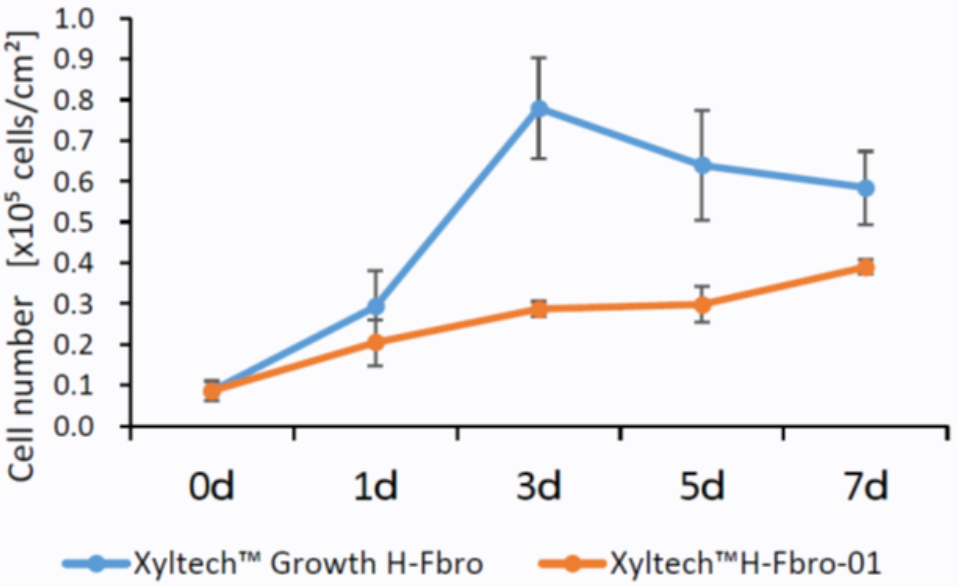
Application Note #1

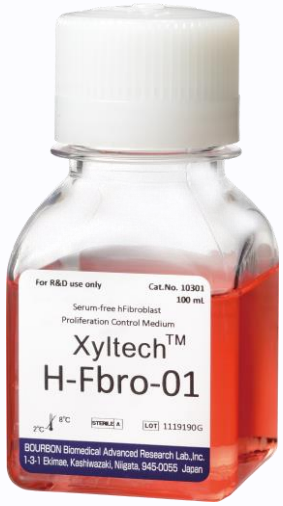
Live cell transport with Xyltech™ H-Fbro-01

June, 2023

Bourbon Biomedical Advanced Research Laboratories, Inc.

Product concept





Xyltech™ H-Fbro-01

Xyltech™ Growth H-Fbro

Figure 1. Changes in the number of human fibroblasts
The cell number of human fibroblasts cultured using Xyltech ™ Growth H-Fbro (for cell proliferation, blue) and Xyltech ™ H-Fbro-01 (for cell proliferation suppression, orange) was determined. (mean ± SD, n=3)

Proliferation of human fibroblasts cultured in Xyltech ™ Growth H-Fbro (growth medium) can be suppressed by using Xyltech ™ H-Fbro-01 medium while maintaining the cell properties.

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-Materials & Methods-

Cells：Normal Human Dermal Fibroblasts（NHDF, Lonza）
Medium: Xyltech™ Growth H-Fbro* (Growth medium, BBARL)
Xyltech™ H-Fbro-01*(Proliferation control medium, BBARL)
*Used with 1% Artificial Serum（Animal-free, CSTI）

For live cell transportation condition

1. NHDFs were seeded at a density of 0.5 x 10^4 cells/cm^2 into transport flasks (iP-TEC) and cultured overnight.
2. The next day, the vessels were filled to capacity with H-Fbro-01. The flasks along with heat-retaining material, were then packaged into the transport box for a two-day transportation period.
3. After arrival, the culture medium was replaced with the growth medium, and the cultivation was resumed.

