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Potential release of trace metal ions from metallic orthodontic appliances and dental metal implants: A Review of *in vitro* and *in vivo* experimental studies

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Abstract

Metallic wear nanoparticles, corrosion products and trace metal ions release from metallic materials, e.g. metallic alloys and pure metals, implanted into the human and animal's body in orthodontic surgery is becoming a major cause for concern. Additionally, metallic wear nanoparticles can themselves undergo a corrosion process contributing to the total level of dissolved metal ions. This review briefly provides an overview of both metallic alloys and pure metals used in implant materials in dental surgery. A short section is dedicated to important biomaterials and their corrosive behavior in both real solutions and various types of media that model human and animal's biological fluids and tissues. The present review gives an overview of analytical methods, techniques and different approaches applied to the measurement of *in vivo* and *in vitro* trace metals released into body fluids and tissues from patients and animals carrying metal dental implants and metallic orthodontic appliances. Reference levels of ion concentrations in body fluids and tissues that have been determined by a host of studies are compiled, reviewed and presented in this paper. This review summarizes data, describing the potential toxicity of metal wear debris, free metallic ions and corrosion products release from metallic orthodontic appliances (metal surgical implants) *in vivo* and *in vitro*. Finally, a collection of published clinical data on *in vivo* and *in vitro* released trace metals from metal dental implants is included.

Keywords: Metallic orthodontic appliances; Dental metal implants; Human biological specimens; Animal model; *In vivo* and *in vitro* metal ions release; *Ex vivo* quantifications

1. Introduction

In dentistry, metallic materials are used as implants in reconstructive oral surgery to replace a single tooth or an array of teeth, or in the fabrication of a dental prosthesis, such as metal plates for complete or partial dentures, crowns and bridges, and are particularly essential for patients requiring hypoallergenic materials. Depending on the different requirements for the wide range of applications, the dental material market offers a large variety of products; various inert metallic and alloy biomaterials are used in these implant systems [1,1a]. Elements which constitute the orthodontic fixed appliances (bands, braces, wires) are manufactured from metal alloys which undergo corrosion in the environment of the oral cavity.

Over the past decades, there have been significant developments in the availability of suitable metals and metal alloys for dental implants. These materials possess excellent corrosion resistance, good mechanical properties, low inherent toxicity and good biocompatibility. However, when pure metal and metal alloys are implanted into a complicated and corrosive physiological *in vivo* environment, the surface oxide film stability may be affected (disrupted) and fresh metal surface must be exposed to release a large amount of metal ions from metallic materials, resulting in increased metal ion release. Metal ions are released to the aggressive body environment (biological fluids and tissues in the body) from metallic biomaterials, used in dental implants. This is accomplished through various mechanisms, including corrosion,

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wear and mechanically accelerated electrochemical processes, such as resulting in stress corrosion, corrosion fatigue and fretting corrosion. A large amount of released metal ions could be harmful to human health and may eventually lead to severe complications and failure of the implant system. As an implant corrodes, either electrochemically or mechanically, metal ions, metal complexes or implant-derived particles (in the nanometer range) are released.

Medical-grade stainless steels and other alloys (Co-Cr; Co-Cr-Mo; Cr-Ni-Cr-Mo), tantalum, zirconium, wolfram, titanium and titanium alloys are all used as biomaterials, while noble-metal-based alloys are used for dental materials because of their high corrosion resistance and durability. Metal ion release from metallic dental implants is inhibited by the surface oxide as a passive film where partial dissolution and reprecipitation are repeated in aqueous solutions, therefore ensuring corrosion resistance. Therefore, the film properties and the potential in change *in vivo* must be considered in order to understand metal ion release.

This paper takes a close look at the mechanisms of metal ion release from metallic implant materials and the behavior of the released ions *in vivo*, presented according to empirical data. After a brief summary of the composition and types of bioimplant materials and biocorrosion, the release of *in vivo* metal ions is discussed. Other aspects leading to the release of *in vitro* metal ions and potential related adverse physiological effects, including toxicity and metal allergy, are the scope of this contribution and will be discussed here.

For the first time, a comprehensive survey of available approaches for the determination of *in vivo* and *in vitro* bioaccessible metallic nanoparticles/ions in the human body and animals is reported. Furthermore a compilation of the results (metal particles and ion studies) that have so far been published in the literature is presented. Other aspects leading to the release of *in vivo* and *in vitro* metal ions and potential related adverse physiological effects, including toxicity, carcinogenicity, genotoxicity and metal allergy, are beyond the scope of this contribution and will not be discussed here. It is not within the scope of this review to evaluate possible toxicological effects produced by the intake of trace metals, nor to estimate tolerable animal body intakes.

2. Metallic orthodontic appliances/metal implants

Biomaterials are either natural or mane-made materials, which are used to aid or replace the functions of living tissues. They must simultaneously meet many requirements and have special properties such as non-toxicity, corrosion resistance, fatigue durability and biocompatibility. Metal and metal alloy biomaterials (stainless steel, Co-, Cr-, Ti and Ti-based alloys) for implants have been widely applied in the dental fields [2,3], while noble-metal-based alloys are used for dental materials, because of their high corrosion resistance and durability [4]. These materials possess excellent corrosion resistance, good mechanical properties and biocompatibility. However, when a metal or metal alloy is implanted into a complicated and corrosive physiological environment, the oxide stability may be affected; resulting in increasing metal ion release. Therefore, the analysis of impurities in steels and alloys used for medical applications is of prominent importance, because of possible health effects from the use of improper materials.

Titanium and titanium-based alloys have been widely used in dental implants and can be considered as the most corrosion-resistant of the alloys described [5]. This is based on the very high stability of the TiO_2 passive film that spontaneously forms on the alloy surface. Ti and its alloys do not provoke allergic reactions and are considered to be highly biocompatible. At present, most of the implanted systems are made of pure titanium (cp-Ti) or Ti-6Al-4V alloy. Cp-Ti does not yield sufficient hardness for load-bearing applications and is therefore mainly used in dental surgery, for the manufacture of acetabular shells and in coatings for joint replacement. An interesting theme of recent debates is the selection of conventional titanium implants or more recently introduced and increasingly popular zirconia implants as dental implants [6,7]. Titanium remains the gold standard for the fabrication of oral implants, even though sensitivity does not occur, though its clinical relevance is not yet clear.

Zirconia implants may prove to be promising in the future; however, further *in vitro* and well-designed *in vivo* clinical studies are needed before such a recommendation can be made. In this review, the terms "zirconia" (zirconium) and "titanium" are used for general and comprehensive material names, while ZrO_2 and Ti are used when the compositions are clear. Stainless steel (SS) is the general name for a number of different steels used mainly because of its resistance to a wide range of corrosive agents. SS (primarily type 316L, iron based) is used cost effective orthodontic fixed appliances [8]. However, major disadvantages of SS are well-documented. Upon prolonged contact with human tissue surface corrosion phenomena takes place resulting in a high rate of locally released corrosion products. Release of large amount of certain metal ions may lead to harmful deceases. The ions released from SS are mostly of iron, nickel and chromium. Specially nickel is recognized as a strong immunological reaction medium and may cause hypersensitivity reactions, contact dermatitis, asthma, and moderate cytotoxicity [9].

3. *In vivo* corrosion and wear behavior of metallic orthodontic appliances/metal implants

Corrosion of metals and alloys used as implants in the body is a complex process where the metal is challenged within the body due to changes of the pH, body fluids, exposure to cellular processes, *etc*., and is due to the chemical environment of the body [10,11,11a]. A metallic foreign body", such as dental implants, may interfere with oxidation-reduction reactions that are the basis of metabolic and growth processes. Red-ox reactions occur at the metal surface and can cause denaturation of the tissue that is in contact with metallic implants. The metals and alloys used as surgical implants achieve passivity by the presence of a protective surface layer. This film inhibits corrosion and keeps current flow and the release of corrosion products at a very low level. Even so, metallic ions from dental metallic implant materials are eventually generated and released into the body by electrochemical corrosion of metal surfaces, chemical dissolution, *in vivo* wear or mechanically accelerated electrochemical processes, such as fretting corrosion, stress corrosion or corrosion fatigue [12-17]. Passi et al. [18] reported diffusion of titanium into the bone bordering dental implants, while when aluminum was present in the fixture; it leaked diffusely into the surrounding bone. Vanadium leakage was not found in the tissues.

Metal implants induce the transfer of some metallic wear particles from dental metal mini-implants to surrounding soft tissues (lungs, liver, spleen and bone marrow) [19,20,20a]. In dentistry nanoparticles are playing an increasing role: they are intentionally embedded *per se* or as byproducts from mailing processes of larger filler particles in many dental materials or are produced by intraoral adjustment of set restorative materials through grinding/polishing regardless whether they contain nanoparticles or not [21]. The fact that nanoparticles can originate from various metallic orthodontic appliances in the human body is well known in dentistry; the question is what is the limit to the level of nanoparticles that is still tolerable in the human body. Mombelli et al. [22] have observed that the sizes of particles produced from titanium-based dental implants are to range from nanometer to micrometer scales.

Corrosion is one of the possible causes of dental implant failure after initial success; corrosion affects the biocompatibility of dental implants, and their functional ability and useful lifetime [23]. Due to its mechanical properties, good resistance to corrosion in biological fluids and tissues and very low toxicity, titanium has been the most commonly selected material for dental implants [24,25]. Pure titanium and titanium alloys have inherent corrosion resistance, and they are considered nontoxic. However, limited information exists on the corrosion characteristics of nanoscale titanium implants. There is some evidence indicating that metallic implants may undergo surface degradation and may become the site of reactions that lead to the release of metal ions to surrounding tissues. This process may cause peri-implant inflammatory reactions and cytotoxicity [26]. The corrosion processes of the orthodontic appliances, when severe, may be observed by the naked eye as a discoloration (change of enamel color around the brackets), [27] as the result of the deposition of corrosion products. Furthermore, corrosion is a continuous process, and its effects are cumulative so that in the bracket slot, there may be a progressive increase in surface rigidity or a corrosion product buildup over time.

4. Metals and metal ions release from metallic orthodontic appliances/metal implants in the animal body models

It is well established that metal ion is released from metallic implant materials. All non-noble metals and alloys from medical metallic implants are release metallic species into the body. This raises the issue of amount and fate, i.e. transport and local and systemic metal storage, of these metal dissolution products [28]. Metals from dental implants are released into surrounding tissues by various mechanisms, including corrosion, wear, and mechanically accelerated electrochemical processes such as stress corrosion, corrosion fatigue and fretting corrosion. This metal release has been associated with clinical implant failure, osteolysis, cutaneous allergic reactions, and remote site accumulation [29]. It is now recognized that biomaterial capacity to perform a specific function in patients cannot be evaluated only *in vitro* or *in vivo*, and that also *in vitro* testing, unfortunately, does not exclude *in vivo* testing [30]. *In vitro* methods provide necessary data to supplement those found *in vivo*, usually accepted, firstly, as a first choice method for testing material safety and, secondly, as a method for testing separate phenomena to gain a deeper insight into the *in vivo* mechanisms of actions that are fundamental in determining failure or success of implantation surgery. In addition, *in vivo* methods can reduce the number of animals used for *in vivo* biocompatibility evaluations and since the implantation of toxic biomaterials in animals can be avoided through preliminary *in vitro* tests, there is a reduction in animal suffering. Biomedical implants should be subjected to both *in vitro* and *in vivo* studies for their application. Although *in vitro* studies give an insight of the behavior of the material under the given conditions, it should not be regarded as the final conclusion for recommending a material as an implant, whereas *in vivo* tests examine the actual performance of the implant within the animal models [31]. Animal models have been widely used in biomedical research and have been crucial for acquiring basic science and clinical knowledge pertaining to medical science. Animal models provide

important biomaterial knowledge that eventually leads to the development of more effective clinical treatments of diseases in both humans and animals, therefore, acting as a bridge between *in vitro* studies and clinical trials; consequently, the data obtained in studies on animals can be transferred to humans [32].

The present section reported the results of *in vitro* and *in vivo* experimental investigations on the release of metallic traces in the biological fluids, tissues and different inner organs from the implantation of the orthodontic mini-implants in the animals.

4.1. Titanium ions release from orthodontic mini-implants

The presence of titanium (Ti) particles around dental implants has been reported in the literature for decades; information on the metal ion release from titanium implant materials into various tissues, fluids and inner organs of animal is presented, according to the empirical data, and location of Ti particles in relation to dental implants [33].

Since the classic work of Ferguson et al. [34,35] numerous studies have shown that metal ion release is influenced by the material-tissue interaction and by the roughness or porosity of the implant surface. In addition, authors showed that ionization of all metal occurs to some extent and titanium may be released in relatively large concentrations into adjacent tissues. Vitalium, 316 stainless steel, A-286 stainless steel, aluminum 2024-T3, titanium, and zirconium were embedded in the skeletal muscles of albino rabbits, one alloy to any one animal. Spectrographic analyses of the trace metal concentration of surrounding muscle, spleen, lung, liver, kidney, and control muscle were done. The spleen was found to be the most active site of trace ion storage, the other organs tending to get rid of an early increase in concentration.

In 1984, Woodman et al. [36] attempted to quantify the amount of titanium released from a Ti-6Al-4V prosthetic segmental replacement in the long bones of baboons. There was no statistical difference between experimental and control animals in serum titanium concentrations. A six-fold increase was measured in the urine of the experimental group (implantation time 36-92 months) compared to controls. Titanium is detected in tissue around titanium implant. There were inconsistencies of titanium levels in local muscle. Lung, spleen, and regional lymph node samples of baboons with implants had consistent increase in titanium levels in comparison to controls. No kinetic data were presented for serum and urine. A kinetic analysis was given for the spleen and lungs. However, interpreting these data is difficult due to several experimental problems. The implant design was complex. It had several parts made of different alloys of titanium. In addition, stainless steel screws were used. Thus, the likely presence of fretting and galvanic corrosion and wear could have been factors that increased the scatter of the data. Time affects the diffusion of metals from implants to bone or other organ tissues such as liver, kidney, and muscles.

Seki [37] used rabbits as an animal model. Author reported the titanium and aluminum concentration in subcutaneous tissue around various implanted metallic plates made of pure titanium, titanium alloy, and titanium nitride. The amount of titanium eluted from cp-Ti, Ti-6Al-4V and TiN, increased with time, although only slightly in all cases. However, the Al concentrations in the tissues surrounding the Ti-6Al-4V alloy are 276, 287, 164, 232 $\mu g\ g^{-1}$ dry weight after 2, 4, 8 and 12 weeks, respectively, and they are considerably higher than the Ti concentrations. Histological examination of the reactions of bone tissue to all titanium implants, showed the formation of new bone between the metallic screw and bone tissue from the 4th week, and it gained a close and dense contact with normal bone at the 12th week after implantation.

Ektessabi et al. [38] reported the results of application of micro ion beam PIXE spectroscopy to detect release of titanium from titanium and titanium alloy implants inserted in the tibiae of rabbits for three months. It was found that titanium ions could be detected in the surrounding tissues, as a gradient from the implant surface and in higher amounts in the bone tissue as compared with the soft tissues.

Moberg et al. [39] investigated the occurrence of corrosion associated with the use of metallic implants to stabilize jaw fractures. Three different types of plates, Co-Cr and Ni-Cr alloys and Titanium, were connected *in vivo* to the mandibular bone surface of monkeys. The animals were killed after 3 and 6 months. The mucous membrane and bone tissue were analyzed for concentrations of Co, Cr, Ni, Mo, Al, and Ti. With the exception of Ti, higher concentrations of all the above elements were found in the tissue near the implants when compared with contra lateral controls. However, no signs of corrosion were observed on the surface of the implants.

Lugowski et al. [40] determined the amount of Al, Cr, Co, Mo, Ni, Ti, and V in organs (brain, lung, kidney, and spleen tissues) in 2 years rabbit from and *in vivo* implantation experiment. Because there were no true controls, i.e. rabbits with no implants, the rabbits with non-titanium containing implants were considered as controls. The authors used

rabbits with hydroxyapatite, Co-Cr-Mo alloy or alumina implants as controls. No differences between either the rabbit with a dense Ti-6Al-4V disc or the rabbits with a porous Ti-6Al-4V disc and the control rabbits were found in any of the analyzed tissues.

Frisken at al. [41] reported a methodology for the determination of titanium at μg g^{-1} level of dissemination of titanium from threaded screw type implants following placement of single implants in sheep mandibles. Twelve sheep were implanted with a single self-tapping implant for time intervals of one, four and eight to 12 weeks. Four un-operated sheep served as controls. Regional lymph nodes, lungs, spleen and livers were dissected and subsequently analyzed. Results associated with successful implants showed no statistically significant different levels of titanium in any organ compared to controls, although some minor elevations in titanium levels within the lungs and regional lymph nodes were noted. Debris from a single implant insertion is at such a low level that it is unlikely to pose a health problem. However, this study suggested that implant failure may result in considerably more titanium release which can track through the regional lymph nodes.

He et al. [42] compared titanium (Ti)- and Zirconia (ZrO_2)-implants in mini pig maxillae with respect to Ti/Zr release into the surrounding bone tissues, the resulting short term tissue responses and the potential toxicity. After 12-weeks of implantation, increased concentrations of Ti and Zr can be detected in bone/tissues near Ti and ZrO_2-implants in mini pig maxillae. Ti content released from Ti-implants in two times higher than the Zr content released from ZrO_2-implants. ZrO_2-nanoparticles showed lower cytotoxicity and DNA damage compared to results reported for Ti-nanoparticles in human cells.

Toledano-Serrabona et al. [43] evaluated the accumulation of ions in blood and organs caused by Ti metal particles in a mandibular defect in rats, together with a description of the local reactions of oral tissues to this Ti alloy debris. 1 month after implantation of Ti metal particles, authors observed a granulomatous inflammatory reaction, and the concentration of Ti ions increased in the liver, spleen, and brain (except lung tissue), as the concentration of vanadium ions in the brain.

