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CRISPRi Screening in Cancer Spheroids to Investigate Factors in Genome Stability

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Advisor: Dr. Paul Campagnola
Date: 10/04/2024

Presentation Overview

- Problem Statement
- Background Research
- Product Design Specifications
- Preliminary Designs & Design Matrices
  - Cell line
  - Spheroid Formation Protocol
- Conclusions
- Acknowledgements
- References

Problem Statement

- **Current Limitations:**

  Existing CRISPR screening in 2D monolayers fails to identify factors that regulate genome stability and DNA damage in the 3D environment of tumors.

- **3D Models:**

  There is a need for a cell culture method that accurately mimics the 3D tumor microenvironment to better assess gene function and cellular responses.

- **Project Objective:**

  The project aims to identify tumor-specific factors that regulate genome stability using CRISPRi screening in optimized 3D cultures to access DNA damage.

Background Research I

2D versus 3D

- 2D monolayer CRISPR screens do not mimic key aspects of tumor biology [1].
- Growth phenotypes in 3D better resemble those seen in tumors [1].

Cell Line

- Derived from tumor [2]
- Can proliferate indefinitely in culture [2]
- Assembled into a 3D structure → spheroid formation [2]

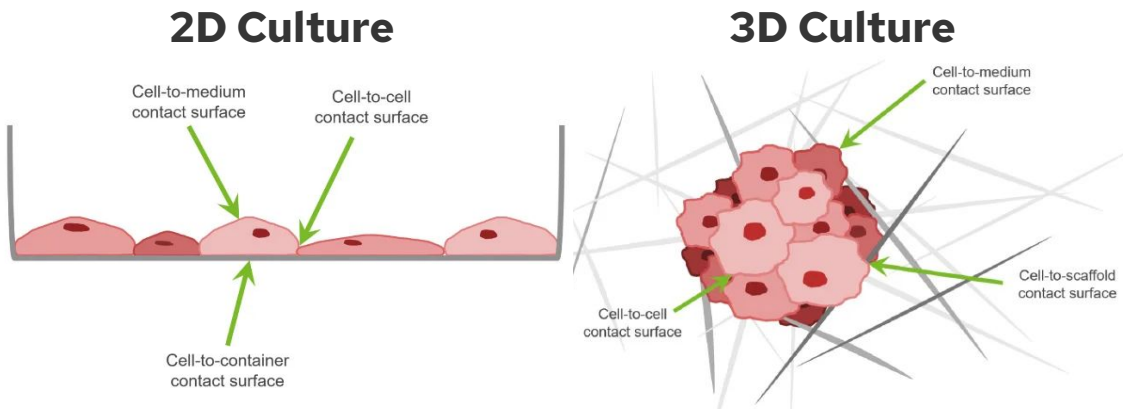

Figure 1: 2D versus 3D cell culture [1]

