Molecular Recognition of VSIG1 in Biological Samples for Fast Diagnosis of Gastric Cancer

Journal of The Electrochemical Society, 2024 **171** 077516
1945-7111/2024/171(7)/077516/7/$40.00 © 2024 The Electrochemical Society ("ECS"). Published on behalf of ECS by IOP Publishing Limited

Molecular Recognition of VSIG1 in Biological Samples for Fast Diagnosis of Gastric Cancer

Raluca-Ioana Stefan-van Staden,[1,2,z] Damaris-Cristina Gheorghe,[1,2] and Ruxandra-Maria Ilie-Mihai[1]

[1]*Laboratory of Electrochemistry and PATLAB, National Institute of Research for Electrochemistry and Condensed Matter, 060021 Bucharest-6, Romania*
[2]*Faculty of Chemical Engineering and Biotechnologies, National University of Science and Technology POLITEHNICA Bucharest, Romania*

VSIG1 is a new biomarker member of the JAM family relevant in gastric cancer diagnostics. Due to its detection and quantification impact for fast and early diagnosis of gastric cancer, two types of intelligent miniplatforms based on stochastic sensors as detection tools, were designed and validated using real samples. A 3D stochastic microsensor based on Nitrogen and Sulfur dopped graphene paste modified with calix[4]arene-25,26,27,28-tetrol, and a 2D disposable screen-printed stochastic sensor based on thin film gold modified with calix[4]arene-25,26,27,28-tetrol were constructed and inserted as working sensors into the miniplatforms. The proposed intelligent miniplatforms shown sensitivities as high as 1.12×10^{10} $s^{-1}g^{-1}$ml, limits of determination of 1×10^{-23}g ml^{-1}, and working concentration ranges between 1×10^{-23} and 1×10^{-8} g ml^{-1}. Recoveries higher than 99.30% with % RSD values lower than 0.05% were obtained when used for screening test of biological samples, for VSIG1.
 [DOI: 10.1149/1945-7111/ad6508]

Manuscript submitted June 17, 2024; revised manuscript received July 6, 2024. Published July 26, 2024.

The glycoprotein A34 (GpA34), known as VSIG1, was first discovered and described by Scanlan et al. in their work.[1–6] The initial findings on the expression of VSIG1 in normal human tissues indicated a significant degree of tissue specificity, with notable upregulation of both VSIG1 mRNA and protein in the stomach.[1] The only method available to date for the determination of VSIG1 is ELISA; this is also the standard method used in clinical laboratories for its determination. While ELISA is quite expensive and needs a high processing of the sample, it cannot be used as mass screening method for the population prone to develop gastric cancer. Therefore there is a high need for tools and screening methods able to be used for its detection and quantification in biological samples.

Stochastic sensors are good candidates as tools for biomedical analysis:[7–10] no sampling is needed for the biological sample; extremely small concentrations (attog/ml and lower) may be determined from very complex matrices; the composition of the matrix does not influence in any way the results of the analysis; a molecular recognition of the biomarkers is possible, based on their signatures; quantitative analysis is highly reliable, and the matrix did not influenced it. Therefore, two types of stochastic sensors were chosen as recognition and analysis tools for the molecular recognition and quantification of VSIG1 in biological samples such as: whole blood, urine, saliva, and tumoral tissues. The novelty of the work is first the utilization of electroanalysis for the qualitative and quantitative assay of VSIG in biological samples (as only ELISA is used to date for its assay in clinical laboratories), and besides the design of the stochastic sensors is their incorporation in a versatile intelligent miniplatform, able to accommodate both 3D and 2D types of sensors. While for the design of the 3D microsensor, a Nitrogen and Sulfur dopped graphene matrix was used, for the disposable 2D sensor, a thin film gold based matrix was used. The calix[4]arene-25,26,27,28-tetrol (C4-tetrol) was used as modifier. Both matrices made of graphene and gold are able to keep in a good shape the channels of the C4-tetrol. Calixarenes are a class of cyclic compounds with cavities whose phenol units are connected by alkylidene groups.[11] They are amphiphilic cyclic nanostructures with a basket-like shape, able to accommodate molecules like VSIG1 in order to produce the stochastic signal. The proposed screening tools will help the early diagnosis of cancer, which is a disease that can only be cured if diagnosed at a very early stage.[12–19]

