Somatic mutation detection from liquid biopsy-derived cellular aggregates formed by magnetic 3D bioprinting

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Background

There is increasing interest in the analysis of circulating tumor cells (CTC) from liquid biopsies to track dynamic changes in mutational profile. One of the major challenges to CTC analysis is the inability to culture them, largely due to their scarcity preventing their survival in culture. CTC culture would allow for their expansion, analysis, and the potential development of companion *in vitro* diagnostics and novel therapies.

Towards that end, we use a novel technique, magnetic 3D bioprinting, to culture CTCs into spheroids. The principle behind magnetic 3D bioprinting is the magnetization of cells and their aggregation into spheroids using magnetic forces.[1,2] We can use this method to actively magnetize CTCs, then aggregate them into close contact for their survival, expansion, and analysis. Magnetization is accomplished via incubation with NanoShuttle™, a biocompatible nanoparticle assembly that is non-specific, and does not interfere with fluorescence or require specialized equipment.

In this study, we demonstrated the ability to aggregate CTCs using magnetic 3D bioprinting, then perform next generation sequencing (NGS) to detect somatic mutations from renal and prostate cancers.

Magnetic 3D bioprinting is the ideal method to culture CTCs for expansion and analysis

Methods

- Liquid biopsies were drawn from prostate and kidney cancer patients according to IRB-approved protocols
- Blood was first enriched immunomagnetically for EpCAM+/EGFR+ cells (Isoflux, Fluxion Biosciences), then enumerated for CK+/CD45- cells
- Microbeads were removed enzymatically off CTCs
- CTCs were concentrated (800 g for 5 min), resuspended (1 x 10^6 cells/mL), and incubated with NanoShuttle™ (1 µL/1 x 10^4 cells, Nano3D Biosciences) for 2 h with constant agitation to magnetize
- Magnetized CTCs were distributed into cell-repellent 384-well plates (Greiner Bio-One), then aggregated into spheroids with a 384-well magnetic spheroid drive (Nano3D Biosciences)
- CTCs were cultured in RPMI-1640 + CTL supplement + 10% fetal bovine serum
- Viability was measured through culture using RealTime-Glo (Promega)
- After 4 d, the cells were lysed and DNA was amplified by whole genome amplification with the NGS kit (Fluxion) and quantified via qPCR
- Targeted libraries were sequenced (PGM, ThermoFisher)
- Data was analyzed using a customized variant calling/filtering pipleine based on standard Ion Reporter alignment tools and VarSeq™ for variant filtering and functional interpretation

Enumeration

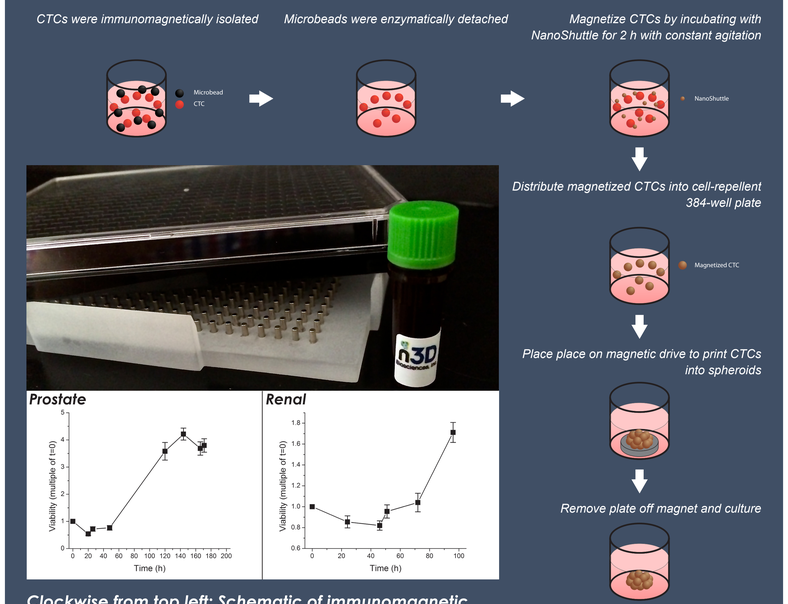
CTCs were successfully enriched from drawn blood for EpCAM+/EGFR+ cells and CK+/CD45- cells were enumerated

IsoFlux system to immunomagnetically isolate CTCs from liquid biopsies via microfluidics

Sample	# CTCs	% CTCs	Notes
Prostate			
001	466	27.1%	Advanced, metastatic, CRPC
002	452	24.6%	Advanced, metastatic, CRPC
003	456	33.7%	Advanced, metastatic, CRPC
004	722	24.3%	Advanced, metastatic, CRPC
005	139	2.7%	Minimal, metastatic, CRPC
006	170	4.3%	Minimal, metastatic, CRPC
007	227	1.9%	Hormone sensitive, metastatic, locoregional recurrence
008	10	0.01%	Minimal, metastatic, CRPC
009	130	13.6%	Advanced, metastatic, CRPC
010	199	14.1%	Advanced, metastatic, CRPC
Renal			
003	143	1.7%	Advanced, metastatic
004	65	5.0%	Advanced, metastatic
005	349	24.1%	Advanced, metastatic
006	661	37.2%	Advanced, metastatic
007	170	8.4%	Minimal, metastatic
008	143	2.7%	Advanced, metastatic
009	765	28.0%	Advanced, metastatic
010	1413	29.8%	Advanced, metastatic

Enumeration of CTCs from enriched blood (IsoFlux, Fluxion Biosciences) drawn from liquid biopsies from patients with both renal and prostate cancer, and their disease states at the time of collection.

Magnetic 3D Bioprinting



Clockwise from top left: Schematic of immunomagnetic sorting, bead removal, magnetization, and spheroid culture; viability of spheroids from prostate and renal cancer patients over time, which increased significantly over time (n = 10 patients); 384-well bioprinting kit used for culture.

NGS

Chr:Pos	Ref/Alt	Alt Allele Frequency	Gene Name	Gene Region	Effect
13:32906729	A/C	77.86%	BRCA2	exon	Missense
9:21968199	C/G	73.70%	CDKN2A	UTR3	Other
3:52436441	C/A	67.99%	BAP1	intron	Other
17:29508775	G/A	30.63%	NF1	exon	Other
9:21970916	C/T	20.67%	CDKN2A	exon	Missense
11:32456694	C/A	12.49%	WT1	exon	Other
16:68855984	C/T	3.32%	CDH1	exon	LoF
5:112175771	G/A	1.79%	APC	exon	Missense

An example of somatic variants found in magnetically 3D bioprinted spheroids of CTCs, isolated from a liquid biopsy from prostate patient 004. These variants were detected using the Oncomine panel (ThermoFisher) and are all found in the COSMIC database

Sample	Variants	In COSMIC
Prostate		
001	8	0
002	17	1
004	40	8
007	27	7
Renal		
003	80	12
005	9	2
006	32	5
007	37	5
010	88	11

Number of somatic variants found for each sample, and the number of those variants found in COSMIC

Future Directions

- Optimize CTC isolation from blood, particularly enzymatic microbead removal
- Improve CTC growth and expansion in culture
- Explore phenotype of expanded CTCs with immunohistochemistry and NGS
- Develop protocol to print CTC spheroids with speed and reproducibility for precision medicine

Acknowledgements

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References

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