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Chitosan's effects on the acidity, copper ion release, deflection, and surface roughness of copper-nickel-titanium archwire

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ABSTRACT

Background: *Chitosan has an antimicrobial effect in oral hygiene control. Orthodontists sometimes prescribe mouthwash to adolescent patients. Copper-nickel-titanium (CuNiTi) orthodontic archwire is widely used in orthodontic treatment. Chitosan's effects on the CuNiTi properties of orthodontic archwire are not generally known.* ***Purpose:*** *This study aimed to measure the acidity, copper ion release, deflection, and surface roughness of CuNiTi orthodontic archwire immersed in artificial saliva and 2% chitosan.* ***Methods:*** *This study comprised experimental laboratory research. Forty-two CuNiTi orthodontic archwires were divided into three groups. Group A consisted of 18 archwires immersed in artificial saliva, Group B consisted of 18 archwires immersed in 2% chitosan, and Group C was six archwires for the baseline sample. The two intervention groups (A and B) were divided into three subgroups of six samples and were subjected to different immersion times—i.e., two, four, and six weeks. Acidity, copper ion release, deflection, and surface roughness were measured using pH meters, atomic absorption spectrophotometry (AAS), a universal testing machine (UTM), and a scanning electron microscope (SEM).* ***Results:*** *The results showed that Group A was more alkaline than Group B, and it was significantly different only in Week 2. Group B's copper ion release was significantly lower than Group A for all the time observations ($p<0.05$), and the deflection analysis showed no significant difference in any of the groups ($p>0.05$). Furthermore, the SEM images showed CuNiTi in Group A at Week-6 had the most porosities and defects.* ***Conclusion:*** *The chitosan produces buffer effects on the pH; it also exhibits lower copper ion release, no differences in unloading forces, and subjectively has better surface roughness.*

Keywords: *CuNiTi; chitosan; copper ion release; deflection; surface roughness*
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INTRODUCTION

Adolescent patients undergoing orthodontic treatment are usually unable to maintain adequate oral hygiene because compliance is a significant issue at this age.[1] Therefore, practitioners recommend mouthwash.[2] One well-known mouthwash contains chlorhexidine (CHX) and is commonly used due to its clinical efficacy in chemical plaque control.[3] However, using chlorhexidine mouthwash daily may lead to undesirable side effects, such as tooth and tongue staining, an unpleasant taste, and a sense of burning or dryness in the mouth.[3,4]

On the other hand, recent studies have found that chitosan has an antimicrobial effect on many organisms.[3,5] Chitosan is a linear and primary polysaccharide produced from the deacetylation of chitin and can be used as a chemical agent for mouthwashes.[3,6] It can be extracted from fungi, the exoskeletons of insects, or the shells of crustaceans.[5] Chitosan is widely used in biomedicals, cosmetics, and foods in agriculture because of its biodegradability, biocompatibility, renewability, absorptivity, non-immunogenicity, non-toxicity, and non-carcinogenicity.[5,6]

Brackets and archwires are essential in orthodontic appliances for moving teeth to targeted positions.[7] Nowadays, archwire made of CuNiTi is frequently used in orthodontic treatment due to its better tooth movement. The addition of copper to NiTi wire creates temperature

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transition range (TTR) control and gives better spring back properties. CuNiTi orthodontic archwire also has greater material strength, produces more constant but gentle force, and is more resistant to permanent deformation.[8–10]

The acid contained in different food and beverages can release nickel ions from the wire.[11] Allergic reactions to these nickel ions are also associated with chrome and cobalt.[12] Moreover, excess copper in the body can cause liver damage and gastrointestinal symptoms. Nevertheless, copper toxicity rarely occurs in individuals who do not have hereditary copper homeostasis defects because copper is an essential mineral in the formation of hemoglobin and thus helps to prevent anemia. It also has an effect as an antimicrobial agent.[13–15] Copper is naturally present in the human body and some foods, such as offal, shellfish, seeds, and nuts.[16] However, copper toxicity has been reported in populations that consume water containing high levels of the element, when the corrosive action of water causes it to leach from copper pipes in buildings. WHO revealed that domestic plumbing can be subject to corrosion. High levels of dissolved oxygen have also been shown to accelerate corrosion in some cases. Water that flows to residents contains copper oxide, increasing daily copper exposure.[14] The copper ions released from dental alloys ranged from 0.045–0.098 ppm/day, and copper ions reached a cytotoxic level at a concentration of 10 ppm.[17]

