NEW ORGANOID SCAFFOLD WITH FIBRILLAR COLLAGEN, LAMININ, AND HYALURONIC ACID

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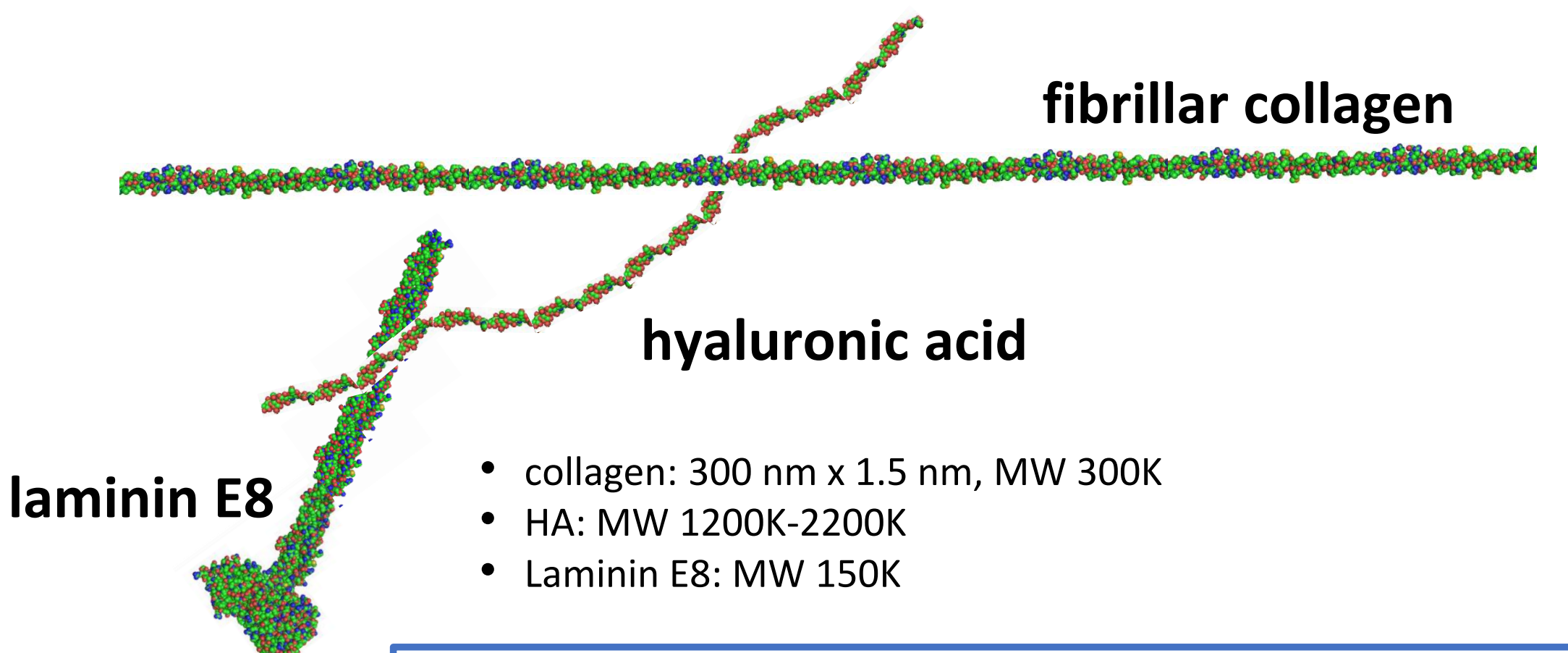
Introduction

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 as in vivo. In order to develop a new substrate, we combined collagens, hyaluronic acid, and laminin E8 fragment.

Materials and Methods

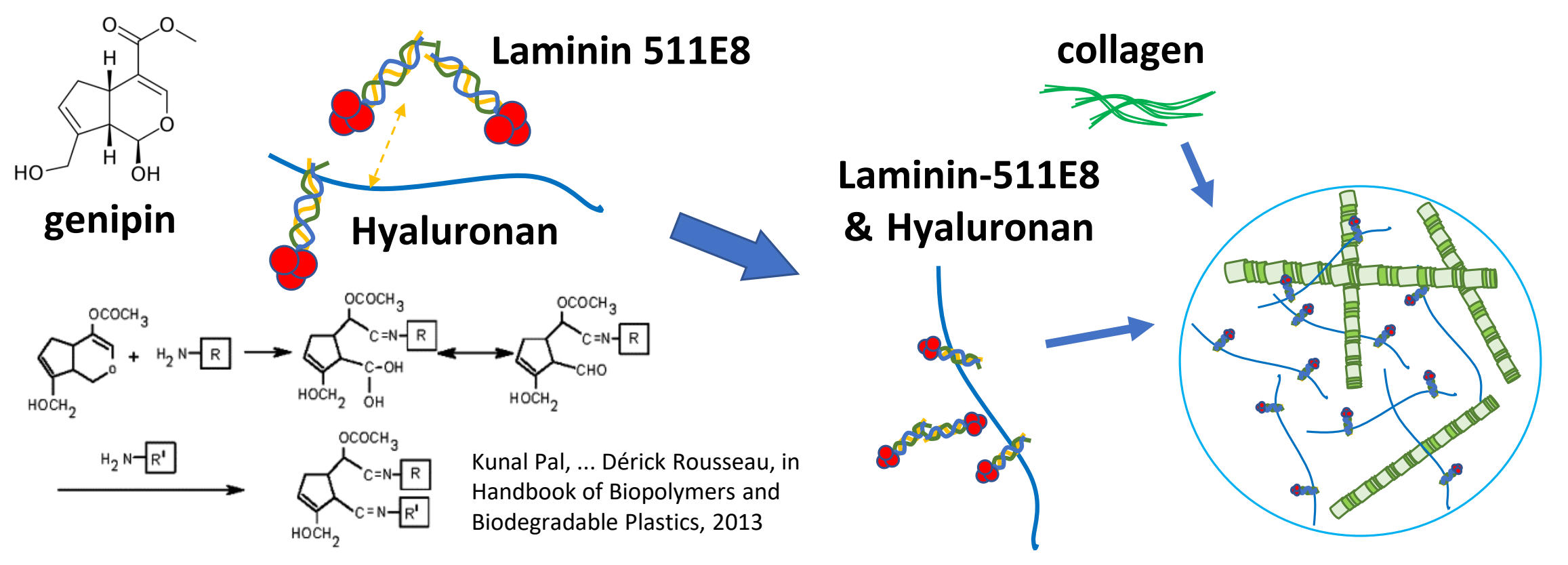
- Sodium hyaluronate (average MW 1,200-2,200 kDa, HA-LQH, made by fermentation method with *Streptococcus zooepidemicus*, Kewpie, Japan) is cross-linked with human recombinant laminin-511 E8 fragment (Nippi, Inc., Japan) using genipin.
- Pepsin-extracted porcine skin collagen (roughly 80-85% of type 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.



