Mouse Intestinal Organoid Culture From Primary Tissues

Introduction

The GelNest™ Matrix by NEST, developed from mouse tumor tissue, enhances cell adhesion, differentiation and proliferation. It mimics the physiological environment, making it ideal for research in tissue engineering, cell culture, and particularly useful in organoid and stem cell culture. It also aids in cancer research, focusing on invasion, angiogenesis, and in vivo tumor formation.

This application note highlights its use in mouse intestinal organoid culture from primary tissues. In 2009, Hans Clevers and his team produced a three-dimensional small intestine organoid structure in vitro from Lgr5+ intestinal stem cells. This structure mirrors the physiological conditions of the intestinal epithelium, enabling long-term in vitro cultivation while maintaining cellular differentiation capacity. This method is increasingly used in stem cell research, disease models, and regenerative medicine.

Small intestine organoids can be cultured either from pluripotent or embryonic stem cells, or from crypt stem cells of the small intestine. The latter is more common due to convenience, technical simplicity, and the potential for multiple passages, allowing for long-term in vitro cultivation for up to two months.

Materials & Methods

1. Euthanize the mouse and collect 15cm of the intestine from the end of the ileum. Remove outer membrane and flush with ice-cold PBS. Use scissors to cut open the intestine segment with the lumen facing up. Gently wash the cut intestine segment with ice-cold PBS.
2. Add 15 mL of ice-cold PBS to a 50 mL centrifuge tube. Cut the intestine into 1~2 mm pieces with scissors and wash with PBS. Repeat the wash step until clear. Remove the supernatant and resuspend the tissue fragments in 25 mL of intestinal crypt digestion solution (contains 2-5mM EDTA), incubate on ice for 20 min, and rotate on a 20 rpm rotating bed for 30 min. Remove the supernatant.
3. Resuspend the tissue fragments in 10 mL of cold (2-8℃) PBS buffer solution with 10% FBS. Filter the supernatant through a 70 μm filter and the filtrate in a clean 50 mL tube. Repeat the filtration 3 times and combine the filtrates obtained.
4. Centrifuge the collected solution at 300×g for 5 minutes at 2-8 ℃. Discard the supernatant.
5. Resuspend the precipitate in 10 mL of PBS buffer solution. Centrifuge at 200×g for 3 minutes. Remove the supernatant. Repeat the centrifuge step if there are excessive single cells in the suspension.

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6. Take 10 µL sample and count crypts under a microscope. Centrifuge at 200×g for 3 minutes and remove the supernatant.

Note: Crypts suitable for culture can come in different sizes and typically have a rectangular or circular shape with pretty smooth edges. They appear like small, folded portions of an epithelial monolayer. Fractions that have a high concentration of villi, single cells, or debris are not ideal for organoid culture. The goal is to achieve the highest enrichment for suitable crypts.

7. Resuspend the crypts in the intestinal organoid complete medium with 8-20 crypts per µL media. To make the complete medium, add:

Content	Working Concentration
N-2 Supplement (NEST 211641 or equivalents)	1X
B-27 Serum Free Supplement (NEST 211611 or equivalents)	1X
Wtn3A, Noggin, R-spondin3 (NEST 211511 or equivalents)	1X
N-acetylcysteine	1mM
EGF	100 ng/mL
DMEM/F-12 Culture Medium	1X

