

Electro-chemical properties of carbon nanotube based multi-electrode arrays

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Abstract. A novel class of micro electrodes was fabricated by synthesizing high density carbon nanotube islands on lithographically defined, passivated titanium nitride conductors on a silicon dioxide substrate. Electrochemical characterization in phosphate buffered saline of these new electrodes reveal superb electrochemical properties marked by featureless rectangular cyclic voltammetry curves corresponding to a DC surface specific capacitance and volume specific capacitance as high as 10 mF/cm^2 and 10 F/cm^3 , respectively. These electrodes are also characterized by a nearly constant impedance magnitude over the range of 200 Hz to 5 kHz. High fidelity extracellular recordings from cultured neurons was performed and analyzed to validate the effectiveness of the fabricated electrodes. The enhanced electrochemical properties of the electrodes, their flexible and simple micro-fabrication preparation procedure as well as their bio-compatibility and durability suggest that carbon nanotube electrodes are a promising platform for high resolution capacitive electrochemical applications.

1. Introduction

Multi-electrode arrays (MEAs) recording systems have long been demonstrated as a powerful platform for the investigation of excitable biological systems [1]. MEAs are often employed to record extracellular electrical activity of biological cells in order to study their collective behavior [2, 3]. These systems, supported by various data analysis algorithms [4], were also proven effective for applications such as cell-based biosensors [5], drug screening [6] and safety pharmacology [7]. The relative small dimensions of the electrodes and their precise positioning support high spatial resolution recording, which is very important when attempting to analyze the activity of complex, many-cell systems. The minimal invasiveness of the MEA recording also allows long term cell recording (as long as several months), which is essential for investigations dealing with adaptation and plasticity [8].

Despite their many advantages, a fundamental impediment of micro-electrodes is their relatively high specific impedance which leads to increased overall electrode impedance values for micro-meter scale electrodes and consequently to increased noise levels. MEAs recording performances are also limited by the electrode-cell coupling which is determined, among other things, by cell-electrode attachment as well as cell-electrode sealing. All these parameters play a crucial role in determining the fidelity of the recorded signal [9, 10, 11].

Therefore, in order to improve the recording performances of MEAs, researchers have labored extensively on improving cell-electrode coupling [10], as well as on reducing the impedance of the electrodes [1, 3]. On the second front, extensive effort have been directed towards increasing the effective area of the electrodes in order to reduce the electrode impedance while maintaining overall small electrode dimensions [12]. The desired increase in the effective area of recording electrodes have been established by various surface treatment methods, such as electroplating (typically platinum, iridium or titanium nitride) [3] and electrochemical etching [13]. Electroplating roughening can produce up to two orders of magnitude improvement in electrode impedance [3].

Carbon nanotube (CNT) coatings offer a new and exciting route to further improve the performances of MEAs due to their many beneficial properties such as mechanical stability, chemical durability, good electrical conductance [14] and their biocompatibility [15]. Recent studies have also revealed that CNTs provide an excellent surface for neural cell adhesion and growth either on uniform surfaces [16] or on isolated CNT islands [17, 18]. Moreover, it was recently shown that CNT substrates may even boost neuronal electrical signaling [19].

In addition to the advantages outlined above, CNT also proved their usefulness as coatings for electrochemical electrode applications: CNT coated electrodes exhibit exceptionally high specific capacitance, well suited for super capacitance applications [20, 21, 22]. Therefore, the integration of CNT coatings on MEA offer many interesting prospects.

In this study we present a methodic DC and AC investigation into the electrochemical (EC) properties of novel micro-fabricated small-scale CNT electrodes. The novel electrodes are based on highly dense CNT mats synthesized by chemical vapor deposition (CVD) directly on conductive titanium nitride traces that were lithographically defined on silicon dioxide substrates. As will be presented below, the highly dense and entangled CNT ensembles form an effective three-dimensional electrode with very high volume specific capacitance. AC measurements reveal very low impedance frequency dependence (e.g. compared with platinum black electrodes). Finally, we demonstrate below that our CNT electrodes can be used as excellent substrates for cell adhesion and facilitators of high fidelity extracellular recordings. Recordings from cultured neuronal

cell assemblies are presented and discussed.