[z]E-mail: ralucavanstaden@gmail.com

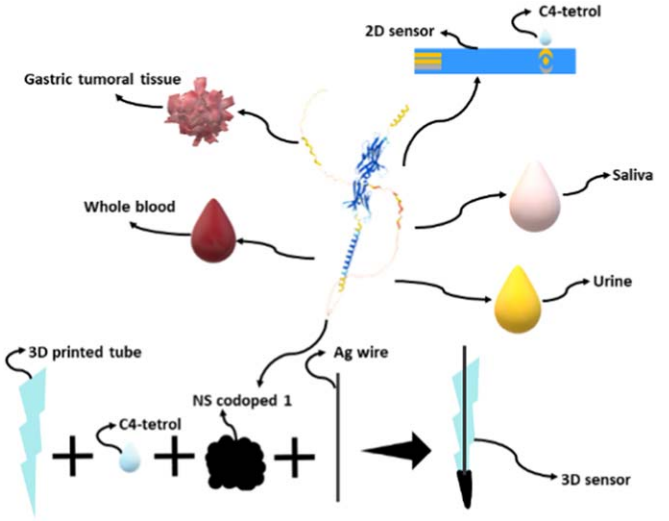
Experimental

Materials and reagents.—All chemicals were of analytical grade. VSIG1, C4-tetrol and the silver wire were purchased from Sigma Aldrich. Deionized water, obtained from a Millipore Direct-Q 3 System was used for the preparation of the VSIG1 solutions, with different concentrations (4.7×10^{-5} g ml^{-1} to 4.7×10^{-23} g ml^{-1}) using the serial dilution method. All VSIG1 solutions were prepared in phosphate buffer solution (0.1 mol l^{-1}, pH 7.4). When not in use, the solutions were kept at a temperature of −20 °C. The screen-printed electrode was purchased from Metrohm, DropSens. Nitrogen (3.61%) and Sulfur (14.57%) codoped graphene (NS-Gr) was provided by the National Institute of Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania.

Apparatus and methods.—To record all measurements, the intelligent miniplatform was connected to a miniaturized potentiostat, which was linked to a smartphone via Bluetooth, using the PSTrace software (Version 5.9). The intelligent miniplatform comprises: the 3D/2D stochastic sensors, a Pt wire (auxiliary electrode), and an Ag/AgCl wire (as reference electrode). The 2D disposable screen-printed gold electrode was obtained from Metrohm DropSens. The 2D disposable screen-printed thin film gold electrode setup comprises of a working electrode, which is made out of gold and subsequently modified with C4-tetrol, an auxiliary electrode, additionally made from gold, and a reference electrode, which is comprised of silver.

Design of the 3D printed C4-tetrol/NS-Gr stochastic microsensor.—To obtain a homogeneous paste, 0.01 g of NS-Gr powder was mixed with paraffin oil. 50 μl of C4-tetrol were added to obtain the C4-tetrol/NS-Gr paste. Subsequently, the paste was transferred into a 3D printed tube with an internal diameter of 150 μm, that exhibited non-conducting properties (Scheme 1). The contact with the external circuit was made possible by inserting an Ag wire in the modified paste.

Design of the 2D C4-tetrol/gold disposable stochastic sensor.—The surface of the stochastic screen-printed electrode and the surface of the auxiliary electrode are based on gold thin films, while the surface of the reference electrode is based on silver. The diameter of the ceramic substrate was 3.4 × 1.0 × 0.05 cm. The active surface of the stochastic screen-printed electrode was modified using a solution of C4-tetrol; 10 μl solution of C4-tetrol (10^{-3} mol l^{-1}) were dropped on the active surface, and left for a period of seven days at room



Scheme 1. Design of the 2D and 3D stochastic sensors.

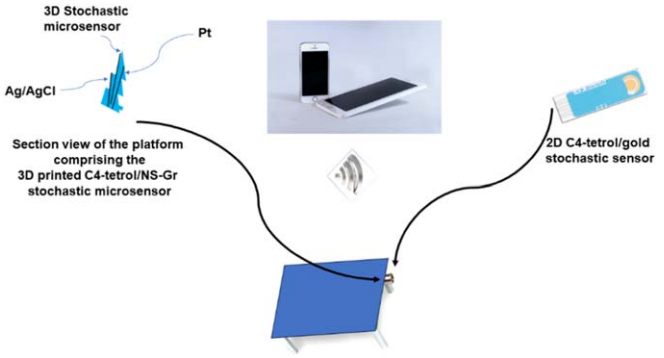
temperature, away from light, to dry (Scheme 1). Before and after each measurement, the sensor was cleaned with deionized water. When not in use, the 2D C4-tetrol/gold disposable stochastic sensor was kept at room temperature, in a dry place.