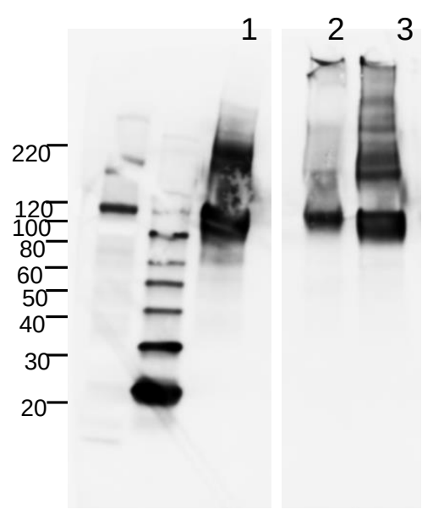
- collagen: 300 nm x 1.5 nm, MW 300K
- HA: MW 1200K-2200K
- Laminin E8: MW 150K

Final concentration of each content of the gel
Collagen: **1,000-2,000 μg/mL**
Hyaluronic acid: **200 μg/mL**
Laminin-511 E8: **5 μg/mL**

Collagen-Hyaluronic acid-Laminin E8 gel



Kunal Pal, … Dérick Rousseau, in Handbook of Biopolymers and Biodegradable Plastics, 2013



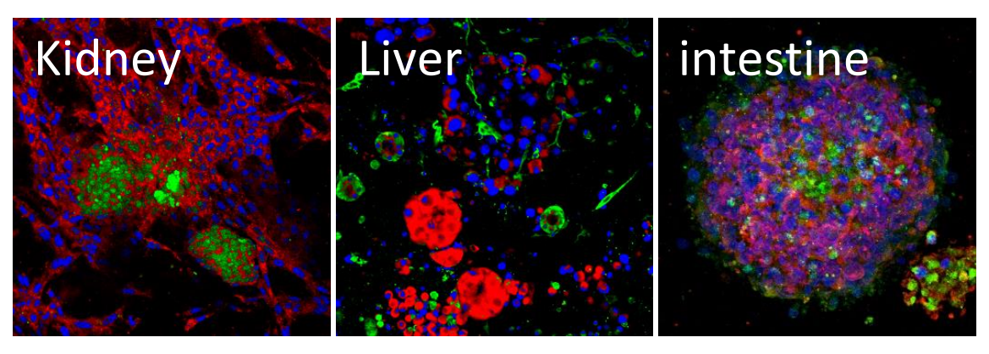
Cross-linking between amino groups with genipin

1) laminin 511 E8
2) laminin 511 E8 crosslinked with HA
3) laminin 511 E8 crosslinked (control)

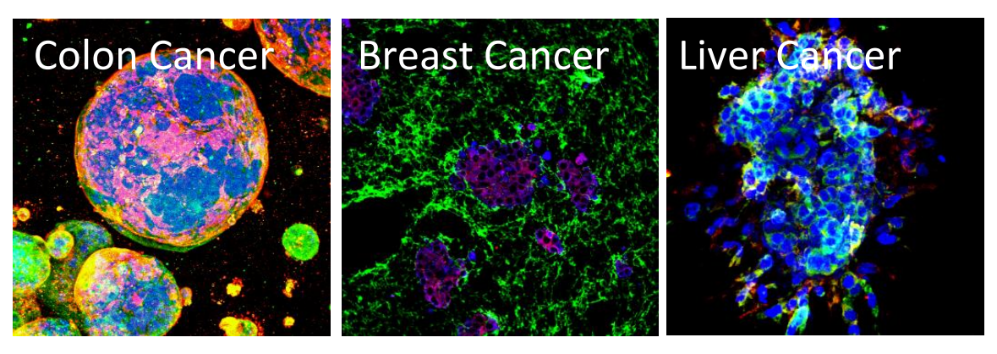
The laminin C-terminal E8 fragment, about 1/5 of the laminin molecule is recombinantly expressed. Laminin-511E8 has a strong interaction with cellular integrin α6β1 and induces cell motility.

