ASSESSING REAL-TIME SICKLING KINETICS IN INDIVIDUAL CELLS FROM ERYTHROCYTE POPULATIONS OF SUBJECTS WITH DIFFERENT HbA, HbS AND HbF PERCENTAGES

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INTRODUCTION

- Polymerization of deoxygenated hemoglobin S (HbS) leads to red blood cell (RBC) sickling and vaso-occlusion
- Allogeneic hematopoietic stem cell transplantation (HSCT) is the only approved curative option available for sickle cell disease (SCD)
- Objective biomarkers are needed to assess disease status, population-scale sickling kinetics and to monitor the pharmacodynamic impact of Hb-modifying and curative therapies
- Functional Fluidics has developed a dynamic sickling assay (DSA) that uses an enzymatic O_2 scavenging system providing tight control over the rate and depth of induced-hypoxia

AIM

Assess real-time RBC sickling kinetics in sickle cell trait (SCT) and SCD patients with variable distributions of HbA, HbS and HbF, and compare pre-and post-HSCT

METHOD

Dynamic Sickling Assay (Figure 1)

- Hypoxia is induced through a timed protocatechuic acid/protocatechuate-3,4-dioxygenase enzymatic reaction (PCD-PCA)
- The fraction of sickled cells is determined by time-lapse imaging, analyzed with AI technology and reported as a function of time
- Measured parameters include:
  - **mPoS@5%**: time to reach 5% induced sickling (min)
  - **mPoS@50%**: time to reach 50% induced sickling (min)
  - **Rate of Sickling**: highest induced sickling rate (%·minute^{-1})
  - **Maximum Sickling**: highest sickling percentage (%)
  - **AUC at 10min**: The area under the curve during 10min of DSA (%·minute)

Sample collection

- SCT and SCD subjects, with and without chronic exchange transfusions were enrolled in The Institute for Regenerative and Cellular Medicine (IRCM) IRB under the RBC health initiative protocol (FF-RBC-003v5)
- SCD subjects were enrolled in NIH haploidentical HSCT protocol 17-H-0069. Blood samples from 4/10 planned subjects were collected pre-and 3, 6, and 12 months after HSCT

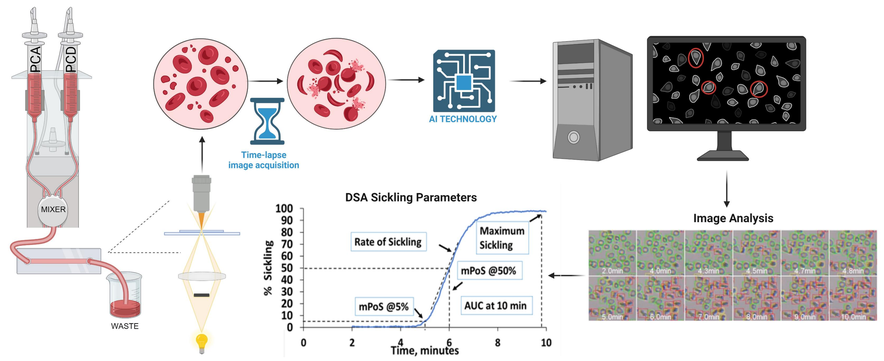

Figure 1. Dynamic Sickling Assay setup

RESULTS

SICKLING KINETICS IMPROVE AS HbS% DECREASES AND HbF% INCREASES

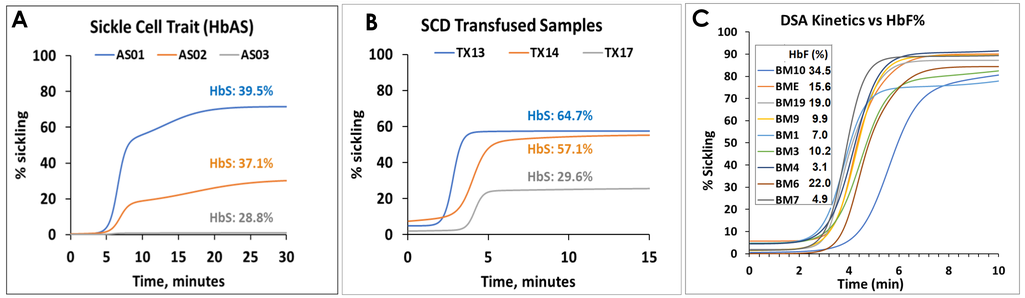

Figure 2. Sickling kinetics in SCT subjects (**A**), transfused SCD subjects (**B**) and SCD subjects with variable HbF (**C**)

RESULTS

POST-HSCT SAMPLES EXHIBIT IMPROVED SICKLING KINETICS

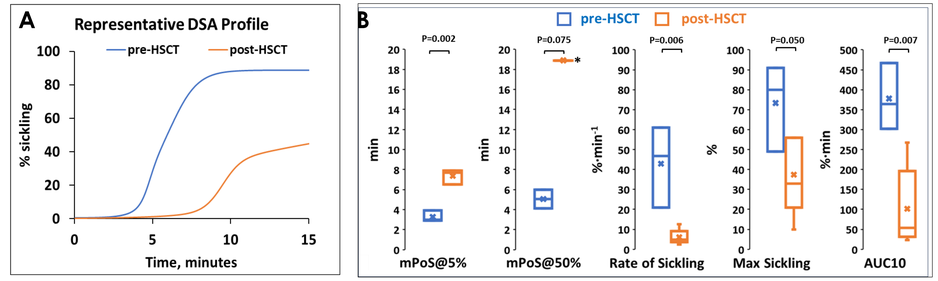

Figure 3. Sickling kinetics in SCD subjects pre-and post HSCT (**A**) Representative sickling profile from 1 patient (**B**) Comparative analyses from 4 SCD subjects
**only 1 post-HSCT sample exhibits >50% max. sickling*

- Compared to baseline values, post-HSCT samples exhibit statistically significant improvement in: mPOS@5%, rate of sickling, maximum sickling and AUC10

Table 1. Sickling kinetics correlations

		mPoS@5%		mPoS@50%		Rate of Sickling		Max Sickling		AUC10	
	n	r	p	r	p	r	p	r	p	r	p
HbS% (Sickle Cell Trait)	3	NA	NA	NA	NA	NA	NA	0.30	0.803	0.86	0.345
HbS% (Transfused SCD)	3	-0.99	0.100	NA	NA	0.626	0.596	0.99	0.107	0.99	0.066
HbF% (non-Transfused SCD)	9	0.87	0.002	0.83	0.006	-0.74	0.023	0.15	0.692	0.73	0.025

- No significant correlation in SCT subjects, although noteworthy differences evident under hypoxic conditions in samples with similar HbS% levels
- HbS% and DSA parameters show a correlation in SCD transfused samples, not statistically significant likely due to limited sample size
- HbF% demonstrates a significantly strong correlation with DSA parameters except for max. sickling

CONCLUSIONS

- SCT subjects with similar HbS% exhibit different sickling kinetics upon exposure to the same hypoxic stress due to intracellular RBC distribution of HbA and HbS
- Transfusion SCD samples show an improvement in sickling kinetics as HbS% decreases approaching a significant correlation with AUC10
- Statistically significant correlations are observed with increasing HbF percentages on 4 out of 5 sickling parameters
- Compared to pre-transplant values, post-HSCT samples exhibit significant improvement in: mPOS@5%, rate of sickling, maximum sickling and AUC10
- DSA can differentiate samples based on Hb distribution and can be used to assess sickling kinetics pre- and post HbS modifying and curative therapies, such as hematopoietic stem cell (HSCT)

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CONTACT INFORMATION