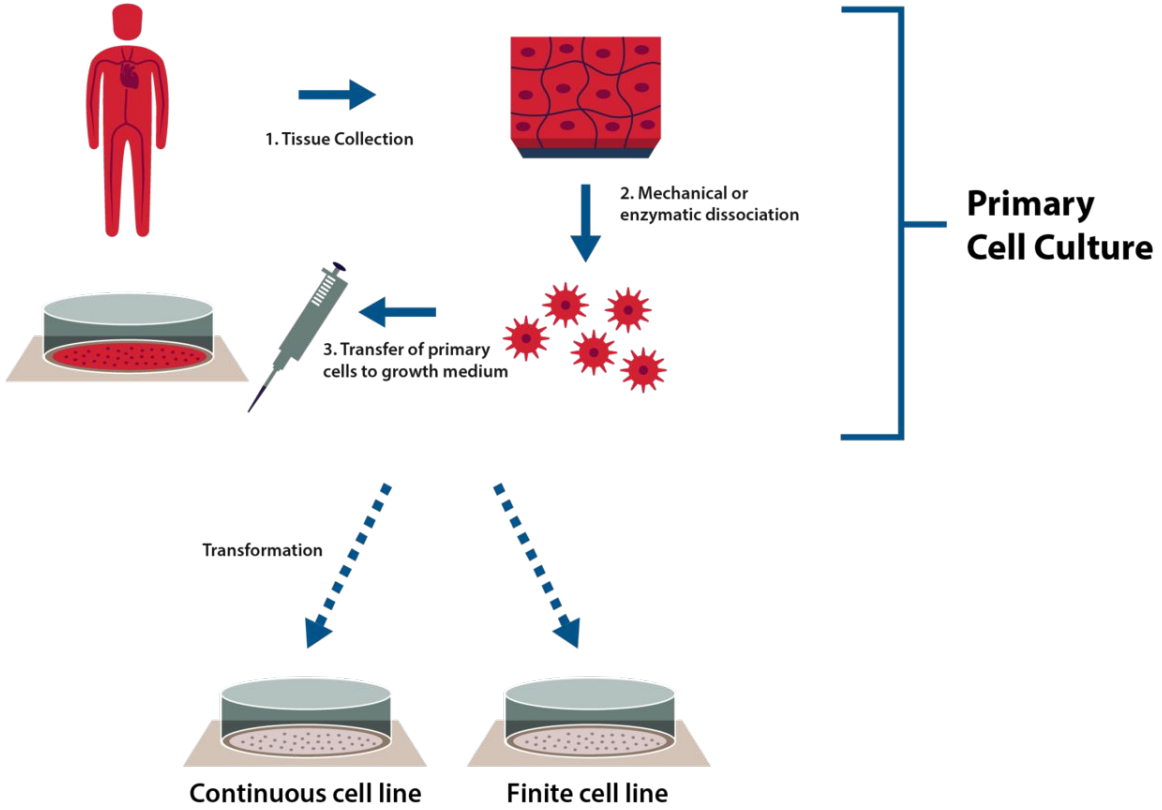

Figure 2: Cell Line Creation [2]

Background Research II

CRISPRi Screening

- Knockdown every gene/segment of sequence available in a full genome [3].
- Knockdown of gene via lentiviral infection gRNA (guide RNA) and Cas9-expressing gene [3].
- Check for DNA damage after knockdown of each gene via γH2AX staining [4].

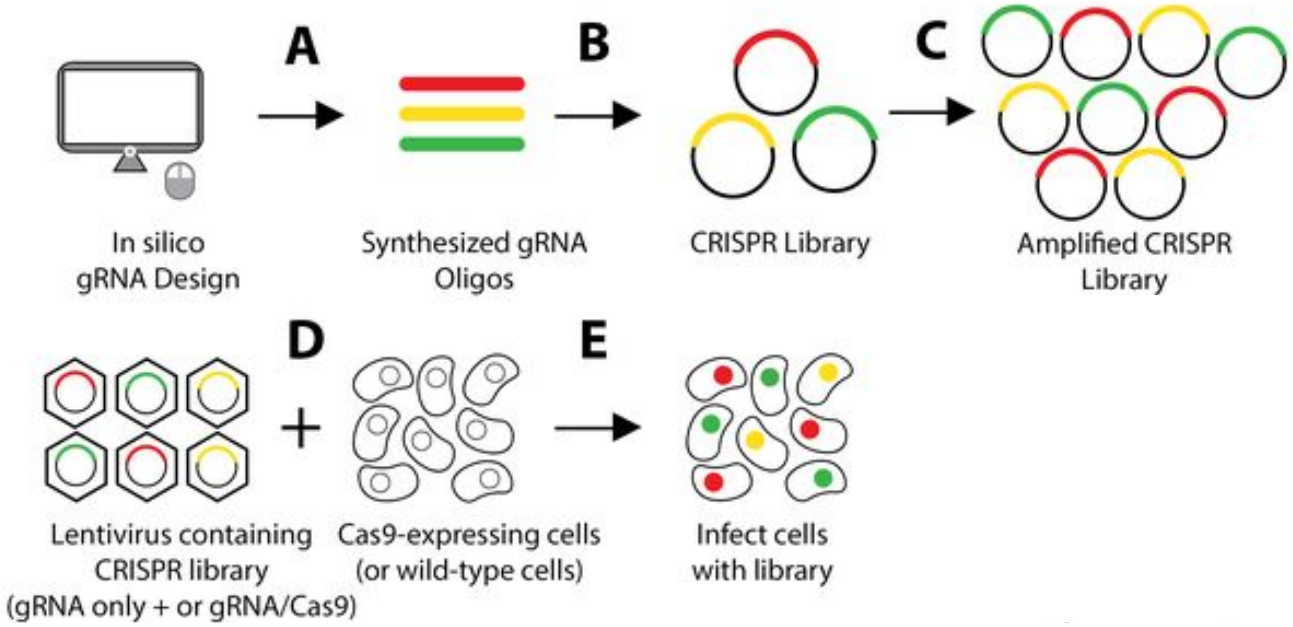

Figure 3: Lentiviral infection pathway [3]

Figure 4: Gene knockdown via CRISPR/Cas9 [4].