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

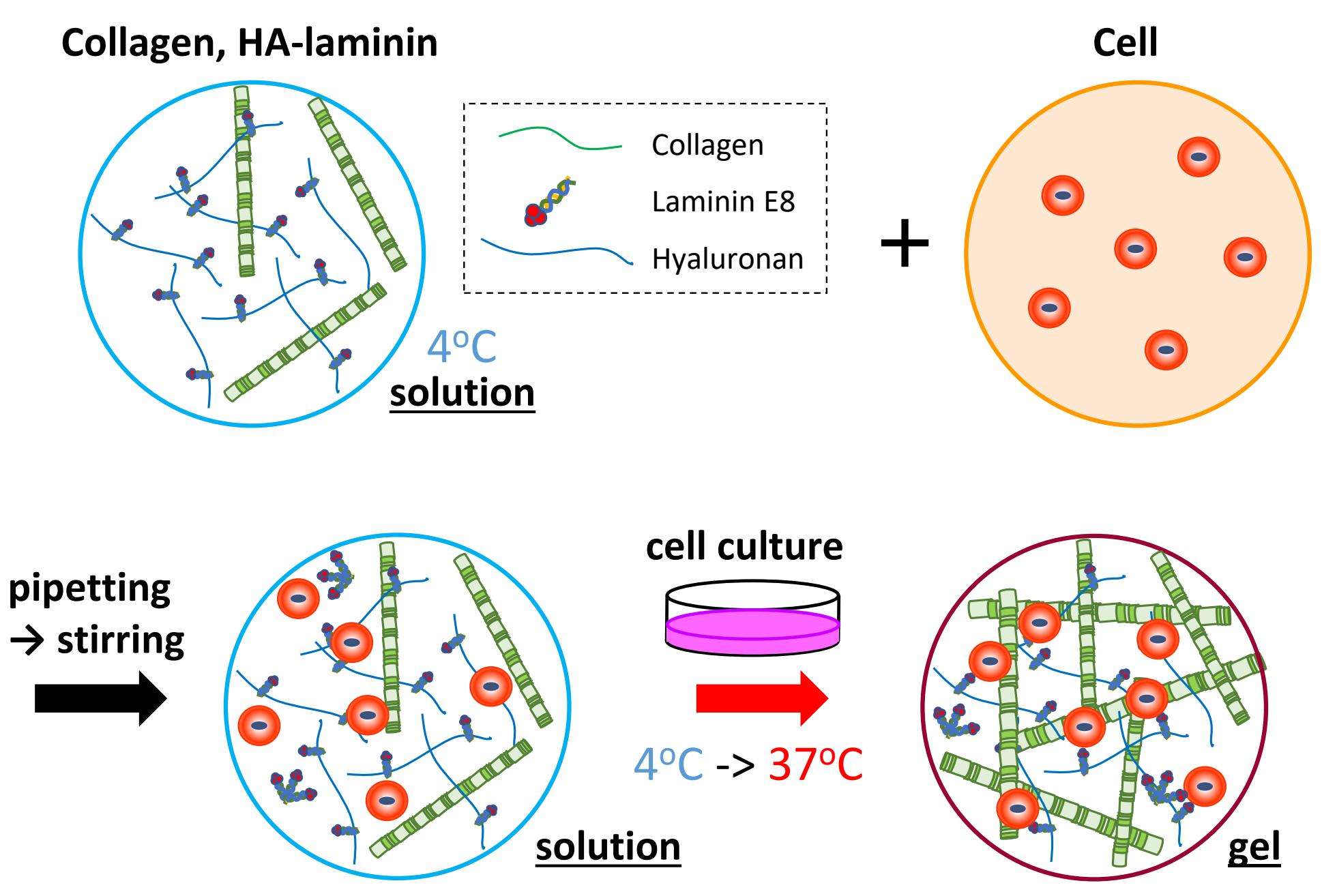


Studies that cannot be carried out with Matrigel
✓Multi-organ organoid formation
✓Organoid formation using adult patient-derived cells

For Organoid research
---- especially for not satisfied with current 3D substrate