For culture condition

1. NHDFs were seeded on a 6-well-plate at a density of 0.5×10^4 cells/cm^2 and cultured overnight.
2. The next day, the medium was exchanged, and the cells were cultured for 2 days.
3. Further, the medium was exchanged again, and the cells were cultured for an additional 2 days.

For static condition at 35˚C (transport control)

1. NHDFs were seeded into transport flasks at a density of 0.5 x 10^4 cells/cm^2 and cultured overnight.
2. The next day, the medium in the flask was replaced, and the vessels were filled to capacity with H-Fbro-01. They were then placed in a 35 ˚C incubator (non-CO_2), and left for 2 days.

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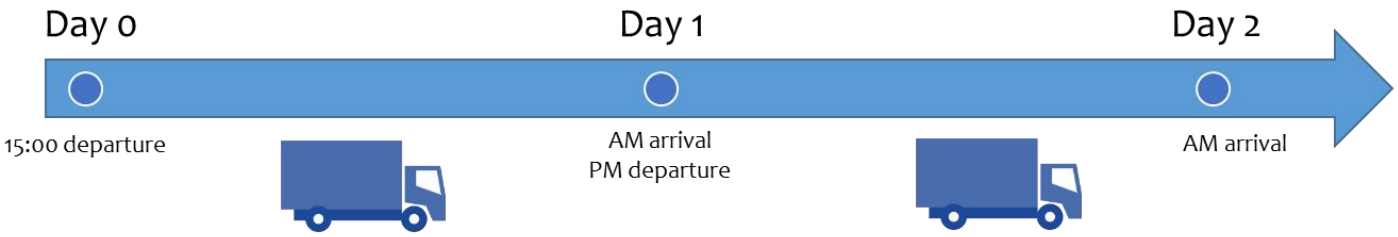
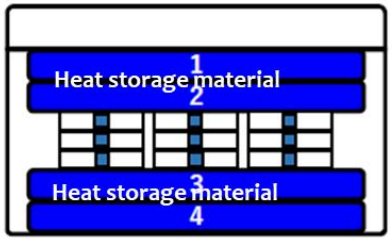


Transport box

Inside the box



Internal Structure

iP-TEC® Flask-25
(Sanplatec Corp.)

Temperature/humidity/shock logger (Fujita Electric Works, Ltd)

Impact threshold: 10G

The two-day transportation scheme and the inside of the transport box.

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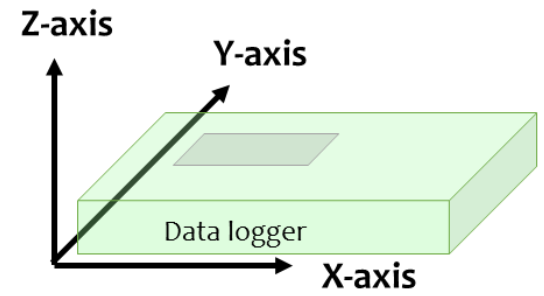
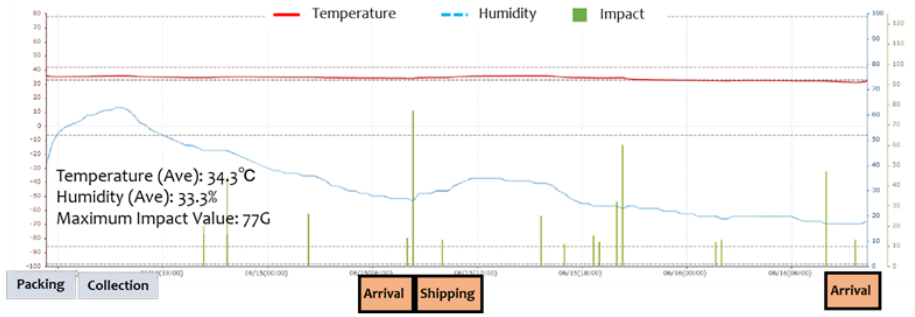
A



