

Culture of Intestinal Epithelial Cell Sheet Models with Cell Culture Inserts using the Tabletop Automated Cell Culture System "MakCell"

The tabletop automated culture system "MakCell" is designed to automatically perform medium changes, contributing to the reduction of labor time required for medium changes essential for daily cell maintenance, as well as minimizing the need for weekend work. In this study, we successfully automated the cultivation of cell sheets using cell culture inserts with "MakCell."

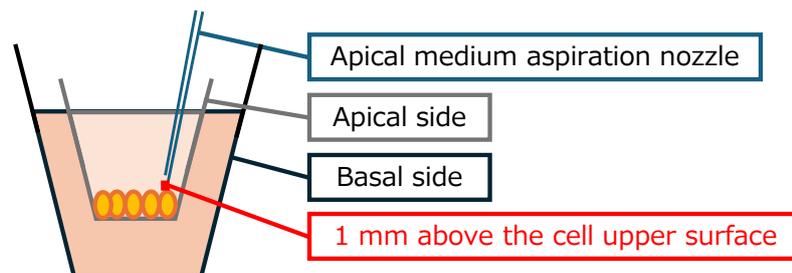
The intestinal epithelial cell sheets cultivated with "MakCell" were confirmed to possess the same barrier function as those cultivated through manual medium change.

METHOD

- Cell Line: Human colon cancer-derived cell line (Caco-2 cells)
- Vessel: SUMILON Companion Plate for Cell Culture Insert (Manufactured by Sumitomo Bakelite #MS-80240C) for 24-well Cell Culture Insert (FALCON #353095)] Coated with HCl-diluted Cellmatrix Type I-C (Nitta Gelatin) CellQART Insert (SABEU #9320402)
- Medium: D-MEM (High glucose FUJIFILM Wako)
- Medium Supplements: FBS (Sigma), Antibiotics (Gibco), NEAA (Sigma)
- Antibodies: ZO1 antibody (Abcam), Fluorescently labeled Phalloidin (Cosmo Bio), Fluorescently labeled secondary antibody (Thermo Fisher)
- Reagents: FITC-Dextran 4 kDa (Chondrex), TNF α (R&D)
- Culture Duration: 21 days with medium changes every 2-3 days (at the same time for both manual culture and MakCell culture)

Order of medium change for each well

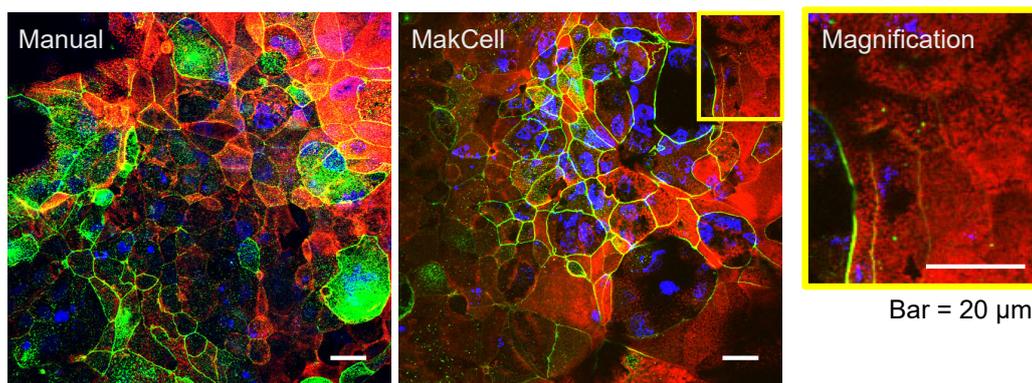
Aspiration of basal medium (Nozzle tip touching the bottom)	➔	Supply of basal medium (Slow dripping)	➔	Aspiration of apical medium (Nozzle tip 1 mm above the cells)	➔	Supply of apical medium (Slow dripping)
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RESULTS

① Cell sheet immunofluorescence staining ZO1+Phalloidin+DAPI

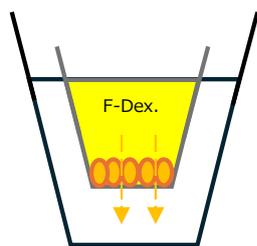
The formation of tight junctions and the presence of microvilli, observed in intestinal epithelial-like structures due to the high-density culture of Caco-2 cell line, were confirmed.



RESULTS (Continued)

② Permeability Test

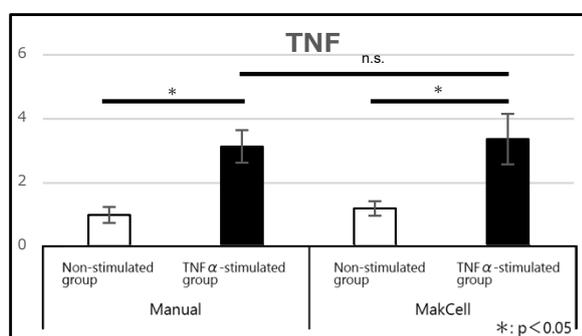
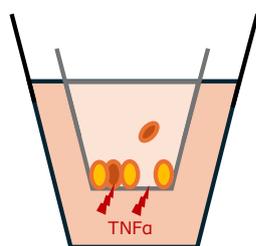
FITC-Dextran 4 kDa (F-Dex.) added to the cultivated cell sheet did not permeate to the basal side.



		F-Dex. Addition Amount (μg)	Results (μg)	Permeability Rate(%)
Cont. (No Culture)	F-Dex.(-)	0	Not Detectable	-
	F-Dex.(+)	3.125	1.56	50
Manual	F-Dex.(-)	0	Not Detectable	-
	F-Dex.(+)	3.125	Not Detectable	-
MakCell	F-Dex.(-)	0	Not Detectable	-
	F-Dex.(+)	3.125	Not Detectable	-

③ Gene Expression Analysis Induced by TNF α Stimulation

The increase in the expression of the inflammatory-inducing gene (TNF) was confirmed following TNF α stimulation.



Using MakCell, we performed the culture of Caco-2 cells. On the 21st day of culture, microvilli indicative of intestinal epithelial-like differentiation were observed due to high-density culture. Furthermore, based on the expression of ZO1, which forms tight junctions, and the results of material impermeability of the cell sheets, we speculate that we successfully cultivated cell sheets with barrier function. We also confirmed an increase in the expression of the inflammatory-inducing gene TNF, which is one of the mechanisms that enhance intestinal epithelial structure permeability and induce inflammation, following TNF α stimulation. These evaluations confirmed that the analytical results for manual culture and MakCell culture were comparable.

DISCUSSION

- Using MakCell, we have cultivated a cell sheet that replicates intestinal function in the small intestine, making it widely usable in fields such as drug discovery research.
- Using MakCell, we can eliminate concerns about damage to the cell sheets caused by operator errors, allowing for stable production of the cell sheets.
- Since operators are freed from the need for frequent and careful medium changes, they can focus on various tests and analyses using the cell sheet model. Therefore, MakCell can be considered a system that contributes to the advancement of research.

(Reference)

- The medium change sequence with MakCell is just one example; the order of suction and supply can be arranged freely.
- It supports not only medium changes using cell culture inserts but also standard culture vessels.
- It is compatible with low-oxygen or hypoxia culture.
- Up to three types of medium can be loaded.

If you have any questions, please feel free to contact us using the information below.

