NOVEL 3D CULTURE SUBSTRATE FOR ORGANOIDS CONTAINING COLLAGEN, LAMININ-E8, AND HYALURONAN

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Abstract

We evaluated gels composed of (1) collagen, (2) laminin 511E8 fragments containing interaction sites with cell surface integrin receptors, and (3) hyaluronic acid as three- dimensional cell culture substrates capable of inducing tissue formation that mimics in vivo conditions. The efficacy was examined using liver organoid formation, cancer organoid formation, and cranial nerve organoid formation as indices.

When primary human hepatocytes were cultured in gel, this gel, hereafter referred to as MM511 gel, showed superior effects on proliferation and hepatic parenchymal differentiation compared to Matrigel culture.

Primary hepatocytes proliferated well in type I collagen gel, but most cells were induced to differentiate into bile ducts. On the other hand, in MM511 gel, despite using the same concentration of type I collagen, inhibited bile duct differentiation, and many cells formed spheroids with positive hepatic parenchymal markers. The above induction of hepatic parenchymal spheroid formation in MM511 gel culture was also notable in MM511 gel culture mixed with pepsin-solubilized type IV collagen.

For developing mouse brain neuron culture, dispersed cells formed spheroids in MM511 gels with additional type V collagen and in MM511 gels with additional hyaluronic acid and proteoglycans. An induction of astrocyte differentiation was observed in addition to neuronal differentiation. In contrast to cultures in Matrigel, numerous large sphere formations positive for astrocytic cell markers were observed in MM511 gel cultures.

Patient-derived colorectal cancer cells in MM511 gel culture showed good proliferation and formation of large colorectal cancer spheroids. In MM511 gel cultures of patient-derived colorectal cancer cells, rapid proliferation and formation of large colorectal cancer spheroids were observed. The spheroids contained cells positive for metastatic markers that were not observed in cultures in Matrigel. Cross-sectional observation of the spheroid structure in frozen sections revealed a two-layered structure with an outer and inner cell populations. The interior of the spheroid was rich in hyaluronic acid, while the exterior of the spheroid was rich in type I collagen.

MM511 gel provides a diverse extracellular environment to induce differentiation.

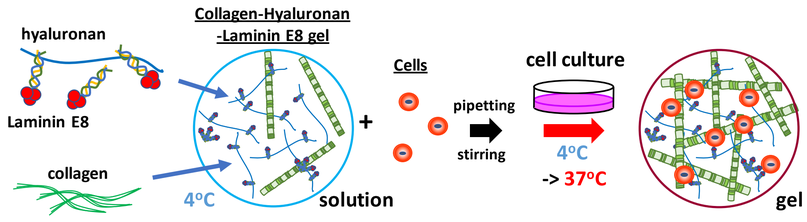
Introduction

Three-dimensional cell culture is essential for mimicking human tissues and organs. Currently, Matrigel is widely used as a scaffold. Although this gel has been used for a variety of applications, the cells often do not exhibit tissue structure and function shown as *in vivo*. In order to develop a new substrate, we combined collagens, hyaluronic acid, and laminin E8 fragment.

Materials and Methods

- Sodium hyaluronate (average MW 1,200-2,200 kDa, HA-LQH, made by fermentation method with *Streptococcus zooepidemicus*, Kewpie, Japan) is cross-linked with human recombinant laminin-511 E8 fragment (Nippi, Inc., Japan) using genipin.
- Pepsin-extracted porcine skin collagen (roughly 80-85% of type I and 15-20% type III collagens, Nippi, Inc.) and acid-soluble bovine skin collagen (Nippi, Inc.) was used.
- Type IV collagen was extracted from porcine kidney with pepsin.
- Type V collagen was extracted from porcine cornea.

