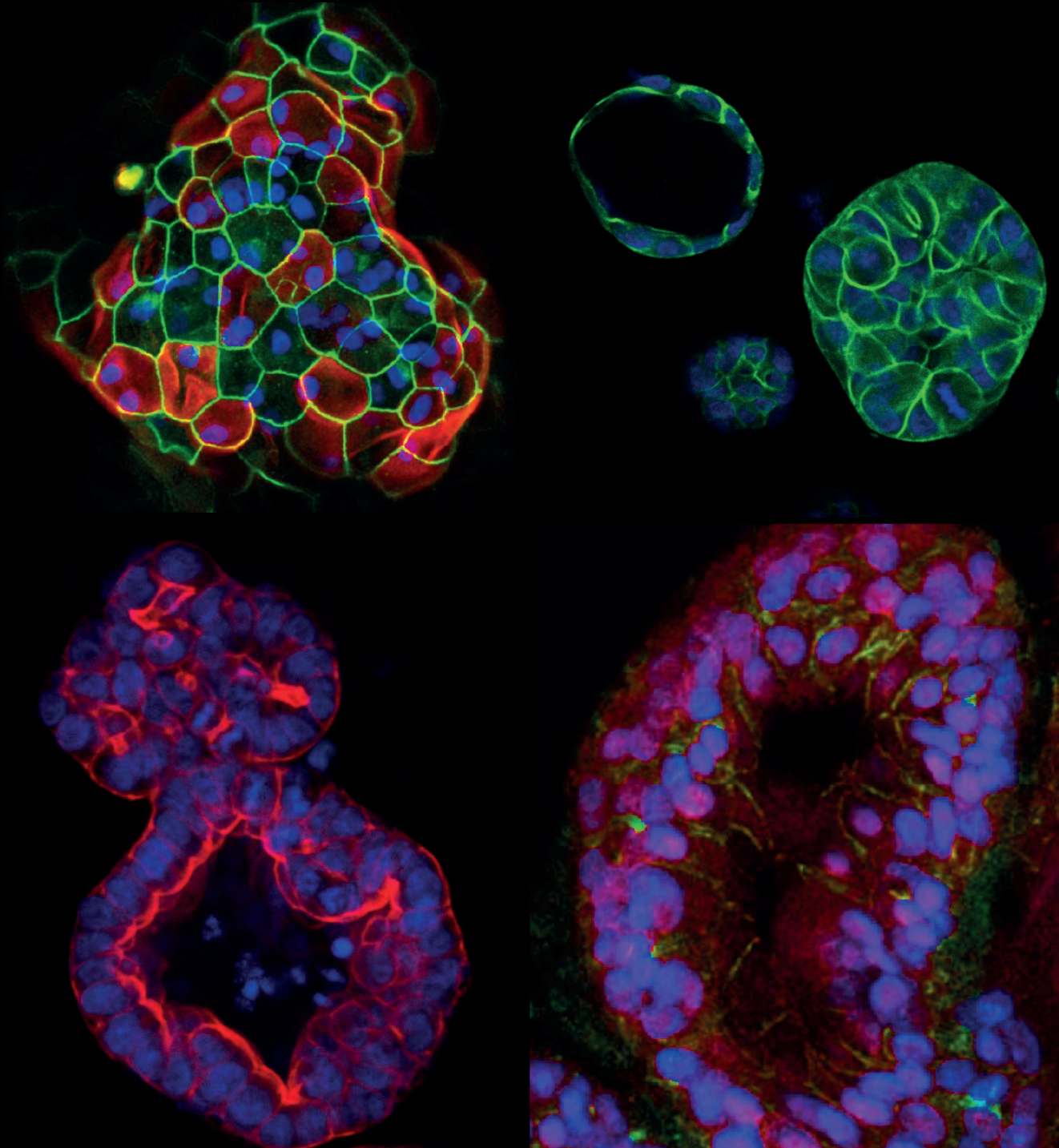


Organoid Growth *on* BME 2



Liver – Gastrointestinal
Oesophagus – Mammary

Liver Organoids

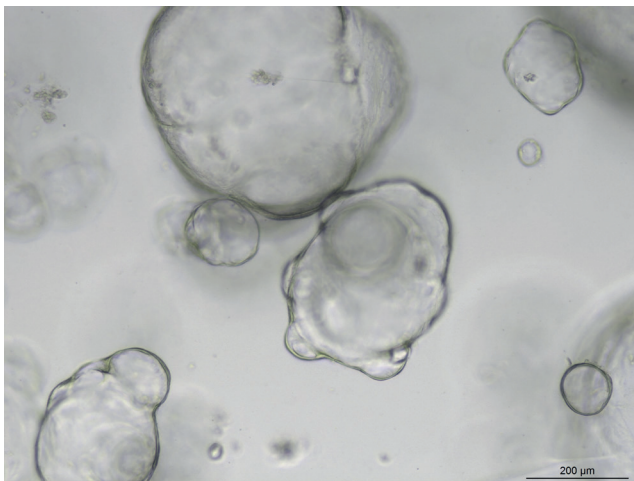
Human Liver

In their recent paper in Cell, Huch, Clevers et al (2015) have shown that:

- Primary human bile duct cells can readily be expanded in vitro into 3D liver organoids using BME 2 RGF
- Adult liver stem cells maintain their ability of self-renewal; differentiating into functional hepatocyte cells in vitro and generating bona fide hepatocytes upon transplantation.
- Expanded cells preserve their genetic integrity over months in culture (agreeing with the authors' previous observations in a mouse model).
