Patient-derived tumoroids

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Optimization of tumor tissue enzymatic dissociation and initial culture conditions for rapid and viable tumoroid generation

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Key Takeaways

- OncoPro™ Tumoroid Culture Medium is a standardized, serum-free and conditioned medium-free media system for patient-derived tumoroid culture
- Optimization of tumor dissociation conditions balances viable tumor cell yield with tumoroid formation in culture
- Addition of growth factors during initial derivation may improve long-term tumoroid establishment efficiency

Introduction

Several studies have indicated that patient-derived cancer models (tumoroids or cancer organoids) recapitulate key genotypic and phenotypic features of patient tumors, making them potentially valuable tools for basic and translational cancer research. OncoPro™ Tumoroid Culture Medium is a serum-free, conditioned medium-free cell culture medium designed to culture tumoroids derived from multiple cancer indications. Here, we highlight studies aiming to improve tumoroid formation during initial culture establishment, focusing on the development of enzymatic dissociation cocktails that maximize tumoroid formation in culture and results of screening experiments designed to improve tumoroid formation during initial tumoroid passaging.

OncoPro™ Basal Medium; OncoPro™ BSA; OncoPro™ Supplement; Gibco™ B-27™

Optimized for epithelial-derived carcinomas:

Demonstrated Compatibility: Colorectal, Lung, Head & Neck, Pancreatic, Breast, Endometrial

Suggested additional growth factors:
Lung, head & neck – FGF10
Pancreatic – FGF10, gastrin
Breast, endometrial – FGF10, β-estradiol

Materials and Methods

Surgical resection samples were placed in supplemented Hibernate™-A Medium containing antibiotics and shipped overnight at 4° C to a central processing lab. Tissues were dissociated via enzymatic and mechanical dissociation using various enzyme cocktails, counted, and plated in OncoPro™ Tumoroid Culture Medium (in the presence of indication-specific factors when applicable). Cultures were analyzed for metabolic activity (using PrestoBlue™ HS Cell Viability Reagent) and tumoroid area (by imaging on an EVOS™ M7000 Imaging System) after 7 days in culture. The workflow is illustrated schematically in **Figure 1**. In separate experiments, dissociated tumor cells from resections or commercial sources were cultured in OncoPro™ Tumoroid Culture Medium supplemented with various factors, and tumoroid size and cell count were tracked over initial passage(s).