8. Extract 150 µL of the crypt suspension, mix it with an equal volume of non-diluted GelNest™ Matrix for Organoid Culture (NEST 211272), then gently pipette up and down ten times for thorough blending.
9. Add 50 µL of the sample to the **center** of each well in a pre-warmed 24-well plate, forming a dome-like structure. Avoid bubble formation.
10. Allow the plate to sit undisturbed at 37℃ for 10 minutes for the matrix gel to solidify. Handle the plate carefully to avoid disturbing the gel.
11. Carefully add 500 µL of room-temperature (15-25℃) organoid culture medium to each well, pouring it down the side to avoid disturbing the gel.
12. Monitor organoid growth. Typically, crypts form a spherical structure around 3 hours into culture. After 2-4 days, the organoid begins to bud, forming a complex structure by the 5th to 7th day.
13. Entirely replace the medium the next day. Carefully remove the old medium from the edge of the well and add 500 µL of fresh, room temperature organoid culture medium.
14. Observe the intestinal organoid under inverted microscope regularly and record.
15. Select suitable antibodies to stain the organoid and visualize under a fluorescence microscope, along with including proper controls for specificity. (Please refer to other protocols for immunostaining steps.)

Results

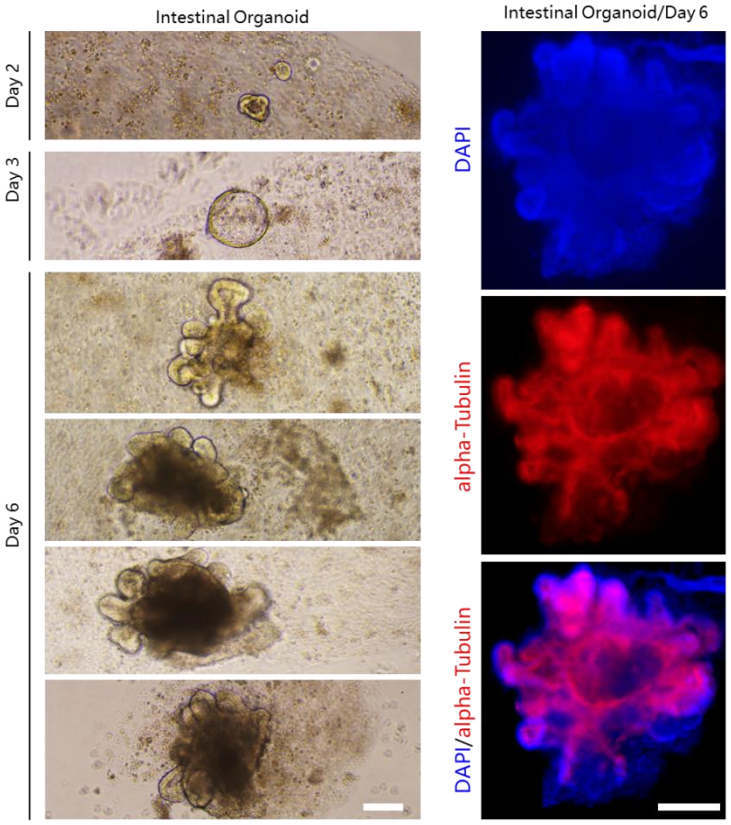
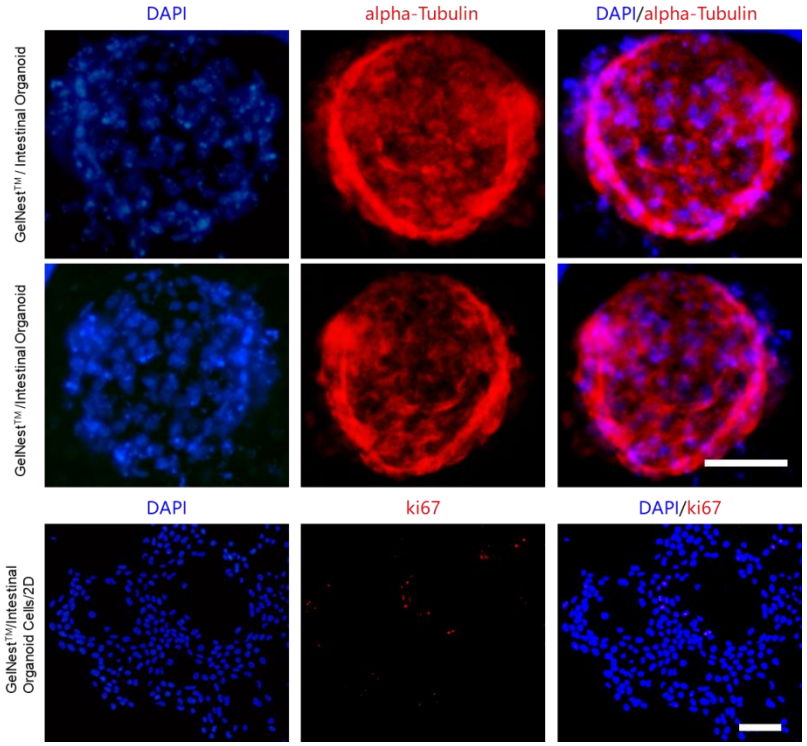


