

Article

Ion Release from Dental Implants, Prosthetic Abutments and Crowns under Physiological and Acidic Conditions

María Arregui ¹, Florian Latour ¹, Francisco Javier Gil ², Román A. Pérez ², Luis Giner-Tarrida ¹
and Luis M. Delgado ^{2,*}

¹ Department of Dentistry, Faculty of Dentistry, Universitat Internacional de Catalunya, 08195 Sant Cugat del Vallès, Spain; mariaarregui@uic.es (M.A.); od078476@uic.es (F.L.); lginer@uic.es (L.G.-T.)

² Bioengineering Institute of Technology, Universitat Internacional de Catalunya, 08195 Sant Cugat del Vallès, Spain; xavier.gil@uic.cat (F.J.G.); rperezan@uic.es (R.A.P.)

* Correspondence: lmdelgado@uic.es; Tel.: +34-935042000

Abstract: Ion release from dental implants and prosthetic restoration can affect osteointegration and implant viability over a long period of time. Therefore, the aim of this study was to study the ion release from implants and crowns, with and without intermediate anodized abutments, in two different media, simulating clinical conditions. The implants, intermediate prosthetic abutments and Cr–Co crowns were divided into two groups depending on the media: Hanks’ solution and 1% lactic acid, simulating body fluids and microbiologically conditioned fluids, respectively. The study followed the ISO 10271:2011 and 10993-15:2000 standards modified to simulate the replacement of fluids in the oral environment. The ions’ release was measured by inductively coupled plasma mass spectroscopy (ICP-EOS), and only aluminum, chromium, cobalt, titanium and vanadium were identified. Ion concentration was higher in lactic acid than in Hanks’ solution at all time points ($p < 0.05$). Only vanadium showed a very low ion release in lactic acid, with no statistically significant differences from the ion release in Hanks’ solution ($p = 0.524$). Both anodized abutments and the immersion medium influenced the release of ions and affected the corrosion of these structures. The presence of an intermediate anodized abutment also affected ion release, as the level of ions was lower in groups with this component.

Keywords: ion release; corrosion; anodization; dental implants; prosthetic abutments



Citation: Arregui, M.; Latour, F.; Gil, F.J.; Pérez, R.A.; Giner-Tarrida, L.; Delgado, L.M. Ion Release from Dental Implants, Prosthetic Abutments and Crowns under Physiological and Acidic Conditions. *Coatings* **2021**, *11*, 98. <https://doi.org/10.3390/coatings11010098>

Received: 31 December 2020

Accepted: 11 January 2021

Published: 18 January 2021

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1. Introduction

In the last 20–30 years, implant dentistry has experienced a strong development, and one of the factors that has contributed to its success is the biocompatibility of its materials. The definition of biocompatibility has been modified and changed over the years as the subject has become better understood, and thanks to the standards and regulations established by the ISO and ANSI/ADA [1,2]. In the tissue engineering field, biocompatibility has been recently redefined as “the ability of a material to perform with an appropriate host response in a specific application” [3], which has been deeply discussed elsewhere [4]. Clinically, dentists associate the marketing of a product with its biocompatibility, because it has passed the required tests prior to its clinical use subject to standards organizations [1].

Titanium and its alloys are highly used materials because of their biocompatibility, good mechanical properties, resistance to corrosion [5–7] and ability to osseointegrate into the body [5]. However, in the oral environment, biodegradation occurs due to the thermal, microbiological and enzymatic conditions therein [8]. One of the consequences of degradation is corrosion, since it is a destructive attack on the metals caused by a chemical or electrochemical reaction within the environment, and it is one of the causes of metallic implant failures in the oral environment [9] such as crevice pitting and galvanic corrosion [10]. Corrosion can be measured in different ways, such as: (1) analyzing the

surface using microscopy; (2) studying the corrosion voltage using electrochemical tests; and (3) measuring direct ion release using spectrometry tests [1,11].

Dental implants are mainly made of commercially pure titanium (cpTi); however, abutments and crowns are usually made of titanium alloys and chromium–cobalt (Cr–Co), respectively. This combination of metals in the oral environment not only causes the appearance of corrosion by friction but also results in galvanic corrosion, which in turn causes the dissolution of the metals in the implant, ion release and, finally, bone destruction [12]. This degradation generates an ion release, which cannot be dismissed even at relatively low concentrations, as it has a relationship with implant failure, toxicity, hypersensitivity or allergies caused by metals both locally and systemically [10,12–14]. The biological response depends on the released metallic ion, the concentration and the exposure time [10,11]. Nevertheless, it is not only metals that can induce cytotoxic effects, as nonmetallic dental materials are also susceptible to inducing cytotoxicity [15].

