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High-Throughput Single-Cell Quantification of HDACi-Based HIV Reservoir Reactivation

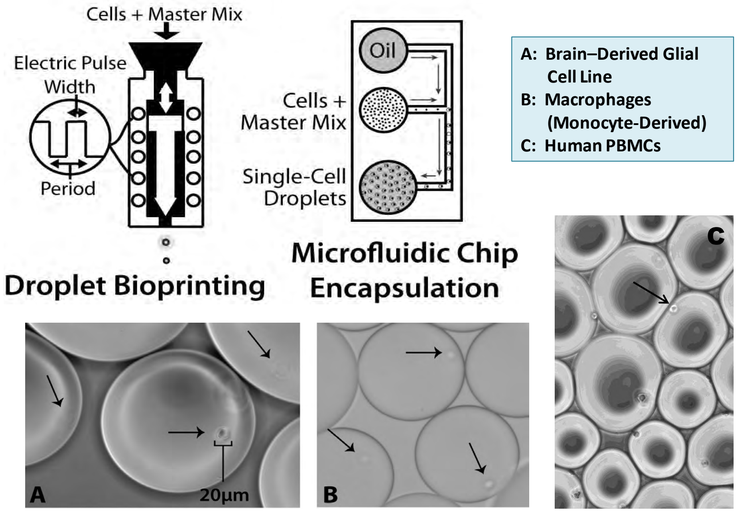
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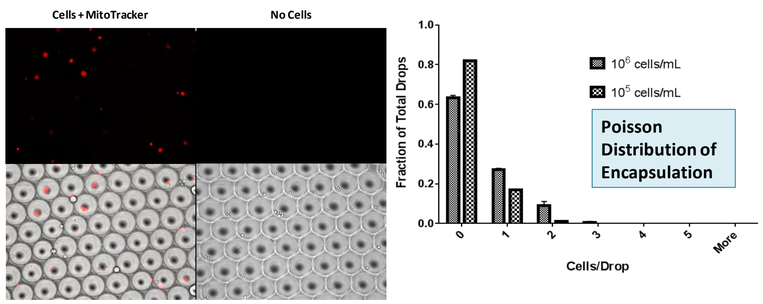
Background and Motivation

Reactivation of latent viral reservoirs is on the forefront of HIV-1 curative strategies. However, little is known about HIV-1 reactivation on a single cell level, such as if reactivation leads to significant increases in the number of transcriptionally active cells, or if increases in cell-associated HIV-1 RNA arise from a smaller, active portion of the reservoir. We therefore designed and implemented a novel microfluidic-based, single cell encapsulation and PCR assay to directly measure the number of cells that produce cell-associated unspliced RNA (usRNA) from HIV-1-infected individuals on suppressive ART before and after HDAC inhibitor and T cell receptor (TCR) stimulation *ex vivo*.

Single-Cell Encapsulation



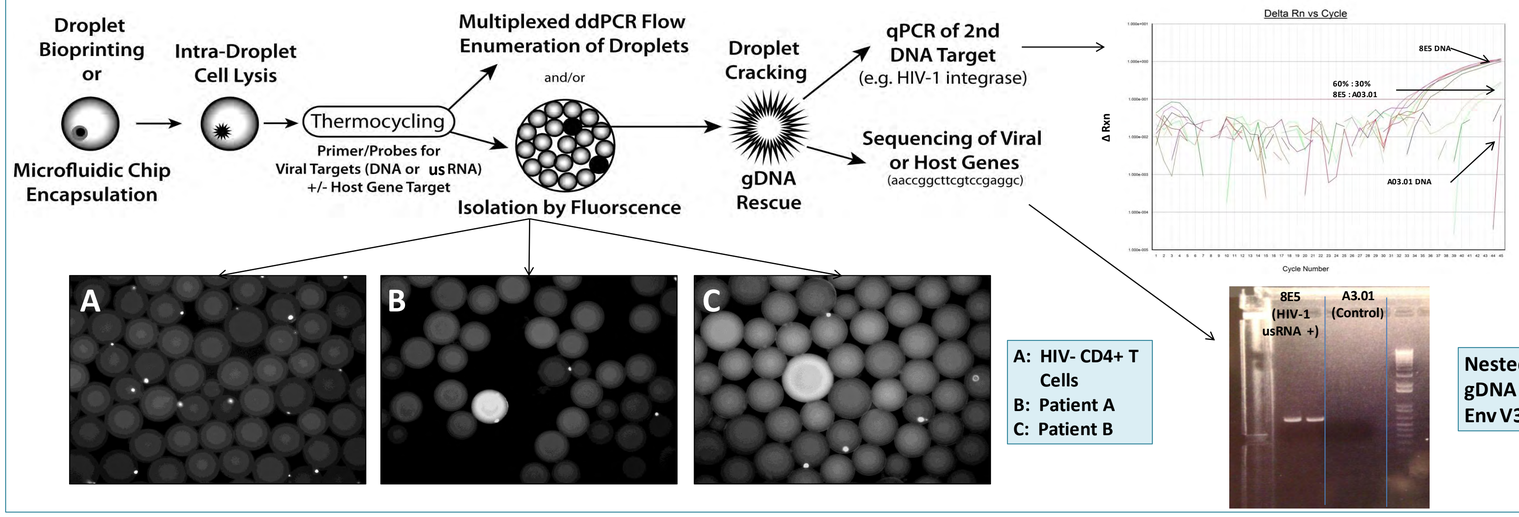
- A: Brain–Derived Glial Cell Line
- B: Macrophages (Monocyte-Derived)
- C: Human PBMCs



Various cell types were encapsulated using either bioprinting or micro-fluidic devices to test the robustness of our homemade water-in-oil droplet cell packaging technique. Additionally, MitoTracker Orange dye was utilized to directly visualize cells for validation of Poisson distribution of encapsulation.

