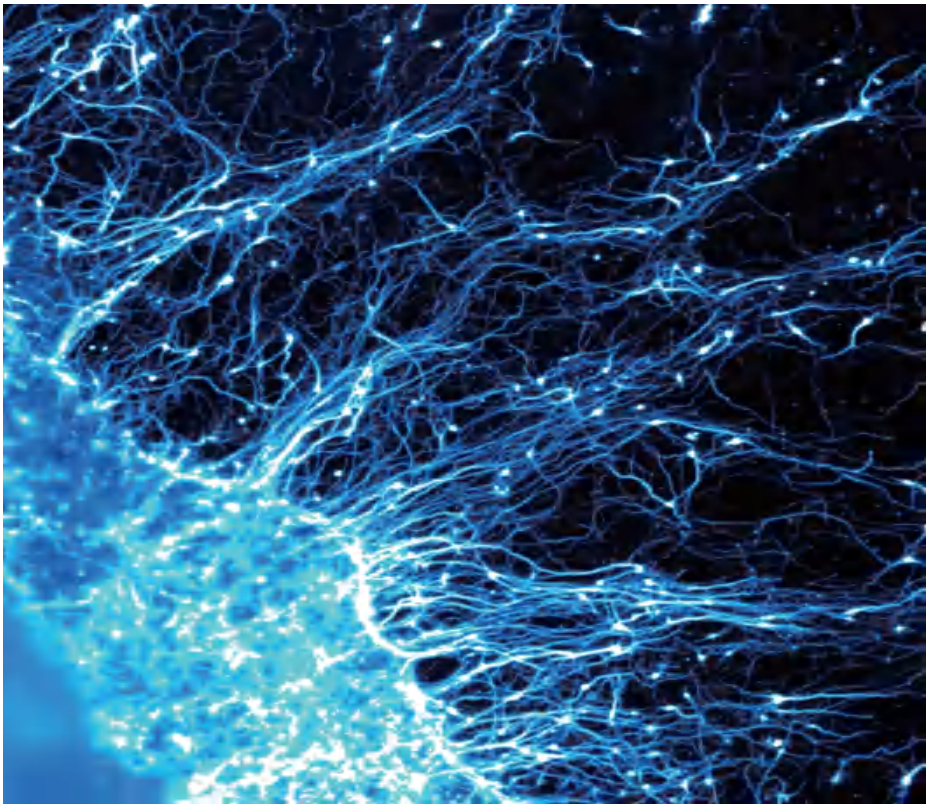
Extragel Matrix Manual



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CONTENTS

Product Introduction

The basement membrane is a matrix under the basal surface of epithelial cells of animals. AMSBIO's Extragel Matrix is a reconstituted matrix hydrogel formed by base-ment membrane components extracted from mouse tumor tissues. This matrixhydrogel is mainly composed of laminin, collagen IV, and heparan sulfate proteo-glycans (Kleinman et al. 1986). Besides, it contains various growth factors, such asepidermal growth factor (EGF), platelet-derived growth factor (PDGF), nerve
growth factor (NGF), basic fibroblast growth factor (FGF-2), transforming growth factor-β (TGF-β), and insulin-like growth factor (IGF) (Vu-kicevic et al. 1992).

Product Characteristics

Extragel Matrix is liquid at 4℃ but gelled when heated to 37℃. This transformation phemomenon is reversible. It can be liquefied again when it is stored at 4℃ overnight. (Tip: It is recommended to store the Extragel Matrix in an ice box in a refrigerator at 4℃ to realize the full liquefaction of the reconstitute matrix hydrogel.)

Dispense Extragel Matrix into appropriate aliquots when first use. Stable for 2 years when stored at -80℃

PRODUCT APPLICATION

Extragel Matrix can be applied to the growth, differentiation, metabolism and toxicology of organoids, in vivo and in vitro angiogenesis experiments, and tumor formation experiment of immunodeficient mice.

PRECAUTIONS

Extragel Matrix would start solidifying after the temperature is higher than 10℃, so the operation should be performed on ice. The matrix hydrogel can be dissolved in basic culture medium pre-chilled at 4℃, and the organoid can be released from the Extragel Matrix.

