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Integrated Electrochemical Dopamine Sensing with Finger Priming Pump on a Chip

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Abstract. Development in microfluidic technology has contributed to increased understanding in neural tissue engineering through the in vitro observation of cell-on-chip (CoC) systems. This has been further helped by the integration with the broader MEMS (micro mechanical and electromechanical systems) technology that offers external devices such as detectors or biosensors to show the characteristics of the observed object. An on-chip microsystem microfluidic platform for dopamine detection is presented here. The microfluidic platform integrates electrochemical detection with finger pumping and a valve system as means to control the fluid flow. This microenvironment offers a quicker result in observing the phenomena related to the neural cell activities with a relatively small specimen volume of 50-100 µL, eases the handling of movement, and consequently reduces the cost of consumable items. The microfluidic platform presented here showed that the pump module that also serves as a mixing point was able to deliver at maximum of 121.36 µL with 2-3 strokes of normal finger pressure priming. A series of valves aids in the termination or isolation of fluid flow in a specific zone for further processing. Ultimately, the microfluidic platform is also equipped with a portable electrochemical detection module that allows us to measure the dopamine concentration up to 1 mM. This development showed that the on-chip testing of dopamine could be conducted easier and be more portable to handle.

Keywords: Cell-on-chip; Dopamine; MEMS; Neural tissue engineering; On-chip testing

1. Introduction

The use of conductive carbon matrix to culture neuronal cells has been demonstrated recently (Whulanza et al., 2022; Sagita et al., 2018). To optimize neuron growth across the matrix, a scaffold was used as a substrate to enhance nerve cell interaction in vitro. The substrate, together with the in-vitro platform was analyzed for cellular viability and

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electrically controlled and release of neuromodulators (Nimbalkar et al., 2019; Vomero et al., 2018). The characterization of neuromodulator, i.e., dopamine ions, in release and uptake studies on the conducting substrate for neural application is ultimately the main interest of this research. Successful optimization of these characteristics will be important for treating injuries to the nervous system. Interactions between neuron cells and extracellular matrix are key factors in studying cell migration, proliferation, differentiation, and apoptosis, all of which are critical functions for a neural-engineered construct (Pavesi et al., 2015; Kotwal & Schmidt, 2001). These requirements are not fully understood by researchers; therefore, study of the complete set of biological requirements must be employed.

The availability of commercial microfluidic devices such as microreactor systems is growing rapidly to carry out various industrial and laboratory processes (Nadhif et al., 2019; Nadhif et al., 2017; Whulanza et al., 2017). The system typically integrates input processes, chemical reactions, outputs, and analyses on a laboratory scale that are carried out continuously. Early developments in microreactor technology mainly focused on the design of microreactor chips with silicon, glass, or polymer materials such as PDMS (polydimethylsiloxane). The design aims to add a small amount of reagent to the reaction zone in a volume of microliters under controlled conditions. Such flows can be clearly observed through the precise control of reaction parameters (Nunut et al., 2020; Whulanza et al., 2019; Whulanza et al., 2014). This recent advancement demonstrates the incorporation of significant microfluidic device work into a functional Lab-on-Chip that addresses the required result in a portable device with minimal laborious work.

The process of developing microfluidic systems has resulted in successful product commercialization. However, some challenges still lie ahead for a Lab-on-Chip (LoC). The most basic thing is in the concept of end-to-end integration. But currently, off-device development research will soon be grafted into a full LoC system. LoC has the same problem in the chip world, namely the interface problem. A Micro-Electro-Mechanical-System (MEMS) sensor package is needed in such a way that it can provide accurate readings. Therefore, this preparation system requires additional systems such as microscopes, pumps, computers, and spectrometers (Charmet et al., 2020; Whulanza et al., 2015; Suwandi et al., 2014).

Finger-powered micropump has been widely developed for variety of application, including operational of finger-actuated, one-way, positive micropump for pathogens detection system (Qi et al., 2022; Jo et al., 2020), finger-actuated, negative pressure chamber for electrochemical detection of ascorbic acid (Liu et al., 2022) and microreactor (Lee et al., 2022; Park et al., 2019; Whulanza et al., 2019). The lab-on-a-chip that is being developed in this study is the integration of microreactor processes such as cell culture or adsorption with the microfluidic component, i.e., finger priming pump and manual valve. Ultimately, the dopamine sensing package is also coupled with the portable electrochemical detection module.

