# COMPARISON OF *IN VITRO* CORROSION BEHAVIOUR AND BIOCOMPATIBILITY OF Ti-13Zr-13Nb AND PASSIVATED 316L STAINLESS STEEL COATED WITH TiCN

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*Abstract.* AISI 316L stainless steel finds widespread use as implant materials. However, it is prone to localized attack in the body fluids. Titanium alloys have the highest corrosion resistance among the metallic materials used as orthopaedic prostheses. Therefore, an implant material that unites the high surface stability of titanium alloys and the low cost of the 316L is of great interest. TiCN films obtained by PVD processes present high wear and corrosion resistances making them attractive materials for orthopaedic applications. However, there is little information about the corrosion behaviour of TiCN-coated stainless steels in physiological solutions. In this study, 316L specimens were passivated in nitric acid solution for 10 minutes and then coated with TiCN films produced by PVD process. The corrosion resistance of the coated specimens was evaluated by Electrochemical Impedance Spectroscopy (EIS) and potentiodynamic polarization tests after 28 days of immersion in Hanks' solution. The results were compared with those obtained from bare, passivated, non passivated and TiCN-coated 316L steel, and bare Ti-13Zr-13Nb specimens. The passivated and TiCN-coated 316L specimens presented corrosion resistance similar to that of bare 316L. The highest corrosion resistance was obtained for Ti-13Zr-13Nb specimens. The *in vitro* biocompatibility of Ti-13Zr-13Nb and TiCN-coated 316L was investigated using a citotoxicity assay. Both materials were not cytotoxic in the conditions tested.

*Keywords*: 316L,Ti-13Zr-13Nb, corrosion, cytotoxicity

## 1. Introduction

Austenitic stainless steels of type 316L are among the most common implant materials for orthopaedic applications. The combination of good corrosion resistance, easy hot and cold workability, satisfactory mechanical properties and low cost accounts for this spread use (Kannan, Balamurugan and Rajeswari 2003). However, the protective oxide layer inherent to 316L SS is not stable during continuous exposure to physiological solutions. Consequently, crevice or pitting corrosion may occur. Several works in the literature report these problems (Sivakumar, Mudali and Rajeswari 1993, Xulin et al. 1997, Arumugam, Rajeswari and Subbaiyan 1998). Metal ions released by corrosion processes may accumulate in internal organs leading to allergic or infectious reactions, which in turn may result in failure of the implant device (Gurappa 2002). Titanium alloys were developed as alternative materials to stainless steels and have since been extensively used as biomaterials. One of these alloys is Ti-13Nb-13Zr, a near-β phase alloy containing elements with excellent biocompatibility (Okazaki et al., 1996). The main advantages of the Ti-13Nb-13Zr alloy, compared to other titanium alloys, such as Ti-6Al-4V and Ti-6Al-7Nb, widely used as biomaterials, are its low elastic modulus, closer to that of bone, and the absence of aluminum and vanadium, which have been associated to short- and

long-term adverse effects (Davidson et al. 1994). The main disadvantage of titanium alloys compared to austenitic stainless steels is its higher cost. An attractive economic alternative is the coatings deposition on 316L SS to improve its corrosion resistance and biocompatibility. Carbonitride films (TiCN) produced by PVD processes present a combination of high wear resistance and biocompatibility. So, its use may be considered for coating orthopaedic implants (Shtansky et al. 2004, Precht et al. 1996, Senna et al. 1997). However, there is little information in the literature about the corrosion resistance of TiCN-coated 316L stainless steel (SS) in physiological medium. Previously reported results showed that no significant improvement was observed on the electrochemical behaviour of TiCN-coated 316L in Hanks' solution at 37ºC comparatively to that of the bare 316L stainless steel (Antunes, Melo and Costa 2004). According to the literature (Shih et al. 2004, Kannan, Balamurugan and Rajeswari 2004, Noh et al. 2000), the passivation of the stainless steel surface in acidic solution may be a cheap and effective method to improve its corrosion resistance.

The aim of this work was to evaluate the corrosion resistance of 316L stainless steel (ASTM F-138) in three distinct conditions: bare, passivated in nitric acid solution, and passivated and TiCN-coated. Bare Ti-13Zr-13Nb alloy was also used for comparison. Electrochemical impedance spectroscopy and potentiodynamic polarization tests were used as investigating techniques. The biocompatibility of the materials was determined using a cytotoxicity assay.

