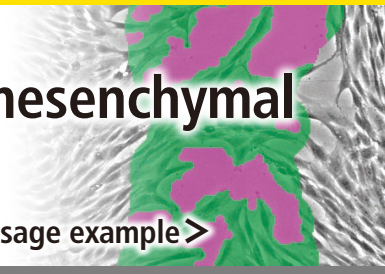
Application Note

CL-Quant Add-on Module

Evaluation of the gap closure of mesenchymal stem cells using "Scratch Assay"

< BioStation CT usage example >



Cell migration is one of the major processes in development and differentiation processes, immune responses, cancer cell invasion and metastasis, and wound healing. Evaluation of cell migration ability is very useful in research into embryology, cell biology and cancer. Recently, cell migration ability has been reported to be one of the important properties of mesenchymal stem cells (MSCs). The BioStation CT, equipped with an incubator for monitoring cells using a built-in microscope and CCD camera, allows automatic long-term time-lapse imaging of MSC. Closure ratio of the scratched area can be automatically measured by digitalizing areas of both the scratched gap and migrating cells into the scratched gap, using the image analysis software CL-Quant and its Add-on Module "Scratch Assay".
To evaluate the migration ability of MSC, a scratch assay was performed, and the closing ratio of the scratched surface area was measured from phase contrast images acquired over time.

Observation device

- BioStation CT (Nikon, MLA10000)

Image analysis software

- CL-Quant ver. 5.02 (Nikon, MLS21000)

CL-Quant Add-on Module

- PC-AR04 Scratch Assay (Nikon, MLS30204)

Cells

- ASC 52 telo, hTERT immortalized adipose derived mesenchymal stem cells (ATCC® SCRC-4000™)

Reagents and materials

- MesenPRO RS™ Medium (Thermo Fisher Scientific, 12746012)
- L-Glutamine, 200 mM Solution (Thermo Fisher Scientific, 25030081)
- Penicillin-Streptomycin (10,000 U/mL) (Thermo Fisher Scientific, 15140122)
- Dulbecco's phosphate-buffered saline, no calcium, no magnesium without phenol red (DPBS, Thermo Fisher Scientific, 14190144)
- TrypLE™ Express Enzyme (1x), no phenol red (Thermo Fisher Scientific, 12604013)
- TPP® tissue culture test plate (6-well) (TPP, 92406)
- Culture-Insert 2 well in μ-Dish 35mm, High (ibidi, 81176)

Methods

MSCs were dissociated using TrypLE™ Express and seeded in each well of a 6-well plate placed with culture insert at a cell density of $9x10^3$ cells/well in MesenPRO RS Medium supplemented with L-glutamine and Penicillin-Streptomycin. The cells were cultured in BioStation CT at 37 ˚C, 5% CO_2. Phase contrast images of the 8x8 fields of view at the center of the wells were captured with a 4x objective lens every hour for 2 days from 16 minutes after removing culture insert. Focus was adjusted by autofocus at the center of each well and kept at the same focus position. After the images were taken into CL-Quant, the area (approximately 1.2 mm x 1.2 mm) including the gap (region without cells) generated by culture insert removal was cropped and analyzed by the Add-on Module "Scratch Assay". "Cell migration Area to Scratch region Area Ratio" [ratio of invading cell area to scratched area (in this experiment, area without cells generated by removal of culture insert)] was automatically digitalized by CL-Quant. The values were converted to percentages as the gap closure ratio and the change over time was graphed.

Results

The phase contrast images were taken every hour for about 2 days after removing culture inserts. The images captured are shown in Fig. 1, demonstrating that the gap area and the area of cells invading the gap are accurately masked out.
Analyzing the images taken over about 2 days, the change over time in the gap closure ratio is shown in the graph (Fig. 2). The table also shows the gap closure ratio which was automatically measured by CL-Quant (Table 1). This experiment demonstrated that it is possible to observe the process of gap closure over time without staining the cells.

