SALIVARY BIOSENSORS FOR GLUCOSE MONITORING IN DIABETES MELLITUS-AN OVERVIEW

Anuradha Ganesan[1*], Gautham Kumar N[2], H Krishnan[3], S Satheesh Khannaa[4]

Article History: Received: 12.12.2022 **Revised:** 29.01.2023 **Accepted:** 15.03.2023

Abstract

Diabetes mellitus (DM) is a group of metabolic diseases characterised by hyperglycemia resulting from an absolute deficiency of insulin secretion or reduction in the biological effectiveness of insulin or both. Even though blood has always been the gold standard for diagnosing and monitoring diabetes, saliva promises to be a reliable biofluid as it transcends several of blood's disadvantages. The limitations faced by various biological fluids like blood, tears, sweat in monitoring glucose are surpassed by using saliva. The use of salivary glucose biosensors for real-time tracking of glucose levels in saliva convenient, and can further be used for both diagnosis of diabetes and glucose monitoring. The various biosensors which have been reviewed in this article have reported excellent detection limit and sensitivity. The devices could also be used as home care devices which could provide the patient with rapid and reliable information which could be corroborated in a healthcare setting.This paper reviews the significance of salivary biosensors for clinical diagnosis and therapeutic applications with focus on the technologies and biosensing platforms that have been reported for screening for diabetes.

Keywords: diabetes; biosensors; diagnosis; saliva

[1*]Professor and Head, Department of Oral Medicine & Radiology, SRM Dental College, Ramapuram, Chennai.
[2]Professor and Head, Dept. of Periodontics, Madha Dental College & Hospital,
Kundrathur, Chennai.
[3]Professor & Head,Professor, Dept. of Conservative Dentistry & Endodontics,Mar Baselious Dental College, Kothamangalam, Kerala.
[4]Senior Lecturer, Dept. of Periodontics, Madha Dental College & Hospital, Kundrathur, Chennai.

DOI: 10.31838/ecb/2023.12.s2.027

1. Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from an absolute deficiency of insulin secretion or reduction in the biological effectiveness of insulin or both.[1] Due to the burden of this disease across the globe and in India, diabetes is identified as one of the four priority non communicable diseases (NCDs) targeted for action by the United Nations. According to International Diabetic Federation, 700 million adults will be affected with DM globally by 2045. Various goals and targets have been put forward to reduce the burden of the disease,controlling diabetes, reducing mortality, enhancing easy access for patients to affordable basic technologies and essential medication.[2]

An effective screening , diagnosing and monitoring the diabetic status in patients helps to decrease the burden of the disease worldwide. Intervention includes the diagnosis of the disease as well and once diagnosed, monitoring of the disease status in affected patients.Interventions to improve diabetes outcomes can be directed at individuals with diabetes, health providers or the health system. Patient level intervention includes those directed at improved self management, including medication taking, diet, exercise, self monitoring and appropriate use of health care services. Self management includes diet, medication, exercise, rest, self monitoring of the diabetic status.[3] The most commonly used diagnostic method and monitoring the diabetic status is by detection of glucose levels in blood. The gold standard for determining a person's diabetes condition has generally been estimating blood sugar levels. Recent advances focuses on various biological fluids other than blood in diagnosing and monitoring the glucose levels in patients.

Even though blood has always been the gold standard for diagnosing and monitoring diabetes, saliva promises to be a reliable biofluid as it transcends several of blood's disadvantages.The limitations of various bodily fluids are all overcome by saliva testing for determining the glucose levels in diabetes individuals.[4]

Saliva-A Reliable alternative to blood glucose?