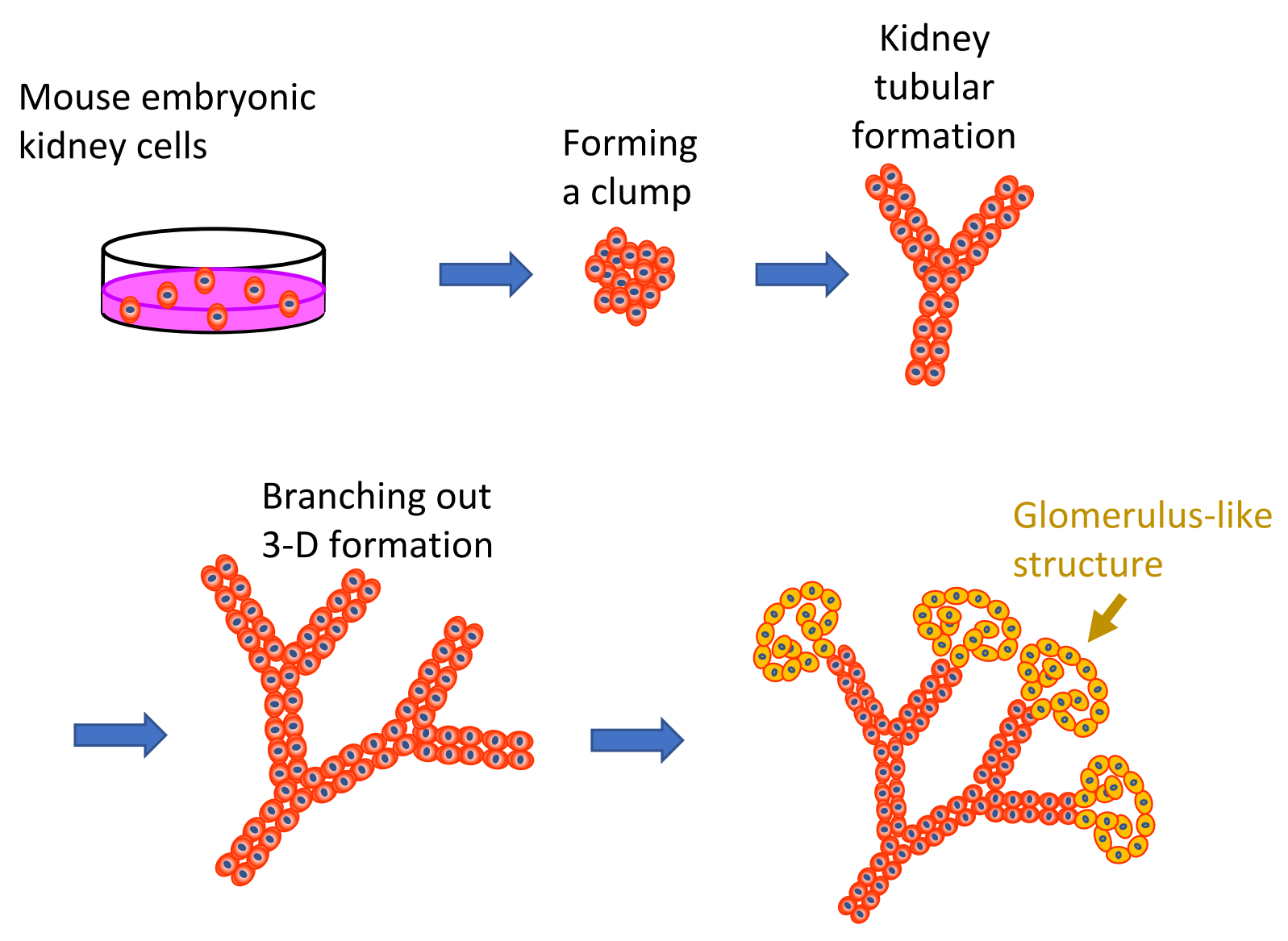
Drug screening
New, more in vivo mimetic in vitro model that is not currently available using Matrigel
✓When culturing cells that are difficult to grow and organize in Matrigel
✓Drug screening that can be completed in vitro without transplantation into a mouse model
✓Screening of drugs for highly malignant cancers with metastatic potential