Impact value[G]=acceleration [a]/9.8

B



C

Impact value analysis

	X-axis	Y-axis	Z-axis
■ 2-day transportation			
average [G]	16.1	14.3	17.2
maximum [G]	41	36	77
number of times	16	24	102
■ Gravity drop test: 10 cm above the floor			
average [G]	3.1	1.3	17.9
maximum [G]	19	4	33
■ Gravity drop test: 40 cm above the floor			
average [G]	5.2	3.9	23.9
maximum [G]	19	13	106

D

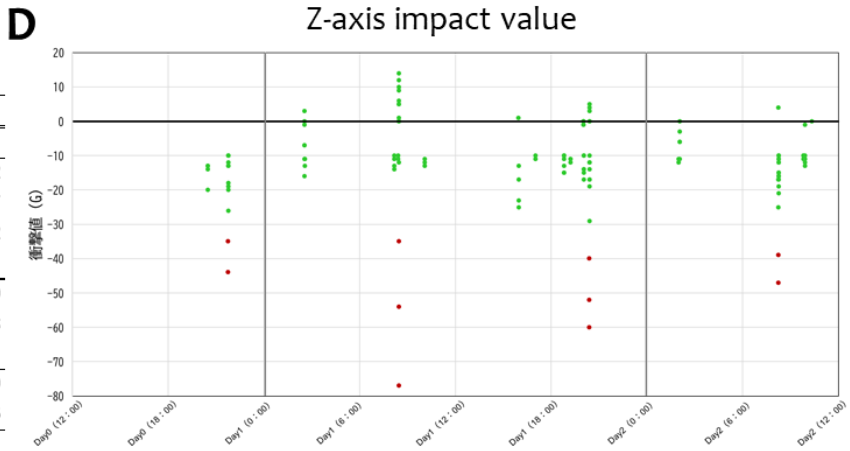


Fig. 1 Analysis of the temperature, humidity and impact value inside the transport box

(A) A temperature/humidity/impact logger was used to record impact values [G] in the XYZ directions. (B) The average temperature inside the transport box was 34.3°C, and the maximum impact value was 77G. (C) As a result of comparing the impact value received during transportation with the free drop test, most impacts were equivalent to dropping from a height of 10 cm above the floor. (D) Additionally, the majority of impacts were detected in the direction of the drop (negative number in the Z direction), only 10 impacts were stronger than the drop from 10 cm above the floor (red).

