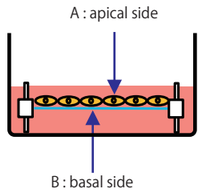
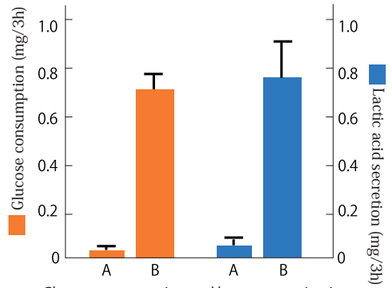
Collagen for Cell Culture
AteloCell®

Example 5 Polarized cell culture using permeable collagen membranes

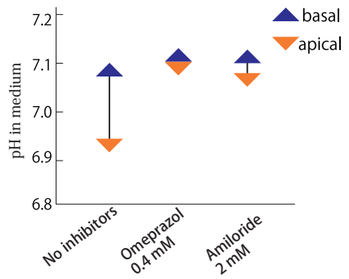
[Reference：Yoshizato K, *et al. J Cell Sci.* 91(Pt 4): 491-499]



Schematic diagram of primary Rat epidermal cell culture



Glucose consumption and lactate secretion in the medium(3 hours after medium change)



Suppression of pH difference in culture medium by membrane transporter inhibitor additives

When cultured using collagen membranes, it was observed that the glucose consumption and lactic acid excretion in the medium on the basal side were significantly higher than on the apical side. On the other hand, a decrease in pH on the basal side was observed, but when a membrane transporter inhibitor was added to the medium, the difference in pH decreased. These results indicate that culture on collagen membranes can maintain cell polarity and maintain a functional membrane transport mechanism. (Reference 5)

Reference

1. Murakami Y, *et al.* Reconstitution of the embryonic kidney identifies a donor cell contribution to the renal vasculature upon transplantation. (2019) *Sci Rep.* Feb 4;9(1):1172.
2. Matsumoto A, *et al.* Induction of enamel matrix protein expression in an ameloblast cell line co-cultured with a mesenchymal cell line in vitro. (2011) *In Vitro Cell Dev Biol Anim.* 47(1):39-44.
3. Ueno T, *et al.* Characteristic Gene Expression Profiles of Human Fibroblasts and Breast Cancer Cells in a Newly Developed Bilateral Coculture System. (2015) *Biomed Res Int.* 2015;2015:960840.
4. Hatou S, *et al.* Functional corneal endothelium derived from corneal stroma stem cells of neural crest origin by retinoic acid and Wnt/β-Catenin signaling. (2013) *Stem Cells Dev.* 22(5):828-39.
5. Yoshizato K, *et al.* Functionally polarized layers formed by epidermal cells on a permeable transparent collagen film.(1988) *J Cell Sci.* 91(Pt 4):491-499.

Cat. No.	Description	Quantity	Storage
KOU-CM-6	Atelocollagen Permeable Membrane (6 well)	12 pcs/box	4℃
KOU-CM-24	Atelocollagen Permeable Membrane (24 well)	24 pcs/box	4℃
KOU-CLF-01	Atelocollagen Permeable Membrane Sheet	1 sheet/box	4℃

World distributor

COSMO BIO USA.