Collagen-Hyaluronan-Laminin E8 gel (MM511 gel)



	Molecular weight	Final concentration of each content of the gel
Collagen	MW 300K, 300 nm x 1.5 nm	1,000-2,000 µg/mL
Hyaluronan	MW 1200K-2200K	200 µg/mL
Laminin E8	MW 150K	5 µg/mL

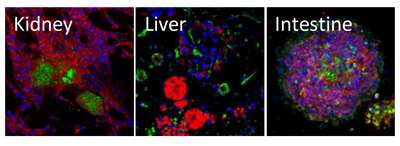
Laminin E8 fragment

The laminin C-terminal E8 fragment, which is about 1/5 of the full-length laminin molecule, is recombinantly expressed. Laminin-511E8 has a strong interaction with cellular integrin α6β1 and induces cell motility.

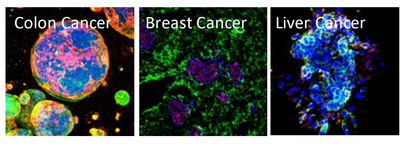
Pros

1. Easy to use
2. Highly versatile (can be used with a variety of cells)
3. Excellent in cell organization

Induction of organoid formation in a variety of internal organs



Induction of organoid formation by cancer cells



Studies that cannot be carried out with Matrigel

✓Multi-organ organoid formation

✓Organoid formation using adult patient-derived cells

For Organoid research

---- especially for not satisfied with current 3D substrate

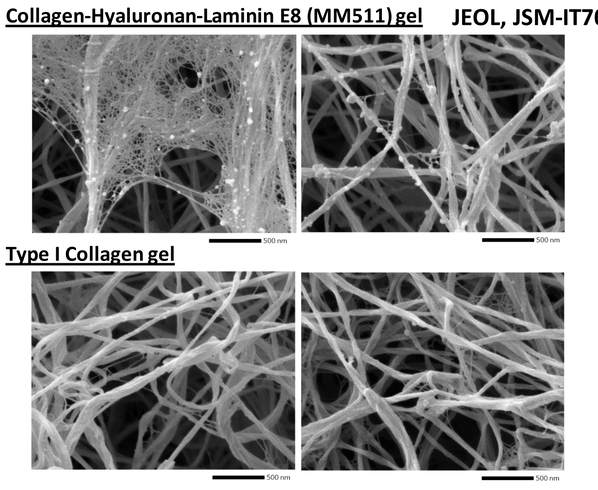
Drug screening

✓ New, more in vivo mimetic in vitro model that is not currently available using Matrigel

✓Screening of drugs for highly malignant cancers with metastatic potential

Scanning Electron Microscope observation

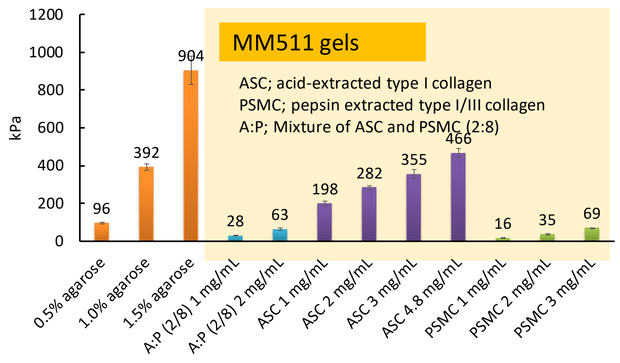
Collagen-Hyaluronan-Laminin E8 (MM511) gel — JEOL, JSM-IT700HR



Type I Collagen gel

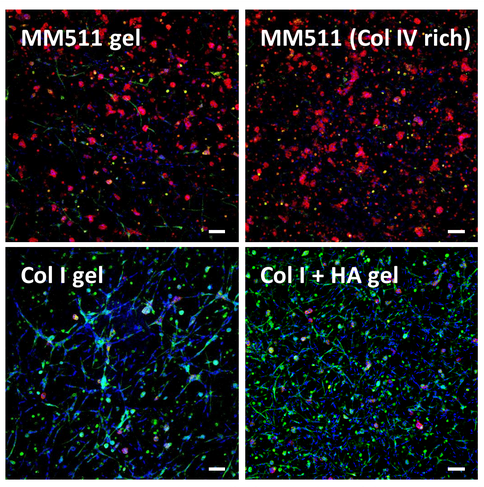
Thinner fibrils and granules were observed in the MM511 gel.

