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EVALUATION OF TITANIUM AND NITINOL STABILITY IN THE ORAL ENVIRONMENT

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Abstract

The interaction between dental alloys and saliva affects both their properties and those of metallic materials. **Aim**: to analyze the in vitro interaction between a series of titanium-based dental alloys and artificial saliva. **Materials and Methods:** Samples made of Titanium and Nitinol were studied. Corrosion under tension was chosen, and a spectrophotometer was used to record the spectra of the solutions after the electrochemical treatment of the samples. In vitro testing of the cytotoxic impact of the studied eluates was conducted through flow cytometry analysis. **Results:** Titanium is by far the most resistant biomaterial, exhibiting the lowest corrosion rate. The cyclic potentiodynamic curve for Nitinol indicates typical pitting corrosion. Flow cytometry shows increased percentages of live cells, very low percentages of dead cells, an almost complete disappearance of pre-apoptotic cells, and an increase in the number of apoptotic cells for both materials. **Conclusions:** Both materials demonstrate increased stability in the oral environment and reduced cytotoxic potential.

Keywords: corrosion; cytotoxicity; saliva.

Prosthetic restorations play a crucial role in maintaining function, aesthetics, and overall oral health, providing durable and functional solutions for replacing lost or damaged teeth, which significantly contributes to patients' quality of life. Titanium is commonly used in alloys for dental implants due to its excellent mechanical properties, low density (4.5 g/cm^3), and high biocompatibility when in contact with bone (1). The most widely used alloy is commercially pure titanium (cpTi), available in four grades numbered 1 to 4, depending on the purity and oxygen content during processing (2). These grades vary in corrosion resistance, ductility, and mechanical strength, with grade 4 cpTi, which has the highest oxygen content (approximately 0.4%) and the best overall mechanical strength, being the most used in dental implants (Table I) (2, 3).

There is also an alloy called Ti-6Al-4V, also known as grade 5 titanium, widely used in orthopedics due to its superior strength and reduced Young's modulus (4, 5). Although this alloy can also be used in dentistry, it releases small amounts of aluminum and vanadium, substances that, at high concentrations, can cause biological changes, such as aluminum interfering with bone mineralization and cytotoxic and allergic effects (6). However, the levels released by this alloy are well below those necessary to produce toxic effects (7). Studies have confirmed that this alloy can achieve satisfactory osseointegration, especially when treated to enhance the oxide layer on the surface (8, 9).

Nickel-titanium (Nitinol) alloys have rapidly become the preferred materials for self-expanding stents, graft support systems, and various other devices used in minimally invasive interventional and endoscopic procedures. Currently, many medical device companies offer products whose performance is based on the unique properties of these

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materials (10). Despite concerns regarding the biocompatibility of nickel-containing devices, NiTi components have been introduced in implant dentistry to secure fixed or removable prosthetic restorations or to eliminate gaps at the implant-abutment interface (11). Nitinol is primarily known for its superelasticity and thermal shape memory. "Shape memory" describes the material's ability to return to a pre-set shape when heated after being plastically deformed, while "superelasticity" refers to the exceptional elasticity of these alloys. This elasticity can be up to 10 times greater than that of the best stainless steels used in medicine and follows a non-linear path, characterized by pronounced hysteresis. Although these properties are impressive, they are not the only important characteristics of Nitinol (10).

Table 1. Composition and properties of titanium alloys used as implants (12)

	cpTi Grade 1	cpTi Grade 2	cpTi Grade 3	cpTi Grade 4	Ti6Al4V
Titanium	ca 99%	ca 99%	ca 99%	ca 99%	90%
Oxygen	0.18%	0.25%	0.35%	0.4%	0.2% max
Iron	0.2%	0.2%	0.2%	0.3%	0.25%
Nitrogen	0.03%	0.03%	0.05%	0.05%	-
Hydrogen	0.15%	0.15%	0.15%	0.15%	-
Carbon	0.1%	0.1%	0.1%	0.1%	-
UTS/MPa	240	340	450	550	900
Yield					
strength/MPa	170	275	380	480	850
Elongation at failure%	25	20	18	15	10

For this reason, the present study aims to analyze the in vitro interaction between a series of titanium-based dental alloys and artificial saliva, focusing on both the effect of saliva on the corrosion of the alloys and the changes produced in the properties of saliva.

MATERIALS AND METHODS

Metal samples, Titan and Nitinol in the form of metal plates with dimensions no greater than 2 cm^2, were examined in order to accomplish these goals. Following the guidelines of the production businesses, the instruments were used successively for the casting of the alloys, processing, and the finishing of the samples.

