Supplementary Material

A Non-Invasive Device for Glucose Monitoring through Saliva– a Paradigm shift in Diabetes Care

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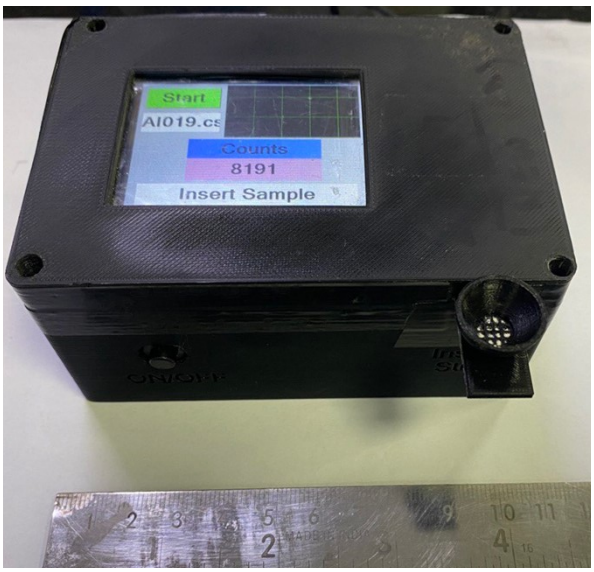
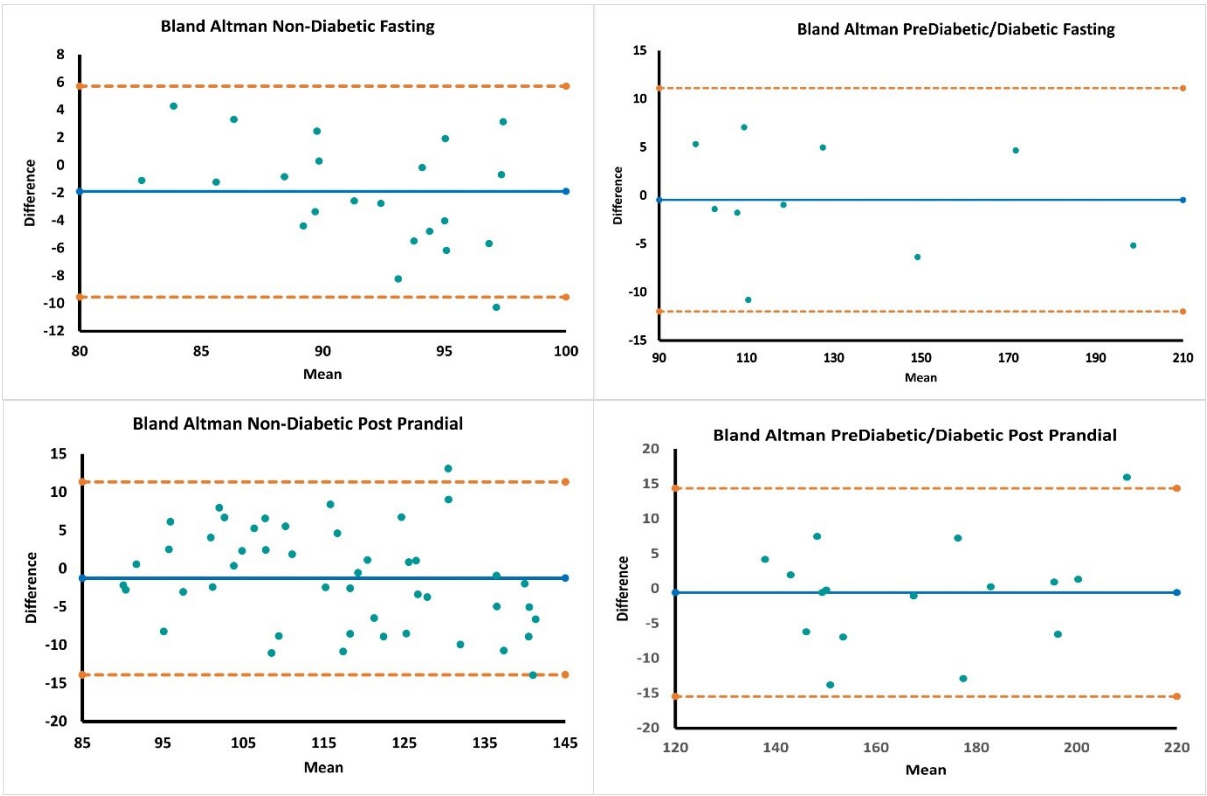
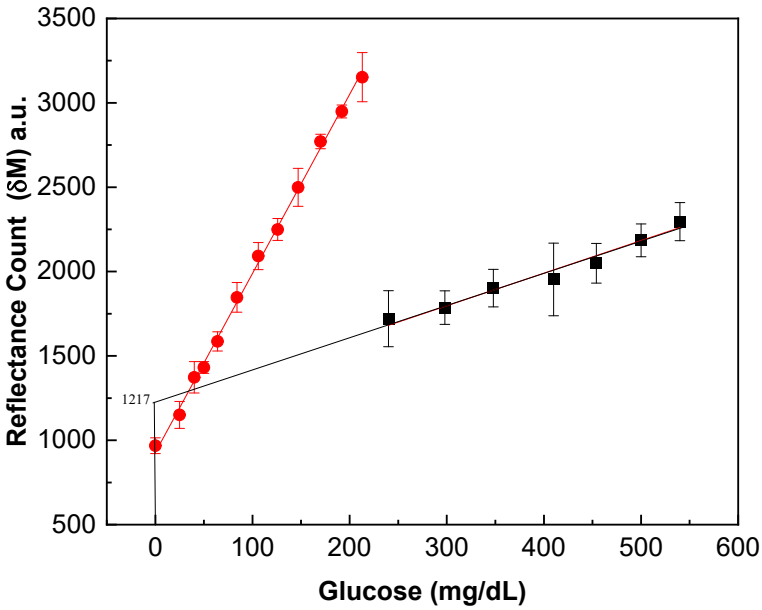