Another factor that influences ion release in dental implants is the type of connection between implant and abutment. There are different types of connection, such as external, hexagonal and internal which can be hexagonal, Morse taper, octagonal or trilobe [16]. In addition to the materials and the type of connection, it is also important to take into consideration the surface treatment of implants and abutments, since in most cases this treatment generates a layer of titanium oxide, which acts as a protection mechanism for the implant and the abutments. The clinical problem is the loss of this protective layer under certain scenarios, which releases ions that can have negative effects on tissues such as bone [17]. Therefore, the development of new strategies to avoid the direct connection of titanium implants and chromium–cobalt crowns could minimize corrosion and its related phenomena, such as ion release.

In addition to the combination of the metals and their connection, the oral environment must also be considered, as its pH can affect corrosion. To evaluate the *in vitro* corrosion resistance of dental implants, it is important to reproduce the oral medium with different types of solutions. The most used solutions, which try to mimic the different pH and clinical situations, are artificial saliva, 0.9% NaCl solution, phosphate-buffered saline (PBS), Hanks' solution [14,18] and lactic and formic acid [19,20].

The aim of this study was to analyze the effects of corrosion and ion release from implants and crowns, with and without intermediate abutments, in two different media simulating clinical conditions. We propose to evaluate the usage of intermediate anodized abutments between dental implants and crowns in order to minimize ion release and its detrimental effects. The null hypothesis was that no significant differences would be found between physiological and acidic conditions in terms of the ion release from metallic dental implant systems.

2. Materials and Methods

Bone level conical implants made of type IV cpTi were used with the clinically common measurements: 14 mm in length and 4 mm in diameter, with an internal hexagonal connection (Naturactis, Eurotekніка Implants Iberia SL, Barcelona, Spain). The intermediate abutments (Straight Plural, Eurotekніка Implants Iberia SL, Barcelona, Spain) were made of titanium alloy (Ti6Al4V) with a height of 2.2 mm, a conical connection at the implant level and an anodized surface. The crowns were made from sintered Cr–Co blocks in a premolar shape with a superstructure length of 5 mm and a mechanized connection, which are clinically common parameters. As a matter of reference and according to ISO 5832-2:2018, ISO 5832-3:2016 and ISO 22674:2006 standards [21–23], the composition of each of the materials studied can be seen in Table 1.

For the anodization, samples were cleaned in ethanol, distilled water and acetone under sonication for 15 min each to remove surface contamination. Subsequently, an electrochemical anodizing process was performed in an aqueous solution with phosphoric acid (Panreac, Castellar del Vallès, Spain) at room temperature. Titanium samples were used as a working electrode, with a platinum sheet as a counter electrode. The working

voltage was 55 V and the time was free until the current dropped to zero. After treatment, all samples were cleaned as explained previously.

Table 1. The composition of the metallic alloys (wt.%), according to ISO 5832-2:2018, ISO 5832-3:2016 and ISO 22674:2006 standards.

Ions	cpTi (IV)	Ti6Al4V Alloy	Cr–Co Alloy
Ti	99	90	-
Al	-	6	-
V	-	4	-
Co	-	-	61.8–65.8
Cr	-	-	23.7–25.7
Mo	-	-	4.6–5.6
W	-	-	4.9–5.9
Si	-	-	0.8–1.2
Fe	-	-	Max. 0.50
Mn	-	-	Max. 0.10

The specimens were divided into two groups: control and experimental. The control group was divided into 3 subgroups according to the material (C1—implant, C2—abutment and C3—Cr–Co). Likewise, the experimental group was subdivided into 4 groups according to whether or not the specimens had intermediate abutments and according to the storage medium, as can be seen in Table 2. All of the experimental groups had a sample size of 3.

Table 2. Group and subgroup distribution.

Group	Study Code	Metals	Storage Medium
Control	C1	cpTi (Type IV)	Hanks' solution
	C2	Ti6Al4V	Hanks' solution
	C3	Cr–Co	Hanks' solution
Experimental	E1	cpTi + Cr–Co	Hanks' solution
	E2	cpTi + Ti6Al4V + Cr–Co	Hanks' solution
	E3	cpTi + Cr–Co	Lactic acid solution
	E4	cpTi + Ti6Al4V + Cr–Co	Lactic acid solution

In the experimental groups, the superstructures were screwed at 35 Ncm for the intermediate abutment, at 25 Ncm for the Cr–Co block coupled directly to the implant and at 20 Ncm for the Cr–Co block coupled to the intermediate abutment. Teflon and composite (Filtek 350XT, 3M Espe, St Paul, MN, USA) were placed in these groups to block access and avoid the screw coming into contact with the storage medium. The torque used to screw on all the structures was in accordance with the manufacturer's instructions.