The grinding of the surface of the metal alloys to be examined was made with granules of different sizes (10–1200 μ). The surface was finally polished in two stages: at first, we polished the surfaces with diamond paste (1 granules) and, in the last step, with diamond paste with lower granulation (0.25 granules) (Diateh, Bucharest, Romania).

The liquid medium used was an artificial saliva proposed by Duffo and Quezada, whose composition is presented in Table II. This composition was chosen because previous studies indicated that it has corrosion properties very similar to natural saliva. The pH value of this solution was determined using an OP-208 pH/mV meter from RADELKIS (Budapest, Hungary): pH = 7.08.

Since the corrosion process occurring simply by immersing the alloy in the corrosive medium is very slow (natural corrosion), the potentiodynamic method was used to study electrochemical corrosion processes.

Table II. Composition of Duffo and Quezada Saliva (13)

Composition (g/L)	
NaCl	0.600
KCl	0.720
$CaCl_2 \cdot 2H_2O$	0.220
KH_2PO_4	0.680
$Na_2HPO_4 \cdot 12H_2O$	0.856
KSCN	0.060
$KHCO_3$	1.500
acid citric	0.030

The PGP201 potentiostat/galvanostat (VoltaLab 21) was employed, and the acquisition and processing of experimental data were performed using VoltaMaster 4 software. The VoltaLab 21 potentiostat has a useful volume of 50...150 ml. A miniature saturated Hg/Hg2Cl2 (calomel) reference electrode with a Luggin capillary was used as the reference, and a platinum electrode with a surface area of 0.19 cm² was used as the auxiliary (measuring) electrode.

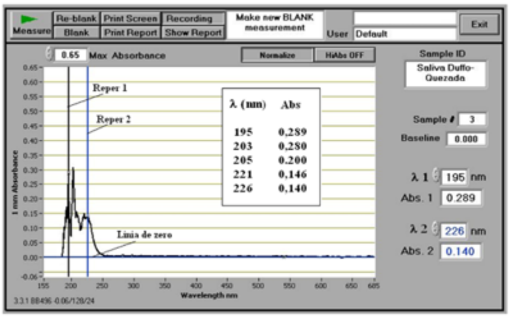
For the study of "forced" (or stimulated) corrosion, cyclic polarization curves (cyclic voltammograms) were used. To obtain these curves, the potential of the electrode formed with the alloy under study was increased at a constant rate in the positive direction to a pre-set value, after which it was scanned in the reverse direction (toward negative values, returning to the initial value or another value). Throughout the potential scan, the current passing through the solution between the working electrode and an auxiliary electrode (platinum) was measured. In this study, cyclic polarization curves (cyclic voltammograms) were recorded in the potential range between (-500) mV and (+2000) mV, with a potential sweep rate of 10 mV/s. The process started from a sufficiently large negative potential to reduce all ionic or molecular species in the solution and possibly from the alloy surface, to carry out the electrochemical process on a "clean" surface. The rate of change of the working electrode potential was relatively high, to obtain sufficiently large current intensities to cover any accidental fluctuations in the system, but low enough to detect all processes occurring in the solution or on the electrode surface.

To determine the pH of the corrosion media before and after the electrochemical treatment of the alloys, a pH/mV meter OP-208 RADELKIS (Budapest, Hungary) was used. A mixed electrode composed of a saturated calomel electrode (SCE) and a glass electrode, mounted together, was used for pH determination. The calibration of the devices was done using three buffer solutions. The experimental results are presented in Table 2.saliva proposed by Duffo and Quezada (Duffo and Quezada), whose composition is presented in Table III.

Tabelul III. The pH values of artificial saliva after the electrochemical attack of the alloys

Saliva	Nitinol	Titan
7,08	7,47	7,44

For recording the spectra of solutions after the electrochemical treatment of the alloys, a NANODROP-1000 UV-VIS spectrophotometer (Germany) was used. When measurements are made in UV-VIS mode, the image shown in Figure 1 is displayed on the monitor. After initializing the device, calibration (recording the zero line → Blank, which appears at the bottom of the image on the screen) is performed. In the present measurements, the zero line was calibrated with distilled water—the liquid component of artificial saliva. After the sample is introduced and the measurement is performed (→ Measure), the spectrum of the used solution appears in the screen window. The image in Figure 1 records the spectrum of the initial artificial saliva. In the result display window, there are two cursors (labeled as Marker 1 and Marker 2 in the figure) that can be manually moved (with the mouse) over various absorption peaks. The wavelength and absorbance corresponding to that peak appear in the boxes at the bottom right of the screen. The spectrum can be printed or saved as a .jpg image. By moving the two cursors,



User: Default
Max Absorbance: 0.65
Sample ID: Saliva Duffo-Quezada
Sample #: 3
Baseline: 0.000
λ 1: 195 nm
Abs. 1: 0.289
λ 2: 226 nm
Abs. 2: 0.140

λ (nm)	Abs
195	0,289
203	0,280
205	0.200
221	0,146
226	0,140

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the absorbances corresponding to all peaks in a given spectrum can be read.

