Synthesis of Target-directed Nanogel Carrier with Glycopolymers and Their Application to Immunotherapy

○Yuki Okuda, Shigehito Osawa, Hidenori Otsuka

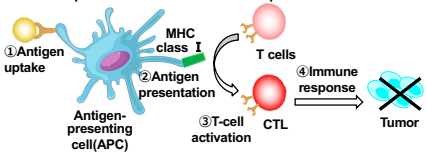
Dept. of Chem., Grad. Sch. of Sci., Tokyo Univ. of Sci., Tokyo, Japan

1. Introduction

■Immunotherapy

Immunotherapy, which is based on antigen-specific immunity, is expected to be an effective and safe treatment for cancer and infectious diseases.

Stimulating cytotoxic T lymphocytes (CTL) is particular important to obtain immune response.



Challenges in CTL induction

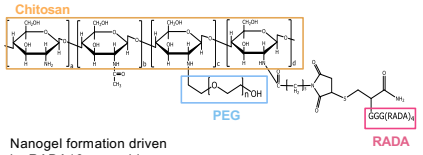
- Less amount of antigen transferred to APC
- Sustaining antigen functionality during in vivo delivery

→ Requiring a carrier that preserves protein function

2. Previous research

■ PEG/RADA-*g*-CS nanogel

Nano-sized gel particles (= **nanogel**) composed of cationic natural polymer of **Chitosan** (**CS**) grafting hydrophilic polymer of **PEG** and self-assembling peptide of **RADA16** on the backbone



Nanogel formation driven by RADA16 assembly

© **Highly efficient protein loading**
© **Slow release of the loaded protein**

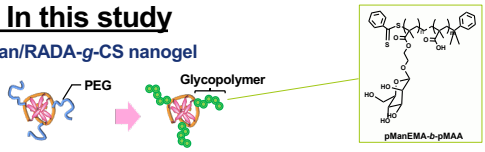
Issues

Low cell affinity due to PEG

→ Poor targeting

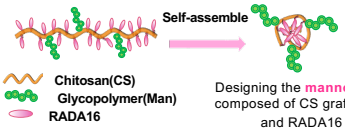
3. In this study

■ Man/RADA-*g*-CS nanogel



We assumed installing a ligand structure, which is widely acknowledged to target dendric cells (DCs) as one of the APCs, on to the nanogel shell.

→Utilizing glycopolymers with mannose (Man) moieties instead of PEG to express binding affinity to DC whereas sustaining the hydrophilic shell character

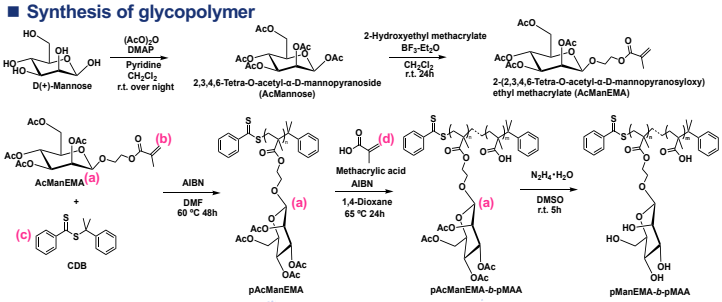


Designing the **mannose-modified nanogels** composed of CS grafting Glycopolymer(Man) and RADA16 on the backbone

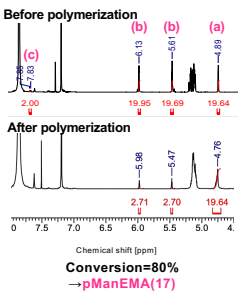
In this presentation, preparation of the glycopolymer containing Man and its molecular targeting property based on Man were investigated.

4. Preparation of glycopolymer and model nanoparticles having Man on the surfaces

■ Synthesis of glycopolymer



【^{1}H-NMR】



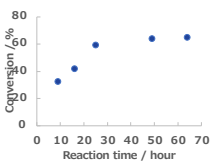
Conversion=80%
→pManEMA(17)

[Tracing monomer conversion]

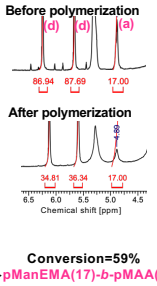
The monomer conversion % was investigated at 0,9,16,25,49,64 hours of reaction periods by ^{1}H-NMR measurements.

The conversion % increased linearly until 25h.