Mikulewicz et al. [44] investigated the release of metal ions from an orthodontic appliance in tests on animals. The trial was conducted on pigs, chosen as a model organism for the exposure assessment. The simulated orthodontic appliance was placed on the inner surface of pig's cheek for 6 months. The invasive (kidney, liver, lungs, aorta, and oral mucosa) and non-invasive (hair) matrices were collected and underwent multi-elemental analysis from the experimental and control groups. The greatest differences in the content of toxic metals were found in the aorta (Ni level was 4.8 times higher in experimental than in the control group), in the cheek (Ni 3.5 times higher), and in the hair sampled after 3 months (Cr 3.4 times higher). The obtained data indicate that the products of corrosion have passed into selected tissues of pigs; however, the doses of toxic metal ions released from the appliance did not reach toxic levels.

Nickel has a number of adverse biological effects that have made the use of nickel in biomedical implants controversial. Sahmali et al. [45] investigated the effects of dental alloys containing Ni on the level of the element in the serum, liver, kidney, and oral mucosa of guinea pigs. The test was conducted for 15 days. It was found that guinea pigs sensitized to Ni had higher levels of Ni in the serum, oral mucosa, liver, and slightly in kidney as compared with the control group. Statistically, significant differences were found between liver and oral mucosa Ni content in the experimental and control groups. Wataha et al. [46] assessed the special distribution of nickel around nickel-containing implants *in vivo*. Pure nickel wire, or a nickel containing alloy (Ni-Cr) were implanted subcutaneously into rats for 7 days. The tissues were analyzed for Ni content and inflammation at 1 mm intervals up to 5 mm away from the implants. The sham surgery sites caused mild to moderate inflammation 1-2 mm from the implant site with no detectable nickel in the tissue. The nickel wire caused severe inflammation up to 5 mm away from the implant site with necrosis for 1 mm around the implant. Nickel concentrations reached 48 μg g^{-1} near the implants, falling exponentially to undetectable levels a 3-4 mm from the implants. The current study showed that the nickel distribution in tissues correlated well with overt tissue inflammation.

Removable osseintegrated titanium mini-implants were successfully used as anchorage devices in orthodontics [47]. The Ti6Al4V alloy was used instead of commercially pure Ti due to its superior strength. The authors analyzed immediately loaded mini-implant fixation and to gauge the vanadium ion release during the healing process. Titanium alloy mini-implants were inserted in the tibiae of rabbits. After 1, 4 and 12 weeks, they were submitted to removal torque testing. In comparison with the control values, the content of vanadium increased slightly after 1 week, significantly increased after 4 weeks, and decreased slightly after 12 weeks, without reaching the 1 week values. The results of this rabbit model study indicate that titanium alloy mini-implants can be loaded immediately with no compromise in their stability. The detected concentration of vanadium did not reach toxic levels in the animal model. The release of Ti particles after treatment of peri-implantations was studied *in vivo* by Deppe et al. [48]. The *in vivo*

studies also focused in models studying the release of Ti particles due to corrosion of the implant surface and frictional wear during implant site preparation and placement, and analyses were restricted to bone (i.e. maxilla and mandible) and distant organs. Notably, Ti particles were found on the implant site (bone surface and bone marrow) as well as in the blood, lymph nodes, and distant organs such as lung, liver, and kidneys. Occasionally, Ti ions were found despite the absence of clinically detectable signs [48]. Higher levels of Ti in the lungs and regional lymph nodes were in animals with failed implants [41].

The concentrations of trace metals in the biological (fluid, organ tissue) samples of animals following ion release from metallic orthodontic appliances and metal implants are presented in Table 1.

5. Release of trace metal ions from metal implants and metallic orthodontic appliances

The discovery of relatively inert metallic and alloy biomaterials has led to their profile use in biomedicine, such as in dentistry. All metallic dental implants degrade to some extent over time, however, resulting in locally and systemically elevated levels of trace metal ions. Several modes of metal ion release exist, including passive dissolution; wear (mechanical), corrosion (electrochemical) and combined mechanical and electrochemical processes (i.e. fretting corrosion). There are three ways of investigating metal ion release: *in vitro*, retrieval (*ex vivo* investigation of *in vivo* aged samples), and *in vivo*. Metal ion release from dental implants has been reported *in vitro* [49-52]*, in vivo* [53] as well *in vitro* and *in vivo* [53a]. A systematic review on release of titanium ions from dental alloys in saliva has been described *in vitro* and *in vivo* [54]. Evaluation of the level of trace elements in patients using orthodontic appliances is a priority [49]. However, *in vitro* tests which evaluate biocompability are used as screening tests and are not able to imitate the clinical environment [52]. Metal ion release from dental implants (orthodontic appliances) has been measured locally, in periprosthetic tissue or, more relevantly, in body fluids, i.e. blood, serum, urine or saliva, which show the systematic impact of metal release. Some experiments evaluating the potential for conventional dental implants to release metallic ions into the body have been performed.

In the two sections below, current information on the trace metal ion release from metallic dental implant materials and the behavior of released *in vitro* and *in vivo* metal ions is presented, according to the empirical data.

5.1. In vitro studies

Metal ions release during orthodontic treatment has become an important issue in the assessment of its biosafety. The most popular assays are batch tests in which the materials are submersed in an environment of artificial saliva, 0.9% NaCl, or organic acids (such as lactic acid) and the released metal ions are quantified by different techniques, throughout the experiment and/or at the end of the experiment. In general, the advantages of these in vitro tests are simplicity, elimination of other factors that are not related to the orthodontic treatment, reproducibility, and lack of ethical problems [55,56]. The main disadvantage of in vitro tests is that they do not reflect the real conditions of the oral cavity and that the materials may respond differently under laboratory conditions vs. clinical conditions. Many of these in vitro studies use different methodological/material approaches, including various types of immersion liquids, different analytical methods (techniques), and materials obtained from several manufacturers [49].

A brief description with the most important findings of these studies is shown below.

The quantities of released metal ions measured in some in vitro studies are useful for relative comparison and for determination of the effect of each individual variable on ions released without the influence of external factors. One of the first studies to research this topic was conducted by Park and Shearer in 1983 [57]. They verified that the amount of nickel and chromium released was about 40 μg nickels and 36 μg chromium's, respectively per day for a full-mouth appliance. Because human dietary intake of nickel is reported to be 300 to 500 μg per day, the release of 40 μg of nickel per day is well below the normal daily intake and may not be of clinical significance. However, the clinician should be aware that release of nickel and chromium from orthodontic bands might sensitize patients to nickel and chromium and may cause hypersensitivity reactions in patients with a prior history of hypersensitivity to these metals.

Kerosuo et al. [58] investigated the release of metal ions under both static and dynamic conditions. A special equipment-oral functional simulator specially designed for the dynamic tests was constructed. As it can be expected, under dynamic conditions, more nickel ions were released. The opposite results were obtained for chromium. The concentrations of Ni and Cr reported by Park and Shearer [57] exceeded the values provided by other investigators. Neither of authors determined the remaining ions (Cu and Fe) in the solution of NaCl.

Table 1 Metal concentration in body fluids and inner organ tissues of animals following in vitro and in vivo trace metal ion release from metallic orthodontic appliances and metal implants

Animal	Study design	Type of metal/alloy	Orthodontic appliance/ implant	Animal body model	Implantation data		Metal ion(s) measured	Conc. (µg L^{-1} or µg g^{-1})	Analytical technique	Research main results	Refs.
					No. animals	Follow-up					
Baboons	*In vivo*	Ti-6Al-4V alloy	Titanium fiber sleeve	Serum	45	3 w to 92 m	Ti V Al	2.71 0.27 427	GF-AAS	Serum chemistry and hematological analysis did not indicate significant differences in any of the parameters measured when comparing the group with implants to control group.	[36]
				Urine			Ti V Al	13.5 1.07 603			
				Lung			Ti V Al	958 2.89 1297		There were inconsistencies of titanium levels in local muscle. Lung, spleen, and regional lymph node samples of baboons with implants had consistent increases in titanium levels in comparison to control. It was found systemic accumulation of titanium in the spleen and lung.	
				Kidney			Ti V Al	1.90 1.81 101			
				Spleen			Ti V Al	90.63 1.78 179.31			
				Muscle			Ti V Al	17.20 3.11 133.71			
				Regional lymph nodes			Ti V	524.33 1.64			

				Liver			Al Ti V Al	573.07 36.3 2.61 139.74			
Rabbits	*In vivo*	SS Pure titanium Ti-6Al-4V alloy TiN	Plates, screws	Subcutaneous tissues	64	2,4,8,12 w	Fe Ti Ti Al Ti	603 11.5 14.4 231.6 8.1	ICP-OES	All experimental materials exhibited excellent resistance to corrosion. Titanium nitride is useful as a biomaterial the same as cp-Ti and Ti-6Al-4V alloy.	[37]
Sheep	*In vitro*	Titanium metal	Titanium screw implant	Node Liver Lung	12	1,4,8,12 w	Ti Ti Ti	100 60 200	GF-AAS	No differences in levels of Ti in any of the examined organs when compared to test sites.	[41]
Mini pigs	*In vitro*	Titanium and Zirconia (ZrO_2) implants	Threaded implants	Bone tissues (maxillae)	18	12 w	Ti Zr	1.67 0.75	ICP-OES ICP-MS	Increased intensity of Ti and Zr can be detected in bone/tissues near Ti- and ZrO_2-implants in mini pig maxillae. Ti content released from Ti-implants is two times higher than the Zr content released from ZrO_2-implants.	[42]
Sprague-Dawley rats	*In vivo*	Ti-6Al-4V alloy	Dental implants	Blood Brain	20	30 d	Ti Al V Ti Al V	145.4 1004.5 1.43 565.6 971.1 6.5	ICP-MS	1 month after implantation of Ti metal particles, the concentration of titanium ions increased in the liver, spleen, and brain, as the concentration of vanadium ions in the brain.	[43]

				Spleen Lungs Liver			Ti Al V Ti Al V Ti Al V	513.3 726.0 44.2 392.7 1610.2 13.4 402.8 723.5 10.8			
Pigs	*In vivo*	SS	Plates	Aorta Cheek Kidney Liver Lung Hair	24	3,6 m	Ni Ni Ni Ni Ni Ni Cr	0.03 0.04 0.01 0.04 <0.001 0.08 0.263	ICP-OES	The obtained data indicate that the products of corrosion have passed into selected tissues of pigs; however, the doses of toxic metal ions released from the appliance did not reach toxic levels. Hair was found to be a noninvasive biomarker of exposure to metal ions released from orthodontic appliances.	[44]
White rabbits	*In vivo*	Ti-6Al-4V alloy	Mini-implant	Kidney Liver Lung	23	1,4,12 w	V V V	0.758 0.785 0.812	GF-AAS	The results of this rabbit model study indicate that titanium alloy mini-implants can be loaded immediately with no compromise in their stability. The detected concentration of vanadium did not reach toxic levels in the animal model.	[47]
Beagle dogs	*In vivo*	Titanium implants	n/a	Spleen Liver Oral mucosa	6	4 m	Ti Ti Ti Ti	4.14 2.01 1.83 1.78	ICP-OES	From these results, it can be concluded that continuous wave CO_2 laser decontamination does not enhance the amount of titanium release *in vivo* and seems to have no adverse effects on the	[48]

				Regional lymph nodes Lung Kidney			 Ti Ti	 1.48 1.30		reaction of local tissues or visceral organs.	
Rats	*In vivo*	Titanium implants	Titanium wire	Liver Kidneys Spleen Lungs Hearth	16	18 m	Ti Ti Ti Ti Ti	78.1 2.10 632 578 160	ICP-MS	Results showed that Ti content in all the selected organ tissues were high, clearly showing both corrosion of the Ti implant and systemic Ti accumulation in target tissues.	[212]

Cortada et al. [59] performed measurements of the ion release in artificial saliva using the titanium implant; they determined the metallic ion release in oral implants with superstructures of different metals and alloys used in clinical dentistry. This study has been realized in a saliva environment at 37°C. The titanium oral implant coupled with a chromium-nickel alloy releases a high quantity of ions and the implant coupled with the titanium superstructure presents a low value of ion release.

Staffolani et al. [60] evaluated the corrosion rate of commercially available orthodontic appliances, obtained from the Leone Company, composed of 304 and 316 steel molar bands, 316 steel brackets and nickel-titanium memory arch wire. Further, in the tested appliances a brazing alloy, composed of palladium, copper and silver, was present between the molar bands and tubes, and between the bracket bases and wings. We evaluated the release, from the metallic implants, of some ions that are known to exert negative effects on cells and tissues. The corrosion products analyzed were nickel and chromium released from molar bands, brackets and arch wires, and palladium, copper and silver released from the brazing alloy. Evaluation was conducted while the appliances were immersed in an inorganic acid solution under controlled conditions of pH, or in organic acid solution contained in food or produced by bacterial flora. It appears that the quantities of metals released in the experiments are low to be cause for concern in utilizing the appliance. Because 316 steel is more corrosion resistant than 304 steel it might be advisable to use molar bands made entirely of 316, rather than 304 and 316 steel. As far as the results obtained by studies conducted in artificial saliva, the titanium alloys were electrochemically stable.

Suárez et al. [61] investigated Ni released in 3 types of lingual orthodontic archwires (stainless steel (SS), NiTi and CuNiTi) after 7, 14 and 30 days of immersion in saliva solution. They demonstrated that SS archwires (8% Ni) released the highest amount of Ni compared to NiTi and CuNiTi archwires (which both have 50% Ni content). This result was also reported previously by Hwang et al. [62] who reported the release of nickel in the solution of artificial saliva. The author's excceded the number of bands (two instead of one) as related to the desired proportion. They checked the release of nickel ions in relation to different types of wires: SS (by different producers), copper NiTi, and Bioforce sent alloy. The results showed that SS wires released ten to 50 times higher quantity of Ni ions than copper NiTi or Bioforce sent alloy. The safest material was found to be copper NiTi. The results of kinetic experiments showed that the rate of ions release and the time necessary for the equilibrium to be reached strongly depended on the material of which elements of orthodontic appliance were made as well as the type of metal ion released [62]. Similarly, Ortiz et al. [63] compared the amount of metallic ions released by 3 alloys (stainless steel, nickel-free, and titanium) in a culture medium over 30 days, and their results reinforce the idea that materials which higher Ni content are more susceptible to corrosion because materials with high Ni content release greater quantities of Ni, Fe, Cr, and Mn into the culture media during the first week of immersion. The titanium brackets and tubes were the most biocompatible of the 3 alloys that were investigated.

Sfondrini et al. [64,65], whose results agreed with the findings of previous studies [66-68], showed that the release of Ni from three types of brackets (new conventional SS, recycled SS, and Ni-free brackets) was higher for recycled brackets, whereas the lowest release of Ni was from Ni-free brackets. Moreover, for all types of brackets, acidic conditions (pH 4.2) produced the highest Ni release, an outcome similar to results obtained by Milheiro et al. [69], and the high release may have occurred because acidic conditions provide a reduced environment in which the stainless steel oxide film required for corrosion resistance is less stable [67]. A significant increase in Ni release was also demonstrated as a function of the immersion period for all of the brackets, except for the time interval between 24 and 48 h. In contrast, Gil et al. [70] reported a significant reduction in Ni ion release from recycled NiTi archwires in the saliva compared to original archwires. For both types of archwires, the concentration of ions released in the medium initially increased very fast, but later in the experiments, the concentration was saturated. The saturation has also been reported by the same authors, who demonstrated a reduction in Ni release from NiTi archwires treated with an oxidation treatment designed to obtain Ni-free surfaces. The treatment resulted in the formation of a titanium oxide protective layer [71].

Because NiTi wires are subjected to mechanical stresses and deformation from tooth movement, the applied forces might induce damage of the oxide film on the surface of the wires and subsequent loss of protection. Therefore, Liu et al. [72] measured Ni release under a continuous bending stress to simulate the intraoral environment, and they demonstrated that bending stress induced greater Ni ion release from NiTi wires compared to unstressed archwires. They suggested that bending stress induced damage of the passivated oxide film on NiTi wires, and the release of ions could be explained by the exposed active metal surface rather than metal-ion transport through the oxide film and hydrolysis of the oxides. Consequently, stress is an important factor to be considered on the corrosion behavior in the design and clinical use of these NiTi wires.

Orthodontic space maintainers were studied by Bhaskar and Subba Reddy [73], who showed in their study that the release of Cr and Ni ions from bands reached maximum quantities at the end of 7 days. Nevertheless, these authors did not find significant differences in ion release when different band materials were used.

Danaei et al. [74] studied the release of Cr, Cu, Fe, Mo and Ni from SS orthodontic brackets after treatment with 3 different mouthwashes was found to reduce the risk of white-spot lesions around the brackets. After 45 days of immersion, they concluded that the highest ion release occured in the presence of chlorhexidine mouthwash, and they recommended avoiding prologed application of chlorhexidine mouthwash in patients who have allergies.

The reconditioning of orthodontic brackets by different technique, including direct flaming, acid bath recycling, or commercial recycling, can degrade the most essential properties of these materials, even though significant differences were not observed in nickel-ion release after static immersion of reconditioned brackets in Fusayama's artificial saliva [75].

Sheibaninia [76] evaluated the effect of pH changes and thermocycling, two main factors that change many times during the day after each meal and drink, on the amount of Ni released from orthodontic appliances. According to the results, thermocycling adversely affected the release of Ni from NiTi wires, and an acidic environment accelerated the rate of Ni ion release. The results showed that pH had some influence on Ni release, but thermocycling was clearly the dominant factor.

The release of Cu ions has been investigated very little in comparison to Ni. Zhang et al. [77] reported that composite archwires (CoAW), formed by solder connection on a NiTi shape memory alloy and stainless steel wire, were resistant to corrosion in simple artificial saliva. The CoAW showed the greatest weight loss and Cu release in chlric solution. The corrosion resistance of the interlayer depends primarily on the initial corrosion. According to these authors, damage to the oxide film would allow active metal to react with the surrounding environment and cause further ion release. However, the greatest level of Cu release detected was lower than the Provisional Maximum Tolerable Daily Intake (PMTDI) limit established for this metal.

Orthodontic appliances and their components such as brackets or arch-wires, are exposed to a variety of intraoral conditions including those of saliva [78]. They degradation may potentially result in leaching of their components (metals) resulting in unintentional human exposure. Some studies have already evaluated the release of selected elements for containing Fe, Cr, Ni, Si, and Mo. The in vitro studies have consistently shown that in case of SS appliances, the elements of concern include Ni and Cr [49,79-83].

