In Vitro - Evaluation of Biodegradation of Different Metallic Orthodontic Brackets

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Abstract

This study aims to evaluate the chemical and structural changes of metallic orthodontic brackets in vitro biodegradation process in artificial saliva.

A total of 240 metallic orthodontic brackets of two different manufacturers were used, American orthodontic (LG Stainless-Steel (AISI 316L), USA) and Dentaurum (equilibirium@mini, Stainless-Steel, Germany) divided into control groupimmersed in distilled water and experimental group and immersed in artificial saliva for a period of 28 days at 37°C under mechanical agitation. The release of ions concentrations determinedusing inductively coupled plasma mass (ICP) spectrometry before and after 1, 7 and 28 days of immersion. Energy Dispersive X-ray (EDX) spectroscopyperformed to detect changes in chemical compositions.

More aggressive chemical and structural changes were apparent in AO orthodontic brackets after 28 daysimmersion in artificial saliva. In general, the biodegradation was increased proportionally with duration of immersion of brackets, the total mean value of ions release in AO (mean=31.04) is higher than in Dentaurum (mean=2.704) particularly nickel ions.The release of ions in tested orthodontic brackets showed a statistically significant difference with P=0.04.

All tested orthodontics brackets in artificial saliva showed less resistant to biodegradation and more obvious in AO bracketsthat might be associated with thetype of stainless steel used by the manufacturer.

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Introduction

The biodegradation process of stainless steel and titanium-based brackets in oral rehabilitation systems commences by corrosion. These wear processes take place simultaneously and influenced by many factors due to the presence of ions, minerals, pH of saliva and solutions[1,2]. The effect of corrosion and resultant release of ions into the oral cavity discussed in the literature because of its biological effects. Biodegradation of orthodontic bracket material been investigated over the past 20 years. However, these studies have given rise to questions without scientific answers.

The introduction of metal ions into human body is an additional hazard to health. These ions released from different metallic orthodontic brackets at different levels in the oral environment resulting from biodegradation process. Depending on a characteristic of metal ions and their solubility of the product containing them, the biological function is affected and leads to systemic and local effects like allergy[3].

Orthodontic metal brackets subjected to biodegradation process in the oral cavity. Biodegradation defined as the series of processes by which living systems render chemicals less noxious to the environment. Living systems in the oral environment, which are microorganisms and enzymatic phenomena, can

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cause corrosion and other associated problems during long time exposure[4,5].

According to Yip et al[6], corrosion is an electrochemical reaction of metals with their surrounding environment, leading to a loss of metals or conversion of the metal into oxide. Corrosion divided into seven types that are pitting corrosion, crevice corrosion, galvanic corrosion, inter-granular corrosion, fretting corrosion, stress corrosion and microbiologically influenced corrosion. Pitting and crevice type of corrosion form on the metallic orthodontic brackets surfaces exhibit many pits and crevices andthought to increase the susceptibility of corrosion because of their ability to harbor plaque-forming microorganism.

In the oral cavity, corrosion takes place by the release of metals ions from orthodontic alloys to form more stable compounds. Thus, corrosion behavior of orthodontic metal brackets also affects their biocompatibility. Biocompatibility is defined as “the ability of a material to elicit an appropriate biological response in a given application” [7]. It is the metallic corrosion products entering the body, such as nickel, titanium, iron and chromium that are being subjects of interest due to their allergenic and cytotoxic effects[8, 7].

An allergic reaction is an acquired condition resulting in an overreaction upon contact with the foreign substance and arises from a genetic predisposition and previous sensitization from exposure to the substance[9,7]. Allergic was divided into four type (Type I to IV), immediate reaction (Type I) and delayed reaction (Type IV) have relevance to orthodontic biomaterials. Nickel and chromium induce Type-IV hypersensitivity reaction in the body. These metals also cause several cytotoxic responses including a decrease in some enzyme activities, interference with biochemical pathways, carcinogenicity, and mutagenicity. Nickel has attracted the most attention as a potential sensitizer in the odonatological setting because nickel atoms are not strongly bonded to form some inter metallic compound, the likelihood of *in vivo* slow nickel ion release from the alloy surface is increased, which may have implications for the biocompatibility of these alloys[10].

