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Supplementary information

Bioengineering human intestinal mucosal grafts using patient-derived organoids, fibroblasts and scaffolds

In the format provided by the authors and unedited

Supplementary Methods: Histology stainings undertaken to produce the immunohistochemistry and immunofluorescence images in the protocol.

Histology

Grafts were fixed in 10% neutral buffered formalin (Sigma-Aldrich) at room temperature for 24 hours and dehydrated in a graded alcohol series, paraffin embedded and sectioned at
4µm.

Reagents:

10% Neutral Buffered Formalin (Sigma-Aldrich, cat.no. HT501128)

!CAUTION Toxic, respiratory, skin and eye irritant. Use a chemical fume hood and appropriate personal protective equipment when handling this material.

Xylene (VWR Chemicals, cat. no. 28973.328)

!CAUTION Use a chemical fume hood and appropriate personal protective equipment when handling this material.

Ethanol absolute (Fisher-Scientific, cat. no. E/0650DF/17)

!CAUTION Flammable and should be kept away from heat, hot surfaces, sparks, open flames and other ignition sources. Use personal protective equipment when handling it.

Alcian Blue-Periodic Acid Schiff (AB-PAS) staining

Reagents:

Alcian Blue 8GX solution (Sigma-Aldrich, cat. no. 66011)

!CAUTION Use personal protective equipment whilst handling it

Schiff's Reagent (Fisher Scientific, cat. no. J/7300/PB08)

!CAUTION Use personal protective equipment whilst handling it

1% Period acid solution (made fresh at room temperature); 1g Periodic acid (Sigma-Aldrich, cat. no. 10450-60-9)/100ml distilled water

!CAUTION Use personal protective equipment whilst handling it

Harris Haematoxylin (Leica Surgipath®, cat. no. 3801561E)

DPX Mounting medium (VWR Chemicals, cat. no. RAYLLAMB/DPX)

Procedure:

1. Bake slides at 60°C for 20 mins.
2. Dewax in xylene and rehydrate in graded alcohol series followed by distilled water.
3. Stain in Alcian blue solution for 5 mins at room temperature.
4. Wash in running tap water for 5 mins, rinse in distilled water.
5. Oxidise with 1% periodic acid for 15 mins at room temperature.
6. Wash in running tap water for 5 mins, rinse in distilled water.
7. Treat with Schiff's reagent for 25 mins at room temperature.
8. Wash in running tap water for 5 mins.
9. Counterstain with Harris Haematoxylin, dehydrate in a graded alcohol series, clear and mount slides.

Alkaline Phosphatase Staining

Reagents:

Vector Laboratories BCIP/NBT Alkaline Phosphatase Substrate Kit cat. no. SK-5400 (RRID: AB_2336236)

VectaMount Mounting Medium (Vector laboratories, cat. no. H-5000)

Procedure:

1. Dewax in xylene and rehydrate in graded alcohol series followed by distilled water.
2. Incubate in the working solution (as per the kit instructions) for 20-30 mins at room temperature.
3. Wash in distilled water for 5 mins.
4. Counterstain with light green for 1-2 mins.
5. Dehydrate in graded alcohol series, clear and mount slides.

Immunohistochemistry staining – E Cadherin, Ki67, Lysozyme, SOX-9, vimentin

E-Cadherin, Ki67, Lysozyme, SOX-9 and Vimentin staining were performed as automated immunohistochemistry stainings on the Ventana Discovery Ultra Slide Preparation System (Roche). Primary antibody dilutions (diluted in Discovery Ab Diluent) and incubation times were all optimised "in house" (The Francis Crick Institute) and are detailed in Supplementary Table 1. Details of reagents are listed below. Slides are first baked at 60°C for 20 minutes. Steps performed on the Ventana Discovery Ultra include de-paraffinization, antigen retrieval (with Discovery CC1; a Tris based antigen retrieval buffer) incubation in primary antibody, incubation in secondary antibody and amplification with DAB (Discovery ChromoMap DAB RUO). Slides are then counterstained with haematoxylin, dehydrated, cleared and mounted.

Reagents for the Ventana:

Discovery Wash	(Roche 07311079001 (950-510))
Benchmark Ultra LCS	(Roche 05424534001 (650-210))
Reaction Buffer Concentrate 10x	(Roche 05353955001 (950-300))
Discovery CC1	(Roche 06414575001 (950-500))
Discovery Ab Diluent	(Roche 05266319001 (760-108))
Discovery ChromoMap DAB RUO	(Roche 05266645001 (760-159))

Immunohistochemistry staining – Sucrase Isomaltase

Reagents:

PO-blocking buffer (16.44g citric acid (Alfa Aesar, cat. no. 36664), 43.04g di-Sodium hydrogen phosphate dihydrate ($Na_2HPO_4.H_2O$) (Fluka Analytical, cat. no. 71662), 4g Sodium azide (Sigma-Aldrich, cat. no. S8032)) in 2l distilled water. Stored at room temperature for up to 6 months.

Hydrogen peroxide (H_2O_2) 30% (Perdrogen™) (Honeywell Fluka, cat. no. 31642) Stored at 4°C.

Tris-EDTA buffer pH 9.0 (2.4g Trizma® base (Sigma-Aldrich, cat. no. T1503), 0.74g EDTA (Fisher Scientific, cat. no. 10213570) in 2l distilled water. Stored at room temperature for up to 6 months.

