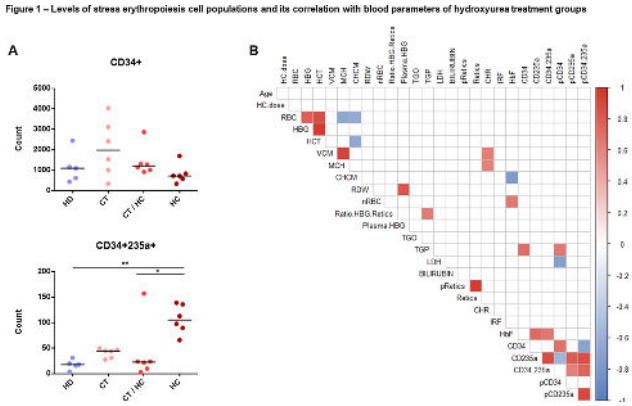
Complete blood count and hemolysis biomarkers of HC and CT / HC groups compared with CT, calculated using Kruskal-Wallis test. '', '**', '***' and '****' indicate p?0.05; p?0.01, p?0.001, and p?0.0001, respectively.*



A) Levels of CD34+ and CD34+CD235a+ cell populations. B) Correlogram of stress erythropoiesis cell FACS data versus hematological parameters of hydroxyurea treatment groups. Levels of stress cells are positively correlated with levels of HbF.

The authors do not declare any conflict of interest

P-029 OCCLUSIONCHIP: A FUNCTIONAL MICROCAPILLARY OCCLUSION ASSAY COMPLEMENTARY TO EKTACYTOMETRY FOR ASSESSING RED CELL HEALTH IN SICKLE CELL DISEASE

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Purpose: Red blood cell (RBC) deformability is a biomarker to assess the clinical status and response to therapy in sickle cell disease (SCD). RBC deformability has been measured by ektacytometry for decades, using shear or osmolar stress. However, ektacytometry averages the RBC population, and cannot report small fractions of abnormal RBCs. A single cell-based, functional RBC deformability measurement provides essential information on the presence of unmodified cell populations that can perpetuate disease complications after the implementation of drug or cell-based therapies. There are two goals in this study: to determine the reproducibility of values generated by the standardized OcclusionChip assay and commercially available Oxygen Gradient Ektacytometry (LORRCA); and to determine if the OcclusionChip assay could complement Ektacytometry for detection of abnormal RBCs in small fractions.

Materials and methods: OcclusionChip microfluidic assay design includes a gradient of capillary network-inspired micropillar arrays embedded into the microfluidic channel forming microcapillaries from 20 µm down to 4 µm. This mimics the non-uniform, continuously changing capillaries in the capillary bed, retaining RBCs with significantly decreased deformability in the upstream array with coarser openings, and those with modestly decreased deformability in the downstream array with finer openings. Micropillar arrays were coupled with two 60-µm side passageways mimicking capillary bed arteriovenous anastomoses. This helps regulate blood flow; when an upstream portion of the array is obstructed, incoming RBCs can flow into the microfluidic anastomosis and re-enter the array downstream. To test the relative merits of the OcclusionChip and Ektacytometry, we measured the elongation index (EI by Oxygen Gradient Ektacytometry) and occlusion index (OI by OcclusionChip) of blood samples containing gradient concentrations of glutaraldehyde-stiffened RBCs. We also tested clinical blood samples obtained from subjects with SCD using oxygen gradient ektacytometry, which measures the EI at a range of pO2 level (159 mmHg to ~5 mmHg), and the hypoxic OcclusionChip assay, which measures OI at physiologic hypoxic pO2 level (45 mmHg).

Results: We tested glutaraldehyde-stiffened RBCs for up to 1% volume fraction; ektacytometry detected no significant change in Elongation Index (EI), while the OcclusionChip showed significant differences in OI (Figure 1). OcclusionChip detected a significant increase in OI in RBCs from an individual with sickle cell trait (SCT) and from a subject with SCD who received allogeneic hematopoietic stem cell transplant (HSCT), as the sample was taken from normoxic (pO2:159 mmHg) to physiologic hypoxic (pO2:45 mmHg) conditions (Figure 2). Oxygen gradient ektacytometry detected no difference in EI for SCT or HSCT.

Conclusion: The OcclusionChip assay is more sensitive to alterations in RBC deformability in SCD, particularly when %hemoglobin S (HbS) is small, thus able to complement Ektacytometry for a more accurate assessment of patient clinical status. These results suggest that the single cell-based OcclusionChip enables the detection of HbS-related RBC abnormalities in SCT and SCD, particularly when the HbS level is low. We conclude that the OcclusionChip is complementary to the population-based Ektacytometry assays and provides additional sensitivity and capacity to detect small but clinically significant populations of abnormal RBC.

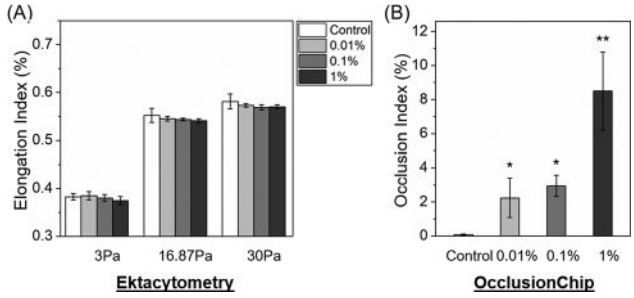

Figure 1. The OcclusionChip enables the detection of small-fraction glutaraldehyde-stiffened RBCs in mixtures with normal RBCs. (A) Elongation Index (EI) by ektacytometry. (B) Occlusion Index (OI) results on the same RBC mixtures.

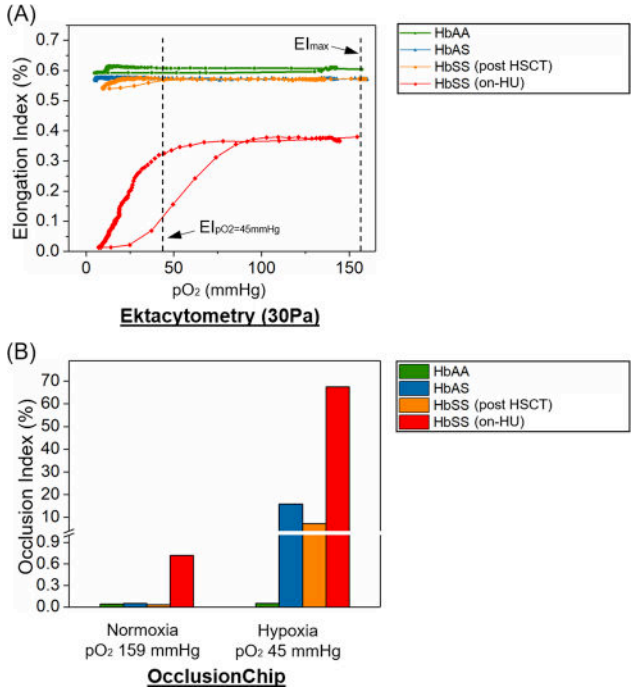

Figure 2. OcclusionChip enables detection of HbS-related RBC abnormalities under hypoxia in SCT and SCD. Shown are (A) Elongation Index (EI) results by oxygen gradient ektacytometry and (B) Occlusion Index (OI) results by the OcclusionChip.

The authors do not declare any conflict of interest