- Organoids derived from patients with genetic disorders can be used to model liver disease in vitro.

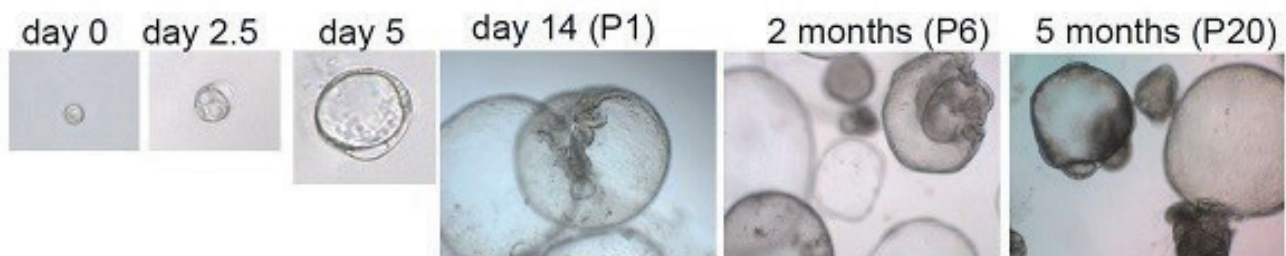
“We have obtained culture conditions that allow us to long-term expand genetically stable human donor liver cells in organoid culture. One of the clues to this success is the use of ECM that allows the cells to grow in 3D. BME 2 has been our ECM of choice for these experiments.”

Dr. Meritxell Huch (Gurdon Institute, University of Cambridge, UK)



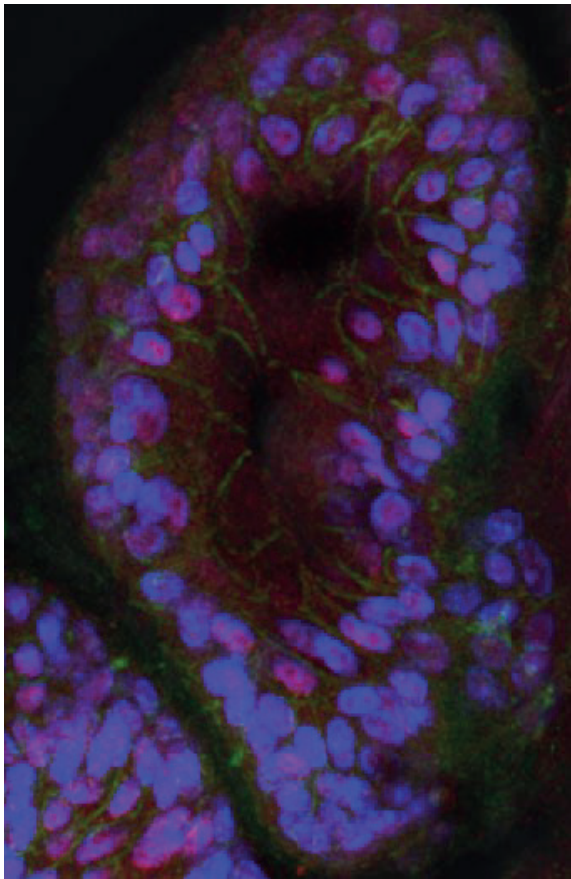
Differential interference contrast (DIC) image of human organoids grown in BME 2 RGF and cultured for >2 months in human liver complete medium. Magnification, 4×.