Bovine serum albumin (BSA) fraction V (Sigma-Aldrich, cat. no. 1073594001)

Normal goat serum (Abcam, cat. no. ab7481)

Liquid DAB + substrate chromogen system (Dako, cat. no. K3468)

Vectastain® Elite® ABC Kit Peroxidase (Standard) (Vector laboratories, cat. no. PK-6100)

Primary and secondary antibodies and dilutions as detailed in Supplementary Table 1.

Procedure:

1. Bake slides at 60°C for 20 minutes.
2. Dewax in xylene and rehydrate in graded alcohol series followed by distilled water.
3. Incubate in PO-blocking buffer (95%) with hydrogen peroxide (5%) (vol/vol) (to block endogenous peroxidase) for 15 mins at room temperature.
4. Wash with distilled water
5. Perform antigen retrieval using Tris-EDTA buffer (heating to a temperature of approx. 100°C for 20 mins)
6. Wash with PBS.
7. Block with 1% BSA (wt/vol) and 10% NGS (vol/vol) (blocking buffer) for 30 mins at room temperature.

8. Incubate in primary antibody (Supplementary table 1) in blocking buffer overnight (approx. 16 hours) at 4°C.
9. Wash with PBS.
10. Incubate in secondary antibody diluted in 1% BSA for 45mins at room temperature.
11. Wash with PBS
12. Incubate with peroxidase conjugated secondary antibody at room temperature
13. Wash with PBS
14. Incubate with Avidin-Biotin complex (instructions as per Vectastain® Elite® ABC Kit) at room temperature for 30 mins
15. Wash with PBS
16. Apply DAB solution and monitor reaction under light microscope
17. Wash in distilled water to terminate reaction
18. Counterstain with Meyer's haematoxylin for 2 mins
19. Dehydrate with graded alcohol, clear and mount slides.

Immunofluorescence staining (UEA and Sodium Potassium ATPase)

Reagents:

Tris-EDTA buffer pH 9.0

Triton X-100 (Sigma-Aldrich, cat. no. T8787)

Bovine serum albumin (BSA)

Normal goat serum

DAPI (Sigma-Aldrich, cat. no. D9542) Aliquoted and stored at -20°C for up to 6 months

ProLong™ Gold Antifade Mountant (Thermo-Fisher Scientific, cat. no. P36934)

Primary and secondary antibodies and dilutions as detailed in Supplementary Table 1.

Procedure:

1. Bake slides at 60°C for 20 minutes.
2. Dewax in xylene and rehydrate in graded alcohol series followed by distilled water.
3. Perform antigen retrieval using Tris-EDTA buffer (heating to a temperature of approx. 100°C for 20 mins)
4. Wash with PBS.
5. Permeabilize with 0.3% Triton X-100 (in PBS) for 30 mins at room temperature
6. Wash with PBS
7. Block with 1% BSA (wt/vol) and 10% NGS (vol/vol) (blocking buffer) for 30 mins at room temperature.
8. Incubate in primary antibody (Supplementary table 1) in blocking buffer overnight (approx. 16 hours) at 4°C.
9. Wash with PBS.
10. Incubate in secondary antibody* (Supplementary table 1) in 1% BSA (wt/vol) and PBS for 45 mins at room temperature in the dark.

*Omit this step for UEA and Sodium Potassium ATPase

11. Counterstain with DAPI (diluted 1:1000 in PBS) for 30 mins at room temperature in the dark
12. Wash with PBS
13. Mount slides

Supplementary Table 1: Antibodies validated for immunohistochemistry and immunofluorescence staining of engineered intestinal mucosal grafts in this protocol.

Antibody	Source and catalogue number (RRID)	Dilution	Antigen retrieval Ventana (mins)	Antibody incubation Ventana (mins)
Collagen-I	Abcam ab34710 (RRID:AB_731684)	1:200	-	-
E Cadherin	Proteintech 20874-1-AP (RRID: AB_10697811)	1:1500	24	32
Ki67	Abcam ab15580 (RRID:AB_443209)	1:1000	48	60
Lysozyme	Dako A0099 (RRID: AB_2341230)	1:2000	48	60
Na^+K^+ATPase	Abcam ab198367 (RRID:AB_2916056)	1:100	-	-
Sucrase Isomaltase	Santa-Cruz sc-393424 (RRID:AB_2891093)	1:100	-	-
SOX-9	Sigma-Aldrich AB5535 (RRID:AB_2239761)	1:250	48	60
UEA	Vector Laboratories RL-1062 (RRID:AB_2336769)	1:200	-	-
Villin	Santa-Cruz sc-58897 (RRID:AB_2304475)	1:50	-	-
Vimentin	Abcam ab92547 (RRID:AB_10562134)	1:500	24	60
Goat anti-mouse HRP	Dako P0447 (RRID:AB_2617137)	1:100	-	-
OmniMap anti-rabbit HRP	Roche 05269679001 (760-4311) (RRID:AB_2811043)	N/A	-	-
OmniMap anti-mouse HRP	Roche 05269652001 (760-4310) (RRID:AB_2885182)	N/A	-	-
Alexa Fluor® 488 goat anti-rabbit IgG	Thermo-Fisher Scientific A-11034 (RRID:AB_ 2576217)	1:1000	-	-
Alexa Fluor® 568 goat anti-mouse IgG	Thermo-Fisher Scientific A-11031 (RRID:AB_144696)	1:1000	-	-