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A Tumor-Acidity-Activated Charge-Conversional Nanogel as an Intelligent Vehicle for Promoted Tumoral-Cell Uptake and Drug Delivery

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1. Introduction

1–1. Drug delivery systems

• Drug delivery systems have gained much attention as a potential tool for enhancing drug efficacy and minimizing side effects.

• Nanomaterials accumulate in tumor cells through enhanced permeation and retention (EPR) effect.[1]

• Positively charged nanoparticles show high affinity for negatively charged cell membranes (Figure 1).[2]

→ Positively charged nanoparticles can be readily internalized by the cells.[3]

• Positively charged nanoparticles interact strongly with serum components, which cause severe aggregation, rapid clearance from circulation and limits their *in vivo* applications (Figure 1).[4]

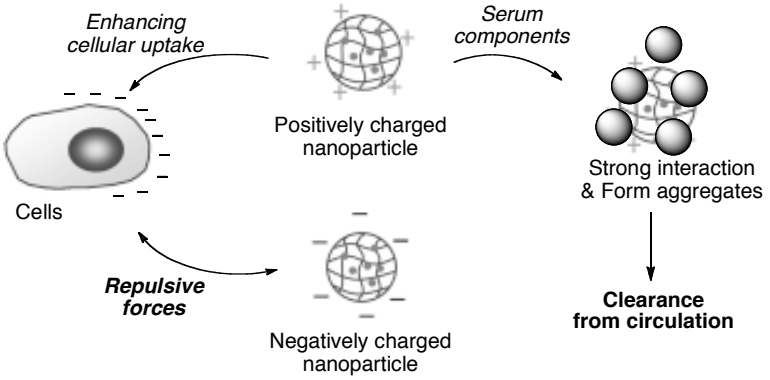


Figure 1. Schematic illustration of the performance of positively charged and negatively charged nanoparticles.

1–2. Intelligent vehicle for promoted tumoral-cell uptake

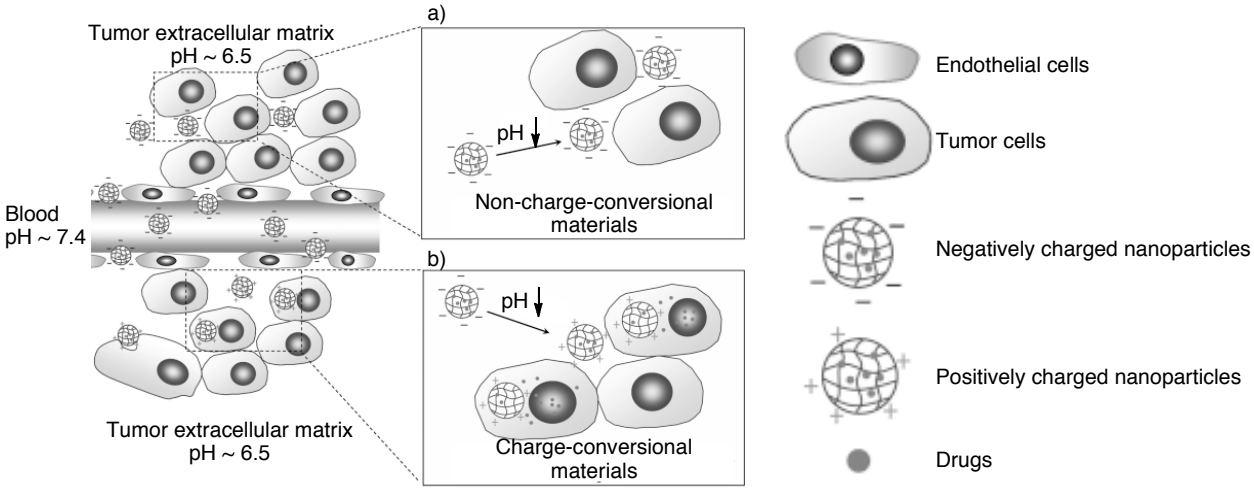


Figure 2. Schematic illustration of the performance of pH-responsive charge-conversional nanoparticles and non-charge-conversional nanoparticles.

• In this work, authors have developed charge-conversional nanogels, which enhance cellular uptake and avoid adsorption with serum components (Figure 2).