Design of the intelligent platforms.—The designed 2D/3D sensors were inserted into the intelligent platforms (Scheme 2) able to perform data acquisition and processing and data submission to portable instrumentation such as smartphone.

The system can also be used to submit the results to the medical doctors and to central acquisition data bases used for monitoring the cancer patients.

Stochastic mode.—The stochastic mode was used in order to perform all measurements. The principle of the stochastic sensors is based on the channel conductivity, when a constant potential of 350 mV vs Ag/AgCl (the value of the potential was optimized to get signatures, t_{off} values able to be reliable read; the optimization was done by applying potentials from 1 mV to 500 mV, and reading the diagrams; the optimum value for which the reading were done reliable was 350 mV vs Ag/AgCl) was applied and the current was recorded, as it can be seen in Figs. 1 and 2. The diagrams were utilized to identify the signature (t_{off} value) of VSIG1, which functioned as the recognition element for the biomarkers. The t_{on} value was used for all quantitative analyses. A calibration process was conducted on the two stochastic sensors utilizing a range of concentrations from a set of solutions of VSIG1. The calibration equation for VSIG1 was established through the utilization of the two stochastic sensors. The equation was derived by determining the t_{on} value, which was read between two consecutive t_{off} values. The parameters a and b were then obtained from the calibration equation $1/t_{on} = a + b \times Conc_{VSIG1}$ using the linear regression method. The biomarker was identified through its signature (t_{off} value) in the screening of whole blood, urine, saliva, and tumoral tissue, as depicted in Figs. 1 and 2. The t_{on} value was subsequently utilized in the calibration equation to determine the concentration of VSIG1 in the aforementioned biological fluids and tissue.

Samples.—A total of 80 samples comprising of whole blood, tumoral tissue, saliva, and urine were obtained from patients who were diagnosed with gastric cancers. The samples were utilized for measurements without undergoing any form of pretreatment prior to analysis. At the time of sample collection, none of the patients were receiving any form of cancer treatment. The samples were procured from the Emergency Clinical Hospital of County Targu-Mures and the Clinical Hospital County Targu-Mures, both of which were authorized by the Ethics Committee under the respective reference numbers 32647/14.12.2018 and 3206/28.02.2019 to carry out the investigation. All patients provided informed consent.



Scheme 2. Insertion of the 2D/3D sensors into an intelligent platform able to submit the signal via wireless to portable phone/tablets.

Results and Discussion

Response characteristics of the intelligent miniplatforms based on 3D printed C4-tetrol/NS-Gr stochastic microsensor and on 2D C4-tetrol/gold disposable stochastic sensor.—The application of a constant potential of 350 mV results in a modification of the current flowing through a channel upon the entry of VSIG1 through said channel and subsequent binding with the channel wall. The ongoing progress occurs in two distinct phases. The initial phase, referred to as the molecular recognition phase, involves the entry of VSIG1 into the channel, resulting in its blockage. This event causes a reduction in the current intensity to approximately 0 value for a specific duration, which is recognized as the signature of VSIG1. The signature is subsequently marked on the diagram as "t_{off}" in Figs. 1 and 2. The qualitative analysis of VSIG1 employs the value of the signature. The subsequent stage is commonly referred to as the reaction phase, as a consequence of the interaction between VSIG1 and the wall channel, which occurs during the equilibrium equation presented below:

$$Ch_{(i)} + VSIG1_{(i)} \Leftrightarrow Ch{\cdot}VSIG1_{(i)}$$

where *Ch* denotes the channel, whereas *i* denotes the interface [11, 12]. Additionally, redox reactions occur. The quantitative parameter, more commonly referred to as t_{on}, denotes the duration required for the reaction phase to reach equilibrium. The concentration of VSIG1 is dependent onto the value of this parameter.

Table I displays the response characteristics of the intelligent miniplatforms based on 3D printed C4-tetrol/NS-Gr stochastic microsensor and on 2D C4-tetrol/gold disposable stochastic sensor. The results indicate that both sensors exhibit distinct signature values (t_{off} values), making them suitable for the detection of VSIG1. The intelligent miniplatforms based on 3D printed C4-tetrol/NS-Gr stochastic microsensor exhibited a significantly higher sensitivity in comparison to the intelligent miniplatforms based on 2D C4-tetrol/gold disposable stochastic sensor. The two miniplatforms exhibited a broad working concentration range, up to 1×10^{-23} g ml^{-1}.