Operation Method

ORGANOID CULTURE (1 HOUR)

1. Thaw the Extragel Matrix (-OM-1 or -OM-1-ph or -OM-3 or-OM-3-ph) in refrigerator at 4℃ overnight.
2. Preheat the 24-well plate in cell culture incubator.
3. Prepare aliquots of Extragel Matrix using pre-chilled tips.
4. Prepare the single cell pellet with 1×10^6 cells derived from patients or animal tissue, centrifuged at 300 g for 5 minutes.
5. Mix the cell pellet with 50 µL Extragel Matrix thoroughly.
6. Add the mixture into the well of plate (50 µL per well).
7. Keep the plate in incubator for 10 minutes, flip and keep after another 5 minutes.
8. Add 500 µL culture medium to the well with matrix and cells.
9. Change the medium every 3 days.

TUMOR FORMATION EXPERIMENT IN IMMUNODEFICIENT MICE (1 HOUR)

1. Mix 1×10^6 cells with Extragel Matrix (volume ratio=1:1) (OM-2 or OM-2-ph or OM-5 or OM-5-ph). The total volume of mixture for each injection should be 100 µL at least.
2. Inject the mixture of cells and Extragel Matrix with a 1 mL syringe mounted with a 16 g needle and inject the mixture into subcutaneous tissues or thigh muscles.

Operation Method

ANGIOGENESIS EXPERIMENT (1 HOUR)

1. Starving cells: replace the complement medium with starving medium: DMEM medium containing 0.2% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/mL penicillin, and 100 μg/mL streptomycin for the incubation for 24h.
2. Uniformly spread Extragel Matrix (OM-1 or OM-1-ph) on the 96-wellplate.
   (Note: The pipette should be pre-cooled for 30 minutes. The operation shall be performed on ice to avoid the gelation of Extragel Matrix and the generation of bubbles.)
   Incubate the 96-well plate in a cell incubator for 30 minutes to solidify the
3. Extragel Matrix.
   Passage HUVECs and count them.
4. Add 200 μL of HUVEC suspension (containing 5×10^4 cells) to a 96-well plate
5. containing Extragel Matrix, and put the 96-well plate in an incubator.
   The vascular network structure will be formed in 3-12 hours.
6. At the optimal time for the vascular network formation, remove the culture
7. medium properly, stain it with the culture medium containing living cell dye 1/1000 Calcein AM (green), and take photos for records with a microscope.

NEURITE OUTGROWTH ASSAY

1. Prepare the rat neural stem cell (NSC) pellet with 1×10^5 cells, centrifuged at 300 g for 5 minutes.
2. Mix the cell pellet with 50 µL Extragel Matrix (OM-1 or OM-1-ph), thoroughly.
3. Keep the plate in incubator for 10 minutes, flip and keep after another 5 minutes.
4. Add 500 µL culture medium to the well with matrix and cells.
5. Change the medium every 3 days.

   Notes: NSC culture medium contains Neurobasal medium, 2% B27, 2mM L-glutamine, 5% FBS, 20 ng/mL EGF, 20 ng/mL bFGF, 100 U/mL penicillin and 100 µg/mL streptomycin. Neurites will be observed from day 2.

hESC AND IPSC CULTURE WITHOUT FEEDER LAYER (1 HOUR)

1. Thaw the Extragel Matrix (OM-4 or OM-4-ph) in refrigerator at 4°C overnight.
2. Mix the Extragel Matrix up&down with Pipette for 5 times.
3. Shortly spin the tube with benchtop centrifuge to remove any bbubles.
4. Preheat the well plate in cell culture incubator.
5. Prepare the diluted Extragel Matrix using basal medium at a ratio of 1:100.
6. Coating the well plate with diluted Extragel Matrix with volume of 300µL/cm^2 for 30 minutes at room temperature.
7. Remove diluted Extragel Matrix after coating and seed the hESC or iPSC solution.
8. Transfer the plate to incubator.

