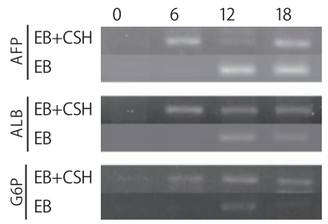
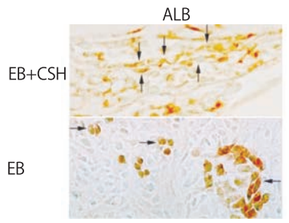
Collagen for Cell Culture
AteloCell®

Example 4 Induction of hepatic diff erentiation of ES cells and transplantation using CSH

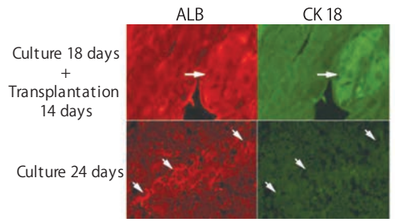
(Imamura T, Department of Urology, Shinshu University School of Medicine)



Expression of various hepatocyte markers by RT-PCR



Immunostaining images [day 18]
(Arrow: ALB-positive cell)



Images of EB+CSH by fluorescence microscope
(Arrows: ALB positive cells)

Embryoid bodies (EBs) were formed from ES cells and then inserted into CSH. EBs both with and without CSH were cultured to differentiate and induce hepatic histogenesis. The EB-derived cells expressed liver-specific genes, and albumin-positive cells formed cord-like structures that were not present in those without CSH. The scaffold including EB-derived hepatocyte-like cells was transplanted into the median lobe of mice. After 14 days, cells positive for both albumin and
cytokeratin 18 appeared in the transplant and formed clustered aggregates. (Ref. 8) [AFP: Marker for initial livers hepatic cells; ALB: Marker for initial to matured hepatic cells; G6P and CK18: Marker for matured hepatic cells]

Reference

1. Mogi K, *et al.* 9-oxo-ODAs suppresses the proliferation of human cervical cancer cells through the inhibition of CDKs and HPV oncoproteins. *Sci Rep.* 2023 Nov 6;13(1):19208. PMID: 37932321.
2. Brizi V, et al. Human iPSC-derived neural crest stem cells can produce EPO and induce erythropoiesis in anemic mice. *Stem Cell Res.* 2021 Aug;55:102476. PMID: 34339993.
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4. Ishikawa M, *et al.* Transplantation of neurons derived from human iPS cells cultured on collagen matrix into guinea-pig cochleae. *J Tissue Eng Regen Med.* 2017 Jun;11(6):1766-1778. PMID: 26205474.
5. Kano F, *et al.* Secreted Ectodomain of Sialic Acid-Binding Ig-Like Lectin-9 and Monocyte Chemoattractant Protein-1 Synergistically Regenerate Transected Rat Peripheral Nerves by Altering Macrophage Polarity. *Stem Cells.* 2017 Mar;35(3):641-653. PMID: 27862629.
6. Ukegawa M, *et al.* Bone marrow stromal cells combined with a honeycomb collagen sponge facilitate neurite elongation in vitro and neural restoration in the hemisected rat spinal cord. *Cell Transplant.* 2015;24(7):1283-1297. PMID: 24911956.
7. Furumoto T, *et al.* Mallotus philippinensis bark extracts promote preferential migration of mesenchymal stem cells and improve wound healing in mice. *Phytomedicine.* 2014 Feb 15;21(3):247-253. PMID: 24182990.
8. Imamura T, *et al.* Embryonic stem cell-derived embryoid bodies in three-dimensional culture system form hepatocyte-like cells in vitro and in vivo. *Tissue Eng.* 2004 Nov-Dec;10(11-12):1716-1724. PMID: 15684680.

	Description	Quantity	Storage
KOU-3D-HCB	3D Honeycomb Boosted	25 pcs/btl	4℃
KOU-CSH-10	Atelocollagen Honeycomb spomge	100 mg/btl	room temprature
KOU-CSH-96	Atelocollagen Honeycomb Disc96	25 pcs/btl	room temprature

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
e-mail : support@cosmobiousa.com
web : https://www.cosmobiousa.com/

Please don't hesitate to contact us with any questions related to product selection or use.