Supplement Fig. S1: The handheld salivary glucometer showing top view with touch screen interface and sensing strip inserted into light tight slot. This is a laboratory prototype and size can be reduced by upto 70% as many components may not be necessary when an ASIC is designed. At present, calibration, correlation and sample condition all had to be optimized and tested.

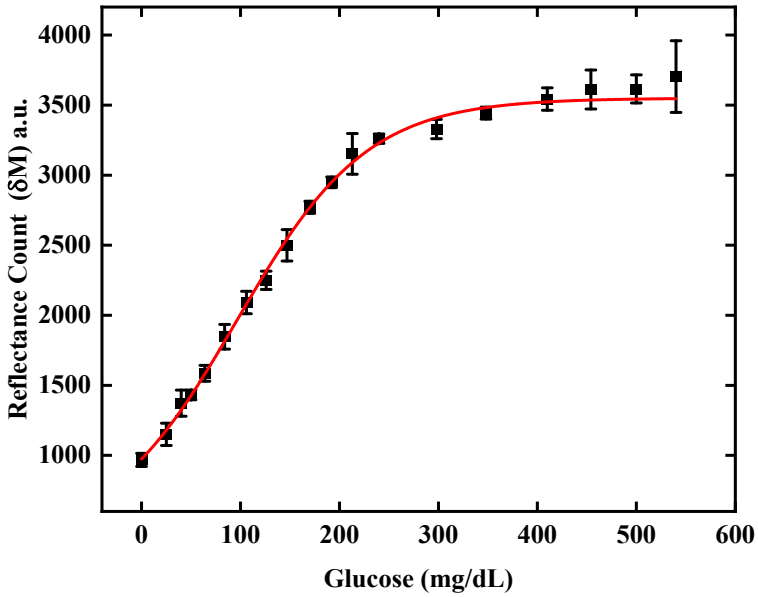
Supplement Fig. S2: Rear view of the strip showing color change with respect to different saliva samples.