Practical Application Cases

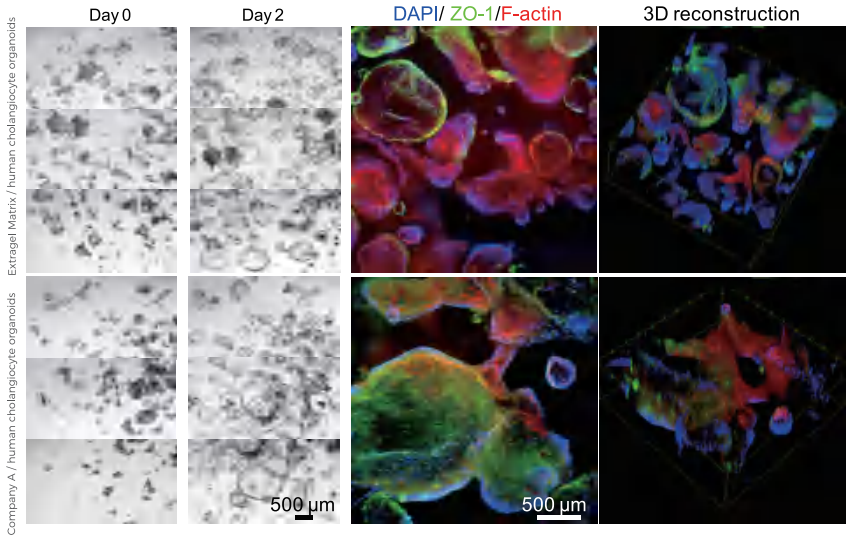
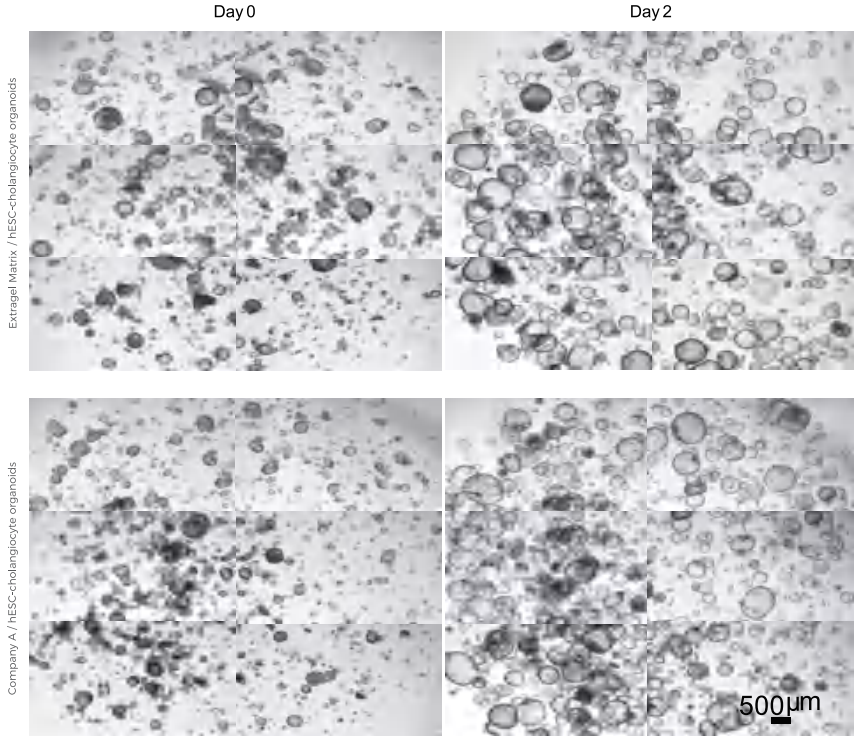


Figure 1.
Establishment of human bile duct organoids in the matrix hydrogel of Company A and Extragel Matrix, respectively. Human bile duct organoids are imaged after being stained with DAPI (nucleus, blue), anti-ZO-1 antibody (tight-junction protein), and Alexa Fluor 647 Phalloidin (cytoskeleton protein F-actin).

Figure 2.
Growth of human bile duct organoids derived from human embryonic stem cells in the matrix hydrogel of Company A and Extragel Matrix, respectively.



Practical Application Cases

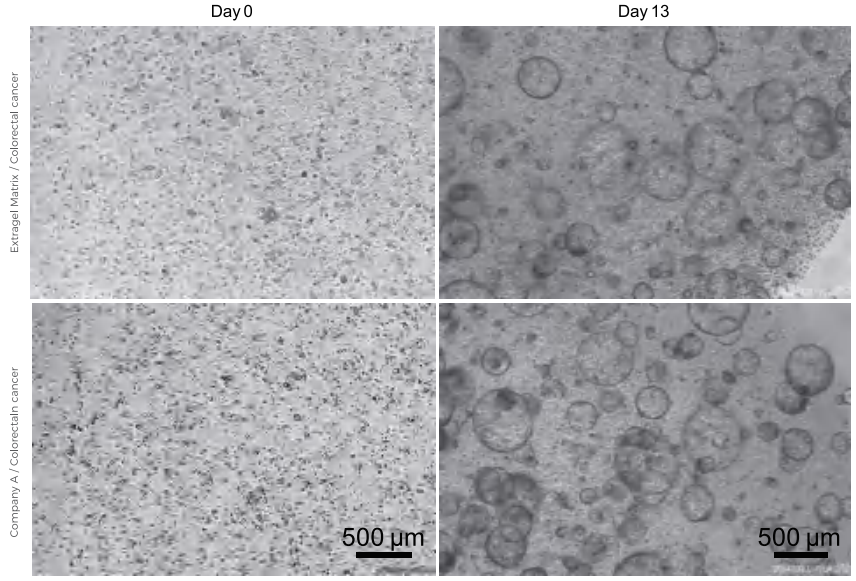


Figure 3.
Growth of human colorectal cancer organoids derived from patients in the matrix hydrogel of Company A and Extragel Matrix for 13 days, respectively.

