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Research Article

Evaluation of the Coating with TiO_2 Nanoparticles as an Option for the Improvement of the Characteristics of NiTi Archwires: Histopathological, Cytotoxic, and Genotoxic Evidence

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For the EPD, different voltages and different times were used. Male rats were used in four groups ($n = 3$) with different treatments. The blood sample was obtained for genotoxic analysis and liver and kidney organs were removed for histopathological analysis. The amount of NPs TiO_2 deposited on the samples of the arches increases gradually in the times of 15 and 30 s. At all voltages, however, at 45, 60, 75, and 90 s, there is an increase up to 25 V. Cell viability in lymphocytes treated with TiO_2 NPs did not cause genotoxicity. In the histopathological findings of hepatic and renal tissue, nuclear alterations and necrosis were observed. The objective of the study was to improve the physical and biocompatibility characteristics of the NiTi arches for which the EPD is used. The technique for the deposition of TiO_2 NPs was used, where this technique could be used as an economical and versatile way to perform homogeneous depositions even on surfaces with the complexity of the NiTi alloy. As for genotoxicity and cytotoxicity, we continue to have controversial results.

1. Introduction

In the recent practice of orthodontics, the use of Nickel-Titanium (NiTi), Nickel-Titanium-Copper (NiTiCu), or Titanium-Molybdenum (TiMo) alloys predominates due to its elastic characteristics that facilitate the alignment and leveling of the arches, improving the elasticity and flexibility [1]. The use of NiTi arches is widely accepted by the orthodontic community and offers biomechanical benefits difficult to match by some other materials on the market; however, a negative aspect of these arches is the roughness which has the ability to retain a greater amount of dental plaque because it favors its adhesion [2]; principally constituted by aerobic bacteria such as *Streptococcus sanguinis* and *Streptococcus mutans*, this plaque propitiates the corrosion of metals and alloys through the formation of organic acids during the

glycolysis of sugars, reducing the pH [3]. Currently, the search for improvement of existing materials in the field of orthodontics has been a subject of study; for example, the organically modified antibacterial silicates (ORMOSIL) such as quaternary ammonium methacryloxy silicate (QAMS) is added to the orthodontic acrylic resins in order to improve antimicrobial activity and toughness [4], resin-based adhesion materials contain Portland-type cement to provide adequate shear bond strength (SBS) and a caries-preventive effect [5], the incorporation of bioactive glass (BAG) into composite resins (BAG-Bonds) showed the capacity for buffering acidic oral conditions through the liberation of calcium in the environment [6], and also the modification of the arches with the application of nanomaterials, for example, nanoparticles (NPs), is an interesting topic due to popularity carried in recent times, and the advantages above the other modification techniques are low cost through the use of simple devices and easy handling. Between the diverse types of nanomaterials used in order to improve the arches' characteristics, the nanoparticles of TiO_2 have benn shown to be cost-effective [7] and they possess a unique photocatalytic property that results in enhanced microbicide activity, principally against bacterial strains of the plaque [8–10] besides their apparently low toxicity and excellent biocompatibility [11, 12]; regardless, there is still controversy about it being harmless [13, 14], since TiO_2 NPs have been related to the induction of cytotoxicity and genotoxicity due to the production of reactive oxygen species (ROS) in different cell types [15–17]; in addition, *in vivo* studies have shown apparently nanoscale and microscale toxicological effects associated with the size of the nanoparticles of TiO_2 [18, 19].

The efficacy of electrochemical methods such as electrophoretic deposition (EPD) is an alternative, versatile, and inexpensive procedure for depositing of these nanomaterials [20] and is a very useful tool for the manufacture of films of nanostructures, where thickness can be controlled varying parameters such as voltage and time, principally with the use of TiO_2 NPs which have been already proven in the literature; moreover, compared with other wet, dry, or plasma deposition methods, attachment could be oriented more effectively due its nondestructive depositing method that does not affect the particle [21]. The efficiency of EPD in the production of homogeneous and reliable films depends to a great extent on the surface chemistry of the particles, the behavior of surface-liquid interfaces under an electric field, and the development of the particle-particle network and particle-substrate network [22]; because of this, the good quality of TiO_2 NPs deposition on stainless steel bars [23] found in the literature does not necessarily correspond to a good deposition quality on other materials including NiTi arches. In our research, we propose an adequate technique for the correct deposition of TiO_2 NPs on the surface of NiTi arches, since the current bibliographic data in this type of material is virtually nonexistent and the methodology is poor described; for this reason, the objective of this study was to evaluate the electrophoretic deposition (EPD) of TiO_2 NPs in arches as well as their histopathological, genotoxic, and cytotoxic effects in Long-Evans rats.

2. Materials and Methods

2.1. Preparation of the NiTi Archwires. Conventional NiTi archwires (0.017 × 0.025 inches) (Ah-Kim-Pech®, México) were sandblasted with aluminum oxide (Zogear Blaster, CHN) and subsequently etched with 10% oxalic acid at 80°C for 60 min followed by immersion in an ultrasonic bath in acetone for 3 min and rinsed with distilled water, according to Paoli et al. [22]. To optimize the adhesion of NPs, later the archwires were cut into pieces of 16 mm.