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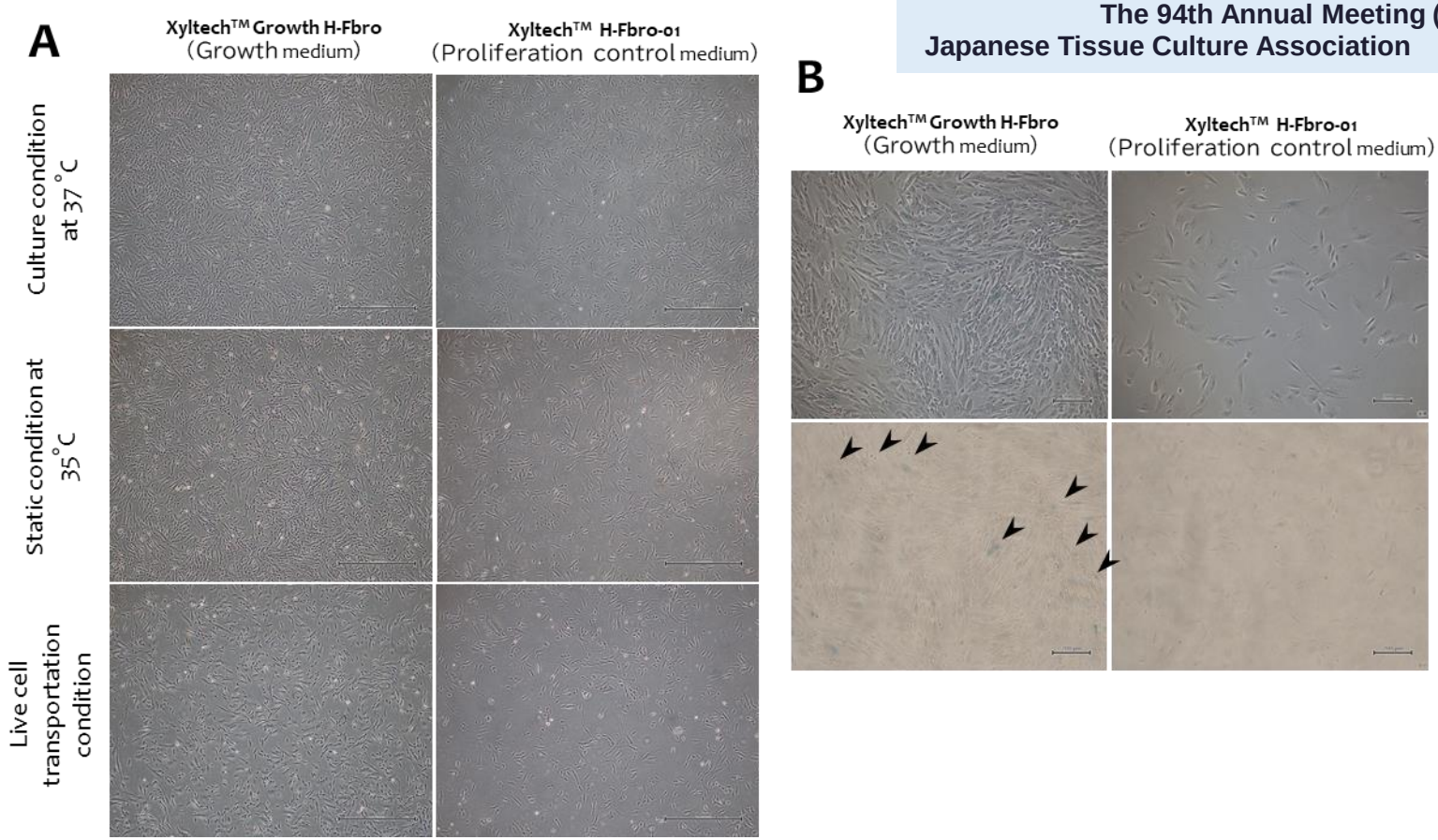

Fig. 2 Morphological observation of NHDF upon arrival and SA-β-GAL staining

(A) Regardless of the conditions, there were no floating cells in NHDFs upon arrival, and no morphological changes were observed when compared to 37°C culture conditions and 35°C stationary conditions. In addition, in the growth medium Xyltech™ Growth H-Fbro, cells reached near confluence in some areas, whereas in the growth control medium Xyltech™ H-Fbro-01, cell proliferation appeared to be suppressed. (B) When the NHDFs at the time of arrival were stained with the senescent cell marker SA-β-GAL, positive cells tended to increase in areas with high cell densities in the growth medium (arrowheads). In contrast, in the proliferation control medium, there were no areas with high densities, and almost no positive cells were confirmed.

