日本放射線影響学会　第64回大会
OS-6-4　放射線応答

Effects of X-ray (electron beam) irradiation at low-dose regions showing different cell viability between frozen and thawed states

細胞培養液、細胞入り容器へのX線照射の影響

平野智子○[1] 、坂井一郎[1] 、塚原正義[2]

1：日立造船株式会社
2：公益財団法人京都大学iPS細胞研究財団

COI開示

演題発表内容に関連し、発表者らが開示すべき
COIに関連する企業等はありません。

In this publication, regarding the effects of X-ray irradiation,
We would be happy to hear the opinions of experts.

Flow of this reports

- Research Background and Purpose
  View of a regenerative medicine site.
  Introduction to Low-Energy Electron Beam Irradiator
- X-ray irradiation test on the culture medium
- X-irradiation test on cells
- Summary

Site view of regenerative medicine.

View of a regenerative medicine site.
Working with isolators provided by CiRAF

Because regenerative medicine products handle living cells and cannot be terminally sterilized,
It is important not to bring bacteria from materials and reagent containers used in the manufacturing process!

In regenerative medicine, aseptic management is one of the most important points.

Like dispensed cultures or frozen vials,
There are materials that are difficult to remove by means other than wiping with disinfectants such as ethanol

Therefore, we focused on the decontamination of the material surface by low-energy electron beam irradiation.

However, there is a concern about the influence of X-rays which are generated secondarily during electron beam irradiation

Therefore, in cooperation with the Institute of iPS-Cell Research (CiRAF), Kyoto University,
The effects of X-ray irradiation on cell medium solutions and cells were investigated.

Electron beam surface decontamination equipment for medium bottles installed in CiRAF

Low energy electron beam irradiator Features of Low Energy Electron Beam Irradiators

・Be used domestically and abroad to sterilize bottles for beverages
Less irradiated odor and coloration

・Voltage is low and no electron beam penetrates into the substrate
Setting Maximum Voltage 125keV,
Transmission Power 20 to 100μm

・Can be sterilized (the surface layer of the substrate) as in the case of radiation

・Can be used only by submitting a notification to the Labor Standards Inspection Office.

Principle of electron and X-ray generation

Filaments in vacuo (tungsten) heated

↓

Thermal electrons are generated

e- e- e-

Applying high voltage

↓↓

Thermal electrons accelerate and electron flow is generated

e-

e-

e-

Electron flow is metal foil (aluminum, titanium) through

Electron and X-rays are emitted into the atmosphere.

Reference

Figure 10 EB,Gammma Ray and X-ray Penetration
Industrial Radiation Processing With Electron Beams and X-rays,
JAEA International AtomicEnergy Agency, 1 May 2011 – Revision 6, pp. 21-22.

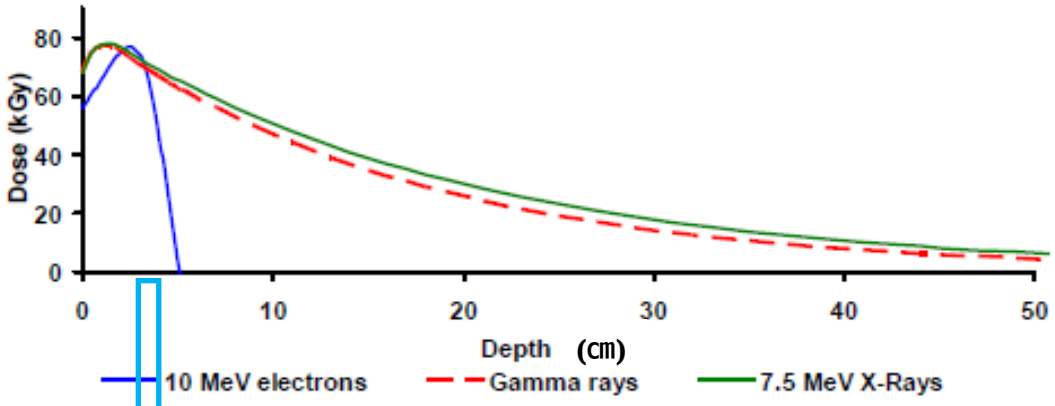
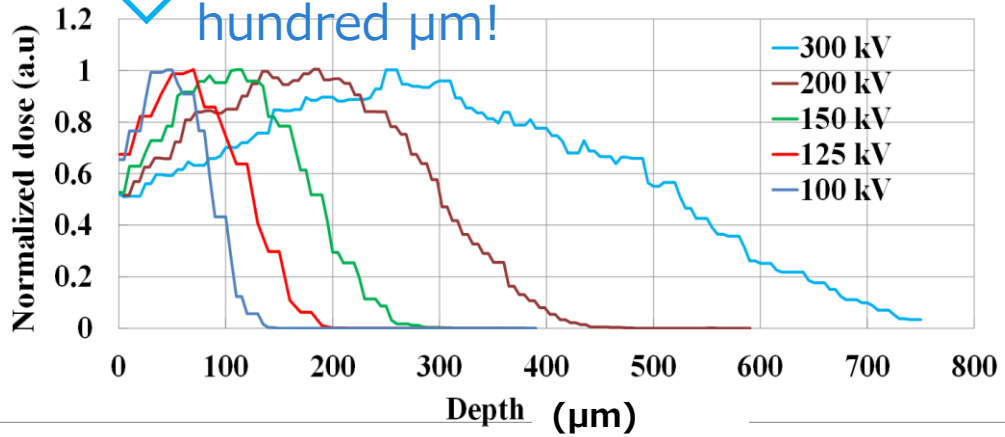


