#2174

Session: 803. Emerging Tools, Techniques and Artificial Intelligence in Hematology

DYNAMIC SICKLING ASSAY: USING ENZYMATICALLY INDUCED HYPOXIA FOR ASSESSING REAL-TIME SICKLING SCALABLE FROM LARGE ERYTHROCYTE POPULATIONS TO INDIVIDUAL CELLS

M. Tarasev[1], X. Gao[1], M. Ferranti[1], R. Goodrich[1], A. Zaidi[1], A. Edenstrom[1], and P.Hines[1,2]

[1]Functional Fluidics, Detroit, MI, [2]Wayne State University School of Medicine, Detroit, MI,

INTRODUCTION

There is a pressing need for assays to monitor patient condition and the progress of sickle cell disease (SCD) therapies affecting hemoglobin (Hb) affinity to oxygen and hypoxia-induced kinetics of Hb-S polymerization. It would be beneficial for such an assay to measure oxygen dissociation under both equilibrium (mimicking longer-term effects) and non-equilibrium (simulating *in vivo* process) conditions.

It is desirable to have a robust and simple to use system suitable for use in clinical trials and ultimately for clinical management of patients receiving SCD therapies, including those targeting Hb-oxygen affinity.

Many SCD clinical trials are presently based in the developing countries, further highlighting the need for a simple and inexpensive system to track treatment progress even in a limited infrastructure environment.

AIM

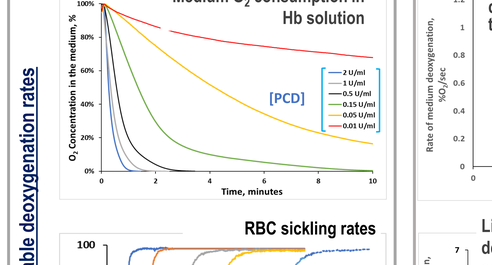
To introduce Dynamic Sickling Assay (DSA) that utilizes enzymatically-induced hypoxia to assess SCD patient condition and can assist in development, monitoring and optimization of SCD therapies.

METHOD

- Diluted blood samples supplemented with Protocatechuic acid and Protocatechuate 3,4-Dioxygenase enzyme (PCA/PCD) are injected into an anaerobic microfluidic chamber
- Medium deoxygenation can be tracked by an oxygen probe
- Hypoxia-induced RBC morphological changes are observed through time-lapsed photography.
- Machine-learning approach for image analysis is used to identify and quantify RBC morphological changes
- Sickling profiles are constructed to represent percent of sickled RBC in a total cell population
- Multiple metrics can be used to comprehensively describe sickling profiles

THE SYSTEM

Design/Schematics



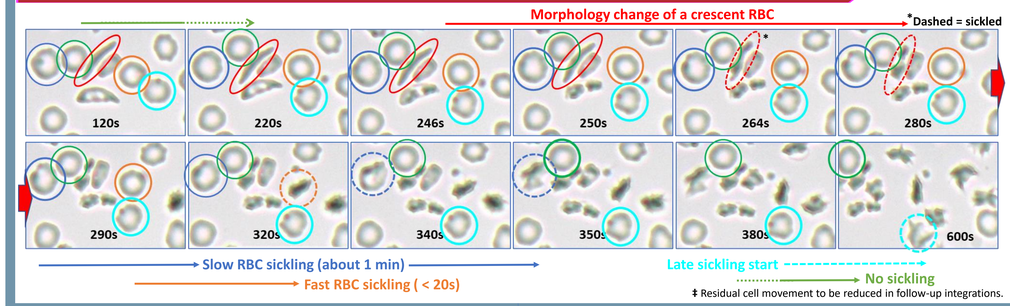
Descriptive results

- RBC fraction sickled at baseline (SF_0)
- Total fraction of sickled RBC (SF_T) /non-sickling fraction (NSF)

- For each sickling RBC fraction (j)
  - ✓ Sickling delay (D_j)
  - ✓ Morphological Point of Sickling ($mPoS_j$): e.g., at 5% or 10% sickling
  - ✓ Sickling rate (R_j)
  - ✓ Fraction size (SF_j)
  - ✓ Area Under the Curve (AUC_j) at a given time post T=0

