SCALABLE ELECTROPOLYMERIZATION OF VERTICAL GRAPHENE OXIDE ELECTRODES AS A PHYSICAL/CHEMICAL BIOSENSOR PLATFORM

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ABSTRACT

SUSANNE, a nanophysical electrochemical synthesis (NES) system, enables scalable production of nanostructured scalable devices (nSD) with high reproducibility. Vertical graphene oxide (VGO) structures on screen-printed carbon electrodes (SPCEs), which are used in biosensors, are called nSDα. Surface morphologies of nSDα vary with input parameters of temperature and electrolyte concentration, each yielding unique redox profiles. The charge transfer resistance (R_{ct}) of nSDα is negligible compared to Pt (161 Ω) and carbon (2 k Ω) suggesting superior electron transfer. Biosensors with nSDα show potential discrimination of 30 µA and ~100 µA at lower and higher potentials, respectively, paving ways for biosensors commercialization.

KEYWORDS

Biosensor Platform, Vertical Graphene Oxide, Electrochemical Sensors, Nanophysical Electrochemical Synthesis

INTRODUCTION

Biosensors based on electrochemistry principles require fast electron transfer between electrodes and electrolytes, and significant perturbations of the electron transfer resistance, to result in change in electrical signal.

Previous studies have reported various methods to modify screen-printed electrodes (SPEs) for biosensors and electrode requirements for biosensing were identified. The electrodes should have good electrocatalytic capability, efficient electron transfer at the electrode-solution interface, and high surface area for biomolecule coverage [1]. To achieve the criteria, SPEs often consist of a support material such as carbon or gold electrodes combined with electroactive coatings such as Pt and Au nanoparticles (NPs) [2], conductive polymers [3], or carbonaceous nanomaterials such as graphene and its derivatives [4]. Graphene's inherent large surface area is believed to improve sensitivity and limit of detection (LOD) by functioning as a template for large surface coverage of bioreceptors, thus more active sites for receptor-antigen interaction and catalytic effect, respectively. Furthermore, graphene oxide edges are full of dangling bonds or unsaturated carbon atoms, thus are more chemically reactive and are more likely to interact with other atoms or molecules. Label-free approaches leverage on graphene's large surface area for effective immobilization of receptors such as DNA/RNA, aptamers, or antibodies [4].

Various methods exist for nanostructuring graphene and its derivatives on substrates, spanning a spectrum from high-vacuum plasma processes to generate defect-free graphene structures to drop casting of randomly oriented graphene and its derivatives from liquid suspension onto device substrates. The former method, while yielding highly sensitive devices, is characterized by its slow and costly nature. Conversely, the latter approach offers rapid implementation at a lower cost but may encounter challenges regarding reproducibility.

One key graphene nanostructures are vertical graphene (VG) [5] where more graphene reactive edges are exposed compared to its basal plane, and VG is believed to enhance mass transport by reducing diffusion path lengths, thus enhancing kinetics of reactions in batteries and supercapacitors. We think that VG on biosensor electrodes as transducers can result in large sensing areas and increased sensitivity. In this work, we demonstrated the scaling up of graphene-composite nanostructuring with controlled vertical graphene oxide (VGO) composites on screen-printed carbon electrodes (SPCEs), using wet electrochemistry or fab-less fabrication on device substrates for the development of affordable diagnostics. We leverage on our understanding of prior work in electrochemistry [3, 6] for the scaling up of the electrochemical deposition process.

SUSANNE

Pilot Plant Design

Sustainable Universal Synthesis of Advanced Nanomaterials through Nanoscale Engineering (SUSANNE) (Fig. 1) is a semi-automated, bench-top, batch nanophysical electrochemical synthesis (NES) system. Designed for the deposition and anchoring of vertical graphene oxide (VGO) nanostructures on conductive SPCEs – the combination of VGO with a substrate is called nanostructured scalable devices (nSD) and the biosensor version of nSD is named nSDα; the NES system is capable of processing 100 nSDs per hour, using four mechanized units: 1. Activation Unit, which functionalizes the SPCE surface with oxygenated groups using 0.1 N H_2SO_4; 2. Rinsing and Drying Unit 1, concluding the acid etching process; 3. an Electrodeposition Unit, where GO is oriented vertically and anchored onto SPCEs resulting in nSDs; 4. Rinsing and Drying Unit 2 for the final rinse and drying of the electrodes. The nanostructured electrodes are subsequently cured in an oven to fix the VGO onto the electrode surface.

