparticles upregulate the expression of the costimulatory signals CD83 and CD86



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

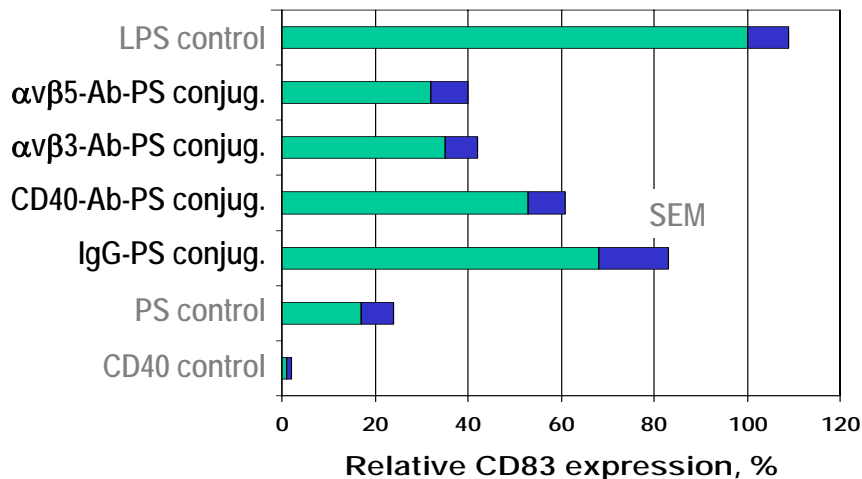


CD83 increase of dendritic cells by cationic PLL surface coatings on PS microparticles



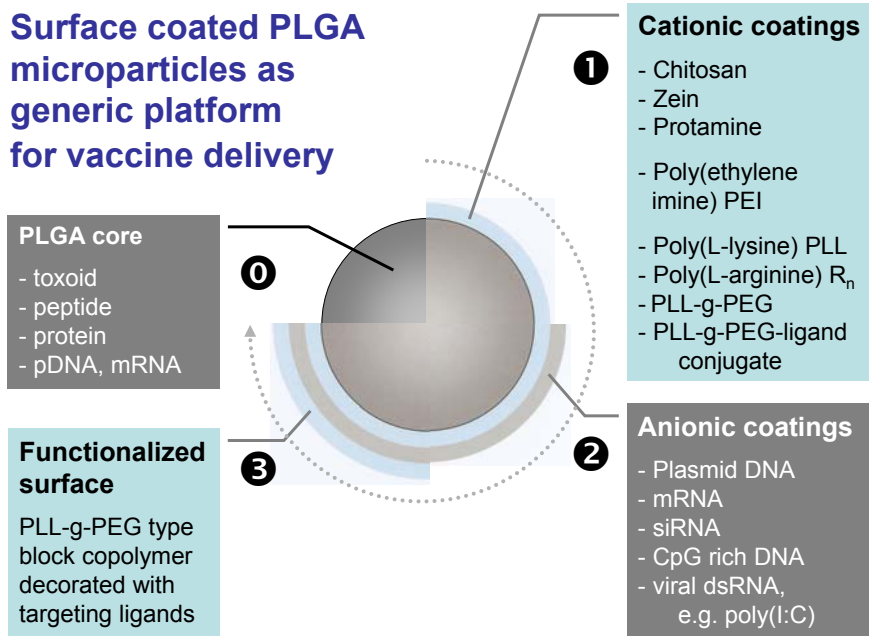
Dendritic cell maturation (CD83) upon surface coating of poly(styrene) (PS) microspheres by conjugation of Ab