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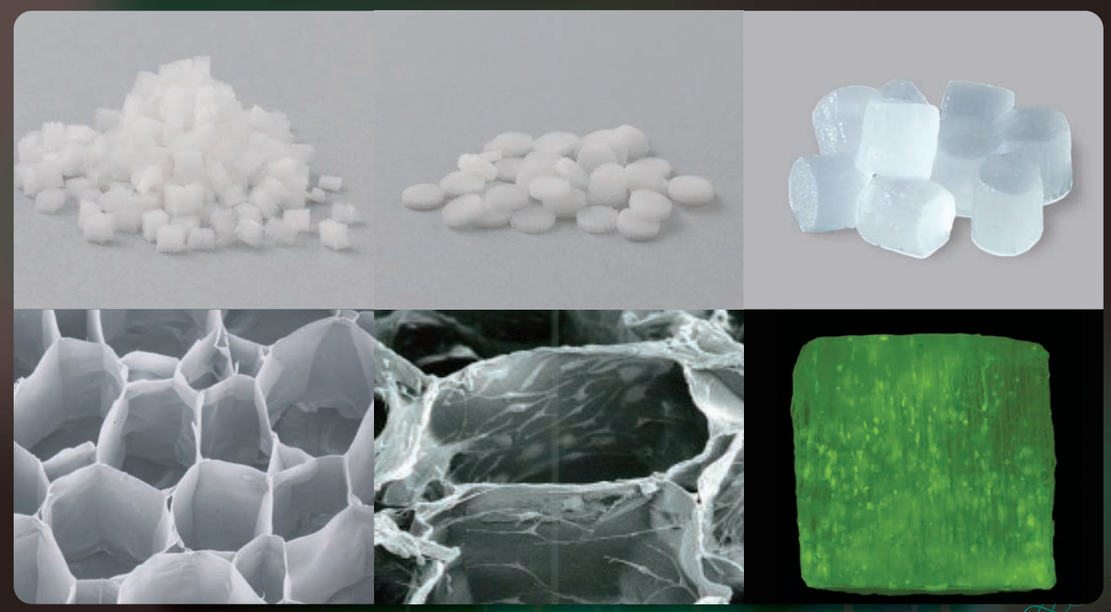
KOKEN

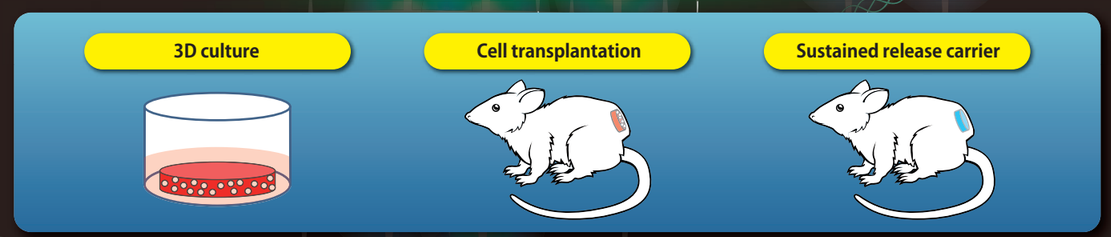
KOKEN

AteloCell® Collagen for Cell Culture

Atelocollagen, Honeycombs Sponge

3D Honeycomb Boosted





Collagen for Cell Culture AteloCell®

Atelocollagen, Honeycomb Sponge CSH-10
Atelocollagen, Honeycomb Disc 96 CSH-96
3D Honeycomb Boosted 3D-HCB