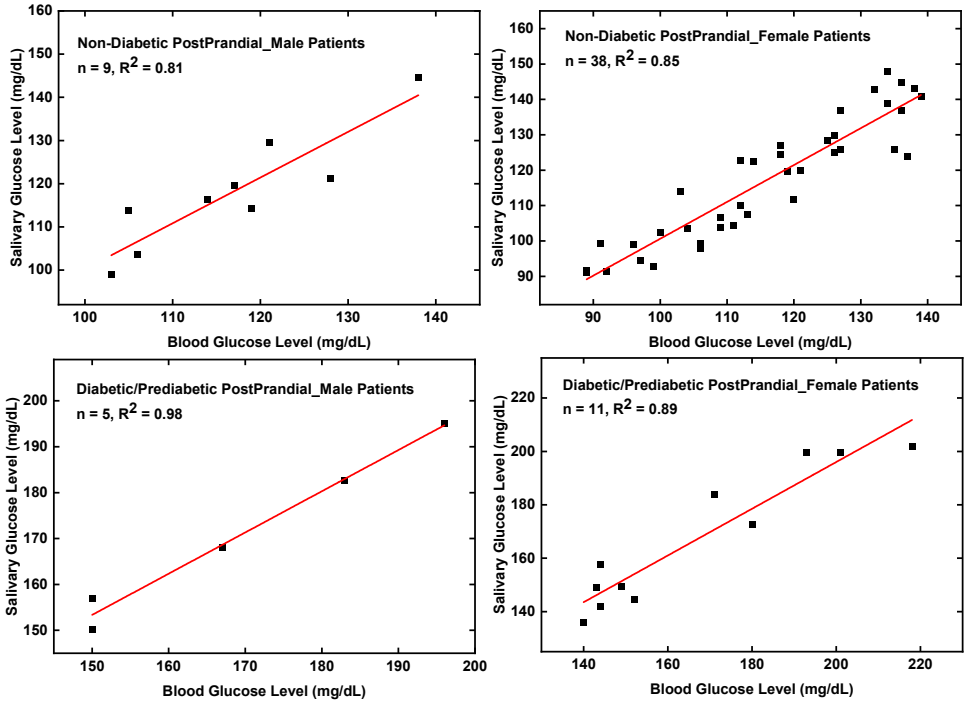
Supplement Fig S3: Bland-Altman analysis for non-diabetic and prediabetic/diabetic fasting groups show most data points within the limits of agreement (dashed orange lines). The mean difference (solid blue line) is near zero, indicating strong agreement between the developed device and Accu-Chek Active.



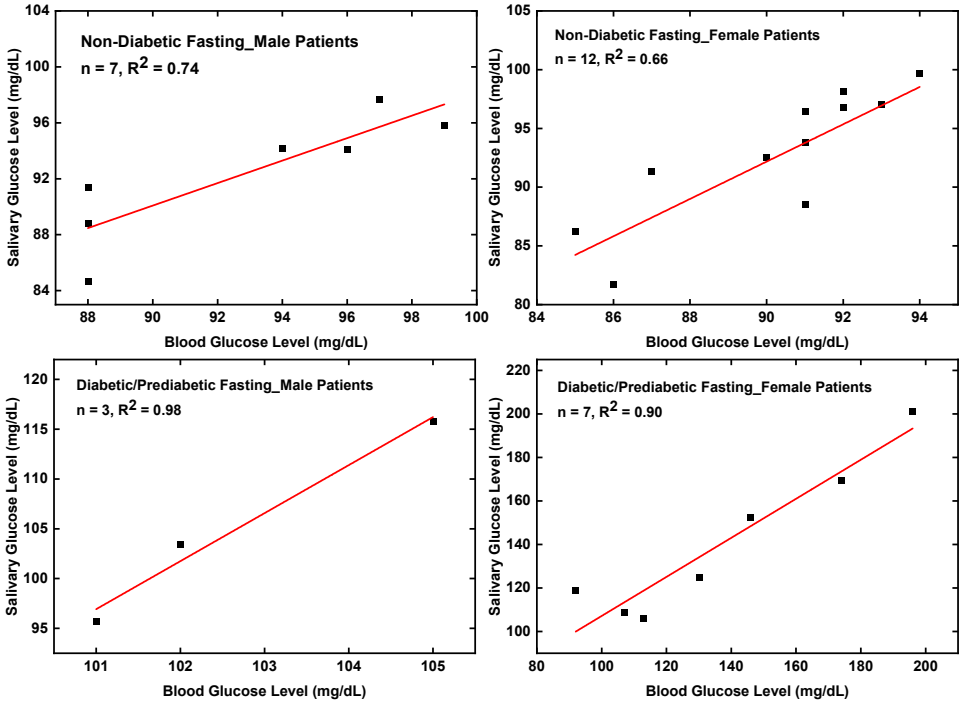
Supplement Fig. S4: Calibration curve of biosensor using 10 U enzyme loading (-●-) versus 2.5 unit enzyme loading (-■-) for each enzyme on the strip. An enzyme loading of 2.5 U or 5 U/strip for both GOx and POx resulted in higher LOQ and LOD than 10 U enzyme loading. Besides, accuracy of sensor was less than approx 50% in case of 2.5 U enzyme loading and average 70% in case of 5 U enzyme loading per strip for both enzyes. This observation can be attributed to slower enzyme kinetics with lower enzyme loading and primarily the diffusion of dye and H_2O_2 onto the cellulose infill leading to lesser amounts of color forming biproduct on strip surface which was detected under reflectance mode. A possible solution could be to enhance the RGB sensor sensitivity or reduce the thickness of infil medium to allow transmittance mode of detection.