In dentistry, a factor in determining the biocompatibility of the alloys used is their resistance to corrosion. Austenitic stainless steel metallic orthodontic brackets have a high stiffness and formability that contains chromium, carbon, and nickel. The chromium helps the brackets become more resistance toward corrosion by forming a passive layer on the metal surface, but this filmdamaged by an aggressive attack by ions such as fluoride and chloride. The same goes for nickel titanium type of orthodontic metallic brackets, where it forms a passivation titanium-oxide layer that provides a protection against corrosion but 1% lactic solution causing this layer is insufficient in reducing the dissolution of metal ions[6,11].

The observation of the surface structure and morphology in order to detect the corrosion of the metallic orthodontic brackets is a straight forward method to evaluate the biodegradation process of the orthodontic brackets. Chappard et al found a positive relationship between roughness analyzed by microscopic images (SEM) and level of roughness measured by contact profilometer.

Despite the limitations of *In Vitro* study design and it does not simulate what is truly happening in the oral cavity, still is better design to show and study the effect of biodegradation and corrosion resistance of orthodontic brackets especially pertaining to measure ion release Not all of the ions released from orthodontic brackets are found in the oral fluids. Some of the ions are absorbed into the oral tissues, while others are ingested and dispersed into distant organs. Thus, the advantage of the in-vitro design is that it presents the total ions released from the bracket.[12]

The objectives of this study are to evaluate the effects of artificial saliva on chemical and structural changes of orthodontic metallic brackets and to compare the changes of different metallic brackets in biodegradation process[13].

Materials and methods

General

Two hundred and forty metallic (Stainless-Steel) orthodontic brackets of two different manufacturers American orthodontic (Stainless Steel(AISI 316L), American Orthodontics, USA) and Dentaurum (equilibirium@ mini, Stainless Steel) were selected for the study. All brackets divided into control and experimental groups. Before testing, they were disinfected with alcohol spray and allowed to dryto avoid any contamination during the procedure as well as their weight was assessed using the electrical

balance to ensure standardization. All brackets tested as received before immersion at T0 and after immersion in different solutions 1 day (T1), 7 days (T2) and 28 days (T3).

Control group

One hundred and twenty metallic orthodontic brackets of the different manufacturer immersed in distilled water for a period 1, 7 and 24 days. Each bracket was mechanically agitated in a salt spray tester machine set at a constant temperature of 37°C in individual 15ml plastic-capped vials containing the15ml solution.

Experimental group

One hundred and twenty metallic orthodontic brackets of the different manufacturer were immersed into 15 ml Modified Fusayama artificial saliva under the same condition for 1, 7, and 28 days.

Preparation of artificial saliva

Artificial saliva formulated according to Fusayama with modification for the biodegradation test. The prepared solution frequently used in previous researches and it generates similar electrochemical behavior of metallic material as in human saliva. The artificial saliva was prepared according to the **Table 1**[14].

Ingredients	Concentration Mg/L
NaCl	400
KCl	400
CaCl2·2H2O	795
NaH2PO4·H2O	690
KSCN	300
Na2S·9H2O	5
Urea	1000

Table 1: Chemical compositions of the stock Fusayama's artificial saliva solution used in the study.

Microscopic brackets analysis (SEM)

Scanning electron microscopy (SEM) used to analyze the surface and structural changes of randomly selected 72 metallic orthodontic brackets at T0, T1, T2, and T3 on the frontal, base and wing surfaces. The micrographs obtained presented at x200, x500, x1000 and x2000 magnification. All images qualitatively assessed and examined by the single examiner under same magnification and sites.

Surface characterization analysis (Profilometer)

The profile meter used to quantify and compare the surface roughness(Ra) of metallic orthodontic brackets of different manufacturer included in this study for all groups at T0, T1, T2, and T3.

Analysis of the chemical composition of the brackets (EDX)

An Energy Dispersive X-Ray (EDX), which is a SEM resource that allows for evaluation of the chemical composition of the brackets. The procedure was standardizing by performed on six brackets from each group on buccal, gingival, base and wing surfaces. In order to quantify the Nickel, Copper, Iron, Cobalt and Chromium ions in metal alloy of the brackets at T0, T1, T2, and T3.

Analysis of the chemical composition of the solution (ICP)

The different immersion solution including distilled water and artificial saliva tested with an inductively coupled plasma (ICP) spectrometer at T0, T1, T2, and T3. Each solution analyzed for release of Nickel, Copper, Iron, Cobalt and Chromium ions in solutions.

Statistical analysis

The result of the present study was subjected to statistical analysis to interpret the differences and the significance between surface roughness from profile meter compared at T0, T1, T2, and T3. The chemical composition of brackets evaluated by the means of ions present in the metal alloy of the brackets from EDX and compared at T0 and T3. On the other hand, means of ions released in distilled water and artificial saliva obtained from ICP were compared at T0 and T3 in each group using SPSS version 20 using ANOVA and t-test.