→ **Controlled by RAFT agents**



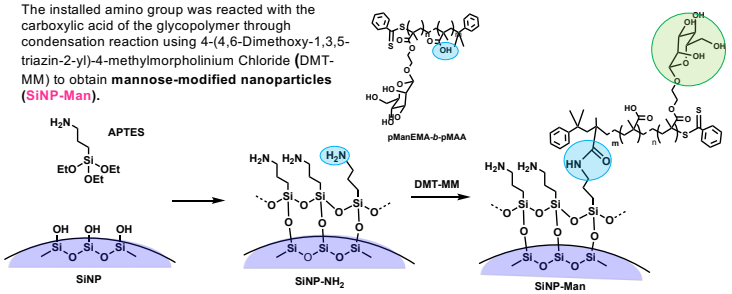
【^{1}H-NMR】



Conversion=59%
→pManEMA(17)-*b*-pMAA(52)

■ Preparation of mannose-modified nanoparticles

A silica nanoparticle (SiNPs) was modified with an **amino-terminated silane coupling agent** (**APTES**). The installed amino group was reacted with the carboxylic acid of the glycopolymer through condensation reaction using 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium Chloride (DMT-MM) to obtain **mannose-modified nanoparticles** (**SiNP-Man**).



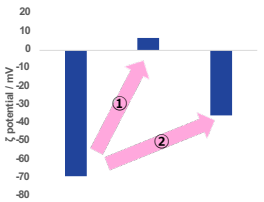
[Zeta potential measurements]

(**Experiment**)

SiNP-Man dispersion; 1 mg/mL in 1 mM PB, pH=7.4

(1) The zeta potential of non-treated SiNPs was -68.8 mV. After silane coupling, the zeta potential increased to +6.81 mV, indicating successful surface modification with amino groups, i.e., obtaining SiNPs-NH2

(2) The zeta potential decreased to -35.6 mV after reacting with the glycopolymers, which is less negative compared to non-treated SiNPs.



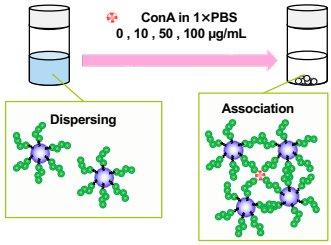
Achieving the glycopolymer modification on silica surface, obtaining SiNP-Man

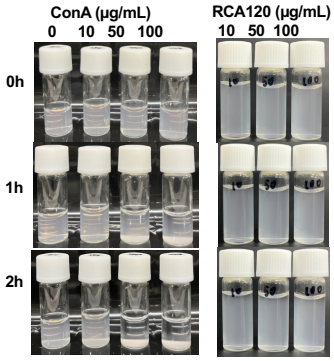
5. Molecular recognition property of SiNPs-Man

■ Aggregation using lectin

We evaluated the aggregation behaviors of SiNPs-Man with the addition of **ConA**, a mannose-recognizing lectin, and **RCA120**, a galactose-recognizing lectin.

1 mg/mL SiNP-Man in 1×PBS

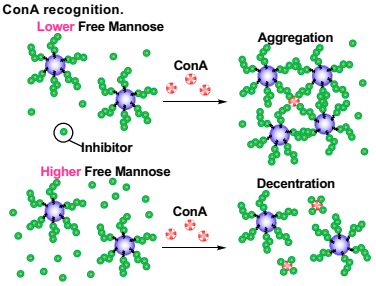




ConA concentrations of 50 and 100 μg/mL deposited the SiNPs-Man after 2 hours whereas RCA120 did not make deposition at any concentration, suggesting mannose-derived molecular recognition work on the SiNPs-Man surface to associate the particles.

■ Aggregation inhibition

SiNP-Man were mixed with ConA under varied **free mannose concentration which would be competitive for the ConA recognition.**



Free Mannose vs. ConA

500eq In the case of 500 eq free mannose to ConA, the sedimentation of the SiNP-Man was still observed. In the case of 100000 eq free mannose, no sedimentation was observed.

100000eq The larger amount of mannose is required for the inhibition, implying high recognition property of SiNP-Man owing to multivalency

[Turbidity measured with spectrophotometer]

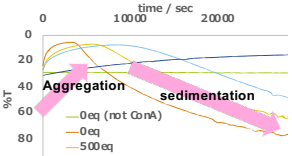
(**Experiment**)

Measurement wavelength: 900 nm

Measurement time: 28800sec

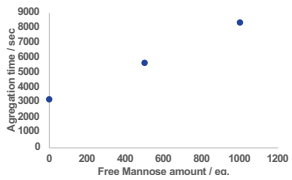
SiNP-Man dispersion: 4 mg/mL in 1×PBS, pH=7.4

ConA concentration: 50 μg/mL



There is no change in transmittance without ConA, , indicating that SiNP-Man has **dispersion stability under physiological condition**.

Time periods starting to decrease transmittance



The increased eq. of free mannose also delayed the sediment formation, suggesting inhibition of the association of SiNP-Man through ConA.

SiNP-Man has a specific molecular recognition property, and the future nanogel containing the glycopolymer is promising to target DCs

6. Conclusion and future work

- **Glycopolymer pAcManEMA-*b*-pMAA block copolymer was prepared via RAFT polymerization**
- **Silica nanoparticles were successfully modified with glycopolymers, which have the well dispersion property.**
- **Specific lectin recognition derived from mannose was observed using mannose-modified silica nanoparticles, showing potency to extended application of nanogels targeting dendric cells.**

- Quantification of requiring mannose density on the surface to show the specific binding
- Synthesis and characterization of Man/RADA-g-CS