2792 Loker Avenue West, Suit 101, Carlsbad, CA 92010 USA
Phone : +1 760-431-4600 / Fax : +1 760-431-4604

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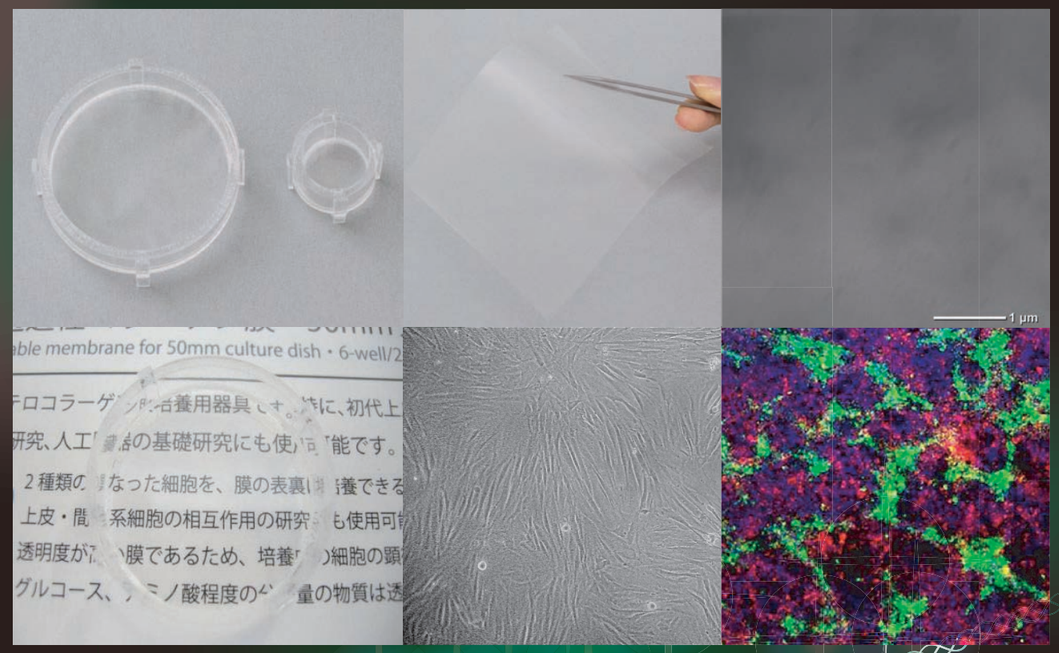
AteloCell® Collagen for Cell Culture

Atelocollagen, Permeable membrane



Collagen for Cell Culture AteloCell®

Atelocollagen, Permeable membrane

CM-6, CM-24, CLF-01

Product summary

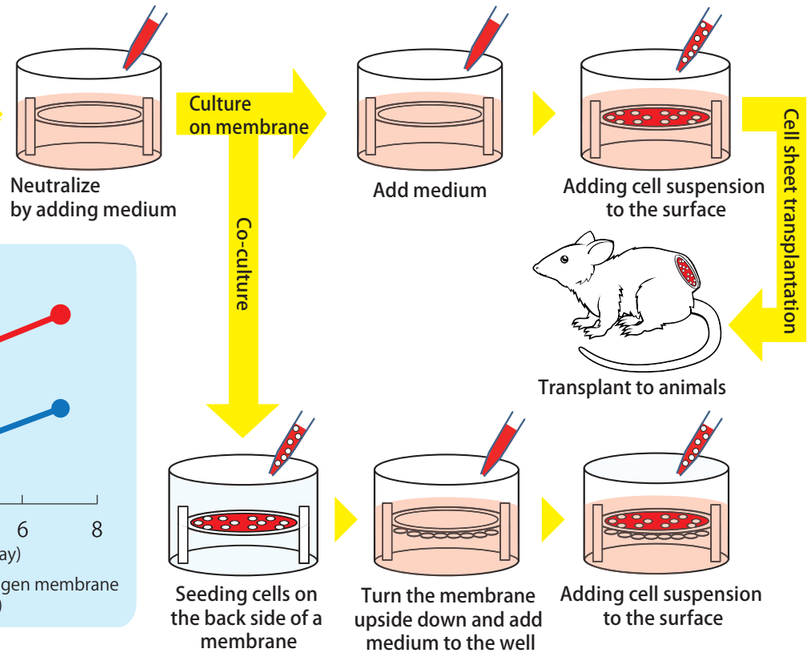
Atelocollagen, Permeable membranes are permeable collagen membranes that are made from atelocollagen and are permeable to substances. By seeding cells on both sides of the membrane, it is possible to observe cell-cell interactions without cells mixing. In addition, when epithelial cells are cultured on this membrane, it is possible to culture them while maintaining the polarity of the cells in the same way as in vivo, such as absorption of nutrients and excretion of metabolic wastes through the collagen membrane.