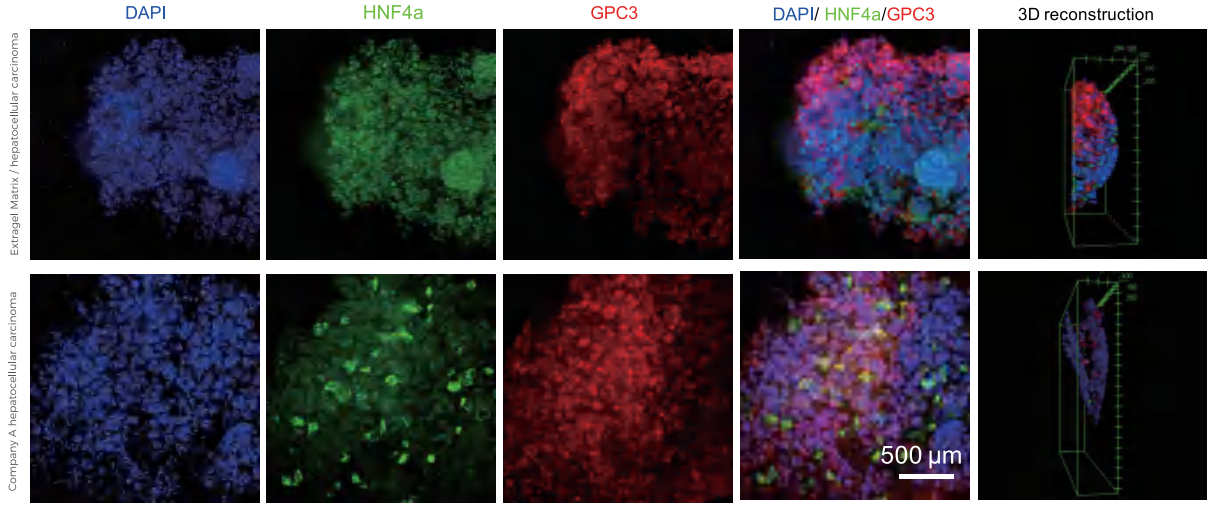


Figure 4.
Characterization of hepatocellular carcinoma organoids derived from patients in the matrix hydrogel of Company A and Extragel Matrix, with immunostaining by DAPI (nuclus), HNF4a (a hepatic biomarker) and GPC3 (hepatocellular carcinoma biomarker).

Practical Application Cases

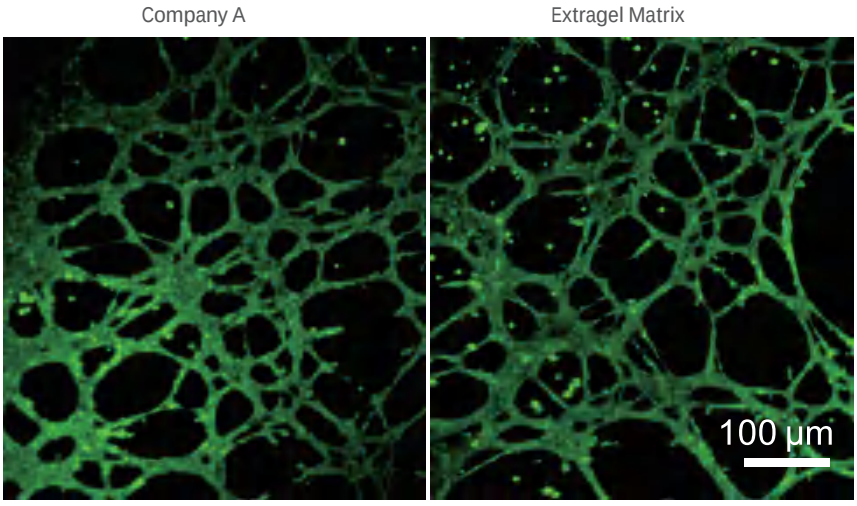


Figure 5.
Human umbilical vein endothelial cells (HUVECs) can form vascular networks in the matrix hydrogel of Company A and Extragel Matrix. HUVEC cells are imaged after being stained with living cell dye Calcein AM (green).