The static immersion protocol was used for this study, following the ISO 10271:2011 and 10993-15:2000 standards [24,25], modified with media replacements to simulate the renewal of body fluids during the analysis period. Two types of solutions were selected for use: a Hanks' solution (Sigma-Aldrich, Madrid, Spain) for the control groups, E1 and E2; and a 1% lactic acid solution for groups E3 and E4. The Hanks' solution (pH = 7.4) was already prepared, and the 1% lactic acid aqueous solution (0.1 mol/L lactic acid and 0.1 mol/L sodium chloride) was prepared immediately before use (pH = 2.3) according to ISO 10271:2011 [25]. Each specimen was placed in a separate polypropylene tube and 1 mL of solution was added per square centimeter of surface area. The specimens were scanned with an extraoral 3D scanner (CS 3600, Carestream Dental LLC, Atlanta, GA, USA) and then the measurements were analyzed using the STL file and the Geomagic Control X software (version 2018) (3D systems, Rock Hill, SC, USA), as well as the information provided by the manufacturer. Table 3 shows the volumes of solution (Hanks' solution or 1% lactic acid) corresponding to each studied group. Subsequently, all tubes were kept in an incubator at a constant temperature of 37 ± 1 °C throughout the study period.

Table 3. The surface area of the components in each group with the corresponding solution volumes.

Group	Surface Area (cm ²)	Solution Volume (mL)
C1	3.5	3.5
C2	0.7	0.7
C3	1.6	1.6
E1–E3	5.1	5.1
E2–E4	5.4	5.4

Following the biodegradation of the alloys, the concentrations of the metallic ions dissolved in the Hanks' solution and 1% lactic acid were measured using inductively coupled plasma mass spectrometry (ICP-EOS). This spectrometric technique facilitates the quantification of chemical elements in dilute solutions, even down to parts per billion (ng/mL). This high sensitivity is due to the use of argon plasma, which operates at temperatures between 8000 and 9000 °C, temperatures at which almost all materials become ionized. This technique has many advantages in the laboratory in terms of identifying the presence of metals, because almost all elements can be determined and identified. This spectrometric technique has a high sensitivity and provides rapid analysis [13].

All solutions were extracted and replaced after 1, 7, 14 and 21 days. The extracted solutions were placed into polypropylene tubes containing 20 µL of 2% HNO₃ solution to avoid ion precipitation and stored in the refrigerator at 4 °C until ICP-EOS analysis. Dissolved ion concentrations were measured using Agilent 7500ce ICP-EOS (Agilent Technologies, Santa Clara, CA, USA).

The surfaces of the components were analyzed at the connection level before (D0) and after (D21) immersion using the SterEO Discovery.V8 stereomicroscope (Carl Zeiss Microscopy GmbH, Göttingen, Germany). The microphotographs were saved by means of a digital camera connected to the stereomicroscope and analyzed with the Zen 2 Lite software (version 1.0, Carl Zeiss Microscopy GmbH, Göttingen, Germany).

All of the experimental groups' procedures were carried out in triplicate. The numerical data were expressed as mean ± standard deviation. The statistical analysis was carried out using MINITAB® (version 16.2, Minitab Inc, State College, PA, USA). A one-way analysis of variance (ANOVA) was performed, followed by Fisher's post hoc test after confirming the normal distribution of each sample (using an Anderson–Darling normality test) and the equality of the variances (using Bartlett and Levene variance homogeneity tests). Nonparametric statistics were used when one or both of the above assumptions were not met and, consequently, the Kruskal–Wallis test was performed for multiple comparison analysis. A significant difference was accepted when $p < 0.05$.

3. Results

In the present study, a higher release of ions was observed in the groups immersed in 1% lactic acid compared to those immersed in Hanks' solution, with statistically significant differences ($p < 0.001$) for aluminum (Al), chromium (Cr), cobalt (Co) and titanium (Ti) ions. The only ion that did not show any statistically significant difference between the two solutions was vanadium (V) ($p = 0.524$). The results for ion release from highest to lowest are as follows: Co > Ti > Al > Cr > V, in which vanadium was not only the least-released element, the amount released was negligible.

When ion release is analyzed over time, it can be seen that all groups showed a higher ion release after 21 days, especially the groups immersed in lactic acid (Figure 1).

In 1% lactic acid, the group with a direct connection between the crown and the implant (E3) presented a higher release of Al, Cr, Co and Ti ions than the group using an intermediate anodized abutment (E4). In Hanks' solution, the release of these ions was lower; however, aluminium release was increased for both experimental groups in comparison to the control group (the materials alone, C1 and C2). On the other hand, the control group showed a higher cobalt ion release than the experimental groups, and the

chromium values were similar between the control and the experimental groups (Table 4 and Figure 2).