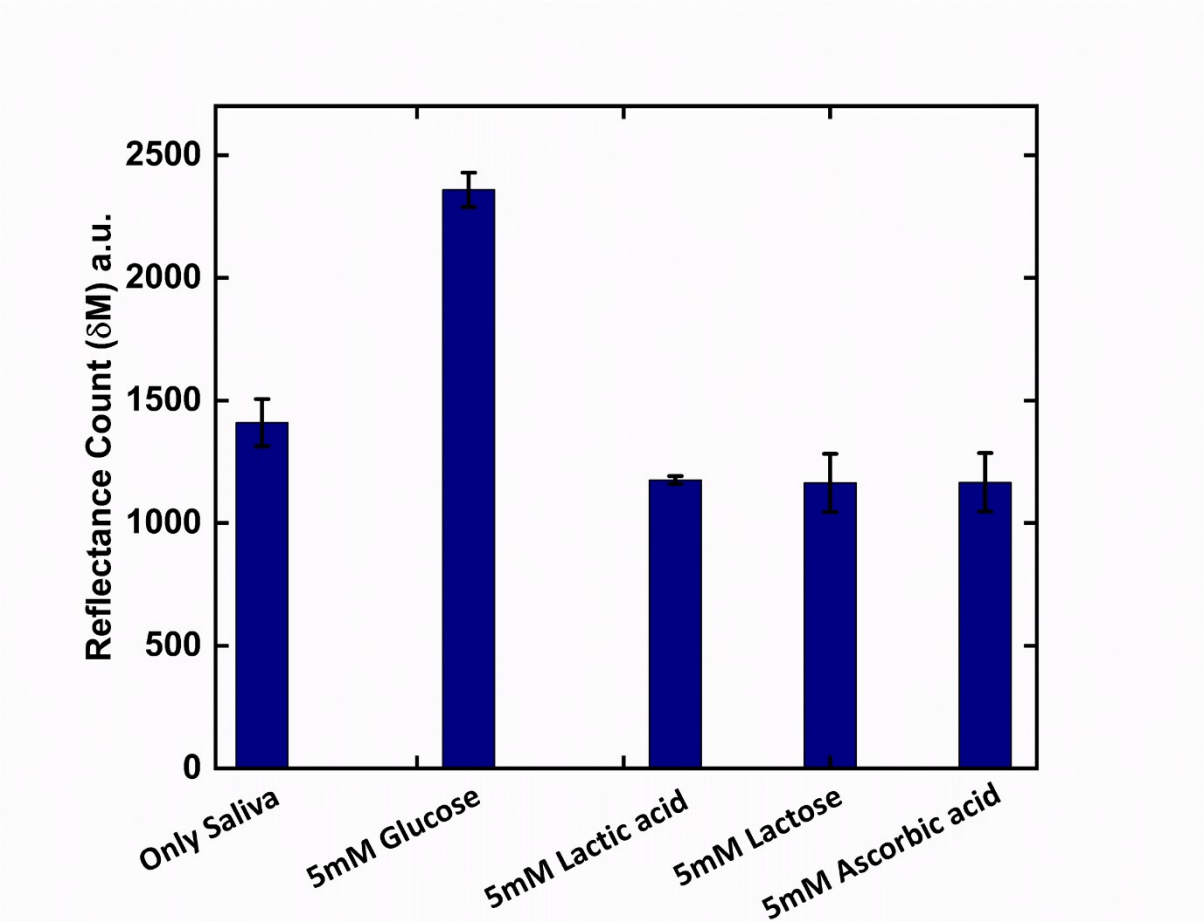
Supplement Fig. S5: Full dynamic range of sensor calibration (extension to Fig. 4) where detection saturation was observed beyond 213 mg/dL glucose concentration. This indicates that either we can make the sensor more accurate at lower concentration ranges or sacrifice LOD to cover wider range of detection. However, for an uncontrolled diabetic patient, it is sudden hypoglycemia that is cause of worry than any persistent BGL equivalent level beyond 213 mg/dL. This version of sensor, hence would not be suitable to adjust insulin dosase but can be a rapid pain free indicator of glycemic levels throughout the day.



