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Biocompatibility of dental alloys used in dental fixed prosthodontics

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Abstract

Many different types of alloys are now available in the market to be used for dental restorations and fixed prostheses. The common criterion for all these fixed prosthodontic materials is the permanent existence of them in the oral cavity for prolonged time without the ability to be removed by the patient. Therefore, knowledge about the biocompatibility of dental alloys is of great importance. This article presents a literature review on the biocompatibility of dental alloys. A PubMed database search was conducted for studies pertaining to the biocompatibility of dental alloys. The search was limited to peer-reviewed articles published in English between 1985 and 2013. Available data revealed that substances are released from alloys into the surrounding tissues; mainly nickel, zinc, and copper. Some alloys such as nickel–chromium alloy have shown to be cytotoxic *in vitro*. Also, elements released from gold alloy showed *in vitro* cytotoxic effect. Therefore, clinicians should give up assuming that gold alloy is completely inert and biocompatible with oral tissues. The clinical relevance of these findings remains unclear. Further *in vitro* studies, as well as controlled clinical trials, are needed due to possible exceptions.

Keywords: Alloy; Biocompatibility; Toxicity; Microstructure; Biodegradation; Ion release

1. Introduction

Many different types of alloys are now available in the market to be used for fixed prosthodontics. In the developed countries like the United States, Europe and Japan, cast gold alloy and all-ceramic materials are the most widely used. In developing countries like the Middle East and South America, base metal alloys and prefabricated stainless-steel crowns are the most prevalent types. However, the common criterion for all these fixed prosthodontic materials is the permanent existence of them in the oral cavity for prolonged time without the ability to be removed by the patient. Therefore, knowledge about the elemental release from

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these materials into the oral cavity in regards to quantification is of great importance.

2. Dental alloy

An alloy is a metallic material formed by the combination of two or more metals or one or more metals with a nonmetal. In their molten state, metals dissolve to various degrees in one another, allowing them to form alloys in the solid state. Just as not all liquids are soluble in one another, not all metals are soluble in one another. This extent of solid solubility depends on the relative sizes of the individual atom species, the crystal structure formed by the pure metal components, and their reactivity [1]. Dental alloys, rather than pure metals, play a prominent role in the treatment of dental disease because pure metals do not have the appropriate physical properties to function in different types of restorations. Other materials may lack a combination of strength, modulus of elasticity, wear resistance and biologic compatibility that a material must have to survive long term in the mouth as fixed prosthesis [2].

3. Biocompatibility and cytotoxicity

The term biocompatibility refers to the ability of a material to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response in that specific situation, and optimizing the clinically relevant performance of that therapy [3]. Therefore, cytotoxicity is the main component of biocompatibility.

4. Why is it significant to study cytotoxicity of dental alloys?

The biocompatibility of dental alloys used in fixed prosthodontics is a critical issue because these materials are in intimate contact with oral tissues for long terms and can not be removed by the patient. Because of this nature of prosthodontic therapies, dentists therefore rely heavily on dental biomaterials. This reliance on materials makes biocompatibility issues especially relevant to prosthodontists and other restorative dentists.

One common misperception of fixed prosthodontic materials is that it may be inert in the oral environment. The placement of a material into the oral cavity creates active interfaces through which the body affects the material and the material affects the body. Regardless of the material placed, these interactions occur depending on the material, the host, and the forces and conditions placed on the material (its function) [2]. Thus, the inertness of fixed prosthodontic materials, such as dental alloys, is not possible and it is unlikely that alloys will be discovered releasing nothing into the body [4].