(Image courtesy of Meritxell Huch, Gurdon Institute, University of Cambridge, UK)



Long-term culture of Human Liver Organoids on BME 2 RGF. (Clonal cultures obtained by seeding sorted cells at one cell per well)

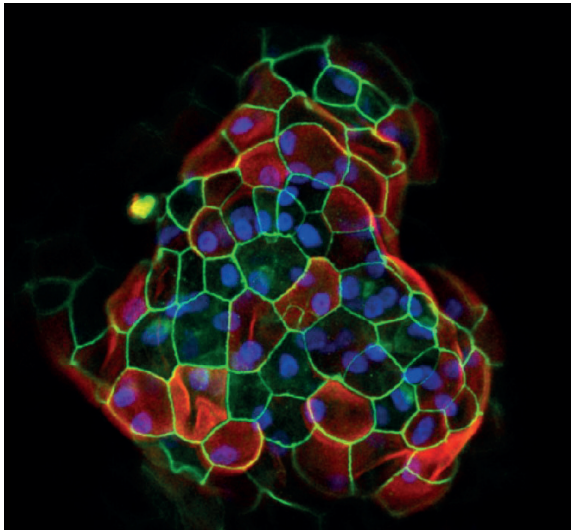
(Image courtesy of Meritxell Huch, Gurdon Institute, University of Cambridge, UK)



Marker Expression in Human Liver Organoids on BME 2 (RGF)

Confocal image stained for ECAD (green) and the hepatocyte marker HNF4. (red); Nuclei counter- stained with Hoechst.

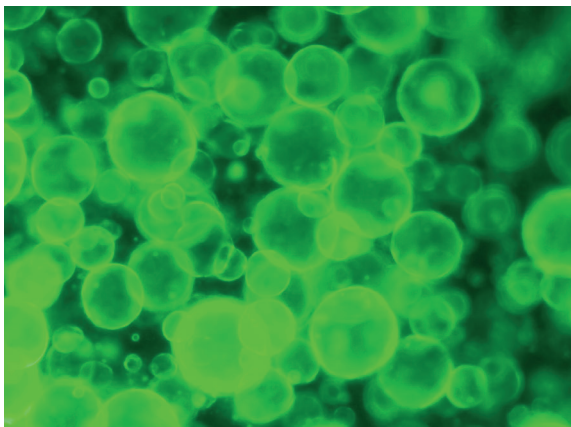
Image courtesy of Dr. Meritxell Huch, Gurdon Institute, University of Cambridge, UK



Differentiation of Organoids into Hepatocytes (liver cells) on BME 2 (RGF).

Expression of hepatocyte genes in human liver organoid after 11 days on differentiation medium (DM). Immunofluorescence for albumin (ALB, red) and ZO-1 (green); nuclei counterstained with Hoechst (Blue)

Image courtesy of Dr. Meritxell Huch, Gurdon Institute, University of Cambridge, UK



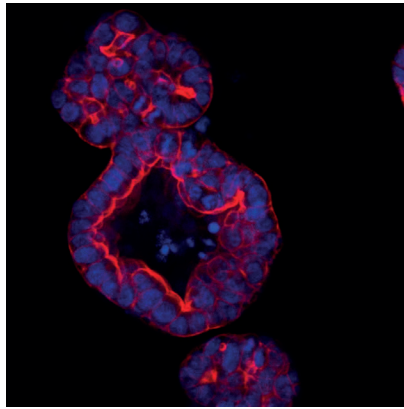
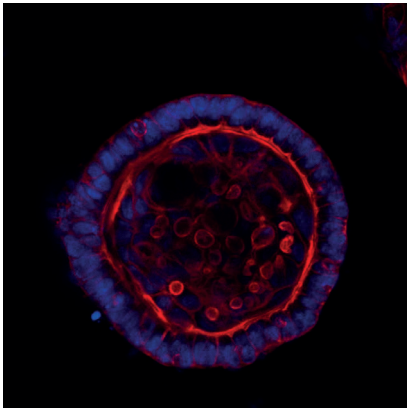
Mouse Liver

GFP-labeled organoids, grown from mouse liver stem cells. grown on BME 2 RGF.

