Keio University

1858

CALAMVS GLADIO FORTIOR

Gene Delivery Systems using GAG

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Gene delivery systems

- Viral vectors

  adeno-associated virus, lentivirus, retrovirus, adenovirus, etc.

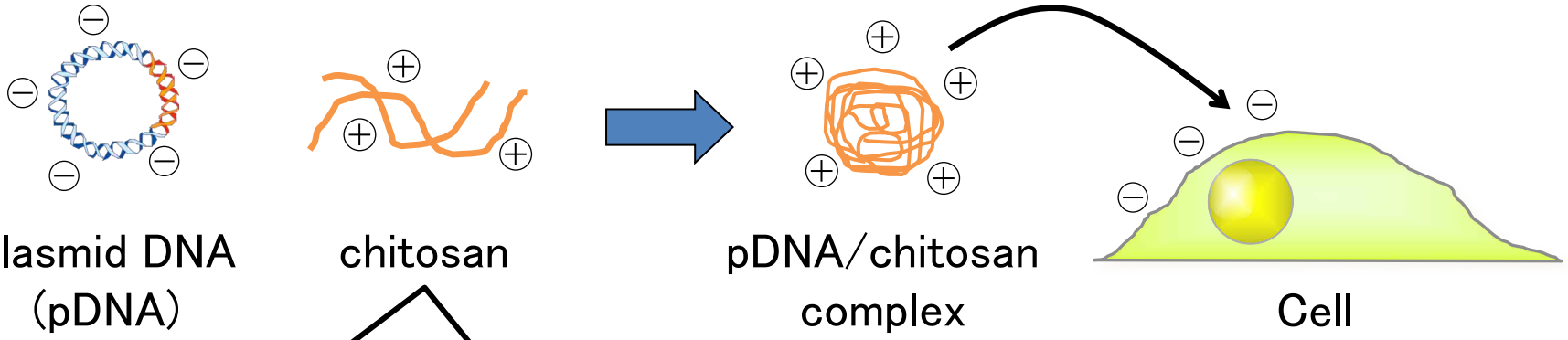
- Non-viral vectors

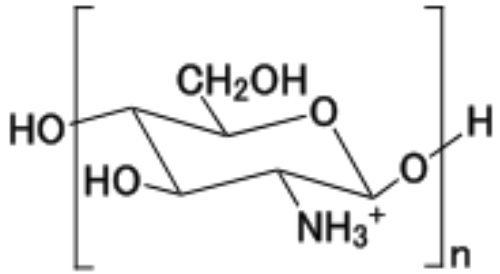
  liposome, cationic lipid, cationic polymer, polymer micelle, etc.

- Physical methods

  hydrodynamic method, electroporation, ultrasonic levitation, etc.

Gene delivery using chitosan





MW = 40k～50k
Deacetylated degree ca. 80%

Natural cationic polysaccharide

Advantages

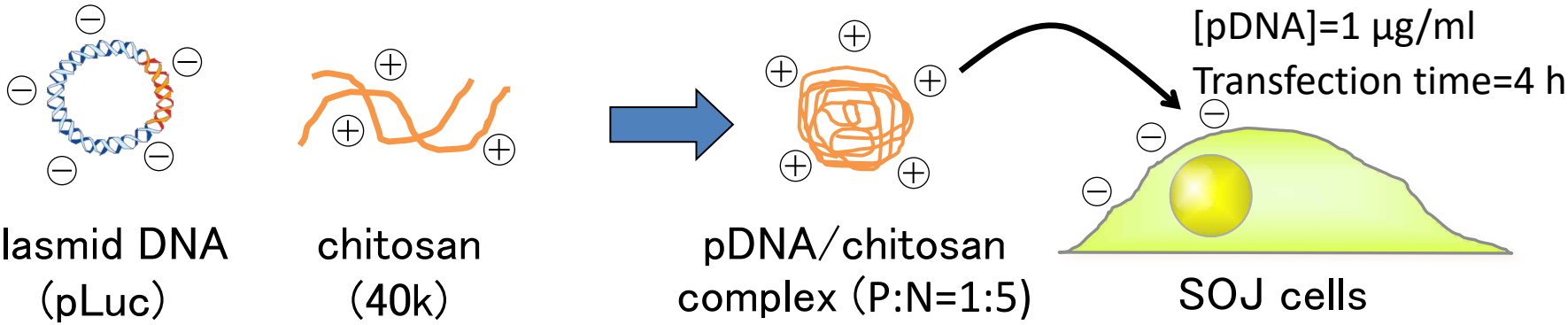
- High transgene expression in the presence of serum
- Low cytotoxicity

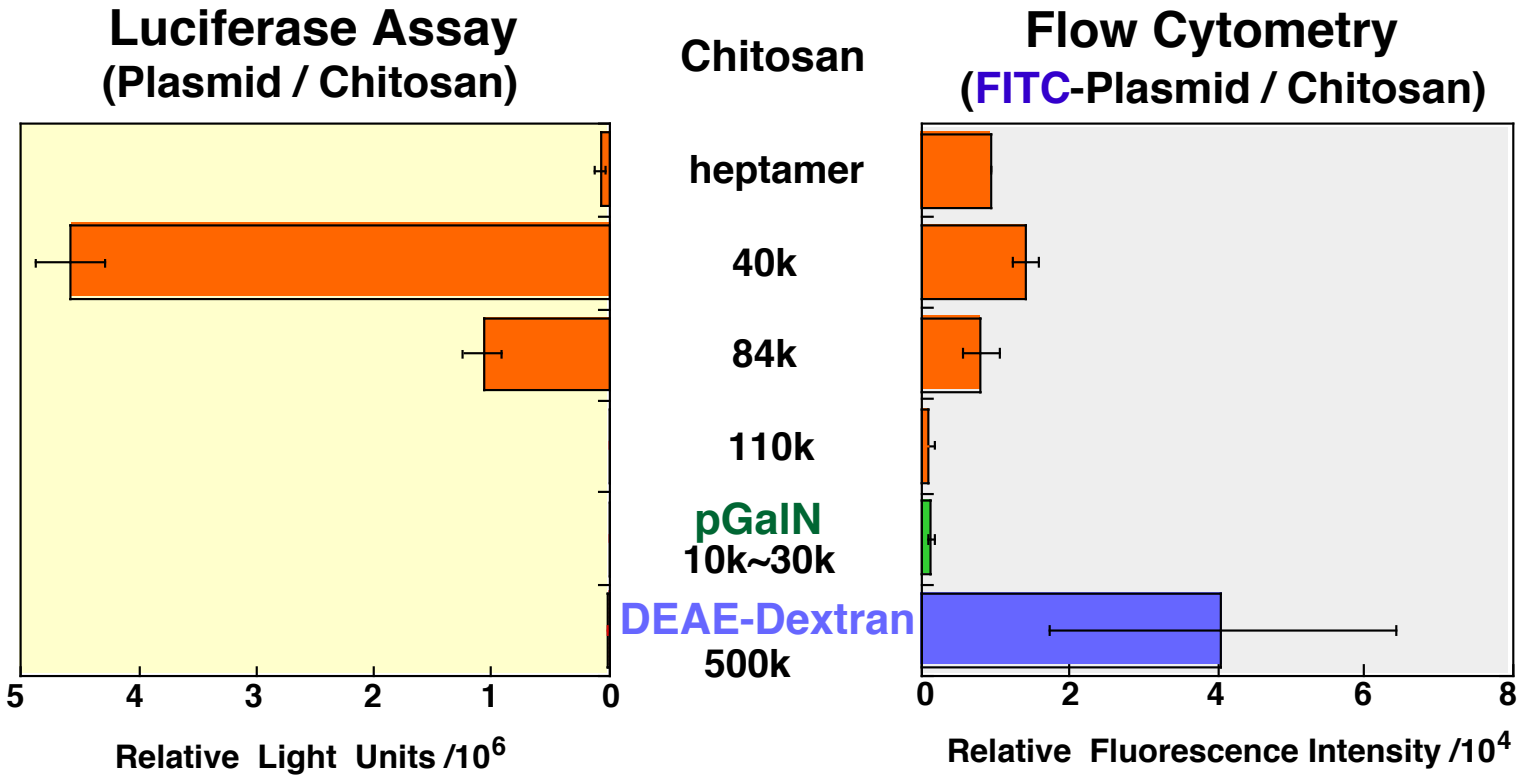
Disadvantages

- Aggregation
- No cell specificity
- Low transgene expression *in vivo*

Biomaterials, **22**, 2075-2080 (2001), Biochim. Biophys. Acta, **1514**, 51-64 (2001)
Biomaterials, **23**, 4573-4579 (2002)