Figure 10. EB, Gamma Ray and X-ray Penetration

The penetration depth of the low-energy electron beam is several hundred μm!



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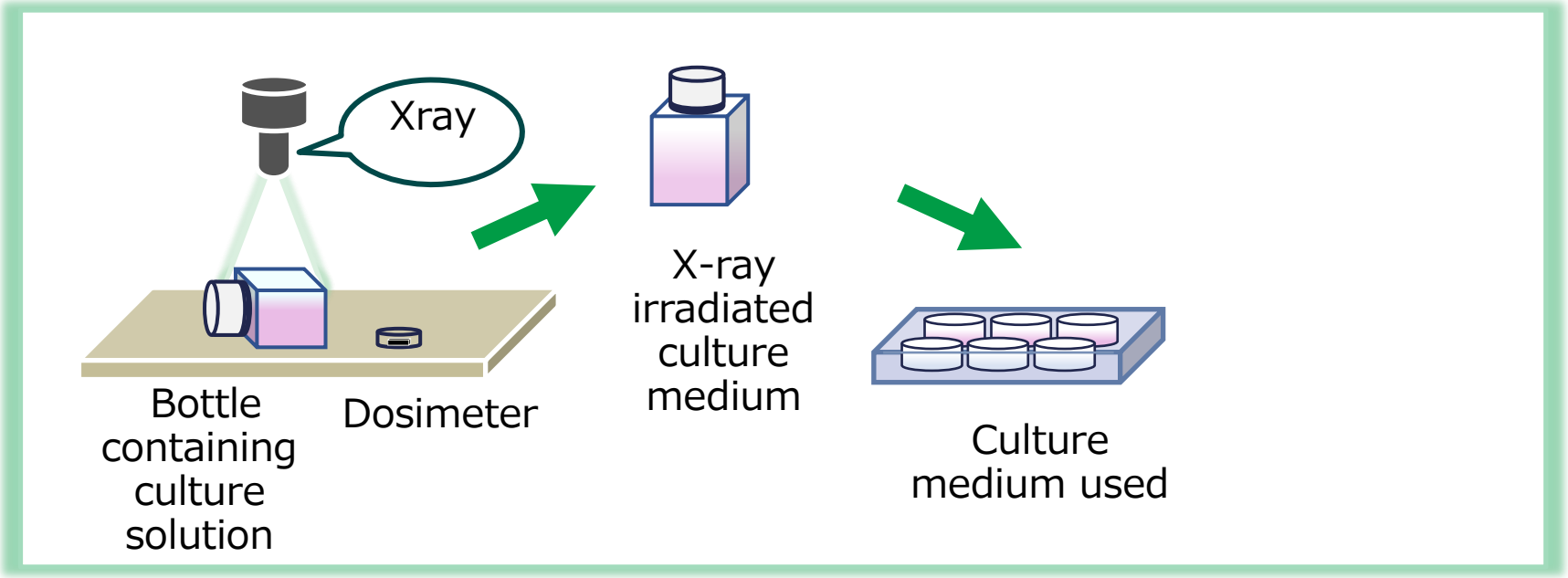
Test method for irradiation of X-rays to culture medium