2. Methods

2.1. Design Consideration

The process of designing this lab-on-a-chip device is based on previously introduced lab-on-a-chip devices, which miniaturized the space of a laboratory onto a single 75 mm x 25 mm x 6 mm chip. This current platform is designed to have a channel width of 1 mm, with its larger contact surface area facilitating the mixing process. To improve the practicality and flow conditions of our previously designed 200 µm x 200 µm channel, the current channel is designed to have a width and height of 1 mm x 1 mm. There are three

main working divisions of this chip, which are the preparation region, the pumping and mixing region, and the detection region.

The preparation region contains two inlets and one intersection merging the inlets. The inlets have a diameter of 1.68 mm to accommodate the Microfluidic Chipshop GmbH. Luer connection, which is used to transfer fluid samples from a syringe pump or micropipette onto the channel of the chip. Two inlets are designed to allow simultaneous insertion of two samples, if necessary.

The pumping and mixing regions contain the liquid sample reservoir, which serves also as the micropump, the meander channel section, and a pneumatic valving mechanism. The reservoir pumping section is a cylindrical space with 6.51 mm in diameter and 1.5 mm high. It is also designed to accommodate the pushing of fingers. Since the microfluidic chip is designed to be 2 mm thick, the ratio of thickness and diameter of pumping reservoir is expected to be capable of pumping operation. The meander section is designed to allow two liquid samples to interact and mix by means of diffusion. Two pneumatic valve points are used to control fluid entering and exiting the pumping and mixing region.

A reservoir covers the area of three electrodes used in the electro-chemical detecting process in the detection region. It has an 8.00 mm in diameter to accommodate the 3 working electrodes designed for electro-chemical detection (Christian et al 2022). On top of it lies the sample outlet twice the size of the inlet.

2.2. Fabrication

There are four important steps to fabricate the lab-on-a-chip device: mold building, polydimethylsiloxane (PDMS) casting and curing, silicone rubber casting and curing, and product assembly. Mold is designed by using Solidworks 2016® software. To minimize the cost of production of the mold, FDM/FFF (fused deposition modeling/fused filament fabrication) was the first method that was adopted, but results show that DLP-SLA (digital light processing stereolithography) is a better fit for the final prototype.

After the mold was completed, PDMS substrate was added to the mold, which is PDMS casting, along with a curing agent equal to 10% of the PDMS weight. The mold will then be put into a vacuum chamber, as air is not wanted in the PDMS mix, which may cause gas bubble formation for 45 minutes, Due to the mold's poor heat resistance, it will then be heated in a heating bed for about 6 hours at 50°C before peeling. On the other hand, similar steps were performed on silicone rubber casting, but it needs about 4% of the curing agent. Also, silicone rubber casting uses a blank aluminum mold, so heating it to 120°C would not be a problem, thereby, speeding up the curing process to about 1-2 hours.

The last step for lab-on-a-chip fabrication is assembling PDMS and silicone rubber to become a single integrated chip. This process requires a plasma bonder from Blackhole Lab., which will be used to stick two different layers, so that they will become one piece of a device.

2.3. Flow Testing

This chip will be tested by its ability to contain and displace fluids, as well as create space to be able to work side by side with the electro-chemical detector. Three tests and three measurements will be conducted to see if the chip is doing its designated job. A flow test will be done by inserting fluid into the chip all the way from the inlet to the outlet to see how well those two layers are attached together so that there will be no leakage. A valve test, on the other hand, is needed to see how well the valves, are inspired by a water gate-work by stopping fluid flow. A mixing test will be conducted to see if the two fluids are mixed consistently.

Finger-pumping force measurement is done as a complementary to see how much force human fingers exerted on the PDMS chip, which will be useful for the next measurement. It is also done because of the fluctuation level on the force gauge meter; hence, measurement will be done 100 times to see the average value of a human finger's force. Force vs. volume measurement is the main thing to do in this research because it shows the characteristics of the finger-pump that is used to displace fluid. It is done by inserting a certain controlled amount of fluid by using a 10-100 µl micropipette, pumping that fluid, and extracting the fluid on the electro-chemical detector section by using a syringe. The fluid will then be put into micro-tubes to be weighed down, where the difference between a liquid-inside-micro-tube and an empty micro-tube is the weight of the water, and hence from its density, the volume of the water can be found.