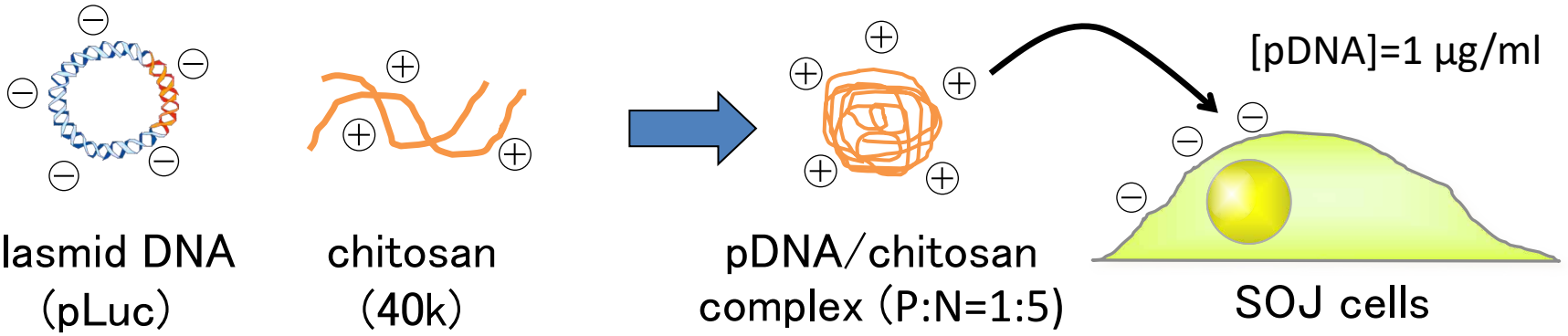
Gene delivery using chitosan

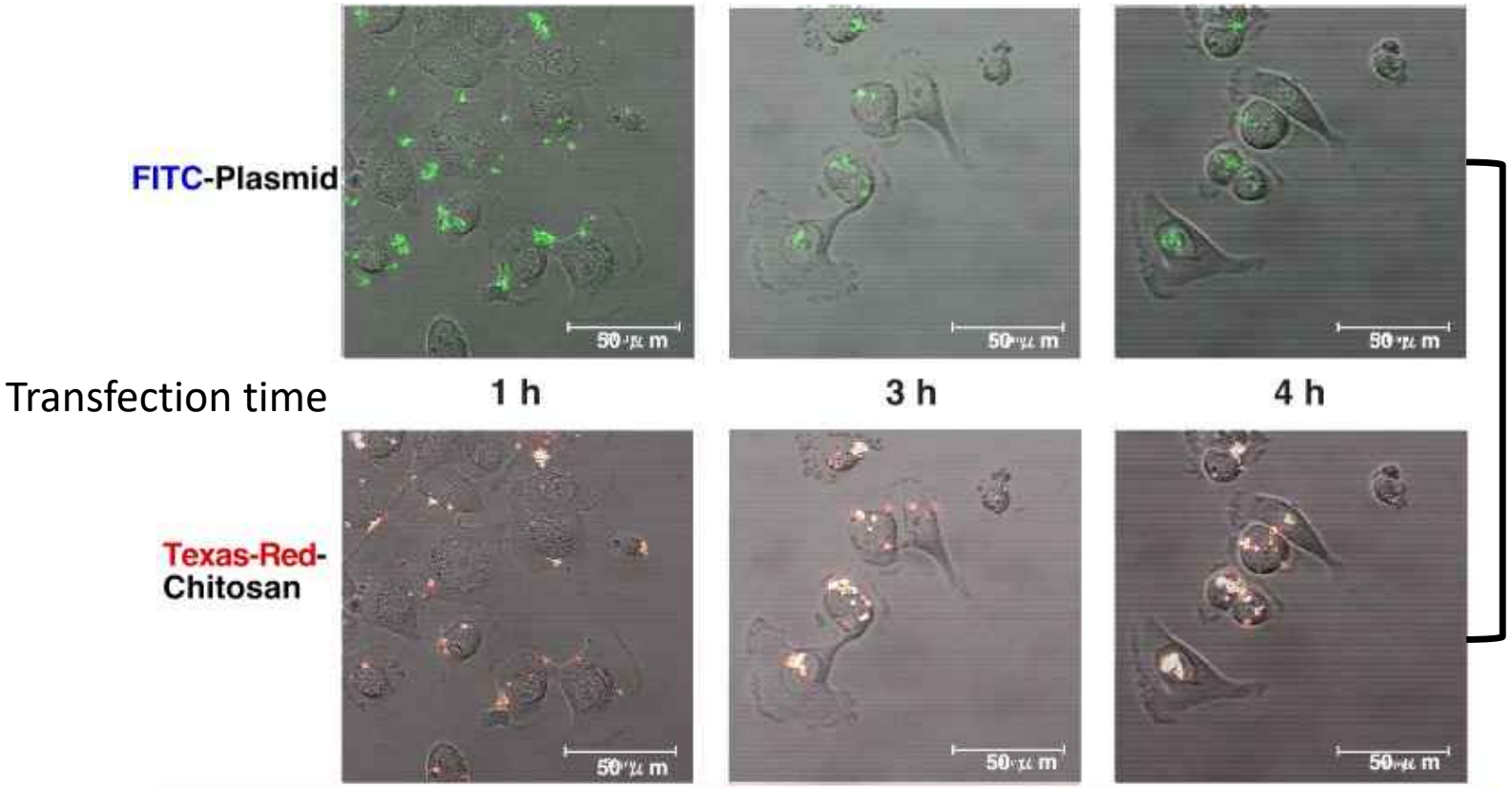




pDNA; normalized to 100, Biochim. Biophys. Acta, **1514**, 51-64 (2001)

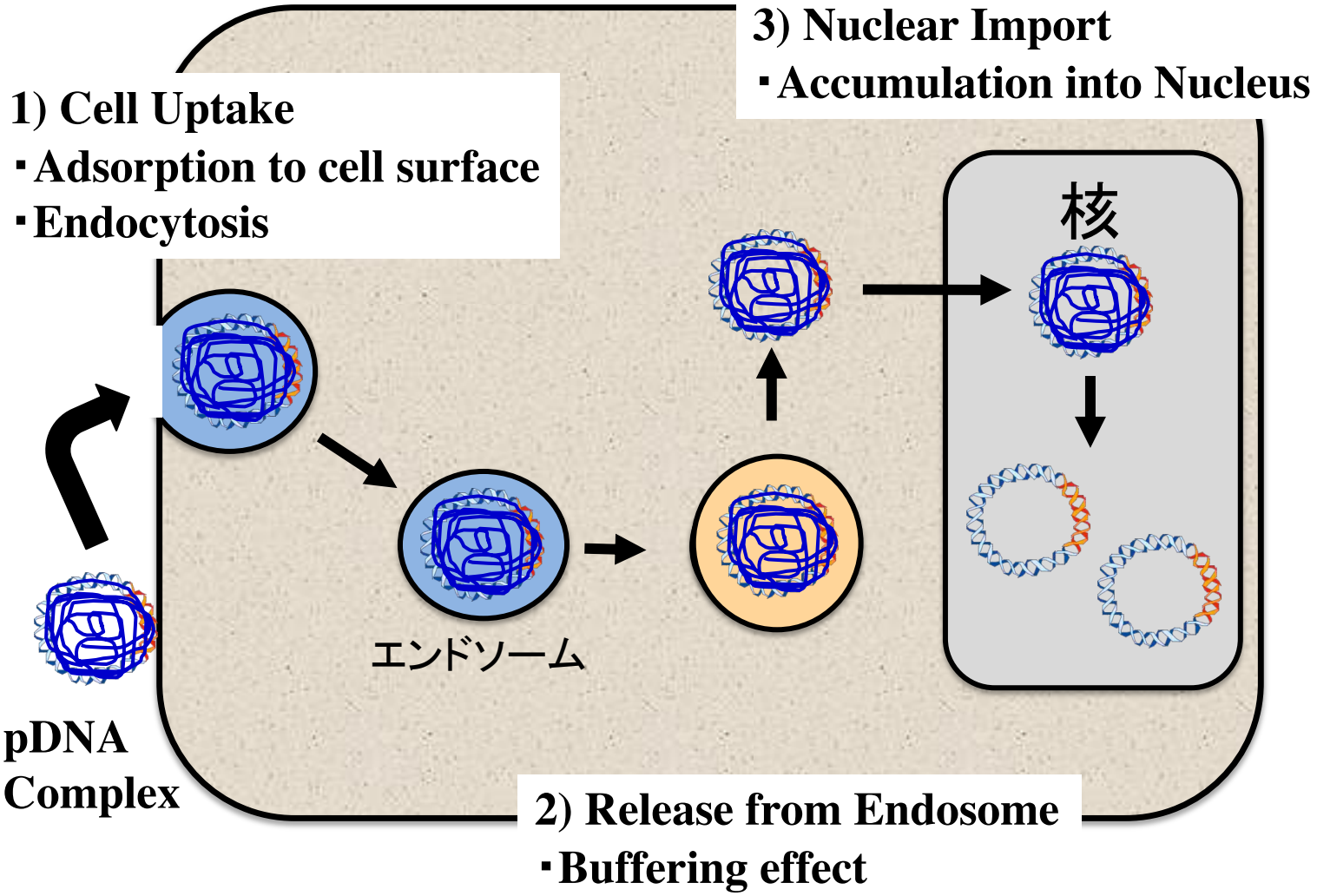
Gene delivery using chitosan





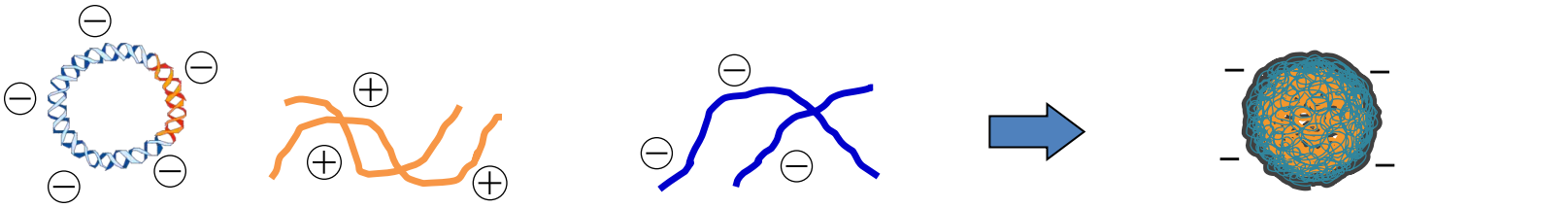
Biochim. Biophys. Acta, **1514**, 51-64 (2001)

Mechanism of cell transfection using pDNA/chitosan complex

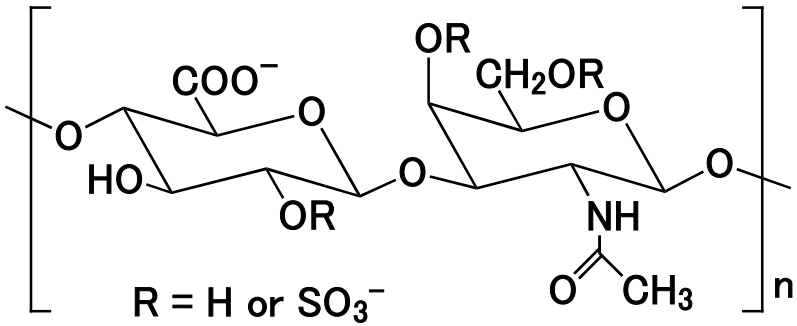


pDNA/chitosan/chondroitin sulfate ternary complex

Improvement of pDNA/chitosan complex



pDNA　chitosan　chondroitin sulfate (CS)　pDNA/chitosan/CS ternary complex



Glucuronic acid　*N*–Acetylgalactosamine

Biomaterials, 33, 7251-7260 (2012), J. Gene Med., 15, 83–92 (2013).