Elastic modulus of MM511 gels and agarose gels



The elastic modulus of MM511 gels can be modified by changing the types and concentration of collagen.

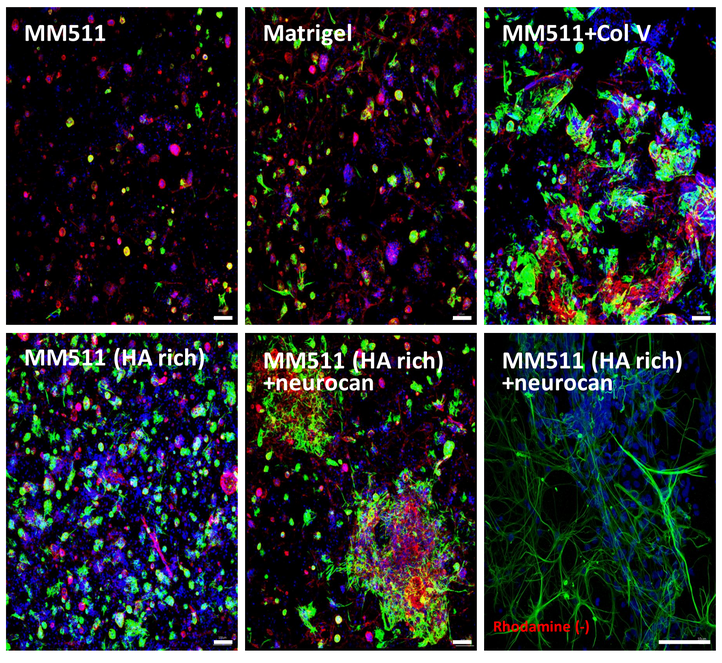
Culture of human adult liver cells in MM511 gels



Culture at Day 7; Hepatocyte (ALB), Bile duct (CK19), Nuclei (DAPI), Bar 100 µm

Bile duct differentiation was suppressed in MM511 gel, and many cells formed spheroids positive for hepatic parenchymal marker.

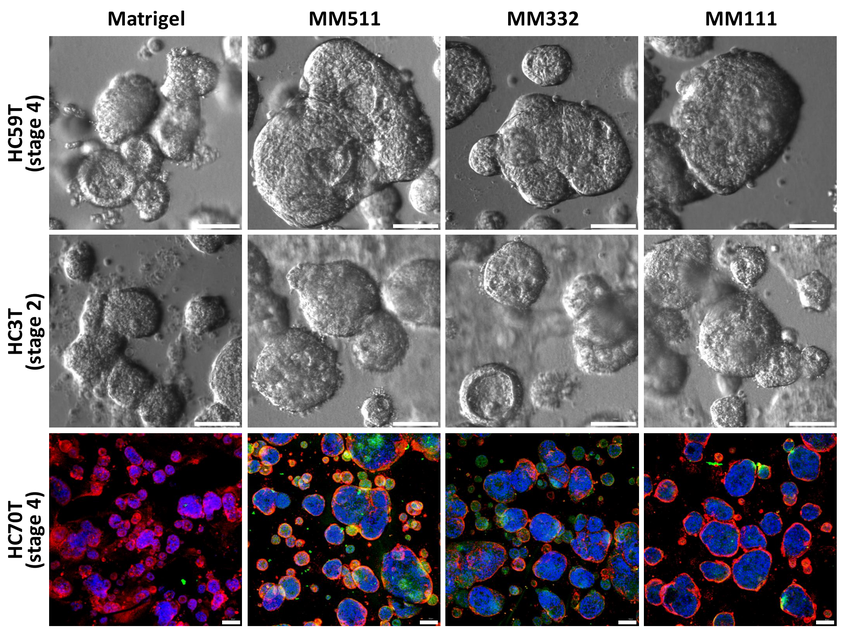
Culture of mouse brain neuron in MM511 gels



Culture at Day 7; Rhodamine, astrocyte (GFAP), Nuclei (DAPI), Bar 100 µm

Numerous large sphere formations positive for astrocytic cell markers were observed in MM511 gel cultures.