Background Research III

γH2AX

- Histone variant which detects DNA damage such as double strand breaks (DSB) [4]
- Early cancer detection
- Cancer treatment
  - Platinum treatments
  - γH2AX facilitates DNA repair

Drug efficiency

- Z-score: sensitivity of cell line to a specific drug when compared to other cell lines [5]

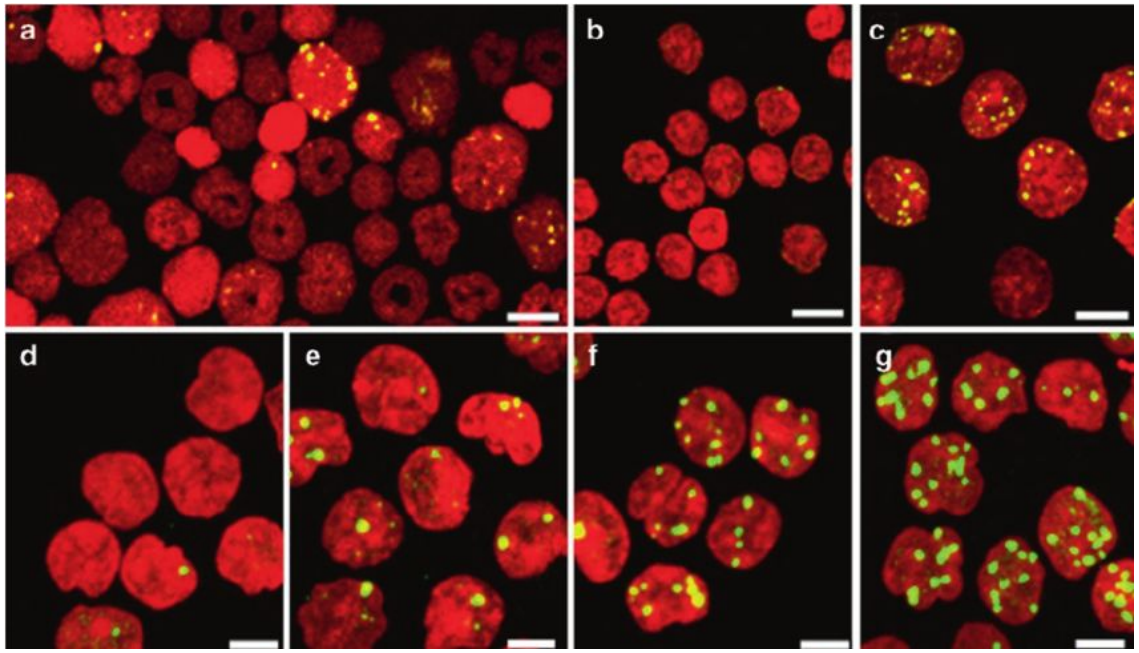

Figure 5: Staining of γH2AX [5]

Product Design Specifications

1

Viability & Adherence:

Human, adherent cell line with good cell-cell interactions, high transfection efficiency, and sensitivity to cisplatin treatment.

2

BSL-2 Safety:

Handling infectious agents must be conducted in BSCs by trained personnel in a restricted laboratory

3

CRISPR Compatibility:

Optimize formation protocol to be used with CRISPR screens [1]

4

Staining:

Develop a protocol to stain for γH2AX: an indicator of DNA damage by DSB [6]

5

Screen:

Perform a high-throughput genome-wide CRISPR screening.