Particle size and zeta-potential of ternary complexes

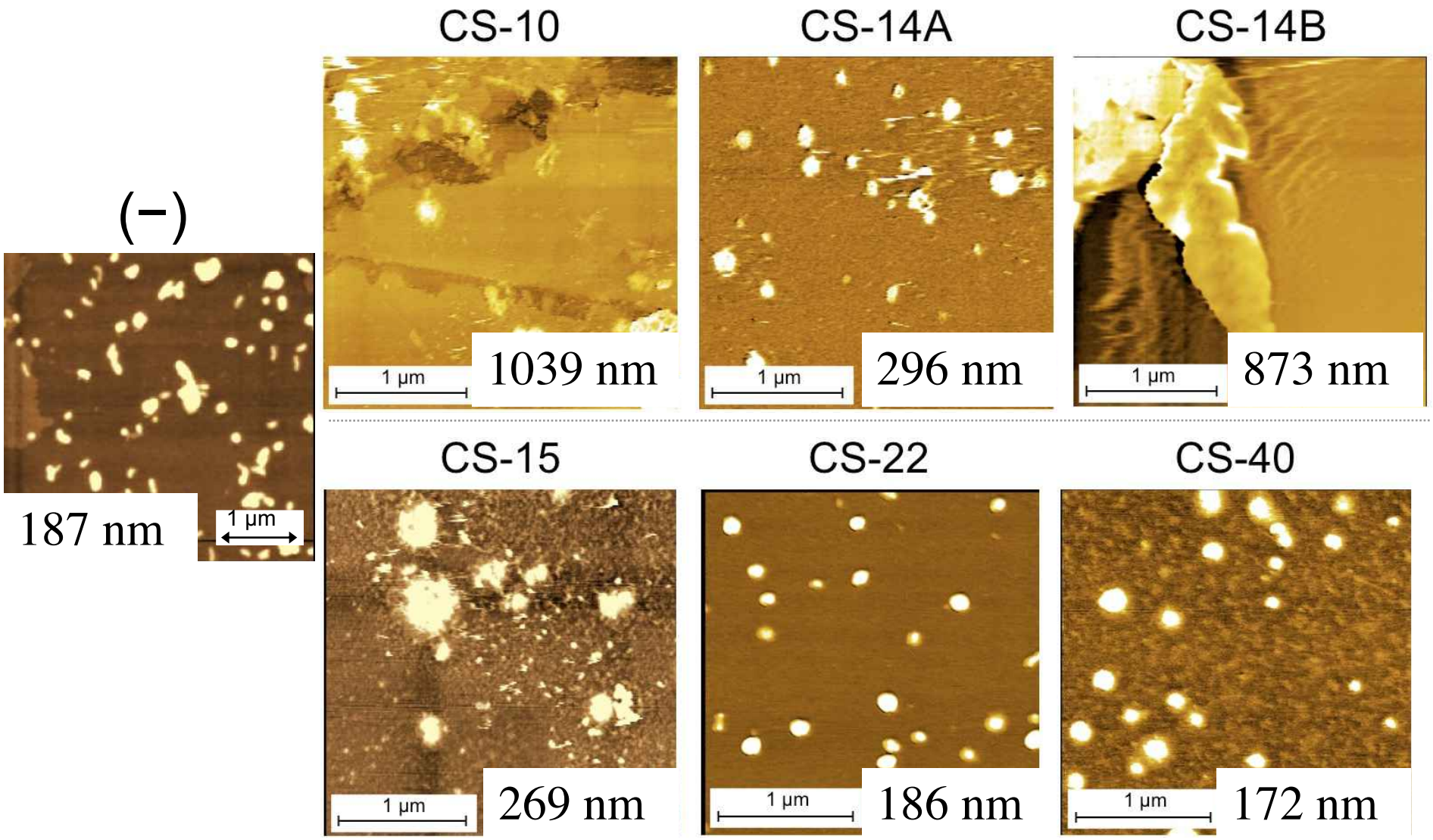
	CS	MW (x 10^3)	Degree of Sulfation (per disaccharide)	Diameter (nm)	Zeta-potential (mV)
pLuc/ chitosan P:N=1:5	-	-	-	187 ± 12	+18 ± 1
pLuc/chitosan /CS P:N:(-)=1:5:16	CS-10	10	1.02	1039 ± 9	-39 ± 3
	CS-14A	14	1.21	296 ± 37	-40 ± 3
	CS-14B	14	0.96	873 ± 18	-39 ± 2
	CS-15	15	1.06	269 ± 3	-40 ± 2
	CS-22	22	1.20	186 ± 8	-39 ± 3
	CS-40	40	1.08	172 ± 2	-39 ± 1

P:N:(-) = phosphate group(pDNA) : amino group (chitosan) : carboxy group and sulfate group (CS) n=3

Ternary complexes of CS-22 and CS-40 formed particles of about 180 nm.

Biomaterials, 33, 7251-7260 (2012)

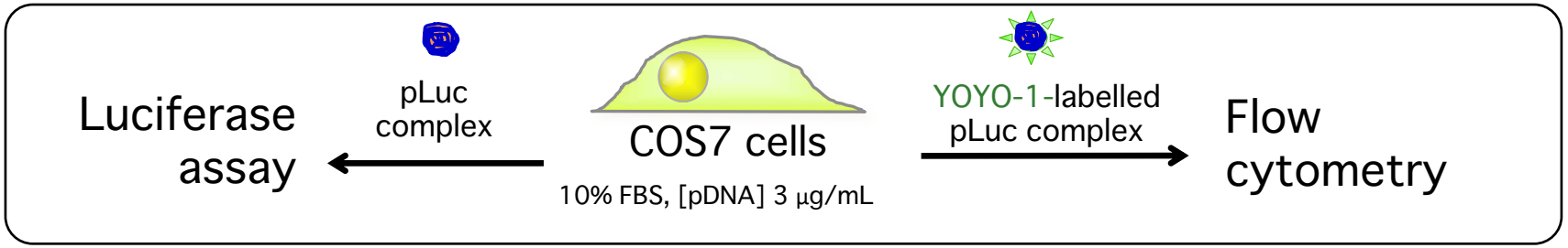
AFM observation of ternary complexes



Ternary complexes of CS−14A, CS−22 and CS−40 formed uniform spherical particles.

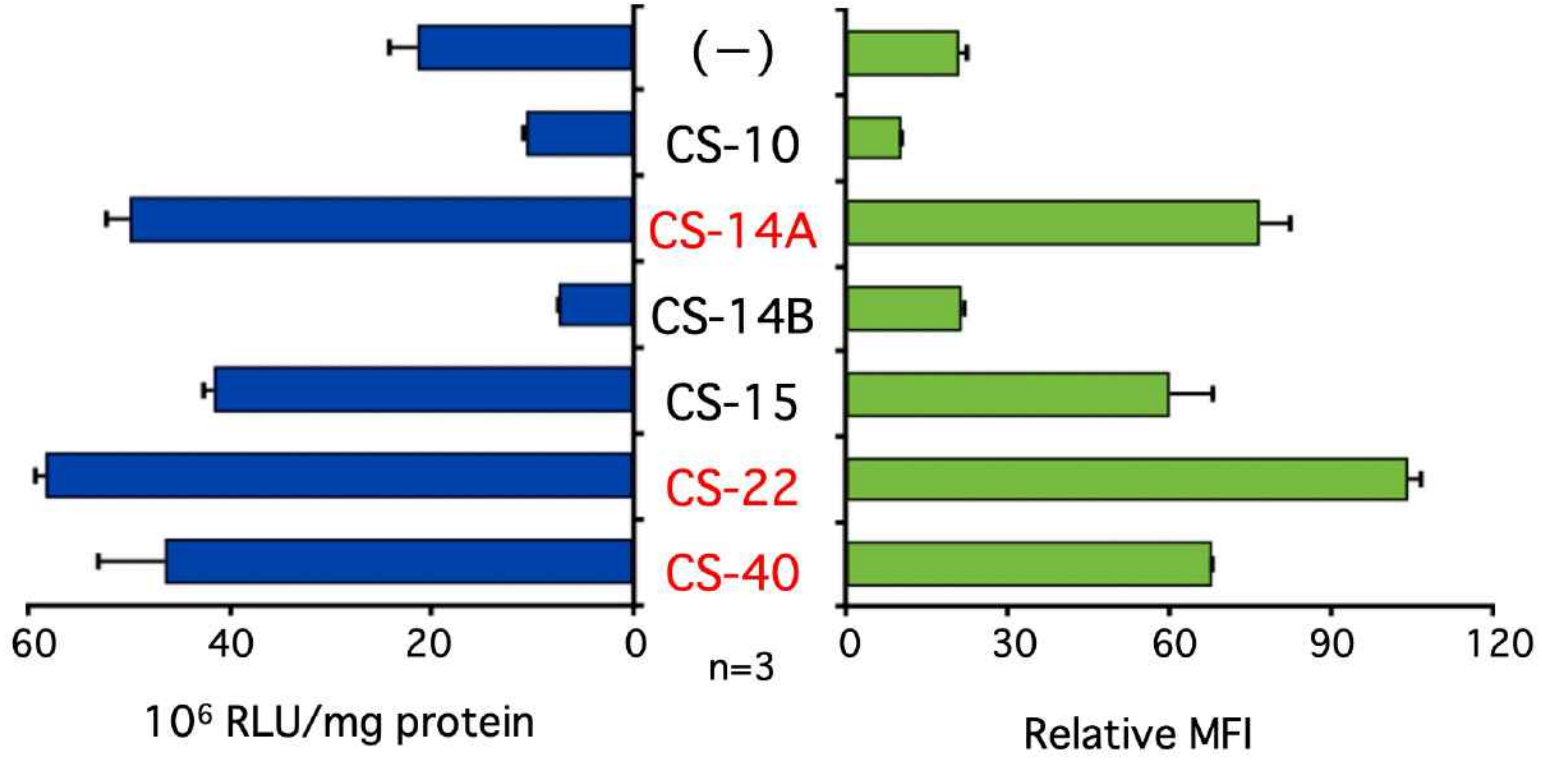
Biomaterials, 33, 7251-7260 (2012)

Transgene expression activity and cellular uptake



Transgene expression activity

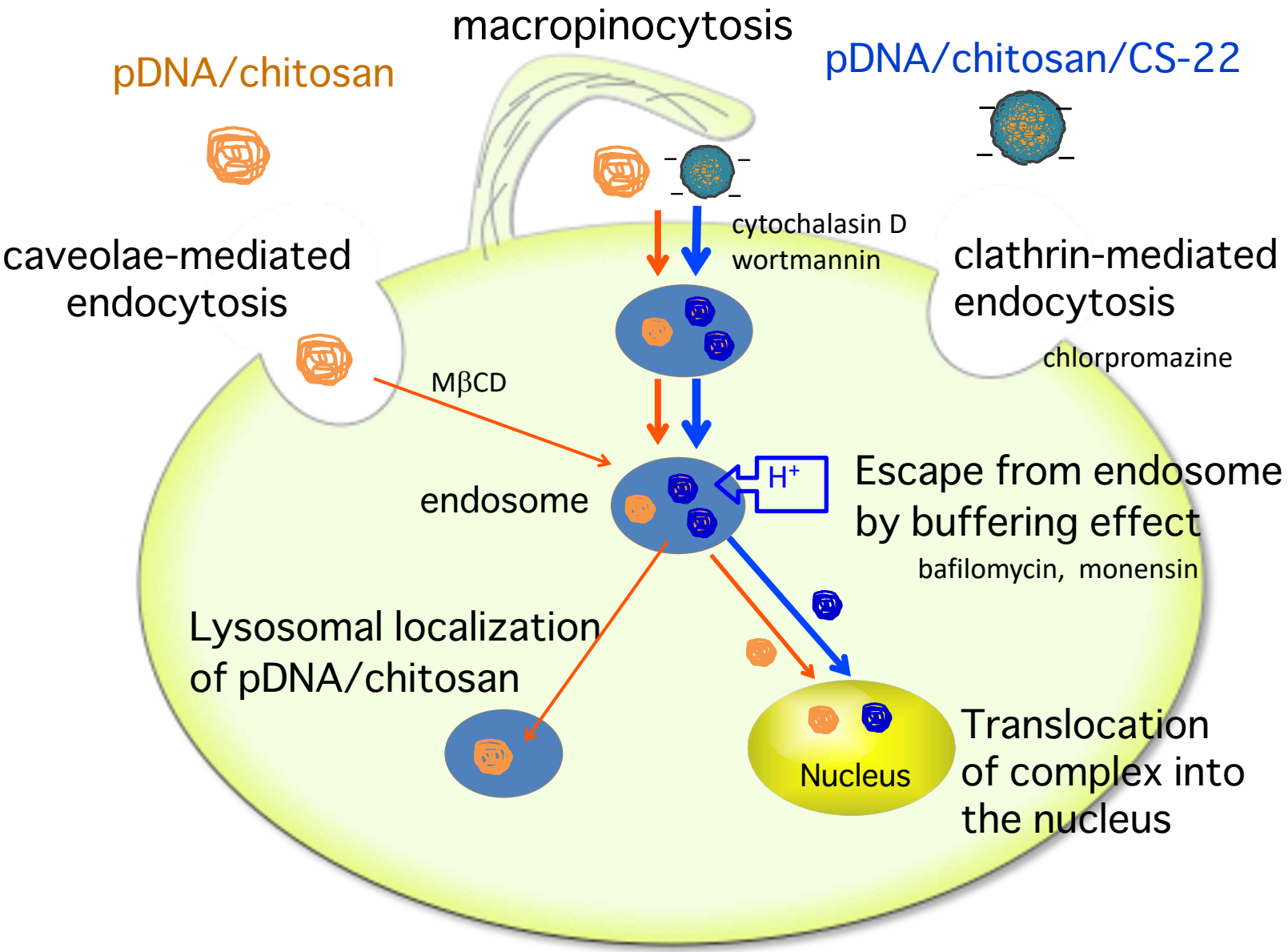
Cellular uptake



Enhanced transgene expression corresponds to increased cellular uptake.