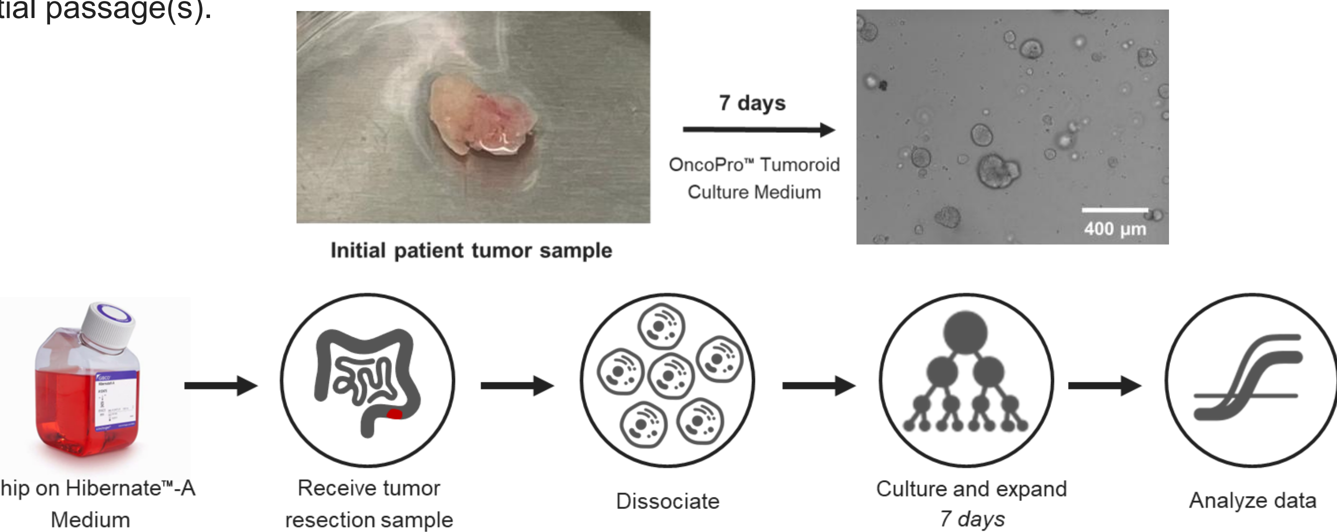


Figure 1. Experimental workflow for generation of tumoroid from cancer tissue samples. Surgical resection samples were shipped overnight in supplemented Hibernate-A medium, dissociated, and plated in OncoPro Tumoroid Culture Medium. After 7 days of culture, the resulting tumoroids were analyzed.

Results

Transport medium design for retained cell viability during tissue shipment and storage

Tissue transport buffer was designed to promote cell survival in CO_2-independent conditions. Selected samples were divided into pieces upon receipt and stored in the tissue transport buffer at 4˚C for various intervals prior to enzymatic dissociation and quantification of cell number and viability. Samples retained similar viability during storage in transport buffer (**Figure 2**).

Tissue Transport Buffer

Component	Amount in ml
Hibernate™-A Medium (1x)	239.5 ml
GlutaMAX™ Supplement (100X)	2.5 ml (1x)
B-27™ Supplement (50X)	5 ml (1x)
Pen-Strep (100x)	2.5 ml (1x)
Primocin® (50 mg/ml stock)	0.5 ml (1x)
Y-27632 (optional; assumes 10 mM stock solution for final 10 µM concentration)	0.25 ml

