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 polymerization 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.

![Scheme 1](image)

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](image)

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) $^3$H NMR
- Intensity of $\text{H}_{\text{amide}}$ ↓ and $\text{H}_{\text{amine}}$ ↑ / the intensity of the PEG mthylene hydrogen atoms ($\text{H}_{\text{PEG}}$)
→ > 60% of the amide bonds had been hydrolyzed after 30 min

2–3. in vitro analysis
a) Confocal laser scattering microscopy (Figure 4)
- $\text{PAMA}$: FITC, green (brightest contrast)
- Nuclei: DAPI, blue
- Membrane (F-actin): rhodamine phalloidin, red
- At pH 6.8, $\text{PAMA-DMMA}$ located in cytoplasm
- At pH 7.4, located adhered to the cell membrane
→ Positively charged nanogel (at pH 6.8 after hydrolysis) has higher cellular uptake than negatively charged nanogel (at pH 7.4).

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.
- Fluorescent-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.

c) Interaction of nanogel with serum 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.
- $\text{PAMA-DMMA @6.8}$: >80% BSA adsorption
- $\text{PAMA-DMMA @7.4}$ & $\text{PAMA-SA}$: almost no adsorption.
→ Positively charged nanogel interacted strongly with BSA
→ Positively charged nanogel rapidly degrade by serum components

d) Release ability of drugs and cell viability (Figure 7)
- Doxorubicin (DOX)-loaded nanogels were prepared by just mixing with DOX at pH 7.4.
- High drug loading efficiency (DLE) of negatively charged nanogel
  - 95% for $\text{PAMA-DMMA}$ (~17 mV)
  - 99% for $\text{PAMA-SA}$ (~25 mV)
  - 19% for $\text{PAMA}$ (~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

- 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

**2-4. in vivo analysis**

- Method —
  i) Tumor was injected into mice (150 mm³)
  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
- **PAMA-DMMA** located in cytoplasm (Figure 9a)
- **PAMA-SA** located in extracellular 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 decreasing protein absorption under neutral conditions.

**4. References**