## 2. Experimental

### 2.1 Materials and surface treatments

The chemical compositions of 316L and Ti-13Zr-!3Nb are shown in Tab. 1.

Table 1. Chemical compositions of 316L and Ti-13Zr-13Nb.

| Element | Composition (mass %) | |
| --- | --- | --- |
| | 316L | Ti-13Zr-13Nb |
| C | 0.007 | 0.035 |
| S | 0.002 | <0.001 |
| Si | 0.037 | ----- |
| P | 0.007 | ----- |
| Cr | 17.40 | ----- |
| Mn | 1.780 | ----- |
| Cu | 0.030 | ----- |
| Ni | 13.50 | ----- |
| Mo | 2.120 | ----- |
| N | 0.070 | 0.004 |
| Fe | 65.05 | 0.085 |
| Ti | ----- | 72.91 |
| Zr | ----- | 13.49 |
| Hf | ----- | 0.055 |
| Nb | ----- | 13.18 |
| H | ----- | 0.011 |
| O | ----- | 0.078 |

*Passivation treatment.* Some 316L specimens were passivated in nitric acid solution (30% v/v) at room temperature during 10 minutes. After passivation the specimens were rinsed in deionized water and dried under a hot air stream.

*TiCN coating.* The TiCN coatings produced by PVD method were deposited on passivated 316L specimens, in a vacuum chamber. The deposition rate was 0.8 - 1.0 μm/hour. The resulting film thickness was in the range of 2-3 μm.

### 2.2 Electrochemical measurements

Bare 316L and Ti-13Zr-13Nb specimens were prepared by epoxy cold resin mounting of both alloys, leaving areas corresponding to 0.33 cm$^2$ and 1.7 cm$^2$, for Ti-13Nb-13Zr alloy and 316L SS, respectively, for exposure to the electrolyte. The surface of bare specimens were prepared by sequential grinding with silicon carbide paper up to #2000 finishing, followed by mechanical polishing with diamond paste of 1 μm. Passivated and passivated and TiCN-coated 316L specimens, with an exposure area to the electrolyte of approximately 1.7 cm$^2$, did not undergo any surface grinding or polishing. All the specimens were degreased with acetone and rinsed in deionized water. After surface preparation and prior to the electrochemical tests, all the specimens remained immersed for 24 hours in Hanks' solution, naturally aerated at 37 $^o$C.

A three-electrode cell arrangement was used for the electrochemical measurements, with a saturated calomel reference electrode (SCE) as reference electrode and a platinum wire as the auxiliary electrode. The EIS tests were

carried out in triplicate to evaluate results reproducibility. The electrolyte used to simulate the physiological medium was Hanks' solution, naturally aerated (pH=6.8). Its composition is presented in Tab. 2. All specimens were immersed in the electrolyte for a period of 28 days.

Table 2. Chemical composition of Hank's solution.

| Component | Concentration (Mol/L) |
|---|---|
| NaCl | 0.1369 |
| KCl | 0.0054 |
| $MgSO_4.7H_2O$ | 0.0008 |
| $CaCl_2.2H_2O$ | 0.0013 |
| $Na_2HPO_4.2H_2O$ | 0.0003 |
| $KH_2PO_4$ | 0.0004 |
| $C_6H_{12}O_6H_2O$ | 0.0050 |
| Red phenol 1% | 0.0071 |
| pH | 6.8 |

EIS measurements were carried out with a frequency response analyser (Solartron SI-1255) coupled to a potentiostat (EG&G PARC 273A). All EIS measurements were performed in potentiostatic mode at the corrosion potential ($E_{corr}$). The amplitude of the perturbation signal was 10 mV, and the investigated frequency range varied from $10^5$ Hz to $10^{-2}$ Hz with an acquisition rate of 6 points per decade.

A potentiostat/galvanostat EG&G 273A was used for the measurements. Potentiodynamic polarization curves were obtained after 28 days of immersion in Hanks' solution at room temperature, using a scanning rate of 1 $mV.s^{-1}$. The measurements started at -800 $mV_{SCE}$ and finished at 3000 $mV_{SCE}$.