A complete in vitro study on the release of 17 elements from SS orthodontic appliances has been carried out by Mikulewicz et al. [79]. In this study, three ion release coefficients were defined and presented show which elements are dissolved to the highest extent and that their release is not proportional to their content in an alloy. The concentrations of Ni and Cr were correlated, which suggests that these ions were release together as a result of corrosion. In a later study, the same authors designed a new continuous-flow system for the in vitro testing of fixed orthodontic appliances (thermostatic glass reactor) to evaluate the release of metal ions [80]. Compared to previous investigations, this innovative study incorporated an approach that is a greater approximation of in vivo assays due to continuous monitoring of ion release over a prolonged period. Sampling was performed at several time points during the 28-day study, and the metal ion concentrations of eight elements were determined. The ions were released in the following order: Si>Cu>Ni>Cr>Mo>Mn>Cd. Fe ions were not released from the appliances, and there was no correlation between the content of a given metal in an alloy and the released quantity. Statistically, significant differences were found for Ni, Cr, and Cu between the experimental and control group. The total mass of released metal ions over 4 weeks was as follows: Ni (18.7 μg), Cr (5.47 μg), and Cu (31.3 μg). All the values were far below the recommended daily doses, so the treatment might not be a significant source of exposure to these metal ions.

Mikulewicz et al. [80a] evaluated of soft drink effect on metal ions release from orthodontic appliances under in vitro conditions, in a continuous flow system. Orange juice/Coca Cola was flowing through the system alternately with artificial saliva for 5.5 and 18.5 h, respectively. The collected samples underwent an analysis in order to determine the metal ions release pattern in time. The total mass of ions released from the appliance into orange juice and Coca Cola (respectively) during the experiment was calculated (μg): Ni (15.33; 37.75), Cr (3.604; 1.052), Fe (48.42; 156.1), Cu (57.87; 32.91), Mn (9.164; 41.16), Mo (9.999; 3012), and Cd (0.5967; 2.173). It was found that orange juice did not intensify the release of metal ions from orthodontic appliances, whereas Coca Cola caused increased release of Ni ions. López-Alias et al. [81] identified and quantified the different ions released by various dental alloys subjected to a continuous flow of saliva and to estimate the nutritional and toxicological implications of such a release. Four pieces of three nickel-based, one noble, one high-noble and two copper-aluminum alloys were cast and then immersed in a

continuous flow of artificial saliva for 15 days. After 15 days, the metallic ions in the artificial saliva were analyzed. The results were compared with the tolerable upper daily intake level of each ion. The copper-aluminum alloys realized Cu, Al, Ni, Mn, and Fe. The nickel-based alloys essentially released Ni and Cr, while the beryllium containing alloy released Be and significantly more Ni. The noble and high-noble alloys were very resistant to corrosion. The amount of ions released remained far below the upper tolerable intake level, with the exception of Ni, released by berylium-containing nickel-based alloy, whose levels approach 50% of this threshold.

Wendl et al. [82] evaluated the temporal release of Co, Cr, Mn, and Ni from the components of a typical orthodontic appliance during simulated orthodontic treatment. Several commercially available types of bands, brackets, and wires were exposed to an artificial saliva solution for at least 44 days and the metals released were quantified in regular intervals. Corrosion products encountered on some products were also investigated. Bands released the largest quantities of Co, Cr, Mn, and Ni, followed by brackets and wires. The use of constant release rates will clearly underestimate metal intake by the patient during the first couple of days and overestimate exposure during the remainder of the treatment which is usually several months long. They data are consistent with heavy metal release by orthodontic materials at levels well below typical dietary intake. Tahmasbi et al. [83] investigated the galvanic corrosion of 24 mandibular central incisors Roth brackets manufactured by four different companies coupled with SS or NiTi wires in an artificial saliva solution. These brackets were immersed in artificial saliva along with SS or NiTi orthodontic wires for 28 days. Corrosion rate was calculated, and release of ions was measured. Among ions evaluated, release of nickel ions from Shinye brackets was significantly higher than that of other brackets.

Barrett et al. [84], Gürsoy et al. [85], Kuchta et al. [86], and Hwang et al. [62] reported the release of nickel in the solution of artificial saliva. In the later work, the authors exceeded the number of bands (two instead of one) as related to the desired proportion. Barrett et al. [84] found slightly higher release of Ni ions in the solution containing immersed appliance with NiTi wire a compared with the appliance with SS wire. The release of chromium ions from orthodontic appliance was also discussed. High metal ion levels are found one to two weeks after exposure to metal appliances, which will later return to their initial levels. Authors concluded that appliances with SS wire released more Cr ions than appliances composed of NiTi wires. Gürsoy et al. [85] compared new vs. recycled brackets and bands and found significantly higher release of Ni and Cr ions by recycled elements. Recycled elements released around 100% more of all metal ions (Cu, Cr, Fe, and NI) as compared with the brand new ones. Kuchta et al. [86] investigated the effect of pH (3.5 and 6.8) on ions release in various types of wires produced by the same manufacturer of NiTi and SS. Dramatically strong effect of pH on release of Ni was found. The concentrations of ions were ca. 30-40 times higher at pH 3.5 when compared with 6.8. The mostly biocompatible was found NiTi alloy. SS was material which released the highest quantities of Ni ions in both pH. Authors in the case of Cr confirmed that the mostly biocompatible material (in artificial saliva at pH 6.8 as well as in 3.5) was NiTi and SS, which released the highest amounts of Cr ions. Hwang et al. [62] checked the release of nickel ions in relation to different types of wires. SS (by different producers), copper NiTi, and Bioforce sentalloy. The results showed that SS wires released ten to 50 times' higher quantity of Ni ions than copper NiTi or Bioforce sentalloy. The safest material was found to be copper NiTi. The authors stated out that Cr, similarly as Ni ions, was released in the highest extent from SS wires (as a part of the appliance) when compared with copper NiTi or NiTi. The difference was 30-90 times higher. Similarly as considering the release of Ni, also for Cr, the safest material was copper NiTi. Corrosion of orthodontic alloys may lead to the release of sizeable amounts of nickel and chromium into the saliva. The results of kinetic experiments showed that the rate of ions release and the time necessary for the equilibrium to be reached strongly depended on the material of which elements of orthodontic appliance were made as well as the type of metal ion released [62].

The release of copper was investigated in two papers [85,62]. In occurred in higher extent in the case of recycled elements (in particular brackets) when compared with the new [83] (two to three times more copper was released by recycled elements). The results of release of copper from different materials vs. pH were slightly different for Cu as for Cr and Ni. The most biocompatible material in the pH of oral environment was NiTi, but under acidic conditions, the lowest amounts of Cu were released by NiTi wires. Similarly as in the case of Cr and Ni, the quantity of Cu released was 30-4- times higher under acidic conditions. Hwang et al. [62] studied the concentration of copper only in the solutions in which the appliance with copper NiTi arch-wires was immersed.

Lucchetti et al. [87] evaluated the ion release of chromium, cobalt, and iron from the Co-Cr alloys used for traditionally cast and computer-aided design/computer-aided manufacturing dental devices after interaction with oral bacteria and different pH conditions. All specimens were prepared from currently available alloys, polished, and immersed in 3 different pH media (artificial saliva [pH 2.3] and 6.5% and 0.9% saline solution (pH 7.1). Solutions were analyzed after 15 and 30 days in the chemical corrosion test days and 30 days in the biocorrosion test to detect ions released in different solutions. The authors showed a higher total ion release from pH 6.8 to pH 2.3, an increased release of Co, Cr and Fe.

Imani et al. [88] reviewed the effect of fixed orthodontic treatment on salivary levels nickel and chromium ions by doing a meta-analysis on cross-sectional and cohort studies. Salivary nickel level was higher in periods of 10 min or less and one day after initiation of treatment compared to baseline (before the insertion of appliance). Salivary chromium level was higher in periods of one day and one week after the initiation of treatment compared to baseline. Corrosion of fixed orthodontic appliances leads to elevated salivary nickel and chromium concentrations early after initiation of orthodontic treatment.

Elastomeric ligatures are increasingly used as a part of esthetic orthodontic treatment, particularly in children. Olszewska et al. [89] investigated the release of 21 metals (Ba, Cd, Co, Cr, Cu, Fe, Li, Mn, Mg, Mo, Ni, Pb, Tl, Ti, Sb, Sr, Sn, Zn, U, and V) and 2 metalloids (As and Ge) from elastomeric ligatures varying in color under in vitro conditions of artificial human saliva. Elastomeric ligatures were incubated in artificial human saliva for 1 month (a typical period of their use) and the release of 21 metals and 2 metalloids was studied. For comparison, SS ligature were incubated for 1, 3, and 6 months (since sometimes their use was prolonged) under similar conditions. From the 23 investigated elements, only Co, Cd, Cr, Ni, Mn, and Sn migrated from elastic ligatures to artificial saliva but the observed levels were always much below safety limits. In turn, stainless steel ligatures released Co, Cr, Fe, Ni, and Sn from which only the level of Ni was of concern given the fact that these appliances are used over a prolonged period, not only in adults but also in children. The present study affirms the safety of ligatures in orthodontic treatment, although tending to support the use of elastic ligatures replaced on a monthly basis over the prolonged use of stainless steel counter parts.

Kassapidou et al. [90] investigated the metal ion release, surface roughness and cytoxicity for five Co-Cr alloys produced by four different manufacturing techniques before and after heat treatment. In addition, to evaluate if the combination of materials affects the ion release in media with different pH. Commercially pure titanium, cpTi grade 4 and a Ti-6Al-4V alloy were included for comparison. All alloys showed a decrease of the total ion release when cpTi grade 4 was present. The total ion release decreased over time for all specimens and the highest ion release was observed from the cast and milled Co-Cr alloy in acidic conditions. The cast and laser-melted Co-Cr alloy and the titanium alloy became rougher after heat treatment. All materials were within the limits of cell viability according to standards.

Standard orthodontic intervention, involves the use of brackets, bands and arch wires. Nickel and chromium containing alloys are present in the manufacture of most of the orthodontic appliances. Haddad et al. [91] has compared the Ni and Cr release from 7 groups of different commercially available bracket models in simulated oral environments. 6 groups of brackets were stainless steel, and 1 group of brackets was made of cobalt-chromium alloy with low Ni content (0.5%). Each bracket was immersed in 0.5 mL of synthetic saliva (SS) or artificial plaque fluid (PF) over a period of 28 days at 37°C. Solutions were replaced every 7 days, and were analyzed. Amounts of Ni release in SS (μg L^{-1} per week) varied between groups from "bellow detection limits) to 694, and from 49 to 5,948 in PF. The group of brackets made of Co-Cr alloy did not release the least amounts of Ni. Amounts of Cr detected in SS and in PF (μg L^{-1} per week) were from 1 to 10.4 and from 50.5 to 8,225, respectively. It was therefore concluded that brackets from different manufacturers present different corrosion behavior. Mutlu-Sagesen et al. [92] evaluated the release of ions from four commercially available dental casting alloys in three different pH media - artificial saliva of pH 2.3 and pH 6.5 and 0.9% saline solution of pH 7.3, for a period of 60 days. A Co-Cr based alloy, a Ni-Cr based alloy, a Ti-based alloy, and a high-Au alloy were selected for this study. The corrosion products, namely the released ions, were identified and quantified. For all ions, pH and period interactions were statistically significant; highest amount of ion release occurred after 60 days of immersion regardless of pH value. For every alloy, ion release results were highest in artificial saliva of pH 2.3 and lowest in 0.9% saline solution. It was concluded that ion release from alloys was pH-dependent. Pillai et al. [93] determined whether the nickel released from the stainless steel brackets have any cytotoxic effects on gingival fibroblast. The results of the study show that the amount of nickel leached is capable of bringing damage to the fibroblast. They study concludes that nickel solution at minimal concentration of 1.18 μg could damage human gingival fibroblast and the nickel released from the different brands of the brackets are not uniform. Jithesh et al. [94] studied nickel ion release from three different orthodontic brackets with metal slot, conventional and recycled stainless steel brackets, in different artificial pH, in different time intervals and reported that metal slot ceramic bracket release is significantly less in case of nickel ions compared to other groups. The nickel release from each subgroup was analyzed. The analysis shows a significant difference between three groups. The study shows that the Ni releases from the recycled SS brackets have the highest at all 4.2 pH except in 120 h. Hence, recycled SS brackets should not be used for nickel allergic patients. Furian et al. [95] evaluated the concentration of nickel, copper, and chromium ions released by Ni archwires with the addition of Cu to their composition Cu-Ni-Ti alloy in neutral and acid media. The archwires remained immersed in neutral or acid solutions for a time interval of 7 days. The immersion of the NiTi Memory Wire and the Flexy NiTi Cu archwires in both solutions presented significantly higher mean Ni concentration values for detectable Cu than that of the other groups. Increased Ni ions are released initially after the orthodontic devices have been fitted, but they decay quickly. This reflected in miniscule corrosion defects as pitting. It is unlikely that orthodontic nickel titanium wires are a relevant additional Ni load for the patient.

A method for Cr(VI) speciation in synthetic saliva after releasing from orthodontic brackets, using silica nanoparticles organo-functionalized with (3-aminopropyl)thriethoxysilane (APTES) for Cr(III)/Cr(VI) separation and in vitro determination is proposed [96]. A corrosion test was carried out on 20 orthodontic brackets, from two different models, after immersion in synthetic saliva (pH 6.0) at 37°C with agitation (125 rpm) for 24 h. It was observed that almost 40% of the total chromium released from the analyzed orthodontic brackets was Cr(VI). Considering the duration of the orthodontic treatment (2 years), the possibility of corrosion of the brackets in the oral environment deserves attention to avoid ingestion of highly toxic specie of chromium.

In a series of papers, Čelebić research group [97-100] study the behavior of a non-noble (base metal) Co-Cr-Mo alloy and high-noble Au-Pt alloy in the solution type in which the alloys are submerged, the pH level and exposure time affect the amount of released metal ions from the alloys. Stipetić et al. [97] investigated how different factors such as the solution type in which the alloy is submerged (phosphate buffered solution at the pH 6 level, whereas lactic acid was added), the pH level and exposure time affect the amount of released Ni ions from the three (Ni-Cr, Co-Cr-Mo, Au-Pt) alloys. The ion release from the above tested dental alloys in the above mentioned solutions was measured over 10 time intervals, i.e. after 1,2,3,4,5,6,7,14,21 days and 30 days respectively. Results show that Ni-Cr alloy and Co-Cr-Mo alloy released Ni ions, but the Au-Pt alloy did not, even at low pH levels. In another paper, the authors [98] examined how Co-Cr-Mo dental alloy behaves in the solutions of different pH value and different composition over a relatively long period of time. Co-Cr-Mo dental alloy was exposed in vitro to either simulated saliva, a highly acidic medium, and in lactic acid. The alloy samples were immersed in three solution for 1,2,3,4,5,6,7,14,21, and 30 days. The analysis showed that during one month Co, Cr, Fe, Zn and Ni ions had been released from tested samples in all three solutions. The results of this study indicate that the leaching of the Co, Cr, Fe, Zn and Ni ions in the solution was dependent both upon the nature of the solution in which the alloy was immersed and the duration of the immersion. In the next two papers [99,100], the authors evaluated the behavior of high-noble Au-Pt alloy samples in a phosphate buffer pH 6. The release of metal ions from the tested alloy was measured in ten time periods (after 1,2,3,4,5,6,7,14,21 and 30 days). Results demonstrated release of only Cr, Cu, Fe, and Zn from tested Au/Pt dental alloy; however, only Cu and Zn were declared. The undeclared Cr from Au/Pt dental alloy or some other element might be responsible for the contact allergy thus far attributed to the gold.

Rai et al. [101] have analyzed the serum fluid levels of nickel and chromium in orthodontic ally treated patients due to biodegradation of the appliance over a period of time. The cross-sectional study included 24 patients undergoing Begg's fixed appliance mechanotherapy consuming mixed diet. The experimental group consisted of patients wearing the appliance from 6 to 24 months with each group consisting of 6 samples. Results obtained indicated that although nickel level in the serum was significant initially in the samples when compared to the controls, there was a gradual decrease of serum nickel level when the appliance was present for a long duration. However, serum chromium levels showed no significant changes with time.

Elshahawy et al. [102] have evaluated the metal ion release from three different commonly used fixed prosthodontic materials (type IV gold alloy, nickel-chromium alloy and stainless- steel alloy) into two different immersion solutions (0.9% sodium chloride and 1% lactic acid); they were kept at 37^{0}C for 7 days. In the gold alloy, there was significant difference between Zn and other released elements in the NaCl solution. The Ni was significantly more released from Ni-Cr alloy than the other elements into NaCl solutions. The same was observed for Fe released from SS alloy. The significant higher release of Ni from the Ni-Cr alloy, Fe from the stainless steel alloy and Zn and Cu from the gold alloy was evident.

One of most recent studies deals with evaluation of the concentration of nickel, copper, and chromium ions released by Ni archwires with the addition of Cu to their composition CuNi-titanium in neutral and acid media [103]. Among the archwires with Cu addition analyzed in this study, the Damon Optimal-Force Cu Ni-Ti archwires were the type that presented the lowest values of metal ion release however, all the archwires evaluated in the present study presented results that showed values below the levels considered toxic to human beings [103].

The concentration of trace metals in the synthetic saliva samples following in vitro ion release from metallic orthodontic appliances is presented in Table 2.

