

Evaluation of polymeric carriers for cell-labeling MRI contrast agent

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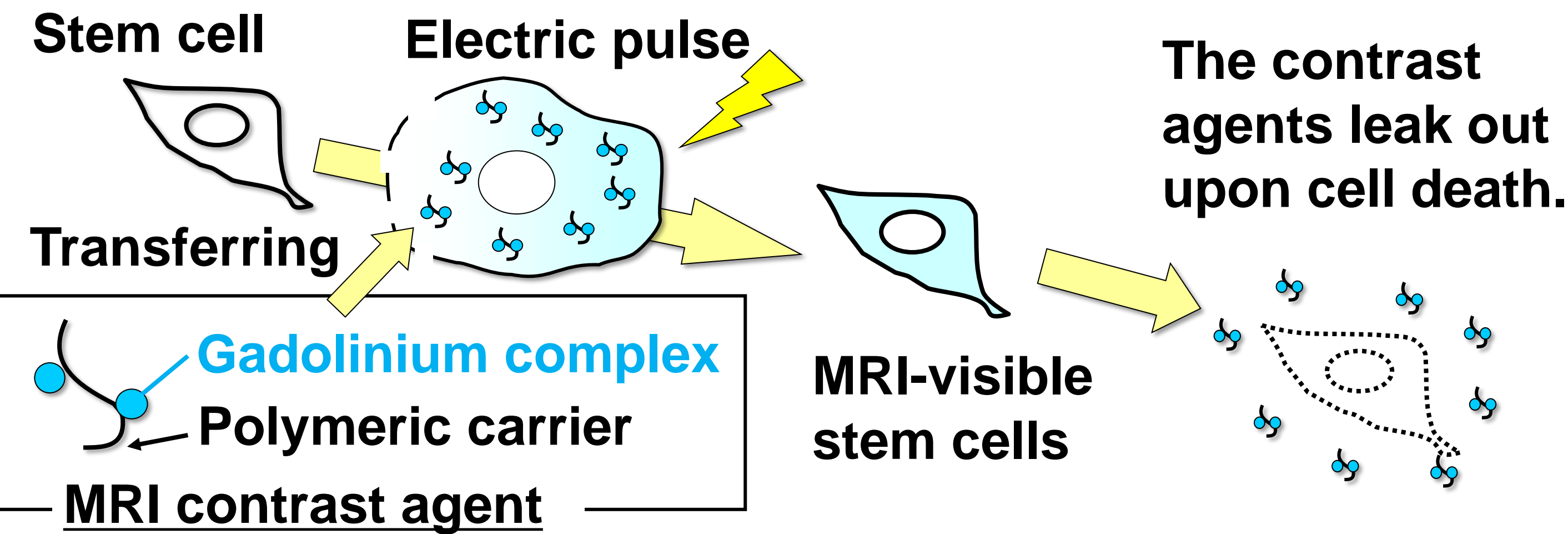
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Introduction

Stem cell transplantation is one of the major strategies in regenerative medicine. However, the efficacy, the role, even the fate of transplanted cells are still unclear. To track their distribution and survival, we have been attempting to label them with novel water-soluble polymeric MRI contrast agents.¹⁾



Required properties

- [1] High Gadolinium content
- [2] Excellent retentivity
- [3] Quick diffusion in the tissue
- [4] Urinary excretion