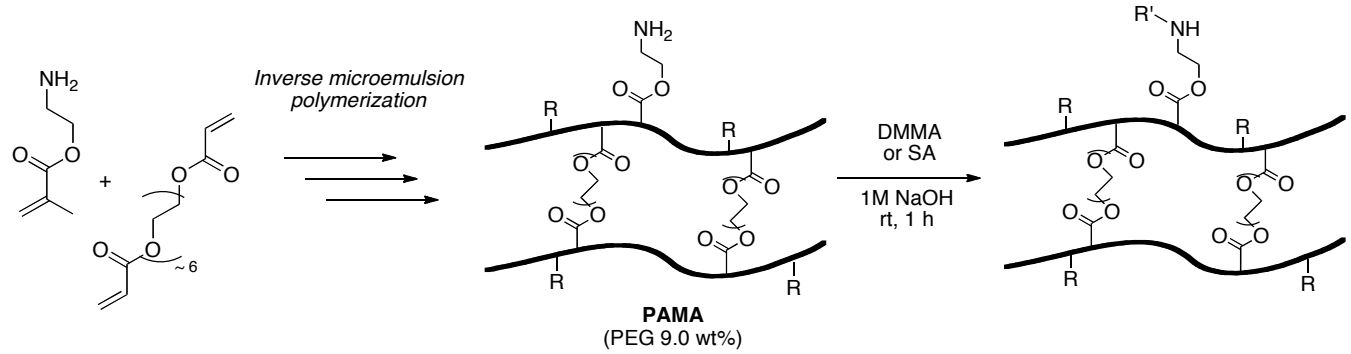
2. Results and Discussion

2–1. Preparation of nanogels

• Poly(2-aminoethyl) methacrylate nanogel (**PAMA**) was synthesized by an inverse microemulsion polymeraization method with poly(ethylene glycol) diacrylate as a cross-linker and potassium persulfate as an initiator (Scheme 1).

• Amine moieties of **PAMA** were reacted with DMMA (2,3-dimethylmaleic anhydride) or SA (succinic anhydride) to afford **PAMA-DMMA** and **PAMA-SA**, respectively.

Scheme 1. Preparation of positively charged (**PAMA**), charge-conversional (**PAMA-DMMA**) and non-charge-conversional (**PAMA-SA**) nanogels.

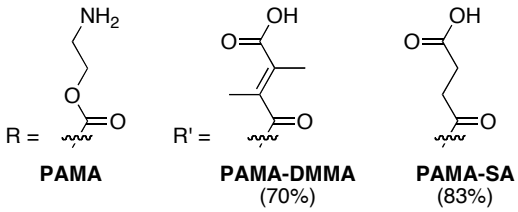


DLS analysis

– **PAMA-DMMA** : charge-conversional (122 nm, –17 mV)

– **PAMA-SA** : non-conversional (??? nm, –25 mV)

– **PAMA** : positively charged (100 nm, +30 mV)



*2–2. Characterization of charge-conversional nanogel **PAMA-DMMA***

• Charge conversion was monitored by zeta potential and ^{1}H NMR (Figure 3)

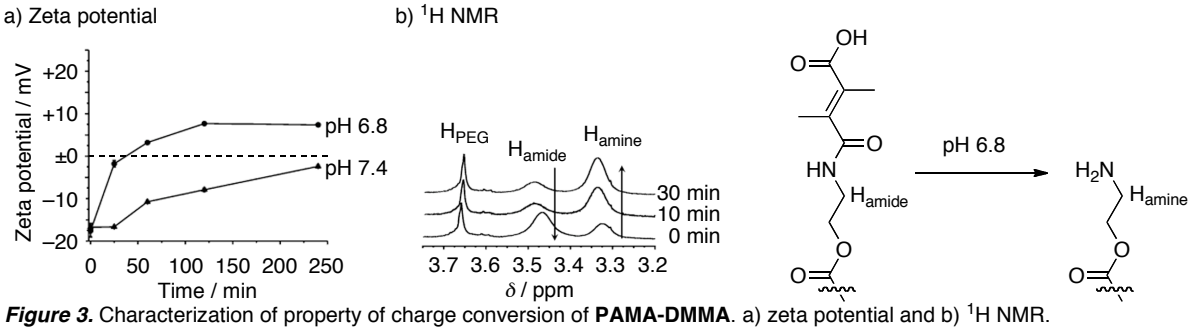


Figure 3. Characterization of property of charge conversion of **PAMA-DMMA**. a) zeta potential and b) ^{1}H NMR.