Figure 1. Growth of mouse small intestine organoids in GelNest™ Matrix gel. On the second day under a 10x objective, small bubble-like structures can be observed; on the third day, significantly enlarged bubble structures can be observed; on the sixth day, small intestine organoids with multiple budding structures can be observed, and the organoids in the culture dish can be directly observed by the naked eye as white granular cell clusters. The scale is 200 micrometers.

Figure 2. Immunostaining results of cytoskeletal proteins and cell proliferation antigens in early small intestine organoids It can be observed that the early small intestine organoids grown in GelNest™ matrix gel are hollow structures formed by a single cell layer, and the small intestine organoids planted in a petri dish coated with 100-fold diluted GelNest™ Matrix gel still maintain good proliferative ability (ki67 positive). DAPI is the cell dye nucleus (blue) alpha-Tubulin is the cytoskeletal protein (red); ki67 is a marker for cell proliferation activity expressed in the nucleus. The scale is 150 microns.



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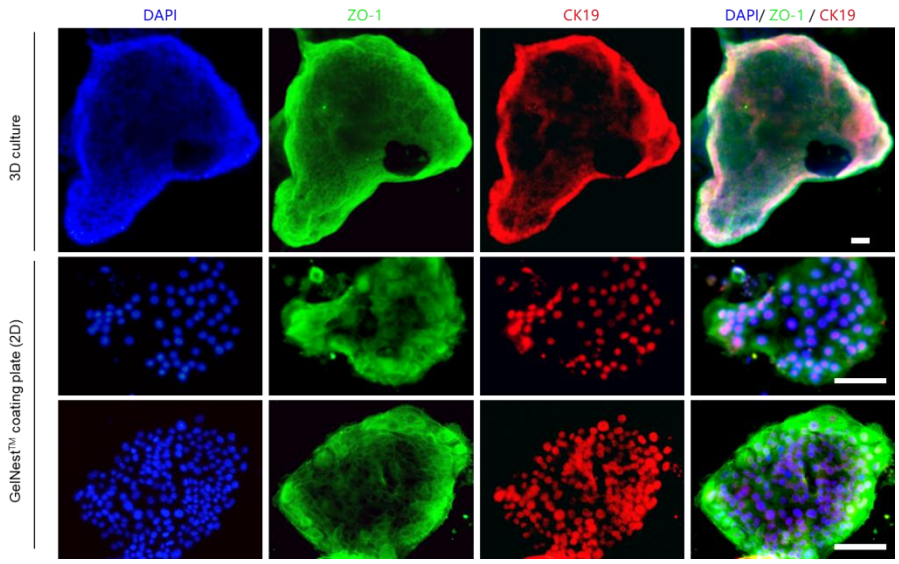

Figure 3. Immunostaining results of tight junction proteins and epithelial cell characteristic proteins in small intestine organoids. It can be observed that small intestine organoids growing in GelNest™ Matrix (3D) or on the thin layer of matrix gel (2D) can maintain the expression of biofunctional cell markers very well. DAPI is a cell dye nucleus (blue); ZO-1 is a marker of cell tight junction proteins (green); CK19 is a marker of epithelial cell characteristics. The scale is 100 micrometers.