Deflection is the response of a wire (bending or twisting) when a force is exerted to move the teeth and needs to be activated periodically.[2] The amount of deflection in each wire may differ depending on its brand, size, and composition.[18–20] Moreover, deflection may alter during application in the oral cavity, after contact with saliva, mouthwash, soft drinks and wire sterilization fluid.[21–24] There is a great deal of interest in investigating and analyzing the properties of orthodontic appliances, such as ion release and surface roughness.[4] Material composition, manufacturing geometry, and surface roughness are considered essential parameters in determining the corrosion behavior of metals. Surface roughness also plays a significant role in the effectiveness of archwires, such as in guided tooth movement, biocompatibility, surface contact and friction, esthetics, hygiene, and color stability.[7,25] The effects of chitosan in the corrosion process of CuNiTi orthodontic archwire have not been studied extensively. Thus, this study aimed to measure the changes in the pH levels, copper ion release, deflection, and surface roughness of CuNiTi orthodontic archwire after being immersed in artificial saliva and 2% chitosan.

MATERIALS AND METHODS

This study was experimental with group control and sought to observe the pH changes for three types of property analysis: copper ion release, the unloading force for deflection, and CuNiTi orthodontic archwire surface roughness. The research was carried out in a number of locations: the immersion process and acidity analysis of the solution were conducted in the faculties of Dentistry and Pharmacy at Universitas Sumatera Utara; the ion release procedure was observed at Balai Standardisasi dan Pelayanan Jasa Industri (Baristand) Medan; the deflection was analyzed in the Impact Fracture Research Center (IFRC) Laboratory, Faculty of Engineering, Universitas Sumatera Utara; and the surface roughness was investigated in the Integrated Research Laboratory at Universitas Sumatera Utara. Ethical clearance had been received in advance from the Health Research Ethics Committee of Universitas Sumatera Utara, Medan, North Sumatra, Indonesia, letter No. 557/KEP/USU/2021.

Forty-two samples of archwire, 4 cm in length, composed of CuNiTi Tanzo® (American Orthodontics) were analyzed. According to Sastroasmoro and Ismael,[26] the calculated minimum number of samples required were six for each group. The size of the CuNiTi orthodontic archwire was 0.016 x 0.025 in. One liter of artificial saliva with a pH value of 7.5, which consisted of $NaHCO_3$ 9.8%, Na_2HPO_4, $12H_2O$ 9.3%, NaCl 0.47%, KCl 0.57%, $CaCl_2$ orp $CaCl_2.2H_2O$ 0.04% (0.045%), MgCl or $MgCl_2.2H_2O$ 0.06% (0.065%) was formulated at Oral Dental Hospital (Faculty of Dentistry, Universitas Sumatera Utara). The chitosan powder concentration was 2% (2/100 ml of distilled water) from prawn shells was formulated at the Laboratory of Research Centre (Faculty of Mathematics and Science, Universitas Sumatera Utara).

The 42 CuNiTi orthodontic archwires were divided into two groups (n=18), and each group was further divided into three subgroups based on observation time—i.e., two, four, and six weeks (n=6). Group A was the control group and used artificial saliva, while Group B used artificial saliva with 2% chitosan. The last six samples formed the baseline and did not undergo intervention. Group B simulated daily use in the mouth (two times a day for one minute). All samples were incubated at 37°C. The 2% chitosan was dissolved in artificial saliva and immersed for 28 minutes for the two-week subgroup, 54 minutes for the four-week subgroup, and 84 minutes for the six-week subgroup.

The acidity of the solution was analyzed by the digital pH meter (Hanna, HI98107) at the end of the observation, atomic absorption spectrophotometry (AAS, Shimadzu AA7000) was used to study the immersed solution of copper ion release after two, four, and six weeks with a wavelength of 324.8 nm and a slit width of 0.5 nm. First, the standard solutions of different concentrations determined the absorbance and continued to make a calibration curve from the value. Then, the measurement of the absorbance and concentration of the sample was determined from the last calibration curve. After this, observation continued with a three-point bending test pressuring a 5-mm load to the wire with a universal testing machine (Tensilon, RTF-1350, Japan) to examine the unloading value of CuNiTi orthodontic archwire deflection in Newtons. The final stage examined the

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surface roughness of CuNiTi orthodontic archwire in three areas with a scanning electron microscope (SEM, Hitachi TM3000) at 2000x magnification.