Fig. 1. Image of the workspace panel for using the device in general UV-VIS mode.

In the box within the monitor window, the wavelengths and corresponding absorbances for the maxima in the UV spectrum of Duffo-Quezada saliva are displayed. In the saliva spectrum, two groups of absorption bands can be distinguished: one group in domain I: 195–205 nm (more intense) and a group in domain II: 220–225 nm (broader and less intense). Depending on the alloy used and its degree of corrosion, changes in the absorption bands may occur, either by shifting their position or by altering their intensity (14).

To highlight and quantify the cytotoxic effect of the chemical components released into the saliva, methods such as protein synthesis and flow cytometry were utilized. The biological material used in the in vitro testing experiments consisted of stabilized, normal kidney cell cultures (obtained from the kidneys of the Cercopithecus aethiops monkey), uncontaminated with mycoplasma. These cultures were maintained in 25 cm^2 plates containing DMEM growth medium (Dulbecco's Modified Essential Medium, Biochrom AG, Germany) supplemented with 2% fetal bovine serum, 100 µg/ml streptomycin (Biochrom AG, Germany), 100 IU/ml penicillin (Biochrom AG, Germany), and 50 µg/ml amphotericin B (Biochrom AG, Germany), placed in an incubator with a humidified atmosphere of 5% CO2 and a temperature of 37°C. After an in vitro development interval that ensured a 48-hour cell treatment duration, the 72-hour-old cultures had their growth medium removed, and the cell layer was washed with PBS (phosphate-buffered saline), then subjected to various methods for the biochemical determination of total protein content using the Lowry method modified by Oyama (14).

To obtain direct indications regarding the interaction of the studied agents with the processes of cell mitosis, apoptosis, and cell viability, we developed a new experimental model compatible with their evaluation during the evolution of various RM cell culture variants, utilizing modern flow cytometry techniques. RM cell cultures aged 24 hours, intended for flow cytometric analysis of cell division, were treated with trypsin, centrifuged at 1800 rpm for 3 minutes, and resuspended in complete growth medium. After determining the number of cells per mL using classic cytometry with the Turk hemocytometer, the cell suspension was diluted to reach a concentration of 106 cells/mL. The suspensions were then preliminarily treated with carboxyfluorescein succinimidyl ester (CFSE) in DMEM culture medium with 10% fetal bovine serum and incubated at 37°C for 10 minutes. Every 24 hours, cells were collected in complete culture medium, centrifuged at 1800 rpm for 3 minutes, washed once with PBS, resuspended in PBS, and analyzed by flow cytometry using a flow cytometer with 488 nm excitation, with fluorescence collected using emission filters specific to fluorescein (FITC) (14).

RESULTS AND DISCUSSIONS

The corrosion parameter values for the studied alloys in Duffo-Quezada saliva are presented in Table IV. It is observed that the instantaneous corrosion rate has relatively low values for the studied alloys, but it varies significantly from one material to another. Thus, Nitinol seems to have increased corrosion resistance, despite the very high corrosion potential (E0 = -579 mV), which indicates a high tendency to corrode. This is due to the very high polarization resistance. Titanium is by far the most resistant biomaterial, having the lowest corrosion rate, under 1 µm/year.

Table IV. Instantaneous Corrosion Process Parameters for Alloy Corrosion in Duffo-Quezada Saliva

The cyclic potentiodynamic curve for Nitinol (fig. 2) indicates typical pitting corrosion, with a breakdown potential (EBD) of +220 mV and a repassivation potential (ERP) of -110 mV. The imperfect passivation range is relatively small, spanning from -110 mV to +220 mV.