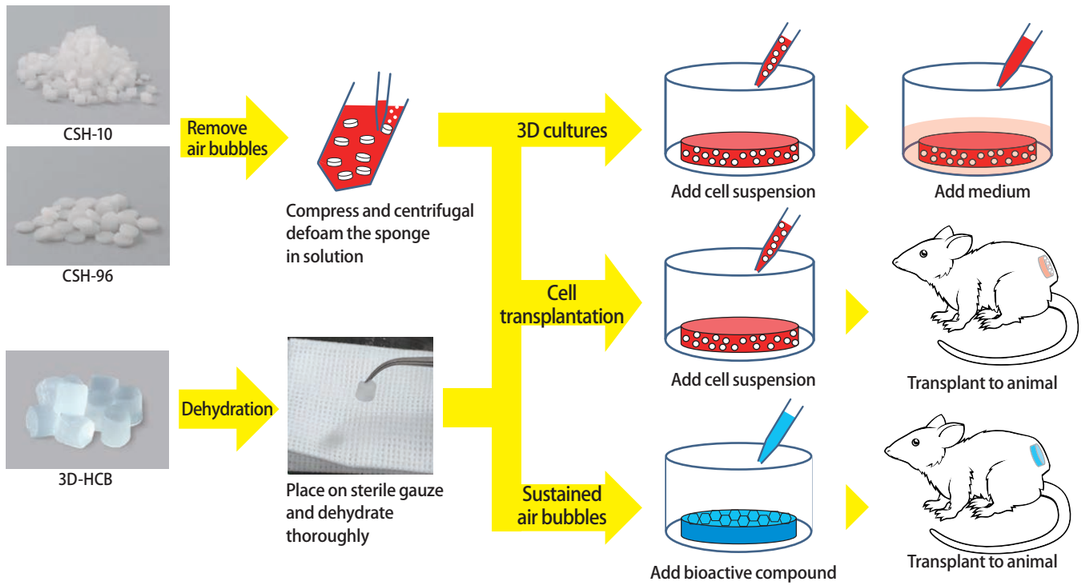
Product summary

Collagen Sponge Honeycomb and Honeycomb Disc 96 are biodegradable sponges with a honeycomb-like porous structure made of atelocollagen with high biocompatibility. The tightly oriented porous structure facilitates the supply of nutrients and release of waste products, allowing cells to grow throughout the pore interior. Cell collection is possible using collagenase. 3D Honeycomb Boosted is an improved product with smaller pore size. By adjusting the height, the adhesion rate at the time of cell seeding has improved, and by immersing in PBS, the trouble of degassing and static electricity countermeasures is eliminated. In addition, since the strength has been improved, we recommend cell recovery with trypsin instead of collagenase.

Applications

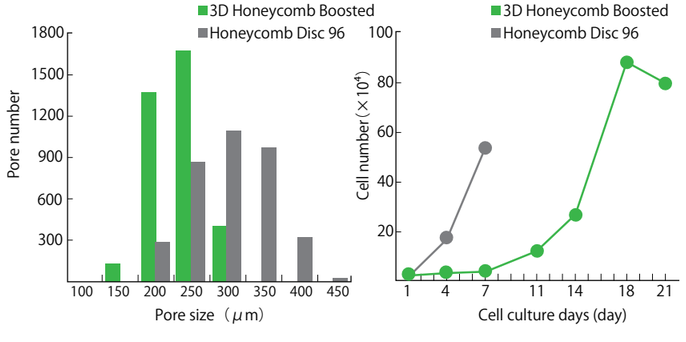
3D cultures, Cell transplantation, Sustained release carrier

How to use



Specification

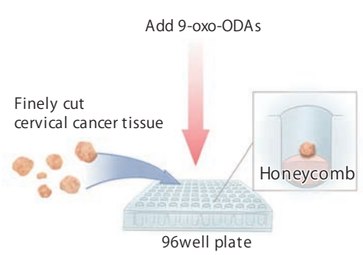
3D Honeycomb Boosted enables high-density three-dimensional culture up to 10^6 cells scale, equivalent to a 35mm dish.



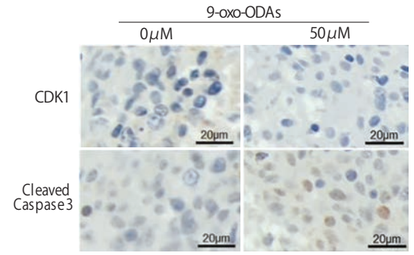
Even though they have the same honeycomb pore structure, Honeycomb Disk 96 (#KOU-CSH-96) showed a broad peak of 250 to 350 μm.
In contrast, 3D Honeycomb Boosted (#KOU-3D-HCB) showed a sharp peak of 200 to 250 μm (based on in-house data).In addition, 1 x 10^4 fibroblast cells were seeded on Honeycomb Disk 96 with a height of 2 mm and 3D Honeycomb Boosted with a height of 5 mm. Both were large enough to fit into a 96-well plate and enabled the seeding and culture of more cells. Comparing these, it was suggested that the 3D Honeycomb Boosted, which is 2.5 times taller, can seed and culture more cells (based on two in-house data obtained at different times).

Example 1 Using Honeycomb: Cultivation of patient-derived cancer tissue and evaluation of antitumor substances

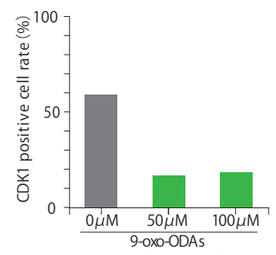
[Reference: Sci Rep. 2023 Nov 6;13(1):19208. Created by modifying figure 6a, 6b and 6c. ©Mogi K., et al. 2023 (Licensed under CC BY 4.0) https://creativecommons.org/licenses/by/4.0/]



