Long-term glycemic markers

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Biomedical Background

Diabetes mellitus, which is characterized by the defective regulation of blood glucose, is the most common disorder of the endocrine system, affecting 25.8 million people in the United States alone (2010). Given the lack of suitable therapeutic options, effective glycemic control is imperative in avoiding acute and chronic complications such as diabetic coma, and microvascular and macrovascular complications. To this end, several research groups, including our own laboratory, have pursued the development of a noninvasive blood glucose sensor, using a wide variety of optical and spectroscopic modalities. Nevertheless, these attempts to monitor glucose levels directly through Raman, infrared or other optical modalities have faced significant challenges and none have been translated to clinical practice. Given this scenario, glycated hemoglobin (HbA1c) presents a new promising target for performing long-term diabetes monitoring. While monitoring blood glucose remains the gold standard for continuous monitoring and evaluation of treatment options, HbA1c has gained approval in the medical community in assessing the long-term history of glycemic control. HbA1c is formed by the nonenzymatic glycosylation (glycation) of hemoglobin exposed to blood glucose and therefore has a strong correlation with the average glucose concentrations in the bloodstream in the preceding three-month period (life span of the erythrocytes). Because of this strong correlation, HbA1c levels have been regularly used to monitor long-term glucose control in established diabetics and has been recently approved for screening for diabetes (HbA1c ≥ 6.5%) and prediabetes (5.7% ≤ HbA1c ≤ 6.4%) in the United States.

Biophotonic Contributions

In this study, we propose and demonstrate the potential of Raman spectroscopy as a novel analytical method for quantitative detection of HbA1c, without using external dyes or reagents. Using the drop coating deposition Raman (DCDR) technique, we observe that the nonenzymatic glycosylation (glycation) of the hemoglobin molecule results in subtle but discernible and highly reproducible changes in the acquired spectra, which enable the accurate determination of glycated and nonglycated hemoglobin using standard chemometric methods. The acquired Raman spectra display excellent reproducibility of spectral characteristics at different locations in the drop and show a linear dependence of the spectral intensity on the analyte concentration. Furthermore, in hemolysate models, the developed multivariate calibration models for HbA1c show a high degree of prediction accuracy and precision—with a limit of detection that is a factor
of 15 smaller than the lowest physiological concentrations encountered in clinical practice. The excellent accuracy and reproducibility achieved in this proof-of-concept study opens substantive avenues for characterization and quantification of the glycosylation status of (therapeutic) proteins, which are widely used for biopharmaceutical development. We also envision that the proposed approach can provide a powerful tool for high-throughput HbA1c sensing in multicomponent mixtures and potentially in hemolysate and whole blood lysate samples.

**Long-term glycemic markers**

**SIGNIFICANCE:** Determination of HbA1c and fructosamine levels

- Extensively used in long and intermediate term assessment of glycemic control (e.g., Diabetes: HbA1c ≥ 6.5%)
- Formed by the non-enzymatic glycation of hemoglobin exposed to blood glucose
- Current assays suffer from lack of sensitivity → Use sensitivity of Raman for protein and protein aggregate structure

**APPROACH:** Principle of glycemic marker detection using drop coating deposition Raman (DCDR) spectroscopy

![Diagram](image)

*Log-term glycemic marker scheme.* Principle of glycemic marker detection using DCDR.
**Drop Coating Deposition.** Composite photograph showing the drop coating ring pattern produced by air-drying Hb and HbA1c mixture solution on a quartz substrate.

**Ongoing Projects**

a. Develop Raman spectrometer based on in blood assay.
b. Monitoring glycemic markers by surface enhancement surface-enhanced Raman spectroscopy (SERS).

**Background Publications**


**Representative Collaborative Publications**