2.3.1. Measurement of Dopamine Ions Using Electrochemical Detection

The dopamine concentration was monitored in situ by measuring the electrochemical detection of the specimen right after the pumping process. Here, the electrodes were connected to an open-source potentiostat (Rodeostat, Pasadena, USA) with a cyclic voltameter (CV) mode. The detection chamber included three electrodes that were screen printed together with the carbon matrix (Arafat et al., 2021; Istiyanto et al., 2019). During the CV tests, the working electrode potential alternated between −1.0 and 1.0 V with a scan rate of 100 mV/s.

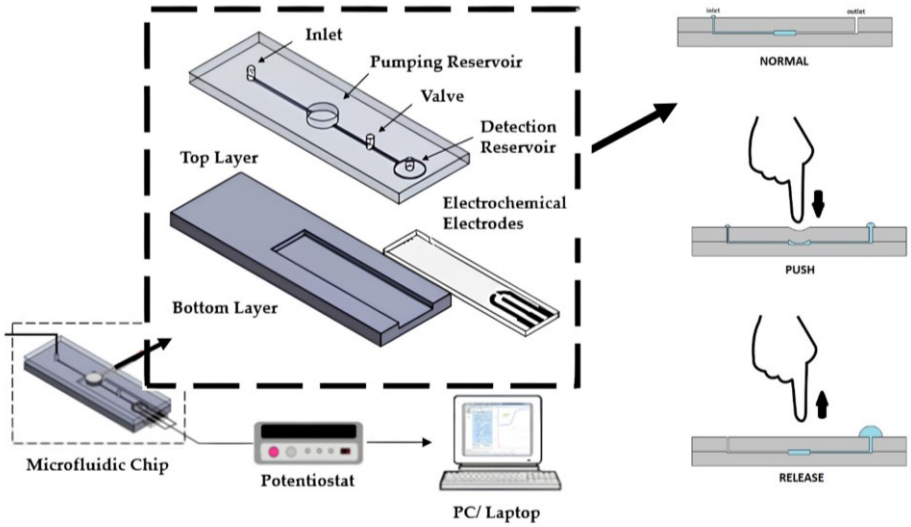

Figure 1 Experiment set-up and apparatus

3. Results and Discussion

A microfluidic platform has been successfully fabricated using DLP/SLA technology (Ahmed et al., 2022). Figure 1 shows the experimental setup for the current setting where the microfluidic platform is positioned on the electrochemical analysis system, which is connected to a tablet as the media for the user interface. The same technology to realize the microfluidic platform, which is more efficient compared to micromilling technology, has been done previously (Whulanza et al., 2018; Whulanza et al 2017). The development of a pneumatically actuated micropump as an intermediary step from bulk external pumping systems such as a syringe or peristaltic pump to an on-chip integrated micropump has also been done previously (Whulanza et al., 2019).

Figure 2a depicts a microfluidic platform unit with an integrated finger priming micropump and dopamine electrochemical sensing. Figure 2b shows the carbon-based

electrodes on PCB substrate realized with the previously developed method. Figure 2b explains the three steps to assembling the PCB with the screen-printed electrode and the covering PDMS layer on top. Figure 2c shows the silicone rubber acting as the base for the microfluidic platform, which enable us to flow the inlet in the reservoir. Figure 2d shows the fully assembled microfluidic device that finger pumped and equipped with the stop valve. Based on the previously mentioned tests, results were produced to see not only how the chip works but also the characteristics of the finger-pump. These results will be placed according to tests and measurements as listed before.