The miniplatforms underwent reproducibility and stability studies. A total of ten miniplatforms of each type were designed as described above. Sensitivity measurements were conducted for each of the miniplatforms designed; a %, RSD value of 0.07% of the sensitivity was obtained for the miniplatforms based on 3D printed C4-tetrol/NS-Gr stochastic microsensor, while a %, RSD value of 0.04% was obtained for the miniplatforms based on 2D C4-tetrol/gold disposable stochastic sensor. These values proved the reproducibility of the design of the miniplatforms based on 3D printed

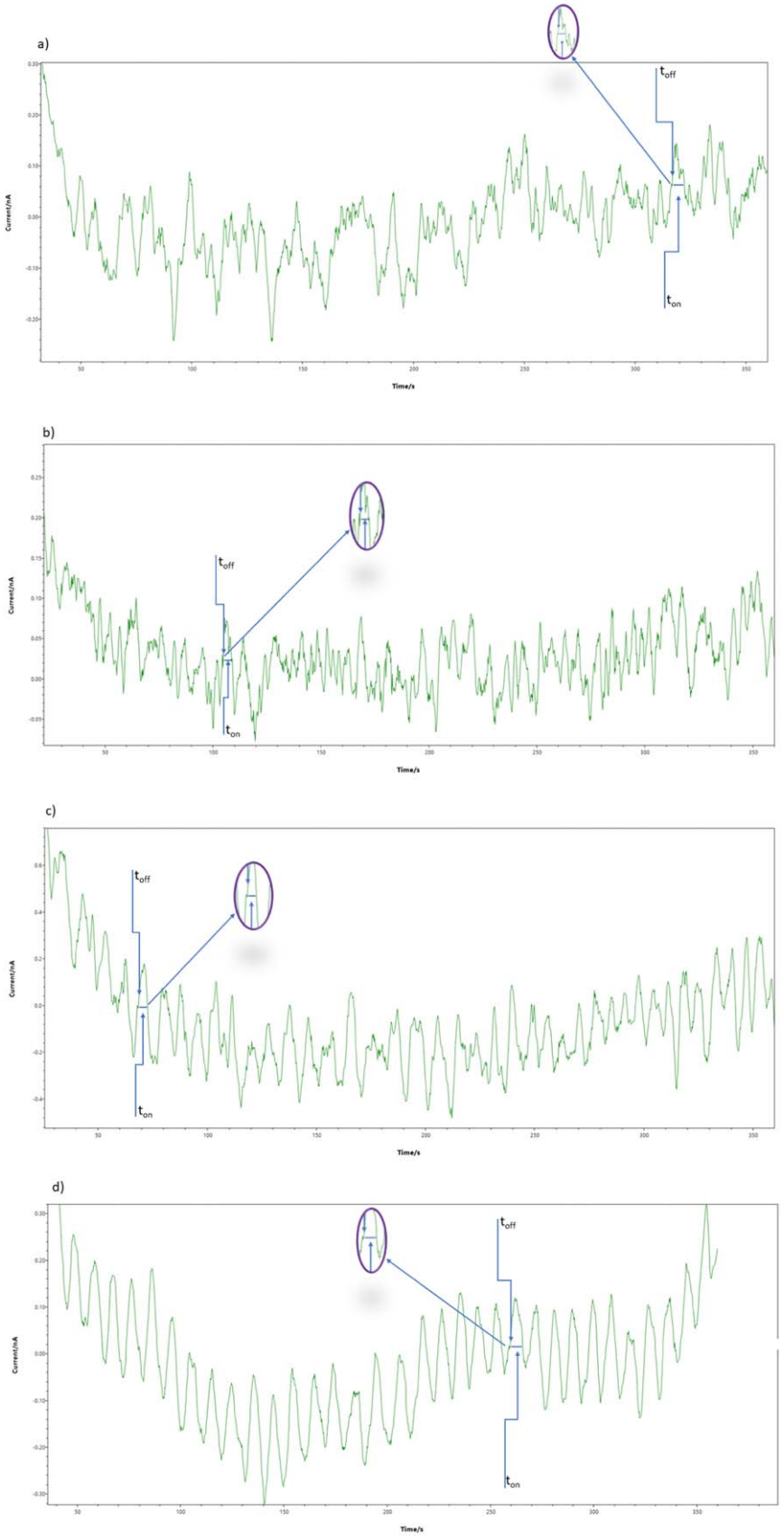


Figure 1. Diagrams obtained by screening (a) saliva, (b), whole blood (c) tumoral tissue, and (d) urine with the miniplatforms based on 2D C4-tetrol/gold disposable stochastic sensor.

