

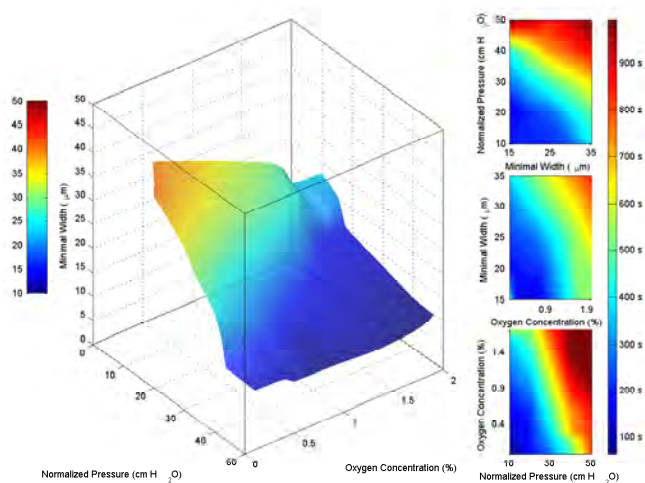
# Collective Hydrodynamics and Kinetics of Sickle Cell Vaso-occlusion and Rescue in a Microfluidic Device

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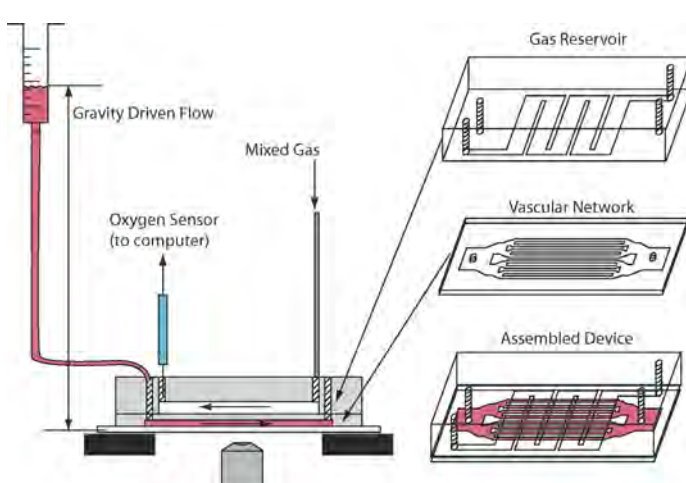
The pathophysiology of sickle cell disease, the first to be implicated with a genetic origin, is complicated by the multi-scale nature of the processes that link the molecular genotype to the organismal phenotype. Here, we show that it is possible to evoke, control and inhibit the vaso-occlusive crisis event in sickle cell disease using an artificial microfluidic environment. We use a combination of geometric, physical, chemical and biological means to quantify the phase space for the onset of a jamming crisis, as well as its dissolution, as shown in Figure 1.

The microfluidic chip designed to independently vary the various parameters that control the onset of vaso-occlusion in a sickle cell crisis is shown in Figure 2. This device allows us to dissect and probe the hierarchical dynamics of this multi-scale process by manipulating the geometrical, physical, chemical and biological determinants of the process. The chip consists of a series of bifurcating channels of varying diameters that grossly mimics the geometry of vasculature. By controlling the physical pressure

gradient across the chip, we can vary the kinetic time scale for transit of red blood cells. The channels are separated from a gas reservoir by a thin gas-permeable polydimethylsiloxane (PDMS) membrane. As the geometries are microscopic, gas diffusion is rapid and the oxygen concentration in the microchannels is governed by the concentration in the gas reservoir. By changing the mixture of this reservoir, we control oxygen concentrations in the channels and hence the onset of microscopic hemoglobin polymerization. By using blood with varying concentrations of HbS and different hematocrits, we can mimic the variability among individuals. This device was used to study the phase space of jamming governed by pressure, channel dimensions and oxygen concentration as shown in Figure 1. Our experimental study integrates the dynamics of collective processes at the molecular, polymer, cellular and multi-cellular level; lays the foundation for a quantitative understanding of the rate limiting processes; provides a potential tool for optimizing and individualizing treatment; and serves as a bench test for dynamical drugs.



▲ Figure 1: Phase space of vaso-occlusion. The colored surface represents a fitted hypersurface in 4-dimensional space: width, pressure, oxygen concentration, and occlusion time. The isosurface was computed from 43 data points using Delaunay triangulation. All points on the hypersurface correspond to triples of height, pressure, and oxygen concentration where the fitted time to occlusion was 500 seconds. The color of each point on the surface characterizes the minimal width in the device and is redundant with the point's vertical (width) coordinate. The filled contour plots represent slices through the fitted volume at specific planes (top: oxygen concentration = 0.5%, middle: normalized pressure = 20 cm H<sub>2</sub>O, bottom: minimal width = 25 μm). This phase space describes the behavior of patients whose samples contained hemoglobin S concentrations of at least 65% (mean 86%, standard deviation 6.7%).



▲ Figure 2: Fabrication and schematic of the device. The oxygen channels and vascular network were fabricated in separate steps. After removal of the device from the SU8 mold master, holes were cored and networks were bonded via oxygen plasma activation and then attached to a glass slide. The widest cross section in the vascular network on the left and right of the device is 4 mm x 12 μm. The vascular network then bifurcates, maintaining a roughly equal cross-sectional area. An open 5 mL syringe was connected to the device and raised and lowered to increase or decrease the flow rates through the device. The gas channels were connected to two rotometers regulating the ratio of 0% and 10% oxygen in the gas mixture that was fed into the device. The outlet of the gas network had an oxygen sensor to validate the oxygen concentration in the microchannels.