Statistical Package for Social Sciences software version 26.0 was used for the statistical analysis. A Shapiro-Wilk analysis concluded that the pH of copper ion release and deflection data were distributed normally with a value of $p>0.05$. Subsequently, these three variables were statistically analyzed using a one-way ANOVA between Group A (the control) and Group B (2% chitosan). This was followed by a least significant different (LSD) post hoc analysis.

RESULTS

The artificial saliva pH baseline was 7.5, but after the CuNiTi was immersed, the pH of both groups rose. The pH for Group A (the control) was significantly more alkaline than that of Group B at Week 2 (Table 1 and Figure 1A). The LSD post hoc test analysis was based on the time intervals and revealed that the alkaline effects were significantly different only in weeks 2–4 for Group A, and Group B did not show any difference in acidity (Table 1). The copper ion release mean levels for both groups were significantly different amongst all the subgroups (at weeks 2, 4, and 6), while Group B demonstrated considerably lower copper ion release than Group A (the control). The copper ion release showed a gradual but significant reduction over time during observation, according to the LSD post hoc analysis, except for Group B from Week 2 to Week 4 (Table 2 and Figure 1B).

The unloading force of the baseline sample was 45.049±2.99N. The deflection test for unloading forces showed that Group B had higher unloading forces than Group A; however, there were no significant differences between both groups in any of the observations over time (Table 3 and Figure 1C). Based on the time interval, the post hoc LSD ANOVA test showed the unloading value of the CuNiTi orthodontic archwire deflection had only altered significantly in Group B, with a p-value of 0.001.

The SEM micrographs of the CuNiTi archwire surface roughness were subjectively analyzed because the exact quantitative result could not be provided. However, the micrograph obtained in this study showed that the immersed CuNiTi archwires in Group A at Week 2 had the fewest porosities and defects. In contrast, the CuNiTi archwires in the control Group (Figure 2) at Week 6 showed the most porosities and defects.

Table 1. Acidity (pH) solution between groups and subgroups (n=6). The baseline pH was 7.5

Solution	Mean ± SD				
	Week 2	p-value**	Week 4	p-value**	Week 6
Artificial saliva	8.29±0.032	0.034	8.13±0.073	0.095	7.99±0.204
2% chitosan	7.82±0.186	0.055	7.99±0.152	0.724	8.02±0.068
p-value*	0.001		0.086		0.754

Significance level of $p<0.05$; * *p-value* from one-way ANOVA; ** *p=value* from the LSD post hoc analysis between time observations.

Table 2. Copper ion release between the groups and subgroups (n=6)

Solution	Mean±SD (μg/L)				
	Week 2	p-value**	Week 4	p-value**	Week 6
Artificial saliva	16.215±0.343	0.001	14.178±0.290	0.001	12.818±0.162
2% chitosan	10.707±0.435	0.952	10.720±0.401	0.001	9.756±0.265
p-value*	0.001		0.001		0.001

Significance level of $p<0.05$; * *p-value* from the one-way ANOVA; ** *p*=value from the LSD post hoc analysis between time observations.

Table 3. Mean levels and independent t-test of the unloading value of CuNiTi orthodonticarchwire deflection (n=6). The baseline unloading force was 45.049N

Solution	Mean±SD (μg/L)				
	Week 2	p-value**	Week 4	p-value**	Week 6
Artificial saliva	47.639±0.866	0.172	45.936±0.699	0.301	47.207±0.933
2% chitosan	49.349±0.299	0.001	45.428±0.698	0.001	48.621±0.352
p-value*	0.092		0.619		0.187

Significance level of $p<0.05$; * *p-value* from the one-way ANOVA; ** *p-value* from the LSD post hoc analysis between time observations.

