Trends in Analytical Chemistry 64 (2015) 100–108

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Emerging trends in optical sensing of glycemic markers for diabetes monitoring

Rishikesh Pandey [b], Narahara Chari Dingari [b,c], Nicolas Spegazzini [b], Ramachandra R. Dasari [b], Gary L. Horowitz [c,d], Ishan Barman [a,e,*]

[a] Department of Mechanical Engineering, Johns Hopkins University, Baltimore, MD 21218, USA
[b] Laser Biomedical Research Center, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[c] Currently at EMC Corporation, Hopkinton, MA, 01748, USA
[d] Division of Clinical Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA
[e] Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA

ARTICLE INFO

Keywords:
Diabetes
Diabetes monitoring
Fructosamine
Glycemic marker
HbA1c
Optical sensing
Photonics
Point of care
Raman scattering
Spectroscopy

ABSTRACT

In the past decade, considerable attention has been focused on the measurement of glycemic markers, such as glycated hemoglobin and glycated albumin, that provide retrospective indices of average glucose levels in the bloodstream. While these biomarkers have been regularly used to monitor long-term glucose control in established diabetics, they have also gained traction in diabetic screening. Detection of such glycemic markers is challenging, especially in a point-of-care setting, due to the stringent requirements for sensitivity and robustness. A number of non-separation based measurement strategies were recently proposed, including photonic tools that are well suited to reagent-free marker quantitation. Here, we critically review these methods while focusing on vibrational spectroscopic methods, which offer highly specific molecular fingerprinting capability. We examine the underlying principles and the utility of these approaches as reagentless assays capable of multiplexed detection of glycemic markers and also the challenges in their eventual use in the clinic.

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1. Introduction

Disorders of glucose homeostasis, including Type I and II and gestational diabetes, represent a leading cause of morbidity and mortality worldwide. Diabetes presents a significant healthcare, economic and societal challenge, as evidenced by its prevalence in nearly 382 million people across the globe – with an expected increase to 592 million diabetic patients by 2035 [1]. Unfortunately, at this time, diabetes has no well-established cure, but must be treated with regular insulin injections and other related medications based on careful monitoring of blood-glucose levels. Maintenance of glycemic control is critical to the patient's quality of life and to avoid serious secondary complications, such as microvascular and macrovascular changes that may result in diabetic neuropathy, nephropathy and retinopathy. Thus, direct quantification of blood-glucose values remains the "gold standard" for diagnosis and monitoring of diabetes. A number of research groups, including our own laboratory, have been engaged in developing a fully non-invasive and continuous glucose monitoring system that can eliminate the inconvenience associated with frequent finger pricks while the patient can readily recognize and predict trends of blood-glucose

* Corresponding author. Tel.: +1 4105160656; Fax: +1-410-516-4316.
E-mail address: ibarman@jhu.edu (I. Barman).

http://dx.doi.org/10.1016/j.trac.2014.09.005
0165-9936/

changes [2]. Despite promising preliminary results, such a method for *in-vivo* glucose tracking in a completely label-free manner has yet to be clinically validated.

In this setting, significant attention has been focused – from both research and clinical standpoints – on the measurement of glycated proteins for monitoring long-term glycemic control in diabetics. These long-term glycemic markers reflect the average value of blood-glucose concentration with the time span of the glycemic history being unique to the biomarker and provide vital information to complement the blood-glucose measurements. These biomarkers, notably glycated hemoglobin (HbA1c) and glycated albumin (GA, fructosamine) [3], provide retrospective indices of integrated blood-glucose values over extended periods of time, with intrinsic half-lives of ~60–90 days and 14–21 days, respectively. Unlike measurement of fasting plasma glucose (FPG), HbA1c and fructosamine do not require an overnight fast, are not affected by short-term lifestyle changes, and show significantly less variability within individuals [4]. Recent studies showed that elevated concentrations of these biomarkers strongly correlate with the presence of diabetic complications [5], such as kidney damage, cardiovascular problems and retinopathy [6] – often predicting the presence of these conditions better than FPG [7]. While affinity chromatography, high-performance liquid chromatography (HPLC) and immunoassays have been employed for analyzing glycemic control, especially for HbA1c, they often suffer from specificity limitations, as the presence of hemoglobin variants, as well as uremia and ethanol ingestion, can result in erroneous readings. Moreover, there is no existing "gold standard" for fructosamine measurement because of the lack of suitability of existing approaches for routine clinical laboratory application. As a consequence, there is an unmet need for sensitive, specific determination of these biomarkers, preferably in a reagent-free, easy manner.