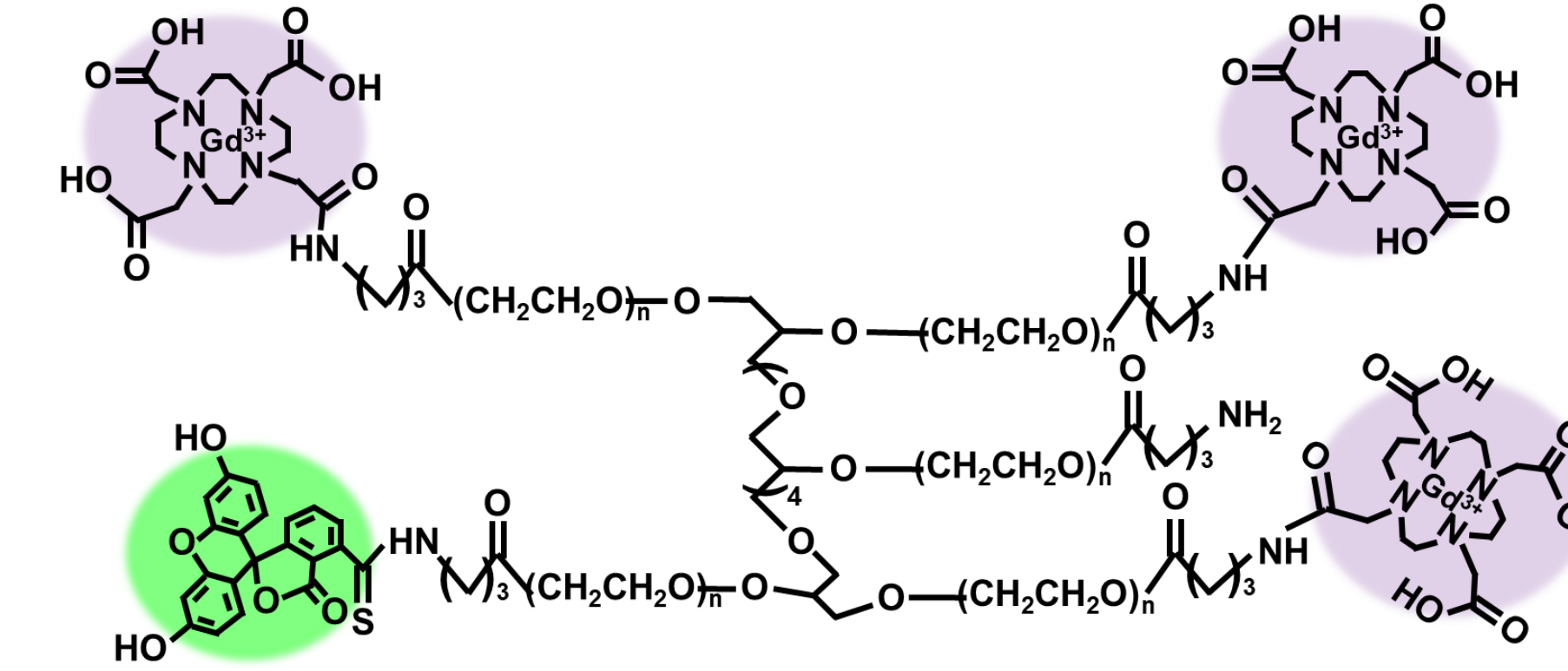
➤ Long-term live cell tracking

Table 1. Previously reported carriers

Carrier	Challenge
PVA	No clinical usage in the blood
Dextran	Slight liver accumulation
PEG	Limitation of Gd content