M Kempf et al. J Drug Target 2003



III

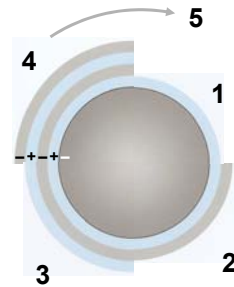
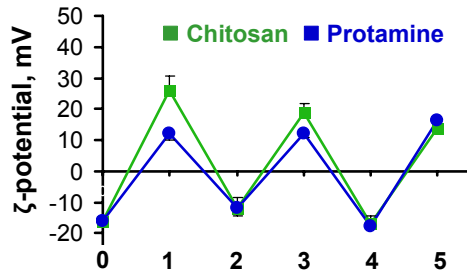
Assembly of surface coatings on PLGA microparticles



Layer-by-layer assembly of functional nanoscale coatings on PLGA microparticles

N Csaba et al. unpublished data

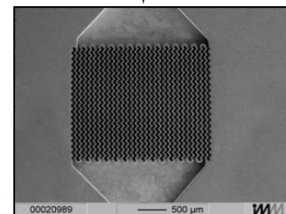
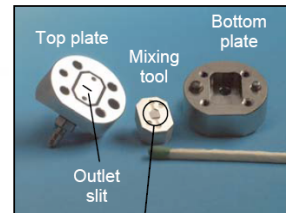
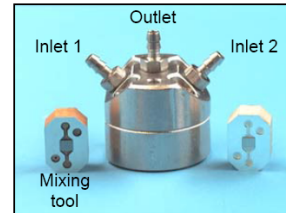
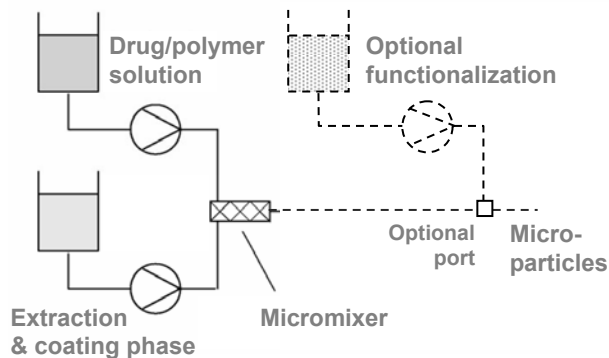
ζ -potentials of stepwise assembled coatings on PLGA microparticles



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

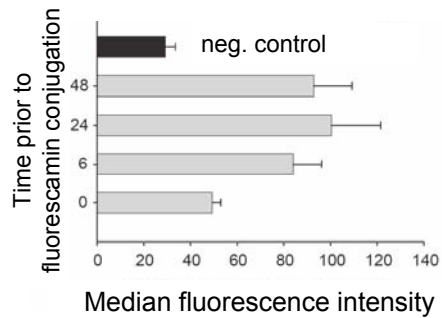
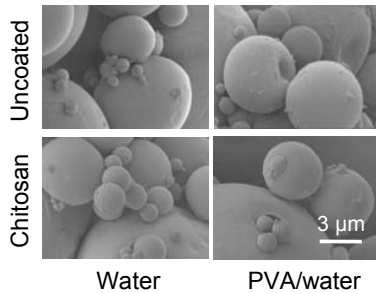
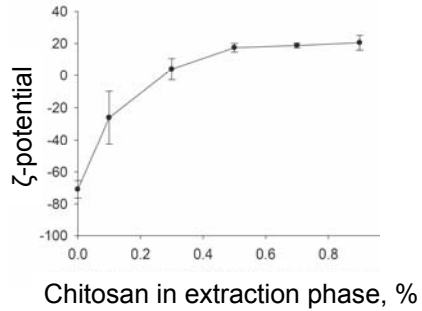
One-step microparticle formation and coating through solvent extraction by static multilamination micromixer

S Freitas et al. J Microencaps 20:67 (2003)
S Fischer et al. J Control Rel 111:135 (2006)