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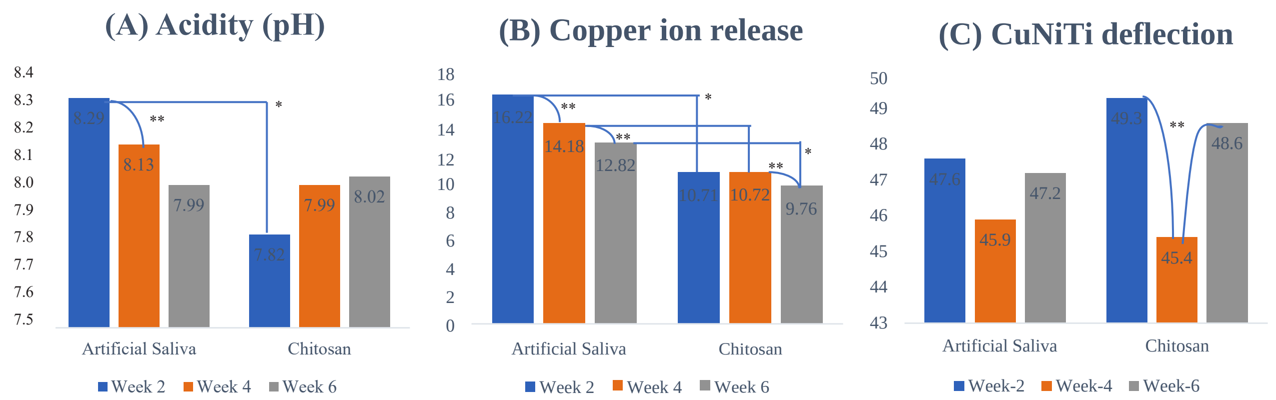

Figure 1. (A) Acidity (pH), (B) copper ion release, and (C) CuNiTi deflection for all groups. A significance level of $p<0.05$; * p-value from the one-way ANOVA; ** p-value from the LSD post hoc analysis between time observations.