C4-tetrol/NS-Gr stochastic microsensor and on 2D C4-tetrol/gold disposable stochastic sensor. In order to determine the stability of the designed miniplatforms based on 3D printed C4-tetrol/NS-Gr stochastic microsensor and on 2D C4-tetrol/gold disposable stochastic sensor for 3 months, measurements of their sensitivities were made during this period of time every day; a %, RSD value of 0.10% of the sensitivity was obtained for the miniplatforms based on 3D printed C4-tetrol/NS-Gr stochastic microsensor, while a %, RSD

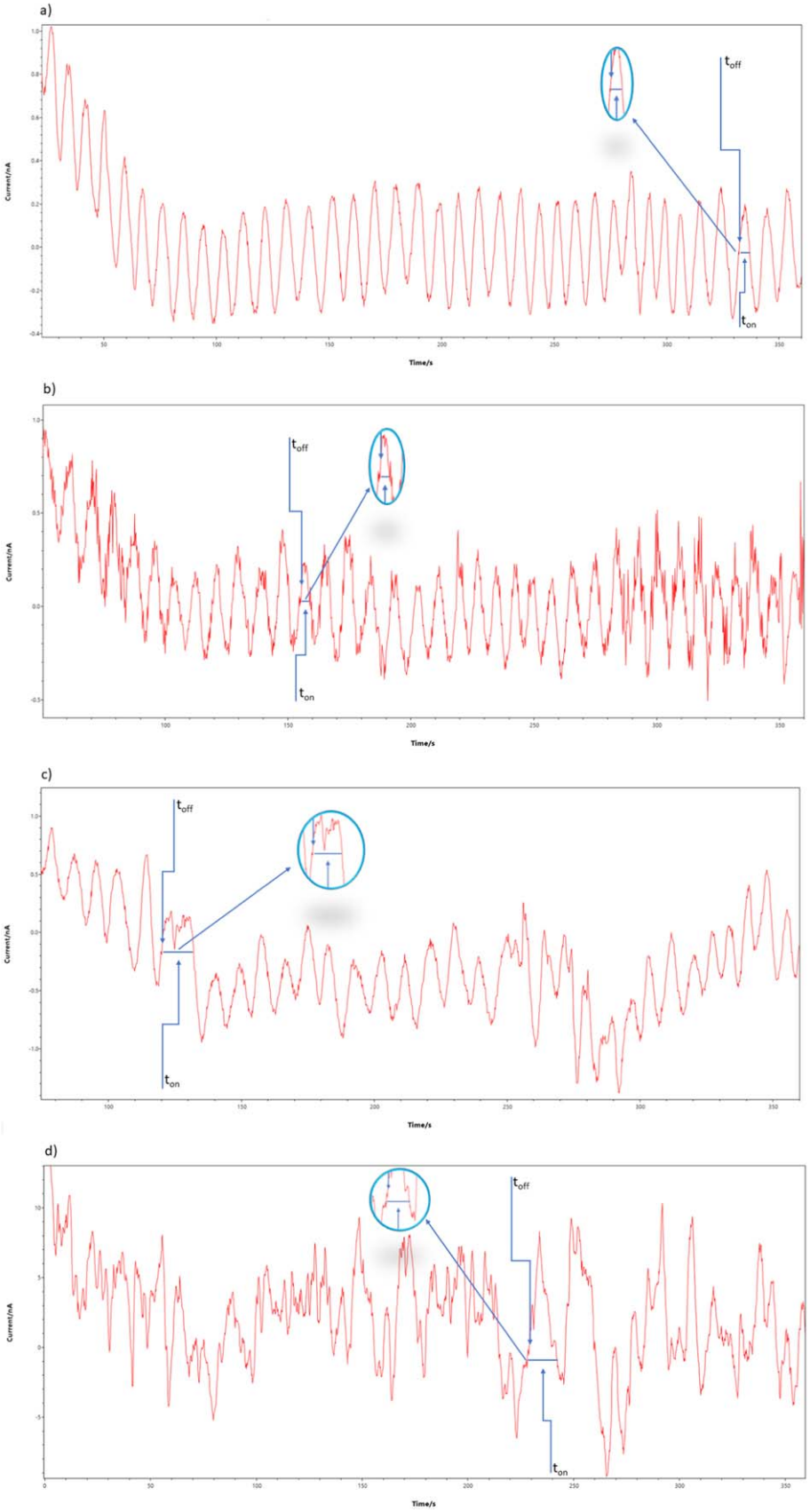


Figure 2. Diagrams obtained by screening (a) saliva, (b), whole blood (c) tumoral tissue, and (d) urine with the miniplatform based on 3D printed C4-tetrol/NS-Gr stochastic microsensor.