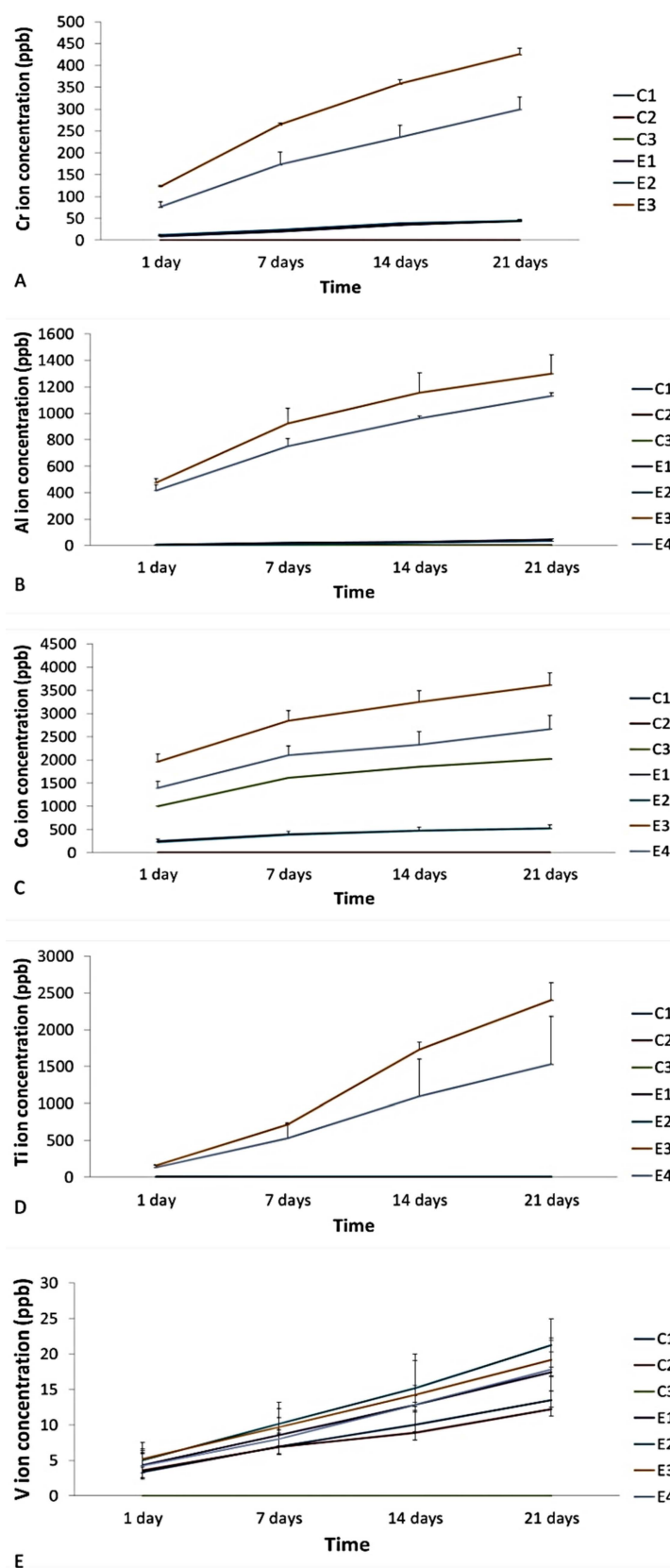


Figure 1. The evolution of ion release over time. (A) chromium; (B) aluminium; (C) cobalt; (D) titanium; and (E) vanadium.