Biomaterials, 33, 7251-7260 (2012)

Intracellular trafficking of pDNA/complexes (COS7 cells)



Expression activity of pLuc complexes after storage

pLuc/chitosan /CS-22

(P:N:(-) = 1:5:16)

pLuc/chitosan

(P:N = 1:5)

storage for 7 days

Freeze-dry(-20 ˚C, 4 ˚C, R.T.)→Rehydration

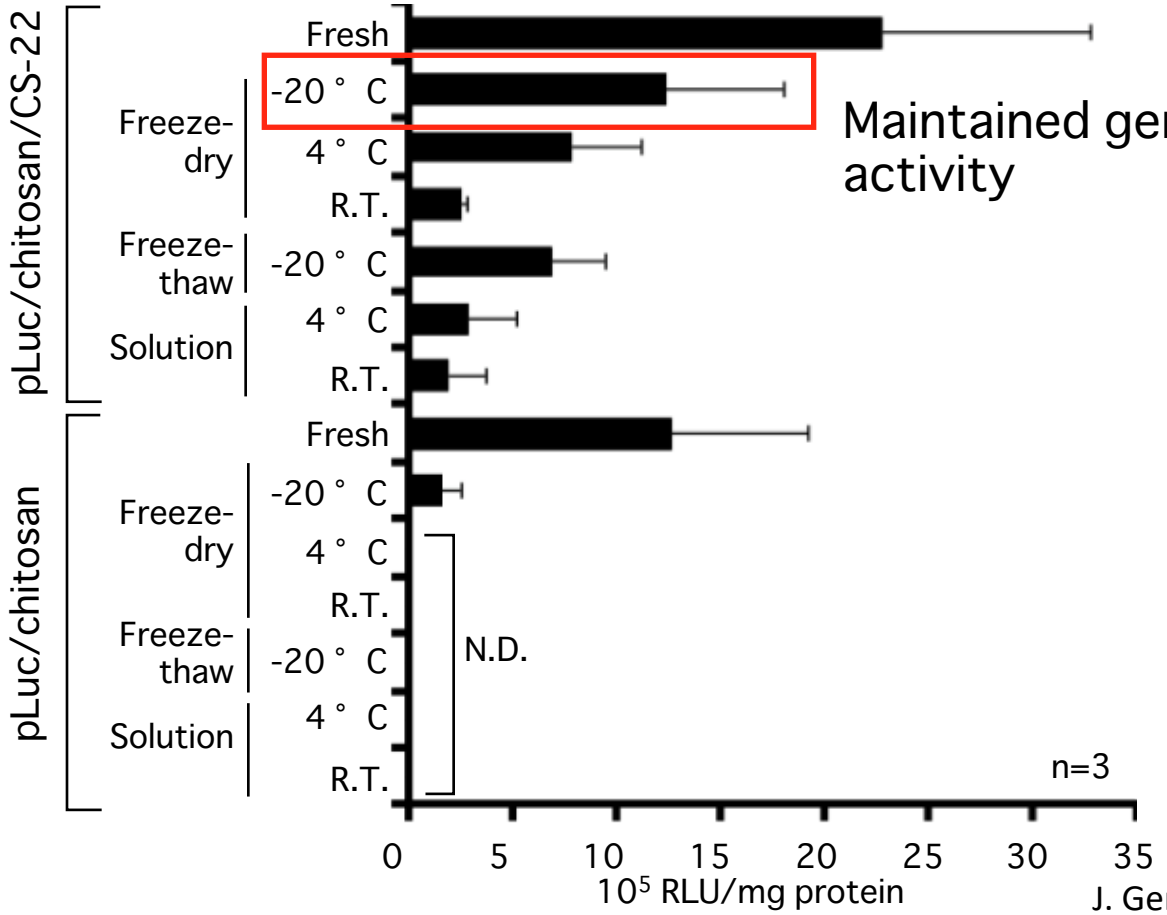
Freeze(-20 ˚C)→Thaw

Solution(4 ˚C, R.T.)

Luciferase assay

Huh7 cells

[pDNA]=3 μg/mL, pH6.5



Maintained gene expression activity

J. Gene Med., **15**, 83–92 (2013).

Suicide gene therapy of tumor-bearing mice

Prodrug: intraperitoneal injection of GCV

(100 mg/kg x 5 days)

0 7 10 14 15 (day)

Euthanasia

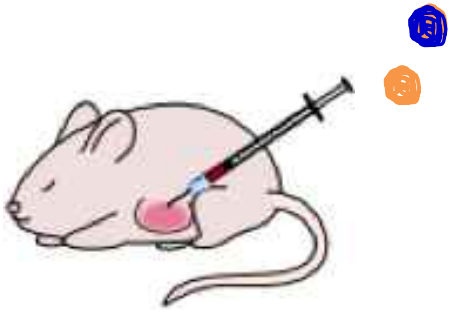
Intratumoral injection of pTK complexes

Subcutaneous inoculation of Huh7 cells

(8×10^6 cells/head)

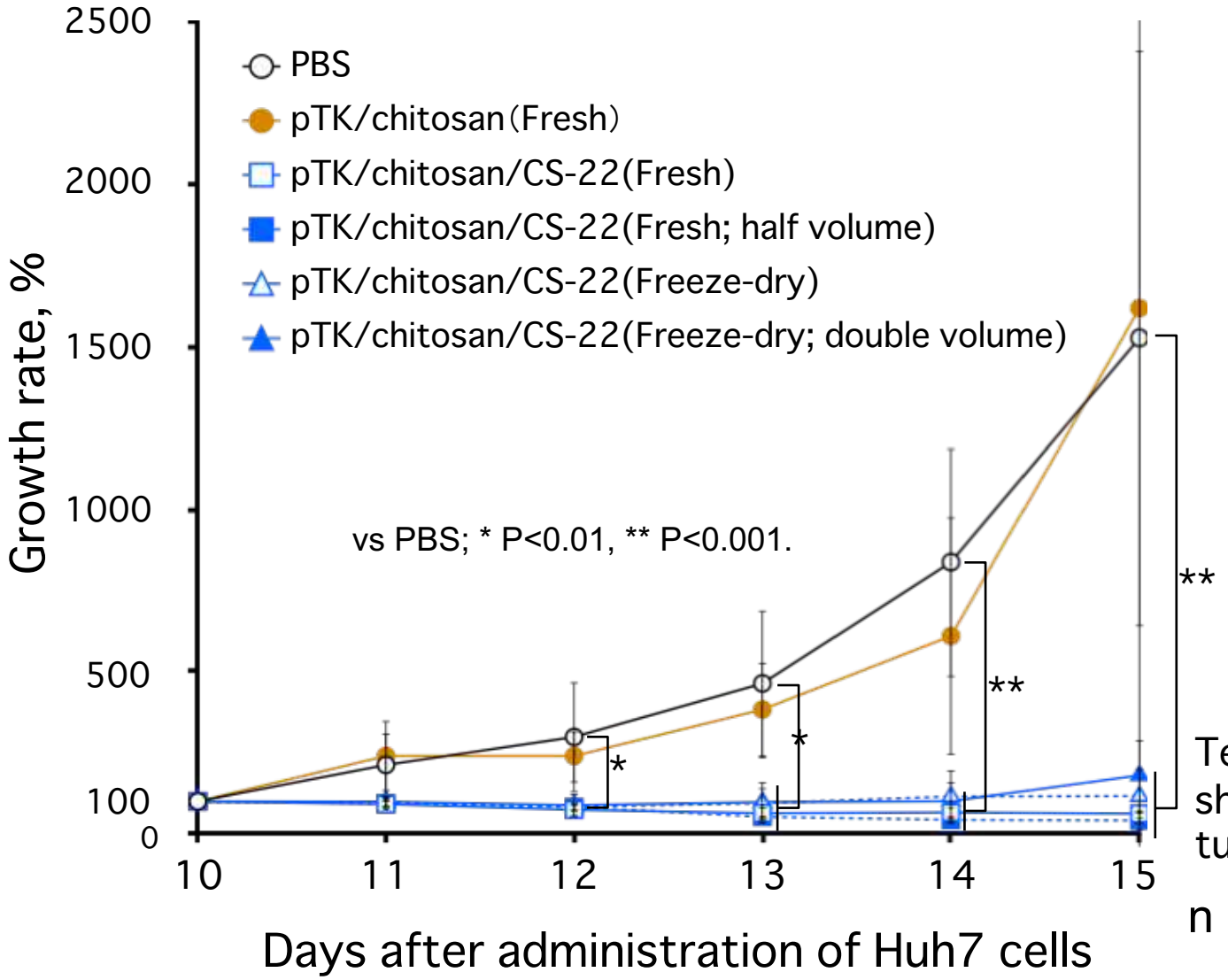
[pTK] = 10 μg/100 μL x 3 days

- pTK/chitosan/CS-22
  P:N:(-) = 1:8:16
- pTK/chitosan
  P:N = 1:8



pTK: HSV-1 thymidine kinase gene

Suicide gene therapy of tumor-bearing mice



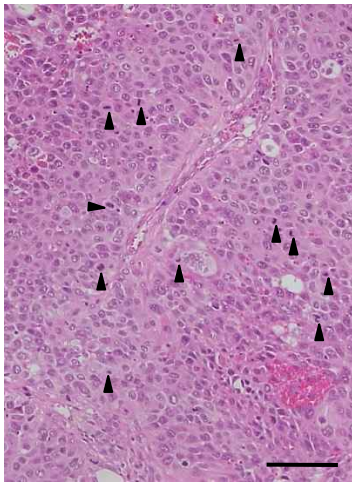
Ternary complexes showed high anti-tumor activity.

n = 4-7

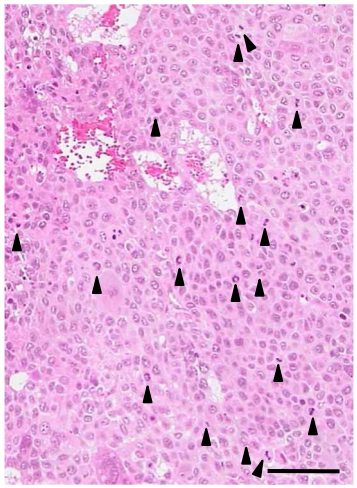
J. Gene Med., **15**, 83–92 (2013).