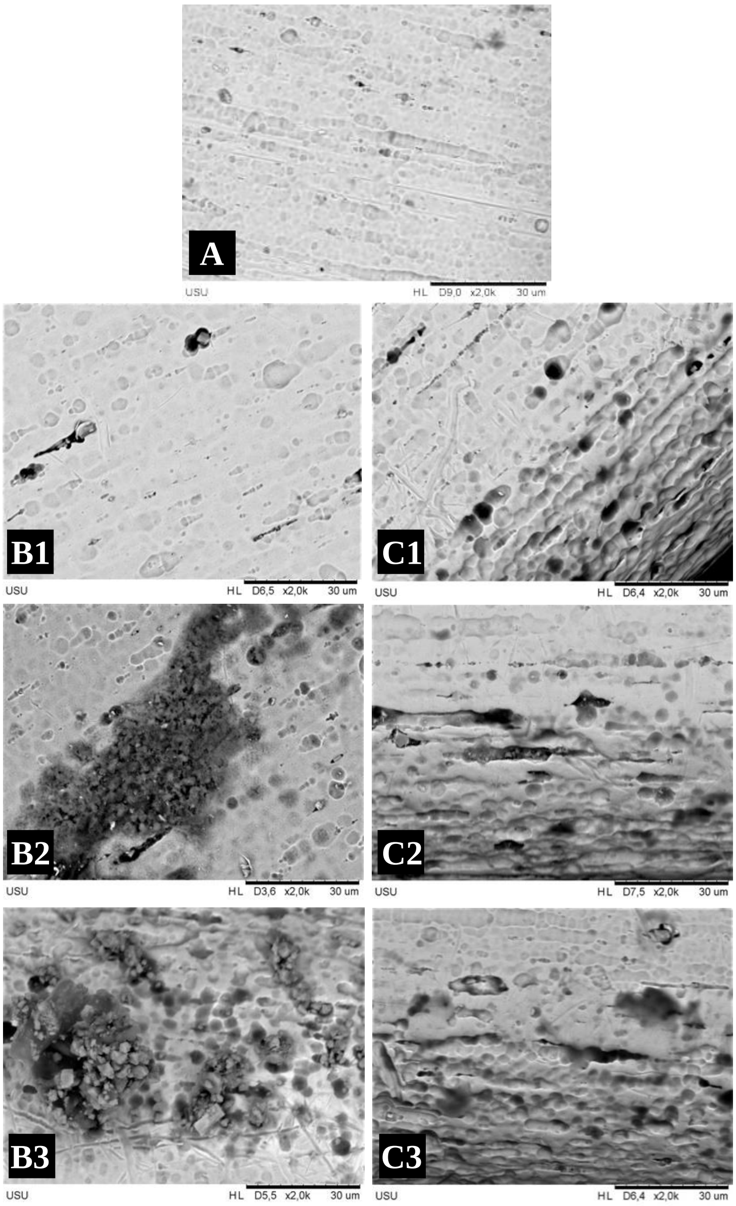

Figure 2. The surface roughness of the CuNiTi archwire. A. Baseline, B. Group A (artificial saliva), C. Group B (2% chitosan), where C1, C2, and C3 are at weeks 2, 4, and 6, respectively.

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DISCUSSION

Corrosion can be interpreted as an electrochemical process in metal orthodontic wire exposed to saliva in the oral cavity. This process causes the material's surface to become rough, weakening the appliance and resulting in dissolution or ion release in the oral cavity, either partially or entirely.[10,27] Orthodontic appliances themselves can alter salivary components and thus characteristics, such as flow rate, pH, buffer capacity, and calcium. The pH and the activity of maintaining the buffer capacity in orthodontic patients were significantly more alkaline than in non-orthodontic patients.[28] This study was an in vitro experiment and simulated the conditions in the mouth. The results were analogous to the previous research in that the pH baseline of the artificial saliva was 7.5, and after the CuNiTi archwire was immersed in the artificial saliva (Group A) and artificial saliva with 2% chitosan (Group B), the pH became more alkaline in all groups at all time observations. Moreover, Group A was more alkaline than Group B. Chitosan is a derivative of chitin with amino groups on its surface and functions by generating positive zeta potential.[29] This means that chitosan's amino groups tend to remain protonated in acidic or neutral pH conditions, so in this experiment, it stabilized the materials. Furthermore, chitosan as a nanolayer particle is used as an efficient pH buffer to reduce metal corrosion caused by the acidity of chemical compounds. Here, the chitosan served as a proton sponge to protect the wire from surface-mediated pH changes.[29,30]

Biocompatibility in orthodontic archwire must be the main requirement of orthodontic appliances. It can be determined by evaluating the amount of metal ion release from the archwire under many different conditions.[31] The release of ion in the oral cavity may cause allergic or even toxic effects both locally and systemically.[12,32,33] Orthodontists usually prescribe a mouthwash to adolescent patients who cannot maintain adequate oral hygiene.[1,32] However, some mouthwashes have been reported to cause the release of ions and decrease corrosion resistance.[34,35] This study observed that copper ion release from the CuNiTi orthodontic archwire in Group B (2% chitosan, which simulated a mouthwash) was lower than that of the control.

The research on copper ion release of different brands of CuNiTi archwire immersed in neutral and acid solutions showed higher release in the neutral solution than in the acid solution.[9] Group A in this study additionally proved more alkaline than Group B; therefore, the copper ion release in the former was higher than the latter. Copper ion release from orthodontic appliances is unavoidable. What should be noted, however, is the safe threshold for ion release in the body.[36] The recommended daily intake of copper ions is 2 mg/day.[13] This study revealed that the highest copper ion release of all the samples was in the artificial saliva at Week 2, with a concentration of 0.016 mg/l or 16 µg/L, which was still lower than the recommended daily intake. Thus, it can be concluded that the copper ion release was within the safe threshold.

The results showed that copper ions released from CuNiTi archwire decreased over time. This study was contrary to another study, which showed that copper ions released from polypropylene composite with copper metal had increased with time and temperature.[37] The differences in the results of this study were due to the research sample and different solutions used. In this research, the copper ion released by the polypropylene composite was predicted to be significant due to antimicrobial activity: higher copper ion release means higher microbial activity. Chitosan can be used as a chemical agent for gel and mouthwashes that provide clinical benefits for plaque control because it has an antimicrobial effect against a wide range of organisms.[3,38] The 2% chitosan mouthwash is not significantly different from chlorhexidine mouthwash on the plaque and gingivitis index.[3] The chitosan gel was also studied for its antimicrobial effects on mini orthodontic implants, and it was concluded that chitosan could reduce the bacterial count at the mini implant site in the patient, even though it was less effective than chlorhexidine. Chitosan could be used as an alternative antimicrobial agent in gel or mouthwash products as it may not cause side effects.[3,38]