2.2. Suspension Design. A suspension was made for the nanoparticle dispersion with a mixture of H_2O and C_2H_5OH (1 : 4) with a concentration of TiO_2 NPs (CAS: 13463-67-7; purity ≥ 99.5%; molecular weight: 79.87; particle size: 21 nm), 1% mass, and poly(diallyl dimethyl ammonium chloride) (PDADMAC) 2% mass [24] (all chemicals were obtained from Sigma-Aldrich Co., Ltd., St. Luis, MO, USA). The stability of the suspension was obtained by magnetic stirring (hotplate stirrer LMS-1003 Daihan Labtech Co., Ltd., Korea) during 12 h before the EPD process. This was carried out with two electrodes in a 500 mL glass vessel; the NiTi archwires were used as deposition substrates and a stainless steel sheet was used as a counter electrode, separated by a distance of 20 mm; both the working electrode and the counter electrode were connected to direct current power supply (Enduro 300V, Labnet International Inc., Woodbridge, NJ, USA). The NiTi archwire samples were weighed on an analytical balance with a sensitivity in μg (Denver Instruments apx-200) and then divided into 6 groups which used a constant voltage (V) of 5, 10, 15, 20, 25, and 30 V, respectively, at different times (15, 30, 45, 60, 75, and 90 s); this was carried out in triplicate. After the EPD the samples were allowed to dry for 24 h at room temperature; the samples were observed under a 10x optical microscope (Labomed S1100, Germany) with an AmScope MD700 digital microscope to determine the degree of TiO_2 NPs deposition. A new weighing of the samples was performed to measure the amount (μg) of TiO_2 deposited. The amount of TiO_2 NPs deposited per area in the NiTi arcs was calculated ($\mu g/mm^2$); the samples were left in a sterile container with 2 mL of PBS solution for 30 days to administer them later to the experimental group (G3).

2.3. Determination of the Genotoxicity of TiO_2 NPs

2.3.1. Animals. Our work was developed under the Official Mexican Standard for use and handling of animals in experimentation (SAGARPA in Mexico, NOM-062-ZOO, 1999). The study was approved by the Bioethics Committee and by the Internal Committee for the Care and Use of Laboratory Animals (CICUAL) of the Faculty of Medicine of the Autonomous University of Coahuila (number CONBIOET-ICA07CEI00320131015). All procedures with experimental animals were supervised by a veterinarian certified by the Secretary of Agriculture, Cattle Raising, Rural Development, Fishing and Food (SAGARPA, key code: MR-0716-33-001-1).

Twelve adult male Long-Evans rats ranging in age from 10 to 12 weeks, with an average weight between 240 and 280 grams, were provided by the Bioterium of the Faculty

of Medicine of the Autonomous University of Coahuila. All animals were maintained under controlled conditions of temperature at 25-26°C in a 12 : 12 h light/dark cycle housed in individual cages with water and food ad libitum.

2.3.2. Experimental Groups and Treatments. The rats were divided into 4 groups; (n = 3) the control group (CG) were given 1 mL of xylocaine®: 2% injectable solution of lidocaine without epinephrine intraperitoneally for 3 days, 1 dose daily; the sacrifice was 24 h after last dose. Group 1 (G1) is given 5 mg/kg body weight of TiO_2 dissolved in 1 mL xylocaine: 2% injectable solution of lidocaine without epinephrine intraperitoneally for 3 days, 1 dose daily. The sacrifice was at 48 h after last dose. Group 2 (G2) is given 5 mg/kg body weight of TiO_2 dissolved in 1 mL xylocaine: 2% injectable solution of lidocaine without epinephrine intraperitoneally for 3 days, 1 dose daily. The sacrifice was at 72 h after last dose. Group 3 (G3) received a solution composed of PBS (2 mL) NPs of TiO_2 plus the arcs NiTi (25 V 90 s). This solution remained at rest for 30 days prior to its administration in the rats; this solution was administered by nasogastric tube for 3 days, and the sacrifice was 72 h after last dose. After the exposure time, the rats were sacrificed by the veterinarian in charge through cervical dislocation; blood samples were extracted by cardiac puncture; the organs (liver and kidney) were removed and fixed in 10% neutral formalin for subsequent histopathological analysis.

2.3.3. Comet Assay in Lymphocytes. DNA fragmentation analysis of individual cells for peripheral blood lymphocytes was performed based on the methodology of Singh et al. [25] with some minor modifications. This allows the differentiation and analysis of cells with fragmented DNA to determine the percentage of fragmentation using specialized software. From each treatment, 10 μL of peripheral blood suspended in 0.5% low melting point agarose was used on slides pretreated with 0.5% normal melting point agarose and then covered with coverslips and the agarose was allowed to solidify at 4°C per 5 min; the slides were placed in a Köplin with lysis solution (2.5 M NaCl, 0.1 M EDTA, 10% DMSO, and 1% Triton X-100). At the end of the lysis, the samples were taken to horizontal electrophoresis chamber and incubated in electrophoresis buffer (NaOH 0.3 M, 200 mM EDTA) at pH = 13.0 to 20 min at 4°C for the unwinding of DNA in a dark room. The electrophoresis was completed with the following specifications: 25 V (1 V/cm), 300 mA for 20 min. After switching off the electrophoresis power source, the electrophoresis chamber slides were carefully removed and rinsed with a neutralization buffer (0.4 mol/L Tris-HCl, pH 7.5) for 5 min. The excess of neutralization buffer was drained off and placed in ethanol and then allowed to dry; once dried, the slides were stored for later reading.