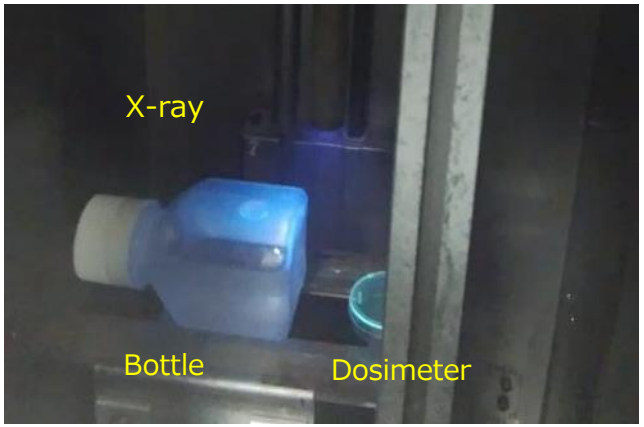


Culture medium used

・ StemFit: 50 mL was dispensed into a 125mL flask

Test Methods

・X-ray irradiated culture medium was used for culturing iPS cells,
The growth of the cells was observed.



Test Methods

Photograph of the chip and the body

Nagase Landaua Corporation

Body : microSTARii

Dosimeter : nanodot (Small OSL Dosimeter)

X-ray irradiation results to the culture medium

Table X-ray dose

Sample	X-ray dose (Gy)
0Gy(No transport)	0
0Gy	0
3Gy	3.1
13Gy	13.7

※Gy(gray) =1Sv (sievert)
Absorbed dose (J/kg)

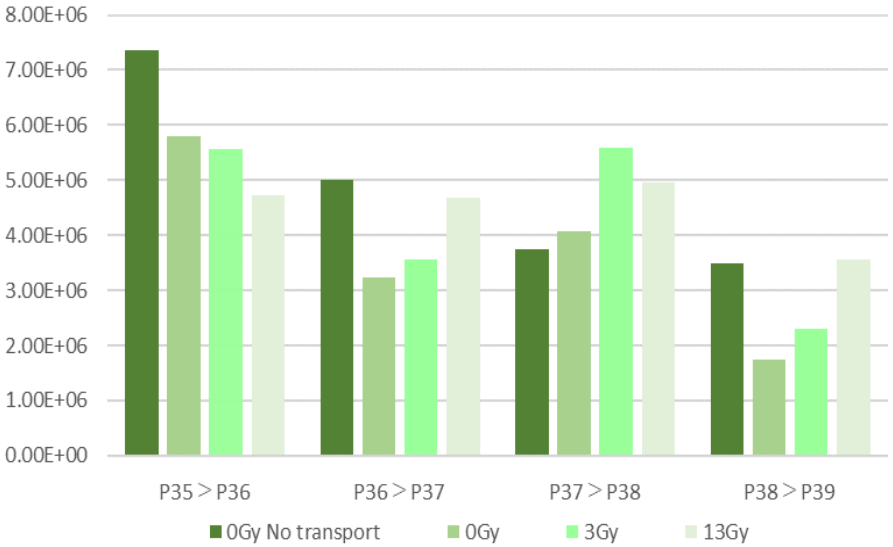
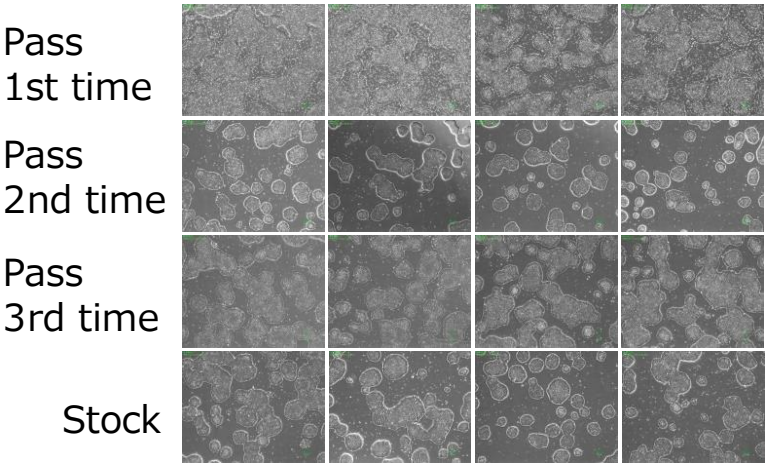


Figure X-ray dose and number of live cells.



Microscopy of photographic cells.

Table cell culture days

days	medium exchange
0	irradiated
1	P35 start using
5	Pass 1st time
6	P36
12	Pass 2nd time
13	P37
19	Pass 3rd time
20	P38, stock

- No growth or morphology defects other than poor growth due to overgrowth were observed.