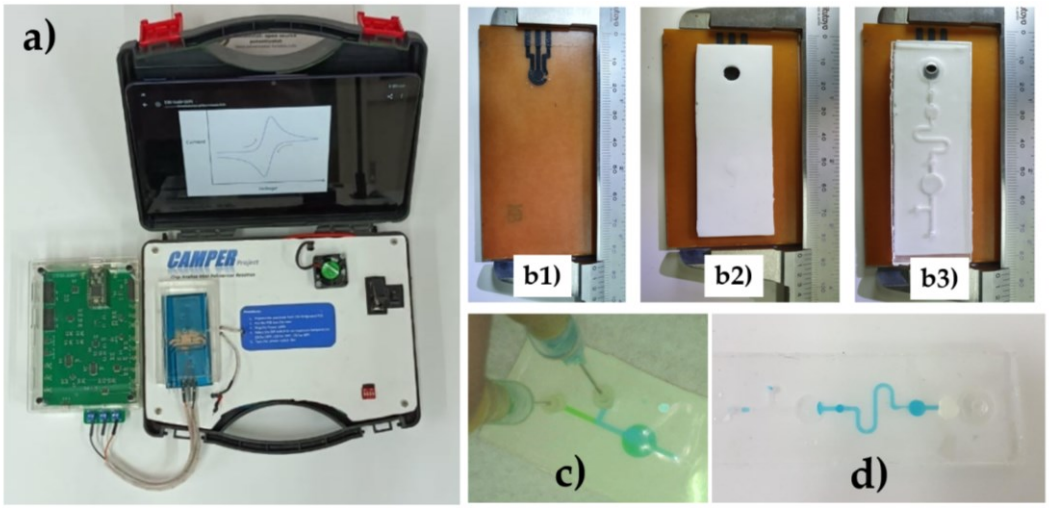

Figure 2 a) Integration of an electrochemical biosensor, a microfluidic platform, and a processor; (b) arrangement of carbon-based electrodes on a PCB substrate in three steps; (c) the silicone rubber as base layer for microfluidic platform as the inlet (d) top layer of microfluidic platform from PDMS as pump priming with a stop valve.

3.1. Flow Test

The flow test is a part of complementary tests to ensure that the chip does what it is supposed to do, especially the most important one as it should not produce any fluid leakage. The test uses a syringe pump, which is set to 7.2 mL/h (a similar value to the peristaltic pump used on the mixing test later). Strength-wise, the bonding of these two layers is excellent, and no leakage is present as the fluid flows until the electro-chemical detector section.

3.2. Valve Test

A valve test is also included in complementary tests to ensure that two valves work properly at the designated valve points. These valve points only exist on the Trial 4 mold and are designed to be a perfect match dimension-wise to the Microfluidic Chipshop Gmbh. Luer, whose original function is to insert a syringe tip onto the chip. While using the Luer in place on the valve point, a syringe pump begins to insert fluid at a normal rate of 7.2 mL/h.

3.3. Finger Pumping Measurement

Finger-pumping measurement is also one of the complementary tasks done to see how human fingers have an effect pressure-wise on the PDMS and silicone rubber prototypes in the lab-on-a-chip. Because of a specific force gauge used, data derived from the device is in grams, so from the measurements above, a couple calculations will be made to derive grams into Newton value. Those values are the forces exerted by human fingers onto the PDMS and silicon rubber chip.

After doing that for 100 times, a pattern of how much force human fingers apply to a PDMS chip is generated by generating a histogram with a range of 19 – 31 N with a value step of 3 N to give a more precise distribution. As seen on Figure 3a, 59% of human finger forces applied to a PDMS chip are between 22 and 25 N, while 35% of human finger forces applied to PDMS chip are between 25 and 28 N. These results in a vast 94% of human finger forces bieng generated between 22 – 28 N.

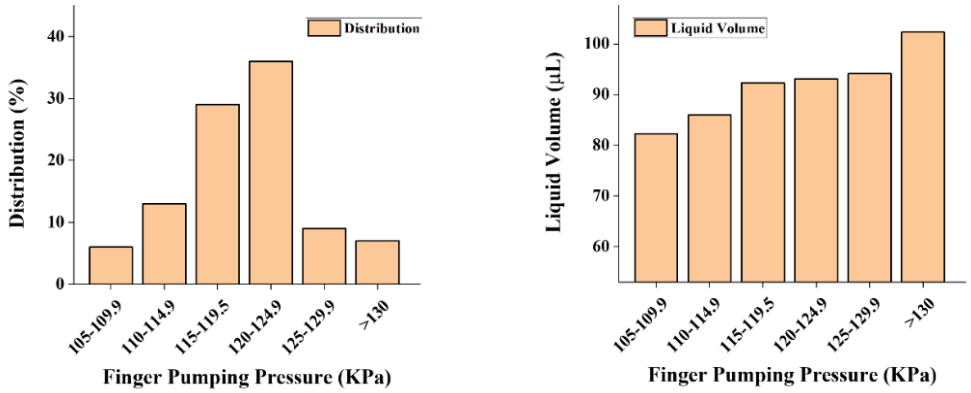

Figure 3 a) Pressure measurement of finger pumping (n=100) and b) the liquid delivered into the sensing zone