6

Budget:

$1000

Cell Lines

NCI-H23

- Adenocarcinoma
  - Non-small cell lung cancer (NSCLC)
- Doubling time: 38 hours [7]
- Mutations:
  - TP53, KRAS, and EGFR [7]
- Z-score average:
  - -0.73 for cisplatin [8]
- $555.00 for 1 vial of frozen cells from ATCC [7]

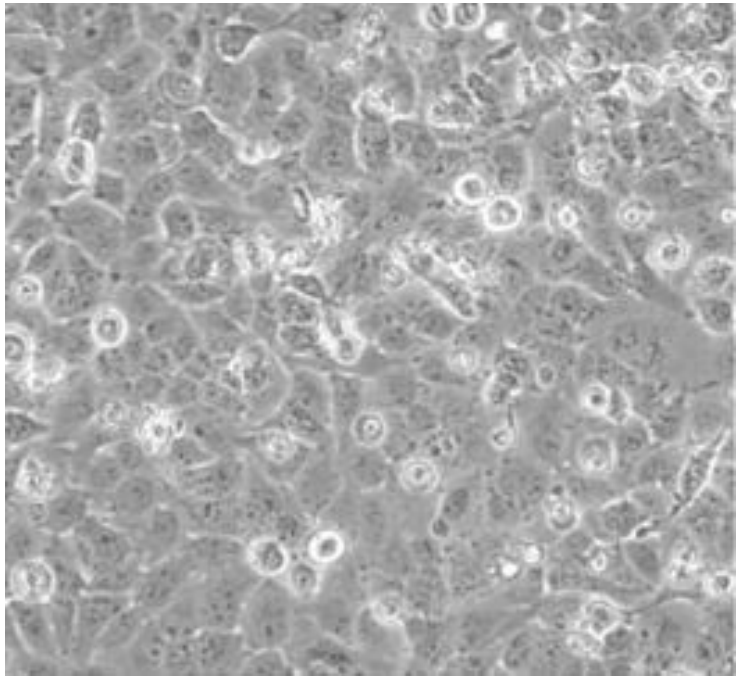

Figure 6: Phase contrast image of high density H23 [6]

A549

- Carcinoma
  - NSCLC
- Doubling time: 22 hours [9]
- Mutations:
  - TP53, EGFR, PIK3CA, ALK, and PTEN [10]
- Z-score average:
  - -0.12 for cisplatin [11]
- $555.00 for 1 vial of frozen cells from ATCC [9]

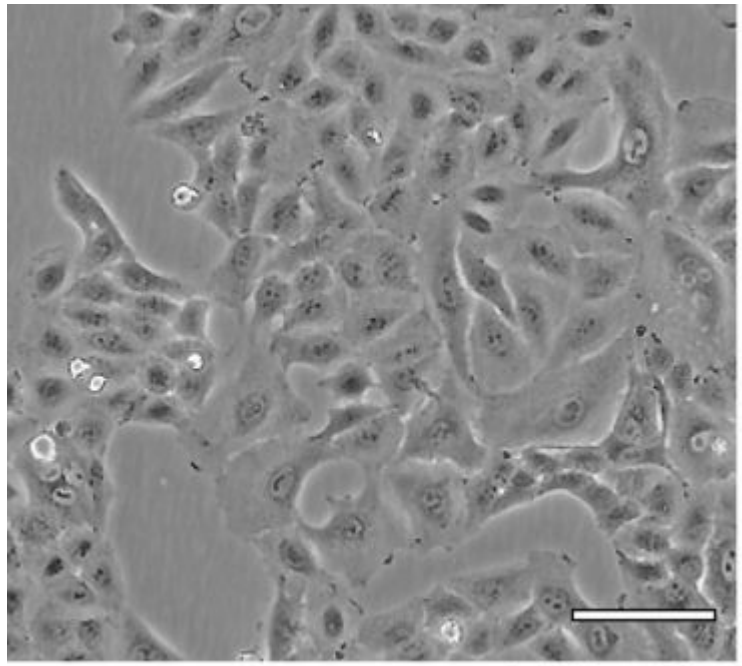

Figure 7: Phase contrast image of high density A549 [7]

OVCAR-5

- Ovarian carcinoma
- Doubling time: 27 hours [12]
- Mutation Summary:
  - KRAS 12, EGFR, and CLAUDIN-4 [12]
- Z-score average:
  - +0.52 for cisplatin [13]
- Product discontinued [14]