2.3.4. DNA Fragmentation Analysis. The DNA was stained in phosphate-buffered saline (PBS) with fluorescent solution of GelGreen™ (Nucleic Acid Gel Stains, Biotium®, Fremont, CA, USA). Comets lymphocytes were evaluated under fluorescence microscope, 40x and 100x (Labomed LX 400, Germany). The images were taken with a fluorescence, 16-megapixel digital camera (AmScope, Digital Camera Microscope # MD700) and were converted into Bitmaps format (*.bmp) and analyzed in TriTek's CometScore Freeware v1.5 software. ImageJ software V.1.8.0 was first used to remove background noise from the DNA images obtained. Automatic image processing software was used for analysis of the comet assay. The software was able to calculate the amount of DNA at specified location based on pixel intensity of images. DNA in the tail was computed as follows:

$$\text{DNA} = \frac{\text{total comet tail intensity}}{\text{total comet intensity}} \times 100. \quad (1)$$

2.4. Histopathological Analysis. At the end of the experimental period, the rats were sacrificed by cervical dislocation; blood samples were collected by cardiac puncture and dissection of the abdominal organs was performed and fixed in 10% neutral formalin for subsequent histological analysis. Representative samples of hepatic and renal tissue previously fixed to be included in paraffin blocks were taken by conventional histological technique, which were cut in a microtome (Leitz 1512, Austria) at a thickness of 5 μm and mounted on slides stained with hematoxylin and eosin (H&E). The stained sections were examined under light microscopy to make the respective observations and to evaluate the morphological changes comparing them with the control tissues.

2.5. Statistical Analysis. We performed Kruskal-Wallis test as a nonparametric test and Dunn test as a post hoc test and unidirectional ANOVA as a parametric test using Tukey as a post hoc test. Measures of central tendency and standard deviation were done. Statistical analysis was performed using the Minitab 17 software for Windows.

3. Results

3.1. Deposition of Arches with TiO_2. The EPD gives us the possibility of generating different rate of amount of TiO_2 NPs deposited per area of NiTi arch (Figure 2) by the modulation of applied voltage and the time of deposition. In our experiment, the amount of TiO_2 NPs deposited on the samples of the arches increases gradually at the times of 15 and 30 s at all voltages (Figures 1(a) and 1(b)); however, at 45, 60, 75, and 90 s, there is an increase up to 25 V (Figures 1(c), 1(d), 1(e), and 1(f)). It was observed that the samples with higher deposition of TiO_2 NPs were 25 V90 s and 30 V90 s (Figures 4(f) and 4(g)). However, in these samples, a large number of fractures were observed in the continuity of the coating; the best ratio between quantity and quality of the coatings obtained by the deposition of TiO_2 NPs in the treated samples, finding the most homogeneous and fracture free coating, was in the sample of 10 V75 s (Figure 4(c)). In the results, a constant increase in the amount of deposited TiO_2 from 15 V to 25 V in all the treated arches could be observed; a variable behavior was found in voltages under 15 V and greater than 25 V. The correlation of the DEF of the TiO_2 NPs at times of 15 and 30 s, 45 and 60 s, 75 and 90 s was highly significant as observed in the correlation