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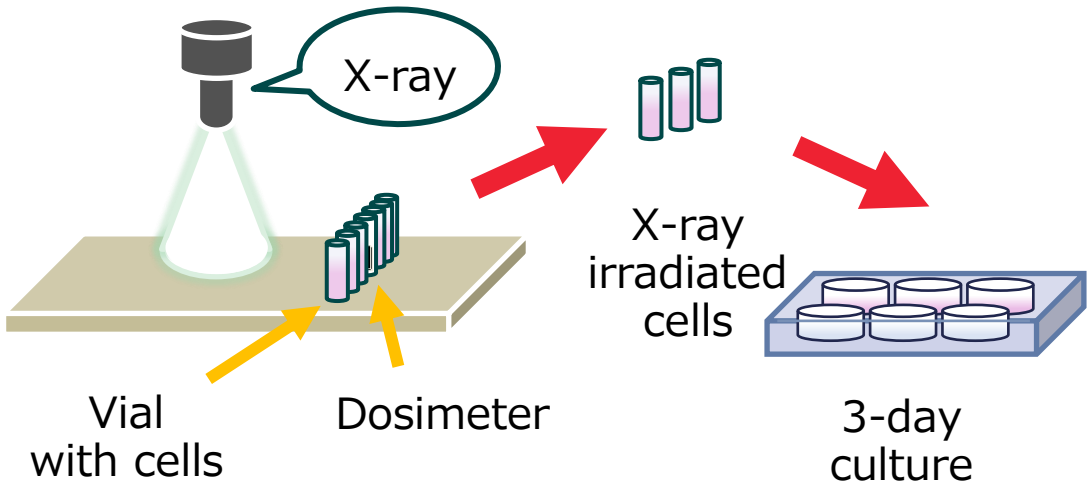
Effect of X-ray irradiation on cells.

Effect of X-ray irradiation on cells.

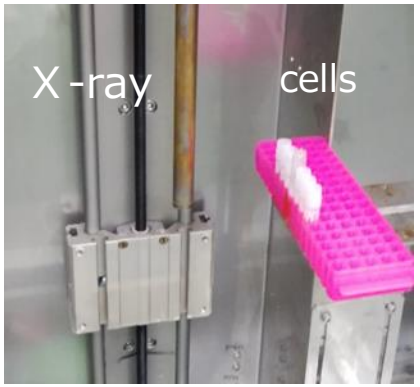


Cells used

①CHO cells: k1 strain (wild strain) N1
②CHO cells: xrs6 line (Ku86 deficient strain) X-ray sensitive N3
③iPS cells: 201B7 (human iPS cells) N3

Test items

・Confirmation of cell proliferation rate



Test Methods

Cell viability after X-ray irradiation.

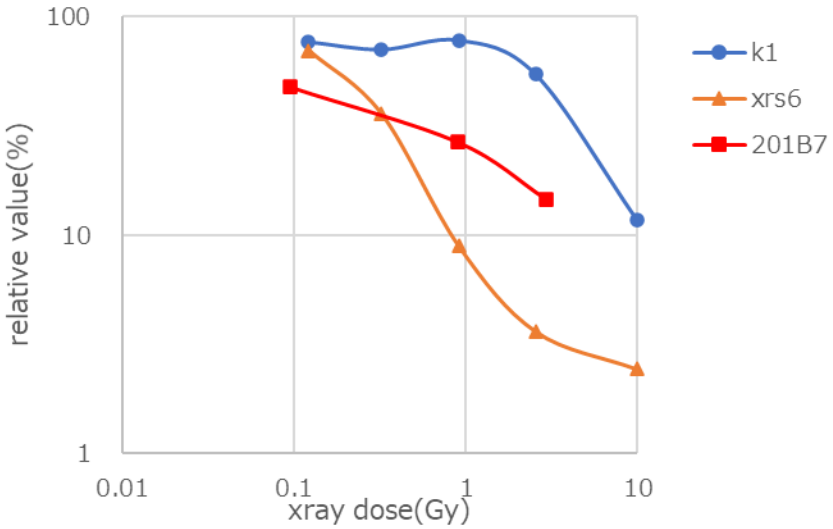
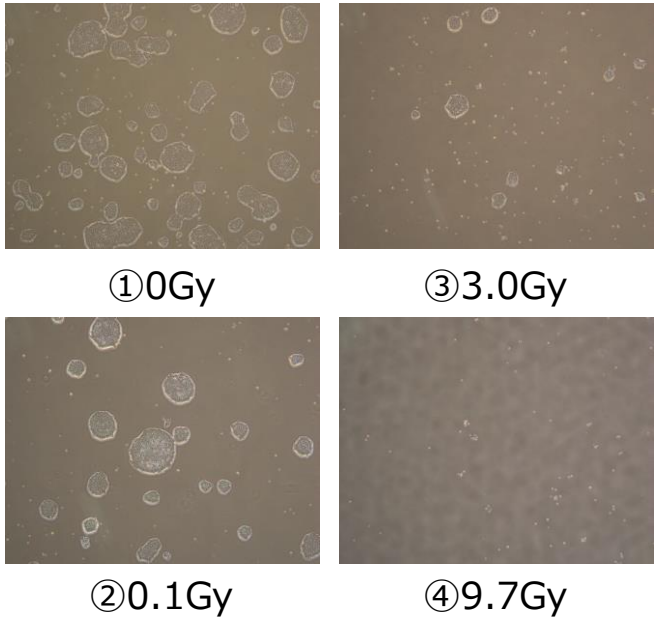