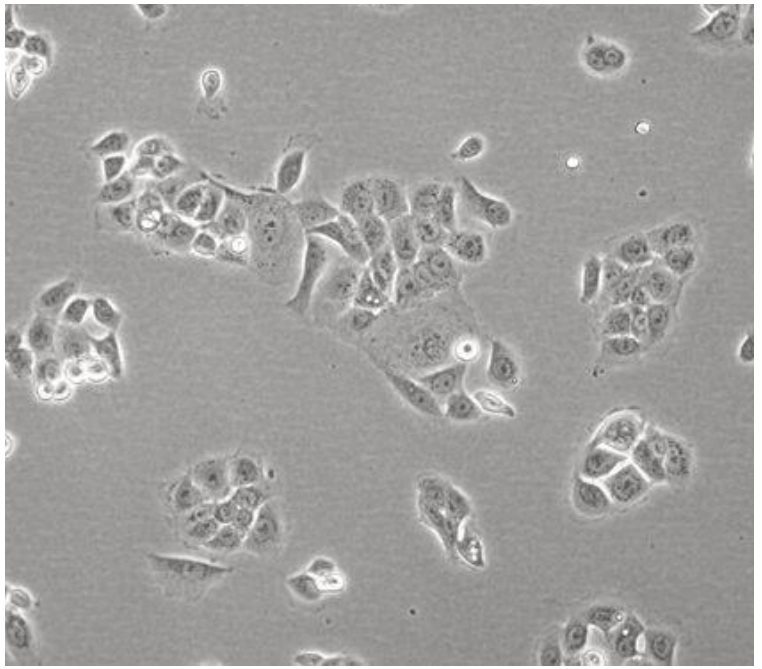

Figure 8: Phase contrast image of low density OVCAR-5 [8]

Cell Line Design Matrix

Table 1. Design matrix for Cell Line.

Design Criteria (weight)	NCI-H23		A549		OVCAR-5	
	Score X/5	Weighted score	Score X/5	Weighted score	Score X/5	Weighted score
Viability (20)	4	16	5	20	3	12
Adhesion (20)	3	12	4	16	4	16
Reproduction Speed (20)	3	12	5	20	4	16
Drug Sensitivity (15)	4	12	5	15	3	9
Genetic Mutation (15)	5	15	3	9	2	6
Ease of Procurement (10)	5	10	5	10	5	10
Total Score (100)	77		90		69	

Spheroid Formation Protocol

Treated Tissue Culture Plates

- Scaffold-free method [15]
- Hydrophilic polymer-coated plates are used to prevent cell adhesion to wells [15]
- Cell aggregate formed by cell-cell interactions [15]
- Spheroid formation after up to 4 days [16]