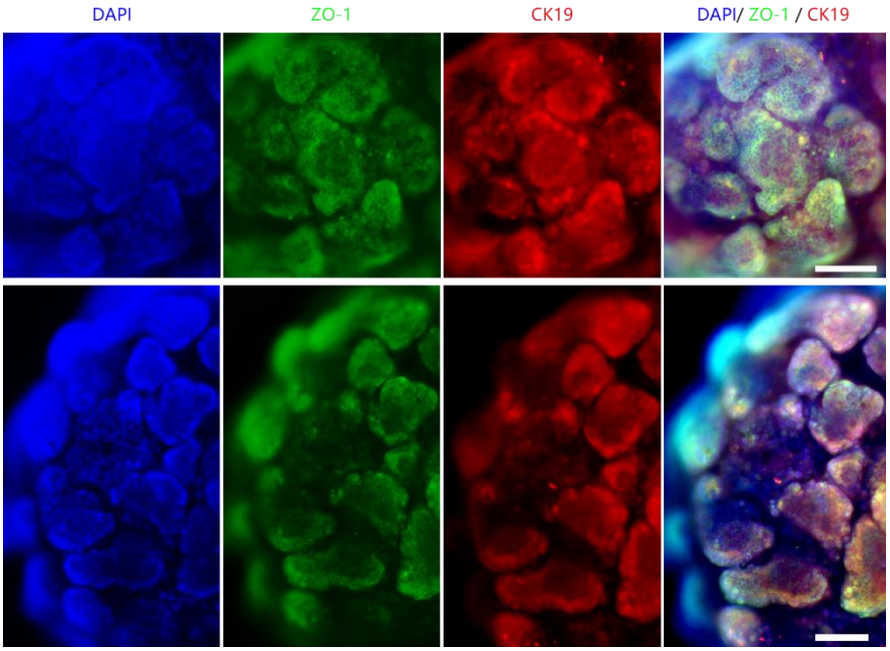

Figure 4. Immunostaining results of tight junction proteins and epithelial cell characteristic proteins in small intestine organoids grown in GelNest™ Matrix gel for 6 days. It can be observed that small intestine organoids grown in GelNest™ Matrix gel (3D) can well maintain the expression of biofunctional cell markers. DAPI is a cell dye nucleus (blue); ZO-1 is a cell tight junction protein marker (green); CK19 is an epithelial cell characteristic marker. The scale is 100 micrometers.

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Other Applications

NEST GelNest™ Matrix is also widely used in the research of cancer development and stem cell. Here we listed some applications where this product could be applied:

Tumor Invasion Assay

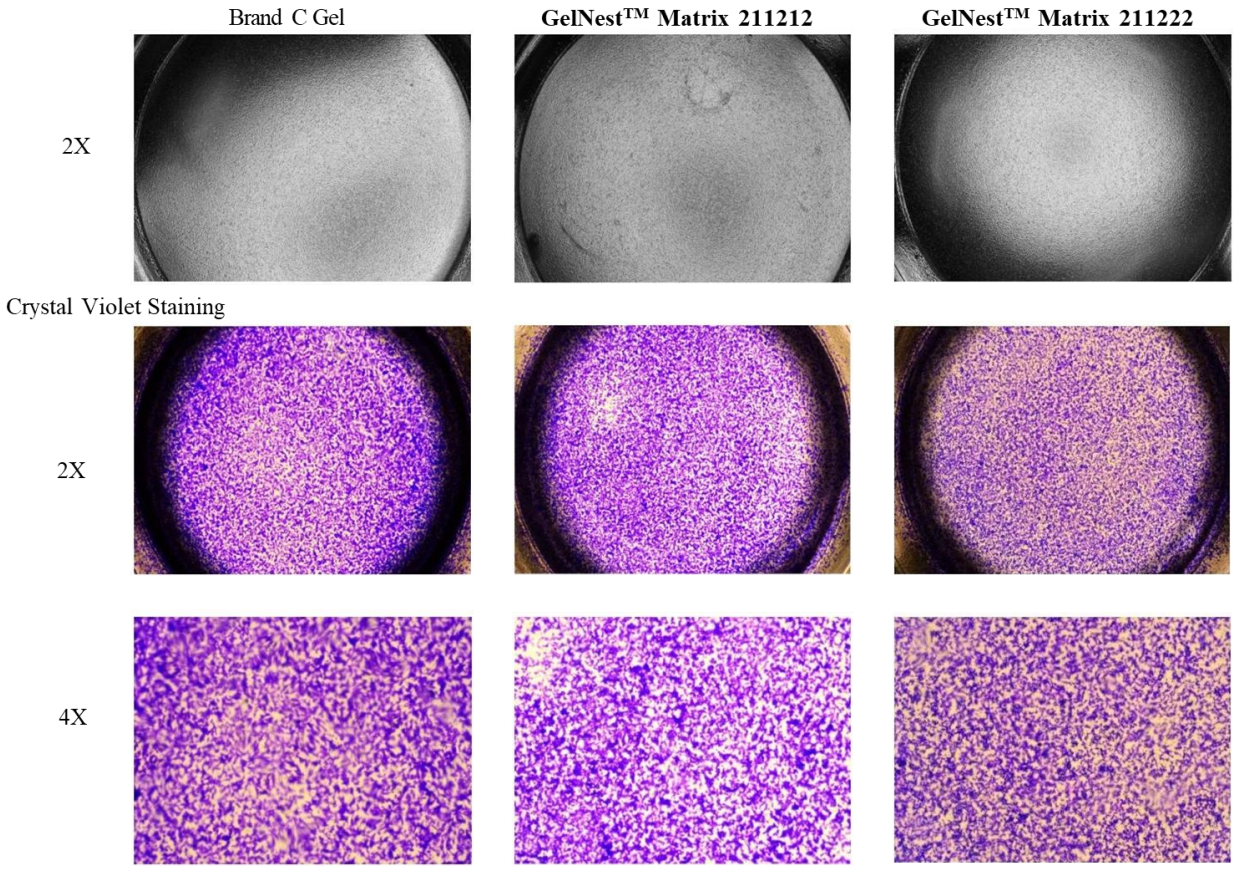

Figure 5. Results of HT-1080 cells cultured on competitor C's matrix gel-modified surface and GelNest™ Matrix Gel-modified surfaces forming a vascular network after 9 hours. Scale bar: 200μm. The results indicate that FBS can significantly induce cells to penetrate the semi-permeable membrane of the extracellular matrix and enter the lower surface of the insert.

Angiogenesis Experiment

Competitor C **GelNest™ Matrix**

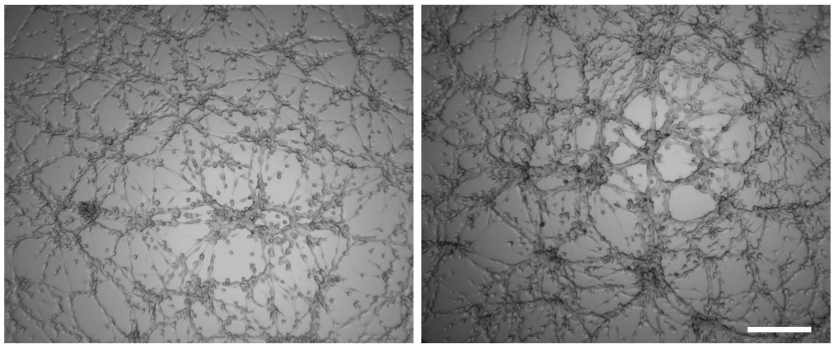

Figure 6. Results of endothelial cells forming a vascular network after 9 hours of cultivation on competitor C's matrix gel and GelNest™ Matrix(211492). Scale bar: 300μm.