The Electrodeposition Unit has a circular layout, accommodates ten SPCE working electrodes (WEs) of 1 mm diameter, is positioned equidistantly from a 10x10 mm Pt counter electrode (CE), where each WEs has a localized Ag/AgCl reference electrode (RE). This circular design fits a standard 140 mm diameter laboratory beaker, though ideally, the distance between the WE and RE should be minimal, and the distance between the WE and CE should be maximized.

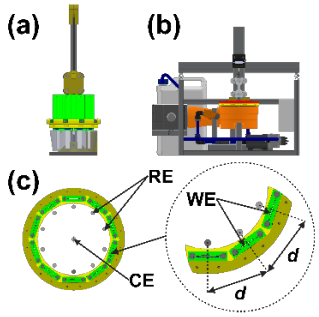

Figure 1. SUSANNE benchtop pilot plant featuring: (a) Electrodeposition and (b) Rinsing and Drying Units; (c) Circular electrode layout with (WE) positioned equidistantly 'd' from each other. The WEs are equidistant from its Ag/AgCl reference electrode (RE) and share a common counter electrode (CE).

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Deposition Process and Reproducibility

The ability of SUSANNE to produce reproducible nanostructured nSD electrodes is clearly shown in Figure 2. The chronoamperometric deposition of nanostructured scalable devices (nSD) is depicted across 20 batches, involving 200 electrodes (Fig. 2, a). Chronoamperometry at 850 mV for 120 seconds successfully covered ten, 1 mm diameter WEs of screen-printed carbon electrodes (SPCEs) with VGO composite. The NES can fabricate 10 nSDs in under 2 minutes per deposition. We have successfully produce sixty thousand (60, 000) of nSDs for our study, promising the potential of scaling up biosensors for commercial use but also providing sufficient data to understand the fundamentals of transduction of the nSD biosensor platform.

Differential Pulse Voltammetry (DPV) graph of nSD in a 50 mM ferricyanide solution exhibits three distinct current peaks (I_p); the tight standard error (SE) of the average current peak ($I_{p,avg}$) values across 100 electrodes (Fig. 2, b) further demonstrates the reproducibility of the electrochemical behavior of the nSDs.

These results collectively highlight the scalability and reproducibility of the SUSANNE deposition process and nSD's electrochemical properties, resulting in biosensors with predictable and reliable performance.

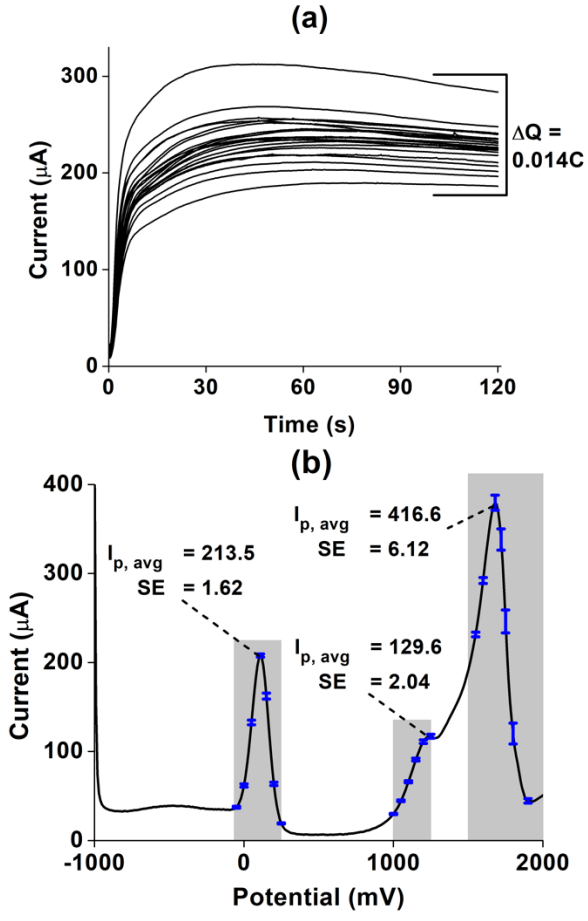

Figure 2. (a) Chronoamperometric deposition of nSD across 20 batches showing small variation in the total charge transfer (ΔQ) across all batches; (b) Differential Pulse Voltammetry (DPV) graph of nSD in 50 mM ferricyanide showing 3 distinct current peaks (I_p); peaks labeled with average current peak ($I_{p,avg}$) values with standard error (SE) across 200 electrodes.