* Extracted from the raw experimental data collected at 2 s/data point

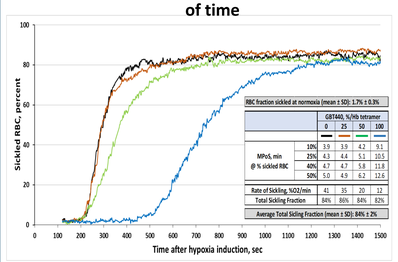
REAL-TIME INDIVIDUAL CELL CHANGES IN MORPHOLOGY



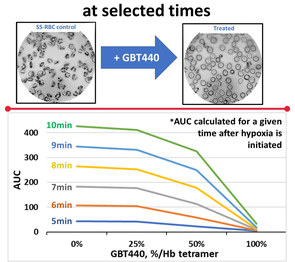
MONITORING DRUG RESPONSE

Difference in timing of RBC sickling (SS phenotype) due to hemoglobin-modifying drug GBT440 (Oxbryta®; GBT) as a function of drug to hemoglobin tetramer ratio

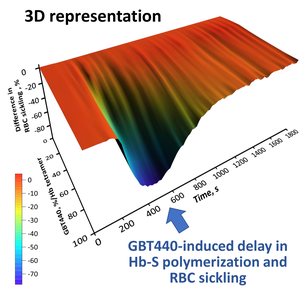
Hypoxia-induced RBC-SS sickling as a function of time



Representation of reduced sickling at selected times



3D representation



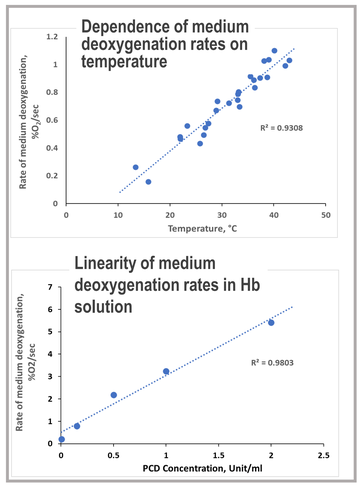
SUMMARY

- Multidimensional profiles allow for fast optimization of assay parameters to target conditions optimal for each application
- Wide range of easily modifiable deoxygenation rates
- Significant sickling delay was observed upon RBC treatment with Hb-modifying drug.
- Total sickling fraction was smaller with sickling significantly delayed for sickle trait (SCT) patients compared to SS-type SCD
- The system allows tracking of morphological status of both populations and individual cells, with individual cells showing differences in their propensity to sickle
- Change in morphology of crescent cells to granular form had been observed upon decreasing medium oxygen tensio.
- The system offers a comprehensive analysis of RBC sickling dynamics
- Dynamic sickling assay can potentially provide a robust and high throughput method for assessing critical RBC response to therapies including that in low resource settings

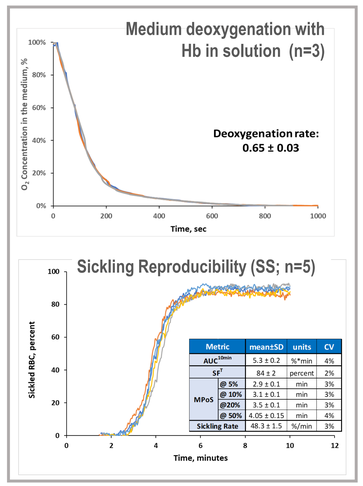
SYSTEM VALIDATION

Modifiable deoxygenation rates

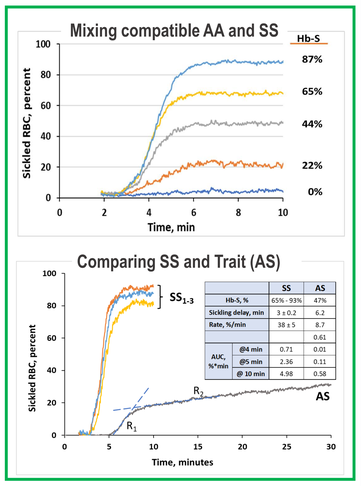
Temperature dependence



Reproducibility



Practical application



DISCLOSURE

Functional Fluidics is a privately held company developing methods for assessment of blood cell function. All authors are employees of the company.

CONTACT INFORMATION

Michael Tarasev, PhD, MBA
michael.tarasev@functionalfluidics.com

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