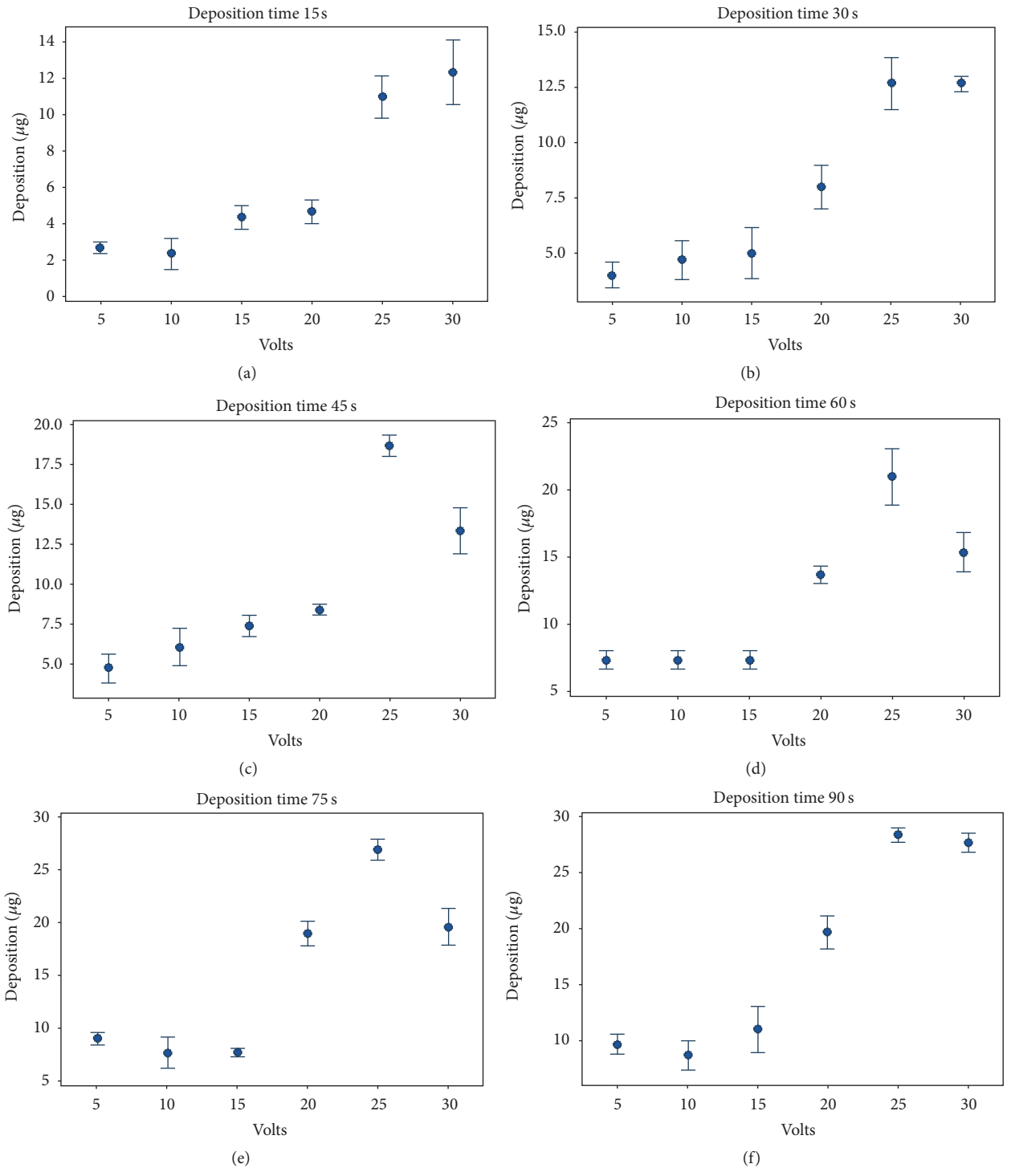

FIGURE 1: Deposition of TiO_2 NPs (μg) voltage (V)/time (s). (a) 15, (b) 30, (c) 45, (d) 60, (e) 75, and (f) 90 seconds versus 10, 15, 20, 25, and 30 V for each deposition. Results are shown in mean and standard error.

graphs (Figures 3(a)–3(c)). This indicates and corroborates what was mentioned above, where, according to the voltage intensity and elapsed time of the DEF, we observed this trend of dependent association because of the voltage with the time of application of voltage to which the arch was submitted. In the microphotographs, we can see the different groups of arcs with their voltages and their effects (Figure 4). In the group of 5 volts (Figure 4(a)), it was observed that the deposition is minimal; increasing the voltage shows a larger deposition, considering the uniformity of the EPD at 10 V 75 s

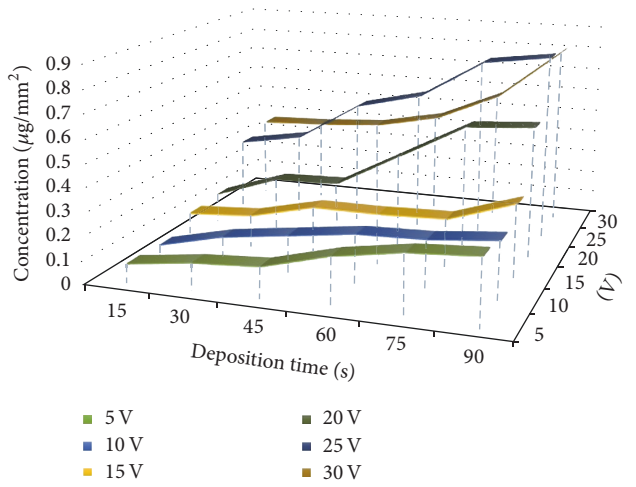

FIGURE 2: Amount of deposition of TiO_2 NPs (μg) per area of NiTi arch (μg/mm^2) (voltage (V)/time (s)). 15, 30, 45, 60, 75, and 90 s versus 5, 10, 15, 20, 25, and 30 V for each deposition.

(Figure 4(c)), the greater amount of deposition is obtained at 25 V to 90 s (Figure 4(f)). The general behavior is that from 15 V there is a sustained increase of NPs deposited up to 30 V where there is a decrease because it exceeds the value of the critical cracking thickness (EAC).

3.2. Analysis of DNA Fragmentation by Exposure to NPs of TiO_2

3.2.1. DNA Fragmentation in Lymphocytes. When the alkaline comet test was performed, a stability was observed in the DNA chain, since there was no significant migration on this (Figure 5); treatment with TiO_2 NPs did not cause cytotoxicity at the concentration of 5 mg/kg for 3 days, 1 daily dose, with the sacrifice being conducted after the last dose at 48 h (G1). There was also no cytotoxicity at the concentration of 5 mg/kg for 3 days, 1 daily dose, with the sacrifice being conducted after the last dose at 72 h (G2) or in the PBS solution (2 mL) in which previously treated arches were submerged (25 V 90 s) and left to rest for 30 days and administered by nasogastric tube for 3 days and with a sacrifice conducted after the last dose of 72 h (G3), suggesting that there were no DNA breaks (see Figure 5(c)). Another parameter delivered by the comet test is the percentage of DNA contained in the comet tail. This parameter is the subtraction of 100% of DNA minus the percentage of DNA contained in the head of the comet. This parameter is the percentage of fragmentation that the evaluated cell has; the group with greater fragmentation was group 1 (G1) which had an average of 6.06 ± 1.49 percent.

