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RAMAN MICROSPECTROSCOPY AND IMAGING PROVIDE INSIGHTS INTO HEMOGLOBIN OXYGEN SATURATION AND METHEMOGLOBIN WITHIN HUMAN ERYTHROCYTES

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BACKGROUND/AIMS： In critical care, an important indicator of disease or dysfunction of erythrocytes is the oxygen (O_2) storage capacity. Resonance Raman microspectroscopy is a particularly sensitive probe for studying the electronic and structural properties of metalloporphyrin complexes including hemoglobin. We present Raman microspectroscopy and images of functional erythrocytes in the oxygenated state (HbO_2Sat) and containing methemoglobin (metHb).

METHODS/RESULTS： The oxygenation processes of a human erythrocyte is monitored using a Raman microspectroscopy and imaging technique. Raman images of the 1638 cm^{-1} band are recorded in the oxygenated and deoxygenated states using only 120s of laser exposure and ~1 mW of defocused laser power. All approaches used to calculate intensity resulted in significant correlation ($r>0.9$, $p<0.01$) between HbO_2Sat values measured by oximeter and HbO_2Sat estimated from Raman spectra. Successive determinations of HbO_2Sat in a given area of constant oxygen level provided results varying ±1-6%. The detection of all physiological HbO_2Sat levels was possible. Methemoglobin recorded images of an erythrocyte with nitroprusside or nitroglycerin using 880 cm^{-1} excitation and an integration time of 480s. MetHb is in the ferric high-spin state; therefore cannot bind O_2. The 880 cm^{-1} excitation spectrum of metHb encapsulated in a single living cell was prepared. The result provided molecular insight into the dynamic nature of the reverse-oxygenated hemoglobin and metHb changing throughout the effect of oxidative stress in erythrocytes and the effects of drugs designed to decrease oxygen affinity in the treatment of such conditions as nitroglycerin and nitroprusside.

CONCLUSION： The results of this study gained by in vivo single-erythrocyte molecular analysis have important ramifications to our understanding of fundamental physiological processes and have important implications for the potential biomedical application of Raman imaging and spectroscopy as a diagnostic and analytical tool in erythrocyte disorders.

Keywords: Hemoglobin. Oxygen Saturation.