

P-64

Fibrous formation observed at the implantation site of undifferentiated iPS cells in NOG mice

NOGマウスにおける未分化iPS細胞の移植部位(背部皮下)に認められた線維形成

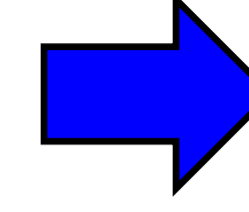
Takafumi Oshikata (押方孝文)¹, Masashi Iida (飯田真志)², Koshiro Katoku (可徳小四郎)¹, Takeshi Kanno (菅野剛)¹

1: LSIM Safety Institute Corporation Pathology Department (LSIM安全科学研究所 病理研究部)

2: LSIM Safety Institute Corporation Safety Assessment Department (LSIM安全科学研究所 安全性研究部)

Background

- NOG mice were subcutaneously implanted with iPS cells and HeLa cells into the dorsal tissue for evaluation of the minimum tumor-producing dose and tumorigenic period.
- Tumor formation was observed in the group implanted with HeLa cells. On the other hand, no tumor formation but fibrous formation was observed in the group implanted with iPS cells.



We examined histopathologically whether this fibrous formation was derived from iPS cells.

Method

Study outline

- Animal: Mouse, NOD/Shi-seid, IL-2RγKO Jic (NOG)
- Implantation site: Dorsal subcutaneous tissue
- Inoculation of cell cultures:
 - Human adipose-derived mesenchymal stem cells (hMSC), Lot No. 0000672320, Lonza KK
 - HeLa cells, Lot No. 61647128, ATCC
 - Human undifferentiated iPS cells (iPS cells), Lot No. P11025, Takara Bio Inc.
- Age at implantation: 7 weeks
- Collection and examination: All organs (including implantation site)
- Tissue fixation: 10 vol% phosphate buffered formalin solution
- Stain:
 - All organs
Hematoxylin and eosin stain (HE)
 - Implantation site in the test article 2 groups
Masson trichrome stain (MT), Anti-human lamin stain (anti-lamin)

Group design

Group	Inoculation of cell cultures	Dose level (cells/body)	Concentration (cells/mL)	Dose volume (μL/body)	Sex	Number of animal
Control	hMSC	1 × 10 ⁶	1 × 10 ⁷	100	Male	7
Test article 1	HeLa cells	1	10			7
		10	100			7
		100	1000			7
Test article 2	iPS cells	1	10			7
		10	100			7
		100	1000	7		

Observation period

Day 1	Day 113	Day 141
Implantation Date	Collection ¹	Collection ²
1: Test article 1 groups		
2: Test article 2 groups		
(Since no tumor formation was observed, the observation period was extended beyond day 113.)		

We report mainly on the Implantation site of Test article 2 groups.

Result

Macroscopic Examination

Group	Control	Test article 1			Test article 2		
Inoculation of cell cultures	hMSC	HeLa cells			iPS cells		
Dose level (cells/body)	1 × 10 ⁶	1	10	100	1	10	100
Number of animal	7	7	7	7	7	7	7
Mass, subcutaneous	0	0	1	7	0	0	0
Nodule, subcutaneous	0	3	5	0	0	0	3