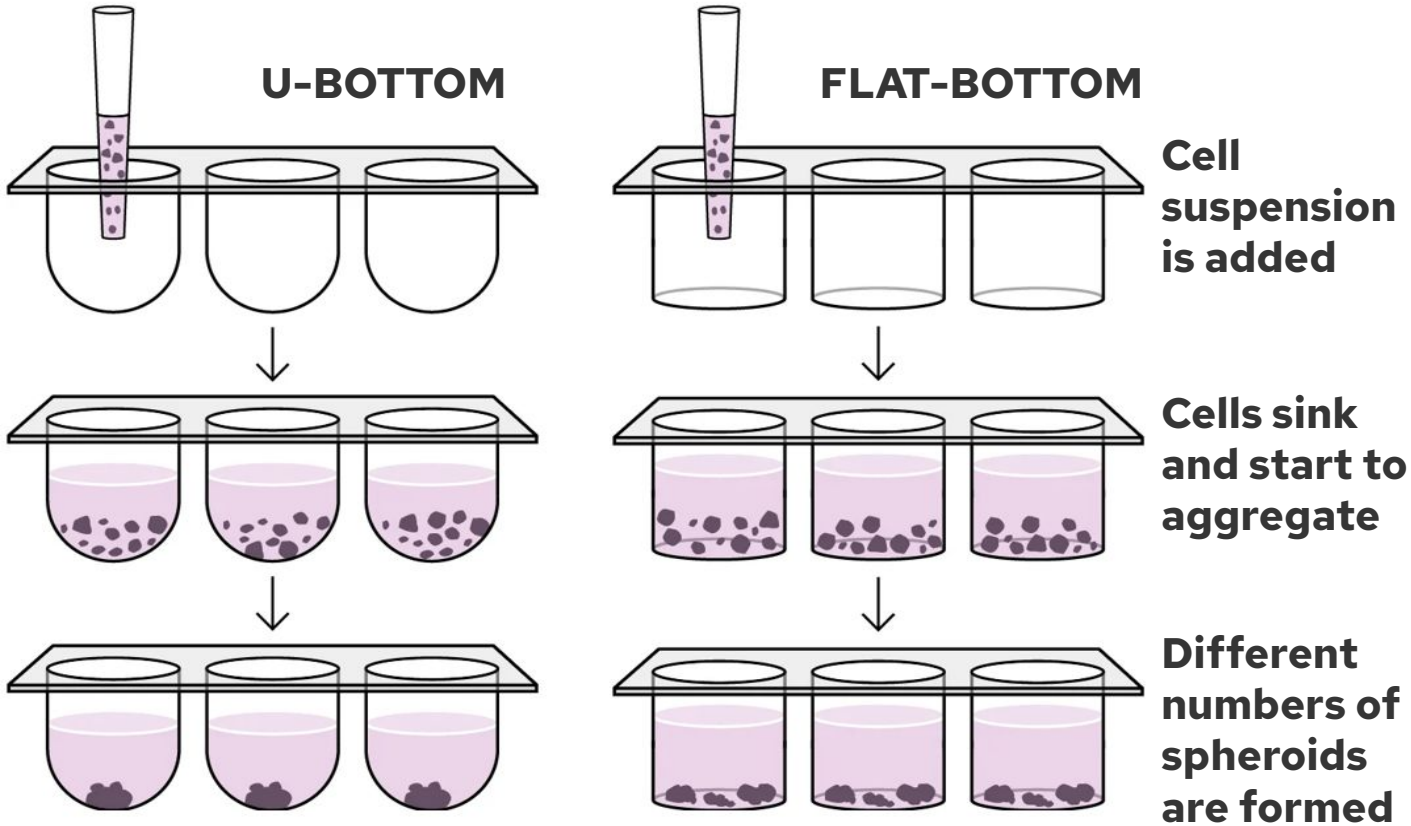

Figure 9: Spheroid formation by treated tissue culture plates/low attachment plates, with U-bottom (left) or flat-bottom (right) wells [9].

Hanging Drop

- Scaffold-free method [15]
- Cells are added to the hanging drop well from the top side of the plate [17].
- Hanging drops are formed at the bottom of the plate [17].
- Spheroid formation after up to 4 days in hanging drops [17].