Also, it has to be stressed that biocompatibility of fixed prosthodontic materials is often overlooked because many practitioners assume that, if the material is on the market, its biocompatibility does not need to be questioned. As mentioned before, two systems are currently responsible for standards that can be used to document products quality: ANSI/ADA and ISO. They do not require specific biologic tests to approve the quality of a new dental material. Rather, they place the responsibility on the manufacturer to present evidence for a compelling case for approval. So, it is up to the manufacturer to defend the substantial equivalence argument [5,6]. The evidences used for approval of quality of a dental material consist of *in vitro* tests (cell-culture), *in vivo* tests (animal tests), and usage tests (clinical trials of the material). However, it is becoming increasingly impractical to test all new materials through all of these stages. The problems of time, expense, and ethics have limited the usefulness of this traditional biologic testing scheme [7]. Therefore, companies market materials with little clinical experience, and may rely heavily on *in vitro* and animal tests.

5. Biologically relevant properties of dental alloys

5.1. Alloy composition and microstructure

It is believed that biologic reactions in general are mainly based on the interaction of a substance eluted from a material with a biologically relevant molecule. Thus, the composition of dental alloy is of importance [8]. In dentistry, alloys usually contain at least 4 metals, and often 6 or more. Thus, dental alloys are complex metallurgically. More than 25 elements in the periodic table of elements can be used in dental alloys. The complexity and diversity of these alloys make understanding their biocompatibility difficult, because any element in an alloy may be released and any influence the body [9].

Dental alloys are commonly described by their composition. However, composition can be expressed in two ways; either as weight percentage (wt.%) of elements or percentage of the number of atoms of each

element in the alloy (atomic percentage = at %). Weight percentage is the most common way of describing an alloy's composition, and is used by alloy manufacturers and by standard organizations. However, biologic properties are best understood by knowing the atomic percentage composition. Atomic percentage better predicts the number of atoms available to be released and affect the body. The wt% and at% of an alloy may be substantially different from each another. For the nickel-based alloy, the atomic percentage of aluminum and beryllium are 2–5 times what would be expected, based on the weight percentages, because aluminum and beryllium are light elements relative to other alloy components [9].

Another way of describing an alloy is by its phase structure (microstructure) which includes the grain structure of the alloy. Phases are areas within an alloy that have the same composition and crystal structure. Single-phase alloys have, more or less, a similar composition throughout their structure. However, elements in multiple-phase alloys combine in such a way that some areas differ in composition from other areas. Thus, the alloy is not homogenous throughout its structure. Whether an alloy is single-phase or multiple-phase is dependent on the solubility of the alloy elements [9]. The phase structure of an alloy is critical to its corrosion properties and its biocompatibility. The interaction between the biologic environment and the phase structure is what determines which elements will be released, and therefore, how the body will respond to the alloy. In general, multiple-phase alloys (such as nickel–chromium alloy) are prone to higher corrosion rates than single-phase alloys because of galvanic effects between the microscopic areas of different compositions [1].

5.2. Biodegradation and corrosion

Biological systems may have harmful or destructive effects on dental materials, classified as biodegradation. In the oral environment, this includes not only the process of destruction and dissolution in saliva but also chemical/physical destruction, wear and erosion caused by food, chewing and bacterial activity [10]. Therefore, it is important to evaluate the material reactivity in the oral cavity, which is governed by thermo-dynamic principles and electro-chemical reaction kinetics. This means that when an alloy is placed in the oral cavity, the alloy-saliva system will be driven toward a state of thermo-dynamic equilibrium. At equilibrium, the alloy either will remain stable in its elemental form or oxidize into its ionic form (corrosion) [11].Thus, the initially uncharged elements inside the alloy lose electrons and become positively charged ions as they are released into solution.

Corrosion is a chemical property that has consequences on other alloy properties, such as esthetics, strength, and biocompatibility. From a biocompatibility standpoint, the corrosion of an alloy indicates that some of the elements are available to affect the tissues around it [9].

Corrosion is measured in a number of ways, such as electro-chemical tests that measure elemental release indirectly through the flow of the released electrons current, or by tests that measure the release of the elements directly by spectroscopic methods. Perhaps the most relevant measure of corrosion from the standpoint of biocompatibility is identifying and quantifying the elements that are released [1].