The cells were infected with a lentivirus expressing GFP

Image courtesy of Dr. Derk ten Berge, Erasmus MC Stem Cell Institute, Rotterdam

Gastrointestinal Organoids

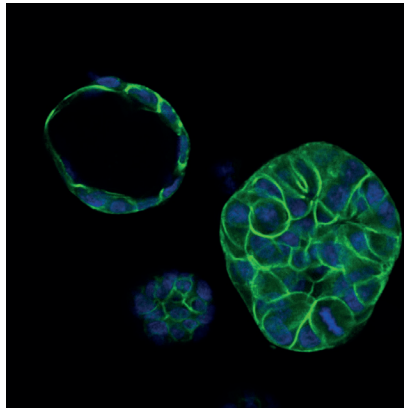
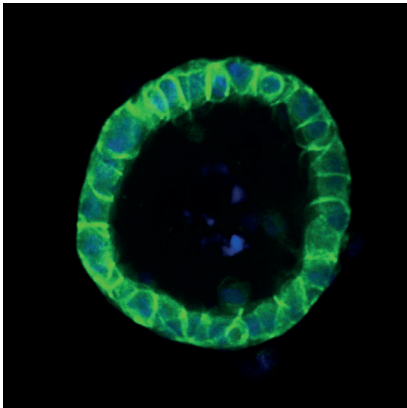


Human Colorectal

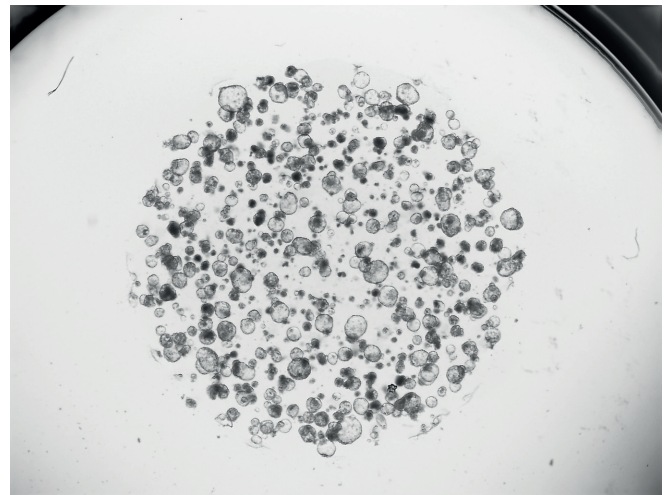
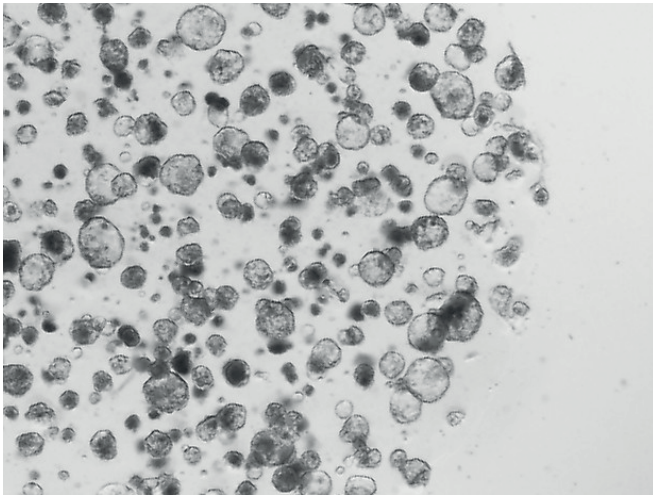
Human Colorectal Cancer (CRC) organoids grown from single cells on BME 2 RGF.

