Using Red Blood Cells to Monitor Blood Analytes

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Measurement of blood analytes provides crucial information about the health of a patient. Many analytes, such as glucose for diabetic patients, require long-term and/or near-continuous monitoring for proper disease assessment and management. However, standard monitoring techniques are inconvenient, do not allow continuous monitoring and/or only allow periodic sampling. Thus, there is a need for a long-term system platform for in vivo sensing. Red blood cells (RBCs) functionalized with optical sensors provide a microscale platform for this type of minimally or noninvasive sensing. With a 120 day lifespan, human RBCs provide a long-term platform that is both biodegradable and biocompatible, thereby eliminating the immune system response common for many implanted devices. Recently our group demonstrated the ability of low hemoglobin RBCs functionalized with FITC to sense extracellular pH.[1] As a step toward in vivo use, we report on the development of high hemoglobin, functionalized RBCs using a near infrared dye to sense extracellular pH.

Our initial work with FITC had an issue because hemoglobin absorption extends through the red and interferes with FITC. Therefore, a pH sensitive fluorophore with response in the near infrared is used in this work.[2] This dye is cell impermeant and can be excited in the 700 - 800 nm spectral region.

Functionalization of the RBCs is accomplished by opening lysis pores (~10s of nm) in the RBC membrane and diffusing sensors into the RBCs. Sensors are loaded using a preswelling technique that yields RBCs with near-normal hemoglobin levels.[3-4] Isolated whole bovine RBCs are suspended in Hank’s balanced salt solution (HBSS) and centrifuged. Following removal of the supernatant, hemolysate solution containing the NIR sensing dye is added to the RBCs until lysis pores are formed and the dye diffuses into the RBCs. HBSS is then added to reseal the RBCs.

When diffused into RBCs, the dye is still responsive to pH in the surrounding medium. The emission is well separated from hemoglobin absorption. In response to changes in extracellular pH, the fluorescence displays both a spectral shift and change in quantum yield.