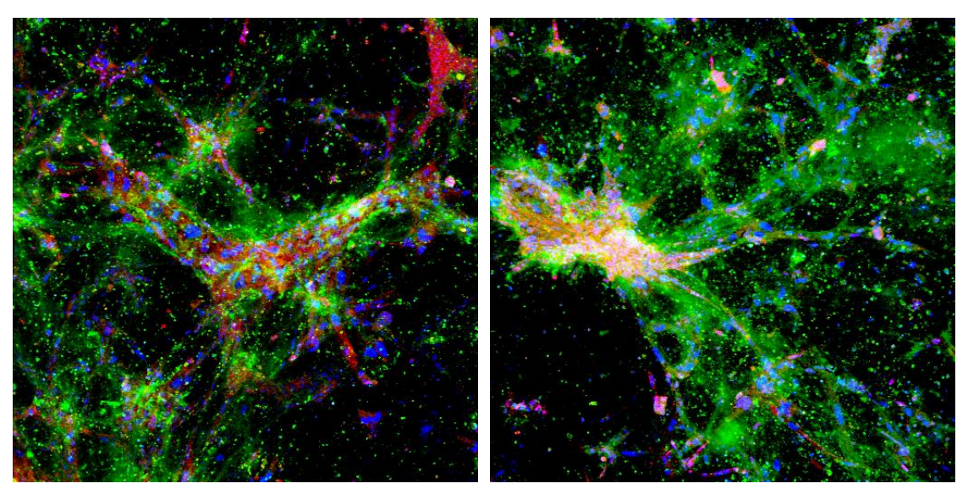
Gel Culture Protocol



Mix with cells for In-gel culture

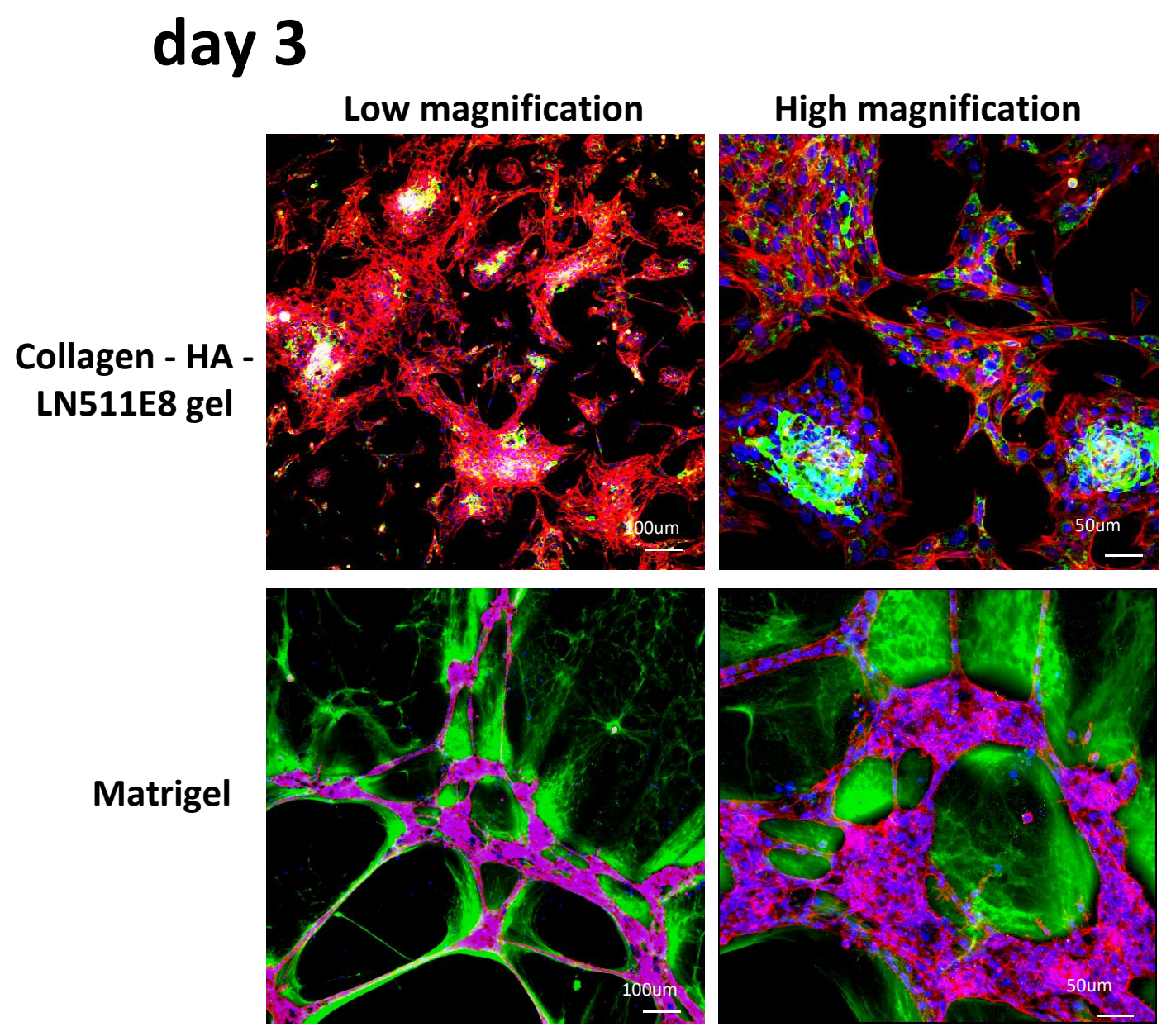
Organization of renal cells induced by 3D culture



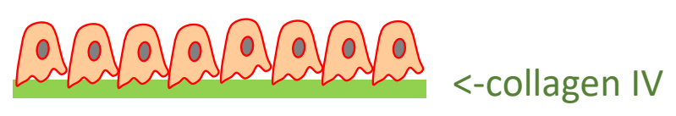


Culture at day 7 DBA (green), PECAM (red), DAPI (blue)

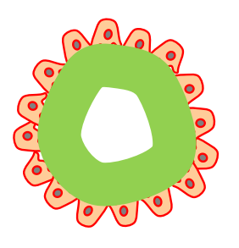
Culture of mouse embryonic kidney cells



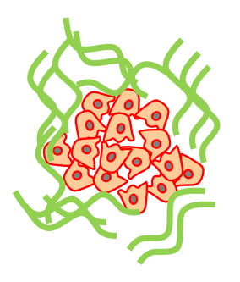
Type IV collagen (green), actin (red), nuclei (blue)



Type IV collagen is a major component of basement membrane.

