Controlling the Surface Properties of Nanoparticles – Bioorganically modified Nanoparticles for Cell Signaling (NANOCYTES)

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NANOCYTES

Outline

- TNF-NANOCYTES Functional Principle
- Nanoparticle Synthesis and Characterisation
- Bioconjugation
- TNF-Nanoparticles in Biological Systems
- Conclusion
Functional Principle TNF–NANOCYTES

Key to Control of TNF Cell-Signalling Behaviour: Surface-Bound TNF

→ Synthetic-Bionic Hybrid-Particles
TNF–NANOCYTES®
Strategy for Immobilization

Requirements
- Immobilization as a Trimer
- Orientation to Bind Receptor
- Suitable Ligand Density
- Minimized Nonspecific Binding
NANOCYTES

Nanoparticle Preparation

1. Core-Shell Nanoparticles

- Free Choice of Size. Here: 100 nm, 1 µm, 10 µm
- Various fluorescence labels integrated in organic shell: green, red, violet

2. Single-Step Bioreactive Polymer Nanoparticles
   Surfmer-Technology

3. Biodegradable Polymer Nanoparticles
Surface Modification of Silica Nanoparticles

1st Shell Reaction: Silanization

$$\text{SiO}_2 + \text{H}_2\text{O} \rightarrow \text{Si(OH)}_2^- + \text{2OH}^-$$

2nd Shell Reaction: Organic Derivatization

$$\text{SiOH} - \text{NH}_2 + \text{COO}^- \rightarrow \text{SiNH} - \text{COO}^-$$

Quality Control

- e.g. by Elemental Analysis and Surface Charge Determination:
- 90 µmol functional groups per 1 g particles
Shell Modification by Polymer Grafting Reaction
Reduction of Nonspecific Binding NSB (e.g. Pegylation)

Low NSB of Proteins

Zero NSB
Shell with Low NSB and Reactive Anchor Groups

Grafting of Functional PEG

Boc-Amino-PEG-Amine (MW 644, n: ~11)

Particle size: SEM

Surface characterization:
ζ-potential determination

 Modifier content (mg/ml)
**NANOCYTES**

**TNF–NANOCYTES®**

Strategy for Immobilization

- **Requirements**
  - Immobilization as a Trimer
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![Diagram](image)
Synthetic Nanoparticle Protein Conjugation

\[ R_1 = \text{NH}_2 \quad R_2 - R_3 = \quad R_4 = \text{SH} \]

Sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC)
TNF-Nanoparticles Bind to TNF-Receptor 2 (TNF-R2)
Cell-Membrane Ligand-Receptor Study by Confocal Microscopy

Ligand TNF-Nanocytés® Binding to TNF-Receptor (HeLa80-R2)

Control: unmodified particles on HeLa80-R2
TNF-Nanoparticles Bind to TNF-Receptor 2
Cell-Membrane Ligand-Receptor Study by Confocal Microscopy

Ligand TNF-Particles Binding to TNF-Receptor (HeLa80-R2)
TNF-Nanoparticles Stimulate Cell Action via TNF-Receptor 2 Signal Pathway Study by Confocal Microscopy

TNF-Particles on HeLa80-R2 with Transcription Factor (NFκB-GFP)
TNF-Nanoparticles Stimulate Cell Action via TNF-Receptor 2 Signal Pathway Study by Confocal Microscopy

TNF-Nanocytes® on HeLa80-R2 with Transcription Factor (NFκB-GFP)
TNF-Nanoparticles Induce Apoptosis via TNF-Receptor 2

TNF-Nanocytes® Action on Target Cell (MF-R2-Fas with FADD-GFP)
TNF-Nanoparticles Induce Apopotosis via TNF-Receptor 2

Bioactivity in Cell Assay

Kym-1 cells: TNF-Receptor 1

MF-R2 cells: TNF-Receptor 2

Cell activity (OD$_{50}$) vs. Concentration TNF (ng/ml)
TNF-Nanoparticles Induce Apopotosis via TNF-Receptor 2

TNF-Nanocytess® Action on Target Cell (MF-R2-Fas with FADD-GFP)

15 min

22 min

30 min

40 min
Conclusions

- Synthesis of Reactive Core-Shell Nanoparticles
- Reduced Non-Specific Binding of Proteins
- Bioactive Conjugation of TNF to Nanoparticles
- Hybrid Particles Mimick Membrane-Like TNF
- TNF-Particles Recruit TNF-Receptor
- TNF-Nanoparticles Stimulates Specific Cell Responses by Receptor-Binding
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TNF-Nanocytes® Action on Target Cell (MF-R2-Fas with FADD-GFP)