a) Zeta potential

• **PAMA-DMMA** at pH 7.4: slightly increased to ±0 mV

• **PAMA-DMMA** at pH 6.8: reached to ±0 mV within 35 min, and then increased over +5 mV

→ Charge-conversional behavior between pH 7.4 and 6.8

• As reference, **PAMA-SA**: no sign of charge-conversional behavior (data not shown here)

→ Negatively charged nanogel

b) ^{1}H NMR

• Intensity of H_{amide} ↓ and H_{amine} ↑ / the intensity of the PEG mthylene hydrogen atoms (H_{PEG})

→ > 60% of the amide bonds had been hydrolyzed after 30 min

2–3. in vitro *analysis*

a) Confocal laser scattering microscopy (Figure 4)

– **PAMA**: FITC, green (brightest contrast)

– Nuclei: DAPI, blue

– Membrane (F-actin): rhodamine phalloidin, red

• At pH 6.8, **PAMA-DMMA** located in cytoplasm

• At pH 7.4, located adhered to the cell mebrane

→ Positively charged nanogel (at pH 6.8 after hydrolysis) has higher cellular uptake than negatively charged nanogel (at pH 7.4).

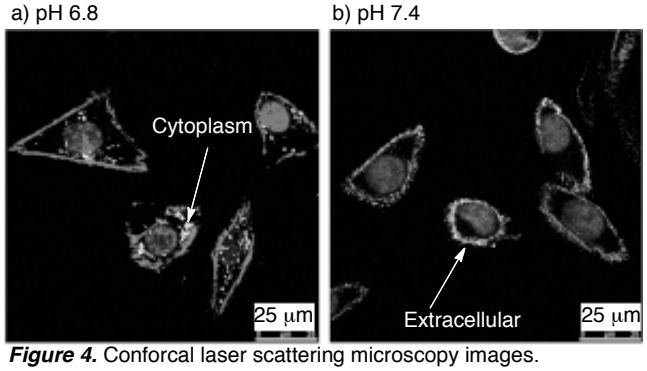


Figure 4. Conforcal laser scattering microscopy images.

b) Fluorescent-activated cell sorting by flow cytometry (Figure 5)

• Fluorescent intensity of FITC at pH 6.8 > pH 7.4

→ Internalization of nanogel at pH 6.8 looks higher than that at pH 7.4.

• Florescent-activated cell sorting by flow cytometry was carried out.

• Cells maintained at pH 6.8 has stronger intensity than that at pH 7.4.

→ Charge conversion facilitated the cellular uptake of the nanogel.

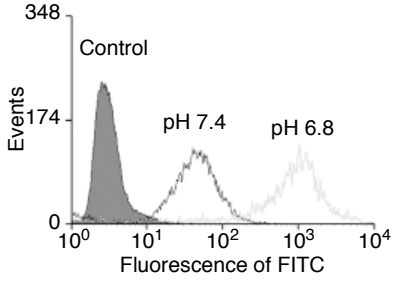


Figure 5. Flow cytometry.

c) Interaction of nanogel with seram component (BSA) (Figure 6)

• Positively charged nanoparticles tend to strongly interact with serum components.

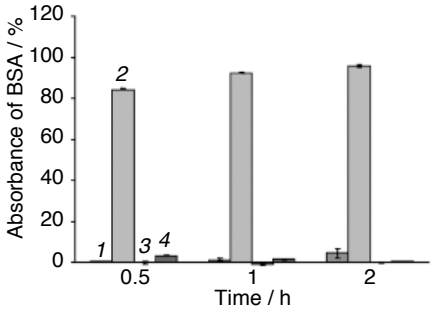
• Bovine serum albumin (BSA) as a model protein was used to examine the potential for *in vivo* application.

– **PAMA-DMMA** @6.8: >80% BSA adsorption

– **PAMA-DMMA** @7.4 & **PAMA-SA**: almost no adsorption.