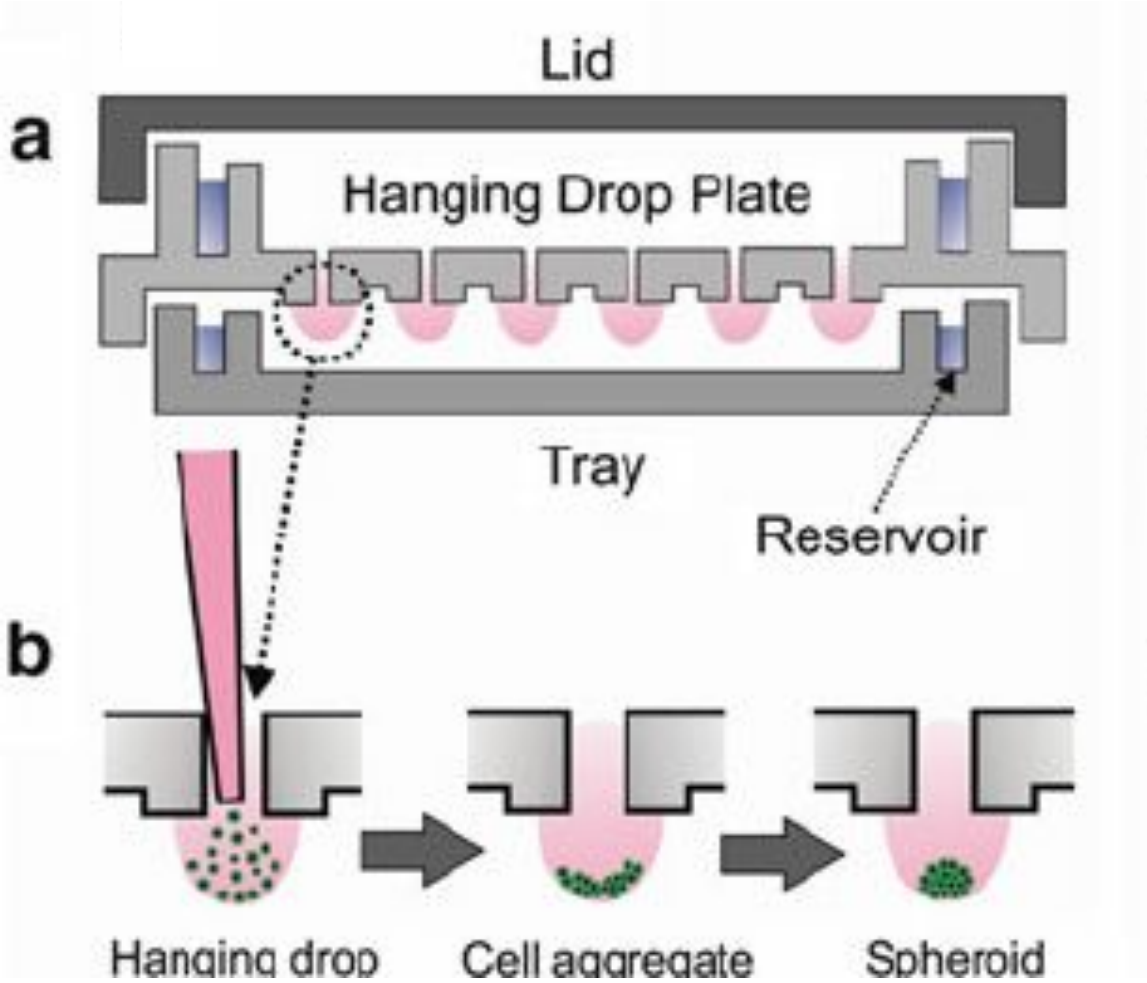

Figure 10: General layout of a hanging drop plate (a) and hanging drop technique (b) [10].

Matrigel

- Scaffold-based method [15]
- Cells are grown in low attachment plates to form aggregates [18]
- Matrigel is added to wells, after when spheroids are formed [18]

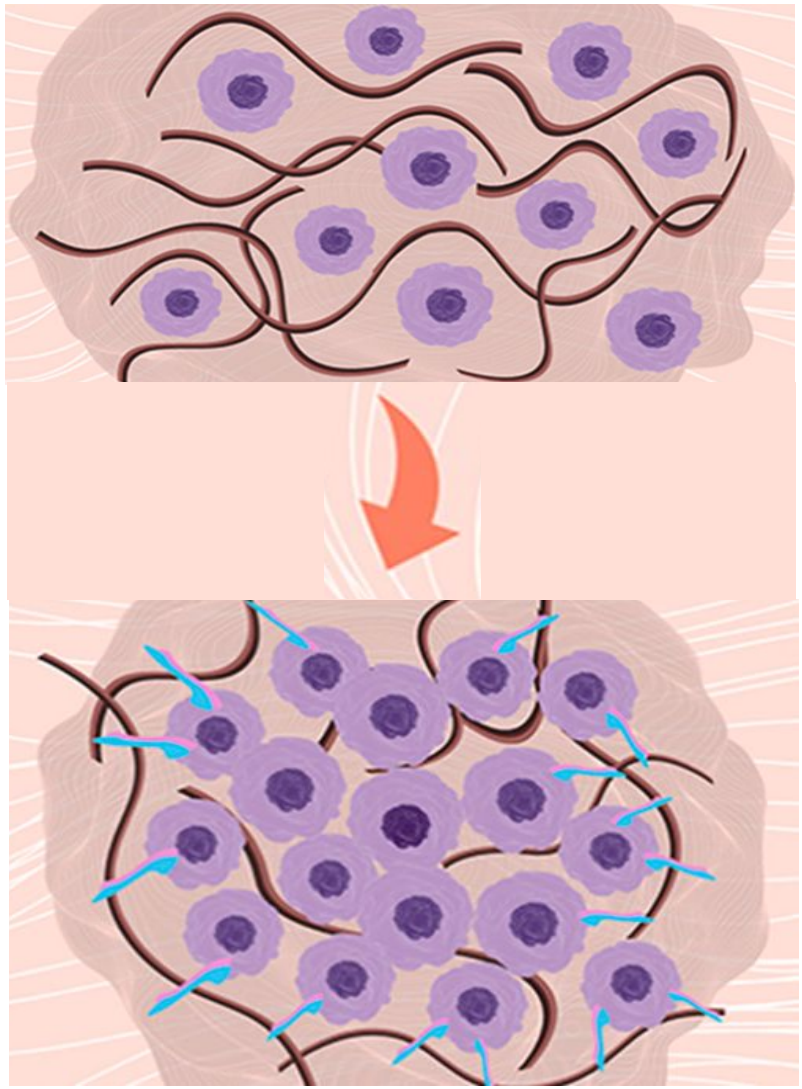

Figure 11: Spheroid formation after addition of matrigel [11].