Table 2 In vitro times and metal concentration in simulated body fluids following trace metal ion release from orthodontic appliances

Type of metal/alloy	Orthodontic appliance	Experimental model	Implantation data		Metal ion(s) measured	Conc. (μg L^{-1} or μg g^{-1})	Analytical technique	Research main results	Refs.
			No. Patients/ implants	Follow-up					
SS	Brackets, bands, wires	0.9% NaCl	6	8 d	Cr Ni	2.5-4.5 17.1-44.3	GF-AAS	Detectable release of nickel and chromium from orthodontic appliances seems to occur and the amount of nickel released increases in dynamic conditions. The amount released may be considered negligible under a toxicological point of view, but can be of considerable importance for individuals with a high degree of sensitivity to nickel.	[58]
SS (304, 316) Ni-Ti alloy	Brackets, bands, wires	Inorganic acid (HCl), Organic acids (TCA, LA)	2	1-28 d	 Cr Cu Ni Cr Cu Ni	**TCA** 1.89 0.49 3.75 **LA** 1.32 0.59 3.27	GF-AAS	It appears that the quantities of metals released in experiments are low to be cause for concern in utilizing the appliance. Because 316 steel is more corrosion resistant than 304 steel it might be advisable to use molar bands made entirely of 316, rather than 304 and 316 steel.	[60]
SS Ni-Ti alloy	Brackets, tubes, wires	Artificial saliva (pH 6.75, 37°C)	320	1 - 84 d	Cu Cr Fe Ni	7.5 20.2-900 3.1-775	ICP-MS	The concentration of nickel ions increased up to the 7th day and then it was consistent after that at 17.03 μg L^{-1} titanium values were	[62]

						17-800		below 0.3 μg L^{-1} which is the titanium detection limit.	
SS Ni-free	Brackets	Artificial saliva (pH 4.2, 6.5, 7,6)	1080	1,24,48,120 h	Cr Cr	0.27-0.52 0.21	GF-AAS ICP-OES	The greatest amount of chromium was released from new stainless steel brackets. The smallest release was measured with Ni-free brackets. The difference between recycled brackets and Ni-free brackets was not statistically significant. For all brackets, the greatest release was measured at pH 4.2.	[64]
SS Ni-free	Brackets	Artificial saliva (pH 4.2, 6.5, 7,6)	3	15 m and 1,24,48,120 h	Ni	0.03-74.2	GF-AAS ICP-OES	Reconditioned brackets released the most Ni, and the highest Ni release was performed at pH 4.2 and lower at pH 6.5 and 7.6. In general, Ni release increased with the immersion time for all brackets.	[65]
Ni-Ti alloy	Wires	Artificial saliva (pH 2.5, 6,25, 37^{0}C)	4	1,3,7,14,28 d	Ni Ti	2.4-55 3,0-31	GF-AAS	The average of Ni ions released per day from the tested Ni-Ti wires in artificial saliva with various acidities, was well below the critical concentration necessary to induce allergy and under daily dietary intake level. According to the release amount of Ti ions, the passive film (mainly TiO_2) on Ni-Ti wires was very protective against corrosion in the slightly acidic artificial saliva.	[67]

SS	Brackets	Artificial saliva (pH 4 and 7, 37°C)	320	1 h to 48 w	Ni Cr Cu Co Fe Mn	60 - 300 3.8 - 15 400 - 700 2.5 - 3.5 8 - 50 100 - 120	GF-AAS	Recycled brackets released more ions than new brackets. Brackets immersed in solution of pH 4 released more ions than those immersed in solutions of pH 7, and the total amount of ions released increased with time over the 48 week period.	[68]
SS Ni-free	Wires	Distilled water of lactic acid 90%	5	24 h	Ni	4.8-239	ICP-MS	All investigated wires release considerable amounts of Ni to which exposure may have biological implications. Acidity has more impact in comparison to mechanical loading.	[69]
Ni-Ti alloy	Archwire	Artificial saliva (pH 7.4, 37°C)	10	30 d	Ni	1.2 - 8.2	GF-AAS	The concentration of Ni ion initially increased very sharply and later reached a saturation level. The film of titanium oxide reduced Ni release. The oxidation treatment avoids the allergic reactions or toxicity in the surrounding tissues produced by the chemical degradation of the Ni-Ti alloy.	[71]
Ni-Ti alloy	Archwires	Artificial saliva (pH 2, 5.3, 37°C)	2	1,3,7,14 d	Ni	4.3-36.8	ICP-MS	Bending stress induced greater Ni ion release compared with unstressed specimens in artificial saliva at both pH 2 and 5.3.	[72]
SS	Bands	Artificial saliva (37°C)	4	1,7,14,21,28 d	Cr	1.70-4.54	AAS	The peak of Ni and Cr released was on 7th day, and	[73]

					Ni	4.95-7.78		then the rate of release diminished, and was very much below the dietary average intake even for four bands used and was not capable of causing any toxicity.	
SS	Brackets	Immersion in mouthwashes	160	45 d	Cr Cu Fe Mn Ni	91 - 838 4.1 - 19.0 53 - 73 207-1065 110-1200	ICP-OES	The highest metals release was found with chlorhexidine compared with the other 2 mouthwashes. The level of manganese release was significantly different in all 4 groups.	[74]
SS	Brackets	Artificial saliva	10	1 w	Ni	0.15-0.18	ICP-MS	Recycling was found to significantly reduce the corrosion resistance and dimensional stability of orthodontic brackets. No significant differences were observed in Ni release with the new brackets, those recycled by direct flaming and in acid bath.	[75]
Ni-Ti alloy	Wires	Artificial saliva (37^{o}C)	40	48 h	Ni	2.01-9.74	GF-AAS	Maximum Ni was released under acidic pH in the thermo cycling group. Thermo cycling and pH can adversely affect the release of nickel from orthodontic wires, while thermo cycling is clearly the dominant factor.	[76]

CoAW (Ni-Ti+SS)	Wires	Artificial saliva	n/a	n/a	Cu	0.2-0.4	ICP-OES	The CoAW samples were most susceptible to corrosion in the chlorine solution (greatest Cu release) and most resistant in the protein containing artificial saliva.	[77]
SS	Wires, brackets, bands	Artificial saliva (0.40 g L^{-1} NaCl)	46	30 d	Al Co Cr Cu Fe Mn Ni Ti Zn	1699 4 101 121 2382 68 573 394 91	ICP-MS	Experimental group contained 39 times more Ni than the control solution; the fixed orthodontic appliances made of stainless steel can be a source of risk exposure to nickel. A correlation between Ni and Cr concentrations in saliva was found.	[79]
SS	Wires, brackets, bands	Artificial saliva	46	28 d	Cu Cr Ni Fe Mo	31.3 5.74 18.7 14.56 1.75	ICP-OES	The estimated doses of Cr, Cu and Ni released during the treatment were far below the toxic dose to humans.	[80]
SS	Wires, brackets, ligatures	Orange juice (pH 3.765) Artificial saliva (37^0C, pH 6.752) Coca Cola (pH 2.405)	46	1 - 28 d	 Ni Cr Fe Cu Mn Mo Cd Ni Cr Fe	**µg** 15.33 3.604 48.42 57.87 9.164 9.999 0.5967 37.75 1.052 >156.1	ICP-OES	It was found that orange juice did not intensify the release of metal ions from orthodontic appliances, whereas Coca Cola caused increased release of Ni ions.	[80a]

		Artificial saliva (37^0C, pH 6.752)			Cu Mn Mo Cd	32.91 41.16 30.12 2.173			
Ni-based alloy Noble alloy Cu-Al alloy	Crowns	Artificial saliva	32	15 d	Ni Cr Al Be Cu Mn Fe Zn Mo	5.22 0.04 0.23 0.41 0.47 0.06 0.32 0.08 0.21	ICP-MS	The daily amount of ions released seems to be far below the tolerable upper intake levels for each ion.	[81]
SS	Bands, brackets, wires	Artificial saliva	22	44 d	Co Cr Mn Ni	0.1 - 0.4 5 - 6 0.8 - 2.0 6 – 30	ICP-MS	The use of constant release rates will clearly underestimate metal intake by the patient during the first couple of days and overestimate exposure during the remainder of the treatment which is usually several months long.	[82]
SS Ni-Ti alloy	Brackets, bands, wires	Artificial saliva (37^0C)	10	1, 7, 14, 21, 28 d	Cr Ni	16.4-233 1.26-8.41	FAAS	Orthodontic appliances release measurable amounts of nickel and chromium when placed in an artificial saliva medium. For both arch wire types, the release for nickel averaged 37 times greater than that for chromium.	[84]
SS Ni-Ti alloy	Brackets, wires	Artificial saliva (pH 7, 37^0C)	60	45 d	Cr Ni Fe	4.5-7.5 10-20 64-140	ICP-MS	There was a significant increase in Ni and Ti ions in group 3 and 4 than the	[85]

								amounts released from the other two combinations.	
SS Ni-Ti alloy	Wires	Artificial saliva (pH 3.5, 6.75, 37^{0}C)	18	1 - 28 d	Cu Cr Fe Ni	15-708 3.3-58 13-406 4.5-194	HR-ICP/MS	Quantities of all released ions were below toxic levels and did not exceed the daily dietary intake. However, these levels are sufficient to cause an allergic reaction because of the high haptenic potential of released elements.	[86]
Co-Cr alloy	Dental prosthesis	Artificial saliva (pH 2.3, 7.1)	3	15, 30 d	Co Cr Fe	5.0 2.6 2.4	AAS	Co-Cr alloys processed with different fabrication techniques show a release of low amounts of ions in all tested conditions. All the alloys are adequately corrosion resistant and well suited for dental usage.	[87]
SS	Ligatures	Artificial saliva (36.6^{0}C)	11	1, 3, 6 m	Co Cr Fe Mn Ni Sn	28.6 21.7 623.5 5.5 1152.7 22.6	ICP-MS	Stainless steel ligatures released Co, Cr, Fe, Ni, and Sn from which only the level of Ni was concern given the fact that these appliances are used over a prolonged period, not only in adults but also in children.	[89]
Co-Cr alloy cpTi Ti-6Al-4V	Cylinders	Artificial saliva (pH 7.03^{o}C)	6	1,4,7,14,21 d	**Total ions** Co,Cr,Al, Ti	0.08-0.65	ICP-OES	The ion release and the surface roughness of the materials tested are influenced by the manufacturing technique of the material.	[90]
SS Co-Cr alloy	Brackets	Synthetic saliva (pH 6.7, 37^{0}C)	280	28 d	Ni Cr Ni	6.93-598 1.6-13.4	GF-AAS ICP-OES	The brackets from different manufacturers present different corrosion behavior. Increasing rates of Ni and Cr	[91]

		Artificial plaque fluid (pH 2.3, 37^{0}C)			Cr	198-7000 103-13.8		release from the studied brackets were observed.	
Co-Cr-Mo alloy Ni-Cr-Mo alloy Ti-6Al-4V alloy Au alloy	Disks	Artificial saliva (0.9% NaCl, pH 2.3, 6.5, 7.3)	32	7,15,30,60 d	Co Cr Mo Fe Ti Au Pt In	0.30 - 4.7 0.09 - 1.7 0.09 - 8.0 1.8 - 18.3 0.0 - 8.0 2.9 - 9.5 0.7 - 53.4 4.9 - 82.7	AAS	For all ions, pH and period interactions were statistically significant: highest amount of ion release occurred after 60 days of immersion regardless of pH value. For every alloy, ion release results were highest in artificial saliva of pH 2.3 and lowest in 0.9% saline solution; ion release from alloys was pH dependent.	[92]
Cu-Ni-Ti alloy	Wires	Neutral and acid solutions	10	7 d	Ni Cu	5.2 - 21.4 <3	GF-AAS ICP-MS	Increased Ni ions are related initially after the orthodontic devices have been fitted, but they decay quickly. This is reflected in miniscule corrosion defects as pitting. It is unlikely that orthodontic nickel titanium wires are a relevant additional Ni load for the patient. Metal ion release by archwires is dependent on the commercial brand and the immersion solution.	[95]
SS	Brackets	Synthetic saliva	20	24 h	Cr_{total}	14.1-20.3	GF-AAS	The results indicated that the Cr(VI) is released from the	[96]

		(pH ~6, 37^{0}C)			Cr(VI) Cr(III)	5.6-7.2 8.5-13		orthodontic brackets of low quality. Considering the duration of the orthodontic treatment (2 years), the possibility of corrosion of the brackets in the oral environment deserves attention to avoid ingestion of highly toxic specie of chromium.	
Co-Cr-Mo alloy	Rollers	Simulated saliva	180	1,2,3,4,5,6,7,14,21,30 d	Co Cr Fe Ni Zn	80 - 500 100-770 90 - 110 20 - 220 190-990	ICP-OES	Leaching of the Co, Cr, Fe, Zn and Ni ions in the solution was dependent both upon the nature of the solution in which the alloy was immersed and the duration of the immersion.	[98]
Au-Pt alloy	Leafs	Simulated saliva (pH 6.0, 37^{0}C)	6	1,2,3,4,5,6,7,14,21,30 d	Cu Cr Fe Zn	32-253 10 31-138 102-211	ICP-OES	The most released ions were Zn, the least released were Cr and Fe. The time of exposure to the extraction solution affects the amount of released ions. All released ions were below the daily dietary level of need for these elements.	[99]
Au-Pt alloy	Leafs	Simulated saliva (pH 6.0, 37^{0}C)	6	1,2,3,4,5,6, 7,14,21,30 d	Cu Cr Fe Zn	210 10 5 150	ICP-OES	Results demonstrated release of only Cr, Cu, Fe, and Zn from tested Au/Pt dental alloy; however, only Cu and Zn were declared. The undeclared chromium from Au/Pt dental alloy, or some other element might be responsible for the contact	[100]

								allergy thus attributed to the gold.	
SS	Tubes, Begg brackets	Serum	25	6,12,18,24 m	Ni Cr	0.40-15.94 0.39-15.75	ICP-MS	The orthodontic appliances corrode in oral environment releasing both nickel and chromium, but in amounts significantly below the average dietary intake.	[101]
SS Ni-Cr alloy	Plates	0.9% NaCl (37^0C) 1% lactic acid	4	7 d	Ni Cr Fe	3-19 0.9-16 39-345	ICP-MS	Transient exposure of tested materials to an acidic environment is likely to significantly increase the element release from them. The significant higher release of Ni from Ni-Cr alloy was evident.	[102]
Ni-Ti alloy	Archwires	Neutral or acid solution	4	7 d	Ni	5.20-21.40	ICP-OES	Metal ion release by archwires is dependent on the commercial brand and the immersion solution.	[103]
SS Ni-Ti alloy	Archwires Brackets	Artificial saliva	60	90 d	Ni Cr Cu Co Fe	1.3-2.5 0.018-0.03 - 0.004-0.012 0.31-0.61	AAS		[103a]

Analytical techniques: FAAS, flame atomic absorption spectrometry; AAS, atomic absorption spectrometry; GF-AAS, graphite furnace atomic absorption spectrometry; ICP-OES, inductively coupled plasma optical emission spectrometry; ICP-MS, inductively coupled plasma mass spectrometry; HR-ICP/MS, high resolution inductively coupled plasma mass spectrometry; Abbreviations: Conc, concentration; CoAW, composite arch-wire; cpTi, pure titanium; SS, stainless steel; TCA, tartaric, citric, ascorbic acids; LA, lactic, acetic acids; TiN, titanium nitride; n/a-data not available; h-hours; d-days; w-weeks; m-months

5.2. In vivo studies

In dentistry, metallic materials are used as implants in reconstructive oral surgery to replace a single tooth or an array of teeth, or in the fabrication of a dental prosthesis, such as metal plates for complete or partial dentures, crowns and bridges, and are particularly essential for patients requiring hypoallergenic materials. Depending on the different requirements for the wide range of applications, the dental material market offers a large variety of products, various inert metallic and alloy biomaterials are used in these implant systems [2]. No metal or alloy is entirely inert in vivo; any metal or alloy implanted in the human body is a potential source of toxicity. Corrosion is one of the possible causes of implant failure after initial

success [11,16]. Due to its mechanical properties, good resistance to corrosion in biological fluids and very low toxicity, titanium has been the most commonly selected material for dental implants and prosthesis [13].

Many types of metals and alloys have been used in dentistry. Possibilities for quantitative *in vivo* diagnosis of early dental erosion and monitoring of the treatment efficiencies *in vivo* are very attractive long-term goals. Therefore, clinical monitoring of dental erosion requires systematic and, preferably, quantitative assessment of dental fluids and tissues over a long period of time. Some experiments evaluating the potential for conventional dental implants to release metallic ions into the body have been performed [49,53]. It has been reported that saliva leakage between the superstructure (Ni-Cr-Mo alloy) and the implant (made of pure titanium) may trigger a corrosion process (galvanic corrosion) due to differences in electrical potential. This generates the passage of ions such as nickel or chromium from the alloy of a crown or bridge to the peri-implant tissues, with consequent bone reabsorption, which may compromise the mobility of the implant and induce a subsequent fracture [104].

5.2.1. Human saliva

Fixed orthodontic appliances including brackets and arches are commonly made of stainless steel (SS) and nickel-titanium (Ni-Ti) alloys and, therefore, have corrosion potential in the oral environment. Several *in vivo* studies have demonstrated the corrosion and release of metal ions from orthodontic appliances through emission of electro-galvanic currents, with saliva acting as the medium for continuous erosion over time [105,106].

The release of Cr and Ni from orthodontic appliances into saliva was assessed by Amini et al. [107]. Samples collected from 28 subjects with orthodontic appliances (stainless steel brackets, orthodontic bands and archwires) had mean concentrations of 2.6 and 18.5 μg L^{-1} for Cr and Ni, respectively, while in samples from same-gender siblings without appliances, concentrations were 2.2 and 11.9 μg L^{-1}. In another study performed by Amini et al. [108] effect of long-term orthodontic treatment on salivary nickel and chromium has been assessed. There have measured salivary levels of these ions during 1 year of orthodontic treatment. Average nickel level changed from 9.75 to 10.37 and then to 8.32 μg L^{-1} in 1 year. Average chromium concentration changed from 3.86 to 4.6 and then to 2.04 μg L^{-1}. The decrease in chromium concentration after 12 months was significant compared to the control. Although slightly increased after 6 months, the concentration of both ions dropped to levels slightly lower than the control groups after 12 months [108].

