Improving Reproducibility and Scalability in Human Organoid Culture Using AI-Engineered Proteins and a Defined Synthetic Hydrogel

Brianna Ettestad, Xi Lu, John Leerar, Emily Freeburne, Dulanjani Rajaguru, Yiao Wang, Barbara Burroughs, Tatsiana Gerassenkov, Christian Erickson, Neil Otto, Didarul Bhuiyan, Hallie Hintz, and Chris Heger
Bio-Techne, Minneapolis, MN

Introduction

Three-dimensional cell and organoid culture systems are key platforms for therapeutic screening and disease modeling. These models rely on animal-derived matrices, notably basement membrane extract (BME), and unstable growth factors such as FGF-10 and Wnt-3a[1]. These reagents pose manufacturing challenges, poor reproducibility[2,3], high costs, and no opportunities for clinical translation due to risk of contaminants. To address these limitations, a two-pronged strategy was used: (1) a novel variant of FGF-10 and a Wnt/RSPO (R-Spondin) agonist[4] with improved thermostability and solubility were developed through AI-driven protein engineering and mutation library screening and (2) a fully defined matrix, Cultrex™ Synthetic Hydrogel, was adopted to replace BME.

Human adult intestinal, liver, and lung organoids and iPSC-derived pancreatic duct-like organoids (PDLOs) were cultured with these reagents. The data revealed robust growth across multiple organoid types while maintaining key markers and morphology. The Wnt agonists more efficiently induced β-catenin signaling at reduced concentrations relative to wild-type Wnt-3a. Further, thermostable FGF-10 supported healthy organoid culture without needing additional supplementation. These findings support the advantages of combining thermostable FGF-10 and Wnt agonists with a synthetic hydrogel to generate supportive growth environments for diverse organoid models.

Materials and Methods

Media and Matrices: Human organoids were thawed in Cultrex UltiMatrix domes (Cat # BME001-05) prior to experiments in Cultrex Synthetic Hydrogel (Cat # CSH-RUO-01). Expansion and differentiation media were composed of Advanced DMEM/F12, GlutaMAX (1X), penicillin-streptomycin (1X), HEPES (1X), N21-MAX (1X, Cat # AR008), nicotinamide (Cat # 4106), and N-acetylcysteine (Cat # 7874). Depending on tissue, cytokines and small molecules include Noggin (Cat # 6057-NG), EGF (Cat # 236-GMP), Prostaglandin E_2 (Cat # 2296), A 83-01 (Cat # 2939), SB 202190 (Cat # 1264), Wnt-3a (Cat # 5036-GMP), R-Spondin 2 (Cat # 3266-RS), Wnt/RSPO2 (Cat # BT-WRSP2), FGF-10 Wild Type (WT, Cat # 345-FG), FGF-10 Heat Stable (HS, Cat # BT-FGF10HS), FGF-7 (Cat # 251-GMP), Gastrin (Cat # 3006), HGF (Cat # 294-HGN), Forskolin (Cat # 1099), R-Spondin 1 (Cat # 4645-RS), and Y-27632 (Cat # 1254).

Specific concentrations of media components for each organoid type can be found on protocols available on the R&D Systems website.

Bioactivity: Bioactivities of the Wnt and FGF-10 HS were assessed by measuring the abilities of the proteins to induce downstream receptor activation using reporter cell lines HEK293 TCF9-SEAP Wnt or Ba/F3 mouse pro-B cells transfected with FGFR2b.

Organoid Culture: Organoids were cultured in Cultrex UltiMatrix or Cultrex Synthetic Hydrogel in 24-well plates. Depending on the tissue type, seeding density varied between 15k-20k cells per 10 µL dome for Synthetic Hydrogel and 10k cells per 10 µL dome for UltiMatrix. Cells were fed every 2-3 days, and split weekly, or at tissue specific intervals. Organoid growth was quantified using an Incucyte® SX5 or MATLAB. Total organoid area was measured using the Incucyte® analytical software.

Immunocytochemistry (ICC): Organoids were fixed overnight in 2% PFA at 4 °C, washed with PBS and sucrose, and cryosectioned. Blocking was performed with 0.2% Triton X-100 and donkey serum. Primary antibodies were applied overnight at 4 °C. Slides were washed and incubated with secondary antibodies, washed, and counterstained with DAPI.