Controllable deposition of VGO composite on SPCEs is achieved by regulating the electrical parameters such as voltage, current, duration of applied potential/current such as the pulse width and frequency. Factors such as electrode area and separation between the WEs, CE, and REs are quantified to control the electric field distribution and the energy density in SUSANNE. Electrode area and separation can affect the reaction kinetics, mass transport, and the efficiency of the energy conversion process. Furthermore, temperature and electrolyte conductivity are monitored throughout the process to maintain reaction selectivity, electrode stability, and electrolyte properties. We developed a feedback control mechanism based on real-time monitoring of the deposition parameters, where chronoamperometry graphs are being quantified as energy is inputted into the system. Owing to the reproducible process, we have utilized SUSANNE for VGO deposition on SPCEs for various biosensors development.

NSD – NANOSTRUCTURED SCALABLE DEVICES

Materials and Structure

NSD flakes size determines the electrochemical properties of the biosensor platform. Figure 3 shows Field Emission Scanning Electron Microscopy (FESEM) images of different types of nSD, exhibiting varied surface morphologies and the corresponding cyclic voltammetry (CV) graphs. NSD consists of large VGO flakes ranging from 500 nm to 1 μm, alongside flat GO structures (Fig. 3, a, b). This peculiar combination of nanoscale to microscale structures has complex defects and inhomogeneities which can be intrinsic to the material and highly dependent on the deposition process.

Temperature and electrolyte concentration affects the nanostructures surface morphology. At elevated temperatures of 70°C, highly porous three-dimensional VGO structures composed of thicker graphene layers are formed (Fig 3, c). Conversely, at reduced temperatures of 30°C, VGO with smaller flake sizes and relatively fewer graphene layers are formed (Fig. 3, d). Electrolyte concentration of 0.1 M gives rise to globular structures with embedded graphene flakes (Fig 3, e), while electrolyte concentration of two orders of magnitude lower than 0.1 M resulted in flatter graphene structures (Fig 3., f). These findings underscore the influence of process parameters on the resulting nSD morphology and structure.

Cyclic voltammetry (CV) measurements were conducted on these various nSD surfaces using a 50 mM ferricyanide solution (Fig. 3). It was observed that a more porous structure with thicker layers of graphene (Fig. 3, c), resulted in higher redox peak currents of 17.7 and -23.3 mA/cm^2compared to structures with relatively fewer graphene layers (Fig. 3, d), exhibited a lower redox peak current of 6.1 and -8.0 mA/cm^2. Globular structures, as depicted in Figure 3(e), displayed a significantly broad CV graph with a bird's peak shape, indicative of a higher capacitive property. Interestingly, the flatter structure in Figure 3(f) exhibited a CV graph profile like that of Figure 3(c) with redox peak currents of 15.7 and -24.8 mA/cm^2, suggesting that factors beyond surface morphology alone contribute to the CV profile and its redox current. For instance, the active sites and effective surface area of both morphologies (c and f) could be similar, resulting in similar redox current peaks.

The results show that surface morphologies significantly influence electrochemical properties. Various nSD surfaces exhibit either capacitive or resistive properties, demonstrating the SUSANNE system's ability to control and customize nSD nanostructuring on SPCEs, thereby influencing their electrochemical properties.