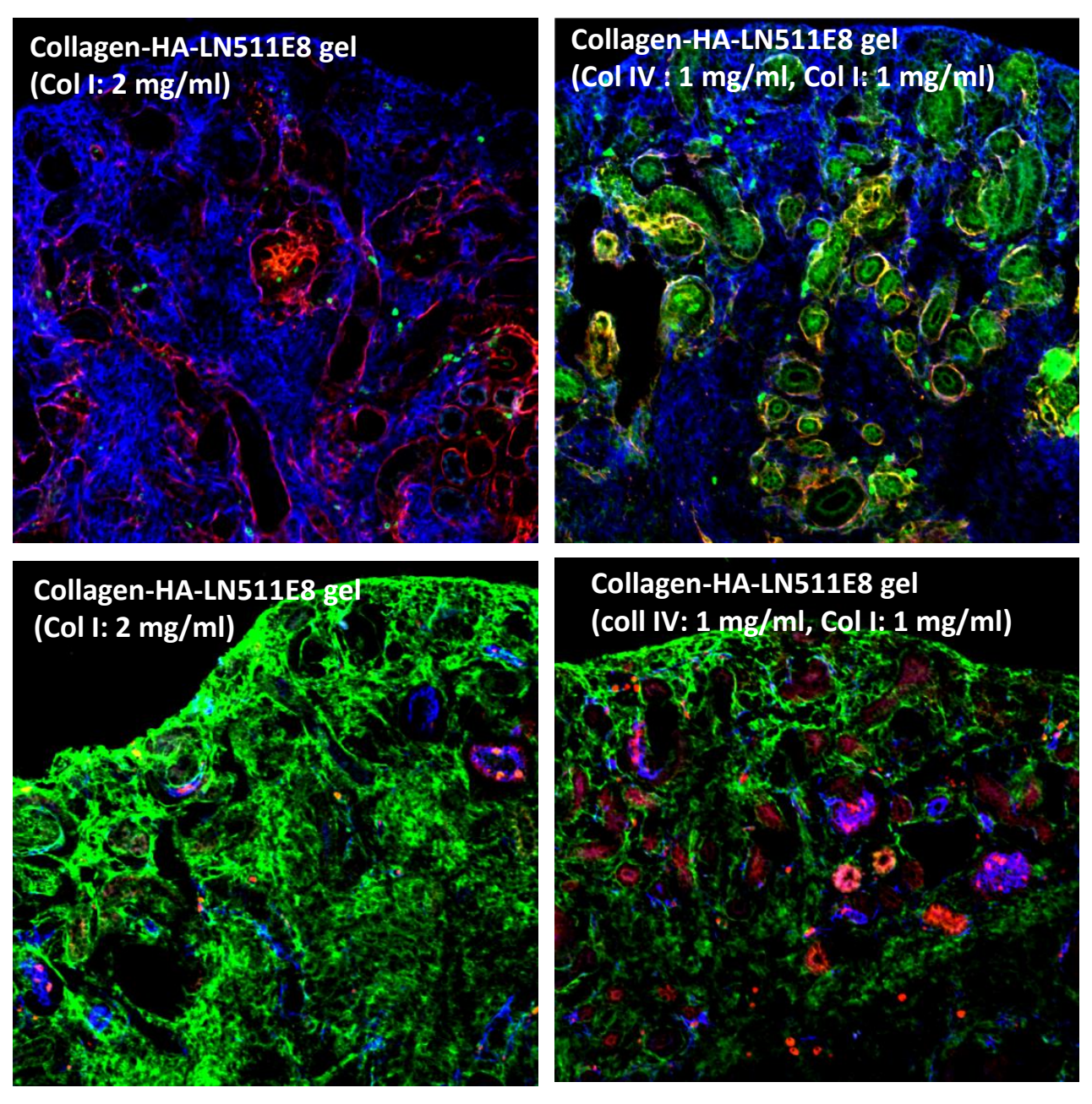


Type IV collagen localizes inside of the cell aggregate cultured in collagen-HA-LN511E8 gel



Type IV collagen localizes on the outside of the cell aggregate on Matrigel

Culture of mouse embryonic kidney cells with Coll I/IV-HA-LN gel



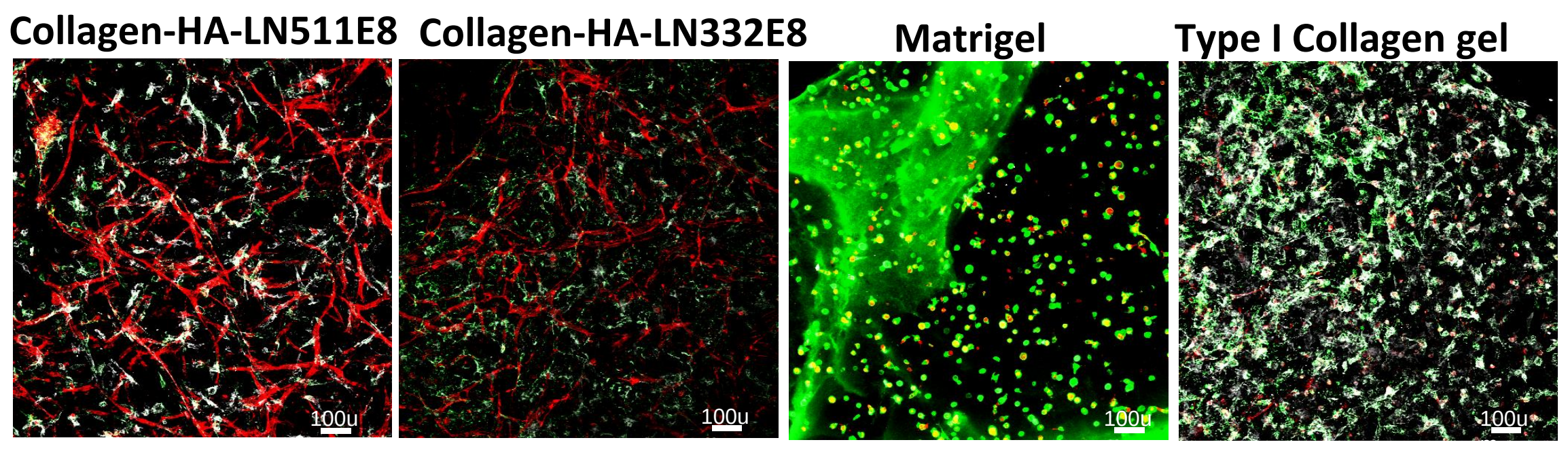
Effect of type IV collagen on organoid culture of mouse embryonic kidney cells.
Type IV collagen was mixed to type I collagen.

Type IV collagen (green)
Type I collagen (red)
HA (blue)

The addition of type IV collagen enhanced differentiation into renal epithelial tissue.