CuNiTi archwire is one of the superelastic archwires and tends to offer a constant, light force, which is needed to move teeth into alignment over a longer activation time.[39] Orthodontic appliances change the salivary conditions in the mouth; however, food consumption, temperature fluctuations, acidity from drinks, and additionally mouthwash used as a biological or chemical agent may also potentially damage the condition of orthodontic archwires.[27,39,40] These factors may alter the stiffness and condition of the CuNiTi archwire, which can be assessed using a three-point bending method to determine its load-deflection properties. These analyses were the most important parameters in identifying the biological environment needed to move the tooth.[25,36]

The deflection for the unloading forces in this study showed that Group B had higher unloading forces than Group A, but there were no significant differences between the groups in any of the time observations. A similar study found an increase in the unloading force after immersion in a NaF solution compared to a saliva group at 2.5-mm deflection.[22] The mechanical behavior of chitosan coated in polyethylene, which is used in biomedical/food packaging, improved the surface hardness of the material as well as its frictional properties, owing to the rough surface of the coating. The study was carried out using nano-indentation, a scratch test to the surface, and measured by an atomic force microscope (AFM).[41]

The unloading force is also affected by friction, which occurs due to the resistance between the wire and bracket; in this in vitro experiment, the effects of friction were not analyzed.[42] Even though the previous study found that the polyethylene coated with the chitosan nanoparticle

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improved friction due to its rough surface, according to the AFM analysis, the archwire with the chitosan nanoparticle coated in stainless-steel (SS) instead reduced friction, as confirmed by a sliding test carried out with a universal testing machine, and exhibited a smoother surface, according to a subjective analysis of the SEM images.[41,43]

The unloading force of CuNiTi archwire decreased from weeks 2 to 4 and increased again from weeks 4 to 6, with both groups exhibiting almost the same force at Week-2. A study was discussed which examined hysteresis or force loss between nickel-titanium (NiTi) and CuNiTi when stored in water, subjected to temperature changes, and in an acidic condition for 60 days, which simulated an orthodontic treatment in the mouth. The data revealed that the control group (not immersed) had the highest loading force, while the immersed condition had a reduced loading force. As chitosan has a buffer effect on CuNiTi in an immersed condition, this study showed that the chitosan groups had higher loading forces than the control group. This parallels the control group of the previous study, which also found that CuNiTi archwire had a lower hysteresis or force loss than NiTi archwire, so that the force remained stabilized throughout the 60 days.[39] The longest observation time in our study was six weeks (42 days), which corresponds to the aforementioned study that noted a reduced force due to intervention, but the CuNiTi archwire had low hysteresis of force, and the force stayed the same throughout the periods of observation. Unfortunately, the chitosan effects of deflection analysis on the mechanical behavior of CuNiTi archwire for observations over several time intervals were still not found.

The last observation of corrosion in the CuNiTi archwire analyzed the surface roughness with an SEM at 2000x magnification. From the beginning of the immersion, Group B showed more porosity and defects, though observations over time showed both of these had increased; however, these features remained the same until Week-6. In Group A, meanwhile, porosity and defects emerged slowly but continued to increase over time. The acidity comparison of groups A and B only found a significant difference from weeks 2 to 4, but the copper ion release in Group A was significantly higher than in Group B for all subgroups. The SEM analysis revealed that Group A at Week 6 had the greatest number of defects and highest porosity. The limitation of this study was that the images for the SEM analysis were analyzed subjectively, and a further study would be required to quantify the surface roughness with an AFM. Another study observed the surface roughness of orthodontic brackets and SS archwire coated in chitosan. They were analyzed with an SEM and a sliding effect test of friction, which concluded that the SS coated with the chitosan nanoparticle had less friction and a smoother surface.[43] On the contrary, it was found that the polyethylene coated with the chitosan nanoparticle exhibited improved frictional properties in the rough surface, which were analyzed with an AFM.[41]

From the results obtained in this study using two solutions, Group B (2% chitosan) demonstrated buffer effects in the pH with lower copper ion release, but there was no significant difference in the unloading force. Further investigation is needed to evaluate the effects of 2% chitosan on another chemical element in terms of ion release, deflection simulating orthodontic treatment, and surface roughness using AFM analysis or an SEM mathematical analysis.

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