2. Experimental

2.1. Microfabrication

CNTs were synthesized by a computer controlled CVD system on p-type silicon substrates with a 500 nm thermal oxide layer (Mitsubishi silicon America), which were lithographically patterned with conductive titanium nitride traces. The conducting tracks are 80 nm thick with an overall measured resistances of 13.7 k Ω (resistivity of $457 \times 10^{-6} \Omega\text{cm}$). Chip preparation steps included TI35-ES positive photoresist lithography (MicroChemicals GmbH) followed by titanium nitride sputtering (MRC 8620). Lift-off and sonication in acetone were employed to remove the residual photoresist, followed by oxygen plasma treatment (Pico-RF-PC, Diener electronics) to remove any remaining organic residues. The entire substrate was then passivated by sputtering a 300 nm Si₃N₄ layer. Reactive ion etching (RIE, Nextral NE860) through the Si₃N₄ passivation layer was performed, following a second lithography step to define exposed resist-free windows on the titanium nitride conductors.

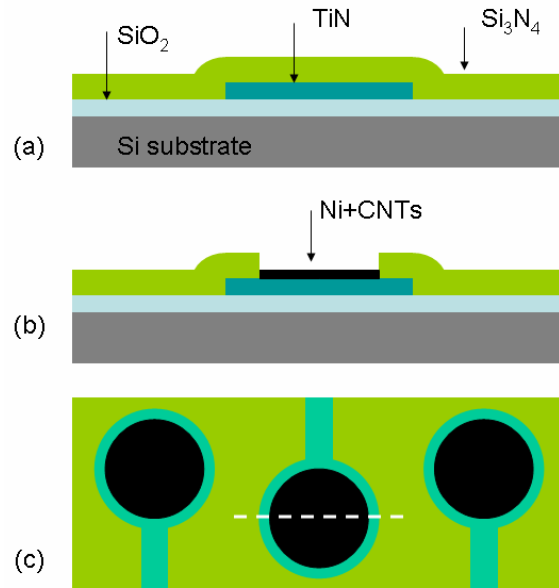


Figure 1. Schematics of the electrode micro fabrication process. The process is based on: (a) a standard micro-fabrication process to define the conducting tracks, a nitride passivation and (b) a self-aligned nano-fabrication step to define the CNT islands by evaporating a thin Ni layer which is used as the catalyst for the CNT growth. (c) Top view of the electrode scheme.

2.2. Carbon nanotube growth

A self-aligned process was used to evaporate (E-beam, VST) a 7 nm thick nickel layer on the exposed titanium nitride conductors to serve as a catalyst layer for the proceeding CNT growth. The Ni island size and thickness define the CNT site dimension and determine the surface packing of the CNT electrodes, respectively. CNT were then synthesized by CVD at 900 °C with hydrogen and ethylene gases in a 1” quartz tube furnace (Lindberg Blue, MiniMite 1100). CVD steps included purging the system at room temperature with hydrogen flow (1000 sccm) for 10 min followed by a temperature ramp to 900 °C at a rate of 35 °C/min at a constant hydrogen flow. At the target temperature, ethylene gas at 20 sccm was added to the hydrogen flow for 10 min, after which the ethylene gas and the furnace were both switched off, allowing the substrate to cool down to approximately 300 °C at a rate of 10 °C/min before the furnace was opened, and the samples were removed.

Surface characterization of the CNT coated surfaces was conducted by high resolution scanning electron microscope (HRSEM, JEOL 6700F) to determine the surface packing scale, and by high resolution transmission electron microscope (TEM, Tecnai F20, Philips) to examine and validate the structure of formed carbon nanotubes (images not shown).

2.3. Electrochemical tests

As fabricated chips were mounted on a printed circuit board using a silver containing adhesive (Elcolit 325) to establish external electrical connections. An EC chamber was created by mounting a glass cylinder (external diameter 20 mm) centered on the chip and sealed with cured polydimethylsiloxane (PDMS, sylgard 184, Dow Corning), the PDMS also serves to isolate the silver electrical contacts from the electrolyte inside the chamber. 3 cm³ phosphate buffered saline (PBS) comprising of 137 mM NaCl, 2.7 mM KCl and 10 mM buffer (pH=7.4 Cat. No. 79382, Fluka) was used as an electrolyte for the electrochemical tests. CNT electrode DC properties were compared against commercial TiN micro electrodes (Multi Channel Systems GmbH).

