Development and Characterization of a 3D-Bioprinted Lung Tumor Model for Preclinical Radiotherapy Studies

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ABSTRACT

Purpose: Develop a 3D-bioprinted lung tumor model and benchmark its radiation response and immunogenic cell death (ICD) signaling against conventional 2D culture.

Methods: A549 cells were extrusion-bioprinted in an alginate–gelatin hydrogel (2×10^6 cells/mL) and matured 14 days. 2D and 3D cultures were irradiated to 0, 2, and 6 Gy using the CellRad irradiator with HVL = 2 mm Al. Endpoints: Clonogenic survival, surface calreticulin (CRT) by flow cytometry, and live/dead confocal imaging.

Results: 3D constructs were consistently more radioresistant than 2D and showed dose-dependent ICD induction:

- Surviving fraction - 2 Gy: **54.5 ± 3.5% (3D) vs 42.9 ± 1.5% (2D)**; 6 Gy: **6.8 ± 1.0% vs 4.1 ± 0.6%**.
- Fold MFI calreticulin (3D) – **0 Gy vs 2 Gy**: $P < 0.05$; **2 Gy vs 6 Gy**: $P < 0.01$.

Conclusion: The 3D-bioprinted model is a feasible, more physiologically relevant platform for studying radiation response and ICD; mixed constructs with fibroblasts and immune cells are planned.

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INTRODUCTION

Lung cancer is the leading cause of cancer mortality worldwide.[1] Many NSCLC patients are surgery-ineligible and rely on radiation therapy, including high-precision SBRT, yet the biology underlying these regimens is incompletely understood.

Preclinical models have key limitations:

- Animal models: Costly, low clinical translation.
- 2D culture: Lacks tumor architecture, cell interactions, and microenvironmental gradients.
- 3D bioprinting: Better replicates these features,[2] but standardized radiation-response assays in 3D are underdeveloped.

We developed a 3D-bioprinted lung tumor model and compared its radiation response (clonogenic survival) and ICD signaling to 2D culture.

METHODS AND MATERIALS

- **Cells:** A549 human lung adenocarcinoma (ATCC), standard conditions.
- **3D construct:** Extrusion-bioprinted alginate-gelatin hydrogel, 2×10^6 cells/mL; 14-day maturation before irradiation.
- **Irradiation:** 0, 2, and 6 Gy delivered with CellRad (2 mm Al beam quality).
- **Clonogenic assay:** Sodium citrate decrosslinking releases single cells from 3D constructs for plating.
- **ICD readout:** Surface calreticulin by flow cytometry.
- **Spatial viability:** Live/dead confocal microscopy.

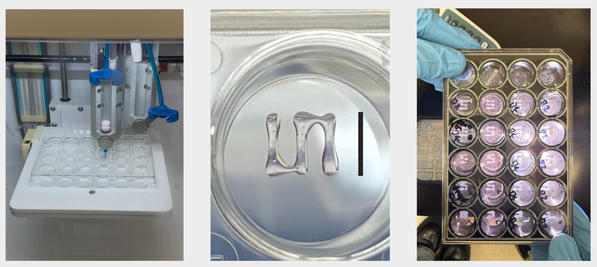


Figure 1. Left-to-right Cellink extrusion bioprinter, close up 3D-bioprinted lung tumor model with 5 mm scale bar, 24-well with 3D constructs in culture.

RESULTS

- **Spatial viability (Fig. 4):** Heterogeneous live/dead distributions within constructs.
- **Clonogenic survival (Fig. 2):** 3D > 2D at both doses.
- 2 Gy → **54.5 ± 3.5% (3D)** vs 42.9 ± 1.5% (2D)
- 6 Gy → **6.8 ± 1.0%** vs 4.1 ± 0.6%.
- **Immunogenic cell death (Fig. 3):** Dose-dependent ↑ surface calreticulin (3D).
- **0 Gy vs 2 Gy**: $P < 0.05$
- **2 Gy vs 6 Gy**: $P < 0.01$

