

celular desencadeiam respostas inflamatórias mediadas por TLR4 e amplificadas por TREM-1. Isso resulta em aumento da permeabilidade vascular, recrutamento de neutrófilos e células mononucleares, e prejuízo na vasodilatação, promovendo morte celular por apoptose. Além disso, HMGB1 contribui significativamente para a inflamação e trombose na anemia falciforme, ativando inflamassoma NLRP3 em plaquetas. **Conclusão:** TREM-1 desempenha um papel crucial como um possível modulador da resposta imunológica em condições de inflamação crônica, sendo ativado por HMGB1. Tanto TREM-1 quanto HMGB1 estão significativamente elevados em estados inflamatórios crônicos e podem piorar clinicamente a AF, especialmente durante crises vaso-oclusivas. Como perspectivas futuras, estudos e ensaios clínicos que visem inibir/bloquear a via TREM-1, são necessários para confirmar esses achados e desenvolver novas estratégias terapêuticas promissoras na redução da inflamação e danos teciduais na AF.

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DEVELOPMENT OF AN AUTOMATED FLUORESCENCE MICROSCOPY ASSAY FOR QUANTIFYING FACTORS AFFECTING RED BLOOD CELL (RBC) SICKLING IN SICKLE CELL DISEASE (SCD)

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Sickle cell disease is the most common monogenetic disease in the world, characterized by a point mutation in the sixth codon of the beta-globin gene, which generates mutant hemoglobin S (HbS). Under conditions of deoxygenation, HbS polymerizes, distorting the discoid morphology of an erythrocyte into a sickle-like shape, responsible for causing vaso-occlusive episodes associated with pain attacks, hemolytic anemia and early mortality. Although the main inhibitor of HbS polymerization is fetal hemoglobin (HbF), many additional factors can contribute to cell sickling, including extrinsic factors from the surrounding interacting cells in the microvasculature environment. However, the lack of straightforward functional assays to quantitate how these factor affect cell sickling, hamper a more profound understanding of SCD physiopathology. Therefore, our objective was to develop an automated fluorescence microscopy assay for quantifying factors affecting RBC Sickling in SCD, without the need of special incubators or microfluidic devices. In order to establish a self-generated hypoxic environment, we cultured

HS-5 stromal cells in 96-well plates at a low-density, and placed a small round (5 mm diameter) glass coverslip above the cell monolayer, thus limiting oxygen diffusion underneath the coverslip. Before placing the coverslips, erythrocytes from patients with different types of SCD (HbSS, HbSC and S-Beta Thalassemia) were fluorescently-labeled with DiD'Oil (red) and seeded along with HS-5 cells. A nuclear dye (Hoechst 33342, blue) and a hypoxia-activated fluorescent marker (Image-iT hypoxia, green) were added, thus allowing the identification of HS-5 cell nuclei and hypoxic regions. Immediately after coverslip placement (0h), and after 1h and 24h, a total of 25 sites from each well were acquired with a 20x objective, using transmitted-light and the three fluorescence channels, using an ImageXpress^{Micro} XLS High-Content-Screening-HCS system (Molecular Devices). Images were exported and analyzed using the open-source software CellProfiler, in order to delineate and segment RBCs. Shape-related morphometric features were extracted for each RBC (including, cell area, perimeter, form-factor, roundness and eccentricity), and used to classify and quantify different sub-populations of erythrocytes. Two form-factor thresholds (of 0.60 and 0.85) were used to classify RBCs into sickled (form-factor < 0.60), abnormally-shaped, and round cells (form-factor > 0.85). An eccentricity threshold of 0.60 was also used to separate round cells from elliptical/sickled cells (eccentricity > 0.60). Data analysis was carried using the open-source software Knime. In the three SCDs evaluated, the percentage of cells with eccentricity > 0.60 increased as a function of time, indicating that sickling occurred. In agreement, the percentage of cells with lower form-factors decreased with time. Importantly, the standard deviation of the percentages of RBCs in each morphological class, as calculated from three replica wells, were around 2.2%, for all three SCD patients and time-points evaluated. The use of advanced imaging tools and analysis software allowed a detailed and quantitative assessment of morphological changes in different hypoxic conditions. The 2D microscopy assay presented here is highly-reproducible and allows a quantitative analysis of the influence of different factors in the kinetics of RBC sickling, thus constituting an important tool to explore the pathophysiology of sickle cell disease ex vivo. **Support:** Grant #2022/12856-6, São Paulo Research Foundation (FAPESP).

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IN VITRO EFFECTS OF HEME ON THE ACTIVATION OF MICROVASCULAR ENDOTHELIAL CELLS, HMEC-1

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Introduction: Sickle cell anemia (SCA) is a hereditary condition characterized by morphological changes in erythrocytes, which increase their fragility and susceptibility to rupture, releasing intracellular components, including the heme