Immunofluorescence for Phalloidin (red) to mark actin filaments; and E-cadherin (green) as epithelial marker; nuclei counterstained with DAPI (blue)

Images courtesy of the Batlle Lab, IRB Barcelona

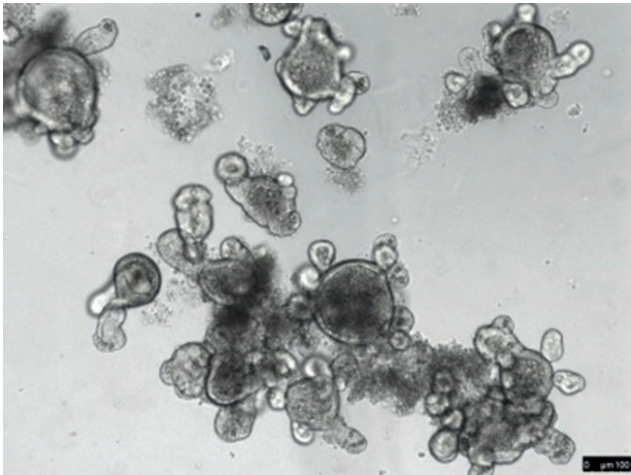


Mouse Adenoma

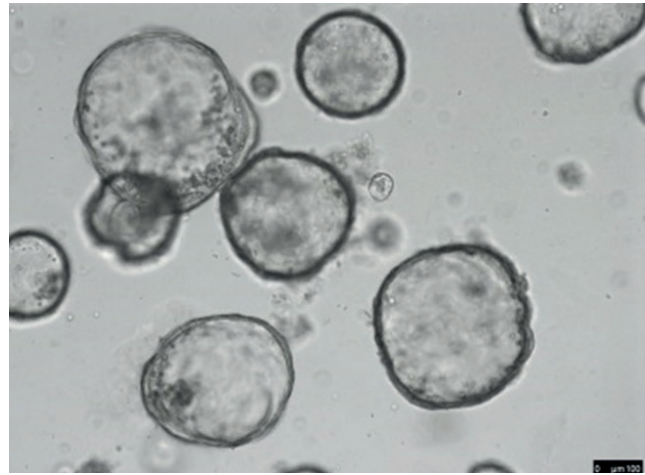


Images courtesy of the Batlle Lab, IRB Barcelona

Mouse Intestine - Colorectal Cancer Model



WILD TYPE

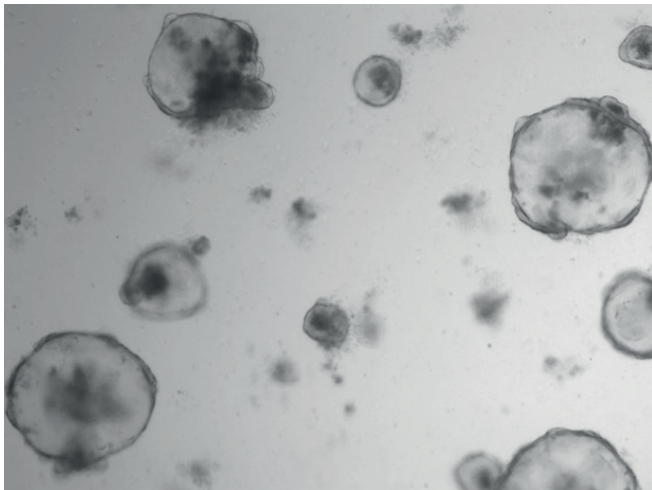


APC-DELETED

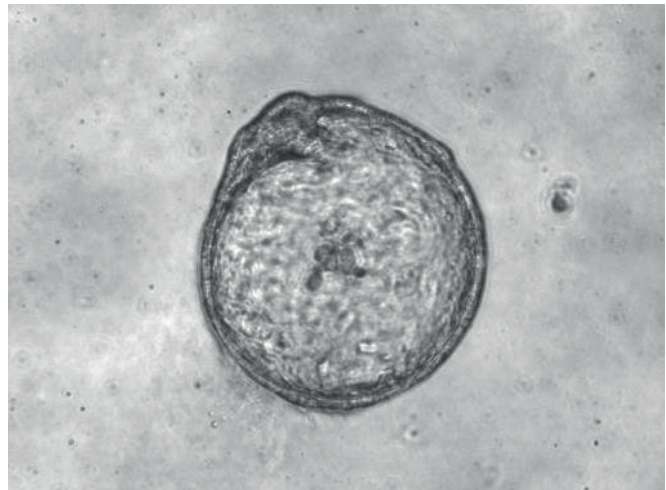
intestinal organoids derived from normal and Apc-deleted mice. (Loss of the negative Wnt pathway regulator APC occurs in the majority of colorectal cancers.)