3.3. Histopathological Findings. Figure 6 shows the microphotographs with the observations found in liver tissue samples. In the analysis of the samples corresponding to the control group, which was administered only with 2% lidocaine for 3 days, no pathological data were found (Figure 6(a)). In those corresponding to group 1, which received 5 mg/kg TiO_2 for 3 days, being sacrificed 48 h after the last dose, the presence of vacuoles included within the hepatocyte cytoplasm was observed (Figure 6(b)). In the case of group 2 samples, they received 5 mg/kg of TiO_2 for three days and were sacrificed 72 h after the last dose; it is observed that the hepatocytes present a foamy cytoplasm and nucleus with granular chromatin (Figure 6(c)). Group 3, which was given PBS solution for 3 days in which the TiO_2 treated arches rested and were sacrificed 72 h after the last dose, extensive areas of cell necrosis were observed with destruction of hepatocytes (Figure 6(d)). Figure 7 shows the representative microphotographs, corresponding to the findings in renal tissue samples. In the analysis of the samples from the control group, no pathological data were found. In the samples corresponding to groups 1, 2, and 3, a slight glomerular retraction and moderate vascular congestion were observed.

4. Discussion

4.1. Electrophoretic Deposition. In the course of achieving the final suspension design, 3 mixtures were used, a mixture of H_2O and NaCl without charging agent (data not shown), a mixture of H_2O and C_2H_5OH (1 : 4) and the polyethylenimine (PEI) charging agent (data not shown), and a mixture of H_2O and C_2H_5OH (1 : 4) and the poly(diallyl dimethyl ammonium chloride) (PDADMAC) charging agent, which proved to be the best combination to enhance the adhesion effect of TiO_2 NPs layers. It was observed that the mixture of H_2O and NaCl because of being a water-based solution produces electrolysis even at low voltages and this causes bubbles to be trapped causing "gaps" in the deposition of the TiO_2 NPs, coinciding with what was commented on in [26–28]. Therefore, it was decided to use an ethanol-based suspension instead of water in order to avoid harmful hydrogen penetration at the working electrode and a charging agent was added to provide an additional surface charge for the stabilization of suspended particles and electrophoretic mobility during the deposition process [29, 30]. To the mixtures based on H_2O and C_2H_5OH (1 : 4) a charging agent of PEI and PDADMAC were added, respectively, having the best adhesion results with the PDADMAC charging agent, coinciding with the results of Lau and Sorrell [29]. In the researches conducted by González-Luna et al. and Wu et al. [12, 31], they suggest a deposition in the range of 20–50 V, and Boccaccini et al. [32] suggest ranges from 10 to 50 V; in our study the voltage range was handled between 5 and 30 V with the largest amount of deposition in the 25 V regardless of time, unlike Paoli et al. [22] who suggest 20 V as the most optimal voltage and Hasegawa et al. [33] who suggest 40 V. This study confirms what other authors observed when the amount of deposited layers was modulated with the deposition time and the voltage, although not necessarily more time and voltage mean a better deposition. In our study, with the combination of the voltage/time deposition, the most homogeneous layers obtained without fractures were with the samples of 10 V/75 s. Samples under this voltage show very little deposition and those superior to it show

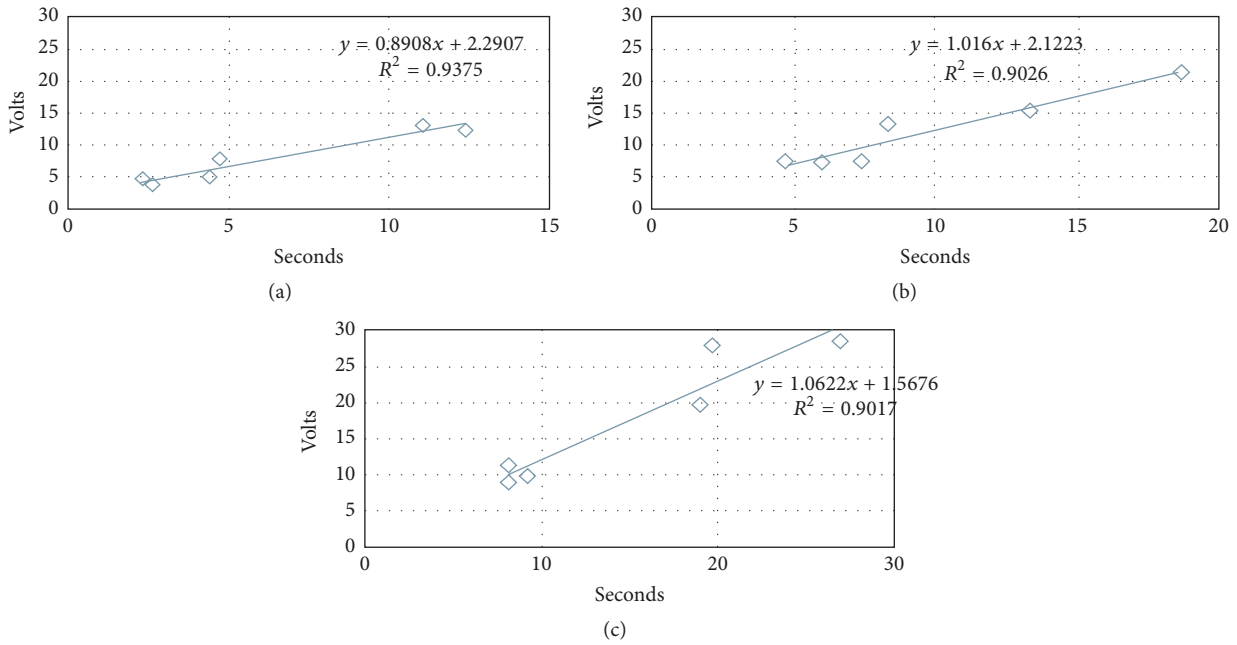

FIGURE 3: Time/voltage correlation. A voltage-dependent association tendency was observed with the time of voltage application. Notes: (a) deposition, 15 and 30 s, (b) deposition, 45 and 60 s, and (c) deposition, 75 and 90 s.