value of 0.15% was obtained for the miniplatforms based on 2D C4-tetrol/gold disposable stochastic sensor, proving the reliability of the sensitivity of the proposed miniplatforms during 3 months period of time.

Selectivity of the miniplatforms designed for the assay of VSIG1 was performed vs other biomarkers such as: IL-2. IDH-1. HRG-α, CEA, and KRAS. The signatures obtained for these biomarkers as well as for VSIG1 are shown in Table II.

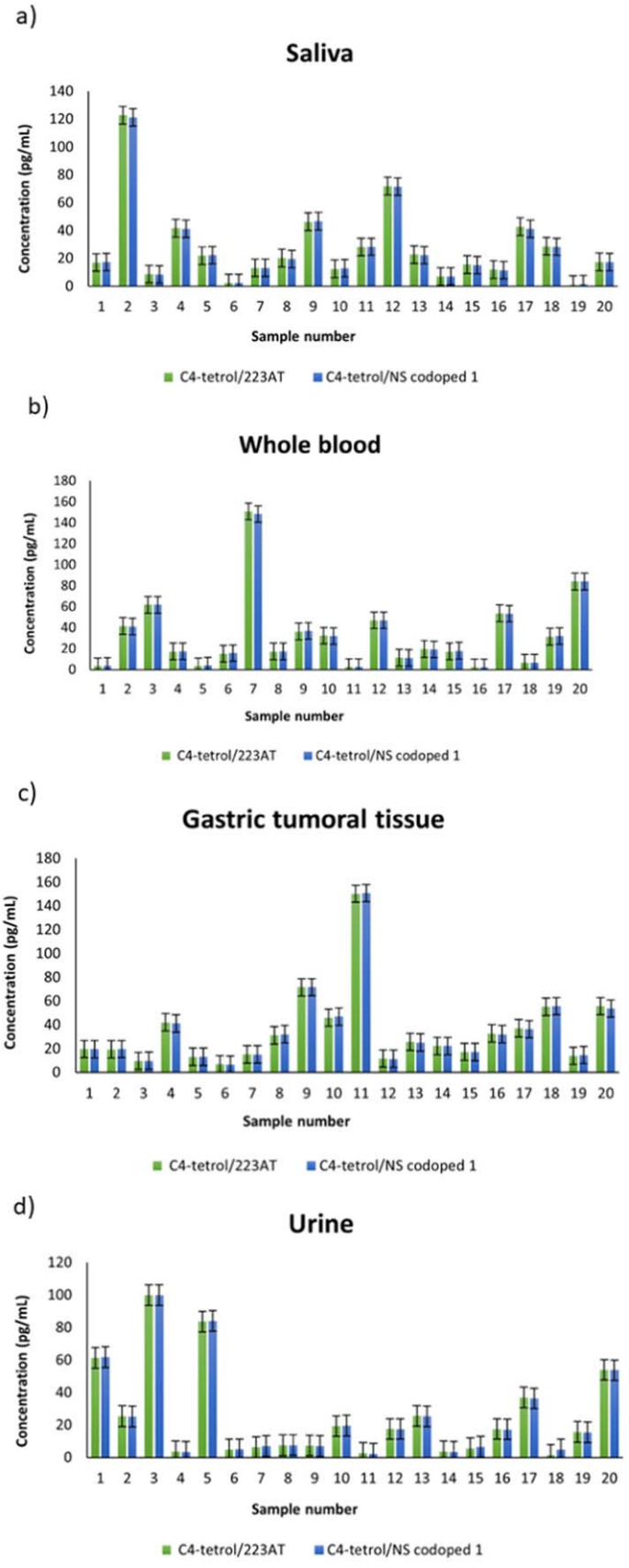


Figure 3. Determination of VSIG1 in (a) saliva, (b) whole blood, (c) gastric tumoral tissue and (d) urine using the miniplatforms based on 3D printed C4-tetrol/NS-Gr stochastic microsensor and on 2D C4-tetrol/gold disposable stochastic sensor.