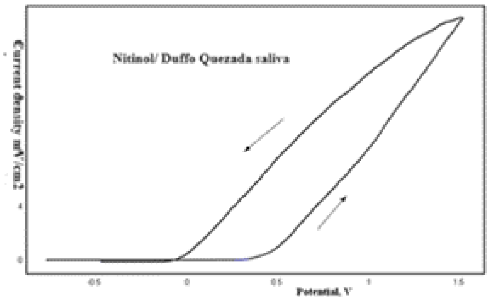


Fig. 2. Cyclic voltammogram for Nitinol in Duffo-Quezada saliva (v = dE/dt = 10 mV/s)

The cyclic voltammogram for Titanium indicates that this material was passivated even before being introduced into the solution (it oxidized slightly in the air during handling). As a result, in the potential range of 0 to 1600 mV, the current density is very low, ranging between 0.05 and 0.3 mA/cm². The increases in current at potentials greater than 1600 mV do not represent any corrosion process but are due to the oxidation of water with oxygen evolution at the electrode. Thus, titanium is the most corrosion-resistant of the metals studied (fig. 3).

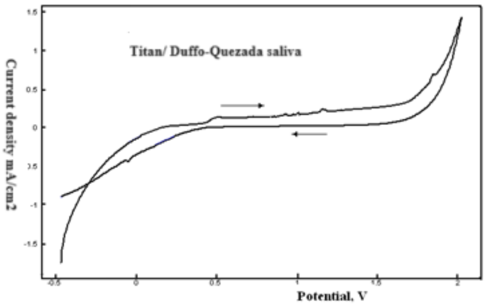


Fig. 3. Cyclic voltammogram for Titanium in Duffo-Quezada saliva (v= dE/dt = 10 mV/s)

The absorption spectrum of the artificial saliva in which the titanium sample was polarized differs from that of the initial saliva and the other samples; the band at 205-206 nm is significantly amplified, and the bands in the second range are merged into a single wide band, but with the same intensity as in the initial solution (fig. 4).

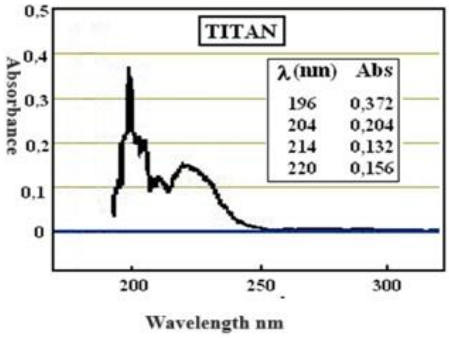
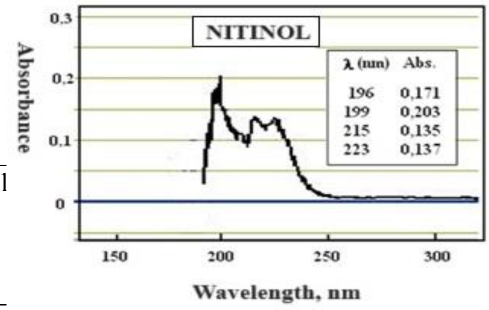


Fig. 4. The UV spectrum of Duffo-Quezada saliva after the electrochemical treatment of Titanium.

In the case of Nitinol, the absorption band at 210 nm is absent, as the alloy does not contain elements that would transition into the solution at this wavelength. The absorption band at 215 nm is attributed to nickel ions that have entered the solution (fig. 5).



All				)
Ti	[illegible]	[illegible]	[illegible]	[illegible]
Niti	-579	141,25	0,32	3,75

Fig. 5. The UV spectrum of Duffo-Quezada saliva after the electrochemical treatment of Nitinol.

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In the case of ionic samples mobilized from Titanium, we observe moderate, statistically significant changes in intracellular protein content, with the disruptive effect on cellular protein biosynthesis being potentiated by 3.74% due to titanium ions. The presence of ionic eluates from Nitinol, following incubation with monkey kidney cells in the culture medium, results in a reduction of cell development ranging from 34.12% to 40%. Excluding the influence of artificial saliva, the degree of inhibition of cellular development solely by the dental materials was: 10.88% (NiTi), 10% (Titan) (fig. 6).

Comparative analysis of the intensity of cellular protein biosynthesis against reference cultures allows for an initial ranking (in descending order) of the dental materials based on their cytotoxic potential as follows: Titanium, NiTi.

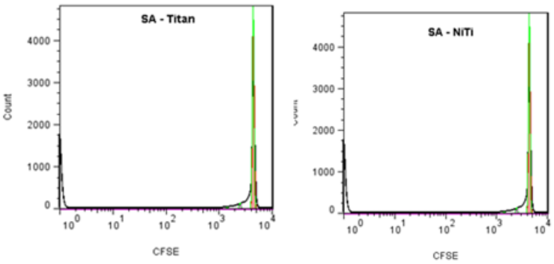

Fig. 6. The process of cellular proliferation in monkey kidney cell cultures, recorded in a continuous flow under the conditions of treatment with ionic eluates resulting from the incubation of Titanium and Nitinol with artificial saliva.

