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Review Article

Organoid Models Derived from Intestinal Stem Cells for Disease Modelling

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Abstract

Background: Intestinal organoids grown from Lgr5-positive stem cells reproduce crypt-villus architecture and regional identity, but contain epithelium alone, without immune cells, fibroblasts, neurons or vasculature.

Objective: To assess where organoid disease modelling is strong and where it is weaker, from culture chemistry to the clinical programmes built on an organoid readout.

Methods: Narrative review of derivation studies, infection models, tumour biobanks and prospective trials.

Findings: Cultures from needle biopsies of metastatic colorectal cancer succeeded in 71% of attempts across 14 patients, copy-number profiles correlating at $r = 0.89$. In the SENSOR trial, 31 cultures came from 54 eligible patients and none of six treated patients responded. In 34 infants with cystic fibrosis, forskolin-induced swelling correlated with sweat chloride at $r = -0.82$.

Conclusions: The evidence supports organoids for mechanism and infection biology, and a functional readout of the causal defect in cystic fibrosis, but not chemosensitivity testing for drug choice.

Keywords: Intestinal organoids; Intestinal stem cells; Disease modelling; Cystic fibrosis; Colorectal cancer

Introduction

The intestinal epithelium renews itself faster than any other mammalian tissue, and for most of the twentieth century that was an obstacle rather than an opportunity: primary intestinal cells died within days in culture. The identification of Lgr5 as a marker of crypt base columnar cells changed the arithmetic. Lineage tracing from the Lgr5 locus showed that a single crypt base columnar cell generated all epithelial lineages over a 60-day period in adult mice [1], establishing these cells as genuine multipotent stem cells rather than a transit-amplifying population with a convenient marker.

Two years later, single sorted Lgr5-positive cells were shown to build crypt-villus structures in Matrigel without mesenchymal support, with roughly six cycling Lgr5-positive stem cells at the bottom of each small-intestinal crypt supplying the founding population in vivo [2]. The mesenchymal niche was replaced by three soluble factors — EGF, noggin and R-spondin — and by Paneth cells, subsequently shown to constitute the niche within the crypt [3]. Extension of the system to human colon, adenoma, adenocarcinoma and Barrett's epithelium followed [4], and with it patient-specific epithelial cultures from routine endoscopic biopsies.

What followed is fifteen years of disease modelling that is strong in some places and weaker in others than the review literature usually admits. This article surveys the culture chemistry, the infection and inflammation models, the tumour biobanks, and the two clinical programmes — colorectal cancer and cystic fibrosis — that have tried to convert an organoid readout into a treatment decision. Only one has succeeded.

Lgr5 Lineage Tracing and the First Crypt-Villus Cultures

Self-organisation is why intestinal organoids are useful and why they are hard to standardise. A crypt or single stem cell placed in a laminin-rich gel generates a closed

cystic structure that buds, and the buds contain crypt-like domains with Paneth cells and Lgr5-positive stem cells at their base, surrounded by differentiated enterocytes, goblet cells and enteroendocrine cells facing a central lumen [2]. No patterning is imposed.

Regional identity survives this process, which matters because duodenal and colonic epithelium are not interchangeable. The mechanism is at least partly epigenetic: DNA methylation defines the regional identity of human intestinal epithelial organoids, so colonic and ileal lines from the same donor remain distinguishable at the level of the methylome [5]. This is the strongest argument for deriving organoids from the anatomical site of the disease under study.

The limits are equally clear. Organoids contain epithelium and nothing else — no immune cells, fibroblasts, enteric neurons, vasculature or microbiota unless deliberately added. Any disease whose mechanism runs through those compartments is modelled only in part. The closed lumen also inverts the geometry of the gut: the apical surface faces inward, inaccessible to anything added to the medium. Much of the past decade's methodological work has been an attempt to get around that single topological fact.

Matrix Dependence and the Limits of Reproducibility

Almost every result described here was obtained in Matrigel, an undefined, animal-derived basement-membrane extract from Engelbreth-Holm-Swarm mouse sarcoma in which proteomics identifies more than 1,800 unique proteins [6]. Growth factor content, stiffness and protein composition vary between lots, and that variation propagates into the biology. Lot-to-lot variability is universally acknowledged and, as far as the primary literature reaches, unquantified — no published coefficient of variation for organoid-forming efficiency across Matrigel lots exists to

cite. The 1,800-protein figure describes composition, not variability, and is no proxy for it.