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Results

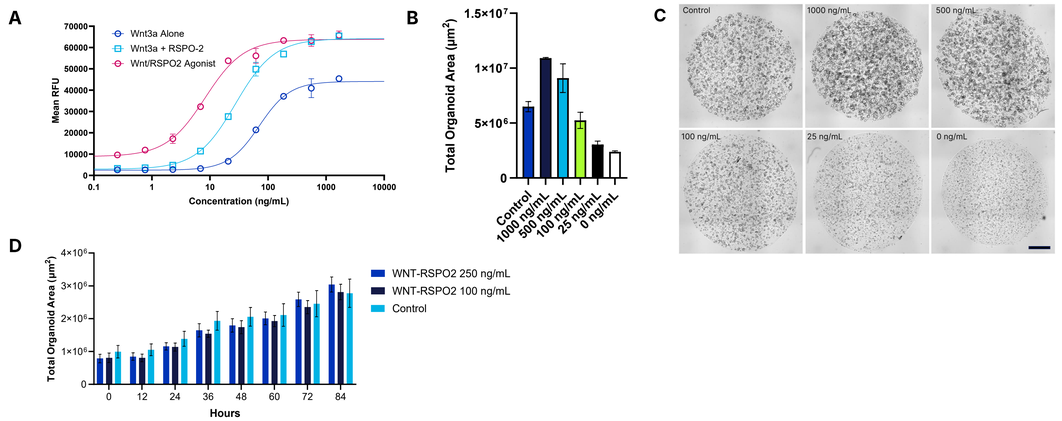

FIGURE 01. (A) Bioactivities of the recombinant human Wnt/R-Spondin 2 agonist compared to human Wnt-3a alone or human Wnt-3a and R-Spondin 2. The agonist exhibits better activity than the Wnt-3a protein alone and similar or better activity than Wnt-3a and R-Spondin 2 together. **(B)** and **(C)** Quantification of total organoid area (µm²/image) and brightfield images of enteroids cultured with UltiMatrix, varying concentrations of Wnt/RSPO2, or control (Wnt-3a and R-Spondin 2). At higher concentrations (i.e., 500 and 1000 ng/mL), Wnt/RSPO2 organoids expanded significantly faster than the control. Scale bar indicates 500 µm. **(D)** When used at 100-250 ng/mL, Wnt/RSPO2 enteroid groups matched the control group's growth rate in Synthetic Hydrogel.

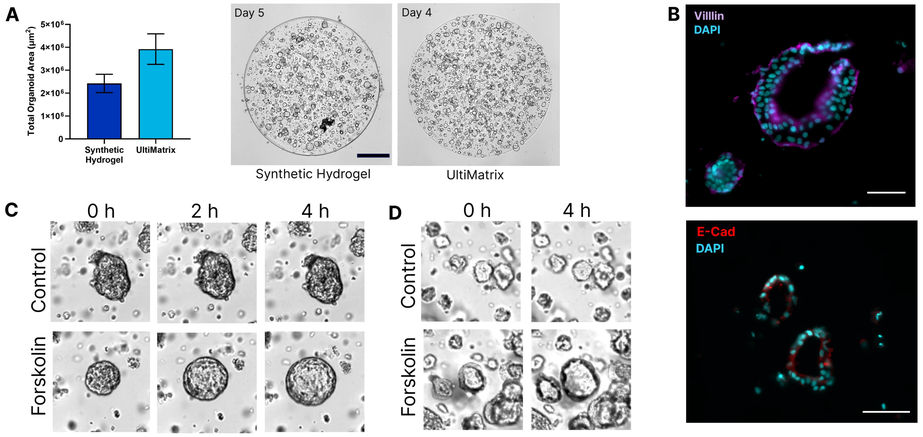

FIGURE 02. (A) Quantification of total organoid area on day 4 of enteroids plated at identical cell densities in Synthetic Hydrogel and UltiMatrix and brightfield images showing organoid size at day 5 (Synthetic Hydrogel) and day 4 (UltiMatrix). Organoids in Synthetic Hydrogel can lag behind BME in terms of size by 24-36 h depending on tissue/donor. Scale bar indicates 1 mm. **(B)** ICC images of enteroids stained with Villin, E-Cadherin and DAPI in Synthetic Hydrogel. Scale bar indicates 50 µm. **(C)** Enteroids and **(D)** Pancreatic duct-like organoids (PDLOs) cultured in Wnt/RSPO2 and Synthetic Hydrogel were differentiated for 7 days and then exposed to Forskolin (10 µM) or DMSO (control) to assess the function of mature CFTR-expressing secretory cells. The presence of Forskolin led to organoid swelling whereas control did not change in size. Scale bar indicates 100 µm.