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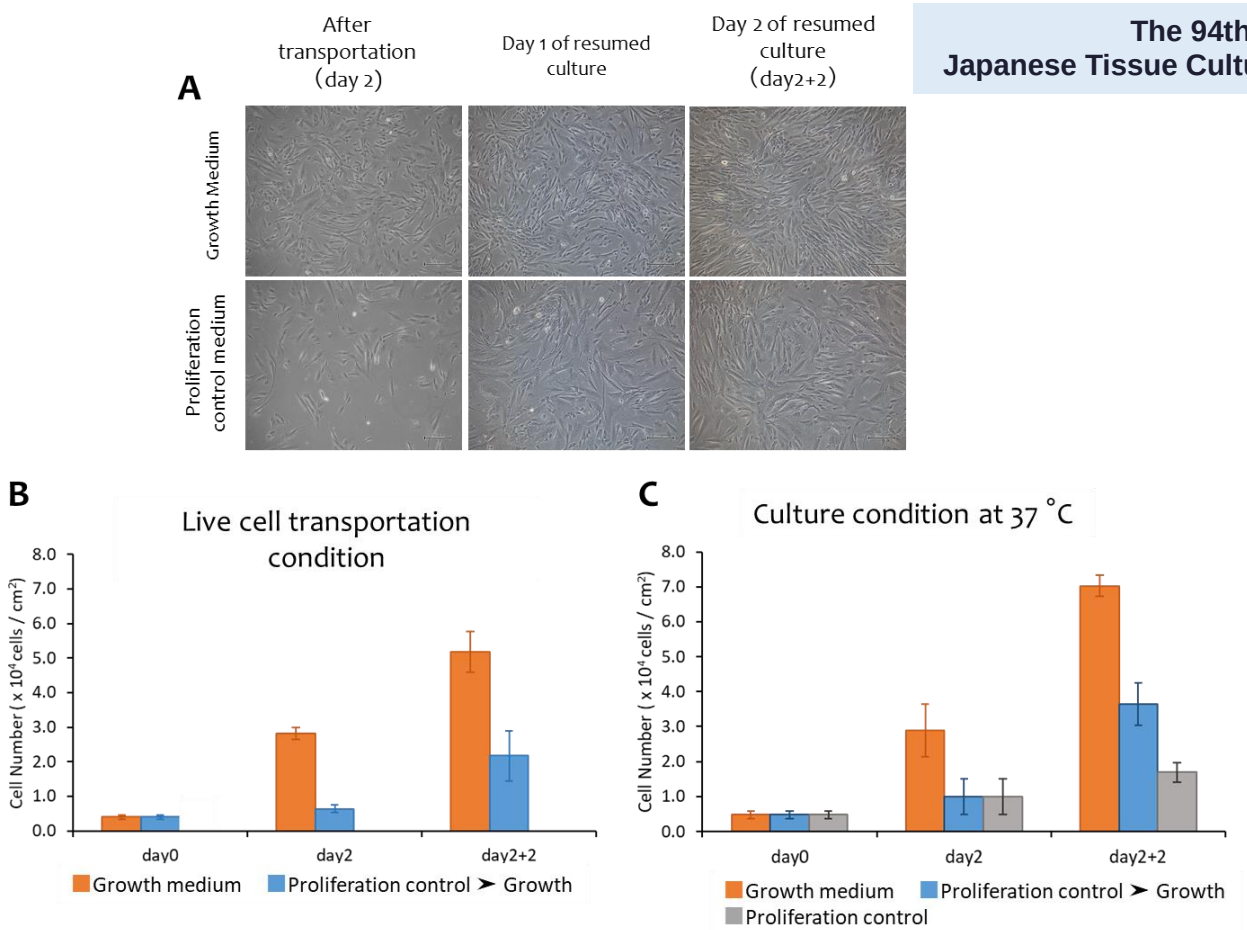

Fig. 3 Changes in cell number during transportation and evaluation of repopulation ability after transportation

(A) After the transportation box arrived, the medium was changed to the growth medium, and the culture was restarted. (B) Following transport (day 2) and after resuming culture (day 2+2), NHDFs showed a suppressed increase in cell number in the growth control medium. However, the cell number increased by 3.4 times on the second day after resuming culture. (C) Under 37˚C culture conditions, growth was suppressed in the growth control medium as in the transport condition. However, the number of cells increased by 3.7-fold two days after switching to the growth medium. Conversely, when the medium was replaced with the growth control medium, the cell number increased by 1.7 times, indicating suppressed growth.