The selected sample size estimated with Power analysis according to confidence interval more than 80%.The data gathered from SEM observation were not interpreted statistically since information involved a qualitative comparison between images.

Results

The result obtained from the control and experimental groups demonstrated in terms of surface changes on the brackets viewed from scanning electron microscope (SEM), values of surface roughness on the brackets surface measured by profile meter, chemical composition of metal ions of the brackets measured with EDX and amount of metal ion release inside each solution quantified by ICP.

After 28 days, all the brackets showed significant changes in surface roughness, their chemical composition, and the amount of ion released into the solutions. AO brackets revealed structural changes compared to Dent aurum brackets in terms of more surface roughness obtained by SEM. These findings obviously supported by a profilo meter.

In addition, artificial saliva caused obvious corrosive effect that is evident from the analysis of all four methods. The time interval also played an important role as the evidence of biodegradation increased proportionally with duration of immersion of brackets.

Microscopic bracket analysis (SEM)

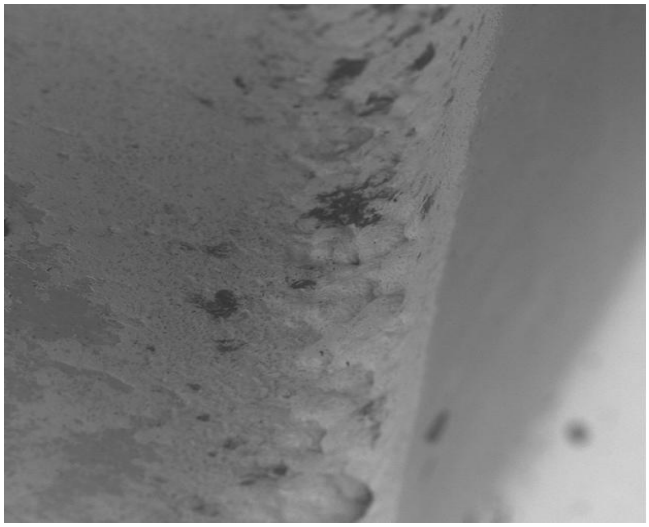
The SEM analysis at T0 and T3 indicated that alterations found on the surfaces of the brackets after the immersion period. In the frontal images, products of corrosion identified in both brands. Once comparing Dent aurum brackets with AO brackets immersed in artificial saliva at T3, the corrosion pattern seen more obviously with AO brackets and deeper effect. The type of corrosion is also different between each brand. Evidence of corrosion observed at the solder junction of the bracket. Analysis of the side images indicated that the region most significantly affected in were the solder junction, especially the angle formed between the wing and the bracket base. **(Figure1and 2)**

Surface characterization analysis (Profilometer)

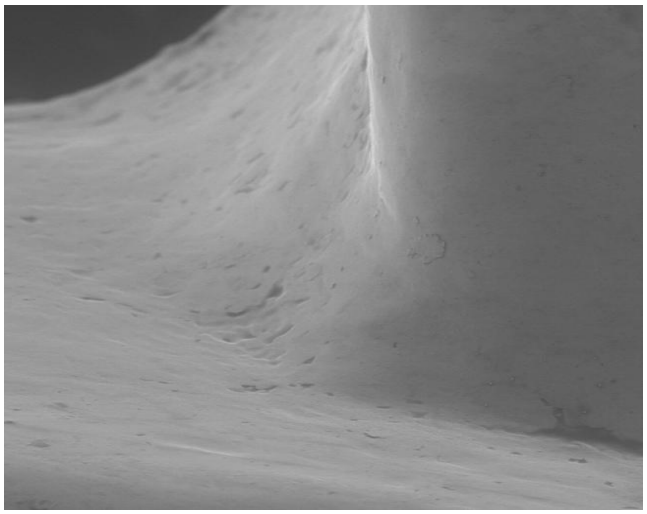
The results showed that there is a statistically significant difference in surface roughness of both brands of orthodontic brackets after 28 days immersion in artificial saliva with P=0.07.