Electrochemical measurements were conducted in a standard three electrode cell, in which the CNT sites served as working electrodes, the counter electrode was made of a platinum wire (area 0.25 cm²) and a Ag/AgCl electrode (194 mV versus SHE) served as the reference electrode. Cyclic voltammetry (CV) measurements were conducted using a potentiostat (263A, Princeton Applied Research) under ambient conditions. Electrochemical impedance spectroscopy (EIS) was conducted under equilibrium conditions by applying a small (50 mV rms) AC signal over the frequency range of 200 Hz to 5 kHz using a lock-in amplifier (SR830, Stanford Research Systems) and a potentiostat.

2.4. Culture preparation and data acquisition

A mixture of cortical neuron and glia cells from rats were plated on fabricated and packaged MEAs at an initial cell count of 0.8×10^6 . Immediately after plating, the culture was mounted onto a 60 channel preamplifier socket (MC-Rack MEA 1060-AMP version 3.2.2.0, Multichannel Systems) for a period of three weeks. Proper culturing conditions (37 °C with 5% CO₂ and 75% humidity) for the entire recording period was assured by a specially designed chamber. Data was sampled at a rate of 10 kHz (gain 1200, inverting amplifier) and stored for off-line analysis. Data was recorded continuously but only spikes exceeding a threshold level of -80 μV were stored in symmetric 20 ms time windows, each containing 200 data points.

3. Results and discussion

The fabricated MEA layout includes sixty 80 μm diameter CNT based micro-electrodes, patterned in a regular $250 \times 250 \mu\text{m}$ array (figure 2a). The effectiveness of the fabrication process in producing high porosity electrodes is readily apparent in optical microscope inspection where extremely black electrodes can be seen, corresponding to a thick CNT layer formed on each electrode. This result is further established in the HRSEM inspection and in particular in the side view image (inset of figure 2b), which reveals that the fabricated electrodes are in fact three dimensional in nature with volume packing very similar to that of previously reported macroscopic CNT electrodes. The surface coverage, density and layer thickness are a direct result of carefully selecting the nickel catalyst layer thickness prior to the CVD step, and to the efficient role of the supporting titanium nitride layer in reducing nickel diffusion [23, 24]. During wetting, the electrode matrix compresses into a more compact structure of several μm in thickness. High resolution TEM imaging of the CNTs after growth reveals a typical multi walled CNT structures, several tens of nm in diameter. Typical tubes consist of wrapped carbon layers, 0.3 nm apart.

3.1. Electrochemical characterization

As prepared CNT electrodes were packaged and were then submitted to CV tests conducted at scan rates between 20 to 300 mV/s (figure 3a). The data reveal featureless rectangular curves for the entire scan rate range and for the selected potential sweep values. No electrochemical activation step was done before conducting the electrochemical tests, thus no broad features related to oxygen-containing functional groups associated with purification steps in nitric acid are observed [25].

The oxidation current versus the scan rate data, derived from the data in figure 3a and presented in figure 3b, exhibit a clear linear dependence corresponding to a DC