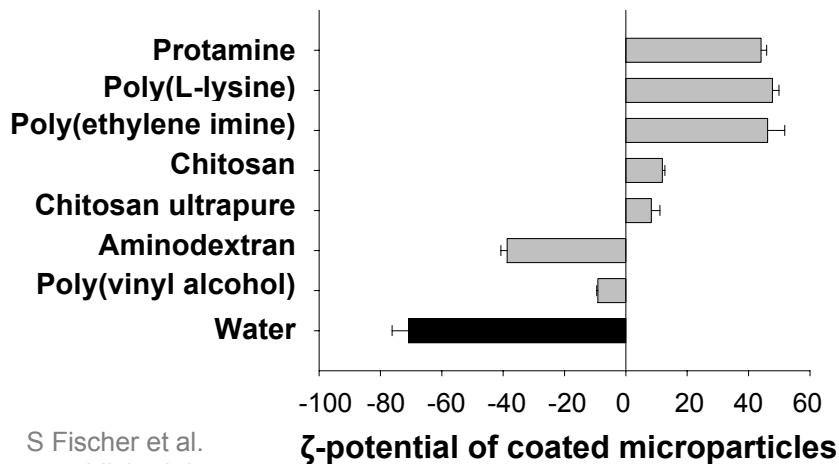


Morphology, ζ -potential and fluorescamine reactivity of a one-step chitosan coating on PLGA microparticles

S Fischer et al. J Control Rel 111:135-144 (2006)

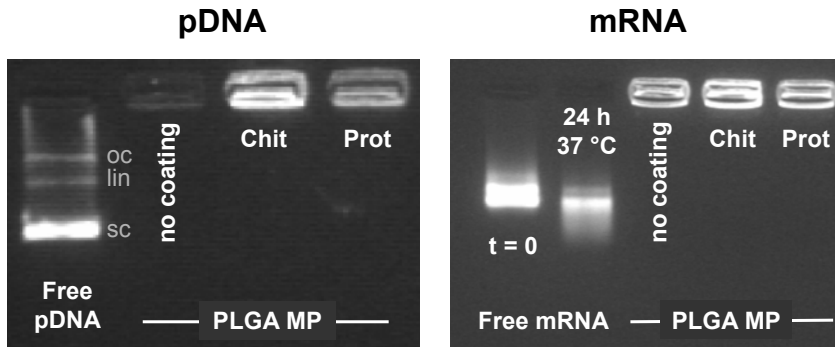


One-step manufacturing and surface coating of microparticles by cationic polyelectrolytes



S Fischer et al. unpublished data

Stable assembly of pDNA and mRNA on chitosan or protamine coated PLGA microparticles

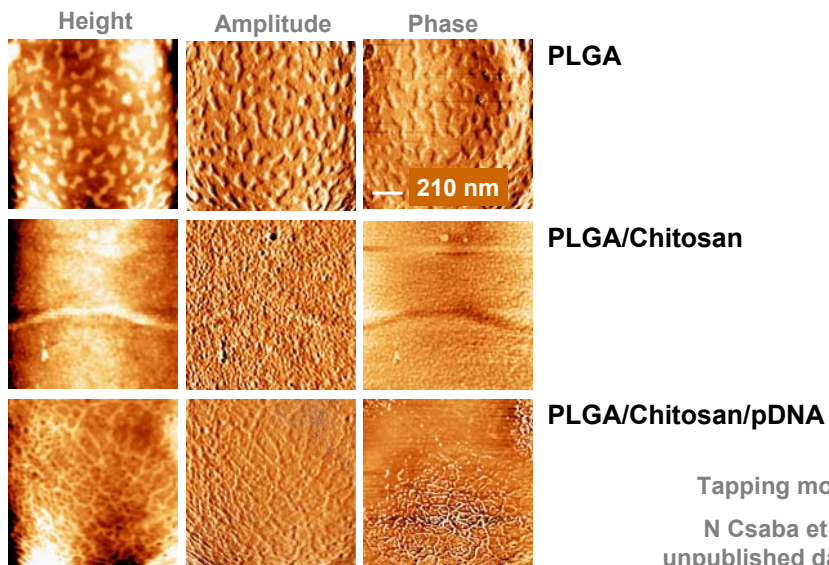


MP loading with 0.5% pDNA, 1 d wash at 37 °C, pH 7.4; gel electrophoresis, 1% agarose, 70 V, 1 h, SYBR Gold detection