Histopathological analysis of tumor sections

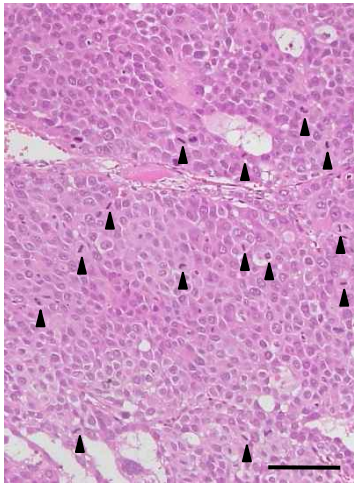
PBS



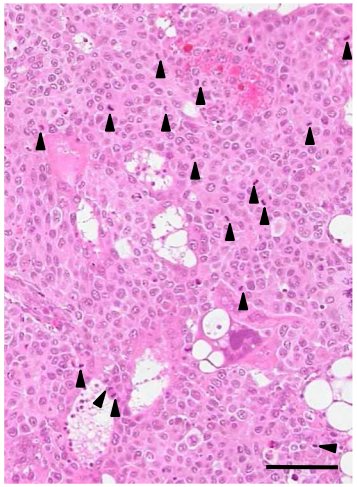
Naked pTK



chitosan/CS-22



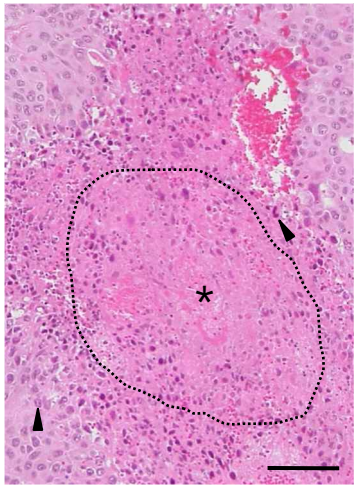
pTK/chitosan



pTK/chitosan/CS-22

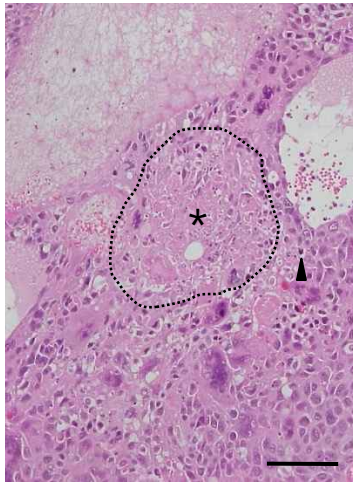
Fresh

Fresh(half volume)



Freeze-dry

Freeze-dry(double volume)

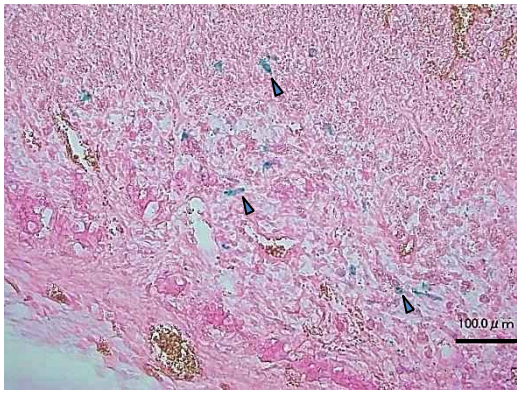


Bar = 100 μm ▲Mitotic figure * Dead cells

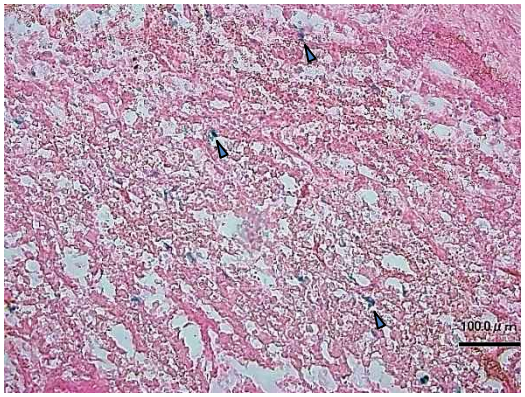
J. Gene Med., **15**, 83–92 (2013).

In vivo β-galactosidase assay

Naked pGal

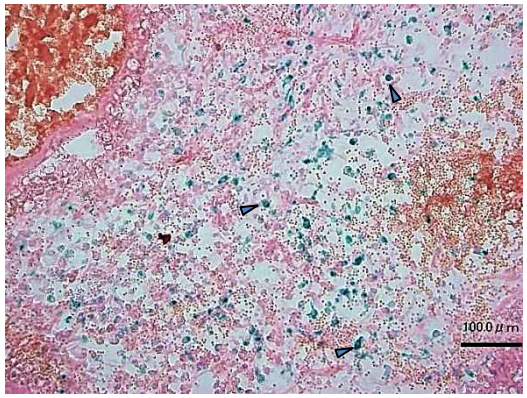


pGal/chitosan

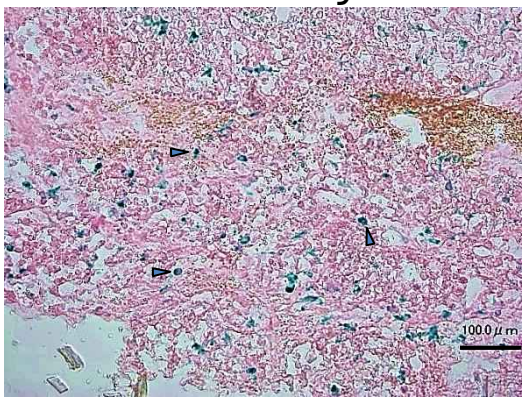


pGal/chitosan/CS-22

Fresh

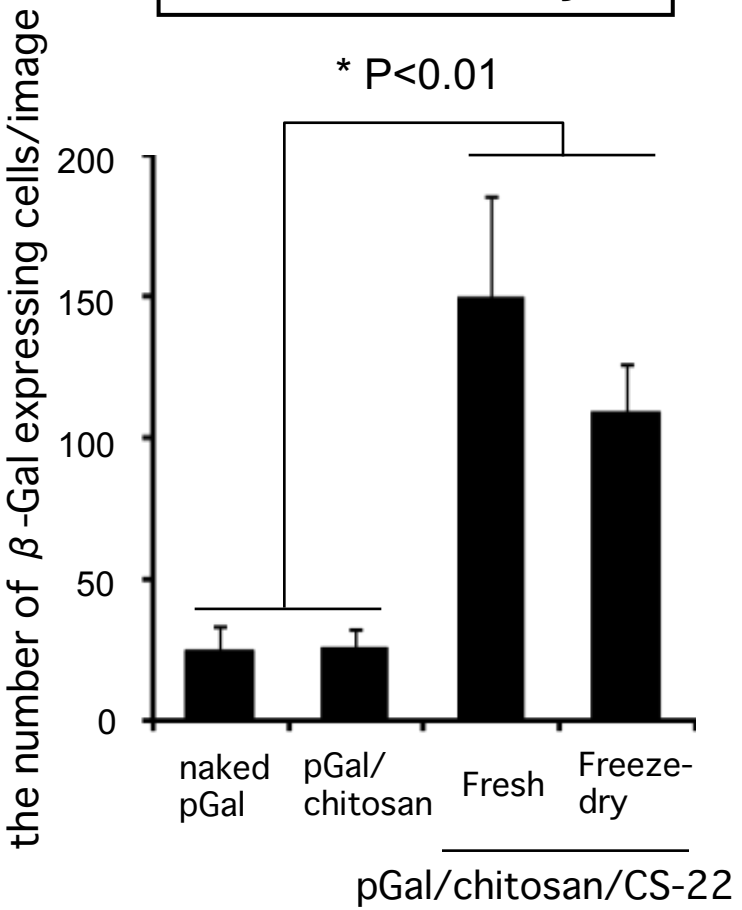


Freeze-dry



▲: Typical β-Gal expressing cells

Quantitative analysis



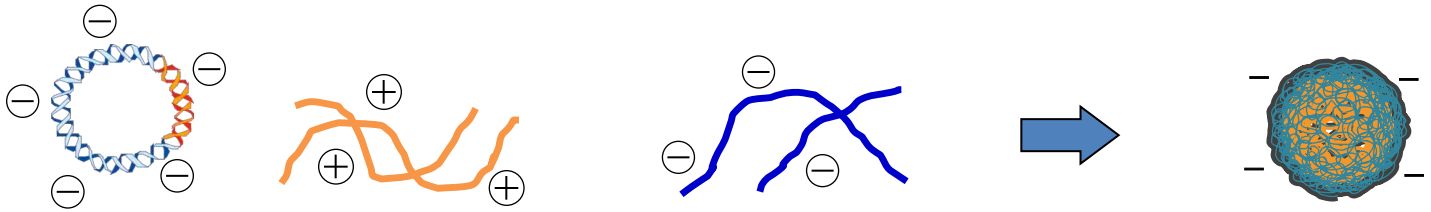
n = 6

Enhanced invasiveness of the ternary complex in tumor tissue

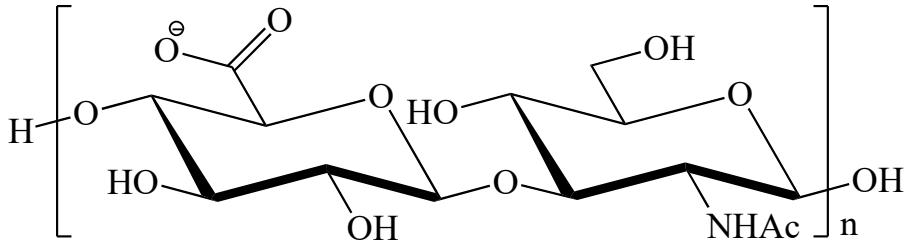
J. Gene Med., **15**, 83–92 (2013).