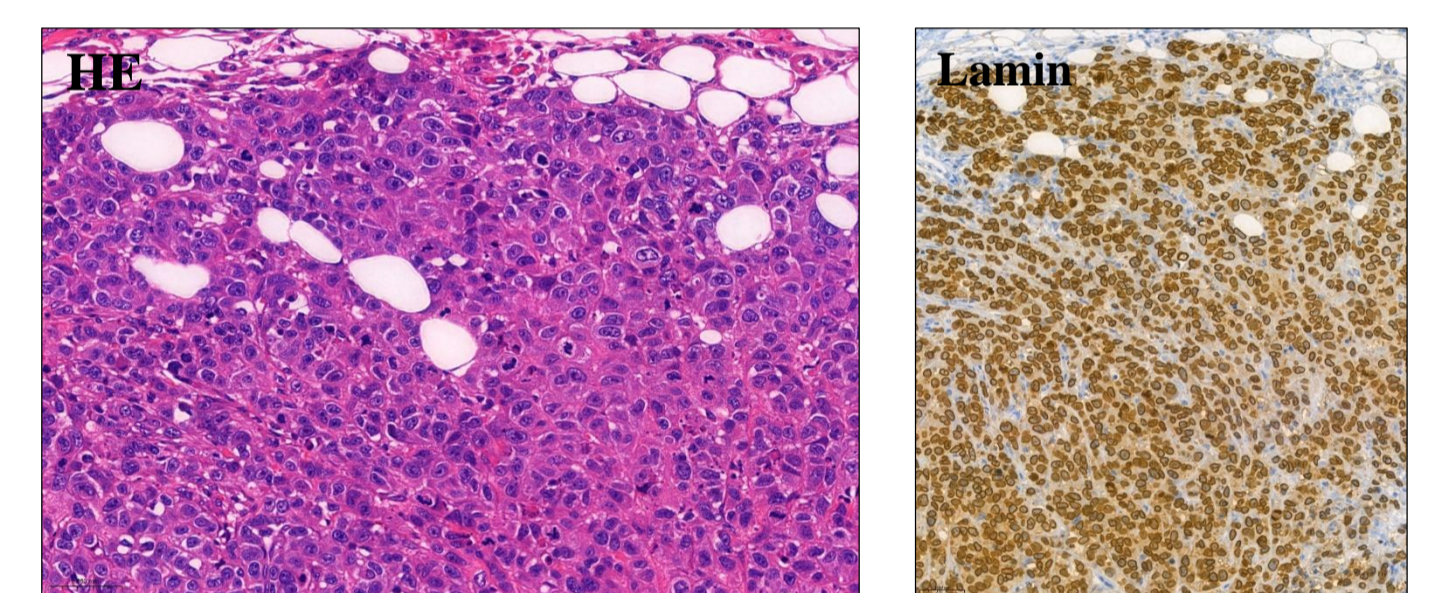
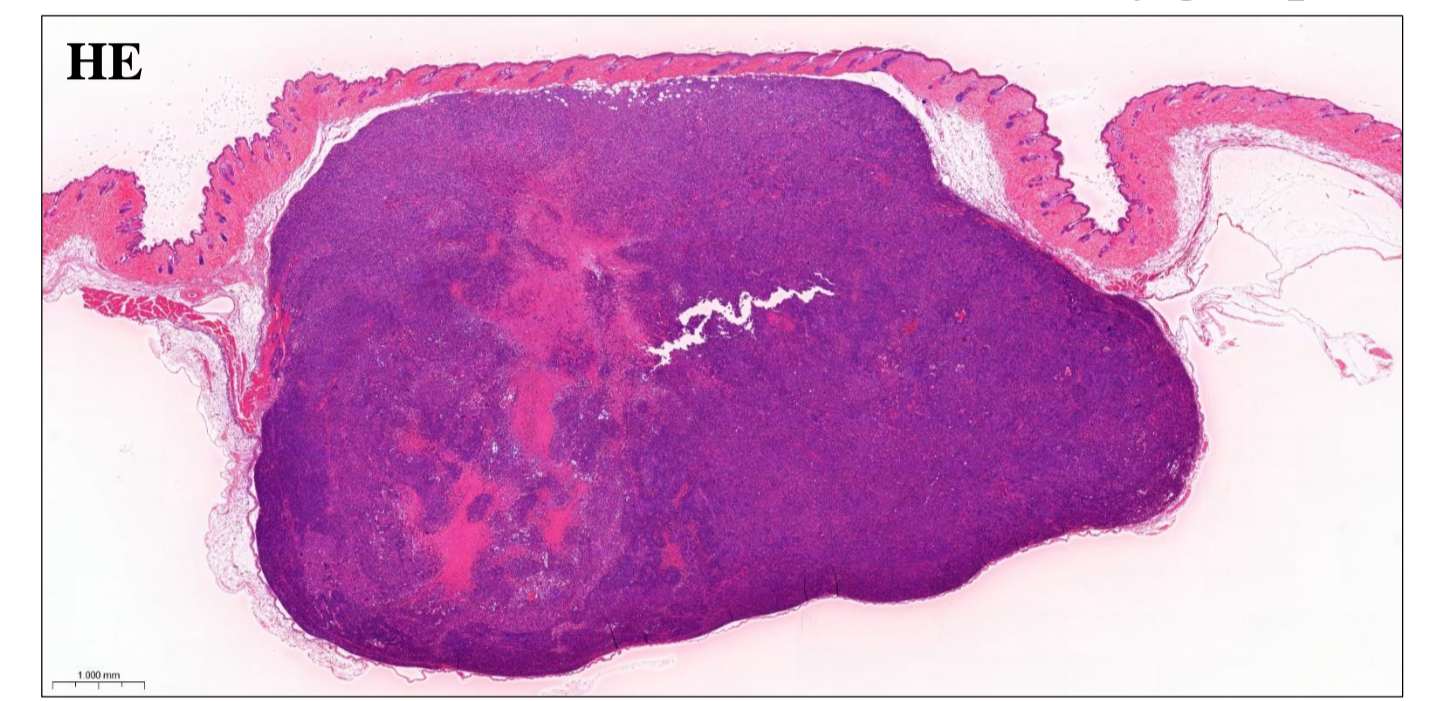
Microscopic Examination (HE stain)