Brand	Solution	Surface roughness (Ra) Mean *
Dentaurum	Distilled water	0.15 (0.03)
	Artificial saliva	0.32 (0.12)
American Orthodontics	Distilled water	0.17 (0.01)
	Artificial saliva	0.40 (0.13)

Table 2: Descriptive statistic of surface roughness value (Ra) in different brand and solution after 28 days (T3) of both control and experimental group.
**Standard deviations are given in parentheses.*



A



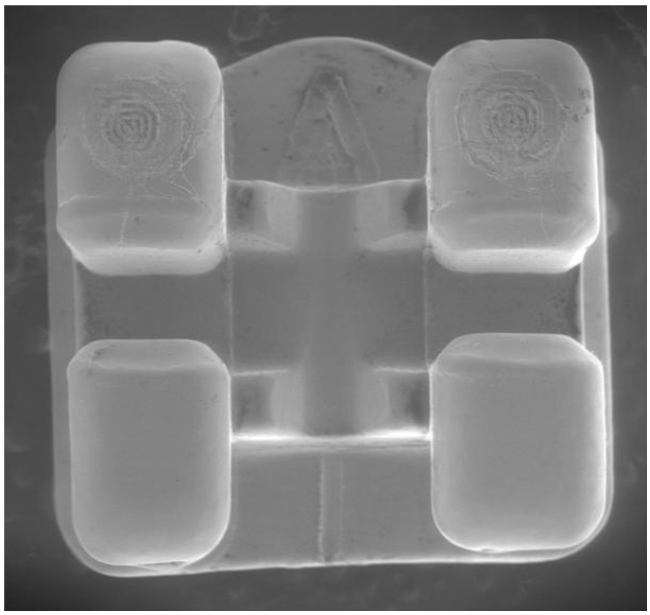
B

Figure 1. Metallic orthodontic brackets under SEM with magnification 300 x after immersion in artificial saliva (T3) showing corrosion on the wings. A: orthodontic brackets (Dentaurum). B: Stainless steel orthodontic (American orthodontics).

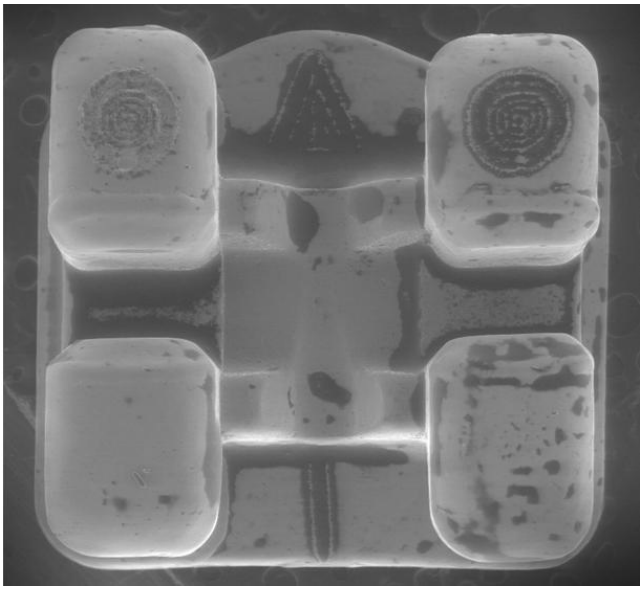
The descriptive statistic for the surface roughness data of metallic orthodontic bracket after the immersion into different solution shown in **Table 2**.

The total mean value of surface roughness in AO (mean=0.57) is higher than Dentaurum (mean=0.45) orthodontic brackets. There was no statistical difference in comparison to both brands of orthodontic brackets (P=0.37). **(Figure3)**

2.A



2.B



2.C

Figure 2. Metallic orthodontic brackets under SEM with magnification 60 x after immersion in artificial saliva (T0: A, T1: B and T3: C).

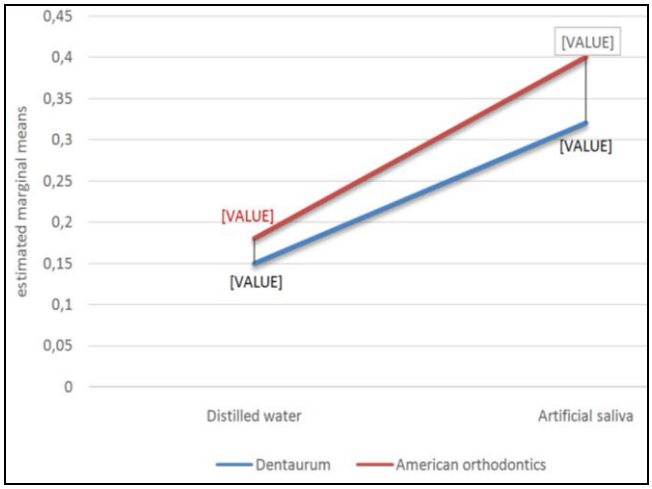


Figure 3. Surface roughness (Ra) in different brands and solutions after 28 days T3 of both control and experimental groups.