greater deposition but with fractures, which increases the time of deposition/voltage. After having a constant increase, the samples treated at 30 V have a decrease in the deposited TiO_2 NPs, as can be seen in the deposition's interval graphs; the possible explanation for this is that it exceeds the value of the critical cracking thickness (CCT) as explained by Sadeghi et al. [30]. When the layer becomes thicker during the deposition process, there is an increase in the resistance because the previously deposited layer is nonconductive, weakening the electric force field and causing weak connections between the particles along the final layer. Therefore, the accumulated NPs on that layer tend to have fractures that cause the detachment of the NPs due to the accumulated excess. It was decided not to perform any sintering technique after EPD because it has been proven that the exposure of the NiTi archwires at temperatures above 500°C alters their mechanical properties [34–36].

4.2. Comet Assay. Although administration of TiO_2 NPs to the experiment animals was intraperitoneally (G1 and G2) and through nasogastric probe (G3), there was no significant induction of DNA breaks in peripheral blood lymphocytes in the results delivered by the comet assay; our negative results were similar to those found in keratinocytes, irradiated with TiO_2 at 20, 40, and 60 min, where there was no evidence of type IV comet damage and the number of comets II and III was approximately 30% [37] also in human diploid fibroblasts and human bronchial epithelial cells that were exposed at concentrations up to 50 $\mu g/cm^2$ where the tail moment does not exceed [15]. But, as mentioned above, there are studies where positive results were found in the comet assays, for example, in lymphocytes, which were treated with TiO_2 at concentrations up to 59.7 μg/mL having a mean of olive moment of 7.30 ± 0.81 [38], human bronchial cell cultures that were treated with 1.77 g/cm^2 TiO_2 NPs causing olive moments up to 90 [17], Rtg cells exposed for 4 h to TiO_2 NPs (50 μg/mL) in MEM cultures PBS, and H_2O solution where there was a percentage of DNA in tail of 32, 45, and 32, respectively [39]. These inconsistent results may be due to the different sizes and structures of the TiO_2 NPs used in the studies as suggested [40], making it difficult to compare the results between the studies.

4.3. Pathophysiological Findings. The results of the present study show the existence of cytotoxic potential of TiO_2 NPs after acute exposure by intraperitoneal injection (G2 and G3) at a high dose as presented in our methodology and coinciding with Singh et al. [25] with progressive damage even after cessation of exposure, supporting the distribution and accumulation data in liver and kidney [41]. In the case of G3, the liver damage was a lot greater, represented by the extensive areas of cellular necrosis. There was not enough time to find fibrous septa (cirrhosis) in the liver parenchyma as in the findings reported by Umbreit et al. [42] where central fibrous septa were found after 7 days of exposure. In the case of renal damage analysis, the findings were more discrete and there was no significant difference in the cases exposed to a controlled concentration compared to those that received the PBS solution in which the deposited archwires rested. In this case, contrary to the findings of Chen et al. [43], what was observed in our study was a glomerular contraction and not a glomerular inflammation. Our observations seem to indicate that, after deposition of TiO_2 NP, NiTi arcs that exceed the CCT, for example, samples of 25 V/90 s, are susceptible to degradation of the coating in an aqueous medium (PBS solution), when ingests are absorbed and TiO_2