Orthodontic appliances (brackets and wires) exposed to the oral environment are affected by thermal alterations in the oral cavity and pH, constant presence of saliva, exposure to foods and drinks, mechanical loads applied to them, and abrasion. They are subjected to aging as such and may undergo dissolution or oxidation [109]. The authors have evaluated the salivary amounts of nickel and chromium in orthodontic patients immediately before treatment, and 2 months after beginning of fixed treatment in two groups of conventional brackets and metal-injection molding (MIM) brackets. Mean nickel level increased from 7.87 (pre-treatment) to 12.57 (2nd month) in the control group, and from 8.62 (pre-treatment) to 8.86 μg L^{-1} in the MIM group. Average chromium level changed from 0.25 (pre-treatment) to 0.35 and from 0.42 to 0.26 μg L^{-1} in the MIM group. Nickel might increase in patients undergoing treatment with both bracket types, although the rate of increase might be greater in patients under treatment with conventional brackets. Using MIM brackets might reduce salivary chromium for trivial but generalizable amounts. Still, ion levels leached from conventional *vs*. MIM brackets might not show a difference after 2 months [109].

Other clinical study has determined salivary concentrations of nickel, chromium and cobalt ions in patients with orthodontic appliances [110]. The study was performed on salivary samples of 60 subjects who were under orthodontic treatment with fixed orthodontic appliance for average of 1.5 to 2 years. Examining the content of nickel in the saliva of orthodontic patients and controls, the nickel content of saliva in study group was significantly higher than the controls. The mean salivary chromium content was 13.60, and 1.40 μg L^{-1} in subjects with and without orthodontic appliances, respectively. Fixed orthodontic appliances release measurable amount of Ni and Co when placed in the mouth however; this increase doesn't reach toxic levels in saliva [110].

It is well established that psychological stress can alter the environment in favor of corrosion of orthodontic alloys by changing the properties of saliva. Amini et al. [1011] aimed to assess the effect of stress induction on salivary nickel and chromium content in fixed orthodontic patients. Thirty patients were enrolled in this experiment. Saliva sample collection was performed at four time points; ion content was measured. The mean amount of salivary nickel increased from 11.9 μg L^{-1} to 14.1 μg L^{-1}. The average salivary chromium content changed from 4.1 μg L^{-1} to 5.1 μg L^{-1}. None of the differences were significant for chromium. Induction of stress in this study led to a significant increase in nickel release from orthodontic appliances into saliva. The salivary chromium content however was not significantly altered, yet gradually increased.

Metal alloy ion release has also been reported [112-121]. Most of these experiments measured metal (Co, Cr, Cu, Mo, Mn Fe, Ni, Ti, V) ion release during the exposure to a biologic medium (blood, serum, urine and mucosa cells) or saliva for periods ranging from 1 day to 1 month. Other studies have also reported the continuing release of metal ions over a 10-month period from a wide variety of dental casting alloys, but the results were not consistent. For example, while some authors have shown an increase in metal ion concentration in the oral fluid of patients with orthodontic appliances [112-121], Eliades et al. [113], Petoumanu et al. [117,118] and Sahoo et al. [120] reported no differences in salivary metal ion concentration between subjects with and without fixed orthodontic appliances. Elshahawy et al. [121] investigated the elemental ion release from two commonly used restorative materials (type IV fixed gold alloy and CAD-CAM ceramic crowns) into natural human saliva of fixed prosthodontic patients. Twenty patients were divided into two equal groups. Saliva collection and clinical evaluation of marginal integrity and gingival health were performed before crowns preparation, 3 months and 6 months after crowns placement. Collected saliva samples were analyzed for element release. The zinc, copper, palladium, gold and silver were released from type IV gold crowns into saliva, while the silicon and aluminum were released from ceramic crowns. Significant increased releases of Zn from type IV gold crown and Si from CAD-CAM-fabricated ceramic crowns into the saliva were evident after three months of clinical service.

In vivo nickel release from orthodontic appliances was reported [122-125]. Nickel-titanium alloys are commonly used in orthodontics, in manufacturing various components of fixed orthodontic appliances. In an effort to understand the issues on ion release and its biological effects, *in vivo* research was conducted. Saliva from 31 subjects, before the placement of brackets and 3 weeks after placement, was collected; the nickel and iron were quantified. The only difference observed for the concentration and volume of nickel and iron, was immediately after placement of the appliance, when there was a significant increase in nickel level in saliva. So it is seems that effect decreases with time [122]. Nickel ion released from fixed orthodontic appliances can serve as allergens or may have serious biological side effects. Ouschal and Lazrak [123] evaluated levels of nickel released into saliva by fixed orthodontic appliances. They performed an *in vivo* study on 16 patients (eight boys, eight girls). Nickel levels in saliva were evaluated before appliance placement, just after placement, and 8 weeks after placement. Results showed a significant increase in nickel levels just after Ni-Ti archwire insertion. However, the difference was non-significant 8 weeks later. To this end, orthodontic appliances release nickel ions mainly at the start of orthodontic treatment. Bengleil et al. [124] evaluated the level of nickel released into the saliva of orthodontic patients using two of commonly used applinaces. Non-stimulated saliva was collected from 18 patients divided into two groups. Nickel concentration value (mg L^{-1}) in first group prior to starting treatment was 0.097. An increase in level of nickel was followed by decrease 4 and 8 weeks after applying the archwire (0.208) and (0.077), respectively. Nickel levels in saliva of the second group were showed minimal variation and ranged from 0.06 to 0.083 throughout period of study. It may be concluded that there could be a release of nickel from the appliances used in first group but it doesn't reach toxic level in saliva. Orthodontic appliances are highly biocompatible, although some side effects associated with the release of nickel ions have been documented [125]. The authors determined the amount of Ni(II) in the saliva of 30 patients treated with Ni-containing self-ligating fixed orthodontic appliances and Ni-Ti archwires. Un-stimulated saliva samples were collected after different time points (before archwire insertion, after archwire insertion, and finally 4 and 8 weeks afterwards) and analyzed. Median salivary Ni(II) concentrations in patients with self-ligating fixed appliances ranged between 13.73 and 85.34 μg L^{-1}, remaining below the daily dietary nickel intake. The highest levels of Ni(II) were measured after the placement of the self-ligating brackets and bands and after Ni-Ti archwire insertion; Ni(II) levels returned to baseline levels after a 2-week period. Self-ligating orthodontic appliances may affect salivary Ni(II) concentrations *in vivo* over the short term.

Various studies have evaluated the discharge of nickel and chromium ions from orthodontic appliances in saliva [126-135]. Baričević et al. [126] have measured metal content of nickel and chromium in un-stimulated whole saliva of 85 patients with and without metal dental appliances. The concentration of metal ions was investigated in correlation to burning mouth syndrome, erythematic of oral mucosa, pH and smoking habit. Results showed a higher Ni concentration in patients with metal restorations, especially wearers of predominantly base metal appliances. The concentration of Cr showed no difference between patient groups. Although burning mouth syndrome was more frequent in the group with dental casting alloys, there was no correlation between higher Ni and Cr concentrations and burning mouth syndrome.

Erythematic of oral mucosa was a common finding in study patients, but did not correlate with salivary Ni and Cr ion concentrations. Salivary Ni and Cr concentrations were not related to either pH or smoking habit. Neamah [127] tested the hypothesis that there is no difference in salivary metal ion content between fifty orthodontic patients with fixed orthodontic appliances and their same-gender sister or brother without any orthodontic appliance. Four samples of saliva were collected from each patient before insertion of the appliance, 1 month after insertion of the appliance, 2 and 6 months after insertion of the appliance; saliva samples were analyzed for Ni and Cr. The mean salivary Ni content in subjects with and without a fixed orthodontic appliance was 16.8 μg L^{-1} and 10.3 μg L^{-1}, respectively. The mean salivary Cr ion level recorded was 2.4 μg L^{-1} in the study group and 2.1 μg L^{-1} in the control group. Nickel and chromium ion concentrations increased immediately after placement of the appliance in the mouth for all study groups. There was no significant difference in the Ni and Cr levels released by the groups of appliances at all study periods. The presence of fixed orthodontic appliances leads to an increased concentration of metal ions in salivary secretions.

Yassaei et al. [128] investigated the salivary concentration of nickel and chromium of 32 patients undergoing orthodontic treatment. The salivary samples were taken from the patients in four stages: before appliance placement and 20 days, 3 months, and 6 months following appliance placement; amounts of metals were determined. It was found that the average amount of Ni in the saliva 20 days after appliance placement was 0.8 μg L^{-1} more than before placement. The average amount of Cr in the saliva was found to be between 2.6 and 3.6 μg L^{-1}. The amount of Ni and Cr at all stages after appliance placement was more than before, but the differences between the chromium and nickel levels of saliva at all stages were not significant.

Talic et al. [129] have measured the amount of nickel and chromium released into saliva of patients treated with fixed orthodontic appliances. Ninety salivary samples were collected in an across-sectional manner. Forty samples were collected from patients with fixed orthodontic appliances after different periods of orthodontic treatment ranging from the first month and up to 32 months into treatment. The fixed orthodontic appliance consisted of 4 bands, 20 stainless steel brackets, and upper and lower Ni-Ti or SS archwires. The other 50 samples were collected from people without appliance; samples were analyzed to measure Ni and Cr levels, respectively. The mean Ni level was 4.197 μg L^{-1} in the experimental group and 2.3 μg L^{-1} in the control group. The mean Cr level was 2.9 μg L^{-1} in the experimental group and 3.3 μg L^{-1} in the control group. Fixed orthodontic appliances resulted in a non-toxic increase in salivary levels of Ni, but no change in Cr levels. Duration of orthodontic treatment did not affect Ni and Cr levels in the saliva.

Mohamed et al. [130] have investigated and compared the salivary level of nickel and chromium in the children having lingual arch space maintainer appliances *vs.* those having stainless steel crowns (SSC). A total 34 patients were selected and allocated into two groups 17 patients each. The first group will be those in need of lingual arch space maintainer without any other metallic restorations. The second group will be patients in need of two SSC restorations also with no other metallic fillings or appliances. The salivary samples were taken from the patients in four stages: before appliances or crowns cementation then after 1 month, 3 months, and 6 months following cementation; the levels of metals was determined. There was a significant increase in the salivary levels of Ni and Cr elements in both groups after cementation of space maintainer appliances or SSC. The amount of released Ni and Cr were significantly higher in group I when compared to group II. The amount of salivary Ni and Cr released after lingual arch space maintainer appliances were more than those after SSC. Dwivedi et al. [131 determined and compared the level of nickel and chromium in the saliva of patients undergoing fixed orthodontic treatment at different time periods. The sample of saliva of 13 patients was taken at different time perios that is: group I (before appliance placement). Group II, III, and IV (after 1 week, 1 month, and 3 months of appliance placement respectively). The fixed appliance comprised of brackets, bands, buccal tubes, lingual sheath, transpalatal arch and wires composed of Ni-Ti and SS; the level of ions was determined. Level of Ni and Cr in saliva was highest in group II and lowest in groups I for both the ions. The release of Ni and Cr was maximum at 1 week and then the level gradually declined. These values were well below the toxic dose of these ions.

Khursheed and Abdulla [132] determined the release of nickel, chromium and iron ions into saliva of patients treated with a fixed orthodontic appliance. Saliva samples from 18 patients were taken at three different time points, group A: before placement of the appliance directly (baseline), group B: one month after appliance placement, group C: four months after appliance placement. The fixed appliance consists of 20 SS brackets, 4 buccal tubes, and superelastic Ni-Ti archwires; level of ions in salivary samples was analyzed. Level of Ni, Cr and Fe ions in saliva was highest in group B and lowest in group A. Nickel, chromium and iron levels in saliva were increased after the placement of fixed orthodontic appliance. Quadras et al. [133] assessed the release of nickel, chromium, and zinc in saliva and serum of patients undergoing fixed orthodontic treatment. The *in vivo* study was conducted on 80 participants. Thirty were included as controls and 50 participants were treated with fixed orthodontic appliances. Saliva and blood samples were collected at five different periods, before insertion of fixed orthodontic appliance and at 1 week, 3 months, 1 year, and 1.5 years after insertion of appliance, respectively; the metal ion content in the samples were analyzed. At the end of 1.5 years, the mean salivary levels of Ni, Cr, and Zn in controls were 5.02 μg L^{-1}, 1.27 μg L^{-1}, and 10.24 μg L^{-1}, respectively, as

compared to 67 μg L^{-1}, 30.8 μg L^{-1}, and 164.7 μg L^{-1} at the end of 1.5 years. Orthodontic appliances do release considerable amounts of metal ions such as Ni, Cr, and Zn in saliva and serum. However, it was within permissible levels and did not reach toxic levels.

Ağaoğlu et al. [134] evaluated saliva samples for the concentration of nickel and chromium ions from patients with fixed orthodontic appliances. The group of 100 patients was divided into five subgroups. The material was sampled from the same patients before and after placement of orthodontic appliance (1 week, 1 month, 1 year and 2 years). The authors stated that in saliva samples nickel and chromium reached the highest levels in the first month after placement of the appliance and decreased in the level before orthodontic treatment. Satija et al. [135] evaluated nickel and chromium ions concentrations in salivary samples from patients treated with fixed orthodontic appliances and their possible influences on hepatic enzyme levels. Saliva was collected from 36 patients; first saliva samples were collected before inserting fixed appliances. Second salivary samples were collected at 1 week, third week and 4 weeks of appliance insertion. In salivary samples, nickel and chromium reached their highest levels in first week. Mean liver function enzymes SGOT and SGPT were also significantly increased in 4 weeks. Fixed orthodontic appliances release measurable amount of nickel and chromium when placed in mouth, but this increase does not reach toxic levels for nickel and chromium in saliva to cause harmful effects in human beings.

They are numerous *in vivo* studies that have demonstrated the release several metals from orthodontic appliances in saliva [136-140]. Arash et al. [136] evaluated the changes of iron, magnesium and chromium in the saliva of 11 patients undergoing fixed orthodontic treatment and had no restorations or crowns. During the fixed orthodontic treatment at successive times (a day, a week, a month, two months and six months) the saliva was collected and evaluated for the amount of Fe, Cr, and Mg. Brackets, band and wire used in all patients was SS alloy. The mean concentration of Fe 66.326 μg L^{-1}, Cr 0.483 μg L^{-1} and Mg 0.552 μg L^{-1} decreased during the study but these results were not statistically significant. Iron, chromium and magnesium concentration do not exceed the standard limits in saliva during orthodontic treatment. Lages et al. [137] have measured the salivary levels of nickel, chromium, iron, and copper released from metal and esthetic fixed orthodontic appliances. Ninety patients were divided into three groups (n=30): control, metal appliance (SS brackets and bands, and Ni-Ti archwires) and esthetic appliance (rhodium-coated NI-Ti archwires). Patients undergoing orthodontic treatment had used their appliances for periods between one and six months. Ni, Cr, Fe and Cu salivary concentrations were measured. Nickel concentrations were significantly higher for patients undergoing metallic orthodontic treatment than for the esthetic group. No significant difference regarding Ni and Cr concentrations were observed between the metal and the control groups or between the esthetic and the control groups. Ni and Cr concentrations were significantly influenced by the type of appliance used. Fe and Cu concentrations were not affected by the type or use of orthodontic appliances. Jurela et al. [138] compared salivary levels of nickel, titanium, chromium, cobalt, copper and zinc prior to and six months after the installment metallic brackets. Salivary levels of metals were measured in 47 patients with metal conventional brackets prior to insertion of orthodontic appliances and six months after insertion of orthodontic appliances. The results showed that salivary level of Ti increased significantly six months after installment of orthodontic appliances unlike salivary levels of Cr and Zn which significantly decreased after installment of orthodontic appliances, regardless of bracket type that was used. Kumar et al. [139] evaluated the release of nickel and chromium ions in human saliva during fixed orthodontic therapy. Saliva samples (ten patients) were collected in three stages: sample 1, before orthodontic treatment, sample 2, after 10 days of bonding sample, and sample 3, after 1 months of bonding. The levels of nickel and chromium were statistically significant, while nickel showed a gradual increase in the first 10 days and a decline thereafter. Chromium showed a gradual increase and was statistically on the 30th day. Nickel and chromium levels were well within the permissible levels. However, some hypersensitive individuals may be allergic to this minimal permissible level. Nayak et al. [139a] evaluated the release of nickel and chromium ions from orthodontic appliances in the oral cavity. The ionic concentration at the end of 10 - 12 months of treatment showed a statistically significant increase in of 17.92 μg L^{-1} for chromium and a statistically insignificant decrease in nickel concentration by 1.58 μg L^{-1}. Pearson's correlation coefficient showed a positive correlation for an increase in nickel concentration after aligning, but not at the end of 10-12 months. A positive correlation was seen for an increase in chromium ion concentration at both time intervals. Nickel and chromium ion concentration in saliva even though below the recommended values per day should not be ignored in light of the new knowledge regarding effects of these ions at the molecular level and the allergic potential. Careful and detailed medical history of allergy is essential.

Kharat [140] have determined the metal (Ni, Cr) ion concentrations in the saliva of patients with and without fixed orthodontic appliances. This retrospective study was carried out on 23 patients who had undergone fixed orthodontic therapy for duration of 12-18 months. The mean salivary Ni content in patients with and without a fixed orthodontic appliance was 17.1 μg L^{-1}. The mean salivary Cr ion level recorded was 2.3 μg L^{-1} in the study group. Although low levels of these metal ions may be of concern to allergy patients, they do not cause problems in most orthodontic patients because toxic levels are never achieved.

Saghiri et al. [140a] evaluated the effect of exposure to radiofrequency electromagnetic fields emitted by mobile phone on the level of nickel in saliva. In addition, the effect of different times of exposure to the RFER was evaluated on the concentration of nickel in saliva.

The concentration of trace metals in the saliva samples of patients following *in vivo* ion release from metallic orthodontic appliances are presented in Table 3.

5.2.2. Oral mucosa cells

Trace metal ions release from fixed orthodontic appliances and their influence on oral mucosa in conditions of *in vivo* are presented, along with a detailed analysis of the exposure of the cells of cheek mucosa epithelium to metal ions [140b]. Several authors evaluated Ni and Co [141,142], Ti, Cr, Mn, Co, Ni, Mo and Fe [143], Ni and Cr [144,145], Ni [146] and Al, Co, Cr, Cu, Ni, Ti and V [147] levels in oral mucosa cells and in dental plaque in the patients with orthodontic appliances (brackets, bands, archwires). In general, it was found that the presence of metals released from orthodontic appliances induced DNA damage and reduced cellular viability of mucosa cells.