Images courtesy of the Li Lab, NIMR, London

Transgenic Mouse



Organoids (passage 1) derived directly into BME 2 RGF. Organoids were split once (using mechanical disruption) following derivation; and the image was taken 3 days later.



Existing organoid culture transferred to BME 2 RGF. Organoids were digested to single cells, which were plated onto BME 2 RGF. The image was taken 6 days after plating.

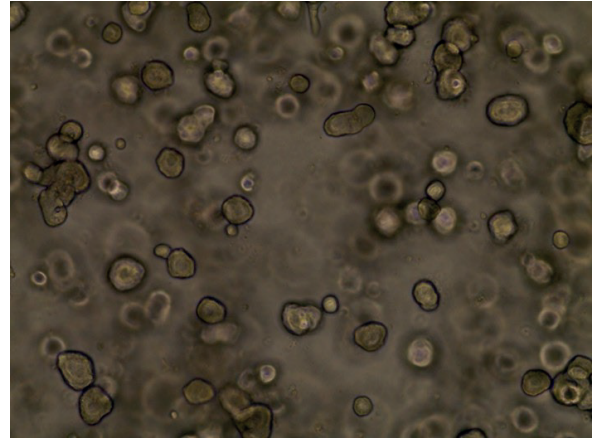
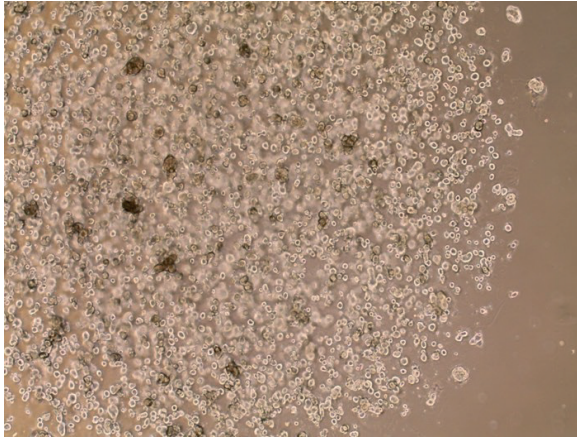
Organoids prepared from the intestinal crypts of transgenic mice:

Images courtesy of the Sansom Lab, Beatson Institute of Cancer Research, Glasgow UK

Oesophageal Organoids

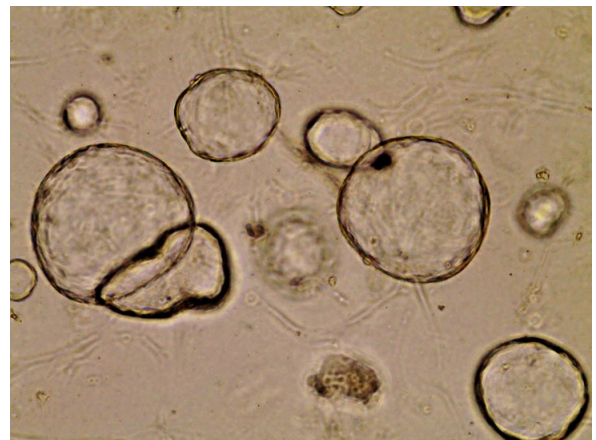
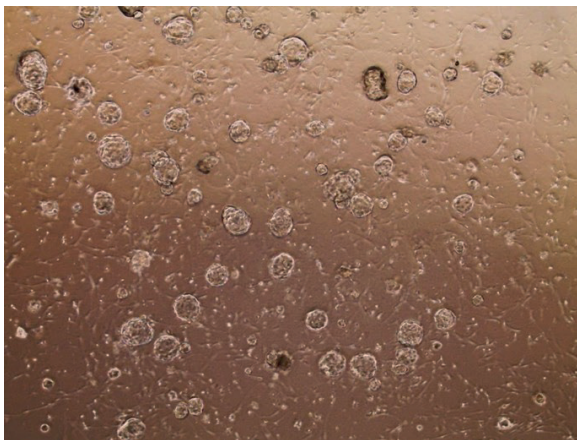
Organoids are from tumours derived from fresh tissue samples in the lab. Images labelled gastric are taken from the lowest part of the oesophagus almost into the stomach