3.4. Force vs. Volume Measurement

The characteristic of the finger pump used on this lab-on-a-chip device will be shown here by how much volume of water is displaced from the mixing point to the electro-chemical detector region to be extracted using a syringe., But before any of that, there is one effect that is vital to the pump's usage, which is the flow's velocity difference. Below is the different velocity caused by pumping the mixing point region of the chip while fluid is constantly flowing.

The velocity generated by a 7.2 mL/h flow from the syringe pump is 1.11 mm/s; it is measured by measuring a certain linear distance on the chip and how much time is needed for the water to reach out that distance. The next step is to measure the linear channel after the mixing point region and determine how much time is needed for it to cover that distance. After 4 measurements, the velocity generated by pumping is 24.00 mm/s with a standard deviation of 2.79. On average, the velocity rise made from this measurement is about 22.89 mm/s.

Figure 4a shows the mapping of points along the pumping region, which corresponds, to the x-axis of Figure 4b. Point 5 and 6 are the points where pumping is happening, and after that, the velocity rises as in Figure 4b. Moving on to the next task, which is to see how much pressure, is needed to displace a certain amount of fluid, Pumping it once will not get the job done, so on average, based on this measurement, it took about 4-5 pumps to displace fluid to the electro-chemical detector region. 30 measurements are taken to determine how the data differs from one another in order to generate a linear graph on its own. Another thing to consider is how well the micropipette works, which in this case is not so precise. Its 100 µL of liquid reading is equivalent to an average of 121.36 µL of liquid by 4 measurements with a standard deviation of 2.46. It is critical to compare the percentage of liquid extracted to the percentage of liquid inserted.

From Figure 3b, the total volume delivered by the pump is extracted when the fluid is in the electro-chemical detector zone and is extracted by a syringe. The maximum average volume delivered is 102.31 µL in class 130 – 135 N, and the minimum average volume

delivered is 83.75 µL in 90 – 95 N class. This represents a directly proportional relationship force and volume.

Figure 3b shows a histogram of displaced volume distribution based on the experimental data. As much as 36.67% out of 30 measurements are in the 90 – 95 µl class. The second highest frequency of displaced volume is in the 85 – 90 µl and 95 – 100 µl class with 20% each, as it is combined for 76.67% of the total in the 85 – 100 µl range.

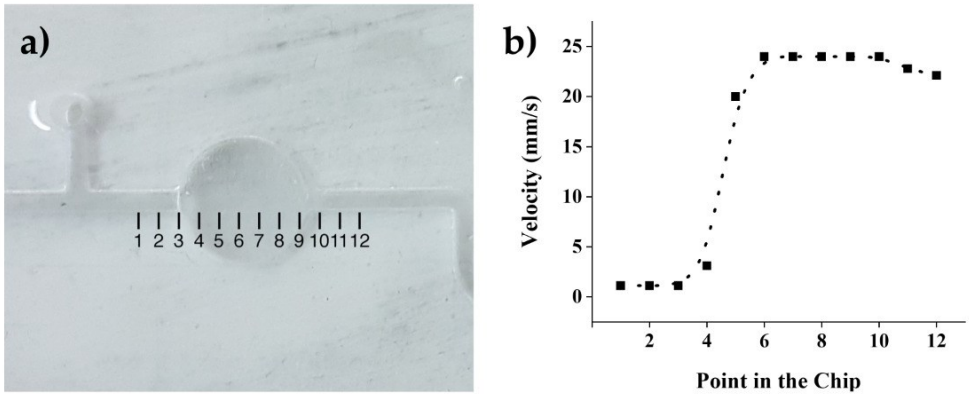

Figure 4 a) The position of measuring point in the pumping zone and b) measurement of velocity in the pumping zone