Particles can be released from metallic devices (due to several mechanisms including corrosion, wear and mechanically accelerated electrochemical processes such as fretting corrosion, stress corrosion and corrosion fatigue) and these metallic particles are accumulated and stored in the surrounding tissues [148-150]. Titanium and its alloys are used for implants and other dental materials. Although titanium dental implants are characterized by great biocompatibility, despite the passive activity of the external layer of oxides, both electrochemical and galvanic erosion may take place in the environment of the oral cavity. A remarkable study dealing with the possible role of Ti in the genesis of yellow nail syndrome was discussed by Berglund and Carlmark [148]. Using EDXRF analyses of nail clippings, Ti was regularly found in fingernails of implant patients but not in control subjects. Titanium dissolves from the implants and is deposited into the nails. Meningaud et al. [149] conducted a study to investigate whether or not a relationship existed between duration of plating and metal release from Ti mini-plates in maxillofacial surgery. Ti was found in the soft tissues in contact with the Ti plates and soluble and non-soluble fractions were distinguished. Their results indicate that almost 100% of Ti is insoluble, most likely corresponding to metallic particles released during the implantation of the plates. Plates, grids and surrounding tissue were investigated to evaluate titanium release and accumulation [150]. The authors concluded that titanium was only present in the interfacial bone, probably due to fretting, and in all fibrous tissue surrounding the devices. High Ti levels were found in blood cells in the connective tissue, however. Cytotoxicity, DNA damage, cellular uptake and size of three kinds of Ti particles were measured [151]. The half-maximal effective concentration (EC_{50}) for Ti nanoparticles in human cells is 2,800 μg mL^{-1}. Therefore, it is assumed that Ti nanoparticles released from dental implants might have no toxicologically clinical effects. Rykowska et al. [151a] determined titanium and other metal (Ni, Al, Zn, and Cu) concentrations in the oral mucosa covering two-stage intra-osseous dental implants. Additionally, authors presented the results of experiments carried out to determine the release of metal ions from two implant systems into a solution of lactic acid (1%).

The concentration of trace metals in the clinical samples of patients following *in vivo* ion release from metallic orthodontic appliances and metal implants are presented in Table 4.

5.2.3. Biological human fluids

Various studies have evaluated the release of trace metal ions from orthodontic appliances in human biological fluids. Blood, serum and urine are the conventional biological samples used for metal content determination. The effects of metal ions release in the biological fluids from implanted metallic appliances in the oral and maxillofacial region have been studied in various conditions [152-158].

Bishara et al. [152] have determined whether orthodontic patients accumulate measurable concentrations of nickel in their blood during their initial course of orthodontic therapy. Blood samples were collected at three different time periods: before the placement of orthodontic appliances, 2 months after their placement, and 4 to 5 months after their placement. The study involved 31 subjects, who had malocclusions that required the use of a fully banded and bonded edgewise appliance. The three blood samples for each patient were analyzed. Patient with fully banded and bonded orthodontic appliances did not show either a significant or consistent increase in the blood levels of nickel. They did not find any difference in nickel levels in blood after 4 - 5 months of treatment. Smith et al. [153] measured blood levels of titanium, aluminum, and vanadium preoperatively and at intervals over a 3-year period for 52 patients, each of whom had 3 mandible porous-surface end osseous dental implants. The results showed that there was no evidence of change from preoperative to long-term values for the 3 metals measured in the study. In that study, the average Ti and V levels detected were 1.03 to 1.68 and 0.10 to 0.11 μg L^{-1}, respectively.

Nickel and chromium levels in serum have been evaluated by previous researchers, after periods of treatment with fixed orthodontic appliances [134]. Nickel and chromium levels in serum increased significantly two years after insertion of orthodontic appliance, however, the levels decreased gradually after the initial increase [101]. A study by Satija et al. [135] showed that significant increase in Ni and Cr ion concentration occurred in blood samples (serum was prepared by centrifuging at 3000 rpm for 10 minutes) collected after 4 weeks. Hence, this should be taken care of in patients having allergy to nickel and chromium. Mudjari and Achmad [154] compared the levels of nickel and chromium in serum and urine in orthodontic 20 patients treated with fixed orthodontic appliances. The samples were taken before treatment (baseline), two months, and six months later during treatment. Average serum nickel level changed from 6.4 to 6.9 μg L^{-1}. Average serum chromium level changed from 5.3 to 5.6 μg L^{-1} in 6 months. Average urinary nickel level changed from 5.3 to 5.6 μg L^{-1} in 6 months. Average urinary chromium level changed from 5.4 to 5.5 μg L^{-1} in 6 months. Orthodontic treatment might raise both urinary and serum nickel levels, but the differences were not statistically significant; the alterations in chromium levels were not consistent; nickel levels were higher in serum than in urine; chromium levels were higher in urine than in serum.

Mercuri et al. [155] had compared metal (Ti, V, Cr, Co, Ni) ion levels in various surgical techniques and has stated that in the dental implant group, one of the patients had elevated levels of serum titanium and another patient showed elevated levels of both serum levels of titanium and chromium. In the orthodontic group, one patient had an increased serum cobalt level. In the prosthetic metallic total temporomandibular joint replacement group, one patient had an increased serum cobalt level and another patient had in increased serum chromium level. All control participants had levels below the normal reference range for all serum markers assessed.

Gopi et al. [156] have evaluated the release of titanium, aluminum, and vanadium from dental implants by comparing the preoperative and postoperatively serum levels of these ions. Serum samples were collected from 30 patients undergoing dental implant placement preoperatively and postoperatively at intervals of 6 weeks, 3, 6, and 12 months; these samples were analyzed for Ti, Al and V levels. There was a slight difference in the postoperative levels of Ti and Al (2.30 and 4.07 μg L^{-1}) as compared to the preoperative levels (2.28 and 2.30 μg L^{-1}). There is no significant difference in the serum metal ion levels before and after the implant placement, although a little increase is observed in the aluminum ion levels after the implant placement.

Menezes et al. [157] controlled nickel level by another biomarker of exposure, urine. The pretreatment and treatment levels of nickel in the urine of 21 orthodontic patients wearing fixed appliances were evaluated. This was done before placement of orthodontic appliances and 2 months after placement. The authors stated that urinary nickel levels increased significantly after 2 months. The biological effect of a systemic increase in urinary nickel is unknown. Begerow et al. [158] investigated to what extent noble metal dental alloys contribute to the total platinum, palladium and gold body burden of the general population. The urinary Pt, Pd, and Au excretion was determined in three non-occupationally exposed before and up to 3 months after insertion of a high-gold dental alloy. The *in vitro* release pf Pt, Pd, and Au from four different types of dental alloys into either artificial saliva or 1% lactic acid solution was additionally investigated; the Pt, Pd, and Au concentrations were determined. Before insertion of the high-gold dental alloy, the Pt excretion of the patients ranged between 1.0 and 7.4 ng L^{-1}. In the immediate post-insertion phase the Pt excretion rose to 10.5 - 59.6 ng L^{-1}. Three months after insertion, the Pt excretion was elevated by a factor of 7. Contrary to Pt, the Au and Pd excretion in urine was not significantly increased after insertion of this type of high-gold dental alloy. There *in vitro* investigations confirm the assumption that Pt, Pd and Au are released from noble metal-containing dental alloys by corrosion. Mudjari and Achmad [154] compared the levels of nickel and chromium in urine in orthodontic 20 patients treated with fixed orthodontic appliances. The samples were taken before treatment (baseline), two months, and six months later during treatment. Average urinary nickel level changed from 5.3 to 5.6 μg L^{-1} in 6 months. Average urinary chromium level changed from 5.4 to 5.5 μg L^{-1} in 6 months. Orthodontic treatment might raise both urinary and serum nickel levels, but the differences were not statistically significant; the alterations in chromium levels were not consistent; nickel levels were higher in serum than in urine; chromium levels were higher in urine than in serum.

As the prevalence of orthodontic malocclusion and the for treatment increases, more and more patients are indicated for orthodontic treatment with fixed orthodontic appliances (brackets). These appliances are composed of metal alloys of Ni, Co, Cr, Ti, Fe, and Cu [159]. Velasco-Ibáñez et al. [159] evaluated the release of metal ions, mainly Ni and Ti, in urine and saliva. Authors selected 35 35 individuals under orthodontic treatment, from whom urine and saliva samples were collected in 3 stages: (a) basal, (b) at 3 and (c) 6 months after the placement of the fixed appliances. A statistically significant difference in the concentration of Ni in saliva was found between 3 and 6 months of intervention and Ti in urine was found 3 and 6 months. Ramadan [160] disclosed the effects of nickel and chromium from fixed orthodontic appliances on the gingival health in a saliva sample of orthodontic patients. Twenty orthodontic patients were treated with fixed orthodontic appliances in the maxillary arch. The concentration of both metals was recorded during pretreatment, at 3 and 12 months into treatment, and 1 month after deboning. The released amounts of Ni and Cr

increased significantly during the treatment period; 1 month after deboning, the levels were not significantly different from those at pretreatment. During treatment, however, the difference was significant.

The systematic review was to analyze the factors affecting nickel release, the amount of nickel being released in commercially available Ni-Ti wires and to also analyze the blood/periodontal evaluation after orthodontic treatment in conventional and nickel free brackets [161].

The concentration of trace metals in the biological fluid samples of patients following *in vivo* ion release from metallic orthodontic appliances is presented in Table 5.

5.2.4. Biological human tissues

In addition to using conventional human biological samples to evaluate metal release from orthodontic appliances, some studies have demonstrated that human hair is an adequate non-invasive matrix for monitoring different ion metals [162].

The first attempt to use human hair to investigate metal release from fixed orthodontic appliances was carried out by Mikulewicz et al. [163]. This preliminary study (28 patients18 controls) demonstrated that stainless steel appliances were the source of significant exposure to Ni and noted that 22% of patients undergoing orthodontic treatment had increased Ni levels in their hair. The highest difference between the groups was found for Ni (39%), Mn (18%), Fe (4.1%) and Cr (2.5%). Furthermore, statistically significant correlations were found between Cr and Fe, which showed that these metals had similar sources of exposure, and multiple regression analysis determined the dependence of Ni content on the level of Co and Mg (synergism) and V (antagonism). Later, a study in human scalp hair from a broad population group with orthodontic appliances (n=70) confirmed that hair mineral analysis is a good method for investigating long-term exposure to different elements (Cr, Cu, Fe, Mn and Ni) [164]. In orthodontic patients, differences in the content of metals in hair were only significantly increased for Mn compared to the control group, but their levels were of the same magnitude to other control populations, and no risks linked to the treatment were found. Moreover, correlations found between Cu/Mn, Cu/Ni and Fe/Ni showed a mutual dependence of these elements in hair (similar chemical structure).

Mikulewicz et al. [165] evaluated metal ion accumulation in the hair of patients with fixed appliances at different time points throughout their treatment (the beginning and in the 4th, 8th, and 12th months of the treatment). These authors reported a peak release of Cr and Fe after 4 months, and the Ni peak gradually increased throughout the year. This study revealed that the Cr content was significantly higher during the treatment, although the doses of the released metal ions did not pose toxicological risks, and the researchers demonstrated that hair mineral analysis permits the study of the kinetics of metal ions and ion transfer to hair tissue. Levrini et al. [166] determined nickel ions released in hair. 100 intact hairs were taken from 15 patients wearing fixed orthodontic appliances. The samples of hair were taken from at least 3 different scalp sites: frontal, vertex and occipital areas. There were no differences in nickel concentrations between the test group (0.50 mg g^{-1} on average) and control group (0.64 mg g^{-1}). It can be assumed that orthodontic appliances do not release significant values of nickel to be a risk factor to the patient's health.

Jamshidi et al. [167] have measured the Ni and Cr ions levels in the scalp hair of patients treated with fixed orthodontic appliances in comparison of the control group. The patient group consisted of 24 patients treated with fixed orthodontic appliances for one year. After one year, the levels of Ni and Cr in two groups showed significant (slightly elevated levels of Ni and Cr ions in the scalp hair of patients) differences (0.086 and 0.258 μg g^{-1} for control group and 0.149 and 0.339 μg g^{-1} for patient group, respectively for Ni and Cr.

Masjedi et al. [168] evaluated the effects of fixed orthodontic treatment using conventional (two-piece) *vs*. metal injection moulding (MIM) brackets on hair Ni and Cr levels. In this double-blind randomized clinical trial, scalp hair samples of 24 + 22 fixed orthodontic patients were collected before treatment and 6 months later. In both groups combined (n=46), nickel increased from 0.1600 μg g^{-1} dry hair mass (pre-treatment) to 0.3199 μg g^{-1} (6th month). Chromium increased from 0.1657 to 0.3066 μg g^{-1}. Both of these increases were significant. Hair nickel and chromium levels might increases about 185-200% after 6 months. They might not be affected by bracket types.

Table 3 In vivo times and metal concentration in human saliva following trace metal ion release from orthodontic appliances

Type of metal/alloy	Orthodontic appliance	Implantation data		Metal ion(s) measured	Concentration (μg L^{-1} or μg g^{-1})		Analytical technique	Research main results	Refs.
		No. Patients/ implants	Follow-up		Metal	Control			
SS	Arches	56	16 m	Ni Cr	18.5 (1.0-49.6) 2.6 (0.3-6.3)	11.9 (1.0-48.9) 2.2 (1.0-7.1)	GF-AAS	A statistical significant difference was found for Ni between both groups (control, study). Fixed orthodontic appliance therapy for an average period of 16 months can lead to increased levels of Ni and Cr ions in the saliva of patients.	[107]
SS Ni-Ti alloy	Brackets, bands archwires	20	6,12 m	Ni Cr	9.75 3.86		GF-AAS	The decrease in chromium concentration after 12 months was significant compared to the control. Although slightly increased after 6 months, the concentration of both ions dropped to levels slightly lower than the control groups after 12 months.	[108]
SS Ni-Ti alloy	Brackets Tubes, archwires	30	2 m	Ni Cr	12.57 0.35	7.87 0.25	GF-AAS	Nickel might increase in patients undergoing treatment with both bracket types (conventional and MIM), although the rate of increase might be greater in patients under treatment with conventional brackets. Still, ion levels leached from conventional *vs*. MIM brackets might not show a difference after 2 months.	[109]
SS	Arches	60	16 - 18 m	Ni Cr Co	17.40 (1.00-40.70) 3.60 (1.30-8.80) 1.64	12.60 (1.00-60.00) 1.40 (0.50-6.00) 1.39	GF-AAS	The salivary content of Ni and Cr ions in patients are increasing after one to two years; this increase doesn't reach toxic levels in saliva.	[110]

					(1.30-6.89)	(0.35-6.06)			
SS Ni-Ti alloy	Brackets, bands Archwires	30	15,30 min 3 m	Ni Cr	11.9-14.1 4.1-5.1		GF-AAS	Significant increase in Ni release into saliva at the stress induction, but Cr content was no significantly altered.	[111]
SS	Brackets	17	1.5 m	Ni Cr Fe	18 27 21		ICP-OES	No statistically significant difference was detected between control and patient groups with respect to salivary metal content, regardless of element. The range of salivary metal levels found did not exceed those of daily intake through food and air.	[113]
SS	Brackets, archwires	47	2 d, 1 w, 1 m	Ni Cr	65 86		GF-AAS	Nickel and chromium concentrations of saliva are not significantly affected by fixed orthodontic appliances during the first month of treatment.	[114]
Ni-Ti alloy	Brackets, buccal tubes	45	1 w, 1,2 m	Ni Cr	0.07-3.32 0.29-8.0		GF-AAS	Fixed orthodontic appliances do not significantly affect nickel and chromium concentrations of saliva during the first 2 months of treatment.	[115]
SS Ni-Ti alloy	Brackets bands Archwires	18	2,4,8 w	Ni	78 (39-170)		ICP-MS	Nickel leaching occurred after placement of the bands and brackets and after placement of the Ni-Ti archwires, associated with and increase of the nickel ion concentration in the patient's saliva.	[117]
SS	Brackets	30	10 m, 24 h, 7,30,60 d	Ni Cr Fe	16.01 1.72 103.58		GF-AAS	Nickel and chromium ion concentrations increased immediately after placement of the appliance in the mouth for all study groups. There were no significant differences in the Ni, Cr. and Fe levels released by the three groups of appliances at all study periods.	[119]
Ni-Ti alloy	Brackets	20	1 h, 1,7,30 d	Ni Cr	2.89-4.95 14.34-36.69		GF-AAS	Both the conventional and the self-ligating brackets did not seem to affect significantly the nickel and chromium concentrations in saliva during the first month of treatment.	[120]

Au alloy	IV Au Crowns	20	3,6 m	Cu Zn Pd Ag Au	10 (2-26) 1048 (308-3337) 26.7 (2-31) 46.7 (0.06-1.3) 24.5 (2-40)	8.3 (0.7-25.2) 695 (91-302) 49 (2-147) 0.2 (0.03-0.7) 38 (2-197)	ICP-MS	Significant increased releases of Zn from type IV gold crowns into saliva were evident after three months of clinical service.	[121]
Ni-Ti alloy	Archwires	16	8 w	Ni	51	32	ICP-MS	Significant increase in Ni levels just after Ni-Ti archwire insertion. However, the difference was non-significant 8 weeks later.	[123]
SS Ni-free	Archwires, brackets	18	2,4,8 w	Ni	mg L^{-1} 0.059-0.208		AAS	There could be a release of nickel from the appliances used in first group but it doesn't reach toxic level in saliva.	[124]
Ni-Ti alloy	Brackets, archwires	30	4,8 w	Ni	21.85 (13.73-85.34)		ICP-MS	Self-ligating orthodontic appliances may affect salivary Ni ion concentrations *in vivo* over the short term. However, levels resembled those documented in conjunction with conventional bracket use and remained below the daily dietary Ni intake.	[125]
SS	Wires, bands, tubes	50	1,2,6 m	Ni Cr	16.8 2.4		GF-AAS	The presence of fixed orthodontic appliances leads to an increased concentration of metal (Ni, Cr) ions immediately after placement of the appliance in the mouth (salivary secretions) for all study groups.	[127]
SS Ni-Ti alloy	Brackets, archwires	90	1 - 32 m	Ni Cr	4.197 2.9	2.2 3.1	ICP-OES ICP-MS	Fixed orthodontic appliances resulted in a non-toxic increase in salivary levels of Ni, but no change in Cr levels. Duration of orthodontic treatment did not affect Ni and Cr levels in the saliva.	[129]