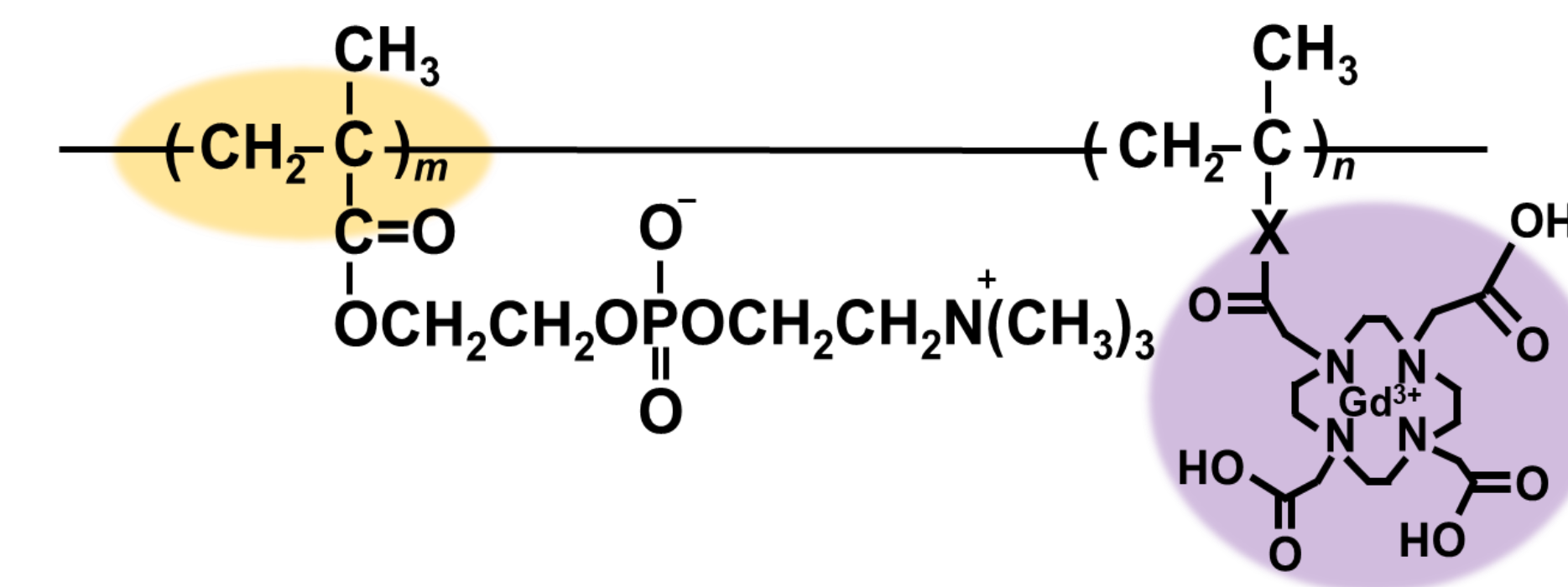
Water-soluble polymeric MRI contrast agent

• **8-arm PEG-FGd₃**, Mw ≈ 17 kDa
(Eight-arm polyethylene glycol conjugated with a Gd chelate and fluorescein)



- Larger Gd content than linear PEG.
- Very low uptake by organs/cells.

• **PMPC-Gd**
(PMPC conjugated with a Gd chelate)

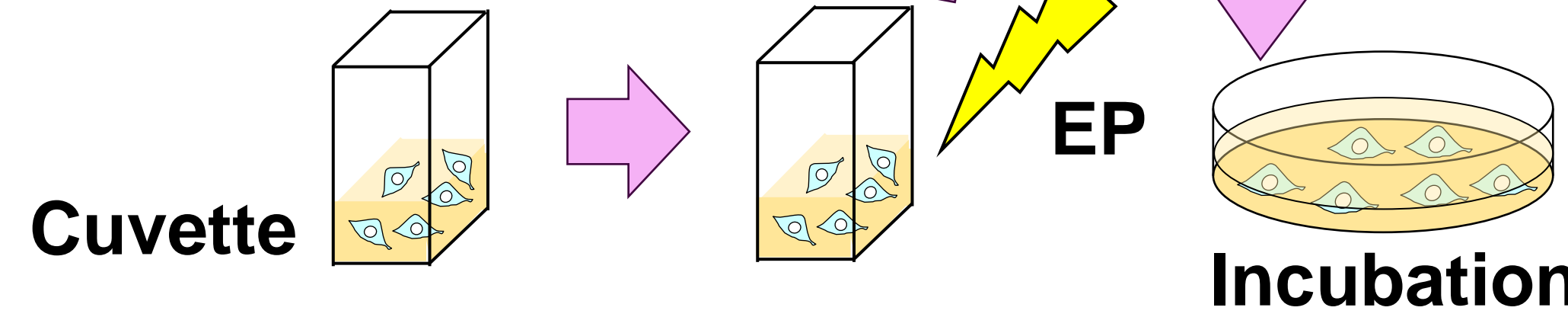


- Larger Gd content by copolymerization.
- Can be used clinically in the blood.