Defined synthetic replacements exist. Polyethylene glycol hydrogels functionalised with RGD adhesion peptides support intestinal stem cell expansion and organoid formation, with stage-dependent requirements: a stiff matrix favours expansion while differentiation and organoid formation require a soft one, so matrix mechanics must change part-way through culture [7]. The materials literature treats Matrigel replacement as solved in principle and unsolved in practice [6].

Practically: the defined matrices work, they have not displaced Matrigel in the disease-modelling literature, and the stiffness values separating the expansion-permissive from the differentiation-permissive regime sit in figure panels rather than abstracts. A reader reproducing a published organoid drug response should assume matrix is an uncontrolled variable across laboratories.

Modelling Infection and Barrier Failure

Infection models forced the field to solve apical access, and produced three distinct workarounds. The first is microinjection. Vegetative Clostridioides difficile injected into the lumen of human intestinal organoids persisted for up to 12 hours, and toxigenic strains — but not non-toxigenic ones — disrupted epithelial paracellular barrier function, an effect reproduced by purified toxin A [8]. The second is the two-dimensional monolayer. An enhanced human gastrointestinal epithelial culture system generated 65 cell lines from patient biopsies, with lines obtained from all accessible tissue sites within two weeks and spheroids requiring passage every three days; on Transwell filters these produce polarised, mucus-covered monolayers with the apical face exposed [9]. The third is polarity reversal: removing the extracellular matrix causes enteroids to evert, presenting the apical surface outward while retaining differentiated cell types [10]. Table 1 sets the three configurations against

the intact organoid, with what each buys and what it gives up.

Table 1: Configurations used to reach the apical surface of intestinal organoids, with the confirmed finding that established each and the feature it sacrifices [2,8,9,10].			
Configuration	Apical access	Confirmed finding	Trade-off
Intact 3D organoid	None; lumen enclosed	Crypt-villus structure with all epithelial lineages from single Lgr5+ cells	Apical surface unreachable
Luminal microinjection	Direct injection into lumen	Vegetative C. difficile viable up to 12 h; toxin A reproduces barrier loss	Low throughput; mechanically disruptive
2D Transwell monolayer	Apical face exposed to medium	65 patient-derived lines; all accessible sites within 2 weeks; passage every 3 days	Crypt-villus architecture lost
Apical-out enteroid	Apical surface everted outward	Matrix removal reverses polarity; differentiated lineages retained	Enclosed luminal compartment lost

These platforms answered questions no cell line could. Human noroviruses, uncultivable for decades, replicate in stem cell-derived human enteroids, with strain-dependent bile requirements [11]. The same infrastructure was redeployed within weeks in 2020: SARS-CoV-2 was shown to productively infect human gut enterocytes [12], and parallel work established infection of bat and human intestinal organoids, supporting the intestine as a genuine site of replication rather than a passive route of shedding [13].

Each workaround carries its own distortion. Microinjection is low-throughput and mechanically disruptive, and delivers an inoculum to a lumen whose composition is not that of the gut. Monolayers restore apical access and transepithelial resistance measurement but abandon the crypt-villus architecture and the stem cell compartment. Everted enteroids keep three-dimensional structure and apical exposure but lose

the enclosed compartment in which secreted products accumulate. No configuration is correct for all questions, and the choice is rarely justified explicitly in published infection studies.

The infection literature is the most convincing application of intestinal organoids, because its question — can this pathogen infect and replicate in human intestinal epithelium, and what does it do to the barrier — is one the model is constituted to answer. The absence of immune cells limits pathogenesis but not tropism.

Inflammatory Bowel Disease and Engineered Oncogenesis

Organoids from patients with inflammatory bowel disease retain phenotypes not obviously explained by the inflammatory environment they came from. Control and IBD-derived colon organoids reached equivalent cell composition after 10 to 12 days of culture, after which the IBD cultures showed reduced size and budding capacity, increased cell death, luminal debris, inverted polarisation and significantly decreased tight-junction protein expression [14]. In ulcerative colitis, alterations in the epithelial stem cell compartment persist in culture and are proposed to contribute to permanent changes in the mucosa [15]. Whether these are cell-autonomous defects or durable epigenetic memory of inflammation is resolved by neither study, and the patient numbers behind the phenotypes are small.