SS	Crowns	34	1,3,6 m	Ni Cr	0.15 0.07		AAS	The amount of salivary Ni and Cr released after lingual arch space maintainer appliances were more than those after SS crowns. Also the maximum amounts of released Ni and Cr were much lower than the dietary intake and were not capable of causing any toxicity.	[130]
SS NI-Ti alloy	Brackets bands, buccal tubes, lingual sheet, archwires	13	1 w, 1,3 m	Ni Cr	6.841 70.386		GF-AAS	The release of Ni and Cr was maximum at 1-week and then the level gradually declined. These values were well below the toxic close of these ions.	[131]
SS Ni-Ti alloy	Brackets Buccal tubes Archwires	18	1,4 m	Ni Cr Fe	34.22 17 99.4	3.95 0.34 45.1	ICP-OES	Nickel, chromium and iron levels in saliva were significantly increased after the placement of fixed orthodontic appliances but were below the toxic levels of the ions.	[132]
SS Ni-Ti alloy	Brackets, Bands, archwires	80	1 w, 3 m, 1, 1.5 y	Ni Cr Zn	67 30.8 164.7	5.02 1.27 10.24	GF-AAS	Orthodontic appliances do release considerable amounts of metal (Ni, Cr, Zn) ions in saliva and serum. However, it was within permissible levels and did not reach toxic levels.	[133]
SS Ni-Ti alloy	Brackets, Bands, wires	100	1 w, 1 m, 1,2 y	Ni Cr	4.12-11.53 0.53-1.53		GF-AAS	The fixed orthodontic appliances release measurable amount of Ni and Cr when placed in the mouth, but this increase doesn't reach toxic levels for Ni and Cr in the saliva and serum and are similar to those found in healthy individuals. However, the placement of archwires can cause an increase in salivary Ni and Cr levels.	[134]
SS Ni-Ti alloy	Brackets, Wires, ligatures	36	1,4 w	Ni Cr	10.372 1.509	5.432 0.596	GF-AAS	The orthodontic appliances corrode in oral environmental and release nickel and chromium. Although, there is initial rise in salivary and serum Ni and Cr levels in 1 week but significantly tapered to permissible blood level in 4 weeks. Also, there was significant rise in hepatic enzymes level from baseline to 4 weeks but it was within normal range.	[135]
SS	Brackets, Bands,	11	1 d, 1 w,	Cr Fe	0.483 66.326		AAS	Iron, chromium and magnesium concentration do not exceed limits in saliva during orthodontic treatment. The concentration of studied elements	[136]

	wires		1,2,6 m	Mg	0.552			(Fe, Cr, Mg) is almost constant after 6 months of bracketing.	
SS Ni-Ti alloy	Brackets Bands Archwires	90	n/a	Ni Cr Cu Fe	221.20 89.45 15.10 517.77	4.14 10.32 11.40 32.04	XRF	Ni and Cr concentration in the saliva of patients submitted to metallic orthodontic treatment was higher than that in the saliva of patients with esthetic appliances, suggesting that these chemical element concentration were significantly influenced by the type of appliance used. Fe and Cu concentrations were not affected by the type or use of orthodontic appliances.	[137]
SS Ni-Ti alloy	Brackets, archwires	42	6 m	Ni Cr Ti Co Cu Zn	5.04 (0.85-12.58) 1.01 (0.41-6.73) 9.29 (0.44-1067) 0.32 (0.01-3.81) 22.19 (8.16-162.6) 168.45 (21.61-3591)	4.24 (0.63-59.94) 1.95 (0.58-32.99) 1.68 (0.42-47.93) 0.46 (0.04-4.77) 23.31 (2.74-2461) 220.67 (32.46-1675)	ICP-MS	The salivary level of Ti increased significantly 6 months after installment of orthodontic appliances unlike salivary levels of Cr and Zn which significantly decreased after installment of orthodontic appliances, regardless of bracket type which was used.	[138]
SS Ni-Ti alloy	Brackets, bands Wires	10	10,30 d	 Ni Cr	mg L^{-1} 0.050 0.016	mg L^{-1} 0.003 0.002	ICP-OES	While comparing levels of Ni in saliva, there was a significant rise from baseline to 10th and 30th-day sample, which was statistically significant. The levels of Cr ion in the saliva were more in 30th day, and when comparing 10th-day sample with 30th day, there was statistically significance.	[139]

SS Ni-Ti alloy	Brackets, wires	30	10 - 12 m	Ni Cr	59.19 102.68	48.78 69.74	ICP-MS	A positive correlation was found in the initial rise in Ni concentration. However, no correlation was found for the change in Ni ion concentration at the end of 10-12 months. A positive correlation was found for the increase in Cr ion concentration after the initial alignment and at the end of 10-12 months.	[139a]
SS Ni-Ti alloy	Brackets, archwires	46	12 - 18 m	Ni Cr	17.1 1.7	11.7 2.3	GF-AAS	The presence of fixed orthodontic appliances leads to an increased concentration of metal (Ni, Cr) ions in salivary secretions.	[140]
RFER	Orthodontic appliances	50	1,2 w	Ni	16.22 (7 - 28)	12.84 (6 - 22)	ICP-MS	Mobile phone usage has a time-depended influence on the concentration of nickel in the saliva of patients with orthodontic appliances.	[140a]

Analytical techniques: AAS, atomic absorption spectrometry; GF-AAS, graphite furnace atomic absorption spectrometry; ICP-OES, inductively coupled plasma optical emission spectrometry; ICP-MS, inductively coupled plasma mass spectrometry; XRF, X-Ray Fluorescence; Abbreviations: n/a-data not available; SS, stainless steel; MIM, metal-injection molding; RFER, radiofrequency electromagnetic radiation; min-minutes; h-hours; d-days; w-weeks; m-months; y-years

Table 4 *In vivo* times and metal concentration in human specimens in patients following trace metal ion release from metallic orthodontic appliances and dental implants

Type of metal/alloy	**Orthodontic appliance**	**Clinical experimental model**	**Implantation data**		**Metal ion(s) Measured**	**Conc. (μg L^{-1} or μg g^{-1})**	**Analytical technique**	**Clinical outcomes**	**Refs.**
			No. Patients/	**Follow-up**					
SS Ni-Ti alloy	Brackets, bands Archwires	Oral mucosa cells	60/30	16 m	Ni Cr Co	21.74 4.24 0.84	GF-AAS	There was no difference in the concentration of Cr and Co in oral mucosa cells of patients with and without fixed appliances. However, a significant higher concentration of Ni can be found in oral mucosa cells of patients wearing fixed orthodontic appliances.	[141]
SS	Brackets, bands	Oral mucosa cells	55/30	n/a	Ni Co	2.521 0.568	ICP-MS	This corroborates that nickel and cobalt released from fixed orthodontic	[142]

Ni-Ti alloy	Archwires							appliances can induce DNA damage in oral mucosa cells.	
SS Ni-Ti alloy Ni-free	Brackets, tubes	Oral mucosa cells	15/15	30 d	Ti Cr Mn Co Ni Mo Fe	0.82-3,04 0.34 0.58-1.08 - 0.04 0.00 1.24-5.36	ICP-MS	Orthodontic apparatus made with titanium are not toxic for the cells of the oral mucosa.	[143]
SS Ni-Ti alloy	Brackets, bands, buccal tubes Archwires	Oral mucosa cells	20/20	30 d	Ni Cr	4.09 3.03	ICP-MS	Nickel and chromium alloys of orthodontic appliances emit metal ions in sufficient quantities to induce localized genotoxic effects, but these changes revert on removal of the appliances.	[144]
SS Ni-Ti alloy	Brackets, bands Archwires	Buccal mucosa cells	40	3,6 m	Ni Cr	0.31 - 0.78 0.52 - 0.78	GF-AAS	Fixed orthodontic appliances decreased cellural viability, induced DNA damage, and increased the Ni and Cr contents of the buccal mucosa cells.; compared to the control group, these changes were not evident at 6 months.	[145]
SS	Brackets Bands	Saliva	24/24	16 m	Ni	25.25 (0.037-51-20) 1.03	GF-AAS	Nickel release occurs into the dental plaque and components of saliva of orthodontic patients, a situation that may reflect time dependence of its	[146]

Ni-Ti alloy	Archwires	Dental plaque			Ni			release from orthodontic appliances into the oral cavity and an aggregation of nickel at plaque sites.	
SS	Brackets, bands, archwires, miniscrews	Oral mucosa cells	60/20	15 m	Al Cr Cu Ni Ti	12.21-12.70 0.18-0.21 1.05-1.13 0.53-0.55 0.33-0.40	ICP-MS	The incorporation of minis crews assayed did not imply a significant increase of metal release.	[147]
Ti metal	Implant	Shed nails Nail clippings	26	n/a	Ti Ti	22 - 48 1.1 - 170	XRF	Yellow nail syndrome is caused by titanium.	[148]
Ti metal	Mini-plates	Soft tissue	51	15 d - 3 y	Ti **soluble** Ti	1306 0.53	ICP-OES	Most of the time, titanium seems to be clinically inert.	[149]
cpTi	Plates, grids	Skeletal tissue	28	6 - 24 m	Ti	detected	EDAX	Titanium release from the devices stops only after bone is laid down on the titanium surfaces. High Ti levels were found in blood surfaces.	[150]
Ti metal	Dental implants	Oral mucosa	n/a	4 - 6 m	Ti Ni Al Zn Cu	1.7-13.1 10.0-0.8 5.3-100.5 16.1-118.8 0.3-13.6	ICP-MS	Corrosion of the implants occurs, thereby Ti and other metal (Ni, Al, Zn, Cu) ions are released into the tissue surrounding the implants.	[151a]

SS Ni-Ti alloy	Archwires, ligatures	Oral mucosa cells	40	n/a	Ti V Zr	3.80-5.23 - 0.54	ICP-MS	The proposed method was suitable for simultaneous determination of Ti, V and Zr in oral mucosa cell samples from patients with orthodontic appliances.	[209]
SS Ni-Ti alloy	Archwires, ligatures	Oral mucosa cells	40	n/a	Co Cr Cu Ni	4.1-25.8 10.9-65.0 5.5-30.0 10.6-63.0	ICP-MS	The proposed method was suitable for simultaneous determination of Co, Cr, Cu and Ni in oral mucosa cell samples from patients with orthodontic appliances.	[210]

Analytical techniques: GF-AAS, graphite furnace atomic absorption spectrometry, ICP-OES, inductively coupled plasma optical emission spectrometry, ICP-MS, inductively coupled plasma mass spectrometry; XRF, energy dispersive X-ray fluorescence. EDAX, energy-dispersive X-ray analysis; Abbreviations: Conc., concentration; n/a-data not available; SS, stainless steel; cpTi, pure titanium; d-days; m-months; y-years

Table 5 *In vivo* times and metal concentration in biological fluids in patients following trace metal ion release from metallic orthodontic appliances and dental implants

Type of metal/alloy	**Orthodontic appliance**	**Human body model**	**Implantation data**		**Metal ion(s) measured**	**Concentration (μg L^{-1} or μg g^{-1})**		**Analytical technique**	**Research main results**	**Refs.**
			No. Patients/ control	**Follow-up**		**Metal**	**Control/ Baseline**			
SS Ni-Ti alloy	Brackets, bands Archwires	Serum	80/30	1 w, 3 m, 1,1.5 y	Ni Cr Zn	81.65 35.6 597.16	8.47 6.02 30.1	GF-AAS	Orthodontic appliances release considerable amounts of Ni, Cr, and Zn into serum during different periods of treatment.	[133]
SS Ni-Ti alloy	Brackets, brands Wires	Serum	100	1 w, 1 m, 1,2 y	Ni Cr	7.87-10.27 6.16-10.98	8.36 6.21	GF-AAS	In the serum, there were statistically significant increases in ion concentration in the second-year groups.	[134]
SS	Brackets, ligatures	Serum	36	1,4 w	Ni Cr	9.948 9.975	8.265 6.477	GF-AAS	There is initial rise in serum Ni and Cr levels in 1st week but significantly tapered to	[135]

Ni-Ti alloy	Wires								permissible blood level in 4th week.	
SS	Brackets, bands, bons	Blood	31	2,4,5 m	Ni	0.4 - 0.9	n/a	AAS	Patients with fully banded and bonded orthodontic appliances did not show a significant increase in the nickel blood level during the first 4 and 5 months of orthodontic therapy.	[152]
Ti-6Al-4V	Dental implant	Blood	52	3,6 m, 1,2,3 y	Ti Al V	3.33 (1.01-7.64) 6.27 (0.80-19.45) 0.20 (0.10-0.73)	3.18 (1.68-6.97) 3.96 (1.26-20.82) 0.20 (0.10-0.44)	GF-AAS	The results showed there was no evidence of change from preoperative to long-term values for the tree metals measured in the study. These findings are reassuring, but do not rule out local or remote accumulation of released ions, which was not measured in this study.	[153]
SS Ni-Ti alloy	Brackets, bonds Archwires	Serum Urine	20	2,6 m	Ni Cr Ni Cr	6.855 5.505 5.610 5.520	6.420 5.305 5.320 5.370	ICP-OES	Orthodontic treatment might raise both urinary and serum Ni levels, but the differences were not statistically significant; the alterations in Cr levels were not consistent; Ni levels were higher in serum than in urine; Cr levels were higher in urine than in serum.	[154]
Ti-6Al-4V	Plates Screws TMJ TJR	Blood (serum)	16	n/a	Ni Cr Co Ti V	0.219-0.477 0.104-0.862 0.146-2.056 0.370-1.798	<0.2 <0.1 <0.15 <0.3 <0.2	ICP-OES	There were compared metal ion levels in various surgical techniques and has stated that in the dental implant group, one of the patients had elevated levels of serum titanium and another patient showed elevated levels of both	[155]

						0.02-0.177			serum levels of titanium and chromium.	
Ti-6Al-4V	Screws	Serum	30	6 w, 3,6,12 m	Ti Al	2.30 4.07	2.28 (1.11-3.77) 4.05 (2.49-5.54)	ICP-OES	There is no significant difference in the serum metal ion levels before and after the implant placement, although a little increase is observed in the aluminum ion levels after the implant placement.	[156]
SS	Brackets, Bands, bonds	Urine	21	2 m	Ni	19.89	17.61	AAS	Urinary nickel levels increased significantly 2 months after the placement of orthodontic appliances.	[157]
SS Ni-Ti alloy	Arches	Urine Saliva	35	3,6 m	Ni Ti Ni Ti	0.015 0.012 0.026 0.029	0.013 0.009 0.022 0.023	ICP-OES	A statistically significant difference in the concentration of Ni in saliva was found between 3 and 6 months of intervention and Ti in urine was found 3 and 6 months.	[159]

Analytical techniques: AAS, atomic absorption spectrometry; GF-AAS, graphite furnace atomic absorption spectrometry; ICP-OES, inductively coupled plasma optical emission spectrometry; Abbreviations: n/a-not applicable; SS, stainless steel; TMJ TJR, total temporomandibular joint replacement; w-weeks; m-months; y-years

Table 6 *In vivo* times and metal concentration in biological tissues in patients following trace metal ion release from metallic orthodontic appliances

Type of metal/alloy	**Orthodontic appliance**	**Human body model**	**Implantation data**		**Metal ion(s) measured**	**Concentration (μg L^{-1} or μg g^{-1})**		**Analytical technique**	**Research main results**	**Refs.**
			No. Patients/ control	**Follow-up**		**Metal**	**Control/ baseline**			
SS	Surgical scissors	Hair	28/18	1.5 - 2 y	Ni Cr Mn Fe	0.5073 0.1331 0.5739 12.22	0.3642 0.1298 0.4850 11.74	ICP-OES	Patient hair analysis revealed that orthodontic treatment with stainless steel appliances is the source of exposure to nickel. Aside from Ni, hair contents of Cr and Fe were also higher in orthodontic patients than in the control.	[163]

SS Ni-Ti alloy	Brackets, tubes, bands, ligatures Archwires	Hair	70/56	24 m	Ni Cr Cu Fe Mn	0.36 0.36 33 25.3 0.23	0.33 0.33 24 24.86 0.42	FAAS GF-AAS	Only significant increased Mn levels in orthodontic patients was detected.	[164]
SS Ni-Ti alloy	Brackets, ligatures Wires	Hair	47	4,8,12 m	Ni Cr Fe	7.42 8.94 131		ICP-OES	The content of Cr was statistically significant higher during the treatment than before the beginning of therapy. However, the doses of released metal ions did not pose a toxicological danger.	[165]
SS	Brackets, wires	Hair	24/28	1 y	Ni Cr	0.258 0.339	0.086 0.149	GF-AAS	Slightly elevated levels of Ni and Cr ions in the scalp hair of patients treated with fixed orthodontic appliances.	[167]
SS Ni-Ti alloy	Brackets Archwires	Hair	46	6 m	Ni Cr	0.3199 0.3066	0.1600 0.1657	AAS	Hair nickel and chromium levels might increase about 185 - 200% after 6 months consisting of either MIM or conventional brackets.	[168]
SS Ni-Ti alloy	Arches, brackets Wires	Gingival crevicular fluid Hair	15	4,8,16 m	Ni Cr Ni Cr	10.410 9.818 0.956 0.295	3.335 1.859 0.125 0.090	AAS	After 16 months of treatment, the hair nickel level was increased by 7.7 times; while for chromium was by 3.3 times. Gingival crevicular fluid nickel level was increased by 3.1 times and chromium level was by 5.3 times.	[169]
SS Ni-Ti alloy	Brackets, ligatures Wires	Hair	47	4,8,12 m	Ni Cr	0.207-0.500 0.124-0.191	0.131 0.013	ICP-OES	The results suggests that consumption of food products of low pH (such as fruit juices, coffee, yoghurt and vinegar) can intensify aggressiveness of conditions in the oral cavity and may have an effect on increasing the release of Ni and Cr ions from orthodontic appliances.	[170]

Analytical techniques: AAS, atomic absorption spectrometry; FAAS, flame atomic absorptions spectrometry; GF-AAS, graphite furnace atomic absorption spectrometry; ICP-OES, inductively coupled plasma optical emission spectrometry; Abbreviations: SS, stainless steel; MIM, metal injection molding; m-months; y-years

Mudjari et al. [169] assessed the levels of nickel and chromium ions in hair and Gingival Crevicular Fluid (GCF) of orthodontic patients and evaluated the corrosion of orthodontic bracket surfaces in 15 patients. The samples were taken before treatment (baseline), 4, 8, and 16 months later during treatment. After 16 months, compared with the baseline, average hair nickel level changed from 0.125 μg g^{-1} to 0.956 μg g^{-1}; average chromium level changed from 0.090 μg g^{-1} to 0.295 μg g^{-1} but no significant difference; average GCF nickel level changed from 3.335 μg g^{-1} to 10.410 μg g^{-1}; average chromium level changed from 1.859 μg g^{-1} to 9.818 μg g^{-1}. Both of these increases were significant. Surface examinations showed that the corrosion on brackets was seen in the fourth month and more severely visible after 6 and 16 months of uses. After 16 months of treatment, compared with the baseline, the hair nickel level was increased by 7.7 times, while for chromium was by 3.3 times. Gingival crevicular fluid nickel level was increased by 3.1 times and chromium level was by 5.3 times.