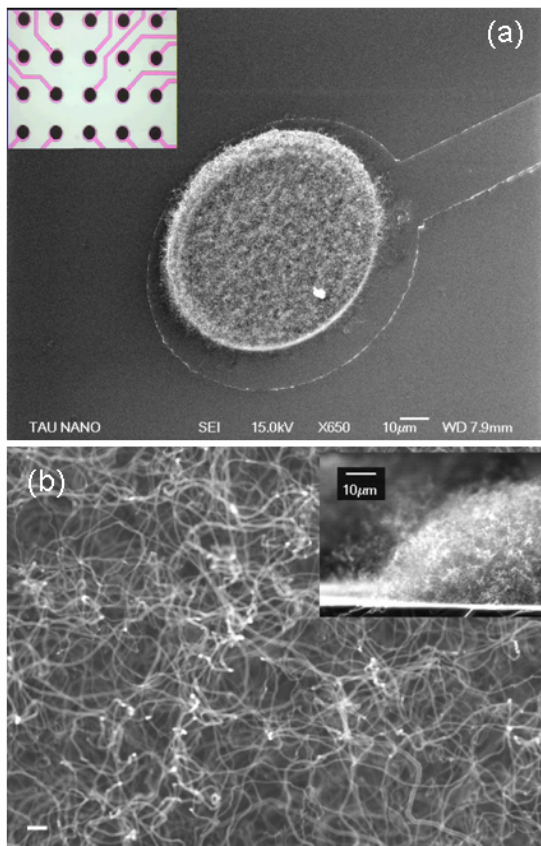


Figure 2. Micro-fabricated CNT MEA (a) Tilted HRSEM image of an 80 μm CNT island centered on a 100 μm conductive titanium nitride pad, patterned on a silicon dioxide substrate. (bar is 10 μm); Inset: Optical microscope image of a regular array of 20 CNT islands out of the total 60 comprising the entire MEA. (bar is 100 μm). (b) HRSEM image of the CNT island surface. (bar is 100 nm). Inset: Side view of a CNT island.(bar is 10 μm)

capacitance of 500 nF, derived from the relation: $i = C \frac{dV}{dt}$ in which i is the oxidation current, C is the linear capacitance and $\frac{dV}{dt}$ is the scan rate. Conspicuous measurable variations in the capacitance of different CNT electrodes on the same chip (up to 20%), correspond to variations in the obtained CNT packing, probably resulting from inhomogeneities in the thin Ni layer and the CVD growth process.

The overall electrode capacitance can then be converted into area as well as volume specific capacitances based on the apparent dimensions of the electrodes (figure 3). Normalizing the obtained capacitance with the area of the electrodes yields a conspicuously high specific capacitance of 10 mF/cm². This value is larger than the DC specific capacitance of commercial TiN micro-electrodes of 2.5 mF/cm². The value for the TiN electrode was derived from a 18 nF total capacitance for a 30 μm electrode measured in similar conditions. By normalizing the CNT electrode DC capacitance with the volume of the electrode (using 10 μm as an average height), a volume specific

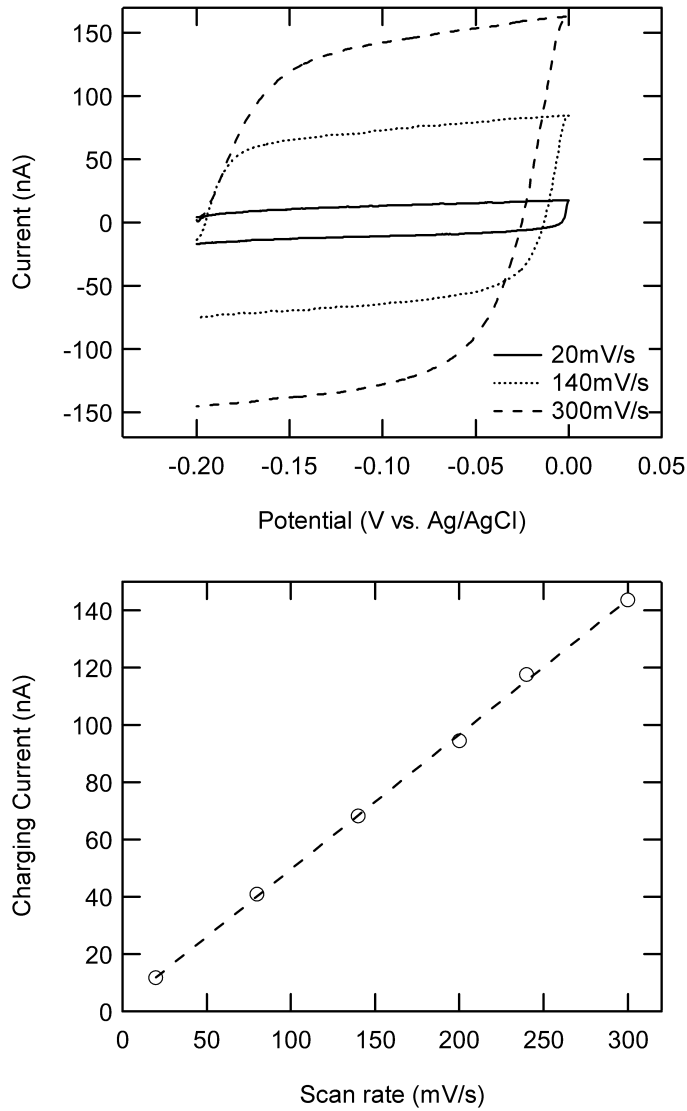


Figure 3. DC capacitance measurement. (a) CV scans of CNT coated electrodes. CVs presented were conducted at 20, 140 and 300 mV/s in PBS. (b) Oxidation current (open circles) versus scan rate for CNT coated MEAs and a linear fit (dashed line).

capacitance of 10 F/cm^3 is obtained, which is consistent with previously reported values of macroscopic CNT electrodes [20]. The extremely high area specific capacitance of the CNT electrodes, as presented here, is thus a direct consequence of their effective three dimensional nature [20].