The cancer literature is more mechanistically decisive because it can build the disease rather than only observe it. Sequential CRISPR editing of four commonly mutated colorectal cancer genes — APC, TP53, KRAS and SMAD4 — in cultured human intestinal stem cells produced quadruple mutants that grew independently of all four stem-cell-niche factors and tolerated the p53 stabiliser nutlin-3, with combined APC and TP53 loss sufficient to generate extensive aneuploidy [16]. Niche-factor independence thus became a functional assay for

transformation, and one that maps onto patient material: a library of 55 colorectal tumour organoid lines spanning histological subtypes and clinical stages showed progressive loss of niche-factor requirements along the adenoma-carcinoma sequence [17].

That correspondence between an engineered genotype and a graded phenotype in patient-derived lines is the strongest internal validation the field has produced. It concerns mechanism, not prediction; the two should not be conflated.

Tumour Organoid Biobanks and Predictive Drug Testing

Patient-derived organoids preserve the genetics of their parent tumours. Cultures established from needle biopsies of metastatic colorectal cancer succeeded in 71% of attempts across 14 patients, and 90% of somatic mutations were shared between organoid and matched biopsy, with DNA copy-number profiles correlating at $r = 0.89$ across a panel of 1,977 cancer-relevant genes [18]. The first prospective living biobank comprised tumour organoids from 20 consecutive colorectal carcinoma patients with matched normal cultures, and its mutation spectrum matched large-scale colorectal cancer analyses [19]. Genomic and histological fidelity to the parental tumour is not in dispute.

Table 2: Cohort size and concordance with patient outcome across patient-derived intestinal organoid studies; cells marked not stated are absent from the source abstracts [18,19,21,22,23,27].			
Study	Setting	Cohort	Concordance with outcome
Weeber 2015	Metastatic CRC biopsies	14 patients	Not assessed; 71% established
van de Wetering 2015	Primary CRC biobank	20 patients	Not assessed
Yao 2020	Rectal cancer, chemoradiation	Not stated	84.43% accuracy
Ooft 2019	Metastatic CRC, chemotherapy	Not stated	Over 80% for irinotecan; failed for 5-FU plus oxaliplatin
Ooft 2021	Organoid-guided off-label	61 enrolled, 54 eligible	6 treated, 0 responses

Study	Setting	Cohort	Concordance with outcome
de Winter-de Groot 2018	Cystic fibrosis, swelling assay	34 infants	$r = -0.82$ versus sweat chloride

Whether that fidelity transfers to drug response is answered inconsistently. Organoids from metastatic gastrointestinal cancers were reported to model treatment response against matched clinical outcomes [20]; that study's frequently quoted concordance statistics lie in the article body and could not be verified, so we do not reproduce them. In locally advanced rectal cancer, organoid responses predicted neoadjuvant chemoradiation outcome with 84.43% accuracy, 78.01% sensitivity and 91.97% specificity, though the published abstract does not state the cohort denominator [21]. A prospective study in metastatic colorectal cancer found organoids predicted the response of the biopsied lesion in more than 80% of patients treated with irinotecan-based regimens without misclassifying any patient who would have benefited, and failed entirely for 5-fluorouracil plus oxaliplatin [22]. A test that works for one regimen and not another is not yet a test.

Forskolin-Induced Swelling and the Attrition Cascade

The SENSOR trial allocated treatment on organoid evidence. Sixty-one patients were enrolled and 31 organoid cultures generated from 54 eligible patients — a 57.4% establishment rate, below the 71% of the earlier biopsy series [18]. Twenty-five cultures were drug-screened, 19 showed substantial in vitro responses, six patients were treated on that basis (three with vistusertib, three with capivasertib), and none achieved an objective clinical response against a prespecified primary endpoint of a 20% objective response rate [23]. Every step was individually defensible and the end-to-end yield was zero. Attrition, not assay accuracy, stands between organoid drug screening and oncological practice.