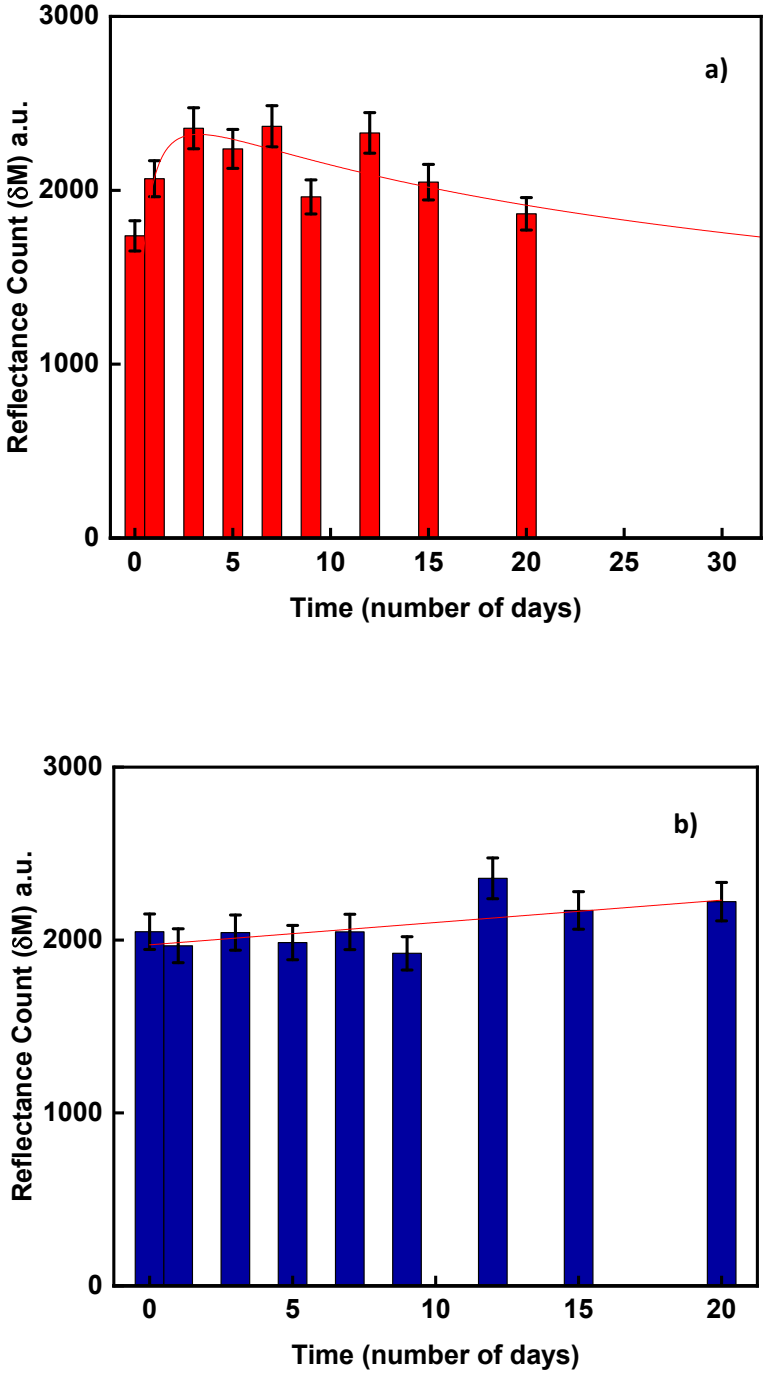
Supplement Fig. S6: Statistical correlation analysis between the SGL and BGL in clinical samples of (A-B) non-diabetic and (C-D) diabetic post prandial subjects, under gender wise distribution of male and female conditions.