3.2. AC impedance

The AC impedance magnitude of the CNT based micro-electrodes was determined by EIS experiment under equilibrium conditions over the range of 200 Hz to 5 kHz. Figure 4 shows the frequency dependence of the impedance of a typical CNT electrode. Two main features are apparent in figure 4: First, is the low impedance of the CNT electrode, 1.1 k Ω at 1 kHz. This value when normalized, compares with best values achieved with other electrode materials such as sputtered Ir or TiN [26]. A second important issue is the weak frequency dependence of the CNT electrode impedance. A power law fit according to $Z_e=R_0f^m$ [3] (dashed line, figure 4) yields m values ranging between -0.11 and -0.36 for CNT electrodes. These values are lower than typical values obtained with platinized electrodes which vary between -0.6 and -0.8, depending on the amount of electro-deposited Pt. The apparat reduced frequency dependence of CNT electrodes reflects higher porosivity, and the variations in m reflect various porosivity levels. As in the DC case, variations in the frequency dependence of different CNT electrodes on the same chip are a result of inhomogeneities in the thin Ni layer and the proceeding CVD growth process.

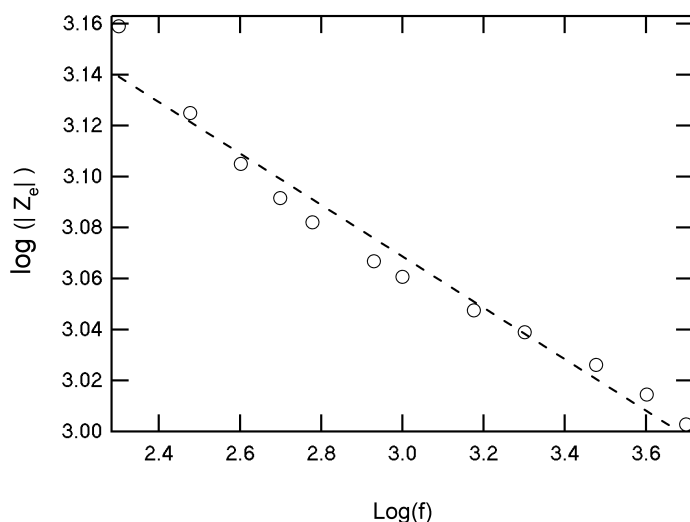


Figure 4. AC impedance magnitude Z_e (open circles) and a linear fit (dashed line) versus frequency in a log-log scale for a carbon nanotube electrodes over the frequency range of 200 Hz to 5 kHz.

We have so far shown that CNT based micro-electrodes benefit from high specific DC capacitance, as well as from weak frequency dependant AC impedance. We now turn to describe how these benefits contribute to the electrical recording performances of the electrodes.

3.3. Extracellular recordings from cell cultures

The superb DC and AC properties of the CNT MEA, as we have demonstrated in the previous sections, are compounded by their interesting interaction with biological cells [16, 17, 18, 19, 27]. As we have shown in previous publications [17, 27], the roughness of the CNT islands acts as an adhesion agent and cells spontaneously migrate and adhere onto the electrode surface (lower inset of figure 5). This property may further contribute to the effectiveness of the electrode as a recording element, as the cell-electrode coupling is very strong. To investigate the electrical recording capabilities of the CNT electrodes, cortical neuronal cells were plated and cultured on the surface of CNT electrode chips as described above. The electrode surface was not treated by adhesion promoting proteins and is maintained pristine.