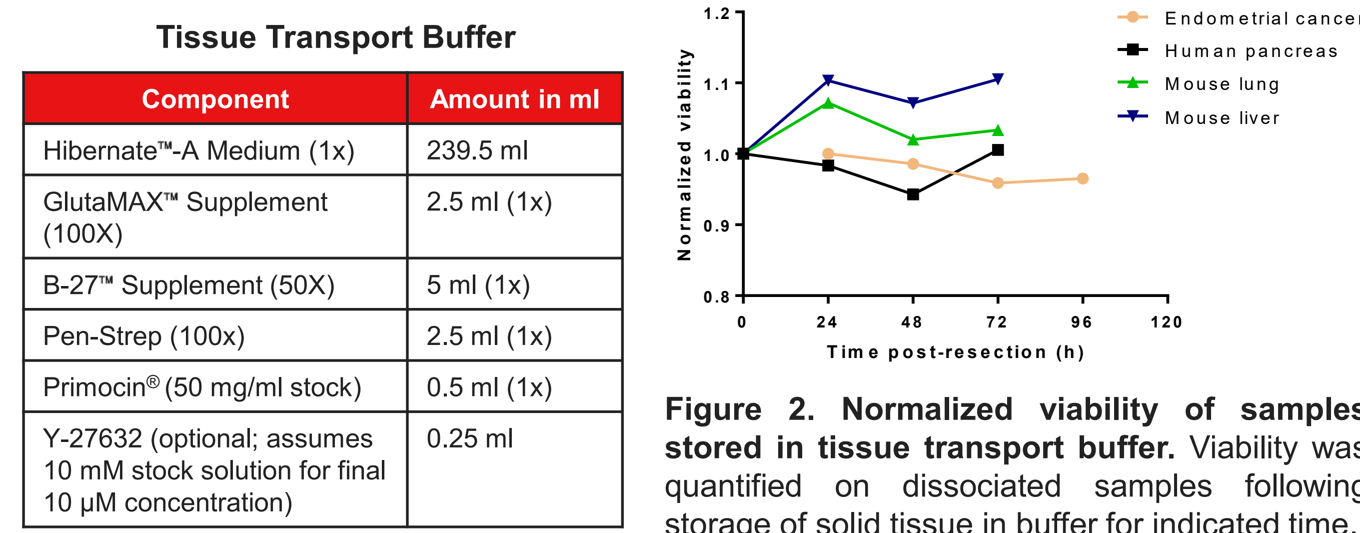
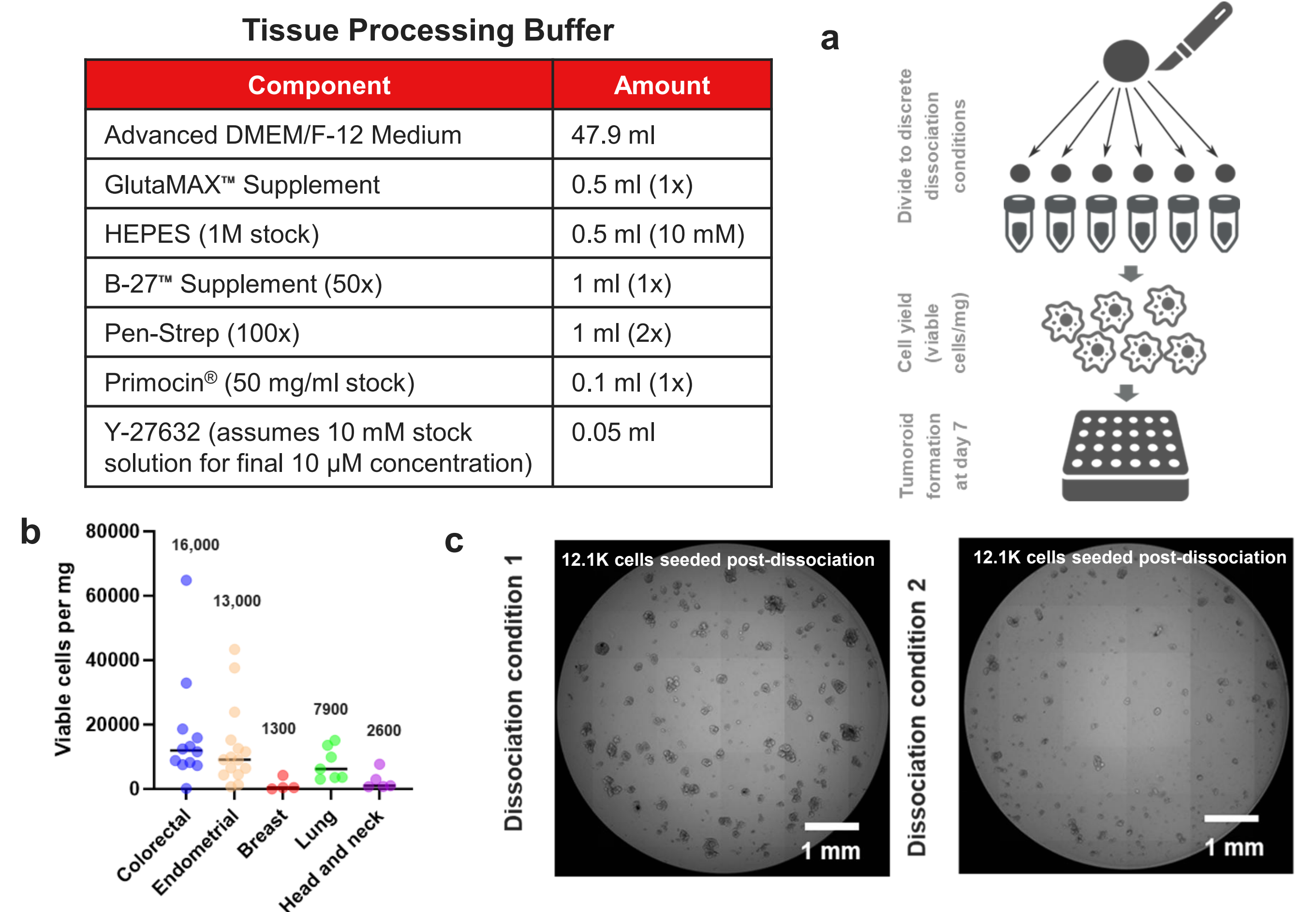


Figure 2. Normalized viability of samples stored in tissue transport buffer. Viability was quantified on dissociated samples following storage of solid tissue in buffer for indicated time.

Tissue dissociation workflow

Following transport, tissues were placed in tissue processing buffer containing enzymes that promote viable cell release. Specifically, received tissues were minced, mixed well, and divided for dissociation in multiple enzyme mixtures as dictated by a design-of-experiment approach to study a range of components and concentrations in a donor-blocked format (**Figure 3**). Dissociation data pooled by donor across conditions indicated effective dissociation, with a wide range in post-dissociation yield based on cancer indication. When an identical number of viable cells were seeded following dissociation in distinct enzyme mixtures, differential tumoroid formation was observed. Further analysis of enzymatic dissociation cocktails leading to high yields and tumoroid formation in culture are discussed in the next section.