Group	Control	Test article 1			Test article 2		
Inoculation of cell cultures	hMSC	HeLa cells			iPS cells		
Dose level (cells/body)	1 × 10 ⁶	1	10	100	1	10	100
Number of animal	7	7	7	7	7	7	7
Tumor formation, subcutis	0	3	6	7	0	0	0
Fibrous formation, subcutis	0	0	0	0	1	2	4

※: Findings of other organs are not shown.

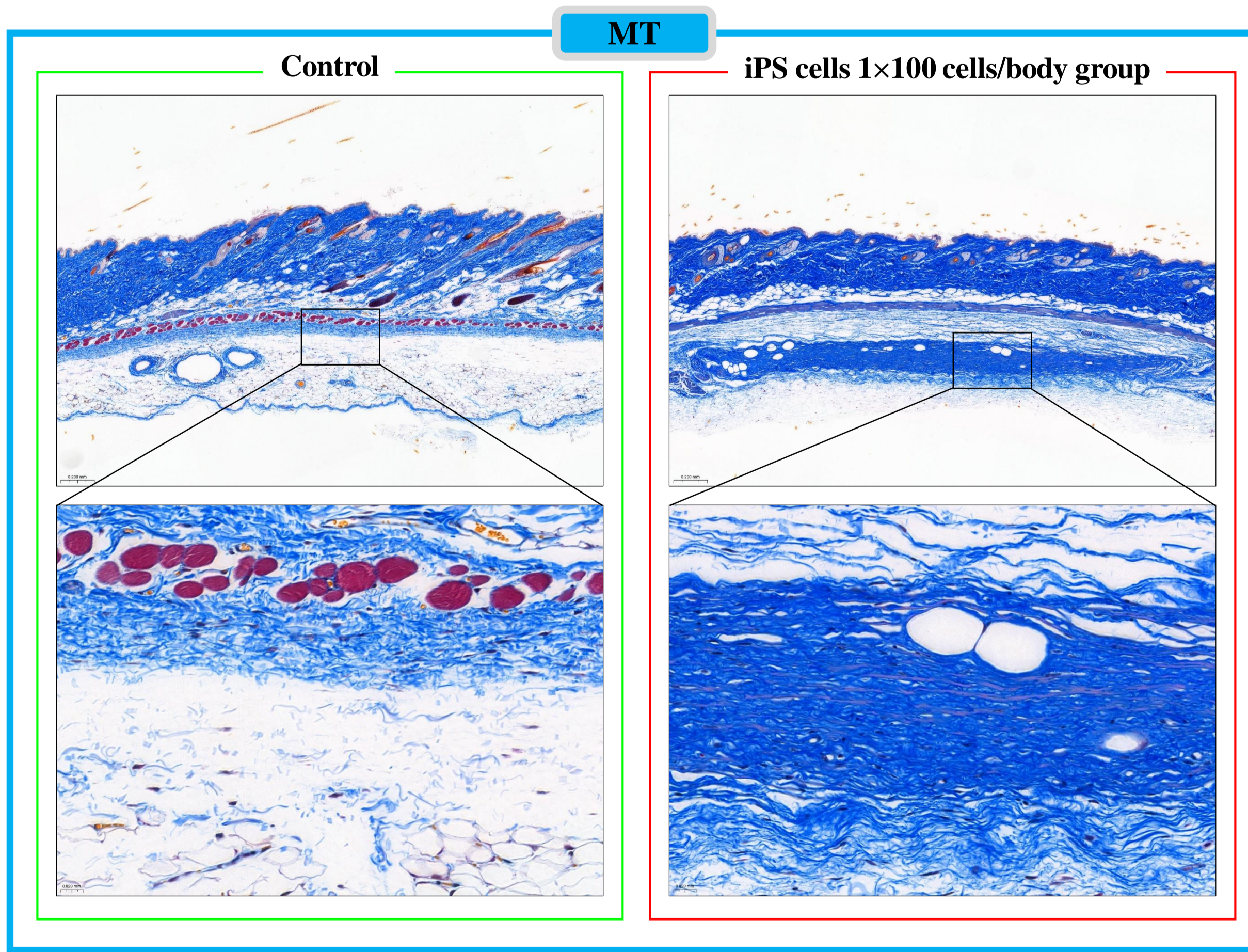
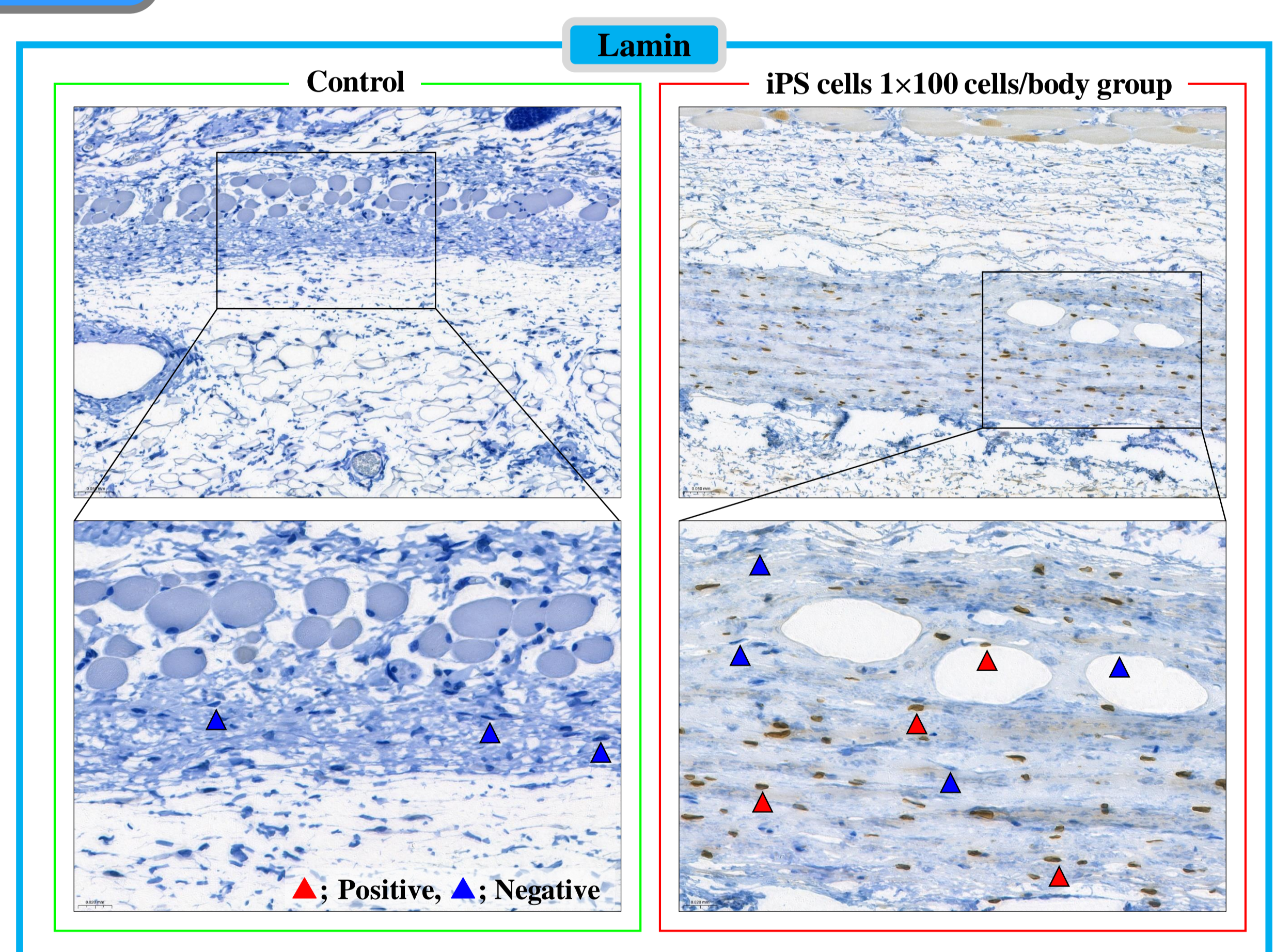
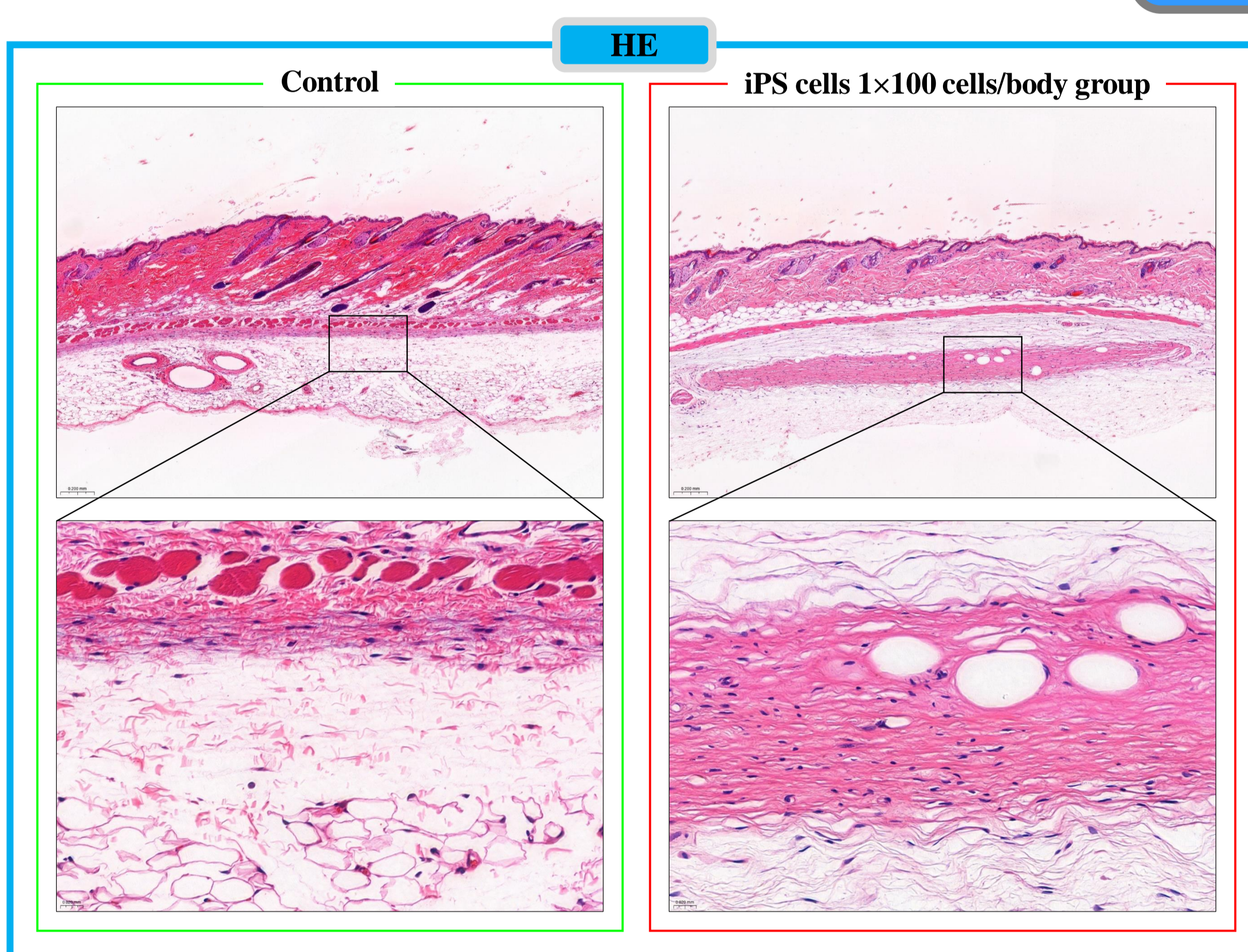
Test article 1

Tumor formation (HeLa cells 1 × 100 cells/body group)



※ The tumor cells were derived from HeLa cells, as they were positive for anti-lamin staining.

Test article 2



Summary

- Necropsy revealed white nodules at the implantation site in the 100 cells/body group.
- Histopathological examination revealed fibrous formation at the implantation site in all groups.
- The fibers consisted of mature collagen fibers with spindle-shaped nuclei, which stained blue by MT.
- Adipocytes were observed interspersed among collagen fibers.
- Anti-lamin staining showed positive in nuclei of collagen fibers and adipocytes, but negative in some nuclei.

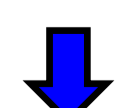
COI Disclosure Information
Takafumi Oshikata

In connection with this presentation, there is no COI to be disclosed with any companies.

本演題に関連して開示すべき利益相反はありません。

Discussion

- Anti-lamin staining showed positive in nuclei of collagen fibers and adipocytes, but negative in some nuclei, suggesting that fibrous formation (with adipocytes) was formed from cells derived from mice and humans.



- These results suggest that when iPS cells are implanted into immunodeficient animals, iPS cells differentiate into mature tissues and grow, and those iPS cells form into mature tissues by engulfing the host-derived cells other than iPS cells.

Image of the possible origin of the fibrous formation