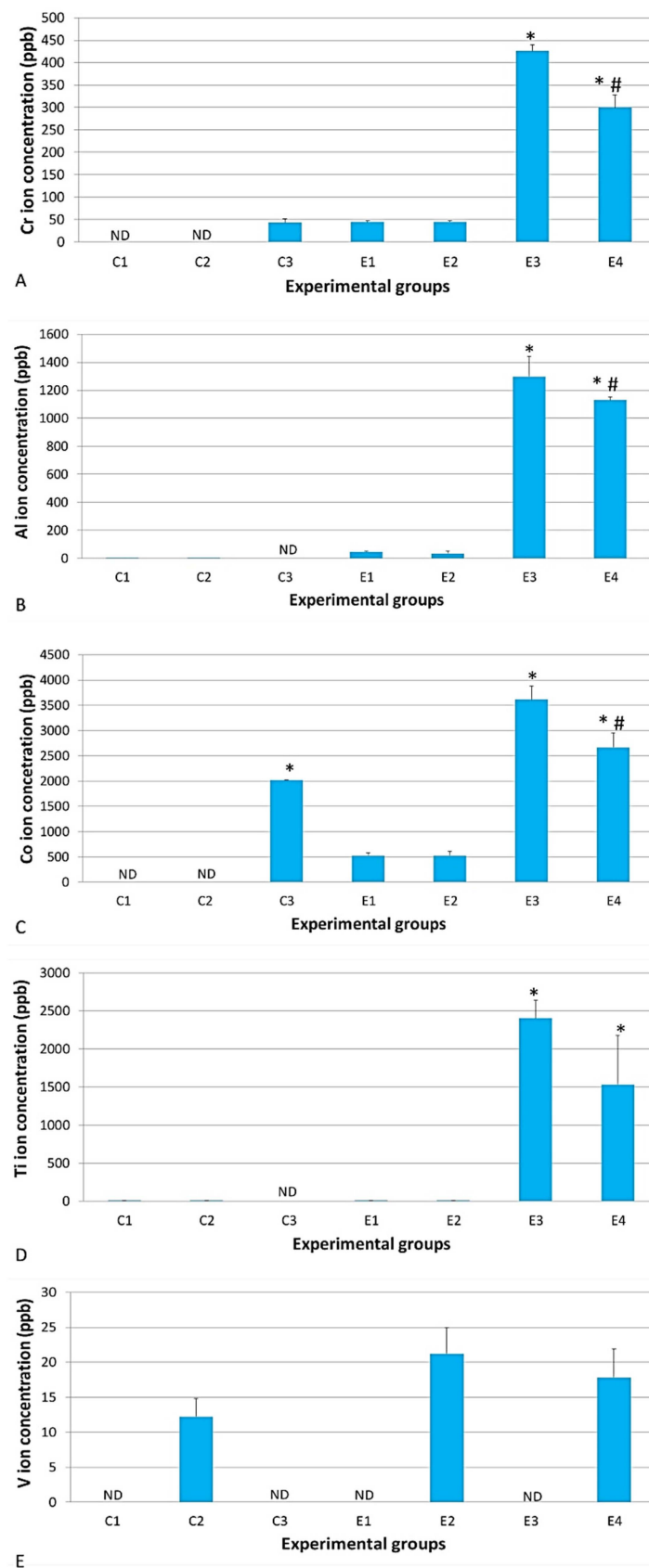


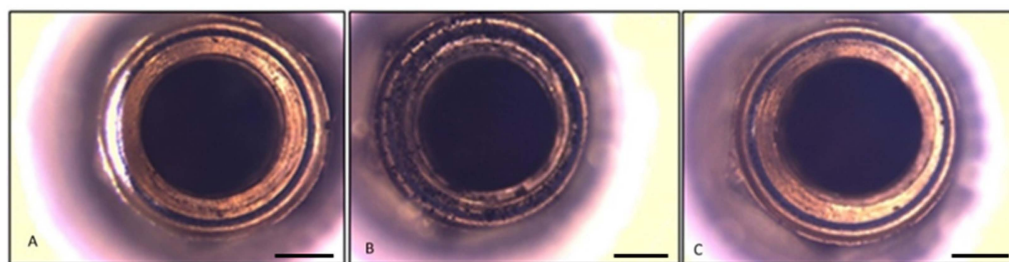
Figure 2. The concentration of ions released at 21 days. (A) chromium; (B) aluminum; (C) cobalt; (D) titanium; and (E) vanadium. “*” in Cr, Al, Co and Ti indicates statistically significant differences to E1; “#” in Al, Cr and Co indicates statistically significant differences to E3; ND: non-detectable.

Table 4. The total levels of metallic ion release from the different groups at 21 days.

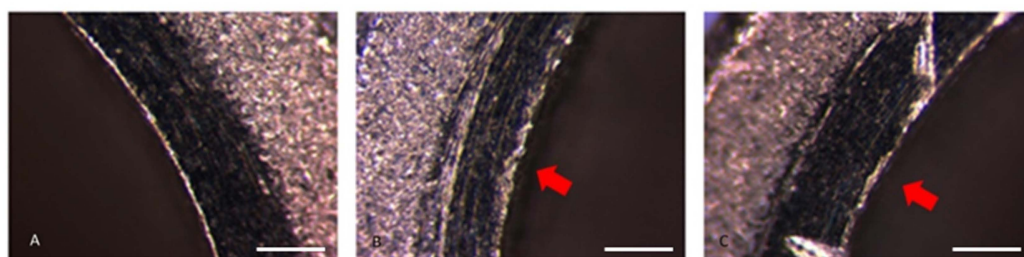
Specimen		Released Ion Concentration (ppb)					
Solution	Group	Ti	Al	Co	Cr	V	Total
Hanks' solution	C1	5.83 (0.05)	2.31 (0.00)	–	–	–	8.14
Hanks' solution	C2	7.79 (1.03)	2.22 (0.00)	–	–	12.23 (2.57)	22.24
Hanks' solution	C3	–	–	2020.68 (2.47)	42.72 (8.21)	–	2063.40
Hanks' solution	E1	6.79 (0.58)	46.41 (2.93)	523.28 (57.61)	43.78 (3.32)	–	620.25
Hanks' solution	E2	6.99 (0.79)	33.26 (16.34)	519.78 (80.89)	44.42 (2.37)	21.25 (3.71)	625.69
Lactic acid solution	E3	2405.49 (236.39)	1299.12 (143.39)	3615.92 (263.35)	426.04 (13.58)	–	7746.56
Lactic acid solution	E4	1531.30 (649.53)	1130.72 (21.98)	2666.21 (287.59)	299.48 (28.52)	17.83 (4.09)	5645.55

Standard deviations in parentheses. “–” indicates an amount below the detection limit.