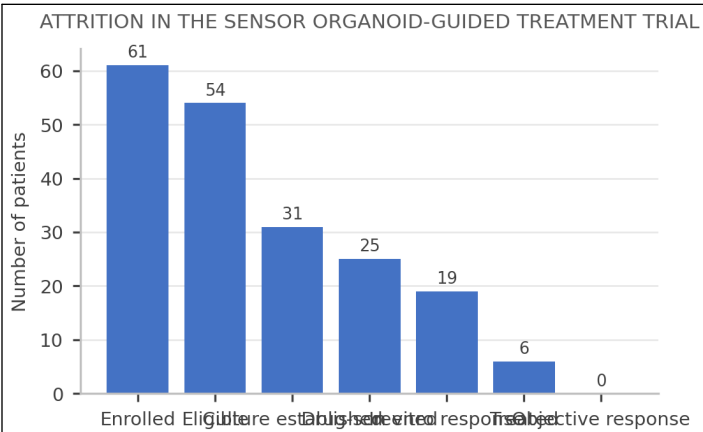


Figure 1: Stepwise patient attrition in the SENSOR trial of organoid-guided treatment for metastatic colorectal cancer, from 61 enrolled patients to zero objective clinical responses [23]; N = 61 enrolled, 54 eligible.

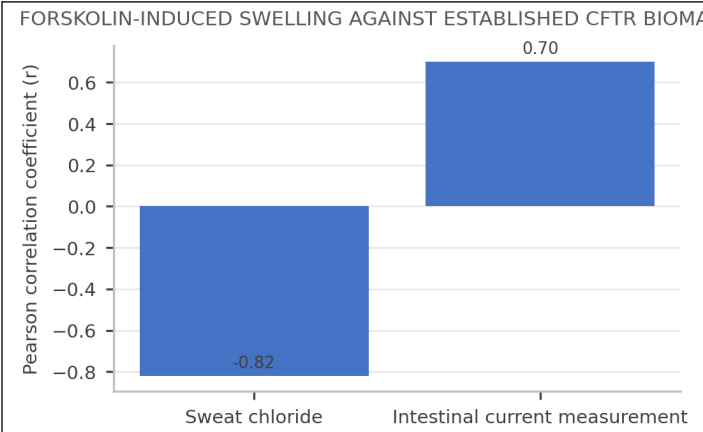


Figure 2: Correlation of forskolin-induced swelling of rectal organoids with sweat chloride concentration and with intestinal current measurement, both $p < 0.001$ [27]; N = 34 infants with cystic fibrosis.

Cystic fibrosis is the counter-example, and instructively the assay there is functional rather than pharmacological. Rectal organoids from patients with cystic fibrosis swell in response to forskolin in proportion to residual CFTR activity, a quantitative primary readout of the causal defect itself [24]. The assay discriminates responses to CFTR-modulating drugs across genotypes, including rare ones for which trial evidence is unobtainable [25], and has been standardised into a documented protocol [26]. In 34 infants with cystic fibrosis, forskolin-induced swelling correlated with sweat chloride concentration at $r = -0.82$ and with intestinal current measurement at $r = 0.70$, both

$p < 0.001$; infants with low swelling had higher immunoreactive trypsinogen ($p = 0.030$) and pancreatitis-associated protein ($p = 0.039$), more frequent pancreatic insufficiency ($p < 0.001$), more chest CT abnormalities ($p = 0.049$) and lower z-scores for maximal expiratory flow at functional residual capacity ($p = 0.033$) [27]. On that basis rectal organoids have been used to select modulator therapy for individual patients [28], though the reported accuracy statistics for that selection are qualitative in the published abstract.

Conclusion

The difference between the two clinical programmes is not organoid quality but what the assay measures. Forskolin-induced swelling reports the activity of a single protein whose loss causes the disease, on a continuous scale; the readout is the mechanism. Organoid chemosensitivity reports a mutant epithelial population in isolation from stroma, immune infiltrate, vasculature and drug pharmacokinetics, against outcomes in patients who have already received several prior lines of therapy. The first is a biomarker; the second is a surrogate for a system it does not contain.

Three things would change the position. Quantified matrix variability would allow cross-laboratory comparison of drug responses that currently cannot be compared. Reporting establishment rates and time-to-result as primary trial endpoints rather than incidental methods statements would make the 57% and 71% figures interpretable as feasibility data [18,23]. And co-culture systems restoring the missing compartments would test whether the oncology failures are assay failures or context failures. Until then, intestinal organoids are an excellent instrument for mechanism, a good one for infection biology, and an unproven one for choosing a cancer drug.

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