Tissue Processing Buffer

Component	Amount
Advanced DMEM/F-12 Medium	47.9 ml
GlutaMAX™ Supplement	0.5 ml (1x)
HEPES (1M stock)	0.5 ml (10 mM)
B-27™ Supplement (50x)	1 ml (1x)
Pen-Strep (100x)	1 ml (2x)
Primocin® (50 mg/ml stock)	0.1 ml (1x)
Y-27632 (assumes 10 mM stock solution for final 10 µM concentration)	0.05 ml

Figure 3. Cell yields and viabilities following dissociation of solid tumor resection samples. (a) Tissue dissociation workflow. (b) Viable cells per mg tissue after tissue dissociation (pooled across conditions for a given donor). Mean viable cells per mg is displayed for each indication. Each point represents one donor. (c) Representative images of tumoroid formation following dissociation of donor-matched tissue with differing enzymes.

Optimization of tissue dissociation cocktails

Tumor tissue dissociation cocktails were determined based on analysis of viable cell yield and health in culture for multiple donors per cancer indication. Example data for colorectal cancer samples are shown below (**Figure 4**). Final enzymatic cocktail formulations for four cancer indications are shown.

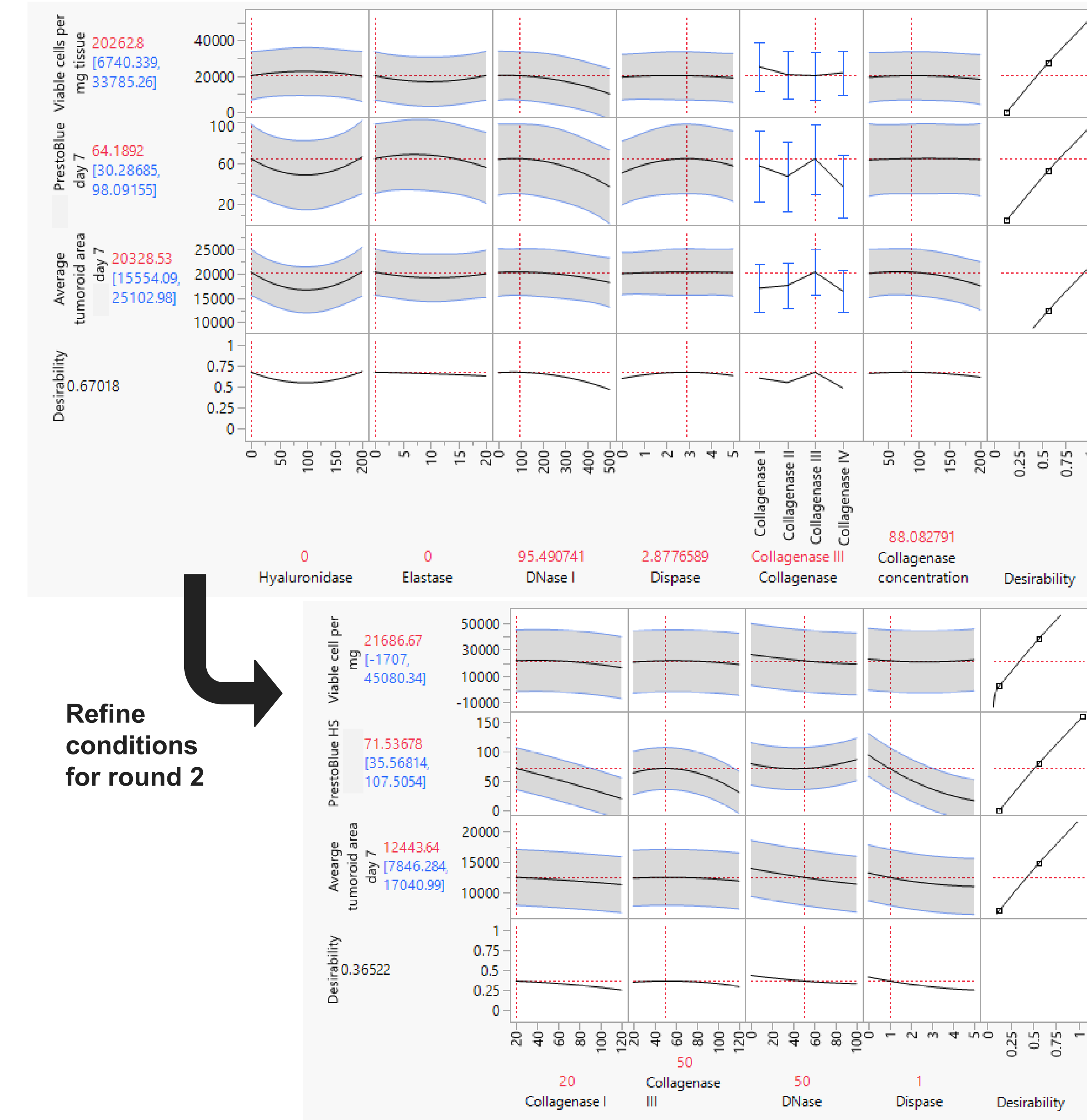


Figure 4. Effect of enzyme concentration on tissue dissociation and initial colorectal tumoroid culture. Impact of enzymes on viable cell yield post-dissociation and tumoroid formation following seven days of culture. Five donors were used for each optimization round.

Colorectal cancer

Component	Amount
Tissue Processing Buffer	2.5 ml
Collagenase Type I	20 U/ml
Collagenase Type III	50 U/ml
Dispase	1 U/ml
DNase I	50 U/ml

Lung cancer

Component	Amount
Tissue Processing Buffer	2.5 ml
Collagenase Type I	80 U/ml
Hyaluronidase	50 U/ml
Dispase	1 U/ml
DNase I	100 U/ml

Breast cancer

Component	Amount
Tissue Processing Buffer	2.5 ml
Collagenase Type III	150 U/ml
Dispase	1 U/ml
DNase I	50 U/ml

Endometrial cancer

Component	Amount
Tissue Processing Buffer	2.5 ml
Collagenase Type III	5 U/ml
Collagenase Type IV	5 U/ml
Dispase	7 U/ml
DNase I	50 U/ml

Screening of factors to promote tumoroid expansion during initial culture

Viably cryopreserved dissociated tumor cell (DTC) samples and dissociated breast cancer samples were cultured in OncoPro medium supplemented with additional factors in a designed experiment. Cells were imaged over time for analysis of tumoroid area, and tumoroids in each conditions were dissociated and counted. The influence of each input supplement was modeled to identify factors that potentially increase tumoroid formation and cell number (**Figure 5**).

