INTRODUCTION

Sickle Cell Disease (SCD) pathology is caused by polymerization of deoxegenated hemoglobin S (HbS) within RBCs. Resultant RBC “sickling” promotes vaso-occlusion and leads to a myriad of clinical complications including vaso-occlusive episodes (VOEs), organ failure, and stroke. The amount of sickling depends on the dose of polymerized fraction and polymerization kinetics, which can be significantly affected by treatments, e.g., by hemoglobin-modifying drugs. There is a need for rapid assays to monitor therapeutic response. Objective: Develop a hypothesis testing and validate a kinetic assay for sickling assessment as a potential CLIA-validated test for use in clinical drug development and medical clinical monitoring of SCD therapies affecting Hb oxygen affinity (e.g., Hb-modifiers, Hb inducers, etc.).

Challenges

SCD therapies altering Hb oxygen affinity must ensure adequate oxygen delivery while reducing RBC sickling associated complications. Conditions that favor oxygen delivery, such as high Hb concentrations or lower Hb-O2 affinity for tissue and more efficient oxygen delivery, would favor RBC sickling. Conversely, therapies that reduce sickling through left-shifting of O2 binding curve by Hb modification or through changes in 2,3-DPG concentration may decrease O2 delivery for patients that already suffer from impaired blood flow and O2 delivery.

Need

There is a pressing need for tests that assess baseline RBC polymerization risk and monitor response to SCD therapies affecting Hb-O2 affinity. Current approaches predominantly use gas exchange to deoxygenate blood samples, which makes it difficult to achieve precise control over the rate and degree of hypoxic conditions.

Solution

Enzymatic systems have advantages offering easier control over the oxygen consumption rates and the level of resultant deoxegenated (hypoxic) Hb, which can be adjusted in the medium. This approach uses Protocatechuate-3,4-Dioxogenase acid (PCPDA) oxygen scrubber system, with the PCP nonmechanical iron core catalyzing the conversion of PCA and molecular oxygen to CO2 and water, the latter unlike other enzymatic oxygen scrapers, the reaction does not generate any reactive oxygen species (like peroxides or superoxides).

Dynamic Sickness Assay (DSA)

Hypothesis is induced in microfluidic channels.

RBC sickling is assessed through time-lapse imaging analysis.

Medium O2 is simultaneously measured for O2 probe. Hypothesis is induced by the addition of PCD to blood sample containing PCA at T=0.

Hypothesis is induced to 0% O2.

Hb hypoxia-stained RBCs quantified at T=0.

Presented sample contains two fractions of sickling RBCs, with SF1 and SF2 representing the total fraction of sickled RBC.

Each fraction is described by Sickness Fraction index (SF), sickling at a given time point.

Differential microfluidic Sickling (DMS) assay: sickling profile and kinetics of sickling at a set oxygen consumption rate.

Novel DSA Assay

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NOVEL HYPOXIA-INDUCED SICKLING ASSAY FOR ASSESSMENT AND MONITORING OF SICKLE CELL DISEASE TREATMENTS AFFECTING HEMOGLOBIN POLYMERIZATION 1Tarasev M, 2Gao X, 1Mota S, 1Ferranti M, 1Edenstrom A, 1Zaidi A, 1,2Hines PC 1Functional Fluidics Inc., Detroit, USA, 2Wayne State School of Medicine, Wayne Pediatrics

DSA: FEATURES

Wide range of oxygen consumption rates

Modest deoxygenation rates in a range from seconds to hours

Assay can be performed in both a steady-state and a kinetic mode

Kinetic assay can model Hb polymerization and RBC sickling as it would occur in vivo in a capillary network.

Wide range of final hypoxia levels

Method allows to modify the severity of hypoxia (partial oxygen pressure) at the end of experiment.

Allows in vivo modeling of in vitro oxygen environment (e.g., in different organs or tissues, or changes due to changes in condition).

Follow-up features

Repeated sickling after one or more cycles of reoxygenation.

Sickling of adhering RBCs (e.g., on VCAM1).

Sickling under flow or under shear.

Simultaneous monitoring of kinetics of O2 release from Hb (hemolytic).

Differential of sickled RBC forms (crecent, hover ball, granular) and transition states (e.g., cell creation).

DSA: ILLUSTRATIVE APPLICATION

Decreased sickling due to in-vitro treatment with Hb-modifying drug (voxelotor)

Unaffected HbSS sample

Delay of 24h to get PO2 to a level inducing RBC sickling

Single phase sickling curve (only one sickling rate)

About 90% RBC are sickled at zero percent oxygen.

HbSS sample (in vitro treated with GBT440)

Two subpopulation with different propensity to sickle

10% of RBC showed sickling similarity to that of untreated cells.

90% exhibited significant increased delay in sickling (about 280 s)

Sickling occurring at a much lower oxygen affinity.

DSA: METHODS

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Figure 2: Hypoxia-induced sickling assay

Figure 3: Hypoxia-induced sickling assay

Figure 4: Hypoxia-induced sickling assay

Figure 5: Hypoxia-induced sickling assay

Figure 6: Hypoxia-induced sickling assay

Figure 7: Hypoxia-induced sickling assay

Figure 8: Hypoxia-induced sickling assay

Figure 9: Hypoxia-induced sickling assay

Figure 10: Hypoxia-induced sickling assay

Figure 11: Hypoxia-induced sickling assay

Figure 12: Hypoxia-induced sickling assay

Figure 13: Hypoxia-induced sickling assay

Figure 14: Hypoxia-induced sickling assay

Figure 15: Hypoxia-induced sickling assay

Figure 16: Hypoxia-induced sickling assay

Figure 17: Hypoxia-induced sickling assay

Figure 18: Hypoxia-induced sickling assay

Figure 19: Hypoxia-induced sickling assay

Figure 20: Hypoxia-induced sickling assay

Figure 21: Hypoxia-induced sickling assay

Supporting Information

Appendix A: Experimental Setup

Appendix B: Data Analysis

Appendix C: Supplementary Figures

Conflict of Interest Statement: Tarasev, Gao, Mota, Ferranti, Edenstrom, Zaidi, and Hines are employees and Tarasev, Gao and Mota are shareholders of Functional Fluidics. 1,2

Conflict of Interest Statement: JSCDH (MPoS), Wayne State School of Medicine, Wayne Pediatrics