Tumour



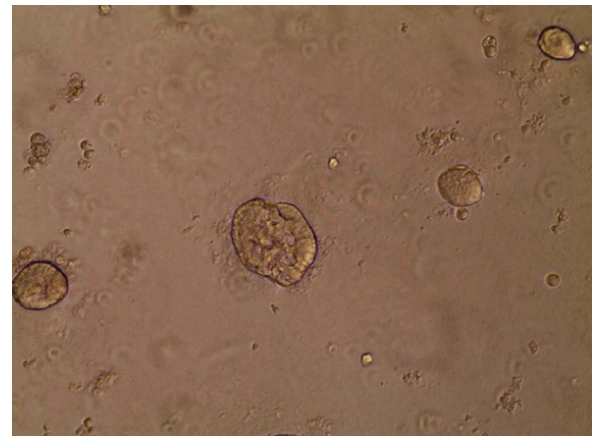
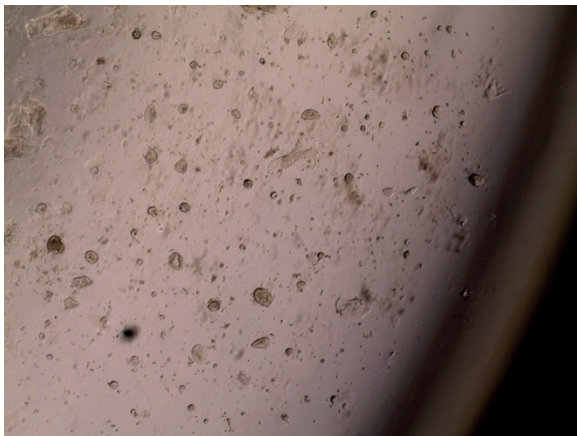
Images courtesy of Dr Mathew Garnett, Sanger Wellcome Trust Institute, Cambridge UK

Oesophageal Barrett's Epithelia



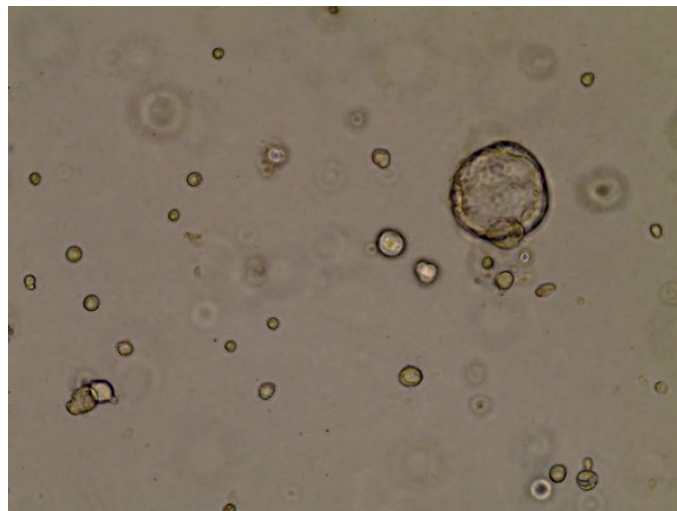
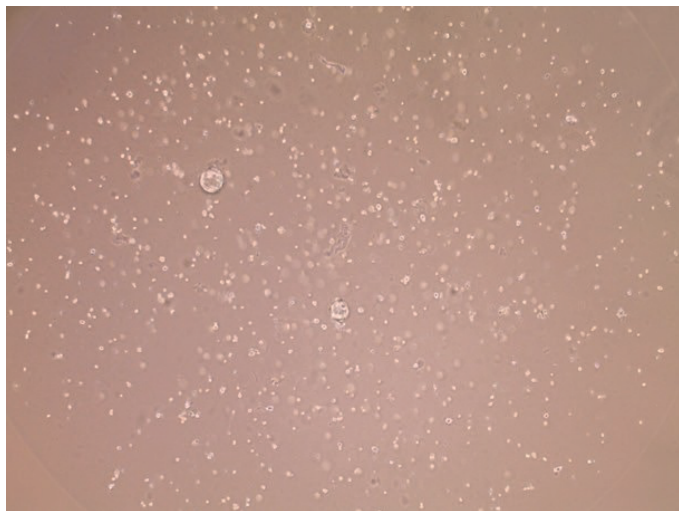
Images courtesy of Dr Mathew Garnett, Sanger Wellcome Trust Institute, Cambridge UK