## 2.3 Cytotoxicity assay

The method is based on the quantitative assessment of surviving viable cells upon exposure to the toxic agent, by incubation with the supravital dye tetrazolium compound MTS (3-(4,5-dimethyltiazol-2-yl)5-(3-carboxymethoxyphenil)-2-(4-sulphophenil-2H-tetrazolium) and an electron coupling reagent PMS (phenazine methosulphate). MTS is bioreduced by cells into a formazan product that is soluble in tissue culture medium, then colorimetric analysis of the incorporate dye is performed. The amount of MTS, the marker of cell viability, taken up by the population of cells, is directly proportional to the number of viable cells in culture. The test sample inducing cell toxicity is measured over a range of extract concentrations of the biomaterial and the concentration yielding a 50% reduction in MTS uptake is taken as the toxicity parameter.

The cytotoxicity assay of bare 316L, TiCN-coated 316L and Ti-13Zr-13Nb was carried out according to ISO 10993-part 5. Chinese hamster ovary cells culture (ATCC CHO K1) was used in this investigation. The CHO cells was cultured in RPMI 1640 medium containing 10% of fetal calf serum (FCS). A 96-well tissue culture microtiter plate was prepared by addition of increasing dilutions (6,25; 12,5; 25;. 50, 100%) of the biomaterial extract (50 µl/well, 4 wells/each dilution). Next, 50 µl of the cell suspension (3000 cells) are dispensed into the wells. Control columns of four wells were prepared with medium without cells (blank) and medium instead extract plus cells (negative control). The microplate is incubated under 5% $CO_2$ humidified atmosphere. After 72 h, 20 µl of a mixture (20:1) of 0.2% MTS and 0.09% PMS in PBS was added to the test wells and left for 2 h. Incorporate dye was measured by reading the absorbance at 490 nm in a microplate reader against the blank column. Cytotoxicity index ($IC_{50\%}$) was estimated by curve interpolation as the biomaterial extract concentration resulting in 50% inhibition of MTS uptake after plotting the mean percentage of surviving cells against the concentration of the extract (%). In this work phenol solution 0.3% and titanium plate were used as positive and negative control, respectively.

## 3. Results and Discussion

Bode plots (phase angle) for bare 316L, passivated 316L, passivated TiCN-coated 316L and Ti-13Zr-13Nb are shown in Fig. 1 for 1 day and 28 days of immersion in Hanks' solution.
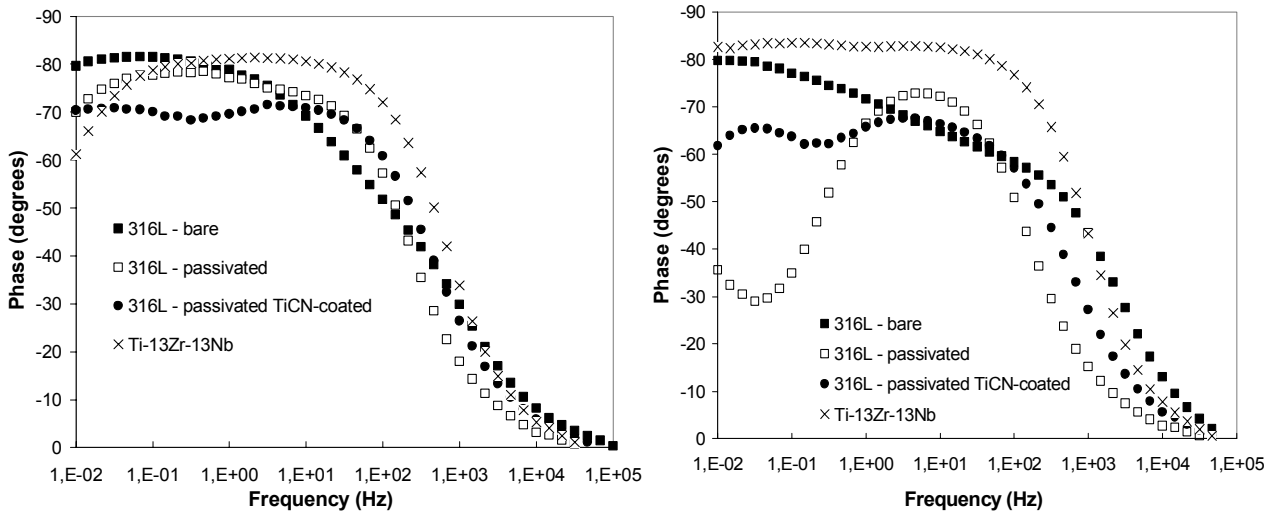
Figure 1. Bode plots (phase angle) for: (a) 1 day and (b) 28 days of immersion in Hanks' solution at 37ºC.