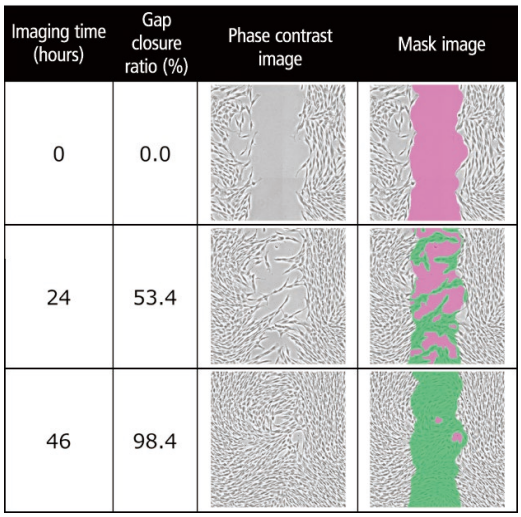


Fig.1 Analyzed result of image and gap closure ratio

The phase contrast images were taken every hour from 16 minutes (imaging time: 0) after removal of the culture insert. In the masked image, the gap area is displayed with a purple mask in the phase contrast image, and the area of the cell that has invaded the gap area is displayed in green. (Image courtesy: Dr. Yuzuru Ito, National Institute of Advanced Industrial Science and Technology)

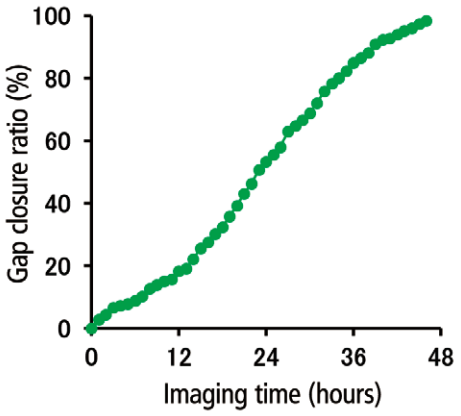


Fig. 2 Time course of gap closure ratio

(Data provided: Dr. Yuzuru Ito, National Institute of Advanced Industrial Science and Technology)

Table 1. Measured values from the phase contrast images

Imaging time (hours)	Cell invasion area (μm²)	Gap area (μm²)	Gap closure ratio (%)
0	-	506,176	0.0
1	14,960	506,176	3.0
2	22,528	506,176	4.5
3	34,128	506,176	6.7
4	37,024	506,176	7.3
5	40,320	506,176	8.0
6	45,040	506,176	8.9
7	52,416	506,176	10.4
8	64,016	506,176	12.6
9	70,848	506,176	14.0
10	76,624	506,176	15.1
11	79,952	506,176	15.8
12	92,960	506,176	18.4
13	96,960	506,176	19.2
14	112,016	506,176	22.1
15	129,856	506,176	25.7
16	139,904	506,176	27.6
17	152,752	506,176	30.2
18	164,224	506,176	32.4
19	181,728	506,176	35.9
20	199,264	506,176	39.4
21	218,112	506,176	43.1
22	234,576	506,176	46.3
23	256,448	506,176	50.7
24	270,080	506,176	53.4
25	281,440	506,176	55.6
26	293,568	506,176	58.0
27	319,248	506,176	63.1
28	327,808	506,176	64.8
29	336,848	506,176	66.5
30	348,064	506,176	68.8
31	364,928	506,176	72.1
32	384,672	506,176	76.0
33	396,656	506,176	78.4
34	405,120	506,176	80.0
35	416,304	506,176	82.2
36	429,984	506,176	84.9
37	438,592	506,176	86.6
38	445,904	506,176	88.1
39	460,256	506,176	90.9
40	467,792	506,176	92.4
41	469,888	506,176	92.8
42	476,128	506,176	94.1
43	482,432	506,176	95.3
44	485,952	506,176	96.0
45	493,568	506,176	97.5
46	498,064	506,176	98.4

(Data provided: Dr. Yuzuru Ito, National Institute of Advanced Industrial Science and Technology)

Summary

- By analyzing the phase contrast image of the scratch assay with CL-Quant, closure ratio of the scratched gap area is calculated automatically.
- By referring to the masked image, you can easily check whether the scratched gap area and the cell migration area are correctly recognized.
- During the cultivation process, the gap closure ratio can be assessed while monitoring the culture status, and the assay termination can be determined.

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