The determining factor for the selectivity of stochastic sensors is the signature of the biomarker of interest, specifically in our case, VSIG1. There are differences between the signature of VSIG1 and of the other biomarkers proving that the proposed miniplatforms are selective.

Molecular recognition and quantification of VSIG1 in biological samples.—80 samples of whole blood, saliva, urine, and tumoral tissues obtained from patients who were clinically diagnosed with gastric cancer were screened for VSIG1. Molecular

Table I. The response characteristics of the intelligent miniplatforms based on 3D printed C4-tetrol/NS-Gr stochastic microsensor and on 2D C4-tetrol/gold disposable stochastic sensor used for the assay of VSIG1.

Intelligent miniplatforms based on	Linear concentration range (g mL^{-1})	Calibration equation and correlation coefficient (r)[a)]	t_{off} (s)	Sensitivity (s^{-1} g^{-1} ml)	LOQ (g ml^{-1})
2D C4-tetrol/gold disposable stochastic sensor	1×10^{-23}–1×10^{-8}	$1/t_{on} = 0.19 + 9.21 \times 10^{7}C$; r = 0.9996	0.8 ± 0.1	9.21×10^{7}	1×10^{-23}
3D printed C4-tetrol/NS-Gr stochastic microsensor	1×10^{-23}–1×10^{-9}	$1/t_{on} = 0.31 + 1.12 \times 10^{10}C$; r = 0.9994	1.1 ± 0.1	1.12×10^{10}	1×10^{-23}

a) $\langle C \rangle$ = g ml^{-1}; $\langle t_{on} \rangle$ = s

Table II. The selectivity of the intelligent miniplatforms based on 3D printed C4-tetrol/NS-Gr stochastic microsensor and on 2D C4-tetrol/gold disposable stochastic sensor used for the assay of VSIG1.

	Signature, t_{off} (s)					
Intelligent miniplatforms based on	VSIG1	IL-2	IDH-1	HRG-α	CEA	KRAS
2D C4-tetrol/gold disposable stochastic sensor	0.8 ± 0.1	1.2 ± 0.1	1.8 ± 0.1	2.3 ± 0.2	0.2 ± 0.1	3.0 ± 0.1
3D printed C4-tetrol/NS-Gr stochastic microsensor	1.1 ± 0.1	1.6 ± 0.1	2.0 ± 0.1	3.2 ± 0.1	0.5 ± 0.1	2.8 ± 0.2

Table III. Recovery tests of VSIG1 in biological samples using the intelligent miniplatforms based on 3D printed C4-tetrol/NS-Gr stochastic microsensor and on 2D C4-tetrol/gold disposable stochastic sensor (N = 10).

	%, Recovery, VSIG1	
Biological sample	Intelligent miniplatforms based on 2D C4-tetrol/gold disposable stochastic sensor	Intelligent miniplatforms based on 3D printed C4-tetrol/NS-Gr stochastic microsensor
Whole blood	99.38 ± 0.02	99.96 ± 0.01
Gastric tumoral tissue	99.95 ± 0.01	99.47 ± 0.03
Saliva	99.87 ± 0.02	99.50 ± 0.02
Urine	99.93 ± 0.04	99.90 ± 0.03

screening was performed, taking into account that this type of sensors allow the molecular recognition of VSIG, based on its signature. Quantification of VSIG1 was performed accordingly with the description of stochastic mode shown above, by reading in the diagrams recorded (Figs. 1 and 2) the t_{on} values associated with VSIG signature in between two t_{off} (signature) values. Examples of diagrams obtained for the molecular screening tests are given in Figs. 1 and 2.

There is a good correlation between the concentrations determined using the miniplatforms based on 3D printed C4-tetrol/NS-Gr stochastic microsensor and on 2D C4-tetrol/gold disposable stochastic sensor (Fig. 3), when used for the quantification of VSIG1 in whole blood, saliva, urine, and tumoral tissue samples.

A statistical analysis was conducted on VSIG1 using the paired Student t-test with at a confidence level of 99.00%. The calculated t-test values were found to be below 3.05, which is lower than the tabulated value of 4.03 at the confidence level of 99.00%. The obtained results of 2.97 for whole blood, 3.01 for gastric tumoral tissue, 2.48 for saliva, and 2.90 for urine indicate that there is no statistically significant difference between the outcomes yielded by the two miniplatforms based on 3D printed C4-tetrol/NS-Gr stochastic microsensor and on 2D C4-tetrol/gold disposable stochastic sensor.