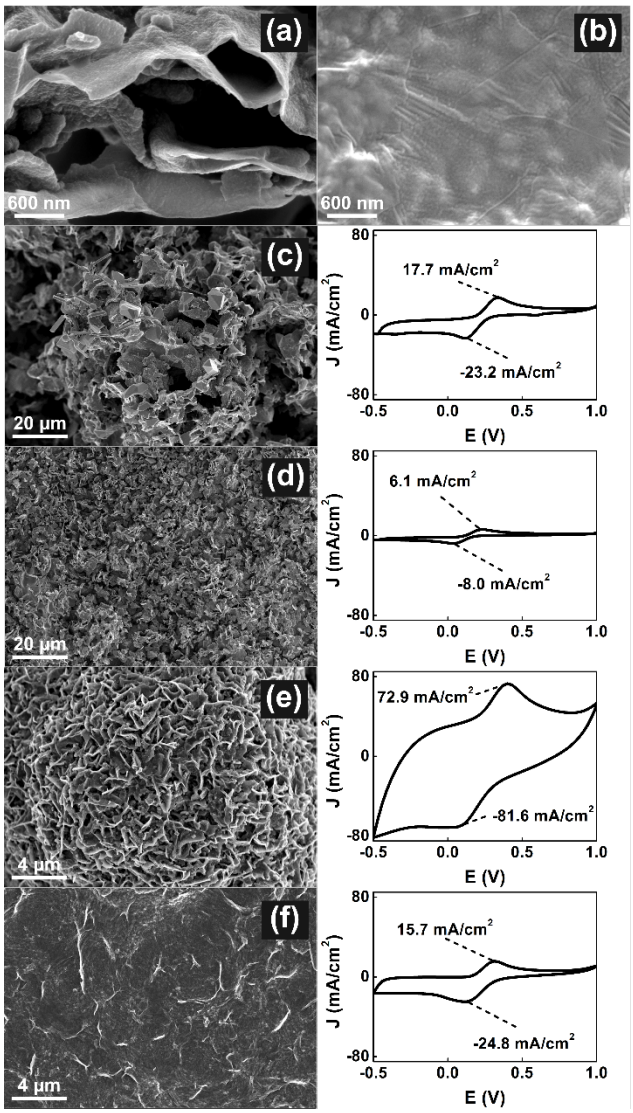

Figure 3. FESEM images showing (a) vertical and (b) flat GO structures of nSD deposited from SUSANNE; varying nSD structures obtained from modifying deposition parameters: nSD solution temperature at (c) 70°C, (d) 30°C; and electrolyte concentration at (e) 0.1M, (f) 0.001M, resulting in surfaces of different morphologies and unique CV profiles.

Electrical Impedance Spectroscopy (EIS) Analyses of nSD

Electrical Impedance Spectroscopy (EIS) was conducted on the SPCEs with various electrode surfaces (carbon, carbon/nSD (α = 210, 1, 2, 3 seconds – the longer the deposition time suggests thicker nSD layer), carbon/Pt, and carbon/nSD/Pt). Redox solution of 50 mM Ferricyanide was drop-casted on top of the electrode with an incubation time of 1 min. A 500 mV potential was applied with a frequency range of 100 kHz to 1 Hz using a commercial potentiostat. Analyses was conducted on the EIS Nyquist plot (Fig. 4) for charge transfer resistance (R_{ct} – width of the semicircle) and the solution resistance (R_s – distance between zero to the starting point of graph on the x-axis) at the higher frequency regions. The size of electrode pores (k = slope of the straight line) and the Warburg impedance (° = angle of the straight line) are identified at the lower frequency region.

Charge transfer resistance (R_{ct}) was evaluated for several electrode materials. As shown in Table 1, nSDα shows negligible R_{ct} in comparison to nSD from shorter deposition time, with decreasing R_{ct} of ~ 1 kΩ for 1 sec increase in the deposition time. A longer deposition time resulted in thicker and porous nSD structures, thus could result in reduced polarization effect, and faster kinetics of electrochemical process. Furthermore, thicker and porous nSD structures can enhance electrolyte accessibility owing to the large surface area, longer diffusion pathways, facilitating mass transport of ions and solvents to active sites. Uniform electrochemical reactions and minimal diffusion gradient can enhance electrochemical transduction. Besides R_{ct}, solution resistance (R_s) for all samples is similar since all the graphs started at almost the same point on the x-axis. In summary, nSDα provides an electrode-electrolyte interface that is highly conductive and favorable for electrochemical reactions, thus is utilized as the biosensor platform.