Result & Discussion

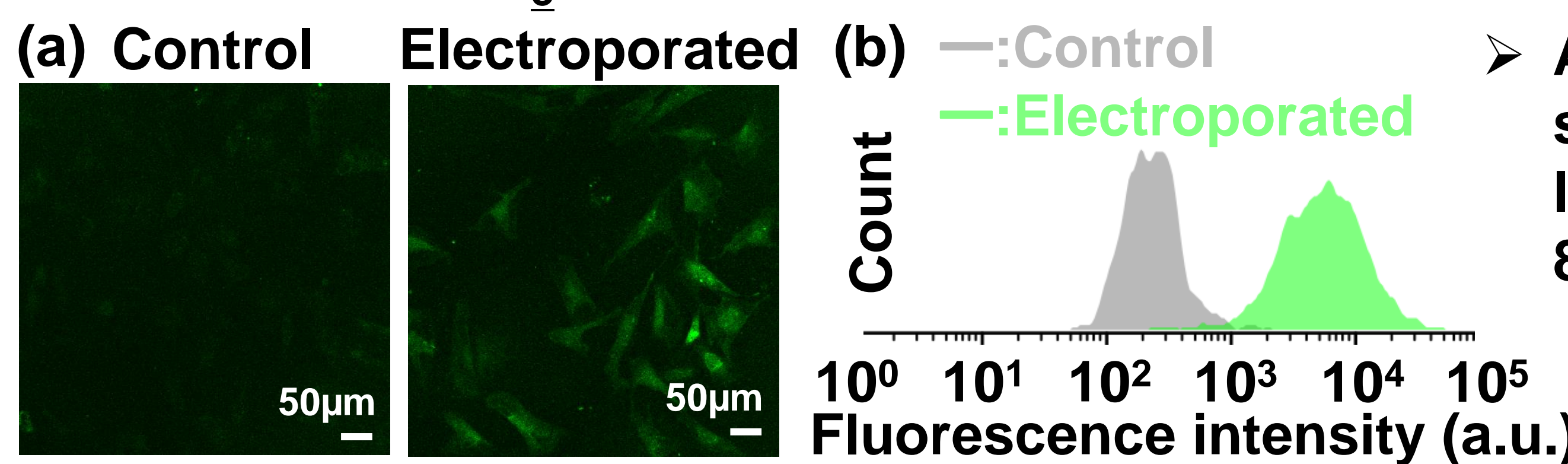
Intracellular delivery by electroporation (EP)

Ad-MSCs suspension in 8-arm PEG-FGd₃ solution



- CLSM
- Flowcytometry analysis
- Fluorescence spectrum of cell lysate
- Cell growth test

~8-arm PEG-FGd₃~



➤ All cells were successfully labeled with 8-arm PEG-FGd₃.

Intracellular retentivity (*in vitro*)