The EIS results of bare 316L SS for one day of immersion show a highly capacitive behaviour at low frequencies (Fig. 1a). The phase diagram presents two time constants that are not clearly separated due to some overlapping, but it is possible though to identify a shoulder around 10 Hz. The second time constant at lower frequencies shows phase angles approaching –80º. Stainless steels have a passive superficial layer consisting mainly of $Cr_2O_3$ (Milosev and Strehblow 2000) that accounts for their relative high corrosion resistance. It is likely that the two time constants are due to the response of the passive layer (still with few defects due to the short immersion period) and to charge transfer reactions at surface of the 316L substrate. After 28 days of immersion (Fig. 1b), the two time constants are more clearly separated. The peak related to the first time constant (at intermediate frequencies) shifts to higher frequencies and decreases with time from one day to 28 days, indicating the improvement of the oxide film protective characteristics, while the second one, at lower frequencies, remains highly capacitive suggesting that significant deterioration did not occur during the whole immersion period. This is likely due to blockage of the defective areas in the oxide as the film grows with time.

The passivation treatment significantly altered the electrochemical behaviour of the 316L stainless steel, especially for 28 days of immersion. The EIS data corresponding to the passivated 316L specimen for 1 day shows that the time constant peak at medium frequencies is higher and also occurs at higher frequencies comparatively to the bare 316L specimen. However, slightly less capacitive angles are associated to the time constant at lower frequencies for the passivated specimen (Fig. 1a). These results could be explained by the growth of a porous film on the passivated specimen, favouring the charge transfer processes at the porous base. The results obtained for 28 days of immersion support this hypothesis showing a significant decrease in the phase angles at lower frequencies followed by the indication of a new time constant at frequencies below 0.1 Hz, that could be indicating localized attack. It is possible that during passivation in the nitric acid solution the fast oxide growth tends to develop a film with more internal defects than that slowly and naturally formed.

The EIS results of passivated and TiCN-coated 316L specimen present two time constants, the first at medium frequencies, with a peak between 100 and 10 Hz, likely due to the defects in the TiCN film, and the second, with peak between 0.1 and 0.01 Hz. From one to 28 days of immersion the phase angles values did not show significant changes. During this period the phase angles slightly decreased and the peak associated to the first time constant (medium frequencies) dislocated to lower frequencies, suggesting slight deterioration of the coating and, consequently, increase of the charge transfer processes on the substrate. This result is supported by the literature (Bull, Bhat and Staia 2003) that reports the presence of intrinsic defects, such as pinholes and voids in PVD TiCN deposited coatings. The first time constant is related to the impedance response of the TiCN film defects at medium frequency values

The Ti-13Zr-13Nb alloy shows highly capacitive response, with phase angles approaching –80º in the medium frequency range and, for one day of immersion, the decrease in the phase angles at frequencies below 1 Hz. From one day to 28 days of immersion the Bode phase angle diagrams indicate the increase in the phase angles at lower frequencies, and the formation of a large plateau from 100 Hz to the lowest frequencies ranged, indicating a highly protective layer on the alloy surface. This behaviour suggests a very stable passive film on the surface of the titanium alloy. Assis et al. (2005) interpreted this result considering a duplex character for the passive film on the titanium alloy composed of an inner barrier layer and an outer porous layer. The barrier layer would account for the corrosion resistance of the oxide film.