We conducted a comparative analysis between nSDα and platinum (Pt) to assess their respective abilities for electron transfer. The deposition of Pt on carbon (carbon/Pt) results in a reduction of more than 90% in the R_{ct} of carbon, implying the significant facilitative role of Pt in electron transfer processes. As previously mentioned, nSDα exhibits a negligible R_{ct}, suggesting minimal resistance for electron transfer across the nSDα-solution interface. Moreover, when 20 nm thickness of Pt is deposited on nSDα, the R_{ct} decreases fourfold compared to Pt alone, thereby reinforcing the efficacy of nSDα as an efficient transducer.

Pore size and shape can influence the surface area for electron transfer and is indicated by the slope (k) and angle (°) at the higher frequency. The corresponding angle values or Warburg impedance are calculated using equation (1). NSDα exhibits a steeper slope (1.51) compared to carbon (0.49) while the nSD (1, 2, 3 sec) have slopes comparable to carbon (0.33, 0.42, and 0.46, respectively). The addition of Pt on nSDα shows slightly higher slope (2.04), suggesting more interconnected pores in comparison to nSDα.

$$angle\ (°) = tan^{-1}\left(\frac{\Delta Z''}{\Delta Z'}\right) * \frac{180}{\pi} \qquad (1)$$

The Warburg impedance (°) at the higher frequency shows the nSDα to have an angle of 56° in comparison to Pt (40°) and carbon (26°). Deposition of 20 nm Pt on nSDα resulted in an increase to 64°. An ideal 45° angle suggests a perfect semi-infinite diffusion process, and a larger angle for the nSDα could indicate various factors such as a slower diffusion process or a diffusion-limited process in the system. It could also suggest the presence of a capacitive element in the nSDα system.

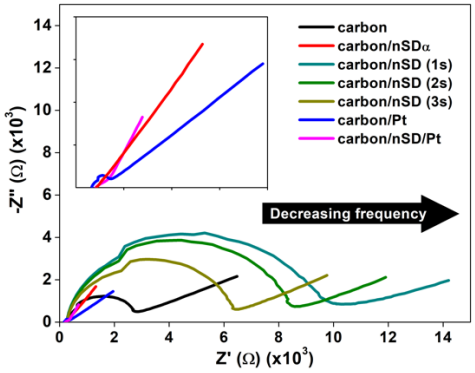

Figure 4. Electrochemical Impedance Spectra (EIS) of carbon, carbon/nSD (α, 1, 2, 3 s), carbon/Pt, and carbon/nSD/Pt in 50 mM ferricyanide over a frequency range from 100 kHz to 1 Hz at 500 mV.

Table 1: Calculated Rct, slope (k) and Warburg impedance (°) values of various transducer surfaces.

Electrode Type	R_{ct} (Ω)	Slope (k)	Angle (°)
Carbon	2265	0.49	26
Carbon/nSDα	-	1.51	56
Carbon/nSD (1s)	8950	0.33	18
Carbon/nSD (2s)	7802	0.42	23
Carbon/nSD (3s)	5804	0.46	25
Carbon/Pt	161	0.85	40
Carbon/nSD/Pt	41	2.04	64

SKUNKBOX DX – A VERSATILE AND SCALABLE BIOSENSOR PLATFORM

Screen-printed Carbon Electrodes (SPCEs) modified with nSDα were used for SkunkBoX Dx for detection of antigen nucleocapsid proteins (N-proteins) from SARS-CoV-2 owing to the negligible R_{ct} and diffusion-limited characteristics. SkunkBoX Dx consists of a multiplex cassette platform that houses three immobilized electrodes (AbN/nSDα) and one nSDα electrode as a control, and an electrical reader (Figure 5).

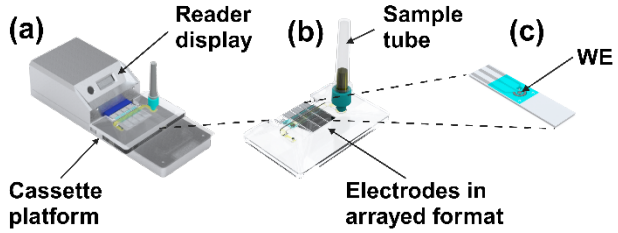

Figure 5. SKUNKBOX Dx biosensor platform with key components: (a) reader unit for multiplexed measurement, (b) arrayed cassette for sample delivery, and (c) SPCE nanostructured with nSDα through the SUSANNE process.