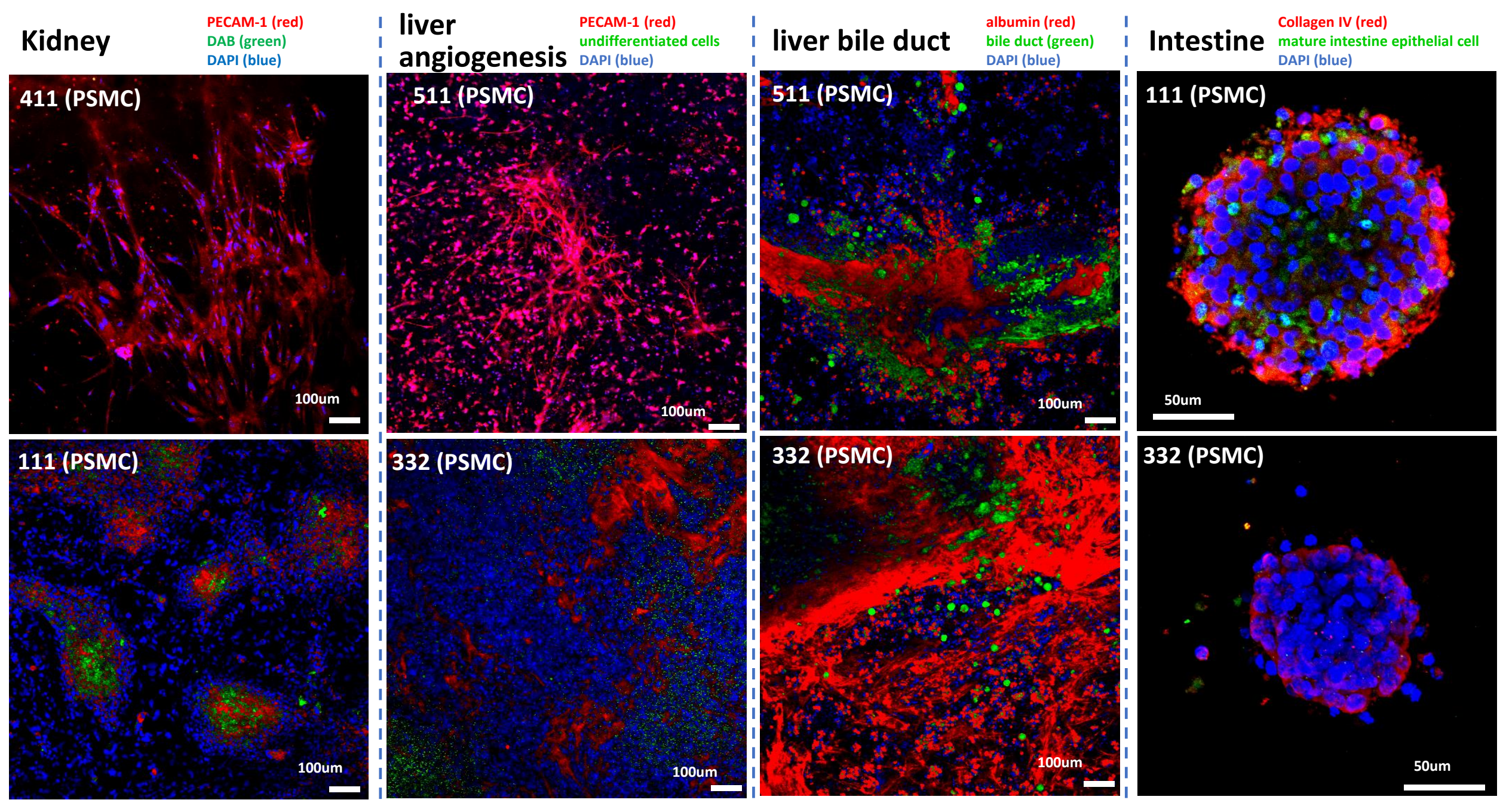
HA (green)
Nephrin (red)
PECAM-1 (blue)

Culture of human aorta endothelial cells

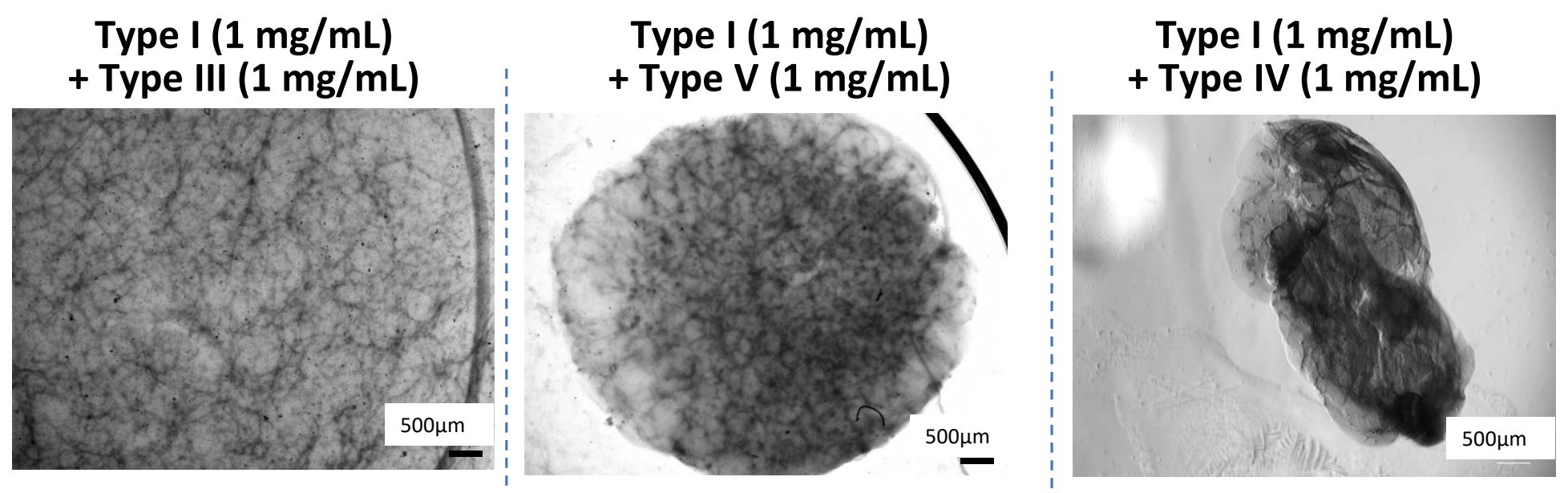


culture at Day 7 Collagen I (white), collagen IV (green), Actin (red)