Spheroid Formation Protocol Design Matrix

Table 2. Design matrix for Spheroid formation.

Design Criteria (weight)	Treated Tissue Culture Plates		Hanging Drop		Matrigel	
	Score X/5	Weighted score	Score X/5	Weighted score	Score X/5	Weighted score
Ease of Fabrication (30)	5	30	4	24	3	18
Scalability (20)	4	16	3	12	5	20
Uniform properties (20)	4	16	3	12	2	8
Cost (15)	3	9	4	12	2	6
Safety (15)	5	15	5	15	5	15
Total Score (100)	86		75		67	

Conclusions on Proposed Design

- Cell line: A549
- Spheroid formation method: treated tissue culture plates
  - Poly-HEMA coated plates [1]
  - Ultra-low attachment plates [1]
- All resources will be provided by Hess Lab

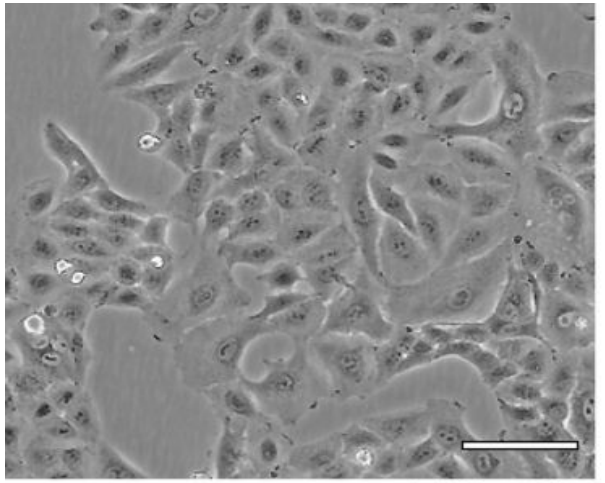

Figure 12: Phase contrast image of high density A549 [7].

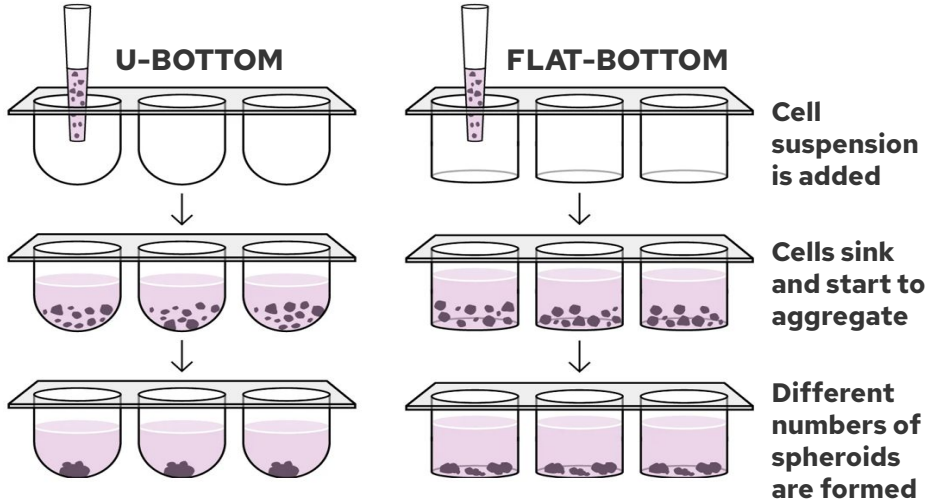

Figure 13: Spheroid formation by treated tissue culture plates/low attachment plates, with U-bottom (left) or flat-bottom (right) wells [9].

Future Work

Next Steps:

- Thaw and begin passaging chosen cell line
- Practice spheroid formation protocol with non-essential cell line
- Fine-tune cell density and viscosity requirements for optimal spheroid formation with chosen cell line
- Test for stemness markers indicative of spheroid formation via qPCR [19]

May 2025 Goals:

- Develop protocol to stain for γH2AX to determine location of maximal DNA damage due to DSBs
- Perform high-throughput genome-wide CRISPR screening to check for effects of different genes on γH2AX detection

Acknowledgements

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Questions?