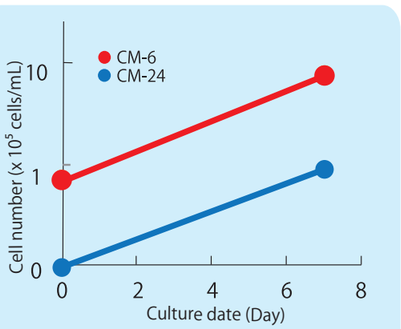
Applications

Culture on membrane, Air liquid Interface culture, Co-culture, Cell sheet transplantation

How to use

CM-6, CM-24

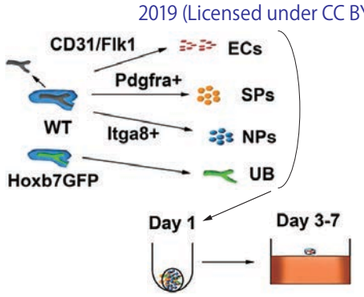


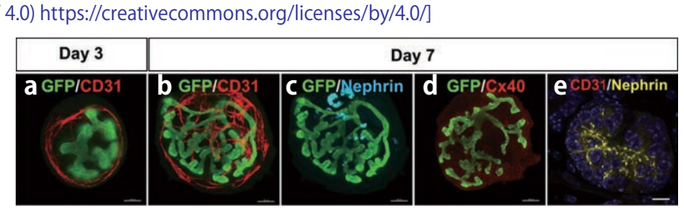


Cell proliferation test using collagen membrane (Using fibroblasts, internal data)

Example 1 On-membrane culture of renal organoids using permeable collagen membranes

[Reference：*Sci Rep.* 2019 Feb 4;9(1):1172. Created by modifying figures 3A and 3C-G. ©Murakami Y., et al. 2019 (Licensed under CC BY 4.0) https://creativecommons.org/licenses/by/4.0/]





Left figure: Schematic diagram of renal organoid reconstruction and *in vitro* culture (ECs: vascular endothelial cells, SPs: interstitial progenitor cells, NPs: nephron progenitor cells, UB: ureteric buds) . Right figure (a-d): Whole mount immunostaining image after renal organoids were cultured at the air-liquid interface on a collagen membrane. Right figure (e): Immunostaining image of kidney organoid section cultured in the same way.
GFP: ureteric bud (only in this figure), CD31: vascular marker, Nephrin: glomerular marker, Cx40: arteriole marker

When kidney organoids reconstituted using a low-adhesion plate were cultured at the air-liquid interface on a collagen membrane, extensive ureteric bud molecules, surrounding vascular networks, and the formation of numerous glomeruli were observed.. The above results show that, although there are certain limitations, it is possible to create renal organoids with vascular structures using this reconstruction method and air-liquid interface culture on collagen membranes.（Reference 1）

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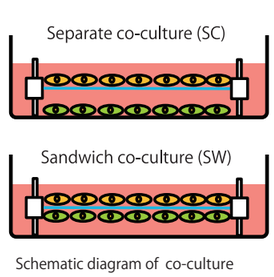
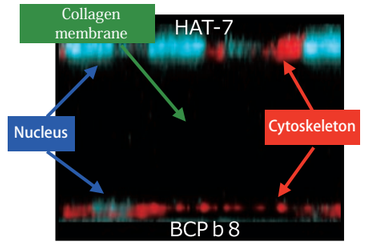
Example 2 Induction of ameloblast differentiation using the permeable collagen membrane

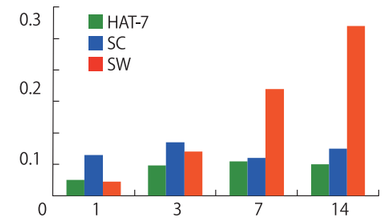
(Taniguchi A, et al., Natl. Inst. for Material Science, Japan.)