Health and disease can be screened effectively by using human saliva, which is an exocrine fluid. It consists of water, electrolytes and variety of proteins like enzymes, immunoglobin, albumin and some polypeptides. Studies also show proteins present in blood are present in saliva as well. Therefore saliva is functionally comparable to blood in reflecting the physiological status of the body.[5]Even though glucose levels in saliva correlates directly with the blood glucose levels, the mechanism of secretion is not very clear. Multiple authors have discussed numerous possible reasons for the higher glucose content in diabetic patients' saliva. There is no single mechanism to explain the appearance of glucose in saliva during periods of prolonged hyperglycemia.Possible damage in the permeability of basement membrane, small molecular size,alterations in the blood vessels, leakage through the gingival crevices and increased leakage from ductal cells all may contribute to the multifactorial cause of increased levels of saliva glucose in diabetics.[6] Saliva is a promising biofluid for early disease detection that leads to more effective treatment, risk assessment for estimation of glucose level and a simple, non invasive alternative to blood and urine tests.Although collecting saliva has less compliance issues than collecting blood, disease diagnosis through saliva analysis has potential value.For fluid collection, there is no particular equipment required.Saliva is whole saliva or saliva specific to a gland. Parotid, submandibular, sublingual, and minor salivary glands can all be directly accessed to collect gland-specific saliva.. The collection of gland specific saliva is useful for the detection of gland specific pathology, like any infection and obstruction. However, whole saliva is most frequently studied when salivary analysis is used for evaluation of systemic disorders. Whole saliva is a mixture of oral fluids and has secretion from major and minor salivary glands. Saliva also has several constituents of non salivary origin and can be collected with or without stimulation. Saliva is collected by gustatory stimulation, application of citric acid on the subject's tongue. Without external gustatory, masticatory, or mechanical stimulation, unstimulated saliva is collected. The best two ways to collect whole saliva is by draining method or spitting method. Draining method is when saliva is allowed to drip off the lower lip and spitting method is when subject spits saliva into a test tube.[7]

This review paper focuses on biosensors in salivary glucose estimation and monitoring in diabetics who can avoid an invasive prick each time they need to test for glucose

Salivary glucose estimation using biosensors

Glucose, being a small molecule moves with ease through membranes of blood vessels; it also diffuses from blood plasma into the gingival fluid into the saliva via the gingival sulcus. It is to be noted that there are certain controversies in the literature with respect to the correlation between capillary blood glucose and salivary glucose. In healthy subjects, there is a lack of relationship between capillary blood glucose and salivary glucose concentrations because insulin from beta cells in the pancreas is released into the bloodstream to normalise the capillary blood glucose level when the glucose concentration exceeds a certain limit. However, salivary glucose concentration is significantly higher in diabetics than in healthy subjects. There is some ambiguity in the association between capillary and salivary blood glucose. Some studies found positive correlation and others showed absence of correlation,hence making the use of salivary glucose concentration as an index for diabetes mellitus remains inconclusive[8]. However, this only means that there is a need for more well designed studies to try and establish a positive correlation between salivary and blood glucose, as the implications for the patient are enormous as salivary glucose collection is non invasive.

Clark and Lyons developed the first glucose biosensor.[9] Their biosensor monitored the consumption of oxygen using an electrode catalysed by glucose oxidase (GOx) enzyme. Yamaguchi et al developed a system to determine salivary glucose concentration that combined flow injection analysis and an O2 electrode.[10]Amperometry was used to detect the amount of oxygen that was consumed, and as the reaction progressed, there was a corresponding decrease in output current with the reduction in the concentration of the dissolved oxygen that reached the sensor. When the amount of O2 consumed on the enzyme membrane and the amount of O2 released from the sensor were in equilibrium, there was a constant current generated in the sample solution, which was proportional to the glucose concentration. The time-course changes of salivary glucose level were monitored in the range of 0.1 to 10mg dL−1 using just200 microlitre of sample solution. Further improvement in the system was achieved by replacing the oxygen electrode with a hydrogen peroxide(H2O2) electrode in the flow cell.In this glucose sensor,an increase in the concentration of H2O2 was observed as the reaction progressed, resulting in an increase in the output current. An amperometric circuit detected a constant current which was proportional to the glucose concentration in the sample solution.The use of H2O2electrode type glucose reduced the influence of dissolved oxygen and was therefore more reliable than oxygen based sensors.Besides, the H2O2-based saliva analysing system required only 50 microlitre of sample solution and it also enabled the monitoring of time-course changes of salivary glucose level in the range of0.1 to 10mg dL−1. There was another approach which used GOx enzyme immobilised on a ferrocene modified gold (Au) film electrode using glutaraldehyde (GA) for the crosslinking method.[11] Ferrocenehas its advantages as it is recognised as a good electron shuttle.Hence, it are commonly used because it eliminated sensor signal interference from O2 level dependence and electroactive biological species. In these studies dental cotton rolls were used to collect saliva samples conventionally and subsequently, saliva glucose sensors were used to analyze the samples.

