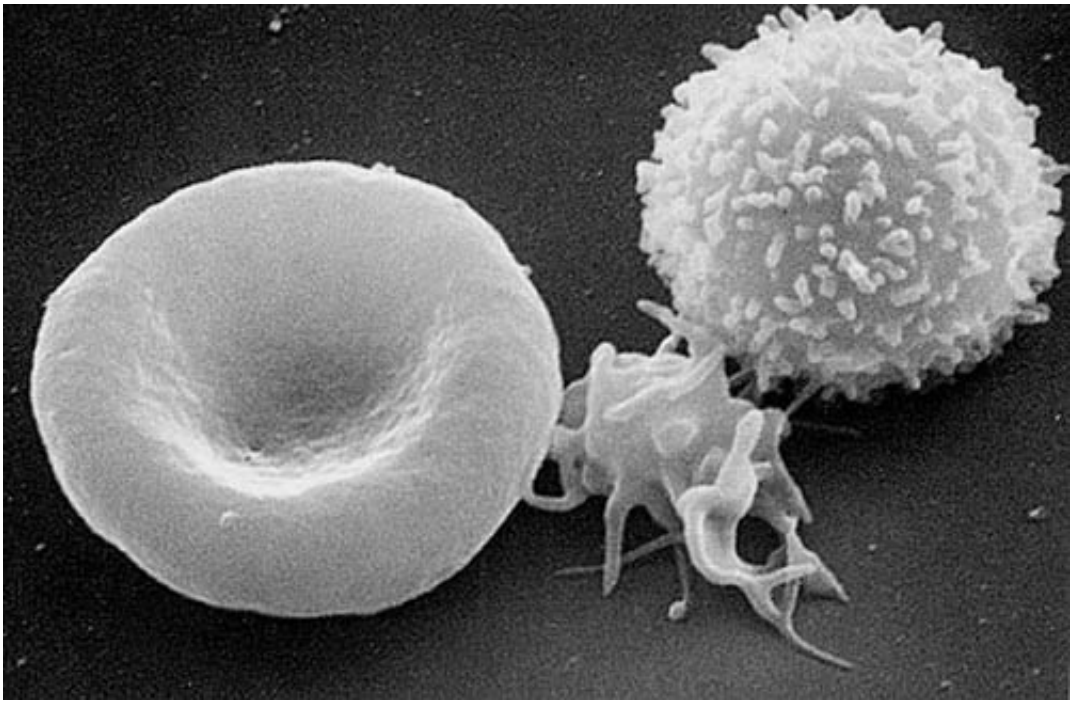
Quantitative measurement of oxygen affinity in a single red blood cell

August 3 2015, by Heather Zeiger



Scanning electron micrograph of blood cells. From left to right: human erythrocyte, thrombocyte (platelet), leukocyte. Credit: public domain

Researchers report a system that quantitatively measures cell volume, hemoglobin mass, and oxygen saturation in individual red blood cells. They found that oxygen affinity is different for individual red blood cells. These differences are likely due to the cellular environment rather than hemoglobin concentration.

According to lead author, Dr. Guiseppe Di Caprio, "We developed an optofluidic measurement system to quantify the oxygen affinity of a large collection of red blood cells, and we find remarkable differences in binding strength on a single cell level relative to the bulk average."

Guiseppe Di Caprio, Chris Stokes, John M. Higgins, and Ethan Schonbrun from the Rowland Institute at Harvard University, Harvard Medical School, and Massachusetts General Hospital have developed a system that mimics the circulatory system and coupled it with an optical technique that can provide quantitative in vivo information of individual red blood cells. " Their work appears in the *Proceedings of the National Academy of Sciences.*

Oxygen affinity is an important clinical parameter that hematologists use to diagnose several conditions. The clinical measure that is often used for this is the p50 number, which is the partial pressure of oxygen when a person's hemoglobin, the oxygen-carrying molecule found red blood cells (RBCs), is 50% saturated with oxygen. Each RBC has a certain amount of hemoglobin in it. If one measures the partial pressure of oxygen and measures the amount of oxygen-filled hemoglobin compared to unfilled hemoglobin, this will provide enough information to make an oxygen dissociation curve. This curve is used to find a p50 number.

Several factors can affect hemoglobin's affinity for oxygen (i.e., shift the oxygen dissociation curve) such as temperature, pH, and the concentration of 2,3-diphosphoglycerate within the RBC. While clinicians can measure individual RBC's mass and volume, this research is the first to look at how these factors affect oxygen affinity in individual RBCs.

Di Caprio et al.'s method combines biomimetic technology with microfluidic cytometry. Biomimetics is an advancing field that combines biology with engineering. As the name implies, scientists build devices

that mimic biological processes or environments. This allows for in vitro studies that mimic in vivo conditions. In this study, Di Caprio et al.'s system mimics the circulatory system.

Their device has three layers: one for RBCs to flow, a gas-permeable membrane, and another for oxygen flow. Importantly, because Di Caprio et al. seek to make quantitative measurements, they designed the system so that the partial pressure of oxygen can be controlled. This allowed them to relate RBC oxygen saturation with pressure. As RBCs flow through the device, they equilibriate with the oxygen, coming to a saturation point. They can then determine cell volume using optical imaging of the saturated RBCs.

Hemoglobin mass can be determined using a quantitative absorption cytometry technique that was previously reported by this group. While previous studies measured the total hemoglobin by looking at a single wavelength (410 nm), the current study looks at a second wavelength (430 nm) to distinguish free hemoglobin with oxygen-bound hemoglobin. By imaging the same cell at both wavelengths, absorbance can be related to oxygen saturation in a single cell.

After testing and confirming their system, the next step was to investigate the kinetics of oxygen saturation. By changing the geometry of their microfluidic analyzer, Di Caprio et al. were able to examine the oxygen binding kinetics for RBCs in fluidic conditions. They determined when and where in their analyzer RBCs reached their oxygen saturation point and confirmed that all RBCs are saturated when they are imaged. This confirms that any variation in oxygen affinity between individual cells is not due to kinetic issues, leaving hemoglobin concentration and/or the cell's cytosolic environment as the probable factors for differences in oxygen affinity.

Because this study deals with high throughput data (approximately 2,000

RBCs at a time) at different concentrations of oxygen, the authors were able to report mean and standard deviation measurements at a particular temperature and pH. Future studies would involve changing temperature and pH to see how this changes individual cell oxygen uptake. They found that standard deviation was highest when the dissociation curve was steepest, which was also at the pressure where oxygen saturation varied the most between individual cells.

Statistical analyses of RBCSs at this level of oxygen showed a correlation between hemoglobin concentration and oxygen saturation: the higher the hemoglobin concentration, the greater the saturation value. However, studies with the same sample using isotonic and hypotonic buffers to create an osmotic gradient showed that changes in hemoglobin concentration did not change oxygen affinity. This means the differences in oxygen affinity between individual RBCs is due to something within the cellular environment. The authors suggest that it is due to differences in the concentration of 2, 3-diphosphoglycerate, a key regulator of oxygen affinity, within the cytosol.

When asked about the implications of their research, co-author Dr. Ethan Schonbrun states, "We believe that the characterization of single cell oxygen affinity could be extremely valuable in hematology, in both anemia diagnostics and to better understand the physiology of the healthy hematological system."

More information: "Single-cell measurement of red blood cell oxygen affinity" Guiseppe Di Caprio, Chris Stokes, John M. Higgins, and Ethan Schonbrun, *PNAS*, DOI: 10.1073/pnas.1509252112