Clonogenic Survival Analysis in 3D

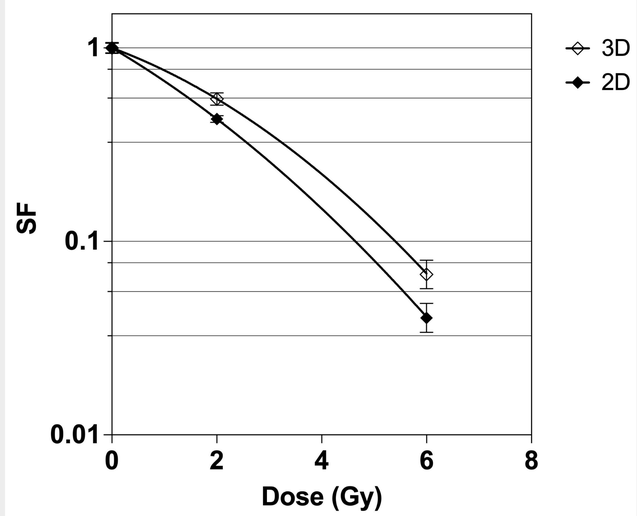


Figure 2. Survival curve after 2 Gy and 6 Gy irradiation, for 2D-A549 and 3D-bioprint derived A549. Represented as mean w/ SD, N=1.

Immunogenic Cell Death in 3D

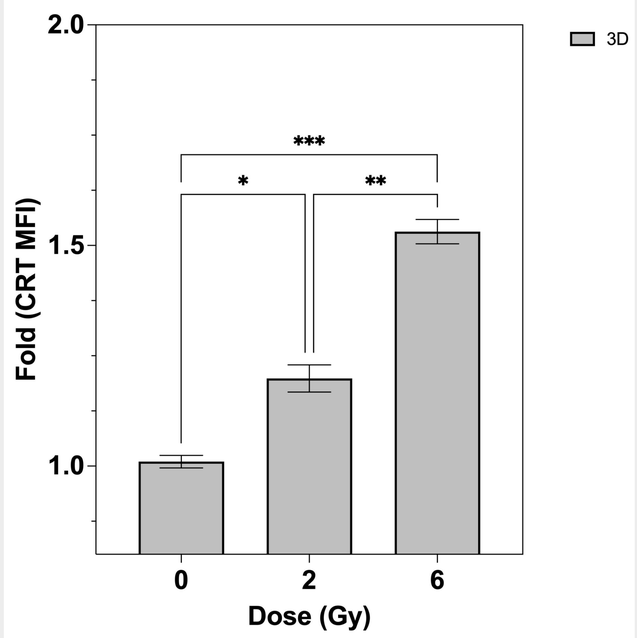


Figure 3. Median Fluorescent Intensity (MFI) of surface CRT expression for live 3D bioprinted cells after 2 Gy or 6 Gy of radiation, normalized to respective untreated controls.

3D Cell Distribution and Viability

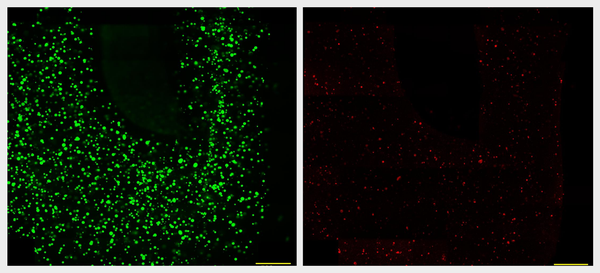
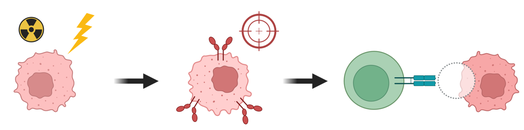


Figure 4. Left-to-right: live and dead cell distribution after 14 days incubation. Images via confocal microscopy (LIVE/DEAD).

DISCUSSION

- **Physiological relevance:** The alginate–gelatin construct reproduces spatial heterogeneity and diffusion-driven gradients of the tumor microenvironment (TME) - features absent in 2D.
- **Clonogenic assay in 3D:** Sodium citrate decrosslinking recovers viable cells efficiently, enabling direct head-to-head comparison with 2D.
- **Radioresistance:** Consistently higher 3D survival with hypoxia-associated radioresistance inside the construct.
- **Immune signaling: 3D** models show dose-dependent ICD via calreticulin externalization.



CONCLUSIONS

- 3D-bioprinted A549 model is feasible for studying radiation response and ICD.
- 3D shows greater radioresistance and stronger ICD signaling than 2D.
- Next: mixed constructs with fibroblasts and immune cells.

REFERENCES

1. American Cancer Society. Cancer Facts & Figures 2025. ACS; 2025.
2. Marciscano AE, et al. Immunomodulatory effects of SBRT: preclinical insights and clinical opportunities. *Int J Radiat Oncol Biol Phys*. 2021;110(1):35–52.