Development of spheroids derived from tumor biopsies and patient-derived xenografts using magnetic 3D bioprinting

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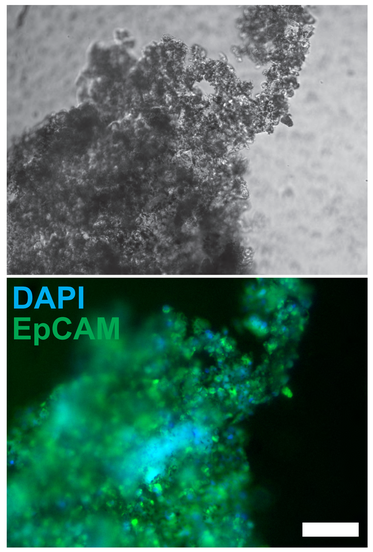
Overview

The successful development of precision medicine assays is highly dependent on the availability of quality biosources. One such biosource is patient-derived tumor tissue, either from primary or metastatic tumors. Cells from these tissues can be easily isolated and contain primary data on the cancer, but these tumors are less accessible than other biosources and scarce. Tumors can be expanded in patient-derived xenograft (PDX) models, but issues still remain on their scarcity and cost. A further limitation on these cells is that traditional monolayer cultures require high cell numbers for confluence and attachment to a stiff substrate that can alter phenotype and poorly represent the native tumor environment. **Improved cell culture platforms that require fewer cells and can recapitulate native tumors are required to take advantage of a scarce resource like tumor tissue.**

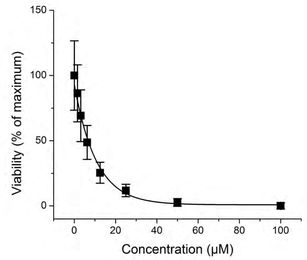
Towards that end, this study uses a 3D cell culture platform, magnetic 3D bioprinting, to print spheroids from tumor tissue.[1] The principle behind this method is the magnetization of cells with a biocompatible nanoparticle assembly, **NanoShuttle™**, and their aggregation into spheroids that represent native tumors using magnetic forces. As these spheroids take the shape of a fixed magnetic field, they can be printed reproducibly with small cell numbers[2], allowing maximum use of tumor tissue. From a technical standpoint, as these spheroids are magnetized, they are **rapid to form** and **easy to handle with magnetic forces**, with **no interference of NanoShuttle™** on fluorescence, luminescence, or other endpoints.

In this study, we demonstrate our ability to print and assay spheroids with cells isolated from metastatic tumor tissue and PDX models.

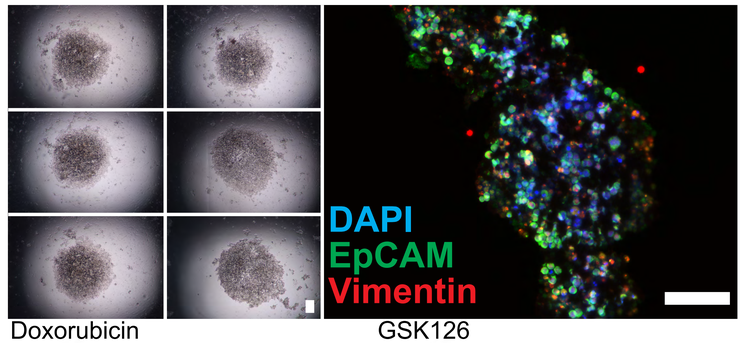
Results

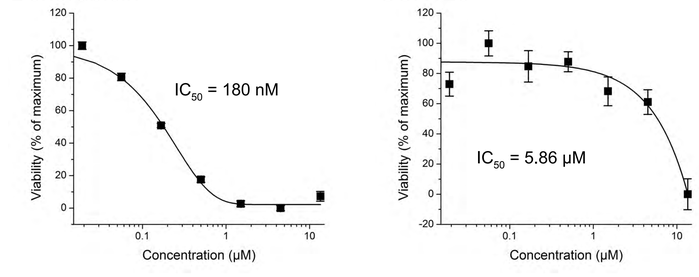


- Advanced metastatic castration-resitant prostate cancer (CRPC) metastasized to the spine
- Cells formed competent spheroids and showed a EpCAM+ phenotype after 72 h of culture
- After 72 h of culture, viability in response to doxorubicin was measured over 72 h of exposure (RealTime-Glo®, Promega, Madison, WI)
- A significant effect of doxorubicin concentration was found ($p < 0.001$)



Brightfield image of a spheroid printed with 10,000 cells isolated from a primary tumor (top), which showed a competent spheroid that stained EpCAM+ (green, center). Nuclei were counterstained with DAPI (blue). After 3 d of exposure to various concentrations of doxorubicin, a signficant dose-dependent response was found (bottom). Scale bar = 50 μm. Error bar represents standard error.