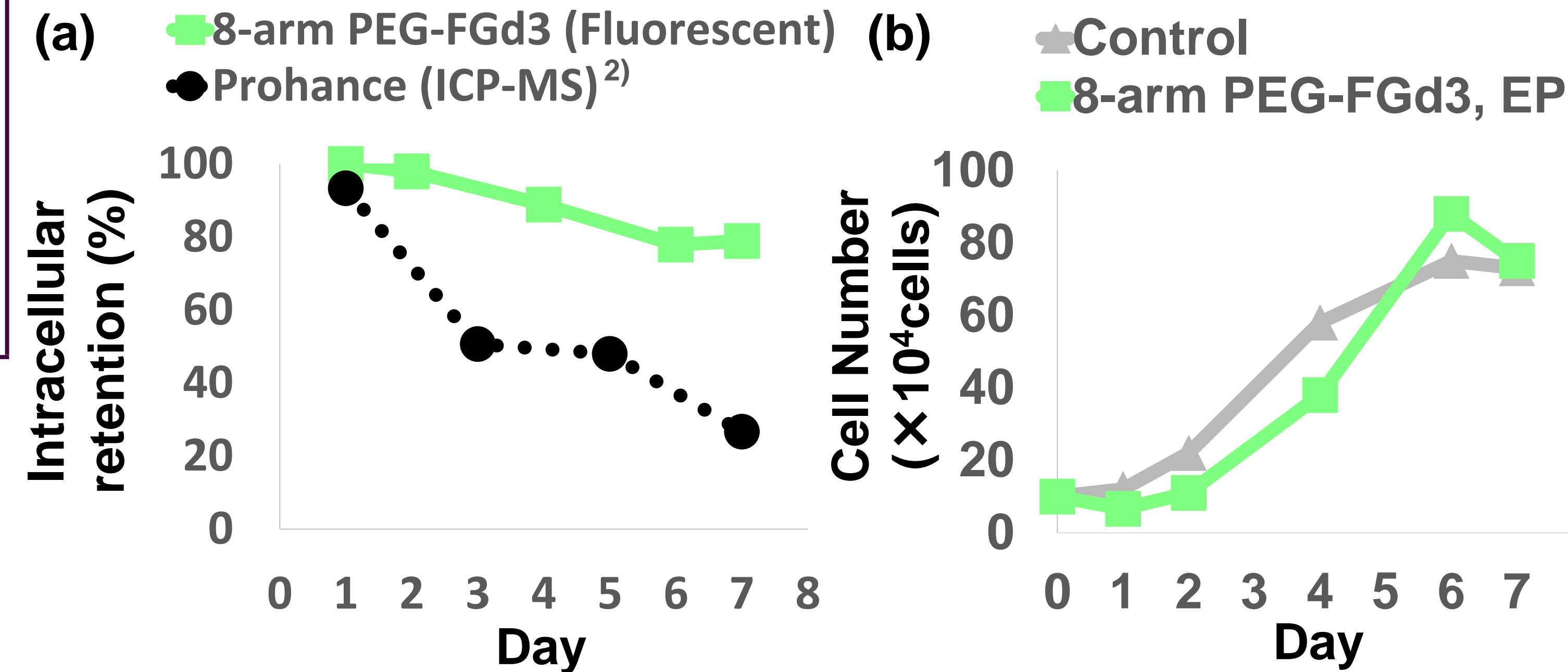


Figure 2. (a) The intracellular retention of 8-arm PEG-FGd₃ and Prohance²⁾ relative to that on at one day post-labeling. (b) growth of labeled and unlabeled cells.

- 8-arm PEG-FGd₃ was retained even after 7 days (a).
- 8-arm PEG-FGd₃ does not adversely affect cell proliferation (b).

Pharmacokinetics (*in vivo*, MRI)