The incubation of RM cultures in a growth medium intentionally contaminated with artificial saliva led to an increased viability of the cell cultures, as indicated by the high proportion of live cells and the extremely low proportion of dead cells. A notable difference was observed between the cytotoxic potential of titanium ions, as estimated by the biochemical protein synthesis test (10.88% in terms of reactivity to artificial saliva) and the results obtained through continuous flow cytometry (nearly zero, indicating very good tolerance by the cells). This conclusion is supported by the high percentages of live cells (88.3% for Titanium and 87.7% for Nitinol), the very low percentages of dead cells (11.7% and 12.3%, respectively), the almost complete absence of pre-apoptotic cells (0.012% and 0.003%), and a slight increase in the number of apoptotic cells (0.049%) (Fig. 7).

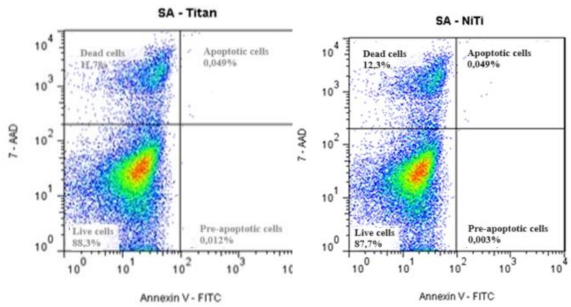

Fig. 7. Flow cytometric analysis of cell viability and apoptosis in monkey kidney cell cultures, grown under normal environmental conditions for Titanium and Nitinol.

Thus, we can conclude that the ions from the dental materials (titanium and nitinol), tested using artificial saliva, can be classified as agents with low cytotoxicity, based on both biochemical and flow cytometric results.

The results of this study suggest that the corrosion of titanium implants under physiological conditions is very low. These results are consistent with those obtained by Greul (15). As is well known, pure titanium in different grades (cp 1-4) and various titanium alloys are frequently used in implantology. For dental implants, pure titanium is most commonly used, thereby avoiding the potential release of ions from alloy components. The mechanical strength of pure titanium grades decreases with purity, that is, from grade 1 to grade 4. However, for dental applications, grade 2 titanium, in its annealed form, represents the standard (17, 18).

Since the deformation phenomena are very small in NiTi-based components, it can be anticipated that wear phenomena in the oral environment of this material can be significantly reduced (18, 19). Of course, additional and more sophisticated investigations are needed to address the long-term stability of such a Nitinol-based attachment system, as well as its biocompatibility (20, 21). Similar to the situation with NiTi-based endodontic instruments, repeated deformation may fatigue the material and ultimately lead to fractures (22).

The tissue reactions of titanium-based alloys depend on the dose, which in turn is affected by the rate of corrosion. Titanium alloys have good corrosion resistance, although this can be altered by the presence of proteins such as albumin, which may lead to an increase in the amount of titanium released into the tissues (12).

In the case of dental implants, complex electrochemical processes involving the implant and its suprastructure are associated with galvanic corrosion leading to a clinically significant situation for two primary reasons: the potential biological effects resulting from the dissolution of alloy components and the bone destruction caused by the electric current generated through galvanic coupling (23, 24, 25, 26).

CONCLUSIONS

Synthesizing the results of the spectrophotometric measurements, the following observations can be highlighted:

Titanium is by far the most corrosion-resistant biomaterial.

The absorption spectrum of Duffo-Quezada saliva exhibits two series of absorption bands that can be grouped into two wavelength ranges: I (195-205 nm) and II (220-225 nm).

Generally, the absorption bands in domain I are attenuated in the spectra of solutions where alloy corrosion occurred, with the most significant attenuation occurring at 195 nm.

Based on the results regarding the impact of ionic eluates from the incubation of Titanium and Nitinol with artificial saliva, we consider that these dental materials can be characterized as having a low cytotoxic potential as expressed through cellular protein synthesis, cell viability, cellular apoptosis, and the development of monkey kidney cell cultures used as biological material in our determinations.

Since variations in the pH of the oral environment, especially those towards acidity, have the capacity to affect the surface condition of dental restorative materials, their wear resistance, absorption capacity, and solubility, analyzing biomaterial changes in solutions with different pH levels is important for future improvements in the materials used in dentistry.

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