Ye et al reported the use of sensors for detecting salivary glucose who developed a CuO nanoneedle/graphene/carbon nanofiber modified glassy carbon electrode biosensor. This sensor, when tested on saliva obtained from healthy volunteers indicated a rapid response as well as a high sensibility.[12]Other glucose-detecting sensors included the ones developed by Li et al. in 2015 (electrochemical sensor using anodized cupric oxide nanowires, which was tested for calibration against serum glucose concentration), by Wang et al. in 2016 (core-shell IrO2@NiO nanowire), and by Du et al. in 2016(a screen-printed sensor chip).[13,14,15]

For detecting surges of glucose intake in a patient over a set period of time, a novel constant-monitoring sensor was also developed. A sensor encased in a mouthguard which is wearable over a prolonged period was developed by Arakawa et al. A platinum and silver/silver chloride electrode in which glucose oxidase (GOD) was immobilised by entrapment with Poly (MPC-co-EHMA) glucose sensor and a wireless transmitter were the main constituents incorporated into this mouth guard. Using artificial saliva, this mouth guard was tested on a phantom jaw and the results showed very high sensitivity and ability to detect glucose in concentrations ranging from 5 to 1000 mmol/L.[16]

Soni et al developed a smartphone auxiliary device and en enzymatic sensor which was paper based which reduced the dependability on expensive auxiliary devices for glucose determination in saliva. This paper-based sensor changed colour in contact with glucose, with saturation being directly proportional to the amount of glucose.Once the sensor came in contact with the sample, a special RGB-analysing software through a smartphone camera was used to scan the sample. The system was tested on both healthy and diabetic subjects and the results showed that there was a strong correlation , between the salivary and blood glucose (0.44 in healthy subjects, 0.64 in prediabetic patients,and 0.94 in diabetic patients)].[17]

Spectrophotometric detection using a low-cost colorimeter was another biochemical sensing method that was employed ;using a colloidal AgNPs/MoS2-based nonenzymatic glucosebiosensor[18]; tested on both saliva and sweat with similar performances; using randomly oriented CuO nanowire networks[19]; using CuO-modified screen-printed carbon electrodes[20]; using molecularly imprinted polymer binding on a conducting polymer layer; tested on both saliva and blood, methylene blue, hydrazine and platinum nanoparticles; and using paper-based sensors[21]standing to prove a high interest in the development of these medical devices.

Yunqing Du et al developed nano structured bio sensors to detect salivary glucose.[22]Their main objective was to develop a biosensor that could detect low-level glucose in saliva (physiological range 0.5–20 mg/dL).On a screen-printed platinum electrode, a layer-by-layer self-assembly of single-walled carbon nanotubes, gold nanoparticles, chitosan and glucose oxidase was used to build the sensor.Quantitative detection of glucose in both buffer solution and saliva samples was done electrochemically. The glucose content of each sample was validated using a standard spectrophotometric technique. The disposable glucose sensors have a detection limit of 0.41 mg/dL, a linear range of 0.5–20 mg/dLin buffer solution, and a response time of 30 s. In a study of 10 healthy subjects,salivary glucose levels between 1.1 to 10.1 mg/dL were detected. The results revealed that the noninvasive salivary glucose monitoring could be an alternative for diabetes self-management at home. The purpose of this study was not intended to replace regular blood glucose tests, but to study salivary glucose itself as an indicator for the quality of diabetes care. The authors concluded that the results of the study could potentially help patients monitor their diabetic status more effectively.

Wenjun Zhang et al also non invasively monitored salivary glucose using a biosensor.[23]To provide accurate, low cost, and continuous glucose monitoring, they developed a unique, disposable saliva nano-biosensor. Two healthy individuals were recruited for the study in which more than eight clinical trials on real-time non invasive salivary monitoring was carried out(a 2–3 h-period for each trial, including both regular food and standard glucose beverage intake with more than 35 saliva samples obtained). As compared to the UV Spectrophotometer, excellent clinical accuracy was revealed. By measuring subjects' salivary glucose and blood glucose in parallel, they found the two generated profiles shared the same fluctuation trend but the correlation between them was individual dependent. There was a time lag between the peak glucose values from blood and from saliva. However,the correlation between the two glucose values at fasting was constant for each person enabling noninvasive diagnosis of diabetes through saliva instead of blood. A good correlation of glucose levels in saliva and in blood prior to and two hours after glucose intake was observed. Since this is the time period of glucose monitoring usually prescribed by doctors, they concluded that this disposable biosensor could be an alternative for real-time salivary glucose tracking at any time.