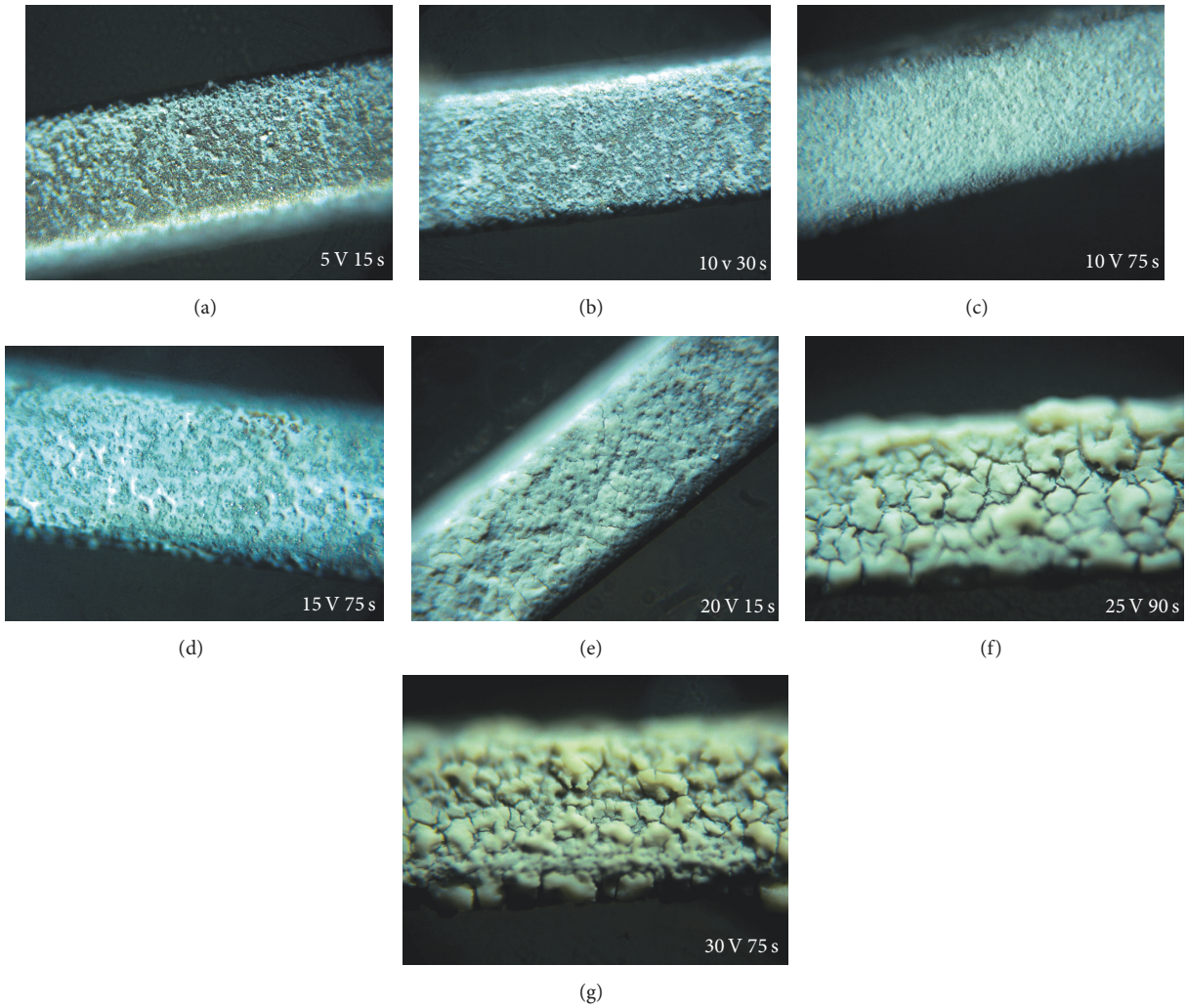

FIGURE 4: Deposited arches. It is observed that, at higher voltage/deposition time, there are more deposited TiO_2 NPs, but there is also an increase in surface fractures ((f) and (g)) (10x).

NPs enter the bloodstream producing a toxic effect on the organs, with the first step being the liver parenchyma, which results in extensive lesions such as those found in our study. Other studies describe the ability of TiO_2 NPs to produce an inflammatory response and induce the production of ROS (reactive oxygen species) inducing apoptosis, as observed in our results [44], in addition to mentioning the relationship between the size of the NPs and their toxicity, suggesting that the smaller the size, the greater the metabolic activity and toxicity. Regarding the intracellular mechanisms of damage [45], when describing the importance of the internalization of NPs of TiO_2, when they accumulate in lysosomes, this leads to their rupture and releases their content, such as cathepsin B with the subsequent activation of caspases to apoptosis.

5. Conclusion

The aim in this study was to improve the physical and biocompatibility characteristics of the NiTi arches; therefore the EPD technique was conducted to deposit TiO_2 NPs, thus proving the success of the method as a low-cost and versatile way of performing homogenous depositions even on complex surfaces such as NiTi alloys; the quality of the deposition was controlled with an adequate voltage, a precise time, and an ideal charging agent. With regard to genotoxicity and cytotoxicity, controversial results were still found, in agreement with other authors of the related literature; for example, our results in the comet assays suggest that there is no genotoxicity in any of the experimental groups under the conditions conducted. Nevertheless, in the cytotoxic findings, cytotoxic potential in the TiO_2 NPs was found, representing progressive damage even after the cessation of exposition and in some cases it was the G3 cellular necrosis. At the end, the use of NiTi arches coated with TiO_2 is not recommended to be placed in the mouth until more experimental research is done about friction resistance and degradation in the oral environment, as well as testing of TiO_2 NPs with long term exposition *in vivo*.