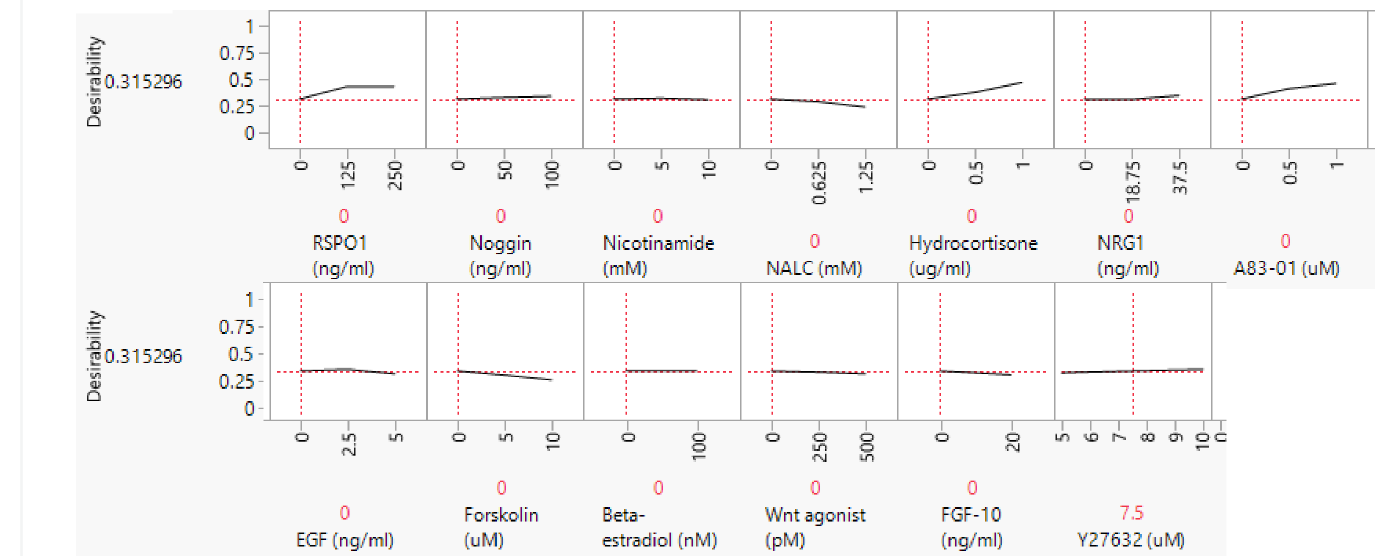
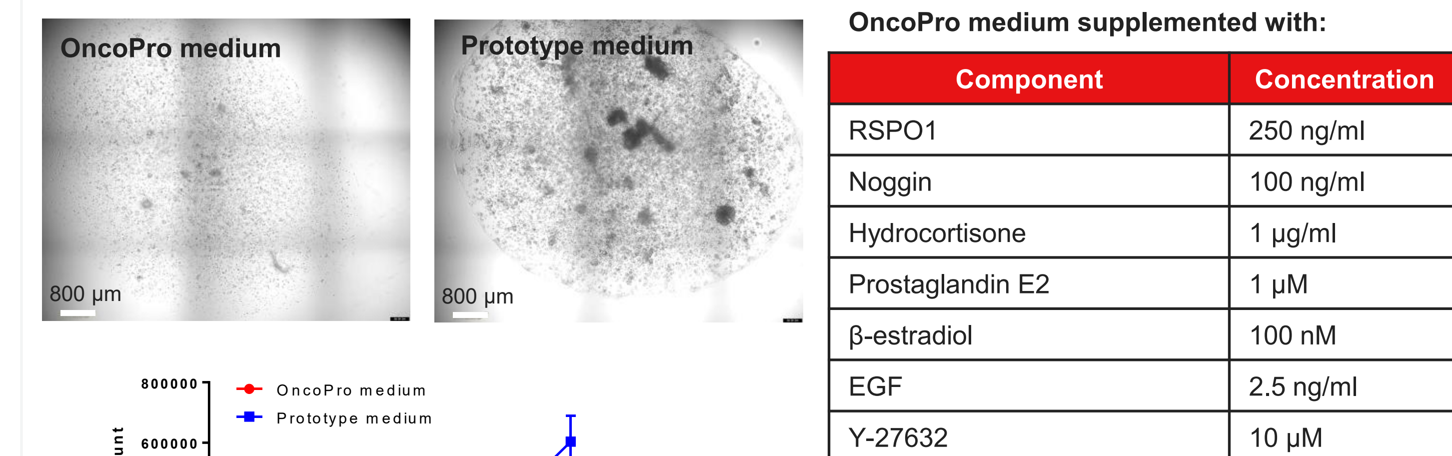


Figure 5. Effects of supplements to OncoPro™ Tumoroid Culture medium. Desirability plots were generated from data on tumoroid area and cell count during initial breast cancer cell culture. Results are consolidated across six donors.

Following screening for factors promoting viable tumoroid cells during initial culture, a prototype formulation was generated and tested on a fresh breast cancer tissue sample, on which cell counts were tracked over multiple passages (**Figure 6**).



OncoPro medium supplemented with:

Component	Concentration
RSPO1	250 ng/ml
Noggin	100 ng/ml
Hydrocortisone	1 µg/ml
Prostaglandin E2	1 µM
β-estradiol	100 nM
EGF	2.5 ng/ml
Y-27632	10 µM

Figure 6. Breast tumoroid expansion in prototype derivation medium. Representative images after 13 days of culture and cell counts over time following culture in OncoPro medium for breast cancer (includes 10 ng/ml heat stable FGF-10 and 10 nM β–estradiol) or supplemented OncoPro medium.

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