The analysis of the corrosion conducted by observing the stereomicroscopic images showed changes in the surfaces. In the internal part of the implant, material transfer could be observed in the groups where the crown was screwed directly to the implant (E1 and E3), whereas no material transfer between the implant and the abutment was observed in the groups with intermediate abutments (E2 and E4) (Figure 3).

**Figure 3.** Pictures ($\times 2.25$) of the internal implant platform: (A) without superstructures; (B) connected to a Cr–Co crown; and (C) connected to an intermediate abutment. Scale bar indicates 1 mm.

At the level of the implant neck, a plastic deformation of the titanium was observed, which caused irregularities in the surface in contact that were more pronounced in the groups in direct connection with the implant (E1 and E3) than in the groups with intermediate abutments (E2 and E4). A greater plastic deformation was also observed in the metals directly connected to the crowns than those using the intermediate abutment (Figure 4).

**Figure 4.** Pictures ($\times 8$) of the external implant platform: (A) without superstructures; (B) connected to a Cr–Co crown; and (C) connected to an intermediate abutment. The red arrows show the deformation of the internal part. Scale bar indicates 200 μm .

When analyzing the corrosion of the unscrewed intermediate abutment, a well-defined line was observed that determined its connection area to the implant, resulting in a deformation of the substrate that could imply material transfer and the loss of the titanium oxide layer (Figure 5).

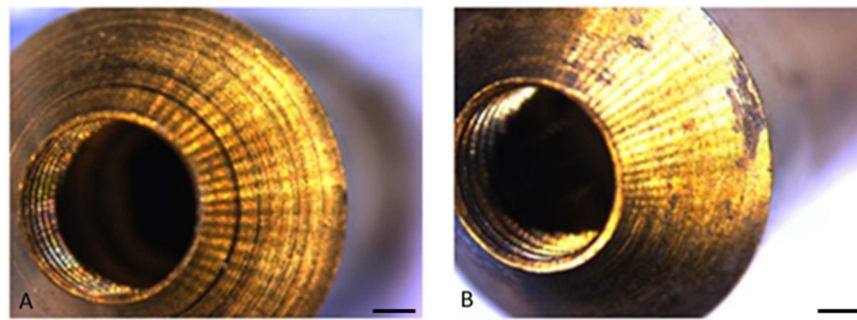


Figure 5. Pictures ($\times 1.6$) of the intermediate abutment: (A) initially, before a connection; and (B) after 21 days connected to a Cr–Co Crown. Scale bar indicates 1 mm.

As can be seen in Figure 6, the implants connected directly to the crown had an exposed area of milled and polished material, whereas it can be seen that the groups with intermediate abutments had a surface treatment; nonetheless, an exposed area can also be seen.