pDNA/chitosan/hyaluronic acid ternary complex

Improvement of pDNA/chitosan complex



pDNA　chitosan　hyaluronic acid (HA)　pDNA/chitosan/HA ternary complex



Glucuronic acid　*N*–Acetylglucosamine

J. Controlled Rel., **112**,382-388 (2006)　J. Gene Med., **19**, e2968 (2017)
Polymer J., **54**, 603-613 (2022)

Characterization of ternary complexes

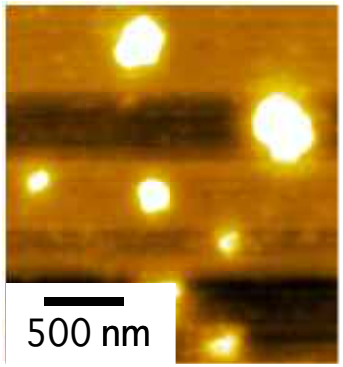
◯ Agarose gel electophoresis

◯ Particle size and zeta-potential

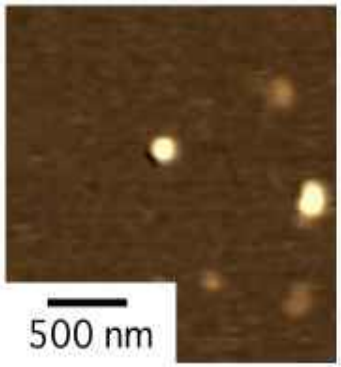
HA	MW	P:N:C	Diameter	Zeta-potential
HA-400	400k	1:5:16	264 nm	-41.6 mV
HA-600	600k	1:5:16	311 nm	-41.3 mV
HA-700	700k	1:5:16	317 nm	-44.8 mV
HA-1300	1300k	1:5:16	336 nm	-45.4 mV

P:N:C = phosphate group(pDNA) : amino group (chitosan) : carboxy group (HA)

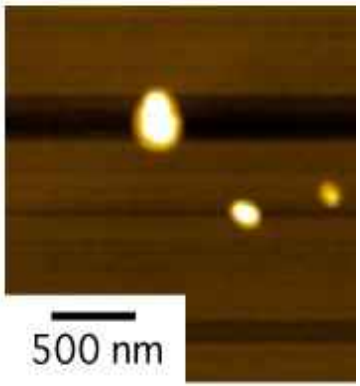
◯ Atomic force microscope



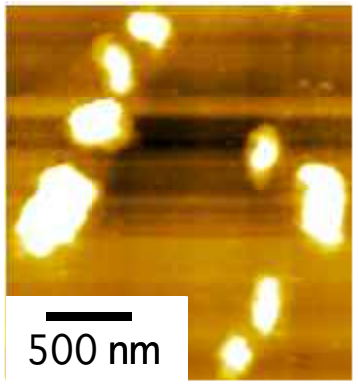
HA-400



HA-600



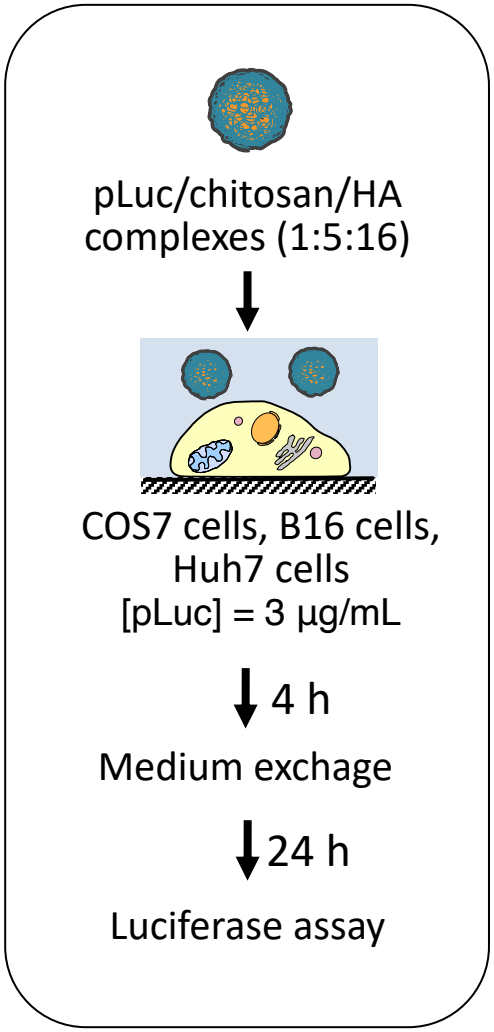
HA-700

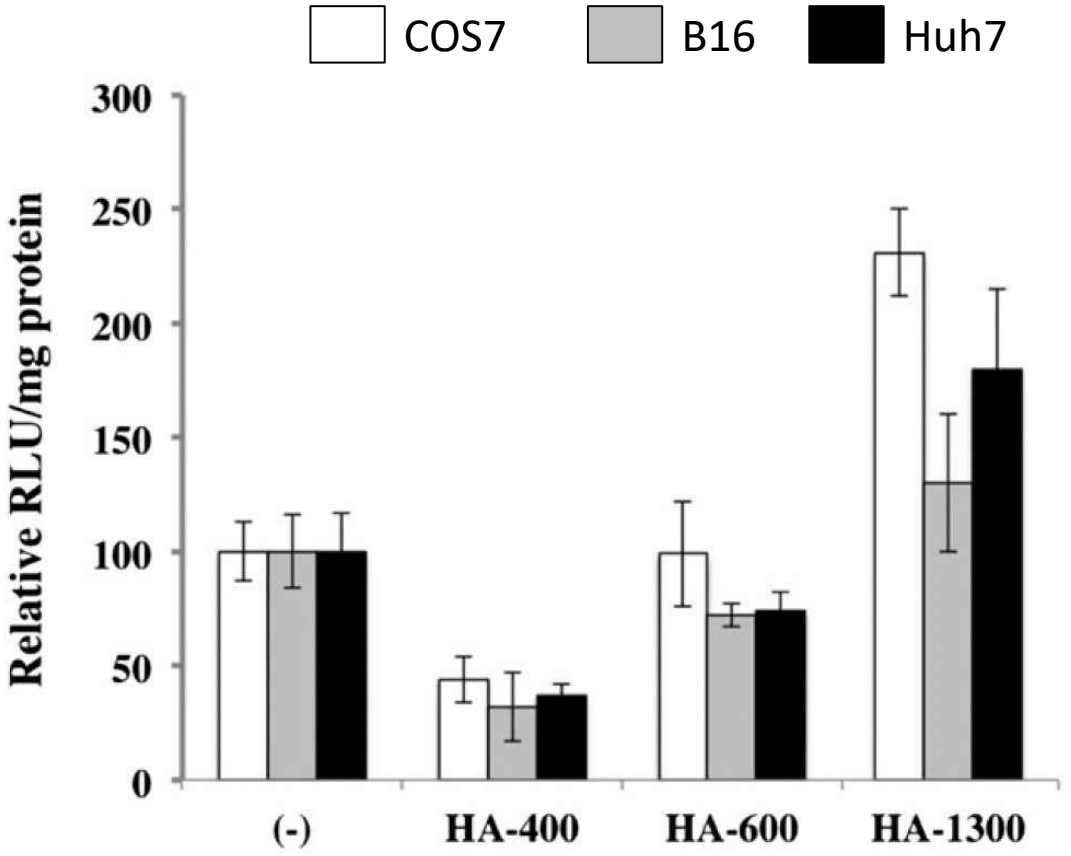


HA-1300

J. Gene Med., **19**, e2968 (2017)

Transgene expression activity using pDNA/chitosan/HA ternary complexes (*in vitro*)





・The expression activity was dependent on MW of HA.

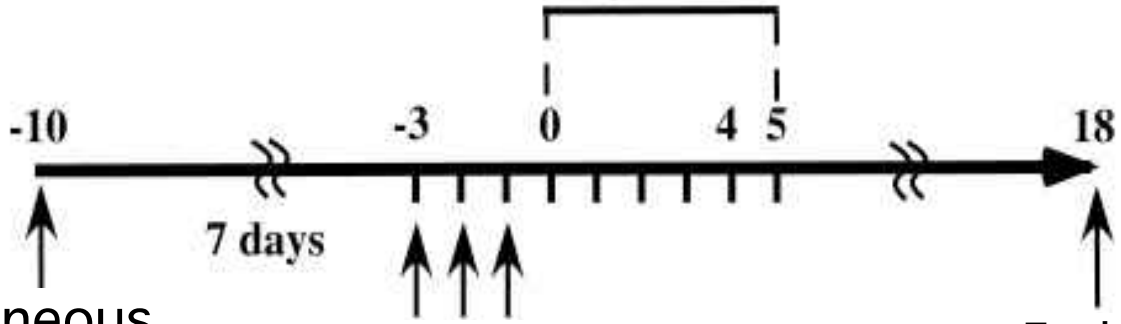
・Inhibition experiment suggested the involvement of CD44 (COS7 cells).

J. Gene Med., **19**, e2968 (2017)

Suicide gene therapy of tumor-bearing mice

Prodrug: intraperitoneal injection of GCV

(100 mg/kg x 6 days)



Subcutaneous inoculation of Huh7 cells

(5×10^6 cells/head)