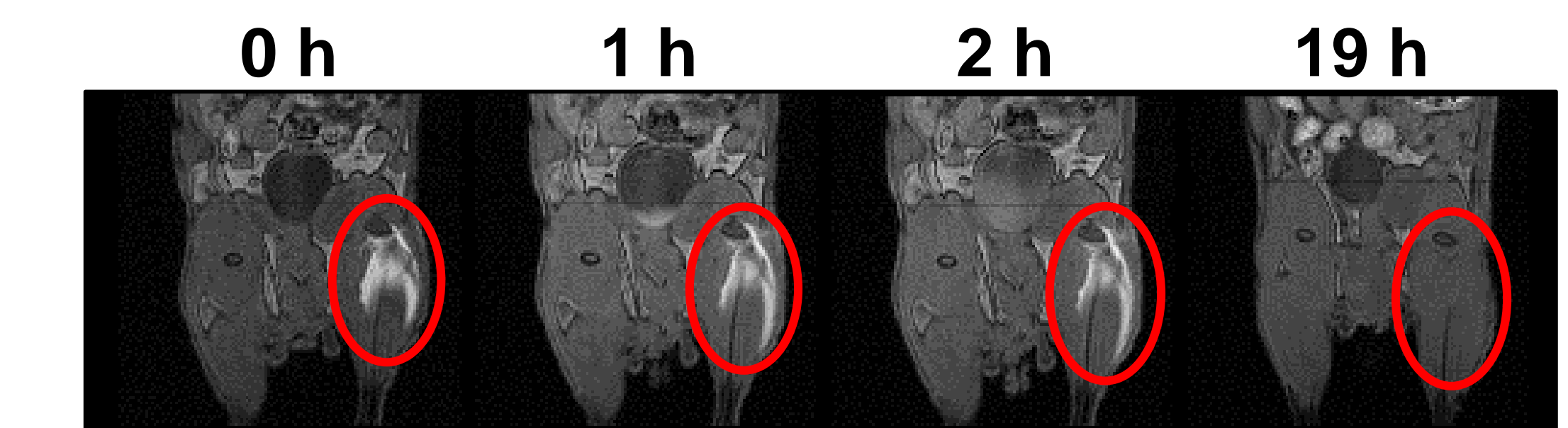
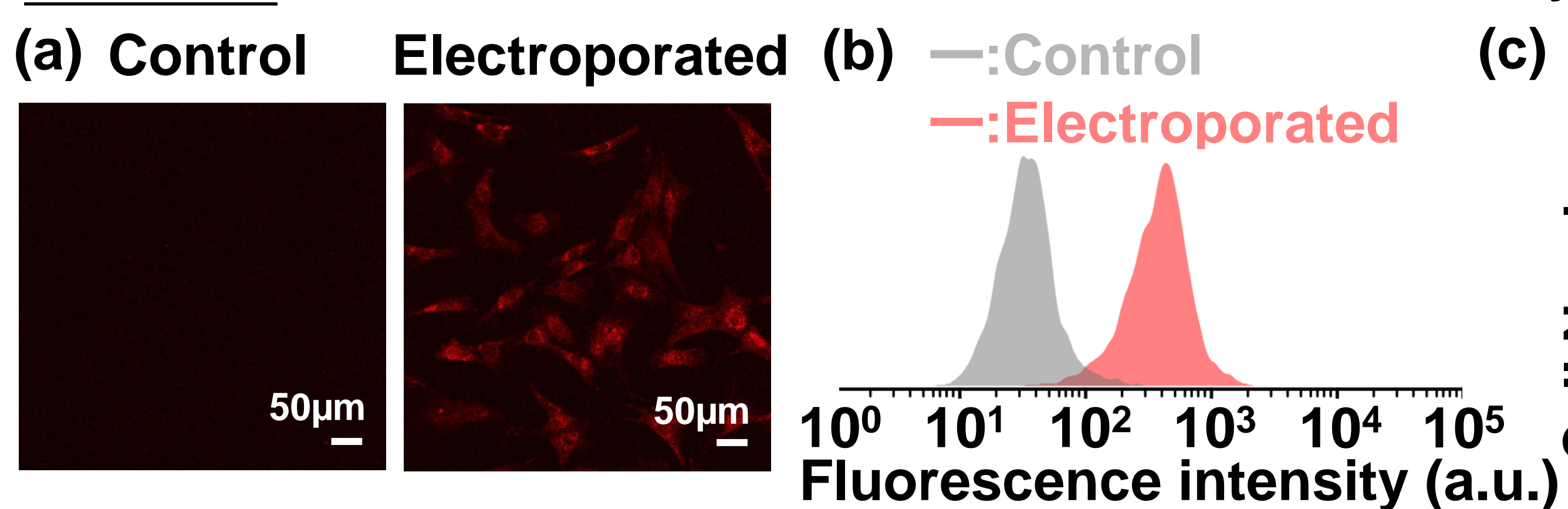


Figure 3. MR image (1.5T) of mouse injected with 8-arm PEG-FGd₃ solution (75 nmole Gd/ head) to intra femoral muscle.