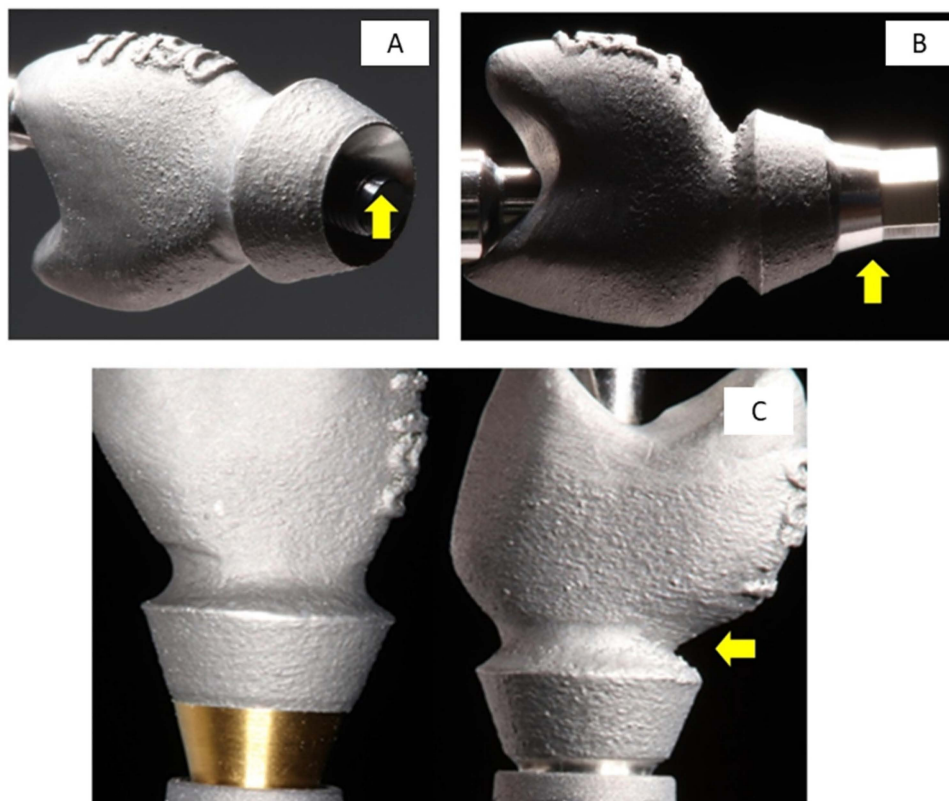


Figure 6. (A) A Cr–Co crown (the yellow arrow indicates the mechanized connection part); (B) A Cr–Co crown with the intermediate anodized abutment; (C) On the left, a Cr–Co crown connected to the anodized abutment and the dental implant. On the right, a Cr–Co crown connected directly to the dental implant with an exposed mechanized surface.

4. Discussion

Ion release can have local and systemic biological effects. In implantology, the release of ions is one of the factors associated with peri-implant inflammatory reactions and subsequent peri-implantitis [16]. To perform this study, the ISO 10993-15 standard was followed, which evaluates the release of ions at the dental implant level [24]; as well as the ISO 10271 standard, which studies the materials at a prosthetic level, simulating the oral cavity [25].

Hanks' solution, which simulates blood plasma with pH = 7.4, and 1% lactic acid, which simulates gingival sulcus fluid in contact with biofilm pH = 2.3, were used to simulate oral fluids. Furthermore, the presence of bacterial plaque was also simulated with this acid after the consumption of food and beverages, which causes a pH drop below 4. This pH variation is due to the presence of acid-forming bacteria, which release lactic acid, among others [6,20].

In the present study, the ions released in higher concentrations were aluminum, cobalt and chromium, especially in the group where the implant was connected directly to the chromium–cobalt crown immersed in lactic acid (E3). In terms of titanium ion release, there were no statistically significant differences when the implant was connected to the intermediate abutment (E4) and the crown (E3), while vanadium ion release was practically undetectable. These results differ from those of other studies [10,14], where vanadium ion release was increased in conditions which were more severe than physiological ones.

One factor that may have influenced these results is the use of an intermediate Ti6Al4V abutment that was anodized as a surface treatment. Yamagami et al. [7] analyzed the nanostructure and the atomic composition of the anodized Ti6Al4V alloy and observed that aluminum and vanadium were not present on the outermost surface of the anodic oxide layer. This could explain why the groups that interposed the anodized abutment (E2 and E4) presented a lower release of ions.

In this study, cobalt was the most released ion, with values ranging from 2600 to 3600 ppb for the indirect crown and the direct crown, respectively, after 21 days in lactic acid. Chromium was released at lower levels, between 300 and 426 ppb. The toxicity of cobalt is strongly related to the dose, and in the case of chromium it is associated with toxicity and mutagenicity [14]. The release of Cr and Co in this study was similar to that noted in Kassapidou et al. [26], where different techniques (cast, milled, laser-sintered and pre-sintered milled) to produce Co–Cr alloys were compared and the cast and milled groups showed the highest ion release in acidic conditions.

The amounts of Cr and Co ions detected can be assumed to induce different local reactions depending on whether or not an intermediate abutment is used and on the medium in which the alloy is placed. The lower Cr and Co ion release in the intermediate abutment group could be related to the surface treatment of the abutment, where anodization led to a reduction in galvanic reactions. Another possibility could be the exposure of a mechanized surface of a crown in the group directly connected to the implant, which was not observed in the intermediate abutment group.

In this study, the presence of aluminum ions released in the group directly connected to the Cr–Co crown (E3) should be highlighted, as they should not be present. A possible interpretation could be the presence of traces from the surface treatment, as aluminum oxide particles were used for sandblasting. This contamination is documented in the literature [5,27]. Jabbari et al. [27] showed that particles adhered to the surface of implants are resistant to severe cleaning. It has also been shown that blasting impurities are difficult to remove by several methods such as acid etching, ultrasonic cleaning and sterilization [5]. Therefore, these particles can cause the release of aluminum ions into the surrounding tissues due to the degradation caused by the physiological environment. Furthermore, it has been shown that these aluminum particles could compromise the corrosion resistance of titanium in a physiological environment [28].

The toxic effect of aluminum is documented in the literature both at a systemic level and at a local level [14,29]. In this last aspect, there is some controversy, as there are authors who consider that aluminum can cause an inflammatory response, altering the healing process [30], as well as an inhibition of the mineralization of the bone matrix [30,31]. In contrast, other authors state that the released aluminum ions do not influence implant osseointegration [32].