Oesophageal Normal



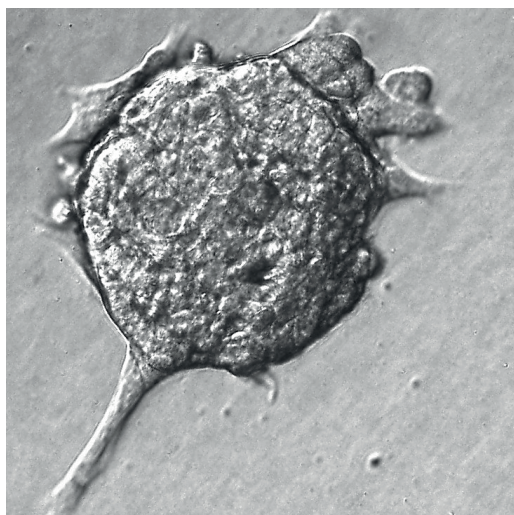
Images courtesy of Dr Mathew Garnett, Sanger Wellcome Trust Institute, Cambridge UK

Oesophageal Normal Gastric



Images courtesy of Dr Mathew Garnett, Sanger Wellcome Trust Institute, Cambridge UK

Breast Organoids



*Mammary cancer organoid from mice,
grown on / invading into BME 2, RGF.*

*Image courtesy of Tuula Kallunki,
Danish Cancer Research Society*

Organoid Protocols & Citations

Andersson-Rolf, Amanda, Juergen Fink, Roxana C. Mustata, and Bon-Kyoung Koo. **"A Video Protocol of Retroviral Infection in Primary Intestinal Organoid Culture."** *JoVE (Journal of Visualized Experiments)* 90 (2014): e51765-e51765.

Huch, M., Gehart, H., van Boxtel, R., Hamer, K., Blokzijl, F., Verstegen, M.A., Ellis, E., van Wenum, M., Fuchs, S., de Ligt, S., van de Wetering, M., Sasaki, N., Boers, S.J., Kemperman, H., de Jonge, J., Ijzermans, J.N.M., Niewenhuis, E.E.S., Hoekstra, R., Strom, S., Vries, R.G.J., van der Laan, L.J.W., Cuppen, E., Clevers, H. **"Long-term culture of genome-stable bipotent stem cells from adult human liver."** *Cell* 160: 299-312 (2015)

van de Wetering, M., Francies, H.E., Francis, J.M., Bounova, G., Iorio, F., Pronk, A., van Houdt, W., van Gorp, J., Taylor-Weiner, A., Kester, L., McLaren-Douglas, A., Blokker, J., Jaksani, S., Bartfeld, S., Volckman, R., van Sluis, P., Li, V.S.W., Seepo, S., Sekhar Pedamallu, C., Cibulskis, C., Carter, S.L., McKenna, A., Lawrence, M.S., Lichtenstein, L., Stewart, C., Koster, J., Versteeg, R., van Oudenaarden, A., Saez-Rodriguez, J., Vries, R.G.J., Getz, G., Wessels, L., Stratton, M.R., McDermott, U., Meyerson, M., Garnett, M.J., Clevers, H. **"Prospective derivation of a 'Living Organoid Biobank' of colorectal cancer patients."** *Cell* (in press)

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- Soluble form of basement membrane from Engelbreth-Holm-Swarm (EHS) tumor.
- Major components include Laminin I, Collagen IV, Entactin & Heparan Sulfate Proteoglycan.
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 - Higher concentration (14 -16 mg/ml)
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BME 2 is a proprietary formulation that has a higher tensile strength than our original BME 1 or similar products such as Matrigel®. The Growth Factor Reduced format (BME 2 RGF “organoid matrix”) has been shown to work well for growing organoids, especially using techniques based on the LGR5 stem cell marker / Wnt signalling system, pioneered by Hans Clevers and co-workers.

Organoid Culture

Organoids are organ-like structures that can be formed by 3D cell culture and differentiation of stem cells or organ progenitors; and are capable of recapitulating aspects of organ function in vitro. Research & therapeutic potential of organoids includes:

Organogenesis Models Drug Testing
Tumor / Disease Models Toxicity Screening
Personalised Medicine Regenerative Medicine / Organ Replacement

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3533-005-02		5 ml
3533-010-02		2x 5ml

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