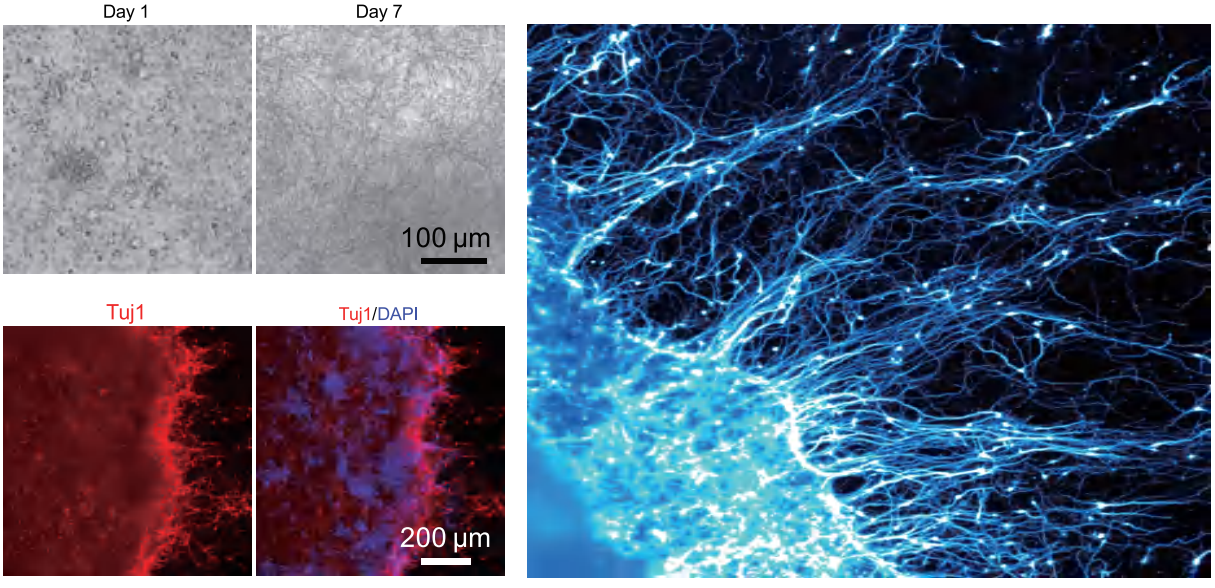


Figure 6.
Rat neural stem cells can grow, differentiate and form neurite networks in the Extragel Matrix. Rat neural stem cells (NSCs) were cultured in Extragel Matrix for 7 days and imaged after being stained with neuron biomarker Tuj1 (red) and nuclear dye DAPI (blue) (A). The right figure presents the neuron with pseudo colour (B). This experiment can be used to evaluate neurotoxicity and the differentiation and development of neurons.

Practical Application Cases

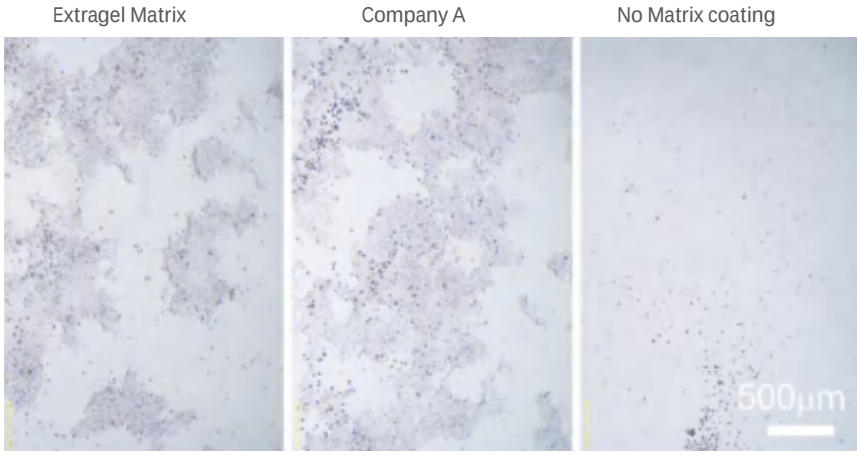


Figure 7.
Growth of hESCs on the matrix hydrogel of company A and Extragel Matrix for 2 days.

Reference

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3. Guillen, K P, et al. A human breast cancer-derived xenograft and organoid platform for drug discovery and precision oncology. Nature Cancer 3: 232 (2022).

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