Chemical composition of the brackets (EDX)

As shown in **Table 3**, differences were found in the composition of the metal alloy used in the brackets after 28 days immersed inartificial saliva (T3) and the statistically significant difference with (p< 0.05) (P=0.01). The brackets in Group of American Orthodontics showed a reduction in the amount of iron, nickel, and chromium ions to Dentaurum brackets.

Solution	Brand	Chromium	Copper	Nickel	Iron
Distilled water (Control group)	American Orthodontics	15.85 (0.99)*	1.59 (0.12)	6.86 (0.63)	67.75 (5.03)
	Dentaurum	18.68 (3.01)	1.66 (0.21)	9.16 (3.88)	62.15 (2.89)
Artificial saliva (Experimental group)	American Orthodontics	16.13 (2.21)	1.29 (0.03)	5.69 (0.86)	70.02 (0.28)
	Dentaurum	16.62 (0.52)	0.43 (0.60)	9.46 (1.35)	54.5 (5.69)

Table 3: Comparison of Chemical composition of orthodontic brackets of different brands: Mean and standard deviations of both control and experimental groups after 28 days. (Mg/L)
**Standard deviations are given in parentheses.*

After 28 days of immersion in both solutions, the comparison of different brands of orthodontic brackets showed a significant difference in Iron (*P*=0.03) however chromium and copper showed no significant difference with *P* value 0.07and 0.16 respectively. In addition, the composition of copper in different immersion solution was a significant difference (*P*=0.03) while no significant difference of nickel, iron and chromium composition in different immersion solution (*P*=0.18, *P*=0.38, *P*=0.87). (**Figure 4**).

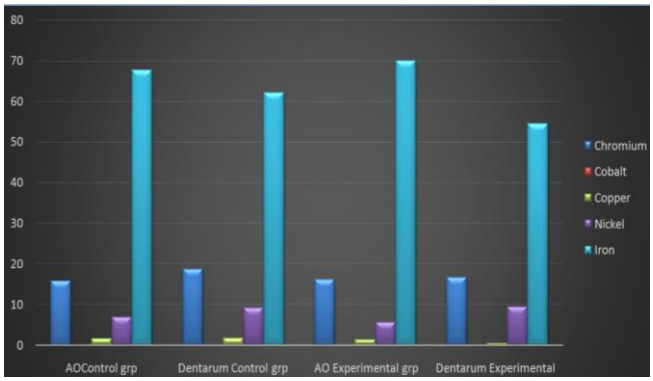


Figure 4. Comparison of chemical composition of orthodontic brackets American orthodontics and Dentaurum metallic orthodontic brackets (Chromium, Copper, Nickel, and Iron) using EDX in all solutions after 28 days (T3).(Mg/L).

Analysis of the chemical composition of immersion solution (ICP)

The descriptive statistic of the mean level and standard deviation of ions released in the groups shown in **Table 4**.The release of ions in different solutions and brands was significantly different after 28 days immersion in artificial saliva (*P*=0.04). In general, the total mean value of ions release in AO (mean=31.04) is higher than in Dentaurum (mean=2.70). In addition, the mean value of nickel release in artificial saliva is increased.

Solution	Brand	Chromium	Cobalt	Copper	Nickel	Iron
Distilled water (Control group)	American orthodontics	-0.29 (0.0005)*	-0.04 (0.0008)	-0.02 (0.002)	-0.04 (0.0005)	-0.008 (0.005)
	Dentaurum	-0.27 (0.005)	-0.04 (0.001)	0.001 (0.06)	-0.2 (0.04)	0.02 (0.05)
Artificial saliva (Experimental group)	American orthodontics	0.006 (0.06)	-0.003 (0.07)	0.09 (0.11)	-0.12 (0.28)	0.029 (0.03)
	Dentaurum	0.005 (0.06)	-0.002 (0.07)	0.05 (0.11)	0.01 (0.08)	0.05 (0.06)

Table 4: Comparison of metal ion concentrations (Mg/L) released from orthodontic brackets of different brands in different solution: Mean and standard deviations of both control and experimental groups after 28 days.
**Standard deviations are given in parentheses.*

Discussion

Over the years, studies have shown alarming reports on the corrosion potential of AISI Type 316L austenitic stainless steel alloy currently used for stainless steel manufacturing. Corrosion resistance of stainless steel formed by the formation of a chromium oxide layer at its surface. Nickel incorporated into austenitic stainless steel alloys to stabilize its formation process.