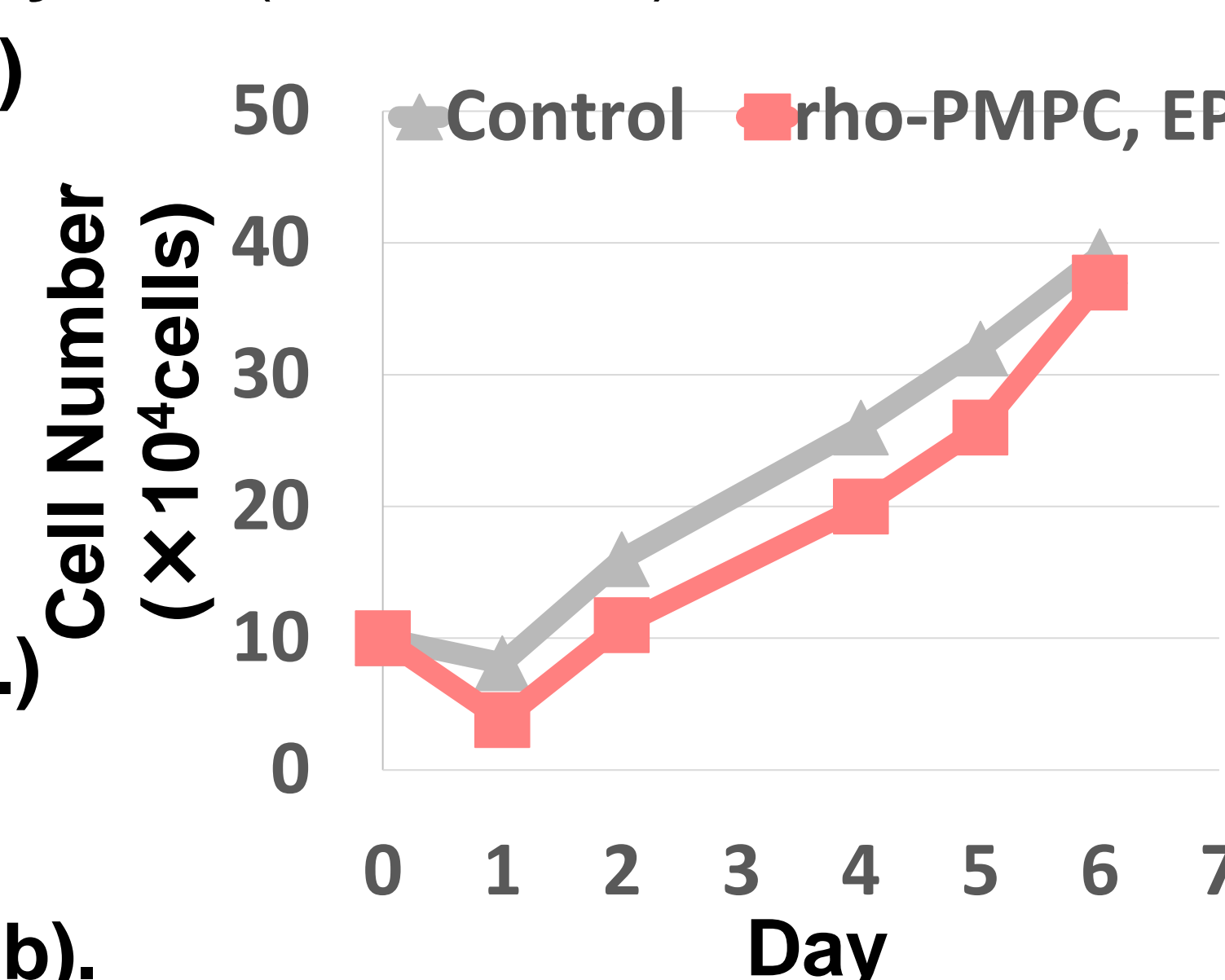
- 8-arm PEG-FGd₃ gradually spread in the muscle, moved to the bladder, and finally excreted within 19 hours.

➤ 8-arm PEG-FGd₃ enables us to visualize the living cell distribution with information on cell survival.

~PMPC~ PMPC was labeled with rhodamine B methacrylate. (rho-PMPC)

