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RESEARCH ARTICLE

Using 3D-bioprinted models to study pediatric neural crest-derived tumors

Supplementary file

Table S1. Designs of bioprinted models for microtumor development and experimentation

Variable		Outcome
Bioink	5% alginate and 5% gelatin	Inadequate consistency for regular bioprinted tumor growth
Bioink	1% alginate and 6% gelatin	Consistent bioprinted tumor growth, adopted for routine use
Printing method	Layered bioprinting	Works well for immunostaining and studies with direct comparison with two-dimensional culture
Printing method	Mixed bioprinting	Works well for studies requiring histology and implantation into animal models; compatible for high-throughput in 96-well plates
Printer settings	Extrusion pressure	Printer-specific, supplied by manufacturer
Printer settings	Extrusion time	Printer-specific, supplied by manufacturer

In order to create three-dimensional (3D)-bioprinted tumors, we utilized a bioink comprising alginate and gelatin that resulted in consistent tumor growth over experiments. The methods were determined based on the goals of each experiment, and the printer settings were aligned with the manufacturer's recommended settings.

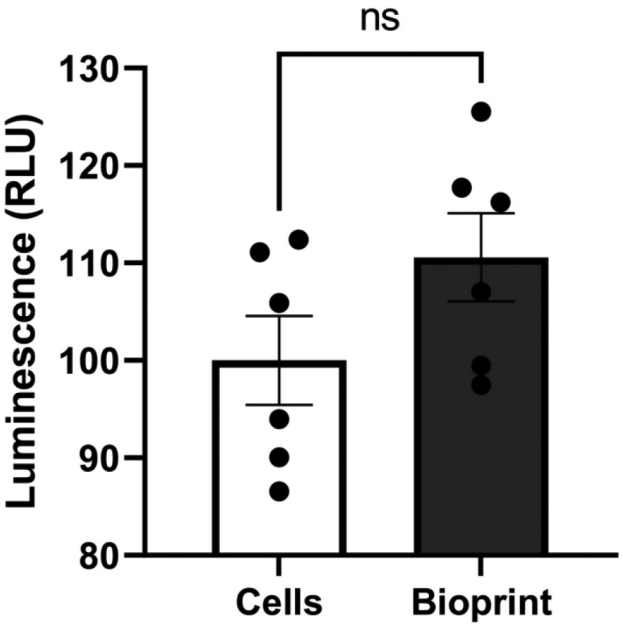

Figure S1. Comparison of cell viability between cells plated via standard pipette and cells plated with bioprinter. JX22P cells (glioblastoma patient-derived xenoline, 7×10^5) in the media were loaded into a 3 mL printing cartridge and placed into a 3 mL pneumatic printhead inCellink's BIO X printer. The cell solution was extruded as a droplet through Cellink's 22-gauge 1/2 inch blunt tip needle at a pressure of 10 kPa for 0.3 s into a 24-well plate. The same cell concentration was plated via pipette (100 µL tip) into a separate 24-well plate. CellTiter Glo Luminescent Cell Viability Assay was performed to ensure that the selected printing pressure did not negatively impact the viability of cells. There was no significant difference in viability between cells that were extruded via the bioprinter (prints) and those that were pipetted by hand onto the plates (cells). These results showed that the printer did not negatively impact the viability of cells at the printing pressure used in the current study.

Figure S2. Bioink does not demonstrate autofluorescence or activate calcein AM. (A and B) Bioink composed of 1% sodium alginate and 6% gelatin without tumor cells was printed onto a 6-well plate. An image of bioink with calcein AM dye added was taken (A) without fluorescence and (B) underfluorescein-5-isothiocyanate (FITC) laser. The bioink did not autofluorescence or fluoresce with calcein AM.

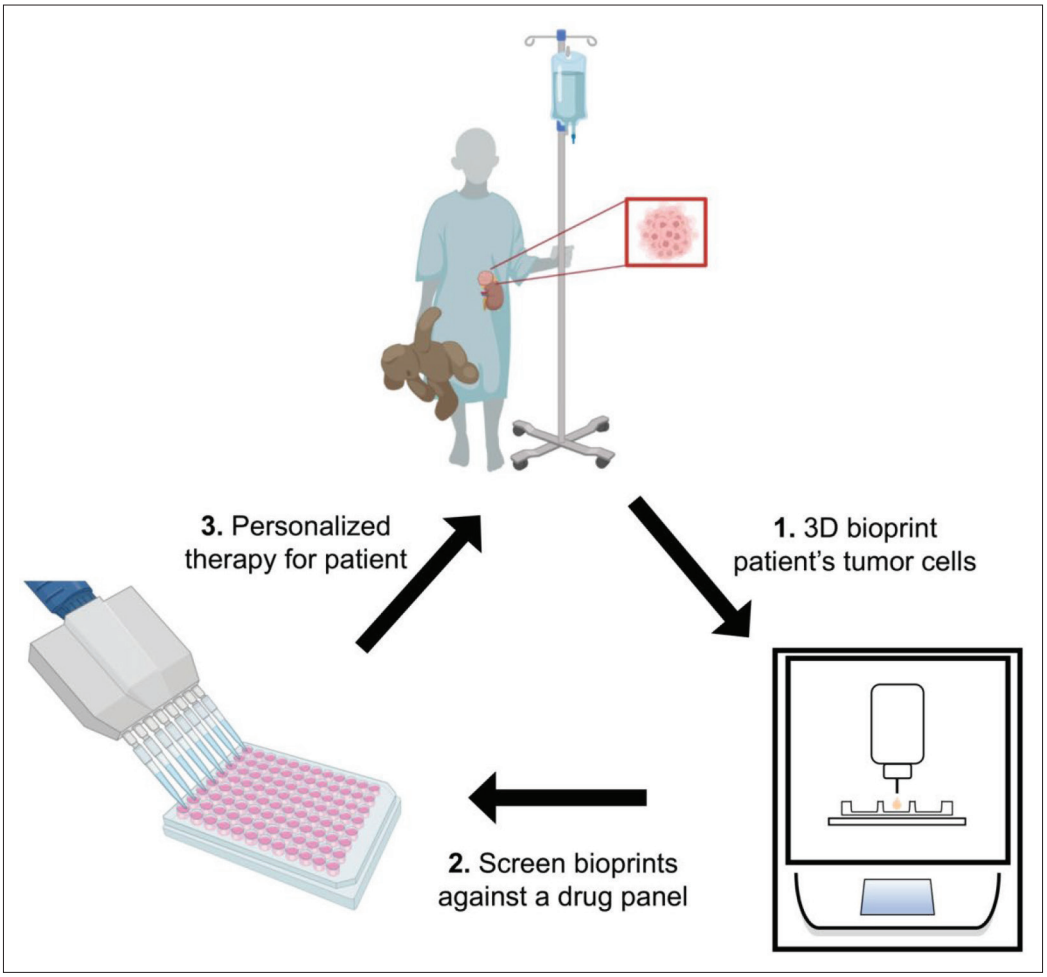

Figure S3. Bioprinted models for personalized cancer therapy. A diagram outlining the schema for which bioprints could be used to generate personalized treatment regimens for pediatric solid tumors. The steps include (i) harvesting a portion of the patient's tumor and constructing 3D-bioprinted tumors, (ii) screening the bioprinted tumors against a drug panel, and (iii) determining the best targeted therapy for an individualized treatment regimen.