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

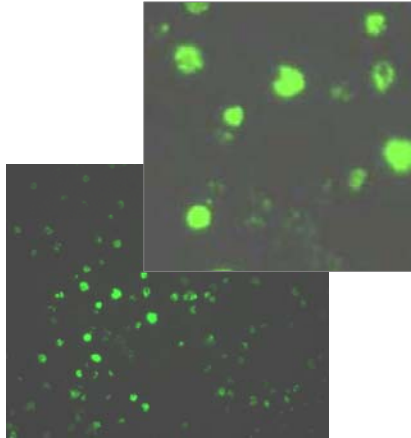


Tapping mode

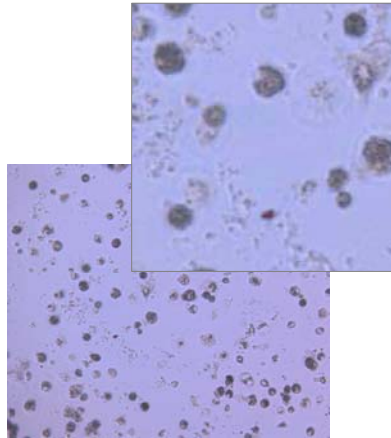
N Csaba et al.
unpublished data

Transfection of Mph with mRNA encoding GFP with chitosan coated PLGA microparticles

N Csaba et al. unpublished data



Fluorescence microscopy

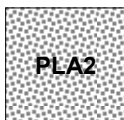


Light microscopy

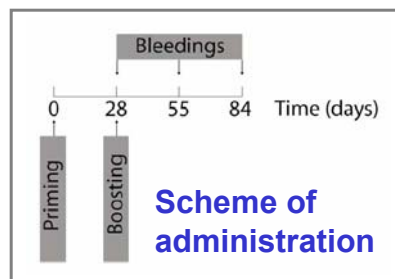
Phospholipase A2 immunotherapy in mice: Experimental setup