Nyquist plots for the bare 316L, passivated 316L and passivated and TiCN-coated 316L obtained after 1 and 28 days of immersion are shown in Fig. 2. All the plots are characterized by a capacitive and incomplete loop, and for 1

day of immersion, the impedance of the bare 316L specimen was slightly higher comparatively to the other specimens tested, passivated and passivated and TiCN-coated 316L specimens. The EIS data for 28 days (Fig. 2b) show that the bare 316L specimen remained highly capacitive throughout the whole test period, while the impedance of the passivated specimen significantly decreased with time. For the passivated and TiCN coated specimen, the impedance also decreased, but this decrease was not as sharp as for the only passivated specimen and intermediate values between those corresponding to the bare and the passivated specimens, were obtained for 28 days of immersion.
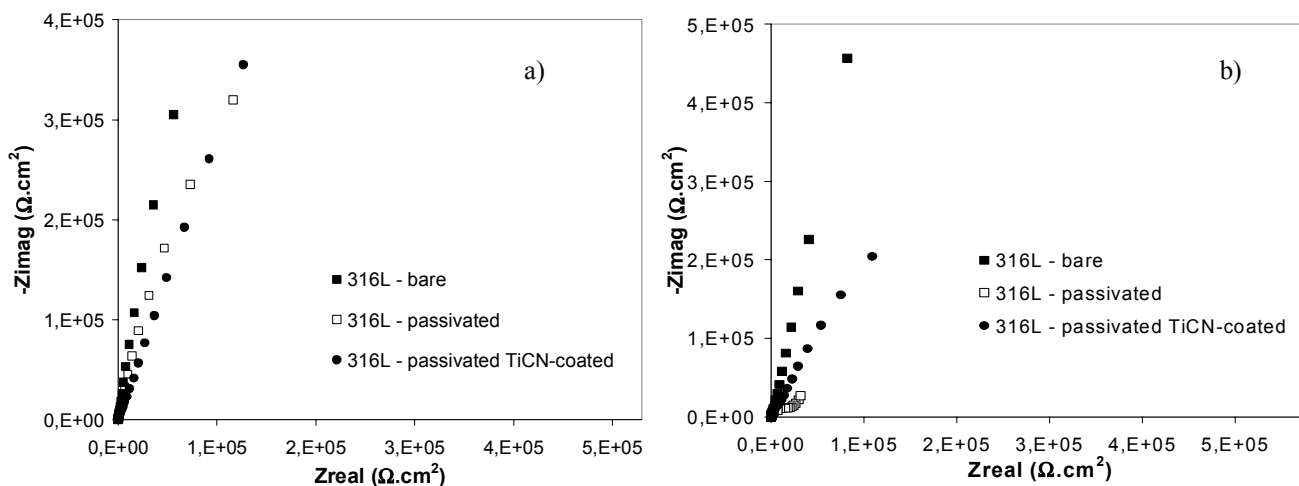


Figure 2. Nyquist plots corresponding to: (a) 1 day and (b) 28 days of immersion in Hanks' solution at 37ºC.

The potentiodynamic polarization curves for all the materials tested after 28 days of immersion in Hanks' solution naturally aerated and at 37ºC are shown in Fig. 3.
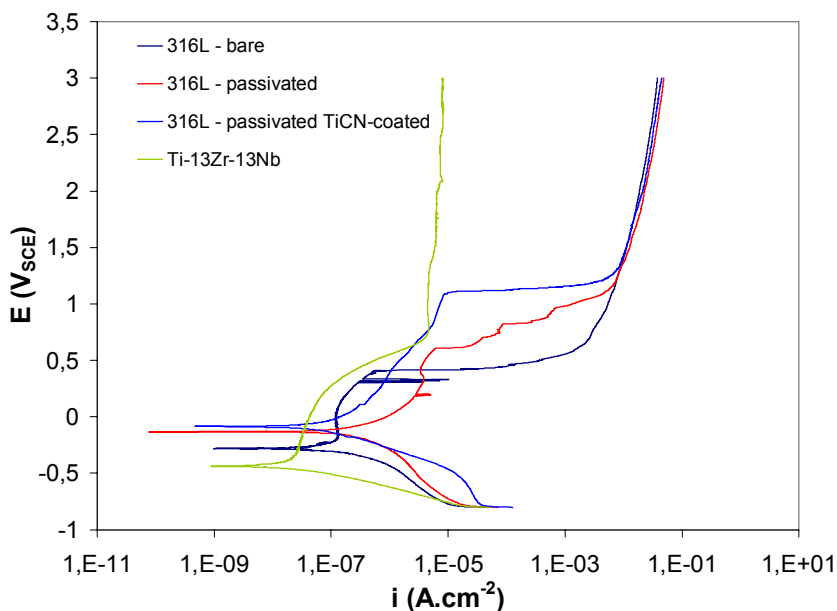


Figure 3. Potentiodynamic polarization curves after 28 days of immersion in Hanks' solution at 37ºC.