• Positively charged nanogel interacted strongly with BSA

→Positively charged nanogel rapidly degrade by serum components



pH	7.4	6.8
PAMA-DMMA	*1*	*2*
PAMA-SA	*3*	*4*

Figure 6. BSA adsorption on the nanogels.

d) Release ability of drugs and cell viability (Figure 7)

• Doxorubicin (DOX)-loaded nanogels were preparaed by just mixing with DOX at pH 7.4.

• High drug loading efficiency (DLE) of negatively charged nanogel

– 95% for **PAMA-DMMA** (–17 mV)

– 99% for **PAMA-SA** (–25 mV)

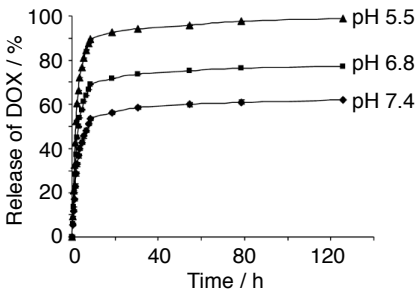
– 19% for **PAMA** (+30 mV)

→ The interaction of negatively charged nanogels with positively charged DOX.

• pH-dependent DOX release

– pH 5.5 > pH 6.8 > pH 7.4

→ The decrease in the interaction between nanogel and DOX accelerated DOX release



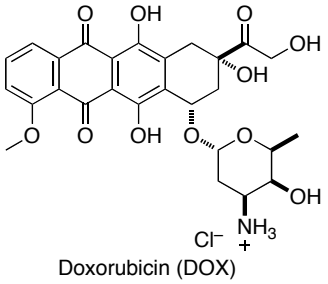


Figure 7. Release of doxorubicin (DOX) *in vitro.*

• Cell viability of DOX-loaded nanogels

– **PAMA-DMMA** shows higher cytotoxicity than free DOX at pH 6.8.

→ Enhancing cellular uptake of nanogel and release of DOX

– **PAMA-SA** shows no cytotoxicity both at pH 6.8 and at pH 7.4.

→ Nanogel itself has no cytotoxicity and low cellular uptakes

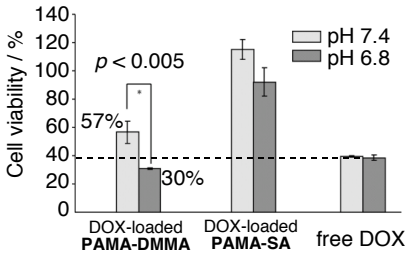


Figure 8. Cell viability of nanogel.

2–4. in vivo *analysis*

— Method —

i) Tumor was injected into mice (150 mm^3)

ii) FITC-labeled nanogels were injected (50 μL)

iii) The mice was sacrificed after 2 h

iv) The solid tumors were harvested

v) The cells and nuclei were stained

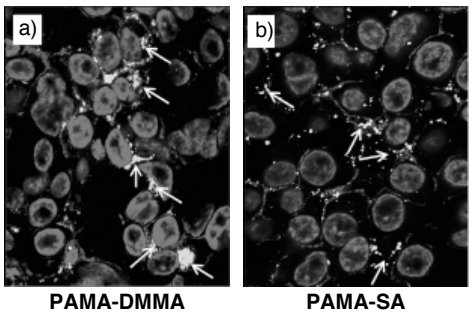


Figure 9. Merged confocal laser scatterin maicroscopy images. White arrows indicates the FITC-labeled nanogels.

• **PAMA-DMMA** located in cytoplasm (Figure 9a)

• **PAMA-SA** located in etxtracellular space (Figure 9b)

– Charge conversional nanogel **PAMA-DMMA** can be internalized more efficiently by tumor cells (slightly acidic tumor extracellular environment)

3. Conclusion

Authors have developed a pH-responsive charge-conversional nanogel for promoted tumoral-cellular uptake and DOX release. The nanogel is transformed from a negatively into positively charged form in the slightly acidic tumor extracellular environment by hydrolysis. This conversion enhanced cellular uptake of nanogels and promoted cargo release. This nanogel is capable of decreaseing protein absorption under neutral conditions.

4. References

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