S Fischer et al. unpublished data

Alum



Formulations and controls



PLGA



$\zeta = -2.7 \pm 4.6$ mV

PLGA+CpG



-7.6 ± 4.6 mV

PLGA/Prot



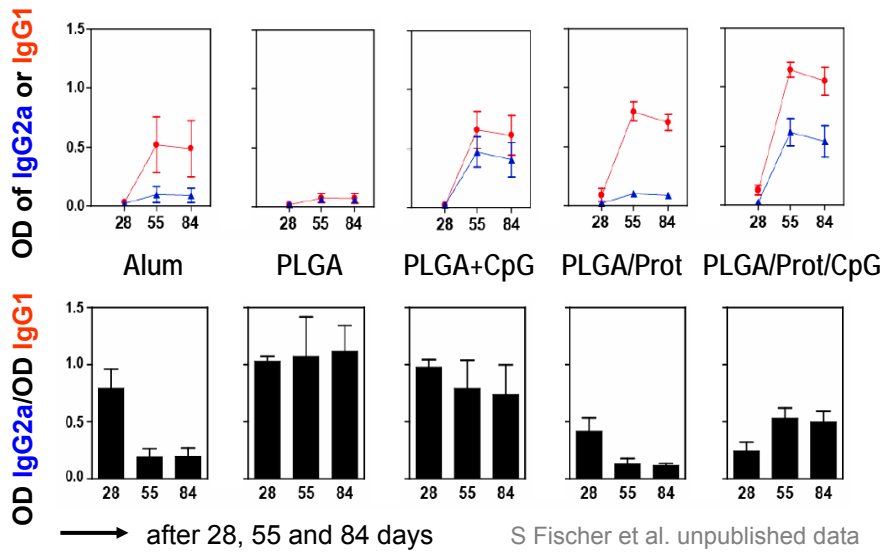
$+10.5 \pm 6.6$ mV

PLGA/Prot/CpG



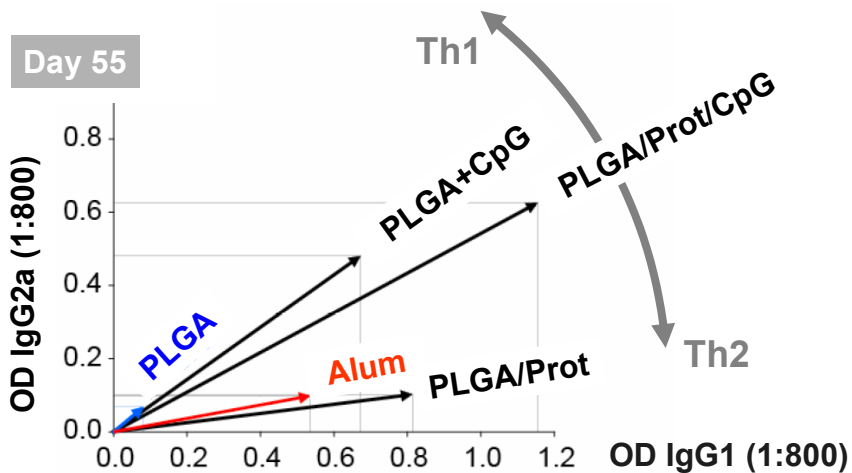
-12.6 ± 5.2 mV

Isotype profile of anti-phospholipase A2 serum antibodies analysed for IgG2a and IgG1



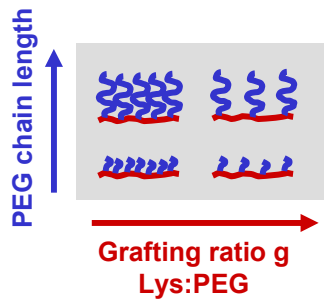
CpG assembly on PLGA MP elicits enhanced and Th1 biased PLA2 immune response

S Fischer et al. unpublished data



IV

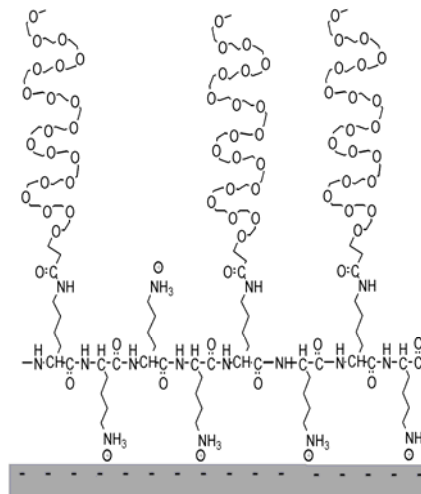
Engineering surface coatings on PLGA microparticles



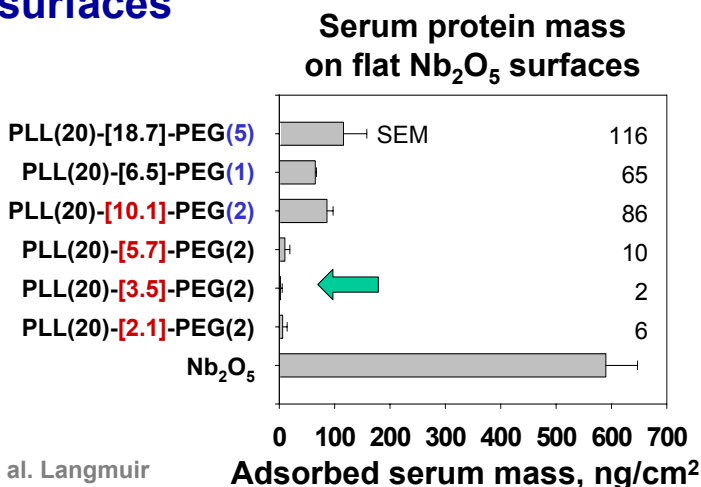
- PLL(20)-[18.7]-PEG(5)
- PLL(20)-[6.5]-PEG(1)
- PLL(20)-[10.1]-PEG(2)
- PLL(20)-[5.7]-PEG(2)
- PLL(20)-[3.5]-PEG(2)
- PLL(20)-[2.1]-PEG(2)