Figure X-ray dose and live cells.



Photographs
Microscopy of iPS cells.

- The iPS cells had zero live cells when irradiated with about 10Gy.

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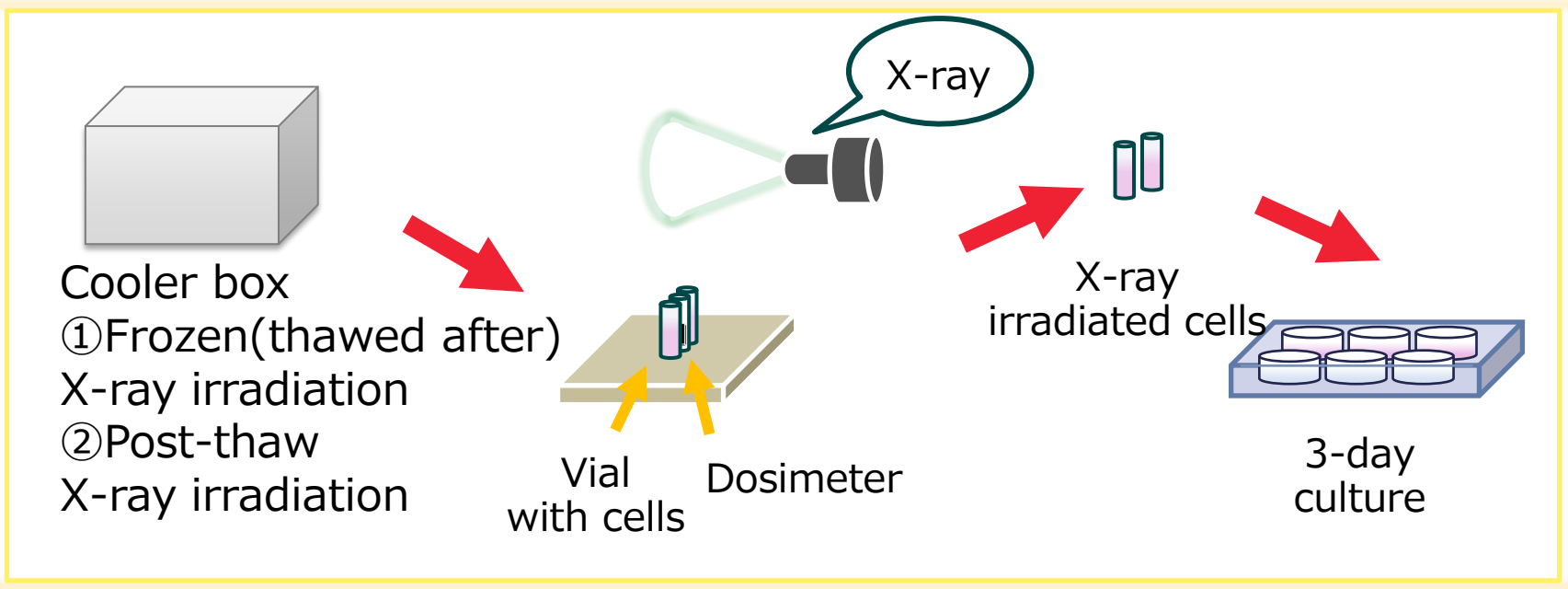
Effect of X-ray irradiation on cells in the frozen state.