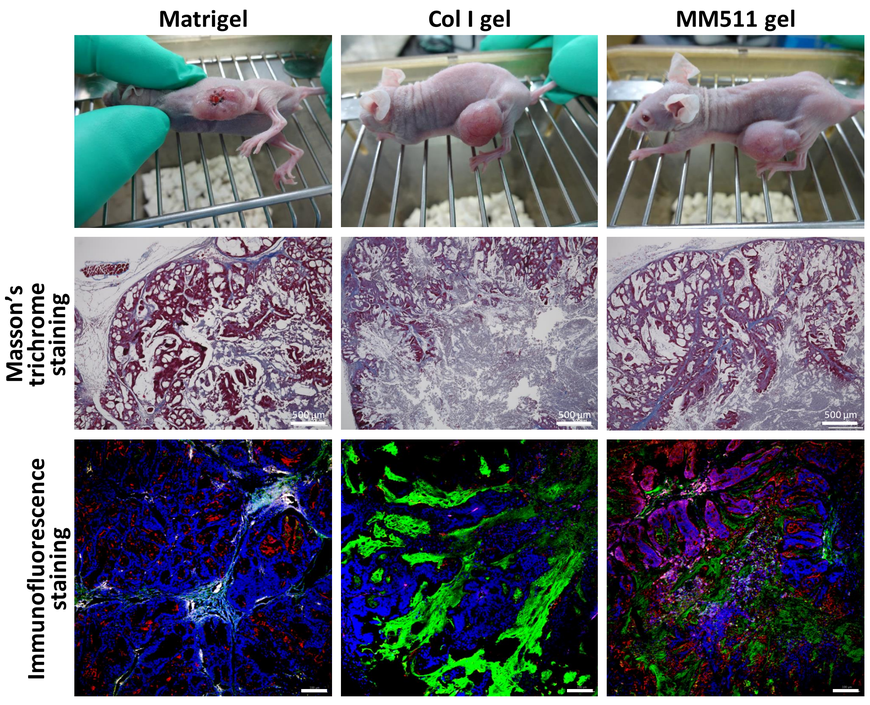
Effects of MM511 gels on colon cancer patients derived spheroid cultures



Culture at Day 8; Colon cancer marker (CEA), Vimentin, Nuclei (DAPI), Bar 100 µm

In MM511 gel cultures, rapid proliferation and formation of large colorectal cancer spheroids contained cells positive for vimentin were observed.

Patient-derived spheroid xenograft (PDSX) model



Colon cancer marker (CEA), Col I, Nuclei (DAPI), Bar 100 µm

- With either gel, patient-derived colorectal cancer cells were transplanted into nude mice, and tumors formed in four weeks.
- When type I collagen gel was used, the tumors containing a lot of stroma formed.
- When type I collagen gel was used, less CEA-positive cells were observed in the tumors. On the other hand, when MM511 gel was used for transplantation, many CEA-positive cells were observed as well as Matrigel.

Summary

- The gel with collagen, hyaluronan, and laminin E8 is suitable for 3D cell culture.
- At 4°C, the gel is in solution, and when incubated at 37°C, it becomes a gel due to the collagen fibril network formation.
- The elastic modulus of MM511 gels can be modified by changing the types and concentration of collagen.
- Cell culture of various organ tissues is possible by adjusting collagen types (I/III/IV/V/ XVIII) and concentration, laminin isoform (111E8/221E8/332E8/411E8/511E8), and various ECM components (proteoglycans etc.).
- It can be used for transplantation of patient-derived cancer spheroids into nude mice as well as BME/Matrigel.

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