S. Pasche et al. Langmuir
19:9216-9225 (2003)

PLL-g-PEG

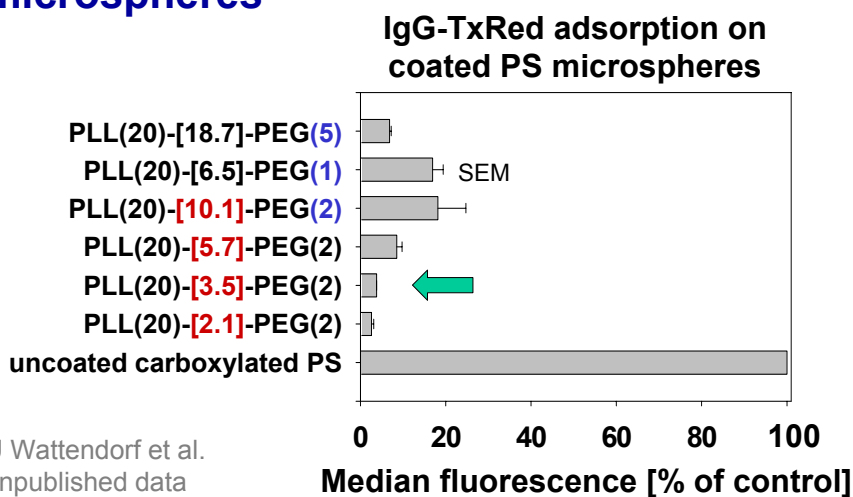


PLL-g-PEG coatings: inhibit serum protein adsorption on flat surfaces



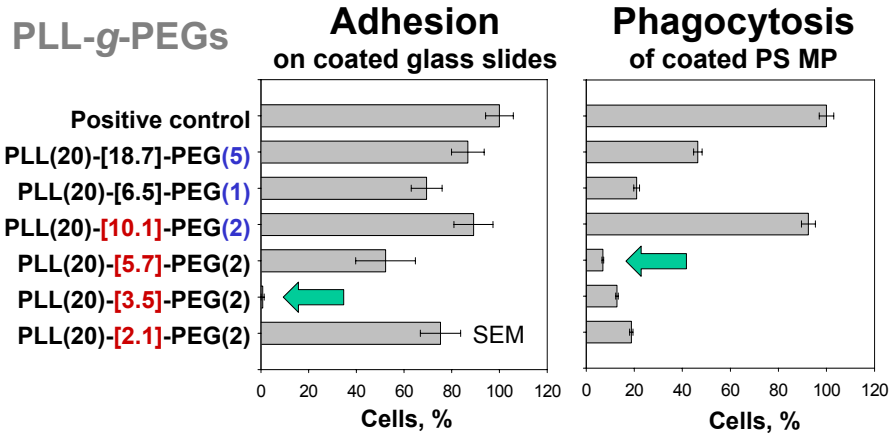
S. Pasche et al. Langmuir
19:9216-9225 (2003)