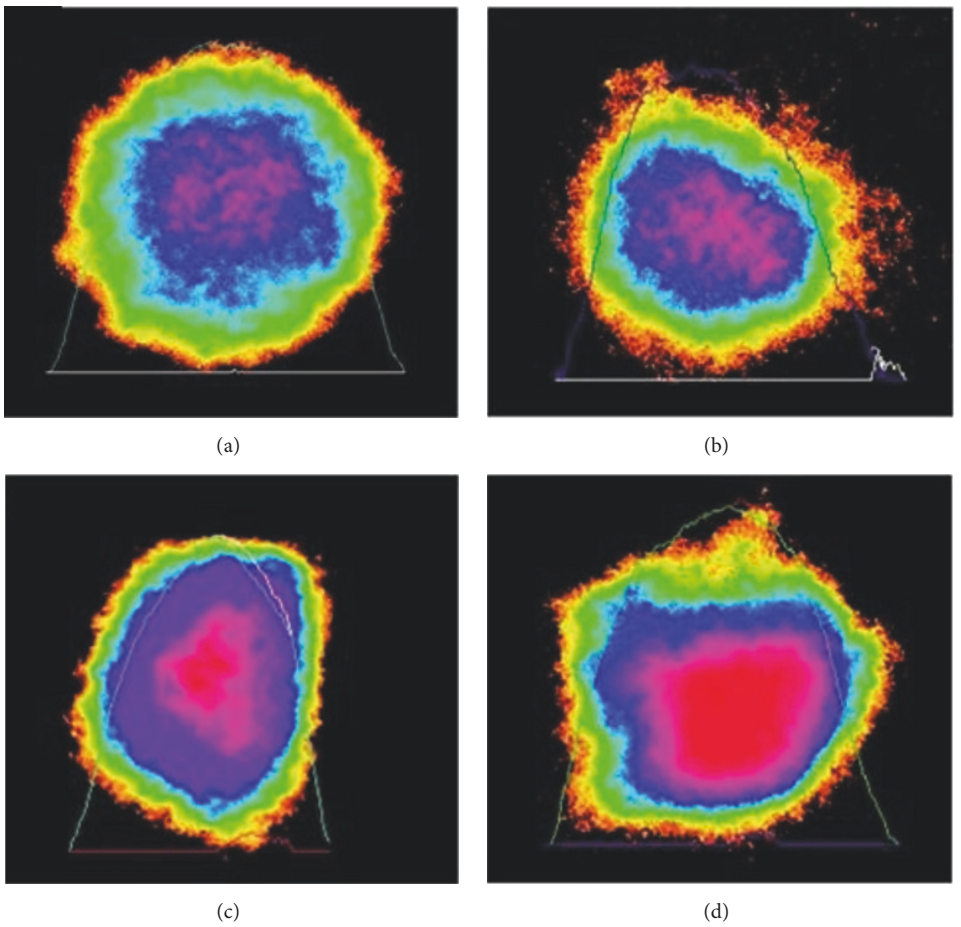

Figure 5: Analysis of the cells with the CometScore^{TM} program for DNA measurement at the comet head, where GC, G1, G2, and G3 ((a), (b), (c), and (d)) showed no significant migration ($P > 0.05$).

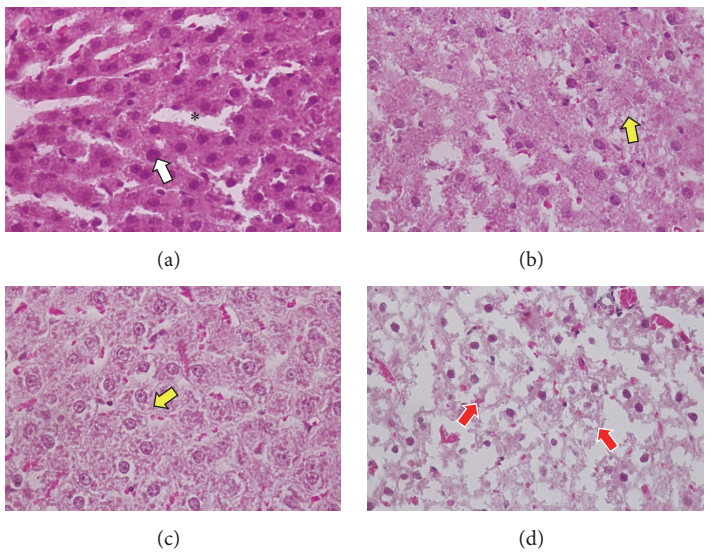

Figure 6: Microphotograph of liver dyed with H&E at 40x. (a) Control group, showing sinusoids (∗) and hepatocyte cords (white arrow) with nucleus and cytoplasm of typical morphological characteristics. (b) In rats of group 1, few vacuoles in the hepatocytes cytoplasm are observed (yellow arrow). (c) In group 2, abundant intracytoplasmic vacuoles are observed (yellow arrow). (d) In group 3, tissue necrosis areas with hepatocytes destruction are observed (red arrow).

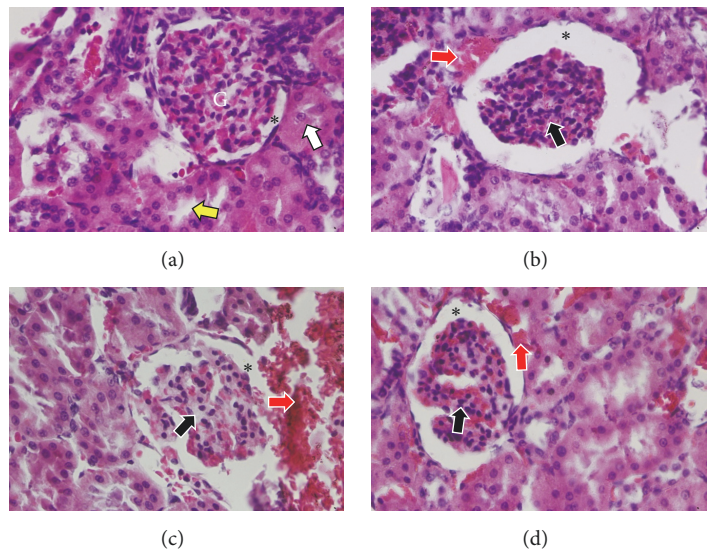

Figure 7: Microphotomicrograph of kidney dyed with H&E at 40x. (a) Control group with normal histopathological characteristics, glomerulus (G), urinary space (∗), proximal convoluted tubule (white arrow), and distal tubules (yellow arrow) are observed. In 1, 2, and 3 experimental groups (microphotographs b, c, and d, resp.) glomerular retraction (black arrows), an increase of urinary space (∗), and mild vascular congestion (red arrow) are observed.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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