Intratumoral injection of pTK complexes

Euthanasia

- pTK/chitosan/HA

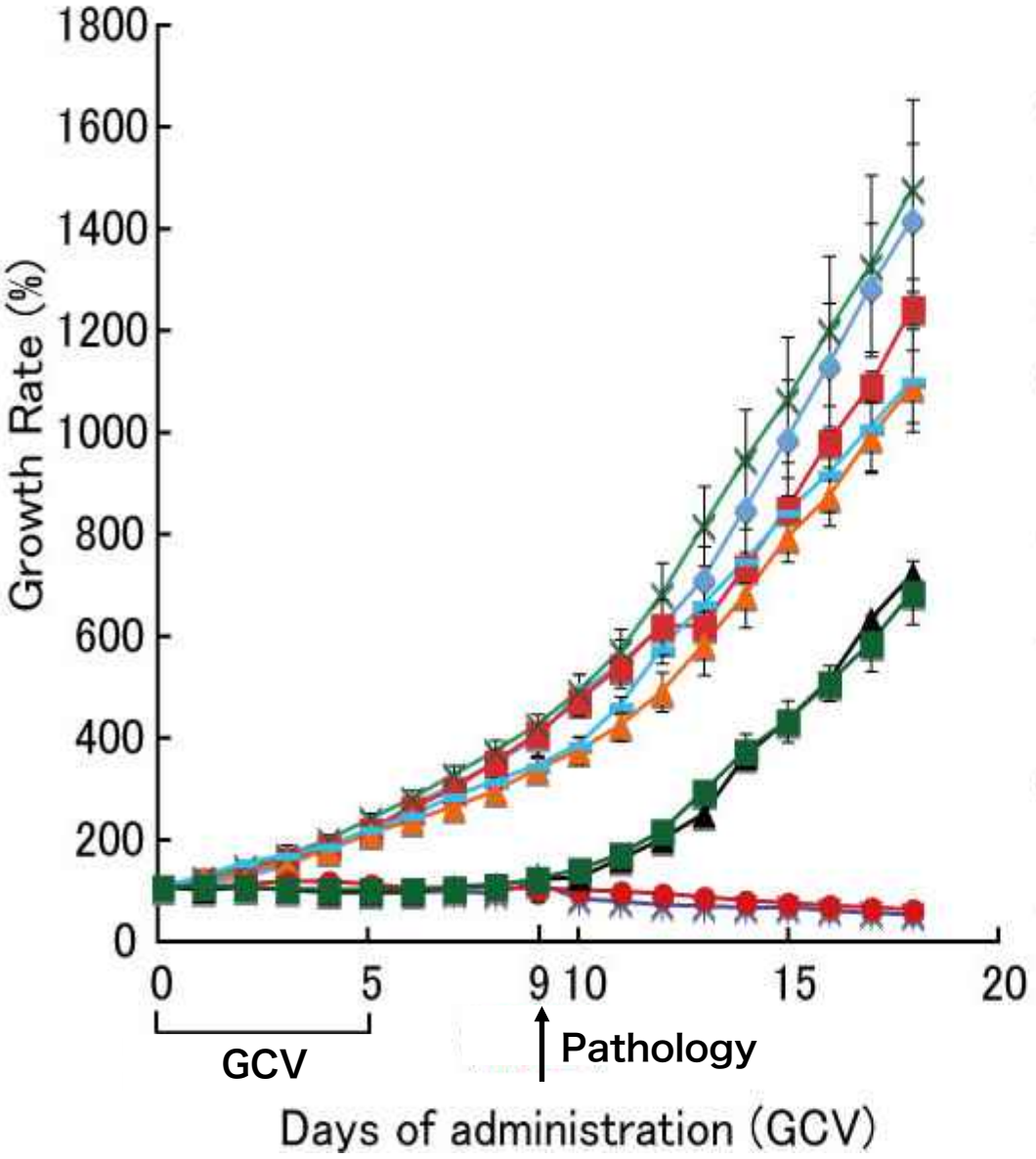
  P:N:C = 1:8:16

- pTK/chitosan

  P:N = 1:8

[pTK] = 10 μg/100 μL x 3 days

Suicide gene therapy of tumor-bearing mice



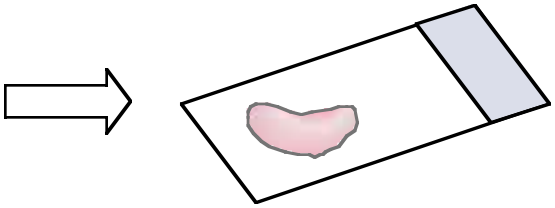
pTK/chitosan + GCV (n = 4-6)
Control (n = 4-6)
Control + GCV (n = 4-6)
Chitosan + HA + GCV (n = 4-6)
Naked pTK + GCV (n = 4-6)

pTK/chitosan/HA-1300 Freeze-Dry + GCV (n = 1)
pTK/chitosan/HA-1300 Fresh + GCV (n = 2)

pTK/chitosan/HA-1300 Freeze-Dry + GCV (n = 3)
pTK/chitosan/HA-1300 Fresh + GCV (n = 2)

J. Gene Med., **19**, e2968 (2017)

Histopathological analysis of tumor sections



Immobilization,
preparation of sections

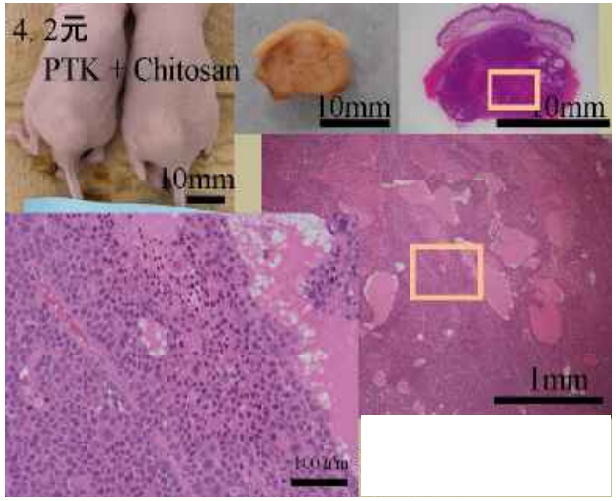
Hematoxylin-eosin stain

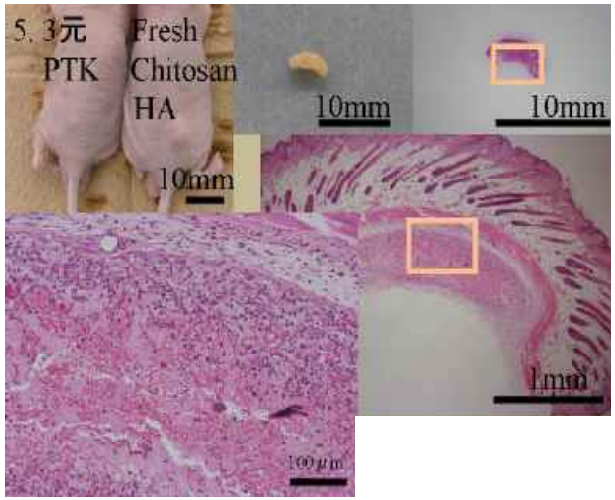
pTK/chitosan (Fresh)

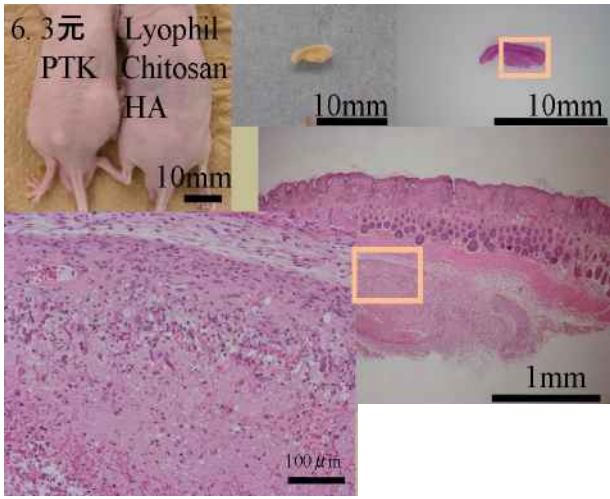
pTK/chitosan/HA-1300

Fresh

Freeze-dry







Tumor growth,
Necrosis in a small part

Complete tumor necrosis

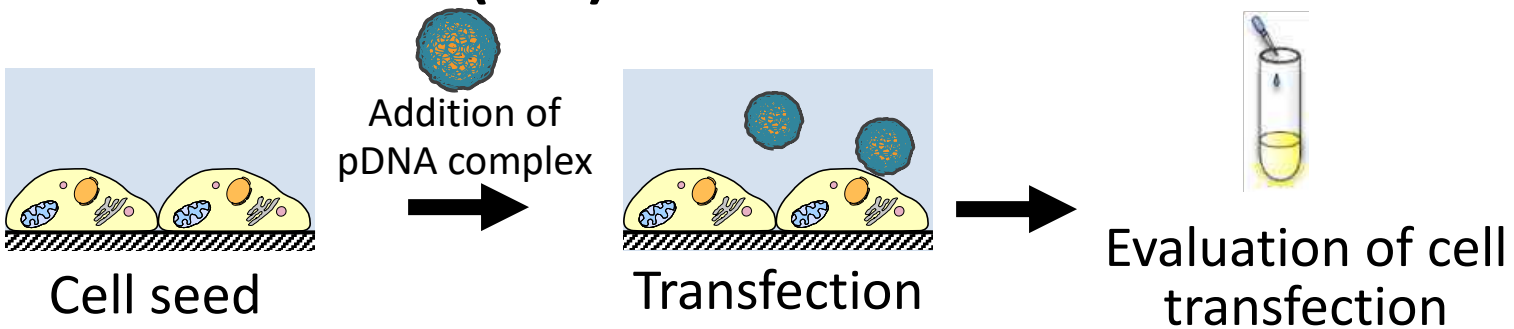
J. Gene Med., **19**, e2968 (2017)

Comparison of binary and ternary complexes

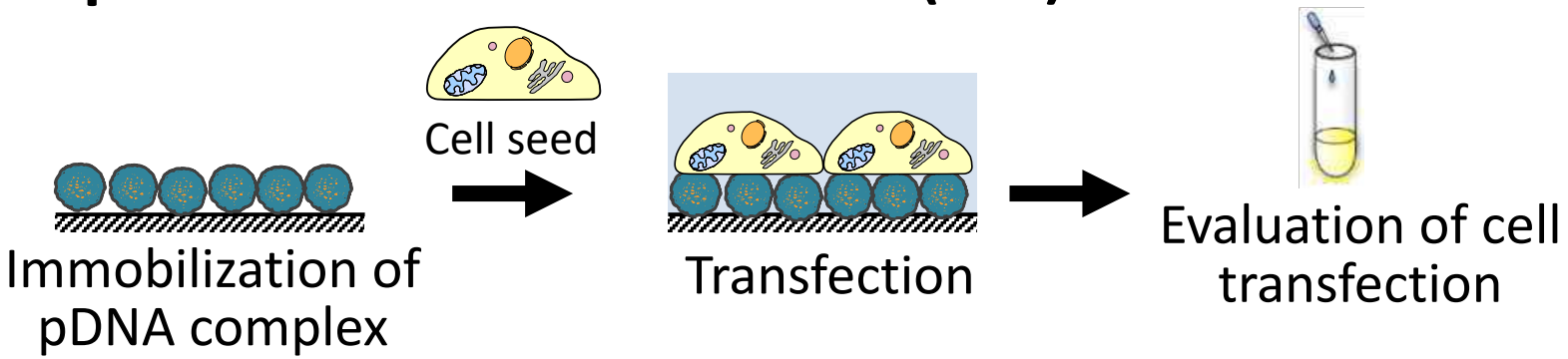
	pDNA	pDNA/chitosan binary complex	pDNA/chitosan/ HA(CS) ternary complex
Stability	×	×	○
Cell transfection activity	×	○	○
Gene transfer into nucleus	×	○	○
Cell specificity	×	×	○
Storage	–	×	○
Suiside gene therapy (*In vivo)*	×	×	○

Solid-phase reverse transfection (RTF)

- **Forward transfection (FTF)**



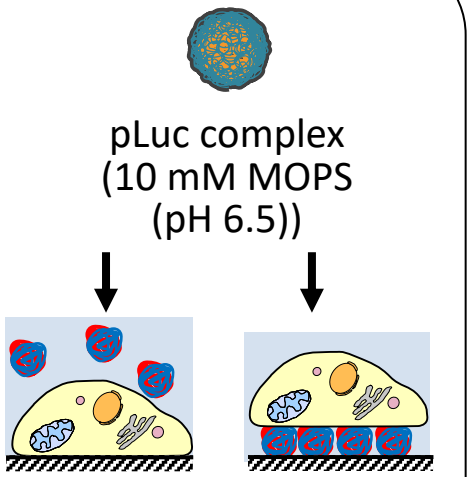
- **Solid-phase reverse transfection (RTF)**