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



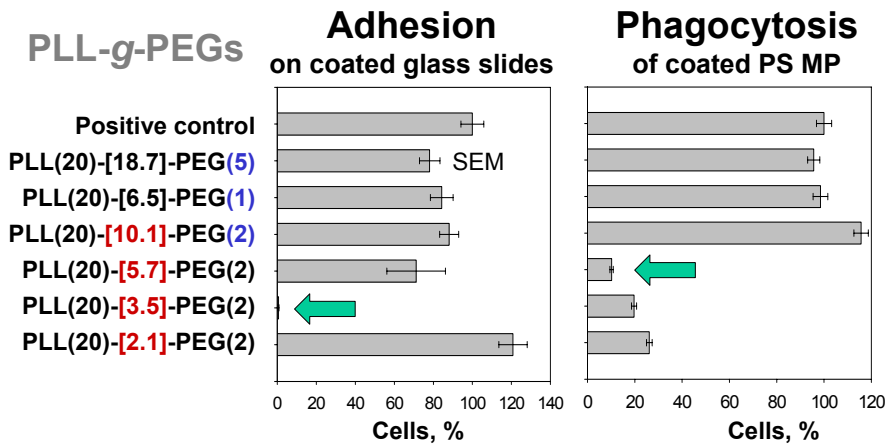
U Wattendorf et al.
unpublished data

DC: Adhesion vs. Phagocytosis



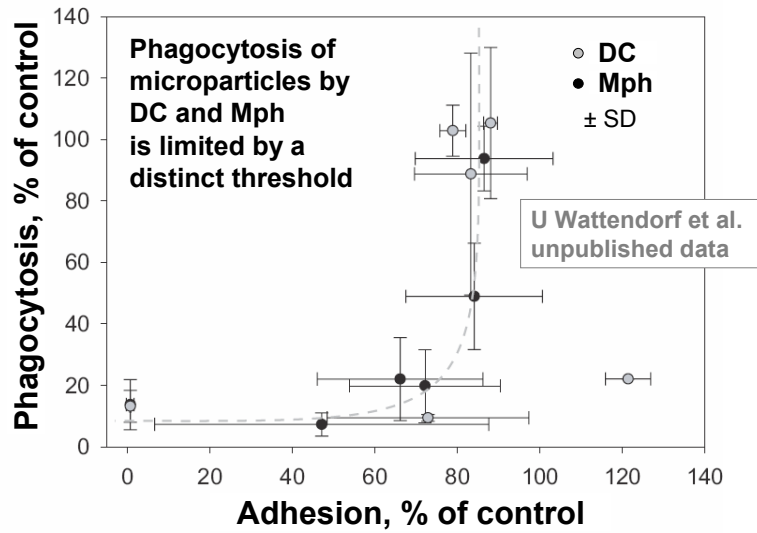
U Wattendorf et al.
unpublished data

Mph: Adhesion vs. Phagocytosis



U Wattendorf et al.
unpublished data

Phagocytosis vs. adhesion of DC and Mph



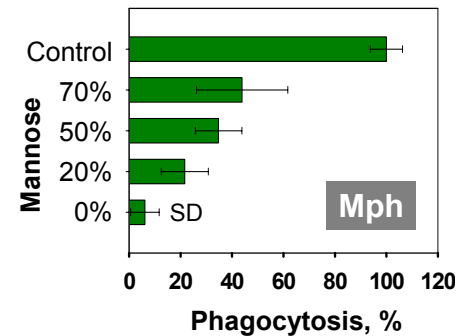
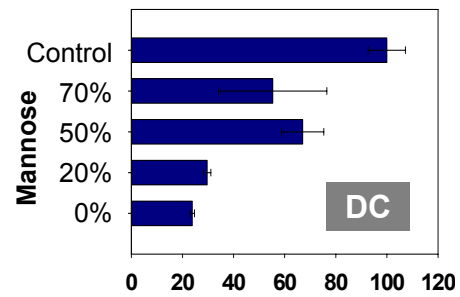
Mannose-mediated phagocytosis of PS microparticles by DC and Mph