After 14 days in culture, spontaneously induced extracellular signals were recorded from cell assemblies formed on the CNT electrodes (figure 5). Recorded signals demonstrate a typical extracellular signal shape reflecting the first derivative of the intracellular action potential signal [11]. Typical recorded signals are in the several hundred μV range. The recorded data is also marked by a signal to noise ratio (SNR) as high as 135. These values should be compared with typical MEA recording depicted in the top inset of figure 5, which are marked by significantly smaller SNR and an overall smaller signal. It is also important to note that the CNT electrodes exhibit a wide repertoire of signals possibly corresponding to several cells forming a cluster on the electrodes.

The quality of the recorded extracellular signals can be evaluated in terms of their SNR, the overall signal amplitude and the shape of the signal. Ideal recordings would be marked by high SNR and high signal fidelity. The better the recording performances of the electrodes, the more feasible it is to separate recorded signals into distinctly recognized signal shapes, which can then be attributed to different neurons in the system, thus enhancing the effectiveness of the recording system [1].

The apparent improvement in the SNR of the CNT MEAs can be readily understood by considering several important factors. First, the improved electro-chemical properties, as demonstrated above, contribute to a lower noise content. This can be seen from the relation between the noise content and the electrode impedance which is given by $U_{th} = \sqrt{4kTB R_{tot}}$ where k is the Boltzman constant, T is the temperature in degrees Kelvin, B is the frequency bandwidth and R_{tot} is the real part of the total electrode impedance. For a 5 kHz amplifier bandwidth, the CNT electrodes presented here are expected to yield a value of 1 μV rms. The total measured noise level of 7 μV rms can be ascribed to the amplifier noise level which can be as high as 6 μV rms. The apparent improvement in the signal amplitude suggests that improved cell-electrode coupling is also playing a vital role. It is important to note that the culturing of the cells was performed directly on exposed CNT surfaces. This fact is possibly contributing to

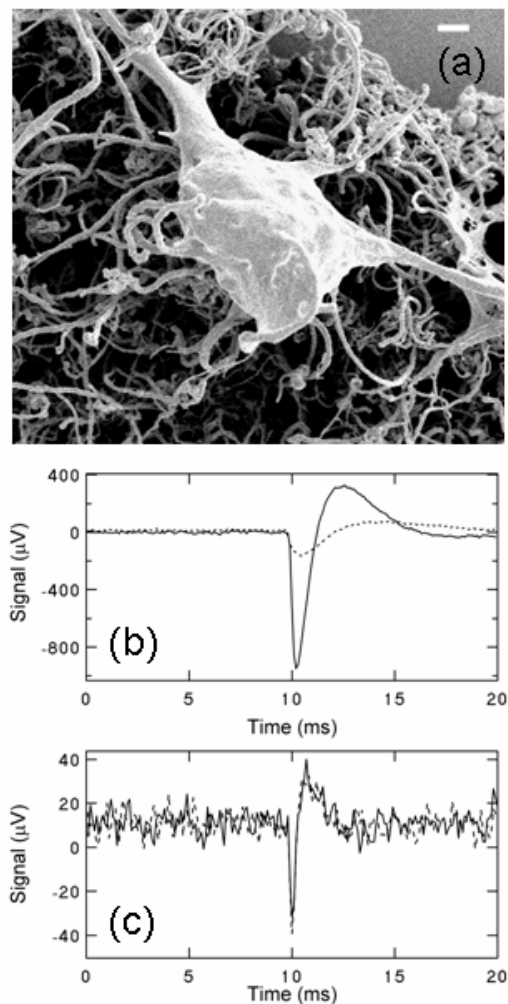


Figure 5. (a) HRSEM image of a neuronal cell on a CNT surface. (b) High SNR extracellular signals recorded from cultured cortical neurons using 80 μm carbon nanotube based electrode. (c) Extra-cellular signals recorded from cultured cortical neurons using a commercial 30 μm TiN electrode.

further improvement in the coupling between the electrodes and the cells.

4. Summary

A novel CNT based multi-electrode scheme was produced and characterized by cyclic voltammetry and impedance spectroscopy experiments. Rat cortical cells were plated and the extracellular signals were recorded using the CNTs as the active electrode component. The enhanced electrochemical properties and simple production process makes the suggested carbon nanotube based micro electrodes superior as electrochemical electrodes. Moreover, with its chemical durability and bio-compatibility the presented ar-

rays can be used as a mediator between biological samples and silicon-based capacitive recording systems.

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