The nSDα electrodes were fixed with Antibody N (AbN), a specific antibody targeting the SARS-CoV-2 nucleocapsid protein. The electrodes were immobilized using EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) and NHS (N-hydroxysuccinimide) coupling chemistry. Next, the N proteins were allowed to incubate on the AbN-immobilized nSDα electrodes for a minimum of one minute. Subsequently, the electrodes were flushed with a background solution for measurement calibration, followed by a two-minute measurement period.

Differential pulse voltammetry (DPV) analyses (-1 V to 2 V, scanning rate 100 mV/s) were conducted for N protein detection. Figure 6 shows magnified regions of the DPV spectrum at low (a) -200 to 500 mV, intermediate (b) 500 – 1200 mV, and high (c) 1300 – 1700 mV potentials with peak current (I_p) and peak potential (E_p) from electrodes of nSDα, nSDα/AbN, nSDα /AbN/NP at 55°C.

As can be seen, the I_p for the positive samples crosses the negative ones at three different potentials, with discrimination most prominent at the higher potentials (~40 µA, 1600 mV) in comparison to the lower potential (10-20 µA, 100 mV).

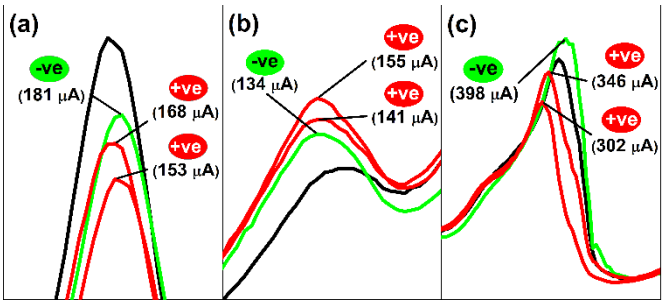

Figure 6. Differential Pulse Voltammetry (DPV) of negative samples (nSDα/AbN) compared to positive samples of SARS-CoV-2 (nSDα/AbN-NP) at 55°C, showing distinct peaks at (a) 100 mV, (b) 1200 mV, and (c) 1600 mV with discrimination at all potentials.

CONCLUSIONS

SUSANNE enables reproducibility of biosensors through scaling up of the VGO deposition process directly on electrode substrates with high reproducibility. Controlling the process input parameters resulted in different nSD surface morphologies with unique redox profiles. Electrodes with nSDα used for biosensors have negligible R_{ct} in comparison to Pt and carbon, where DPV results revealed discrimination towards N-proteins at varying potentials. SUSANNE has the potential for controlled nanostructuring of graphene on electrode/device substrates, opening ccommercialization potential for nanotechnology-based devices.

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REFERENCES

[1] D.M. Heard, and A.J.J. Lennox, "Electrode Materials in Modern Organic Electrochemistry", *Angew. Chem. Int Ed.,* 59, 43 (2020), pp. 18866-18884.

[2] J.C. Claussen, et al. "Nanostructuring Platinum Nanoparticles on Multilayered Graphene Petal Nanosheets for Electrochemical Biosensing", *Adv. Funct. Mater.*, 22, 16 (2012), pp. 3399-3405.

[3] A. Benoudjit, M.M. Bader, and W.W.A. Wan Salim, "Study of Electropolymerized PEDOT:PSS Transducers for Application as Electrochemical Sensors in aqueous media", *Sensing and Bio-Sensing Research*, 17 (2018), pp. 18-24.

[4] S. Eissa, et al. "Probing the Influence of Graphene Oxide Sheets Size on the Performance of Label-free Electrochemical Biosensors", *Sci Rep*, 10, 13612 (2020).

[5] Z. Bo, et al. "One-step Fabrication and Capacitive Behavior of Electrochemical Double Layer Capacitor Electrodes using Vertically-Oriented Graphene Directly Grown on Metal", *Carbon*, 50, 12 (2012), pp. 4379-4387.

[6] J. Park, et al. "An Autonomous Lab on a Chip for Space Flight Calibration of Gravity-Induced Transcellular Calcium Polarization in Single-Cell Fern Spores", *Lab on a Chip*, 17, 6 (2017), pp. 1095-1103.

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