Anuradha Soni et al developed a smartphone based non invasive salivary biosensor.[24] Glucose biosensor was developed using saliva samples for diagnosis of diabetes.Glucose oxidase enzyme along with pH indicator was immoblilised on a paper strip to create this bio sensor.An in-house developed android app measured the colour changes of pH indicator upon reaction with glucose. In healthy and diabetic participants, clinical validation of the biosensor was done to correlate blood and salivary glucose levels. They concluded that use of smartphone enabled on-site determination of glucose levels could be done without involvement of any specialized instrument.

Yunqing Du et al developed an on chip disposable salivary glucose sensor for diabetic control.[25]As a solution for real-time glucose measurements using saliva for diabetic care, they developed an on-chip disposable glucose nano-biosensor through a layer-by-layer assembly process. In this study, a clinical study of 10 healthy subjects was carried out to assess the effectiveness of salivary glucose sensors in glycemic control.Findings revealed (1) the individual blood glucose/salivary glucose ratio at fasting was consistent for an entire year when the test subjects were in good health. (2) the individual salivary glucose levels correlated closely with blood glucose levels after meals; (3) the peaking time of blood glucose and salivary glucose was 15-30 minutes apart (4) the blood/salivary glucose ratio returned to a similar value as fasting, 2 hours after a meal. The results of the study indicated that salivary glucose itself could be used as means for reliable diabetes monitoring and a potential fluid for prognosis of future disease.

2. Conclusion

Non invasive diagnosis of diabetes through saliva is possible because of the constant correlation between blood and salivary glucose.Although individual variations exist in this correlation, there is a good correlation between glucose levels in saliva and in blood before and 2 h after glucose intake. This makes the use of salivary glucose biosensors for real-time tracking of glucose levels in saliva convenient, and can further be used for both diagnosis of diabetes and glucose monitoring.. The sensitivity can be optimized by adjusting the coating procedure for the working electrode and by precise automatic production. The sensors can be used as a lone stand alone device or can be incorporated into other devices like a smart phone.In effect, salivary glucose testing using advanced biosensors definitely holds a promising future and further research is warranted to develop a cheap, sensitive and reliable biosensor which would without doubt ameliorate the patients' compliance in self monitoring diabetes mellitus.

3. References

Pippitt K, Li M, Gurgle HE. Diabetes mellitus: screening and diagnosis. Am Fam Physician. 2016;93(2):103-9. PMID 26926406.

WHO. Global report on diabetes. Geneva: World Health Organization; 2019.

Madana Gopal V, Singaravel Chidambaranathan A, Balasubramanium M. Estimation of alpha amylase, cortisol, and pH level in saliva of patients wearing conventional and biofunctional Prosthetic System complete dentures: A parallel randomized clinical trial. J Prosthet Dent. 2022 Jul 13. doi: 10.1016/j.prosdent.2022.05.015, S0022-3913(22)00341-9. PMID 35842282.

Rajkumar et al. Estimation of serological and salivary biomarkers in patients with oral squamous cell carcinoma, premaliganat lesions and conditions. SRM Univ J Dent Sci. 2010;1(1):14-9..

Mitra Loka A. Comparision of salivary CXCL10 levels in smokers and non-smokers with Stage III / IV generalized periodontal disease. Int J Dent Oral Sci. 2021:4032-5. doi: 10.19070/2377-8075-21000823.

Ganesan A, Muthukrishnan A, Veeraraghavan V. Effectiveness of salivary glucose in diagnosing gestational diabetes mellitus. Contemp Clin Dent. 2021 Jul-Sep;12(3):294-300. doi: 10.4103/ccd.ccd_530_20, PMID 34759688.

Ganesan A, Muthukrishnan A, Veeraraghavan VP, Kumar NG. Effectiveness of Salivary Glucose as a Reliable Alternative in Diagnosis of Type 1 Diabetes Mellitus: A Cross-Sectional Study. J Pharm Bioallied Sci. 2022 Jul;14(Suppl 1):S557-S562. doi: 10.4103/jpbs.jpbs_666_21. PMID: 36110787; PMCID: PMC9469233.

Karthikeyan V, Chander NG, Anitha KV. A salivary sensor for the management of xerostomia in edentulous patients. J Prosthet Dent. 2019 Mar;121(3):384-386. doi: 10.1016/j.prosdent.2018.07.003. Epub 2018 Dec 13. PMID: 30554820.

Clark LC Jr., Lyons C. Electrode systems for continuousmonitoring in cardiovascular surgery. Ann N Y Acad Sci. 1962;102:29-45.