The results of Fig. 3 show that the bare 316L specimen is prone to pitting corrosion in the Hanks' solution with a breakdown potential around $+0.4V_{SCE}$. However, the tendency to pitting corrosion appears at lower potentials characterized by current instabilities at potentials around +0.35V. The polarization curve of the passivated specimen shows increased corrosion current and passive current densities comparatively to the bare specimen, supporting the EIS results. Although a higher breakdown potential (+0.58V) was obtained for the passivated specimen relatively to the bare one, the overpotential for passive film breakdown was similar for both specimens, bare and passivated. As mentioned previously, it is likely that the faster kinetics of oxide film growth on the 316L surface passivated might have led to a less compact passive layer. For the passivated and TiCN coated specimen, the anodic current densities obtained at low

overpotentials, were intermediate between the bare and the passivated specimens, also confirming the EIS results. However for the TiCN coated specimen, the passive range extended from +0.15 up to +1.1V. At this last potential a sharp increase in current density occurred that may be due to the oxygen evolution reaction rather than to the onset of pitting corrosion. This observation must be confirmed by SEM observation of the surface after polarization.

As expected, the Ti-13Zr-13Nb alloy presented the best corrosion behaviour among the tested materials showing no tendency to pitting corrosion in the whole potential range investigated. The passive current and corrosion current densities related to the Ti alloy are significantly lower compared to 316L specimens in the three conditions investigated.

The cell viability curves of the different biomaterials investigated are shown in Fig. 4.
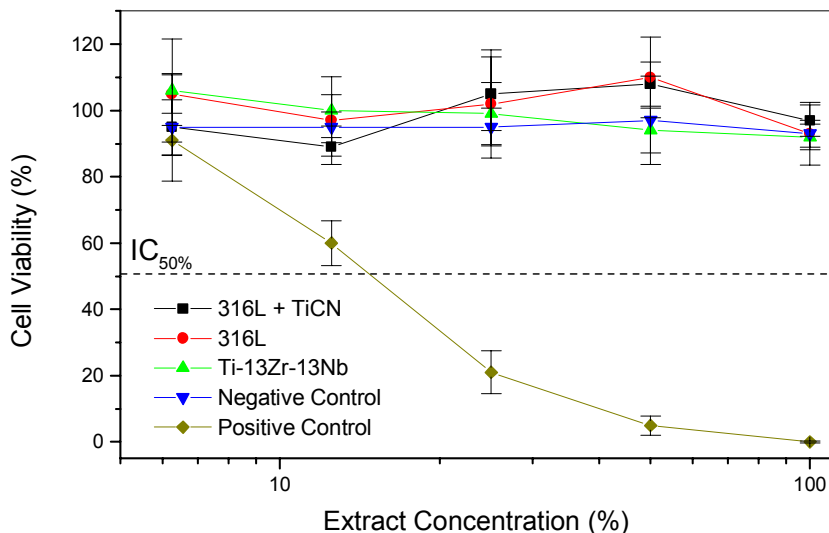


Figure 4. Cell viability curves for the different biomaterials tested.

All the materials tested showed no cytotoxicity, similarly to the negative control ($IC_{50\%} > 100$). To confirm the reliability of the method, the positive control showed a cytotoxic effect over the cells ($IC_{50\%} = 15$). The cytotoxicity test in the biological evaluation is a toxicological screening test that can predict the biocompatibility of 316L stainless steel in the two distinct surface conditions studied. This is a primary indication of the suitability of TiCN-coated 316L stainless steel for biomedical applications.

## 4. Conclusions

The results presented here suggest that the passive treatment used in this work is not appropriate to increase the corrosion resistance of 316L stainless steel used as biomaterial. The deposition of a TiCN coating on the passivated 316L surface, however, improved the resistance to localized corrosion of the 316L surface, either bare or passivated. Furthermore, the non-cytotoxic character of the TiCN coating is an important feature when considering its use for implant materials. Future investigations are necessary to develop a more suitable passivation pre-treatment that produces a very stable and corrosion resistant surface of 316 stainless steel .

## 5. Acknowledgements

## 6. References

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