Artificial saliva used in this study to demonstrate their effects to the structural and biological integrity of metallic brackets. Artificial saliva is included in this study to best simulate the process of corrosion in the oral cavity. The samples were also mechanically agitated. Mechanical agitation is achieved by the salt spray test machine which is a standardized test method used to check corrosion resistance of samples. It works by production of electrical currents due to the dynamic movement of water inside the machine's test chamber. The electrical currents will further enhance the corrosion process of the metallic brackets.[15]

Microscopic analysis can provide a qualitative measure and visualization of surface morphology[16]. The surface finish of the metal alloy is an important factor to prevent corrosion pits and cracks. Surfaces that are rough and irregular predispose to corrosion process and increase the area of metal dissolution. [1718] It was stated that the solder used in the bracket is the significant factor for the onset of the corrosion process [19]

A profilometer is a widely accepted method to assess the surface condition and a common method to analyze surface configuration with a non-invasive approach[20]. Moreover, it provides a quantitative scale and used to compare surface roughness of the treated surfaces.

The results showed that artificial saliva produced marked changes in surface roughness and evidence of corrosion after 28 days. American orthodontic brackets had an evidence of pitting corrosion under SEM and higher values of surface roughness in profilometer. AO brackets are more prone to corrosion compared to Dentaurum brackets. That may be associated with the manufacturing process and heat treatment of orthodontic brackets. Dentaurum Brackets (equilibirium family Stainless Steel) manufactured in one-piece. They manufactured in one piece – body and base and no solder are involved (Monobloc) using one single type of metal alloy. While American orthodontic brackets are manufactured in two pieces (body and base) joined by silver solder.

The EDX is a SEM tool that detects and quantifies the metals comprised in an alloy, and this enables us to measure the release of metallic ions in an indirect fashion. This method according to Eliades et al has clinical relevance and produces results with high reliability[2,17]. AO brackets showed less corrosion resistant where the metallic ions released into the solution. The chemical composition of nickel and iron between different brands was significantly different. The mean value of nickel composition of metallic orthodontic bracket immersed in artificial saliva was higher in comparison to distilled water. This indicates that more nickel ions were lost from the brackets by corrosion in artificial saliva.[22]

ICP analysis is a different method from AAS (Atomic Absorption Spectrometry)analysis where it has the advantage of extracting each metal ions and detecting them without causing any interference to other ions. Therefore, ICP analysis selected to detect the level of ion released in the resulting solutions after mechanical agitation. The amount of ion released was higher in AO brackets compared to Dentaurum brackets regardless of its immersion solution leads to conclude that this is due to a more corrosion resistant characteristic of Dentaurum bracket and its manufacturing process. The amount of nickel ion released is highly significant in artificial saliva compared to distilled water explaining the corrosive effectof the artificial saliva and the release of harmful ions like is nickel into the living systems even in small amount.

According to Meijer, and Smith[23] the solder used in bracket manufacture appears to be a significant factor in the onset of the corrosion process. In 2001, Lee and Chang[22] found that heating orthodontic wires (NiTi and Optimally) to 250ºF for 20 minutes leads them to develop an increased number of pits, worsening corrosion.

Conclusions

In general, biodegradation and corrosion resistance depends on the manufacturing process, nature of alloy and surface features of the appliance. This study showed that release of ions does not depend on the amount of this ion in orthodontic brackets. Rather, it depends on the nature of the alloy and the manufacturing process. Biodegradation more dependent on bracket manufacturing rather than bracket composition.

One-piece orthodontic brackets with one single type of metal alloy are highly recommended and showed superior biocompatibility as in Dentaurum Brackets (equilibirium family Stainless-Steel) than orthodontic brackets in two pieces (body and base) joined by silver solder as American orthodontic brackets.

Despite the limitation of the *In-Vitro* study, the outcomes of this study need clinical attention. It is recommended to orthodontists to select high biocompatible orthodontic brackets that biologically safer for the patient withhigh corrosion resistance and minimal or none ion release in a biological system. Even a small amount of ions released in a biological system may provoke sensitivity reaction when

orthodontic appliances and placed in the oral cavity for several years.

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Declaration of Interest

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