The vanadium released in this study did not reach detectable values for analysis. This result contrasts with the study by Alrabeah et al. [10], where vanadium was released in all groups where the Ti6Al4V alloy was used. This could be explained by the lack of vanadium

in the most superficial layer of anodized abutments. Vanadium is considered an essential element in the body [8,14], but it becomes toxic at high levels, as it can cause both local and systemic reactions [8,33].

The release of titanium ions is higher in the lactic acid immersed groups than in the Hanks' solution group, as in other studies [6]. As described by Delgado-Ruiz et al. [34] in their systematic review, the release of titanium ions is due to the presence of an acidic medium that causes the implant to lose part of its titanium oxide layer that it is unable to recover, causing the metabolites to be deposited in the surrounding tissues. Moreover, the present results show a lower ion release than studies in which the implants were thermally cycled [17]. Therefore, thermal stress could be considered a relevant factor that may affect ion release.

In the present study, it was observed that the longer the time studied (maximum 21 days), the higher the ion release produced in all groups (control and experimental), being the highest in the experimental groups immersed in lactic acid. Similar results were observed in other studies [10,14,19,20].

Okazaki et al. [14] stated that the amount of released ions changes depending on the nature and bonding strength of the metal oxides, the structure (vacancies, interstitial elements and degree of ordering), the role of the alloy elements as well as the composition and thickness of the oxide layer. Additionally, Suito et al. [6] considered that the release of ions was influenced by immersion time, the pH, acid type, the mechanical stimulus and contact with different metals. Furthermore, the corrosion behavior and ion release could be related to the chemical composition of the materials and the differences between the processing techniques of the different parts (e.g., milling of the Cr-Co crown and anodization of the abutment) [35].

The Vickers hardness value is 160–200 HV for pure titanium, 350–430 HV for Ti6Al4V alloy [36] and 420–450 HV for Cr-Co, according to the manufacturer. This variation in hardness between the material of the dental implant, the intermediate abutment and the crown can cause the wear of the internal surfaces of the implant connection during the application of torque to fix the different structures, or even during biting that causes micromovements [36]. As a result, there is a loss of coupling and a reduction in the mechanical integrity of the interface between the implant and the abutment [34].

In the present study, this deformation was observed at the interface of the implant with its superstructures after the application of torque. However, the extension varied depending on the type of metals connected; a greater deformation was observed in the groups where the crown was directly attached to the implant. This could be related to the fact that these groups release more ions due to a higher loss in the titanium oxide layer, causing instability in the alloy elements. It could also be related to the fact that Cr-Co has a greater hardness, thus generating a greater tension in the internal part of the implant and causing a transfer of material from the implant to the crown, since the transfer is from the softer material to the harder one.

One limitation of the study was that we chose to keep the immersion temperature constant at 37 °C, taking into account that the temperature in the oral cavity may fluctuate due to liquid consumption, body temperature, medicine consumption, age and breathing, among other factors [37,38]. It has been observed that these temperature changes influence the behavior of metals and change the amount of ions released. Studies confirm that an increase in temperature causes a decrease in the corrosion resistance of metals used in the oral cavity [9,17].

Future research should study the effect of temperature changes on ion release and corrosion, and should evaluate the effects of tribocorrosion, as this could better simulate the interactions present at the oral cavity level. Furthermore, the effect of the amount and type of ions released on certain cell lines in the oral cavity could also be studied. Clinically, our results suggest that intermediate anodized abutments should be considered when designing a dental prosthetic reconstruction. Moreover, the release of metal ions

under acidic conditions should be considered for patients with previous episodes of metal sensitivity and inflammatory responses.

5. Conclusions

Within the limitations of this study, we conclude that the surface treatment and the anodization carried out on implants and prosthetic abutments, as well as the immersion medium, influence the release of ions from dental implants and prosthetic elements. The presence of an intermediate anodized abutment reduces ion release. Finally, the metallic ion release under acidic conditions was significantly increased in this study, which could have clinical implications, such as revealing increased toxicity, metal sensitivity and inflammatory response in these conditions.

Author Contributions: Conceptualization, M.A. and L.G.-T.; methodology, M.A., F.L., and L.M.D.; formal analysis, M.A., L.G.-T., and L.M.D.; resources, M.A.; data curation M.A., L.G.-T., and L.M.D.; writing—original draft preparation, M.A.; writing—review and editing, F.J.G., R.A.P., and L.M.D.; visualization, R.A.P.; supervision, L.M.D.; funding acquisition, L.G.-T. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors would like to thank Euroteknika Implants Iberia SL for providing the materials for the study.

Conflicts of Interest: The authors declare no conflict of interest.

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