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

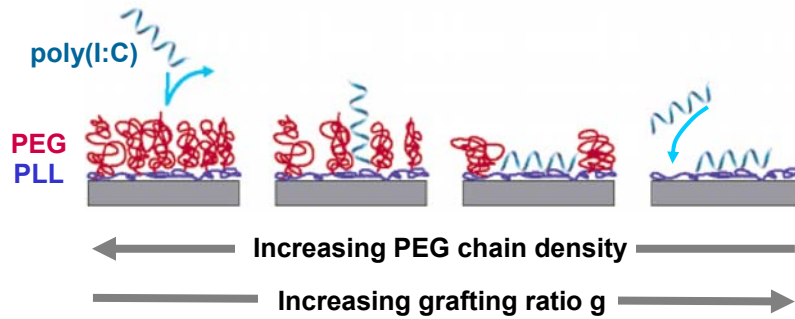
U Wattendorf et al. unpublished data



Embedding poly(I:C) into PLL-g-PEG coatings

access vs. protection

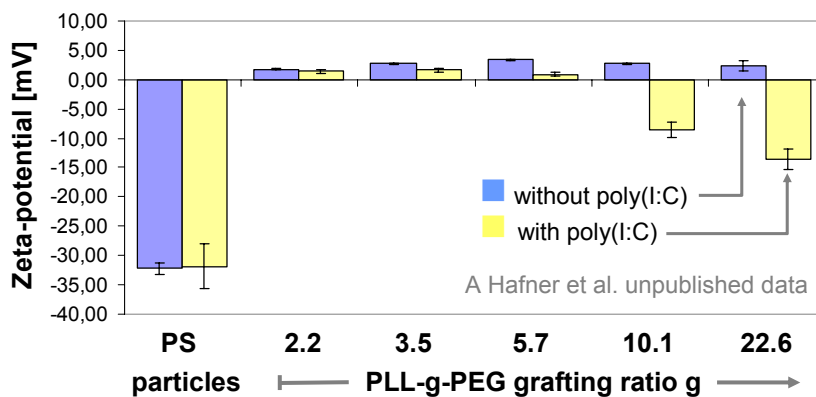
poly(I:C) = poly(inosine)-poly(cytidylic) acid (dsRNA)



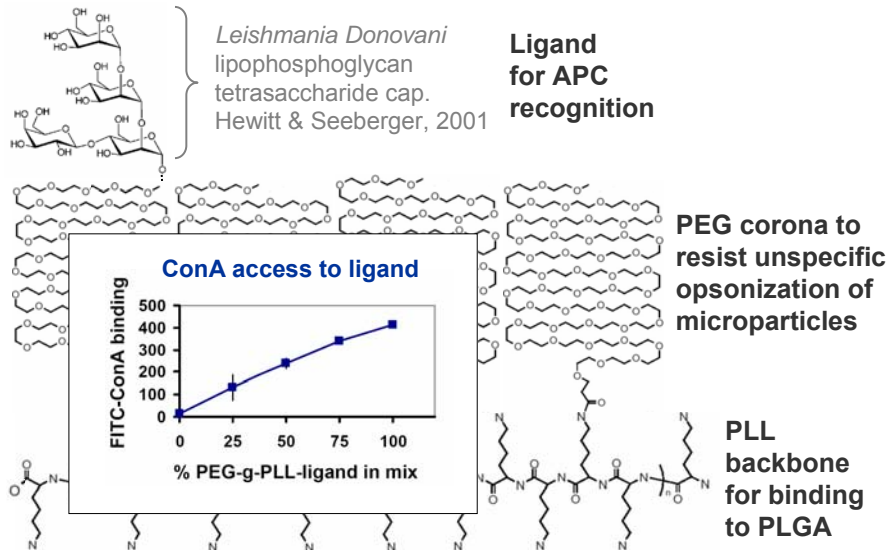
modified from SM de Paul et al. *Analyt Chem* 77:5831-5838 (2005)

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)



Coating with PLL-g-PEG-tetrasaccharide



- PLGA microspheres have potential to enhance and prolong antigen presentation by APC
- PLGA microspheres can be surface coated and accommodate
 - 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 (?)

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The end
Thank you