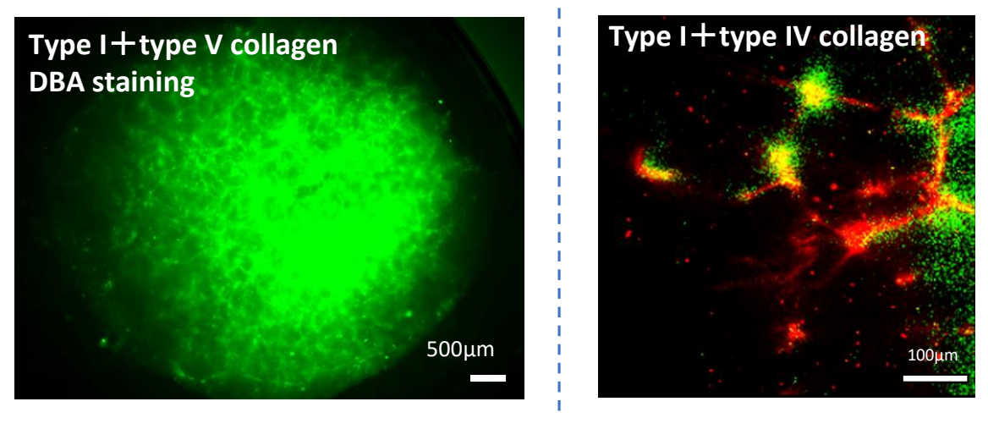
Effect of laminin E8 isoforms on organoid



Effect of collagen types on mouse 13d embryonic kidney organoid in collagen-HA-LN511E8 gel

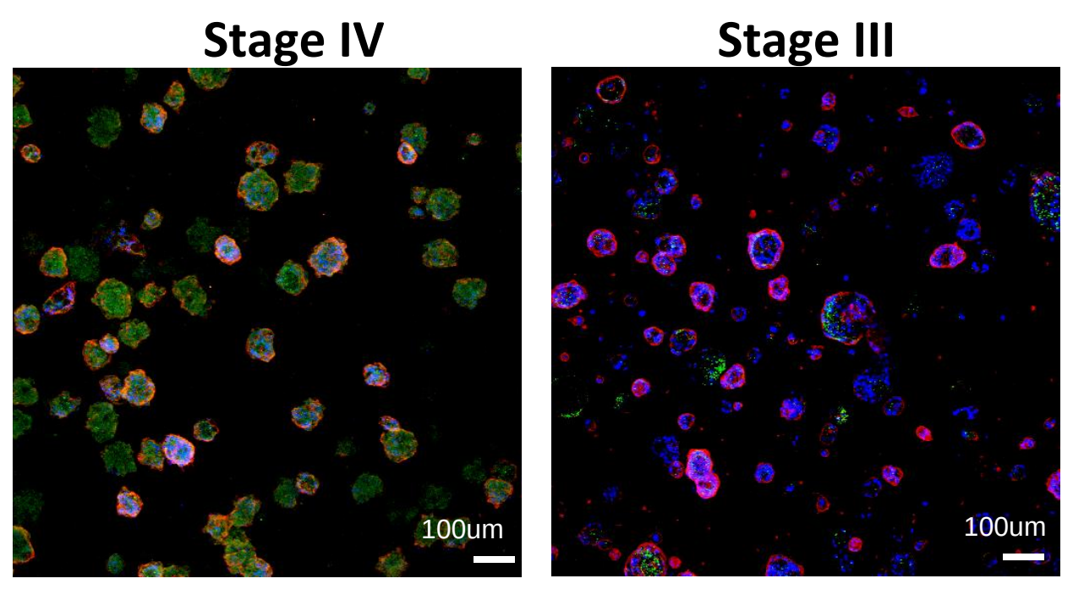


Type V collagen mixed gel is superior in inducing ureterogenesis.
On the other hand, Type IV mixed gel is difficult to observe internally due to severe shrinkage, but is excellent in inducing glomeruloid-like tissue formation by fluorescent observation using antibody staining.



PECAM-1 (red)
Nephrin (green)

Effects of collagen-HA-LN511E8 gels on colon cancer patients derived spheroid cultures



In Collagen-HA-LN511E8 gels, cells derived from patients with Stage 4 metastatic to other organs showed cell population positive for the metastatic potential marker ZEB-1.
In contrast, cells derived from Stage 3 patients without metastasis derived cells, ZEB-1 expression was suppressed.

ZEB-1 (green), E-cad (red), DAPI (blue)

This collagen-HA-LN511E8 gel can be used for transplantation of cancer spheroids into nude mice as well as Matrigel.

Summary

- The gel with collagen, hyaluronic acid, and laminin E8 is suitable for 3D cell culture.
- At 4C, the gel is in a solution state, and when incubated at 37C, it becomes a gel.
- The composition of the gel is clear, and the raw materials are inexpensive.
- Cell culture of various organ tissues is possible by adjusting collagen type and concentration, laminin isoform, and various ECM components.
- For collagens, in addition to major interstitial type I collagen, fibrous collagen types III and V, as well as collagen types IV and XVIII of the basement membrane.
- As laminin, it is also possible to mix 111, 221, 332, 411, 511, etc. in appropriate proportions according to the integrin receptors of the cell membrane.
- It can be used for transplantation of cancer spheroids into nude mice as well as BME/Matrigel.