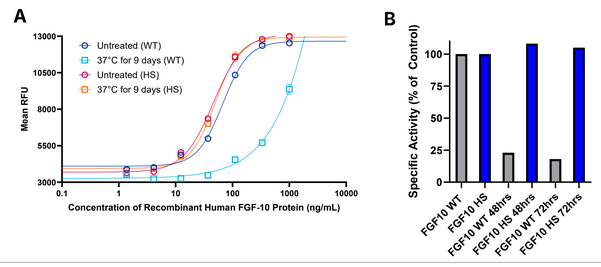

FIGURE 03. (A) FGF-10 WT or FGF-10 HS were either untreated or incubated at 37 °C for 9 days. FGF-10 HS retained similar bioactivity after incubation compared to the untreated, indicating that the HS protein has increased thermal stability. WT showed a significant loss of activity. **(B)** SEAP bioassay of HEK293 reporter cells that were incubated in DMEM media with FGF-10 HS or WT (600 ng/mL) at
37 °C for 48 or 72 hrs. FGF-10 HS maintains activity at 48 and 72 hrs at 37 °C whereas WT loses activity.

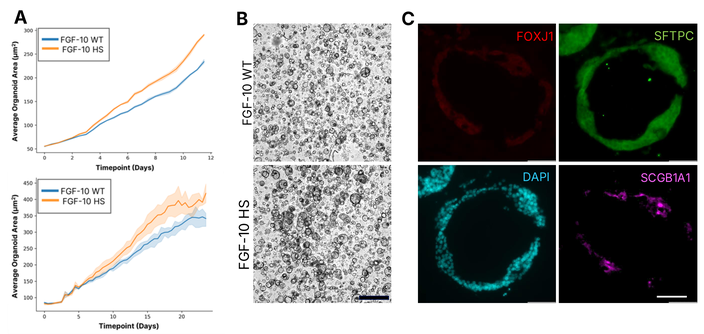

FIGURE 04. (A) Size curve of lung organoids grown with either FGF-10 HS or WT in UltiMatrix (top) or Synthetic Hydrogel (bottom). **(B)** Brightfield images of lung organoids grown in Synthetic Hydrogel with either FGF-10 WT (top) or HS (bottom). Culture with FGF-10 HS led to larger organoids compared to FGF-10 WT in both matrices. Scale bar indicates 1 mm. **(C)** Sections of lung organoids (cultured for 25 days) in Synthetic Hydrogel with FGF-10 HS stained for SFTPC, SCGB1A1, FOXJ1, and DAPI. Phenotype is indicative of alveolar cell type. Scale bar indicates 50 µm.

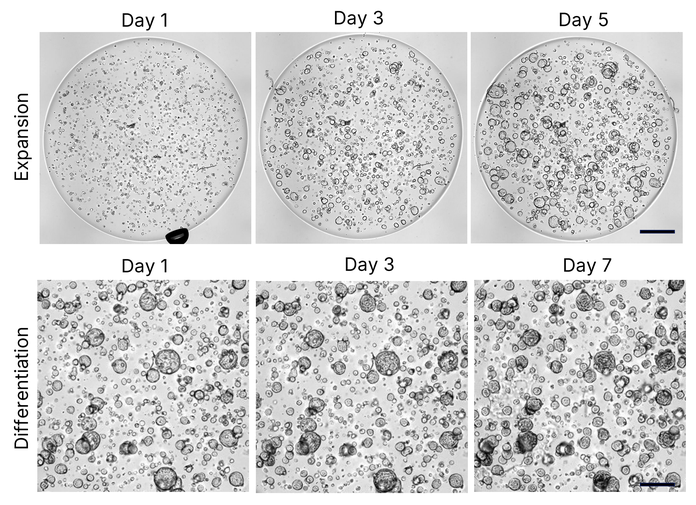

FIGURE 05. Brightfield images of liver organoids cultured in Cultrex Synthetic Hydrogel under expansion (top row, scale bar indicates 500 µm) and differentiation conditions (bottom row, scale bar indicates 100 µm). Expanding liver organoids exhibit typical cystic morphology and can be passaged at least three times in Synthetic Hydrogel (data not shown). Upon differentiation, the organoids transition to a more complex structure, displaying folding and wrinkled morphologies.

Conclusions

- Designer proteins, including FGF-10 HS and Wnt/RSPO2 agonist exhibit thermostability in culture systems leading to improved organoid expansion.
- Cultrex Synthetic Hydrogel effectively supports the growth of stem cell-derived enteroids, lung, and liver organoids, providing a reliable and animal component-free ECM alternative.
- The combination of AI modified proteins with Synthetic Hydrogel establishes a reproducible and robust platform for organoid research.

References
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2. Shin W et al. iScience. 2020.
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4. Janda et al. Nature 2017.