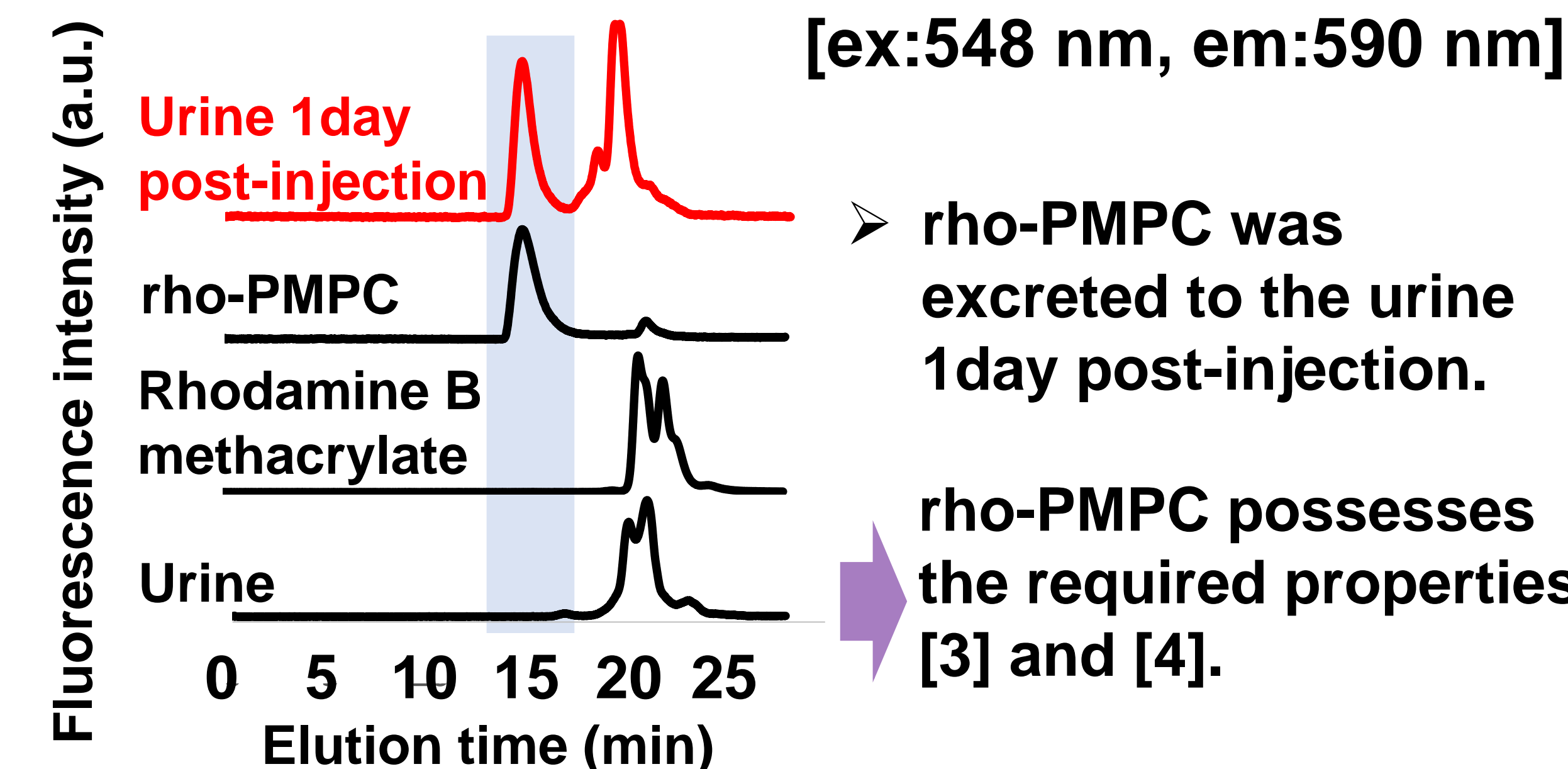


(c)



- All cells were successfully labeled with rho-PMPC (a, b).
- rho-PMPC does not adversely affect cell proliferation (c).

Urinary excretion (*in vivo*, GPC)



➤ rho-PMPC was excreted to the urine 1 day post-injection.

➤ rho-PMPC possesses the required properties [3] and [4].

Figure 5. Elution profile of urine of mouse injected with rho-PMPC (Mw = 88 kDa) solution (0.11 μmole / head) to subcutaneous. (GPC eluent: Methanol:H₂O=7:3, 10mM LiBr+H₂O)

Conclusion

8-arm PEG-FGd₃

- High Gd contrast
- Excellent retentivity
- Quick diffusion in the tissue
- Urinary excretion

PMPC

- Quick diffusion in the tissue
- Urinary excretion

➤ 8-arm PEG-FGd₃, PMPC is expected to be highly potent as MRI contrast agent in tracking transplanted stem cells.

References : (1) C. A. Agudelo, et al., *Biomaterials*. 2012, 33, 2439-2448.
(2) Y. Tachibana, et al., *Bioconjugate Chem.* 2014, 25, 1243-1251.