Yamaguchi M, Mitsumori M, Kano Y. Noninvasivelymeasuring blood glucose using saliva. IEEE Eng Med Biol Mag. 1998;17(3):59-63. doi: 10.1109/51.677170, PMID 9604702.

Priya Hari,Kannan A,Krithika L.Metagenome analysis of buccal mucosal biofilms to identify bacterial prevalence in subjects with and without Type 2 Diabetes.Int J Res Pharmaceutical Sciences.2018;11(2).2029.10.26452/ijrps.v11i2.2132

Ye D, Liang G, Li H, Luo J, Zhang S, Chen H et al. A novel nonenzymatic sensor based on CuO Nano needle/graphene/carbon nanofibermodified electrode for probing glucose in saliva. Talanta. 2013;116:223-30. doi: 10.1016/j.talanta.2013.04.008, PMID 24148397.

Du Y, Zhang W, Wang ML, On-Chip A. An On-Chip Disposable salivary glucose sensor for diabetes control. J Diabetes Sci Technol. 2016;10(6):1344-52. doi: 10.1177/1932296816642251, PMID 27053256.

Li Z, Chen Y, Xin Y, Zhang Z. Sensitive electrochemical nonenzymatic glucose sensing based on anodizedCuO nanowires on three-dimensional porous copper foam. Sci Rep. 2015;5:16115. doi: 10.1038/srep16115, PMID 26522446.

Wang J, Xu L, Lu Y, Sheng K, Liu W, Chen C et al. Engineered IrO2@NiOCore-Shell nanowires for sensitive non-enzymatic detection of trace glucose in saliva. Anal Chem. 2016;88(24):12346-53. doi: 10.1021/acs.analchem.6b03558, PMID 28193044.

Arakawa T, Kuroki Y, Nitta H, Chouhan P, Toma K, Sawada S-I; et al. Mouthguard biosensor with telemetry system for monitoring of saliva glucose: A novelcavitas sensor. Biosens Bioelectron. 2016;84:106-11. doi: 10.1016/j.bios.2015.12.014, PMID 26725934.

Soni A, Jha SK. Smartphone based non-invasive salivary glucose biosensor. Anal Chim Acta. 2017;996:54-63. Jundishapur Journal of Microbiology Research Article Published online 2022 April Vol. 15, No.1 (2022) 1616. doi: 10.1016/j.aca.2017.10.003, PMID 29137708.

Anderson K, Poulter B, Dudgeon J, Li S-E, Ma X, Highly A. Sensitive nonenzymatic glucose BiosensorBased on the regulatory effect of glucose on electrochemical behaviors of colloidal silver nanoparticles onMoS2. Sensors. 2017;17:1807.

Bell C, Nammari A, Uttamchandani P, Rai A, Shah P, Moore AL. Flexible electronicscompatiblenon-enzymatic glucose sensing via transparent CuO nanowire networks on PET films. Nanotechnology. 2017;28(24):245502. doi:

10.1088/1361-6528/aa7164, PMID 28475106**.**

Velmurugan M, Karikalan N, Chen SM. Synthesis and characterizations of biscuit-like copper oxidefor the non-enzymatic glucose sensor applications. J Colloid Interface Sci. 2017;493:349-55. doi: 10.1016/j.jcis.2017.01.044, PMID 28119246.

Lee MH, Thomas JL, Tseng HY, Lin WC, Liu BD, Lin HY. Sensing of digestive proteins in saliva with a molecularly imprinted poly(ethylene-co-vinyl alcohol) thin film coated quartz crystal microbalancesensor. ACS Appl Mater Interfaces. 2011;3(8):3064-71. doi: 10.1021/am2005724, PMID 21736294.

Du Y. 1, Wenjun Zhang and Ming L. Wang 1. Sensing of salivary glucose using nanostructured bio sensors. Biosensors.2016;6:10. doi: 10.3390/bios6010010.

Anuradha G,Arvind Muthukrishnan,VishnuPriya Veeraraghavan.Saliva as a Diagnostic fluid in Diabetes Mellitus-A Review.Drug Invention Today.2020

Soni A, Kumar Jha S. Smartphone based non-invasive salivary glucose biosensor. Anal Chim Acta. 2017;996:54.e63.

Du Y, Zhang W, Wang ML. An on-chip disposable salivary glucose sensor for diabetes control. J Diabetes Sci Technol. 2016;10(6):1344-52. doi: 10.1177/1932296816642251, PMID 27053256.