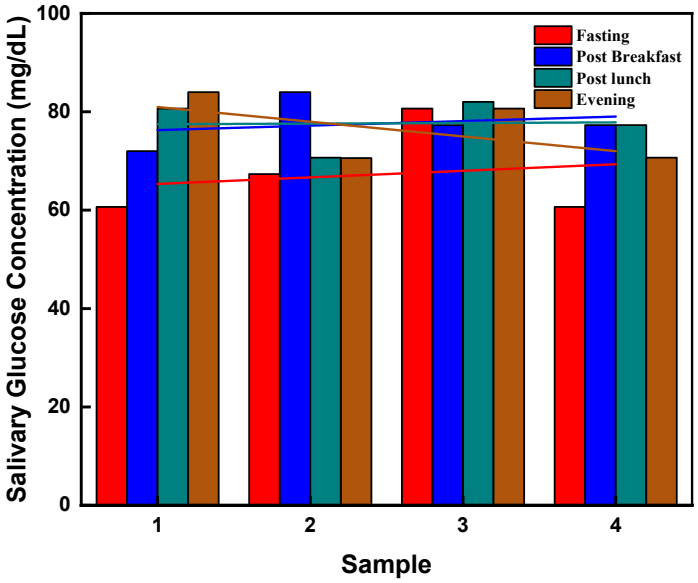
Supplement Fig. S7: Statistical correlation analysis between the SGL and BGL in clinical samples of (A-B) non-diabetic and (C-D) diabetic fasting subjects, under gender wise distribution of male and female conditions.



Supplement Fig. S8: -Interferent study with different interferents at 5mM concentrations along with same spiked salivary glucose concentration of 54 mg/dL, showing no interference from the interferents, as signal was lower than the LOD.



Supplement Fig. S9: The shelf of the glucose strips for 20 days: a) without and b) with 1 mM β-Mercaptoethanol (β-ME) added along with enzyme solution during strips preparation. Analyte used was spiked saliva sample with final 100 mg/dL glucose concentration. The sensor output was completely stable with β-ME and can be extrapolated beyond several months with same reproducibility level.



Supplement Fig. S10: - Reproducibility of the biosensor was estimated with for four different samples at four different time intervals. The biosensor was highly accurate throughout the day, especially with fasting, post-breakfast, and post-lunch samples, but slight variability was found in evening random samples, but it was still within the tolerance level of ±10% on these handmade or lab-made strips. Which, otherwise, was minimum ±5.68% while maintaining a quality control level of ±5% variation between the strips without adding any sample.

S. No.	Biosensors	Detection range	Limit of Detection (LOD)	Sensitivity	Response time	Reference
1.	Paper-based wearable sensors	0 to 2.0 mmol L^{-1}	27 µmol L^{-1}	50.29 AU/mmolL^{-1}	11 minutes	[1]
2.	Paper-based sensor using bienzymatic reaction	0 mgdL^{-1} to 22.5 mgdL^{-1}	0.84 mgdL^{-1}	1.81 AU/mg	N/A †	[2]
3.	CuO nanoneedle/graphene/carbon nanofiber modified glassy carbon electrode	(1 µM to 5.3 mM	912.7 A·mM^{-1}·cm^{-2}	N/A	<2s	[3]
4.	Paper-based sensor and smartphone RGB analysis	50 – 540 mg/dL	24.6 mg/dL	0.0012 pixels s^{-1}/mg·dL^{-1}	20 sec	[4]
5.	Spectrophotometric detection	0 to 18 mg/dL	0.17 mg/dL	N/A	N/A	[5]
6.	pH-based glucometer	32 – 516 mg/dL	32 mg/dL	1.0 sensor count/mgdL^{-1}	15 sec (total time 80 sec)	[6]
7.	Electrochemical sensor using anodized cupric oxide nanowires	1.0µM to 18.8mM	0.3/µM	2217.4/µA·cm^{-2} mM^{-1}	N/A	[7]
8.	Core-shell IrO_2@NiO nanowires	0.5µM to 2.5 mM	0.31 µM	1539.0 µA·mM^{-1} ·cm^{-2}	N/A	[8]
9.	Screen-printed sensor chip	0.5-20 mg/dL	0.41 mg/dL	N/A	N/A	[9]
10.	Mouthguard glucose sensor	5-1000 µmol/L	5 mmol/L	N/A	N/A	[10]
12	4-AAP + Phenol dye-based sensor	14.5 - 213 mg/dL	14.5 mg/dL	10.6 sensor/count/mgdL^{-1}	2.5 secs	Present Work

Supplement Table 1: Comparison of sensors for the saliva-based detection of glucose.