Wołowiec et al. [170] investigated the effect of dietary habits on the release of Cr and Ni ions from orthodontic appliances by hair mineral analysis. The research was carried out on hair sampled from 47 patients at the beginning and in the 4th, 8th, and 12th months of the treatment. The study showed that consumption of acidic dietary products may have the effect on increasing the release of Cr and Ni ions from orthodontic appliances. The release of Cr from orthodontic appliances in patients who consumed fruit, juice, coffee, yoghurt, and vinegar was higher. Mikulewicz et al. [80a] also confirmed that Coca Cola caused increased release of Ni ions from orthodontic appliances under *in vitro* conditions.

The concentration of trace metals in the biological human tissue samples of patients following *in vivo* ion release from different metallic orthodontic appliances is presented in Table 6.

5.3. Mercury dental amalgam

Mercury dental amalgam is a widely used restorative dental material that was introduced over 150 years ago; amalgam is a dental filing material contains approximately 50% mercury (Hg) by weight, as well as other metals, including silver (Ag), tin (Sn), copper (Cu), and zinc (Zn). Elemental mercury (Hg^0) has been used in clinical dentistry since 1830s when it began to be used in filings. Amalgam is a dental filling material contains approximately 50% mercury by weight, as well as other metals, including Ag, Sn, Cu and Zn.

It is well known that all dental materials release ions into the oral environment and have the potential to interact with the oral tissues and fluids. During the last 20 years, some remarkable changes in restorative dentistry have occurred. The employment of amalgam and different

kinds of alloys has dropped dramatically. The main reasons were connected with the aesthetic aspects and the controversy over amalgam employment and metal toxicity, but also because of environmental pollution from mercury waste. However, there is no convincing evidence pointed out to adverse health effects due to dental amalgam restorations, except in rare instances of an allergic reaction, and can be used as a preferred restorative material where aesthetic is not a concern [171-176].

At present, research is being undertaken to evaluate the potential correlation between Hg release from dental amalgams, and a number of chronic and degenerative human diseases [177]. In the past two decades, the safety of amalgam as restorative material in dentistry has been discussed controversially, and this has led to the result that several countries (Norway and Sweden) banned this type of dental filling material [178,179]. Germany and Canada advise against its use in pregnant women and children [180]. The American Dental Association (ADA) has opposed a complete ban on dental amalgams [181]. The World Dental Federation (FDI) is still in favor of the continued use of mercury dental amalgam [182]. The US Food and Drug Administration (FDA) have also considered the question of banning the use of Hg-containing dental materials; acknowledge that dental amalgam releases low levels of elemental mercury vapor [183]. The FDA says mercury from dental amalgam can bioaccumulate in bodily fluids, tissues, kidneys, and the brain, but then states that "*studies have not shown that increased mercury levels and bioaccumulation due to dental amalgam result in detectable damage to target organs*. On one side of the debate, the FDA and the ADA support amalgam as a safe and effective material for dental restorations [184,185], and amalgam continues to play a major role in dentistry today [186]. Results from the only two randomized, controlled, clinical trials on dental amalgam, known as the Children's Amalgam Trials, were first reported in 2006 [187,188]. Both studies found no difference in neurobehavioral outcomes between the amalgam group and the composite (non-amalgam) group; although in both trials the amalgam group showed a statistically significant increase in urinary mercury levels. These two studies, in addition to being widely cited in the literature, are cited by the FDA and the ADA as providing evidence for the safety of amalgam [189,190]. However, indirect evidence for a link between Hg exposures from dental amalgams has been reported [191,192].

The World Health Organization (WHO) deemed the first route of mercury exposure to humans is from dental amalgam. The WHO estimates that the typical absorbed dose of mercury from amalgams is 1 - 22 μg/day, with most people incurring doses of less than 5 μg/day [193]. Considerable variation exists, with an upper range of 100 μg/day associated with gum chewing. Exposure variables include the total amalgam surface area, the physical and chemical composition of the amalgam, the mechanical stresses of chewing and bruxism, the proximity to other metals, and the oral conditions of temperature, pH, and negative air pressure. The FDA assumes and exposure of 1 - 5 μg/day in its current amalgam rule [189].

In November 2013, the Environmental Protection Agency (EPA) released a study of the National Health and Nutrition Examination Survey (NHANES) dataset claiming that mercury concentrations were decreasing in the human population over time [194]. In fact, the opposite conclusion is supported by their data. The unreported rise in blood inorganic mercury levels (IHg) indicates a rise of chronic mercury exposure in the population over time. Blood inorganic mercury level is the best biomarker available in the NHANES dataset to estimate chronic mercury exposure. It is clear that a significant increase in blood IHg has occurred. In fact it had more than doubled. The EPA study on mercury concentrations in NHANES is available for public analyses [195,196]. However, a date certain to ban mercury dental amalgam's use globally has not yet been achieved.

From a risk assessment point of view, it is of interest to reveal the impact of extensive dental treatment, i.e., amalgam removal, on the Hg levels in biological fluids, and to relate this to the influence of the daily Hg uptake from amalgam fillings. It is known that amalgam fillings release mercury; therefore, its concentrations increase in blood, urine, saliva, and intra-oral air of subjects with amalgam fillings [197-208]. Mercury levels in blood and in mouth air before and after chewing were measured in 47 persons with and 14 persons without dental amalgam restorations. Differences in the mouth air mercury levels before and after chewing were statistically significant in the group with amalgams, but not in the group without amalgams. Blood mercury concentrations were positively correlated with the number and surface area of amalgam restorations and were significantly lower in the group without dental amalgams. [197]. Ganss et al. [198] determined the relationship between mercury content of resting and stimulated saliva, and blood and urine. Eighty subjects participated; 40 of them attributed their self-reported complaints to dental amalgam (patients), the others were matched with repsect to age, sex and amalgam restorations (controls). Serum, 24-h urine, resting and chewing stimulated saliva were analyzed for mercury. Median mercury levels in serum were 0.67 μg L^{-1} for patients and 0.60 μg L^{-1} for controls. In urine levels were found to be 0.77 μg L^{-1} and 0.94 μg L^{-1} creatinine, respectively. Resting saliva contained 2.97 μg L^{-1} in patients and 3.69 μg L^{-1} in controls. Chewing mobilized and additional amount of 16.78 μg L^{-1} in patients and 49.49 μg L^{-1} in controls. Only a weak correlation was found between mobilized mercury in saliva and serum. Saliva testing is not an appropriate measure for estimating the mercury burden derived from dental amalgam. Sandborgh-Englund et al. [199] have obtained data on changes in Hg levels in blood, plasma, and urine following removal of all amalgam fillings during one dental session in 12 healthy subjects. Frequent blood sampling and 24-h urine collections were performed up to 115 days after amalgam removal, and in 80 subjects additional samples of plasma and urine were collected up to three years after amalgam removal. A transient increase of Hg concentrations in blood and plasma was observed within 48 hours after amalgam removal. No increase in the urinary Hg excretion rate was apparent after amalgam removal. Sixty days after the amalgam removal, the Hg levels in blood, plasma, and urine had declined to 60% of the pre-removal levels. There was evidence that correlation between mercury levels in saliva, serum, and urine (i.e. the absorbed metal) is weak. Thus, saliva testing to estimate the mercury burden caused by amalgam restorations is also not appropriate. Berglund and Molin [200,201] performed a study to determine whether removal of all amalgam restorations might significantly affect mercury levels in plasma and urine and whether the use of rubber dams might reduce patient exposure to mercury during amalgam removal. The study showed that dental amalgam had a statistically significant implact on the mercury levels found in plasma and urine in the patients tasted, and that the use of a rubber dam during removal of all amalgam restorations significantly reduced the peak of mercury in plasma following removal. Although the composition of saliva does not permit a reliable estimation of body burden of mercury and it is, therefore, far from being of the same importance of blood and urine as a diagnostic tool. A highly subjective and intersubjective variability of results has been reported [202] and there is evidence that some elements such as Hg may also occur in saliva in a particulate form [203]. Monaci et al. [204] assessment of a possible role of human saliva in the diagnosis of some physiological and pathological changes in oral and body functions. Total concentrations of major cations (Ca, K, Mg, Na) and Hg in whole saliva from 33 healthy adults showed that concentrations of Hg were positively correlated to the number of amalgam fillings and increased at a rate of about 1.9 μg L^{-1} for each filling. No correlations were found between Hg concentrations and those of major elements. Data reported in this study, although preliminary, contribute to the assessment of levels of major cations and Hg in whole unstimulated human saliva. A recent report from Turkey showing *ex vivo* mercury release (increased) from dental amalgam fillings after high-powered MRI [205].

Dental amalgam restoration occupies a unique position in dentistry. One outdated member of the family of mercury containing filling materials is the copper amalgam. These amalgam alloys are broadly known as low-copper (5% or less copper) and high-copper alloys (13% to 30% copper). A study reported that through the increasing of their copper density, conventional dental amalgam alloys improved their microstructural and mechanical properties. Reports have also revealed the disappearance of the gamma-2 phase in copper content with more than 20 wt%. Both low and high copper amalgams undergo a transformation process for several years after placement, resulting in a substantial reduction in mercury content, but there exist no limit for maximum allowed emission of mercury from dental amalgams. These modern high copper amalgams are nowadays totally dominating the European, US and other markets, resulting in significant emissions of mercury, not considered when judging their suitability for dental restoration [206].

Bjørklund et al. [207] says "*Despite this, many environmental toxicology researchers still question whether the adverse human health effects of dental amalgams have fully been considered and appropriately addressed by dentists, dental laboratories and government.* Although, However, very recently, Tibau and D Grube [208] stated that "*The latest significant findings on human exposure to mercury dental amalgam using the "Gold Standard National Health and Nutrition Examination Survey (NHANES) database, may finally be the catalyst that will achieve the goal and "Make Mercury History" in the dental sector".*

6. Instrumental techniques and procedures for the measurement of trace metal ions release from metallic orthodontic appliances/metal implants

Chemical trace element analysis is a many-faceted and important problem. Trace and ultratrace elements may be both toxic and essential to life. Taking this account the monitoring of the release of metal ions from metallic orthodontic appliances is of interest.

The main analytical problem is determining these ultratrace metals in clinical samples (human and animal biological specimens) as they are present at extremely low (sub-μg/ng L^{-1}) concentrations in very complicated matrices: human saliva, biological fluids (blood, serum, urine) and tissues. The release of metal ions has been mainly measured using either electrothermal atomic absorption spectrometry (ET-AAS) also known as graphite furnace atomic absorption spectrometry (GF-AAS), inductively couple plasma optical emission spectrometry (ICP-OES), or inductively couple plasma mass spectrometry (ICP-MS). Although flame atomic absorption spectrometry (FAAS) can be, and sporadically is, successfully used for determination of trace metal ions in human saliva [73,84,87], dental implants [92,124,130,136], blood [152], urine [157] and hair [168,169], is not discussed in this paper, because of its relatively limited applicability and the fact that these metals are also easily measured with more robust techniques, such as ET/GF-AAS and ICP-MS.

Atomic absorption spectrometry (GF/ET-AAS) is still the dominant analytical technique used for ultratrace metal analysis in clinical laboratories. Although GF/ET-AAS allows direct sample analysis, most of the works reported in the literature perform specimen's analysis after the digestion of the sample. However, more and more clinical laboratories are transitioning away from graphite furnace AAS techniques toward those based on ICP-MS, which is a form of inorganic MS measuring metal ions rather than molecular ions.

ICP-MS is the method of choice for potentially toxic trace and ultratrace metal determination, in clinical specimens analysis, due to its extremely low limits of detection for most elements (<0.001-10 μg L^{-1}), which are about 1 - 3 orders of magnitude lower than GF/ET-AAS, very high multi-element coverage, outstanding accuracy, an extremely wide linear dynamic range of up to 12 orders of magnitude in the same run and also provides isotopic information, making high accuracy calibration *via* isotope dilution mass spectrometry available. Another advantage of using ICP-MS over ICP-OES is the sample size. 0.1-1 g of sample is needed for analysis with ICP-OES, whereas 0.01-0.1 of sample is adequate for analysis using ICP-MS. ICP-MS has become increasingly applied to clinical samples using a digestion procedure and validated methodology by ICP-MS [209,210]. However, ICP-MS still has some limitation, mainly polyatomic interferences, that can seriously affect its analytical performance. Thus, the main components (Ti, V, Cr, Co, Ni and Mo) of metallic alloys currently used in dental implants have been simultaneously determined in human biological fluids (whole blood and urine) of implanted people by high resolution ICP-MS (HR-ICP-MS) [211] and titanium levels the organs and blood of rats with a titanium implant by double-focusing ICP-MS (DF-ICP-MS) [212].

In general, two major analytical techniques are used, ET-AAS (GF-AAS) and ICP-MS.

Usually digestion procedures, standardization of the methodology, evaluation, analysis (determination of metal ions) are carried out in external, highly specialized laboratories, since expected concentrations of analytes are very low (sub-μg/ng L^{-1}) and for this reason required the use of instruments with very low detection limits (e.g. ICP-MS).

Once a method has been developed it must be validated, ensuring that it produces data in agreement with the true value of the metal in the sample. This is accomplished by calibrating with the method of standard additions, analyzing the given sample with two or more analytical techniques based on various physicochemical principles or by participating in an interlaboratory comparison. One of the best validation methods in the analysis of reference materials (CRM/SRM/RM) of the matrix type and the concentration level of the metals close to those in the tested clinical samples, in order to see whether or not the method is providing accurate results [26].

7. Conclusion

Experimental data show that the biocompability of orthodontic appliances depends on their composition and their corrosion behavior. This can lead to the release of different metallic ions, some of them with known toxic effects. Nickel was the most common ion investigated, followed by chromium in this review of trace metal release from orthodontic appliances during the past 20 years. The trends and needs in wearing orthodontic appliances should receive a different perspective with regards to metal leaching, which may give rise to adverse effects in long-term application. The *in vivo* study reflected the real oral environment for determining fixed orthodontic appliances. *In vivo* studies provide valuable information on the effects of orthodontic materials in real clinical exposure scenarios. Therefore, further clinical studies considering longer populations and longer treatment periods are necessary. Regarding to metal release, *in vivo* monitoring studies of metal ions (such as Ni and Cr), are needed to investigate cause-effect relationship. No longer is it sufficient to simply analyze for the total element amount, but we need additional information as to chemical form, oxidation state, organometallic nature, *etc*. Thus is a critical need to develop (ultra)trace element analysis methods that allow separation of the different elemental species prior to (ultra)trace element detection. In addition, we must analyze at environmental of biological concentration levels, often with subnanogram to picogram quantities. This challenging task requires state-of-the-art multi-method analytical approaches in which atomic spectrometry will play an important role and/or will describe the novel uses of traditionally atomic sources. The elemental distribution and their chemical speciation analyses of the biological and environmental systems will be stressed from the viewpoint of analytical atomic spectrometry and mass spectrometry. To achieve reliable and comparable results, *in vivo* tests should be carried out under strictly controlled conditions so the results would be reproducible and meaningful. There is a need to elaborate certain rules and standards for *in vivo* studies in this area of interest. However, the majority of studies covered 1-2 months long period and did not reflect long-term changes or the impact of the complete treatment, the duration of which is several years, on the whole organisms and the overall accumulation of metals from orthodontic appliances. In addition, the process would not provide a reliable result, when it comes to changes in the release of metal ions as patient usually attends review appointments every one to two months. In such situations, an *in vitro* study has the advantage with the capacity to frequently and quickly measure the release of metal ions in a matter of hours or days. *In vitro* studies are valuable because they are performed under controlled laboratory conditions. However, the obtained results (concentrations of metal ions) may not correspond to metal ions release in *in vivo* conditions. The disadvantage is the variety responses of animal and humans; hence, extrapolation may not deliver accurate findings. Nevertheless, animal studies allow for a controlled environment throughout the study period. Analyzing the results of both *in vitro* and *in vivo* tests, it seems that there is a necessity to elaborate standardized analytical procedures (immersion media-- the type and volume, incubation conditions, static/dynamic conditions, and the duration of the experiment), in particular, to elaborate certified reference materials with detailed methodology provided. This would make the results obtained by various analytical techniques comparable. Regarding the adverse human impact of mercury used in dental amalgam, "*both epidemiological research and scientific reviews about the potential human adverse consequences of Hg exposure from dental amalgam should take into account socioeconomic and environmental conditions. We hope such efforts will help to reduce and prevent toxic Hg effects in human communities*" [2007]. Finally, it has been observed that most of the experimental studies have been performed with traditional orthodontic appliances (i.e. arches or bandwires), but other appliances that are more invasive, but widely used and relatively new (i.e. mini-screws), novel procedures and novel adhesion materials are also worthy of investigation.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares no conflicts of interest associated with this manuscript.

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