Furthermore, the validation process was carried out utilizing the conventional addition method approach, which involved the addition of established amounts of VSIG1 to every biological sample category, namely whole blood, tumoral tissue, urine, and saliva. Table III displays the recovery tests obtained for each type of sample.

The identification of VSIG1 from diverse biological materials yielded remarkably elevated recovery rates, with very low RSD values. The presented data indicate that the proposed stochastic sensors have the potential to effectively molecularly recognize and quantify VSIG1 in biological samples with a high degree of accuracy and reliability.

Conclusions

The miniplatforms designed based on 3D printed C4-tetrol/NS-Gr stochastic microsensor and on 2D C4-tetrol/gold disposable stochastic sensor have potential applications in surgical procedures for conducting molecular screening tests on tumoral tissues. They are capable of providing both qualitative and quantitative data on VSIG1, which can be of great value for medical practitioners in making prompt decisions regarding the surgery, personalized treatment, and the patient's health status.

Acknowledgments

This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS/CCCDI—UEFISCDI, project number PN-III-P2–2.1-PED-2021–0390, within PNCDI III.

ORCID

Raluca-Ioana Stefan-van Staden https://orcid.org/0000-0001-8244-2483
Damaris-Cristina Gheorghe https://orcid.org/0000-0002-6430-2177
Ruxandra-Maria Ilie-Mihai https://orcid.org/0000-0002-2285-9583

References

1. M. J. Scanlan et al., *Cancer Immun.*, **6**, 2 (2006).
2. Y. Inoue et al., *Cancer Sci.*, **108**, 1701 (2017).
3. Y. Jung et al., *Animals*, **11**, 1037 (2021).
4. E. L. Snyder et al., *Molec. Cell*, **50**, 185 (2013).
5. J. H. Kim, K. J. Kim, Y. Y. Rhee, J. M. Bae, N. Y. Cho, H. S. Lee, and G. H. Kang, *Human Pathol.*, **46**, 643 (2015).
6. Y. Chen et al., *J. Surg. Oncol.*, **106**, 286 (2012).
7. D. C. Gheorghe, R. I. Stefan-van Staden, R. M. Ilie-Mihai, and P. Sfarloaga, *Nanotechnol.*, **34**, 345101 (2023).
8. R. I. Stefan-van Staden, M. I. Bogea, R. M. Ilie-Mihai, D. C. Gheorghe, H. Y. Aboul-Enein, M. Coros, and S. M. Pruneanu, *Anal. Bioanal. Chem.*, **414**, 6521 (2022).
9. R. I. Stefan-van Staden, A. A. Bratei, R. M. Ilie-Mihai, D. C. Gheorghe, B. M. Tuchiu, and S. Gurzu, *J. Electrochem. Soc.*, **170**, 057510 (2023).
10. R. I. Stefan-van Staden, C. C. Negut, and P. Sfirloaga, *J. Electrochem. Soc.*, **170**, 037503 (2023).
11. K. Iwamoto, A. Ikeda, K. Araki, T. Harada, and S. Shinkai, *Tetrahedron*, **49**, 9937 (1993).
12. I. Ali, W. A. Wani, K. Saleem, and M. F. Hsieh, *RSC Adv.*, **4**, 29629 (2014).
13. I. Ali, W. A. Wani, A. Haque, and K. Saleem, *Future Med. Chem.*, **5**, 961 (2013).
14. I. Ali, H. Y. Aboul-Enein, and A. Ghanem, *Curr. Pharm. Anal.*, **1**, 109 (2005).
15. I. Ali, W. A. Wani, K. Saleem, and D. Wesselinova, *Med. Chem.*, **9**, 11 (2013).
16. I. Ali, W. A. Wani, K. Saleem, and A. Haque, *Curr. Drug Ther.*, **7**, 13 (2012).
17. I. Ali, M. Alsehli, L. Scotti, M. Tullius Scotti, S. T. Tsai, R. S. Yu, M. F. Hsieh, and J. C. Chen, *Polymers*, **12**, 598 (2020).
18. V. Chugh, A. Basu, A. Kaushik, and A. K. Basu, *ECS Sens. Plus*, **2**, 015001 (2023).
19. N. M. Cusack, P. D. Venkatraman, U. Raza, and A. Faisal, *ECS Sens. Plus*, **3**, 017001 (2024).