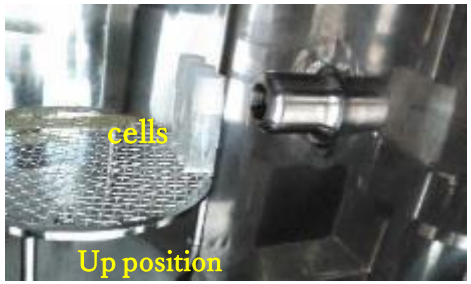


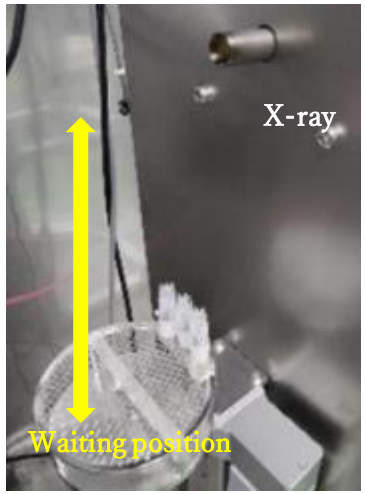
Cells used

①Frozen iPS cells: 201B7
(human iPS cells)
②Thawed iPS cells: 201B7

Test items

・Confirmation of cell proliferation rate





Method of irradiation

X-ray irradiation results on frozen and thawed cells.

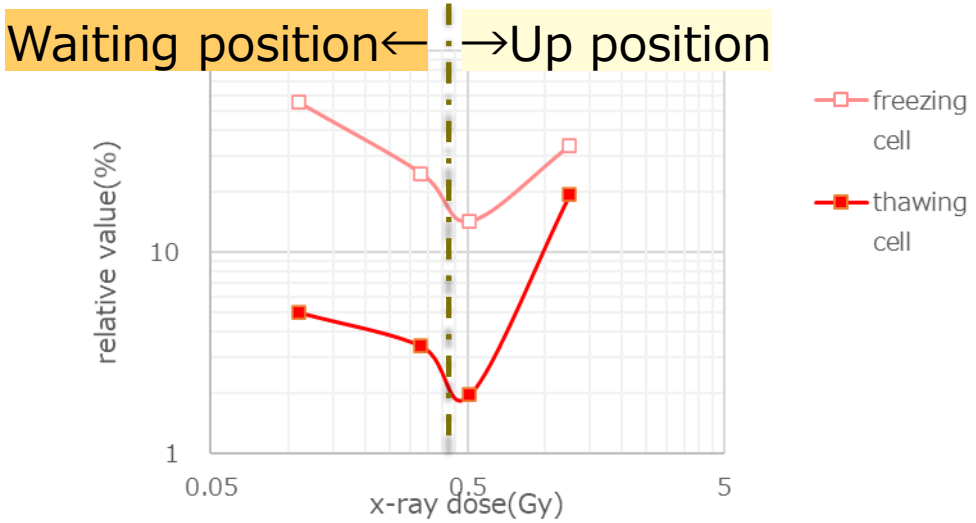


Figure X-ray dose and live cells.

Table X-ray irradiation conditions

X-ray dose (Gy)	electric cirrent (mA)	irradiation time (s)	position	time to thaw (frozen cell) (s)
0	0	0	-	60
0.11	0.5	70	Waiting	318
0.33	1.0	106	Waiting	335
0.51	0.1	7	up	231
1.25	0.1	12	up	292

- Cells in the frozen state are more live cells after X-ray irradiation than those in the thawed state
- There is a phenomenon in which the number of living cells increases in spite of an increase in the irradiation dose

Summary

1. Culture broth after X-ray irradiation is used for cell culture.
   →There were no defects in growth and morphology other than over growth
   Is the protein in the culture actually affected by X-rays?

2. X-ray irradiation to various cells.
   →Compared with CHO cells, iPS cells tend to have a faster reduction in proliferation rate
   Are there cells susceptible to X-ray irradiation?
   Is there a guide or standard for determining the threshold value of X-ray irradiation?

3. X-ray irradiation to iPS cells in the frozen state.
   →The reduction in cell proliferation rate was dramatically improved compared with that at thawing.
   In addition, there is a tendency that the cell proliferation rate increases
   Is it less susceptible to X-ray irradiation in the frozen state?
   Is there an X-ray dose that activates the cells?

We would like to hear your opinions on the above.

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