This need has led to a surge of emerging techniques that exploit an array of chemical and optical contrast mechanisms. Of these, the photonic and direct spectroscopic routes appear to be the most promising, as they could offer simultaneous determination of both biomarkers, without necessitating significant sample preparation. Based on recent research articles in this area, we discuss the emerging optical approaches, especially those employing direct spectroscopic routes to glycemic marker determination. More importantly, we examine in detail the outstanding properties of these sensing mechanisms, their future prospects and research opportunities, while critically analyzing the potential challenges on the path to use in the clinic.

2. Markers of glycemia

Since the first results from the comprehensive Diabetes Control and Complications Trial (DCCT) regarding the importance of complementary biomarkers for diabetes screening and monitoring, opening up and development of this domain have been of great significance both to care for diabetic patients and to assist in unraveling the cause of diabetic complications. While a number of such biomarkers of glycemic variability have been proposed, HbA1c and fructosamine have exhibited stronger correlations with diabetes complications, macroangiopathies and microangiopathies across a larger cross-section of studies. In this review, our discussion therefore focuses on the detection and the quantitation of these markers, which are formed by non-enzymatic glycation of proteins *in vivo* and, as such, are also relevant to the research thrust in advanced glycation end products (AGEs).

HbA1c is the major glycohemoglobin species in human blood [8] and has been employed for close to three decades for long-term assessment of glycemic control in diabetic patients. The American Diabetes Association (ADA) recently added HbA1c to screening for prediabetes ($5.7\% \leq \text{HbA1c} \leq 6.4\%$) and diabetes ($\text{HbA1c} \geq 6.5\%$) [9]. Glycated hemoglobin is produced from the multistep condensation reaction of glucose with a hemoglobin amine moiety [10]. The formation process is initiated by the condensation of a free primary amine on hemoglobin with the glucose carbonyl resulting in the formation of an unstable Schiff base, which may dissociate or undergo an Amadori rearrangement to form the final ketoamine, a conversion that is irreversible. HbA1c is formed by the specific reaction of glucose with the amino-terminal valine in one or both of the hemoglobin (Hb) β-chains, as shown in Fig. 1. The HbA1c value is, thus, directly proportional to the mean concentration of glucose in the bloodstream over the preceding two-month period, which is a direct function of the lifespan of erythrocytes. HbA1c is traditionally reported as a percentage of total hemoglobin; the range of HbA1c values seen in practice is 4–20%. It has been determined empirically that each percentage point increase in HbA1c concentration roughly corresponds to a 2 mM rise in mean blood-glucose level [11]. Driven by the pre-analytical stability of HbA1c and the ability to provide a measure of chronic rather than acute dysglycemia, HbA1c testing has become an integral part of routine clinical check-ups when assessing glycemic control in patients with diabetes mellitus. In addition to offering an estimate of the risk of microvascular and macrovasuclar complications and the risk of severe hypoglycemia, HbA1c measurements allow the setting of appropriate population and individual targets – and, by extension, enable comparison of efficacy of old and new treatments [12].

In selected diabetic patients, there is also a clinical need for biomarker measurements that are more sensitive than HbA1c to shorter-term alteration in average blood-glucose levels. Fructosamine has received increased attention as an intermediate indicator of glycemic status because of its shorter half-life compared to hemoglobin [13]. It is important to recognize that fructosamine is not a specific molecule but refers to the aggregate of all serum proteins without distinction. Nevertheless, because all glycated serum proteins are fructosamines and albumin is the most abundant serum protein, measurement of fructosamine was previously considered to be an indirect measure of glycated albumin. The wisdom of this was questioned recently, spurring the development of more specific glycated albumin assays [14]. In contrast to HbA1c measurements that have been employed extensively in clinical laboratories as an adjunct to blood-glucose determinations, glycated albumin has remained an "underestimated marker of diabetes" [13]. Its recent importance and support in the clinical community stems from the profound impact of erythrocyte-lifespan variability on HbA1c levels, which, in turn, renders the glycated hemoglobin determinations ineffective in patients with hematological disorders (e.g., sickle cell anemia and autoimmune hemolytic anemia). Indeed, even with an accurate HbA1c measurement, the red blood count (RBC) lifespan issue means that the correct value will be clinically misleading, as detailed in one of our prior studies where both a method interference and an RBC lifespan issue were found to confound the true concentration values [15].

While further clinical studies are necessary to define which biomarker works best for specific classes of patients (since it is clear that none of the markers can provide the complete picture in all diabetic patients), measurement of glycated albumin has the potential to provide more accurate results in patients with certain hemoglobin variants and in patients whose RBC lifespans are altered, and to help implement therapy by providing a shorter term response. In the light of the growing recognition of the limitations of HbA1c and the lack of clinically available methods for glycated albumin estimation, development of robust, sensitive glycated albumin assays is extremely desirable in order to fill the gap in the present diagnostic landscape.

3. Determination methods of glycemic markers

The current diagnostic assays for HbA1c determination can be mainly segmented into methods employing charge differences and