(Top) Brightfield images of spheroids (10,000 cells each) formed from PDX tumors. Theses spheroids were competent and reproducible, similar to those derived from tumor biopsies. We found significant dose-dependent responses to GSK126 and doxorubicin, after adding the drugs at 72 h of culture and exposing the spheroids for 72 h. Scale bar = 50 μm. Error bar represents standard error.

- Cells isolated from PDX tumors formed competent spheroids and showed a mostly EpCAM+ phenotype
- There was a significant effect of doxorubicin (IC_{50} = 180 nM) and GSK126 (IC_{50} = 5.86 μM) on cell viability within these spheroids ($p < 0.001$)

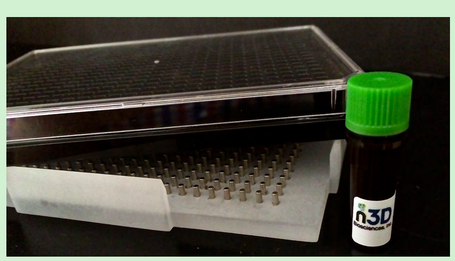
This preliminary data demonstrates our ability to process tumor biopsies and PDX tumors into multiple spheroids that retain phenotype and respond to therapies. These methods form the foundation for developing precision medicine assays using magnetic 3D bioprinting that take advantage of a scarce resource to optimize cancer treatment.

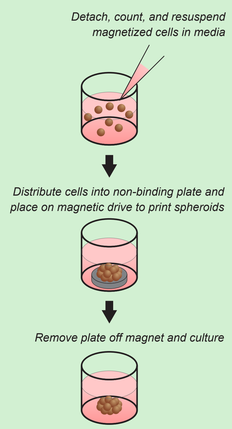
Methods

- Metastatic tumor tissue was obtained from patients according to IRB-approved protocols (HSC-MS-15-0783, University of Texas Health Science Center at Houston)
- PDX tumors were obtained from PDX models generated from MDA PCa-133
- Cells were isolated from these tissues with mincing and without enzymatic digestion
- Cells were magnetized by adding NanoShuttle™ (Nano3D Biosciences, Houston, TX) to the cell suspension and centriguging cells
- Once magnetized, cells were resuspended and distributed into a 384-well cell-repellent plate (CELLSTAR®, Greiner Bio-One, Frickenhausen, Germany)
- Spheroids were then printed by placing the plate atop a magnetic drive of 384 cylindrical magnets underneath each well
- Spheroids were left to print on the magnet overnight to establish cell-cell interactions and build a competent spheroid
- After printing, the plate was removed off the magnet for long-term culture

Cells we have successfully printed

LN-229	H-4-II-E	HeyA8
LNCaP	U251-MG	PC3
HeLa	PANC-1	SUM159
A549	Caki-1	MDA-MB-231
HCT-116	HOS	REN
MCF-10A	DU-145	CTCs
Primary bladder cancer	Primary renal cancer	Primary prostate cancer





Top: Schematic of magnetic 3D bioprinting spheroids.[2] Left: 384-well Bioprinting Kit with NanoShuttle, spheroid drive, and non-binding microplates

Acknowledgements

We thank **Geoff Bartholomeusz, University of Texas MD Anderson Cancer Center**, for his help in optimizing cell isolation from tumor tissue; **Sheri Skinner, Betty Arceneaux, Janis Burley, Michael Garcia, and Pauline Dang, University of Texas Health Science Center at Houston** for help in obtaining biopsy samples

References

1. Souza GR et al. *Nat. Nanotech.* (2010)
2. Tseng H et al. *Sci. Rep.* (2015)