Single-Cell-In-Droplet PCR (scidPCR)

Lab cell lines A3.01 (HIV-) and 8E5 (HIV+) or primary CD4+ T-cells, 10^5-10^6 cells/mL in PBS, were mixed 1:1 with qPCR master mix for usRNA (including in-house intra-droplet lysis buffer), droplet encapsulated, thermocycled, and imaged for HIV-1-specific probe fluorescence. DNA was isolated from a subset of droplets: 1) HIV-1 integrase gene quantification from gDNA, and, 2) V3 loop rescue, purification and sequencing.



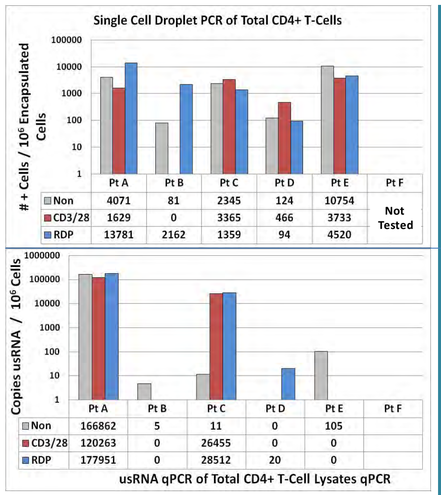
- A: HIV- CD4+ T Cells
- B: Patient A
- C: Patient B

HIV-1 integrase real-time qPCR from rescued gDNA

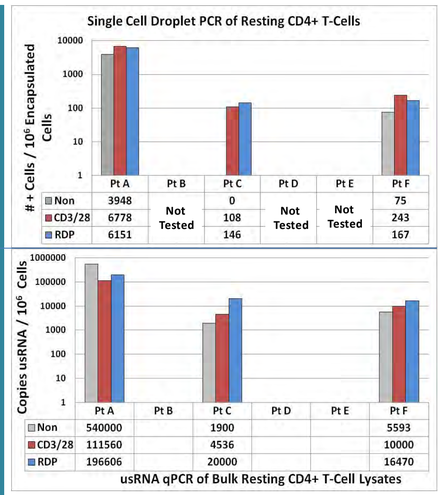
Nested PCR for gDNA HIV-1 Env V3 Loop

Quantifying Single-Cell HIV-1 Reservoir Reactivation

Resting and total CD4+ T-cells were isolated from HIV+ donors on suppressive ART, cultured in the presence of 8nM efavirenz and 10nM darunavir, and activated with either HDACi [50nM romidepsin (RDP), 4 hr pulse] or TCR (100 ng/mL anti-CD3/28 antibodies, 12-24 hrs) stimulation. Cells were evenly distributed for usRNA scidPCR, traditional CCR5 qPCR (for cell enumeration) and usRNA qPCR methods.



Total CD4+ T Cells



Resting CD4+ T Cells

Fold Change of Stimulated / Non-Stimulated CD4+ T Cells [scidPCR (droplet) assay vs. qPCR]

ND: Not Detected; INC: Infinite Increase (Non-Stim = Undetected)

	Total CD4+				Resting CD4+			
	CD3/28		RDP		CD3/28		RDP	
	Droplet	qPCR	Droplet	qPCR	Droplet	qPCR	Droplet	qPCR
Patient A	0.40	0.72	3.39	1.07	*1.72*	*0.21*	*1.56*	*0.36*
Patient B	ND	ND	*26.59*	*ND*	N/A		N/A	
Patient C	1.43	2312.54	*0.58*	*2492.39*	INC	2.39	INC	10.52
Patient D	*3.77*	*ND*	*0.76*	*INC*	N/A		N/A	
Patient E	0.35	ND	0.42	ND	N/A		N/A	
Patient F	N/A		N/A		3.24	1.79	2.23	2.94

All patients were male, 27-56 years of age, and were on suppressive or near-suppressive ART. Pt A was s/p autologous HSCT for Hodgkin disease; pt B completed chemotherapy for B cell lymphoma; pts C and D were s/p allogeneic HSCT, and pts C and F were otherwise healthy HIV-infected individuals.

- scidPCR is sensitive at detecting changes in HIV-1-reactivated cells
- There is discordance between the number of reactivated CD4+ T cells and total usRNA production from some individuals
- HDACi and TCR stimulation had variable effects on HIV-1-reactivation, including differences between resting and total CD4+ T cells

Summary and Conclusions

- We have demonstrated a novel ***Single-Cell-In-Droplet PCR (scidPCR)*** method for detection and quantification of HIV-1 infected, reactivated cells
- T-Cell Receptor and HDACi stimulation was ***variable*** between patients and showed ***discordances*** between resting and total CD4+ T-cell responses
- Direct single-cell analysis of HIV-1 reactivation is required to fully understand the impact of latency-reversing agents on HIV-1 reservoirs

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