Schematic diagram of co-culture



Sectional view 4 days after SW culture

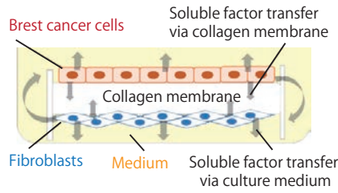


Ameloblastin expression after the co-culture

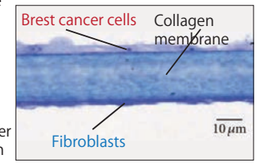
For SW culture, rat odontogenic epithelium cell line (HAT-7) and bovine dental follicle cell line (BCPb8) were seeded on opposite sides of a collagen membrane. As a result, expression of Ameloblastin and Amelogenin (important proteins for enamel formation) were markedly increased compared to SC culture or monolayer culture.
These experiments revealed that the permeability of collagen membrane is advantageous for remodeling cell-cell interactions in a manner similar to in vivo. (Reference 2)

Example 3 Co-culture of Cancer Cells and Fibroblasts Using a Permeable Collagen Membrane

[Biomed Res Int. 2015;2015:960840. Created by modifying figure 1 and Tables 5-6. ©Ueno T., et al. 2015 (Licensed under CC BY 3.0)]



Schematic illustration of co-culture using a collagen membrane



Cross-sectional view after 3 days of co-culture (H&E staining)

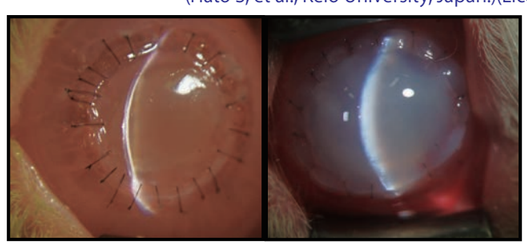
	Upregulated Gene Ontology (GO) terms	Downregulated Gene Ontology (GO) terms
Brest cancer cells	・Cell cycle ・Cell division ・Carbohydrate metabolism, TCA and amino acid metabolism	・Functional process ・Phosphatides acid pathway ・Glucuronic acid pathway
Fibroblasts	・Single organism cellular process ・Carbohydrate transport ・Amino acid transport	・Immune response regulation ・Cholesterol biosynthesis ・Lipid metabolism

Major Gene Ontology (GO) terms with increased or decreased expression following co-culture

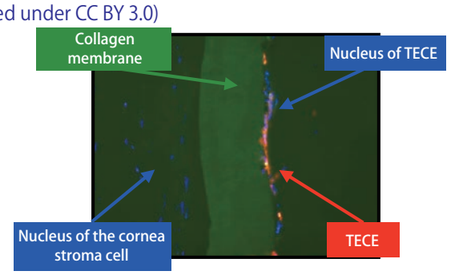
Using a collagen membrane, three types of breast cancer cell lines—HCC1937, MCF-7, and SK-BR-3—were co-cultured with fibroblasts, and gene expression changes were evaluated via microarray analysis. As a result, among the top 100 genes that were upregulated or downregulated in HCC1937 cells, 77% of the upregulated genes showed downregulation in fibroblasts, and 82% of the downregulated genes showed upregulation, indicating a reciprocal effect through co-culture. Furthermore, while all breast cancer cells exhibited reprogramming toward aerobic metabolism, the fibroblasts appeared to be reprogrammed toward a Warburg-like phenotype. These findings suggest that the collagen membrane is effective for evaluating intercellular interactions under non-contact co-culture conditions (Reference 3).

Example 4 Induction of corneal endothelial differentiation and transplantation with the permeable collagen membrane

(Hato S, et al., Keio University, Japan.)(Licensed under CC BY 3.0)



Left panel: Rabbit corneal after tissue-engineered corneal endothelium(TECE) transplantation. Right panel: control group



Vertical section of harvested corneal graft with TECE on day 8 post-transplantation

Multipotent cornea-derived precursors isolated from mouse and human corneal stroma were cultured on collagen membrane and differentiated to functional corneal endothelium. Transplanting TECE attached collagen membrane to donor cornea where corneal endothelium was stripped suppressed edematous and notably increased the transparency of cornea compared to control groups. These results show that collagen membrane, with its high transparency and biocompatibility, is suitable for transplantation, such as corneal transplantation. (Reference 4)