Schematic diagram of culture of patient- derived cancer tissue



Immunostaining of CDK1 and cleaved caspase 3

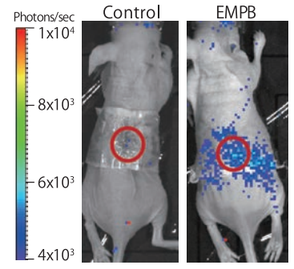


Quantification of CDK1 immunostaining images

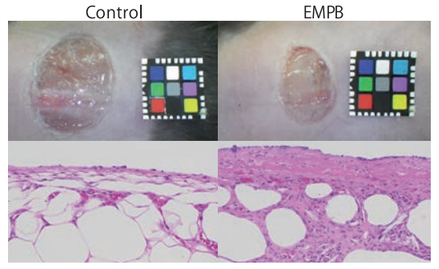
Eggplant calyx has been used in Japan as a folk remedy for verruca vulgaris caused by human papillomavirus. Recently, 9-oxo- ODAs with cell proliferation inhibitory effects were identified in eggplant calyx extracts. In this study, to verify the antitumor effect of 9-oxo-ODAs *ex vivo*, finely sliced cervical cancer tissue was placed on honeycomb (CSH) and cultured with 9-oxo-ODAs. Immunostaining showed that the percentage of cells positive for CDK1, which is involved in cell cycle progression, decreased in the 9-oxo-ODAs-treated group, and the percentage of cells positive for cleaved caspase 3, an apoptosis marker, increased. This experiment suggests that CSH may be useful for *ex vivo* culture of patient-derived cancer tissues and for evaluating antitumor substances (Reference 1).

Example 2 Administration of medical plant extract and cell migration using CSH

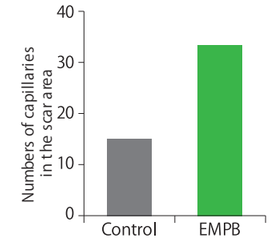
(Maeda A, Skin Regeneration, PIAS Collaborative Research, Osaka University)



Homing assay of Luc-MSCs at the wound site 6 days after intravenous injection using IVIS



Images of wound and H&E stained wound area 17 days after EMPB administration

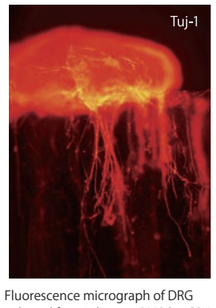


Quantitative evaluation of capillary number in the scar area

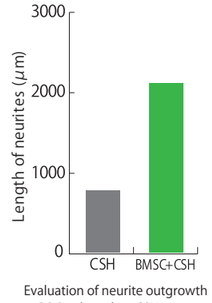
The ethanol extract from Mallotus philippinensis bark (EMPB) is impregnated to CSH and topically applied to the wound of mouse. Then, luciferase-expressing MSCs (Luc-MSCs) were intravenously injected. As a result, accumulation of Luc-MSCs at the wound area was promoted in EMPB treated group. Further evaluations at 17 days after EMPB administration to the wound area revealed that accelerated wound healing and increased numbers of capillaries and granulation tissue. These results suggests that CSH is useful for sustained release of bioactive substance (Ref. 7).

Example 3 Implantation of bone marrow stem cells (BMSCs) into hemisected spinal cord using CSH

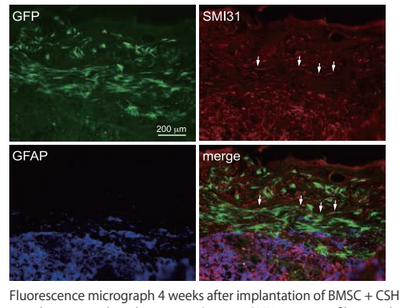
(Enomoto M, Department of Orthopaedic Surgery and Hyperbaric Medical Center, Tokyo Medical and Dental University)



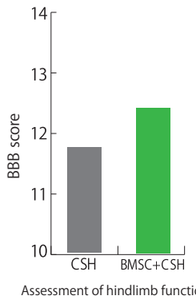
Fluorescence micrograph of DRG cultured for 10 days on BMSC + CSH



Evaluation of neurite outgrowth in DRG cultured on CSH or BMSC + CSH



Fluorescence micrograph 4 weeks after implantation of BMSC + CSH into the rat spinal cord injury site (arrows: SMI31-positive fibers within GFAP-negative areas)



Assessment of hindlimb function 4 weeks after transplantation into the rat spinal cord injury site

A previous report demonstrated that the pore structure of CSH enhances nerve regeneration (Ref. 9). In this research, a remarkable increase of neurite growth was observed when dorsal root ganglia (DRG)s were cultured on GEP-expressing BMSCs contained CSH (BMSCs+CSH) for ten days compared to DRGs cultured on CSH alone. Improved locomotor and sensory function was also observed four weeks after implantation of BMSC+CSH into hemisected spinal cord (Ref. 4). [Tuj-1: A maker for neuron; SMI31: A marker for neurofi lament; GFAP: a marker for astrocyte; BBB score: an evaluation method for hindlimb motor function]