3.5. Dopamine Detection Using in Situ Measurement

Microfluidic platforms to detect/measure dopamine have been widely developed, including paper-based systems (Liu et al., 2019; Manbohi et al., 2019; Feng et al., 2015) and polymer-based systems with integrated electrochemical sensing (Yue et al., 2019; Liu et al., 2018; Yu et al., 2016; Maminski et al., 2005). Electrochemical detection was carried out to measure the dopamine concentration once the uptake process was complete. Note that the liquid specimen was transferred first from the uptake chamber to the detection chamber. Here, the electrochemical behavior of the electrodes is observed using cyclic voltammetry (CV) study. Figure 5(a) depicts the voltammogram of three different concentrations of dopamine. It is possible to see a peak cathodic current at a potential of around 0.2 V vs. Ag/AgCl. Only an oxidation peak appears in Figure 5. Therefore, the possible dopamine reaction that occurred on the surface is irreversible. The measurement shows that those various dopamine concentrations were clearly distinguishable. This could be the best candidate for a non-enzymatic dopamine biosensor (Hardi et al., 2020; Rahman et al., 2016).

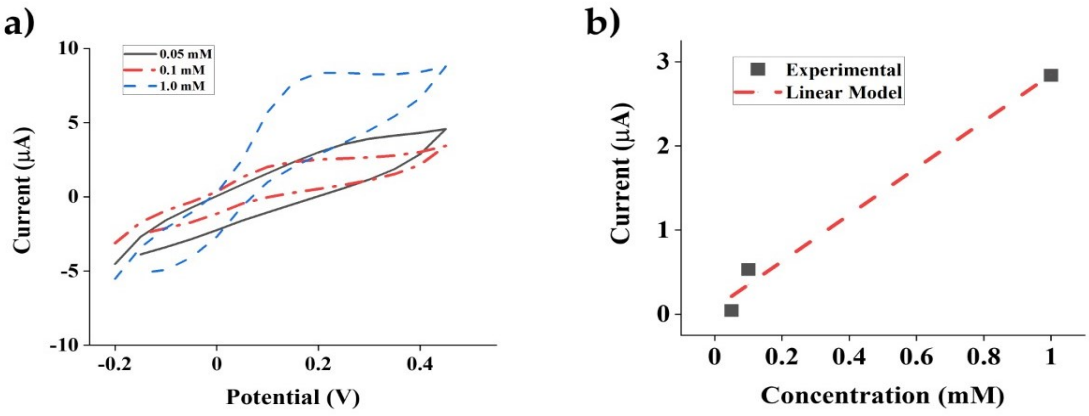

Figure 5 Electrochemical detection plot result

Figure 5(b) shows that the peak dopamine concentration was linearly dependent on the concentration that was prepared (from 0.05 mM to 1 mM). Figure 5(b) also shows the regression plot of the dopamine solution, which is expressed as I (μA) = 2.7752*Concentration Dopamine (μM) + 0.0742 with a Pearson correlation of 0.986. The sensitivity and linear detection range of the dopamine solution were specifically calculated at 0–1 mM. It can be estimated that the limit of detection was around 0.20 mM.

The linear model is used since the most suitable model for measurement or detection is when the parameters are linearly related. The linearity analysis suggested that the measurement using this setup has a plausible result provided by the calculated detection limit. The margin error compared to the linear model showed a lowest value of 0.3% at a concentration of 1 mM, whereas at a concentration of less than 0.1 mM showed an error of 24%. his high error was consistent with the calculated limit detection at 0.2 mM. Hence, this finding showed that our setup enables us to work with a dopamine detection range of 0.2 – 1.0 mM.

4. Conclusions

It can be concluded from this research that the appropriate rapid prototyping method for the current chip mold is DLP-SLA. Although its production cost is more expensive than FFF, the result is much better. DLP-SLA shows a little deviation among the design, the mold, and the product. Moreover, combining PDMS and silicone rubber is a perfect match to create one whole lab-on-a-chip. For the channel size, a 1 mm x 1 mm channel produces a good mixing with two simultaneous flows. For the finger pump, it creates a rise of around 22.89 mm/s to the fluid flow velocity. Overall fluid displacement can be concluded by an average of 75.43% of liquid being displaced from the mixing point region.

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