Biliary Organoid Culture

Competitor C **GelNest™ Matrix**

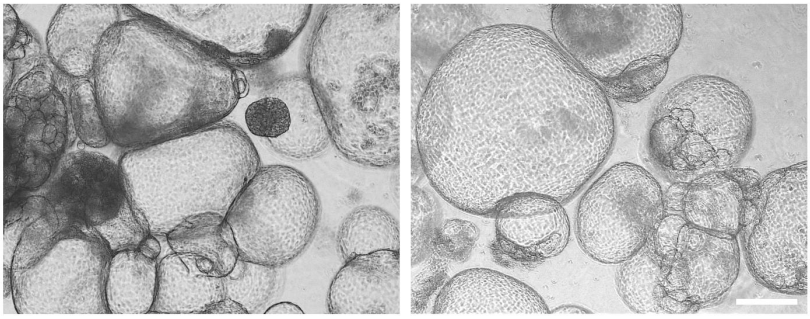

Figure 7. Growth of Human Biliary Organoids in competitor C' s matrix gel and GelNest™ Matrix(211282) after 5 Days. Scale bar: 300μm.

iPSC Culture

Competitor C **GelNest™ Matrix**

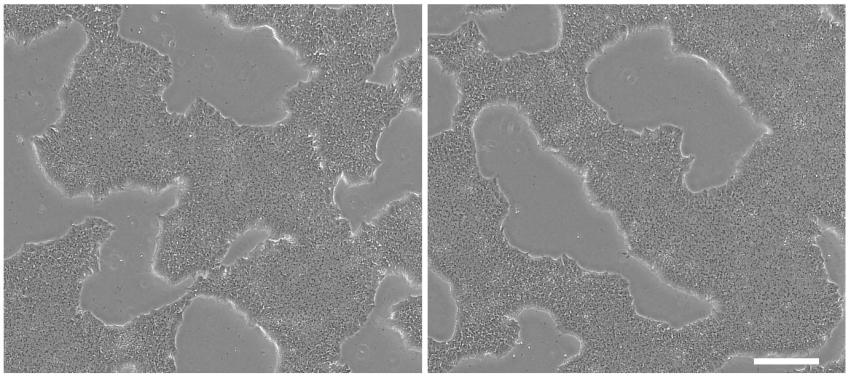

Figure 8. Results of human embryonic stem cells grown on competitor C' s matrix gel-modified surfaces and

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GelNest™ Matrix(211282)-modified surfaces for 3 days. Scale bar: 300μm.

Order Info

Product	Recommended Applications	Specifications	Cat.NO
GelNest™ Matrix	General 2D, 3D cell culture	5 mL/bottle, 1 bottle/pk	211212
GelNest™ Matrix, without Phenol Red		5 mL/bottle, 1 bottle/pk	211222
GelNest™ Matrix, low growth factor	2D, 3D cell culture with higher accuracy requirements for matrix components	5 mL/bottle, 1 bottle/pk	211232
GelNest™ Matrix, low growth factor, without Phenol Red		5 mL/bottle, 1 bottle/pk	211242
GelNest™ Matrix, high concentration	In vivo tumor formation, angiogenesis experiment, general cell culture, etc.	5 mL/bottle, 1 bottle/pk	211252
GelNest™ Matrix, high concentration, without Phenol Red		5 mL/bottle, 1 bottle/pk	211262
GelNest™ Matrix, for stem cell culture	hESC stem cell culture	5 mL/bottle, 1 bottle/pk	211272
GelNest™ Matrix, for organoid culture, without Phenol Red	Organoid culture and differentiation	5 mL/bottle, 1 bottle/pk	211282
GelNest™ Matrix, for Angiogenesis Experiment, with Phenol Red	Optimized for angiogenesis experiments	5 mL/bottle, 1 bottle/pk	211492

*For experiments that use colorimetric identification(such as fluoresence) or sensitive to steroids, we recommend phenol red-free gels.

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