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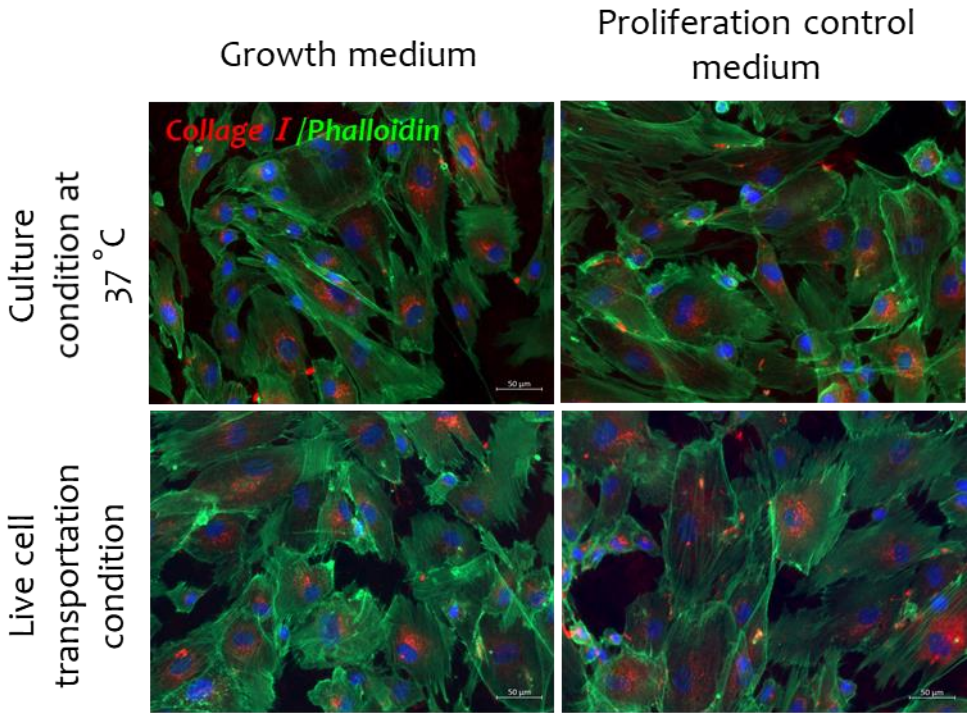

Fig. 4 Collagen production in NHDF after transportation

The transported NHDFs were reseeded 2 days after resuming culture, and immunostaining of collagen I, a fibroblast-specific protein, was performed. The results revealed that collagen I-positive cells were confirmed in both transport condition with the growth medium "Xyltech™ Growth H-Fbro" and the proliferation control medium "Xyltech™ H-Fbro-01", same as NHDF cultured at 37°C.

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-Conclusions-

・It was observed that Xyltech™ H-Fbro-01, a proliferation control medium, could effectively maintain NHDF growth in a suppressed state and inhibit the fomation of SA-β-GAL positive cells during the post-transport increase in cell number.

・It was suggested that the NHDF cells transported in the proliferation control medium "Xyltech™ H-Fbro-01" demonstrated rapid proliferation when the medium was switched to the growth medium, and culture was resumed.

・ NHDF cells maintained their ability to produce collagen I even after transportation.

・During the 2-day transportation period using transport box, it was confirmed that the box was subjected to many impacts in the falling direction. However, there was no observed changes in cell morphology compared to cells kept under static condition, and no floating cells were observed.

Xyltech™ H-Fbro-01

Product information

※For Research Use Only. Not for use in diagnostic procedures

Cat. No.	Product name	Features/Applications	Package
10101	Xyltech™ BOF-01	Human pluripotent stem cells proliferation control medium [Cell proliferation suppression]	100 mL (P)
10301	Xyltech™ H-Fbro-01	Serum-free complete synthetic culture medium for human fibroblasts [Cell proliferation suppression]	100 mL (P)
10311	Xyltech™ Growth H-Fbro	Serum-free complete synthetic culture medium for human fibroblasts [Cell proliferation]	500 mL (P)
10401	Xyltech™ MSC-01 Xeno-Free	Serum-free culture medium for human Mesenchymal Stem Cells [Cell proliferation suppression] (Xeno-Free)	100 mL (P)
10411	Xyltech™ Growth MSC Medium	Serum-free basal culture medium for human Mesenchymal Stem Cells [Cell proliferation]	500 mL (P)
10412	Xyltech™ Growth MSC Supplement XF	Supplement for Xyltech Growth MSC Medium [Cell proliferation] (Xeno-Free)	10 mL (P)

For inquires about product

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