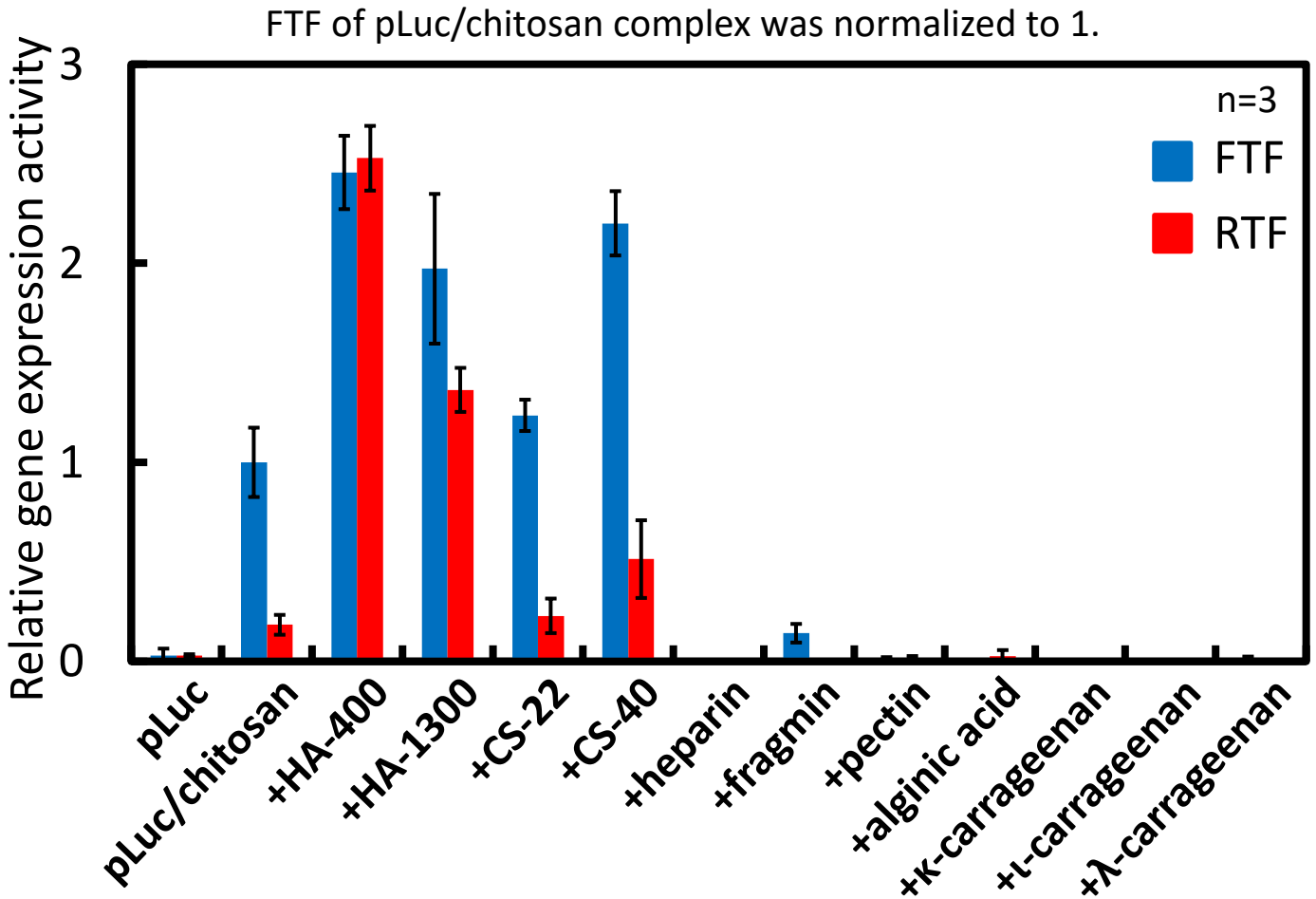


- Useful for cell array development
- Gene carriers are limited and the gene expression mechanism is not elucidated.

Polymer J., **54**, 603-613 (2022)

Cell transfection activity of pDNA/polysaccharide complexes in RTF

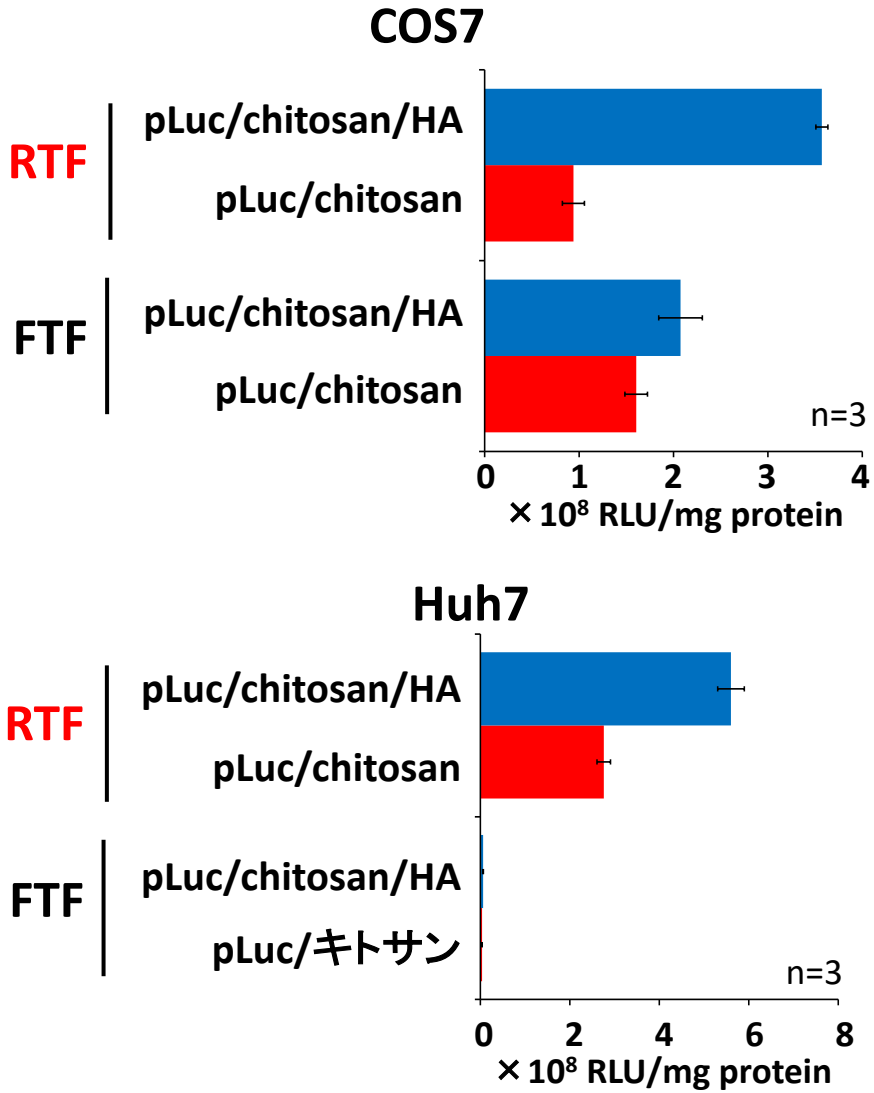




HA ternary complexes are suitable for RTF.

Polymer J., **54**, 603-613 (2022)

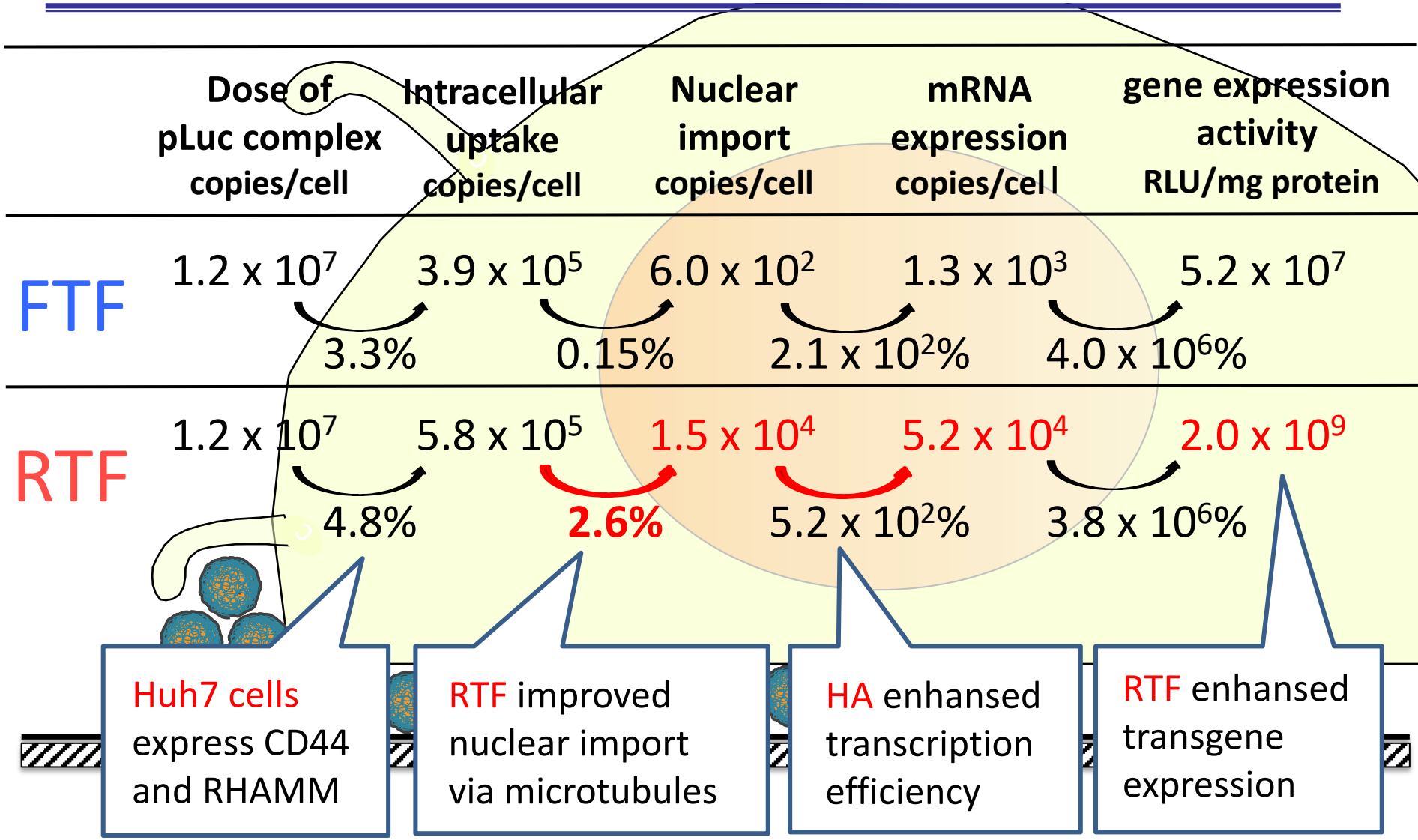
Cell dependence of trangene expression in RTF



Gene expression of the HA ternary complex was enhanced in RTF.

Polymer J., **54**, 603-613 (2022)

Intracellular delivery of pLuc/chitosan/HA-840 compexes (Huh7)



Polymer J., **54**, 603-613 (2022)

Summary of pDNA/polysaccharide complexes

pDNA/chitosan/chondroitin sulfate ternary complex

- Cell uptake by macropinocytosis
- Storage stability by lyophilization
- Transgene expression and anti-tumor activity *in vivo*

pDNA/chitosan/hyaluronic acid ternary complex

- HA receptor-mediated endocytosis
- Nuclear import mediated by microtubule
- Storage stability by lyophilization
- Anti-tumor activity *in vivo*
- Improving transgene expression by solid-phase reverse transfection