Corrosion of an alloy is of fundamental importance to its biocompatibility because the release of elements from the alloy is nearly always necessary for adverse biologic effects such as toxicity. The biologic response to released elements depends on which element is released, the quantity released, the duration of exposure to tissues, mechanical aspects of function, and the local and systemic host environment. Thus, corrosion is a necessary but not a sufficient condition for adverse biologic effects of dental alloys [8,9].

Regarding noble alloys, corrosion is variable; it depends on the microstructure and the presence of corrosion-prone micro-structural phases such as silver and copper [9]. Corrosion of noble alloys may be clinically visible if it is severe, but more often, the release of elements continues for months or years at low levels and is not visible to the eye [12]. Gold-based alloys are referred to as noble alloys, based upon their electrochemical properties. The corrosion resistance of the alloys is due to the high thermodynamic stability of the gold in the alloys [13]. In simulated body fluids and oral environments, gold alloy would not be prone to pitting or crevice corrosion [14]. Corrosion resistance of dental casting alloys with reduced noble metal content is generally inferior to that of alloys with noble metal content greater than 75 wt% [15,16]. Increased corrosion can occur because of lower noble metal content, formation of multiple phase microstructures, or segregations of elements such as silver and copper [17]. In addition, gold-based alloys were not significantly affected by low pH [19].

Nickel–chromium alloys are not as thermodynamically stable and a major aspect of their corrosion resistance is related to the formation of a thin,

protective oxide film (passive film) on the metal surface. If the oxide film is disrupted, then the metal or alloy must re-passivate in order for the material to be protected [13]. Nickel–chromium alloys showed unstable galvanic corrosion behavior [19]. They do corrode in physiological solutions, such as balanced salt, artificial saliva, human saliva, and artificial sweat solutions [20]. Specifically, some of the nickel-based alloys have been shown to be susceptible to pitting and/or crevice corrosion phenomena. There is characteristic hysterias behavior which indicates that once the oxide film on the alloy has been disrupted, the alloys are difficult to re-passivate [13]. In addition, incorporation of elements such as beryllium may reduce the corrosion resistance [21]. The corrosion behavior of beryllium-free (Be-free) nickel–chromium alloy and beryllium-containing (Be-containing) nickel–chromium alloy was investigated by Johansson et al. [15] who found that beryllium-containing nickel alloy was susceptible to localized corrosion and scanning electron microscope revealed an etched surface with corrosion of certain micro-structural features. No significant corrosion was predicted or observed for the non-beryllium nickel alloy.

Regarding Stainless-steel alloys, the coating which is formed by chromium oxides, is extremely thin and transparent. It can not be seen by the naked eye, but it provides protection from corrosion for the metal it covers. When scratched, the surface oxide usually can reform to protect the underlying metal. This protective layer does not form as easily in a solution containing chloride ions. Because saliva contains high levels of chloride ions as the result from the presence of sodium chloride, stainless-steel surfaces can be corroded in the mouth when they are scratched or nicked. Since repassivation does not readily occur, the corrosion can be accelerated in the area of the scratch, producing a small but deep pit. This process is called pitting corrosion, which may be sufficient to weaken the metal to the point of failure by fracture if this occurred in a thin section of the restoration [22].

5.3. Ion release

Corrosion is always accompanied by a release of elements and a flow of current. Release of metallic ions from the metallo-lattice of dental alloys into the oral cavity occurs, and thermo-stable substances such as chlorides, sulfides, and oxides, are formed during this process. The release of substances from dental materials is considered to be gradual and to occur in small amounts [10].

Several statements can be made about the release of elements from dental alloys based on measurements of elemental release from many different alloy compositions, although these generalizations are sometimes not accurate. First, multiple phases will often increase the elemental release from alloys [9]. Second, certain elements have an inherently higher tendency to be released from dental alloys, regardless of alloy composition. This tendency of an element to be released is sometimes referred to as its lability, such as nickel and beryllium which are labile elements. For nickel–chromium alloys, nickel ions were released at a slightly higher rate than bulk alloy compositions, while beryllium ions were released at four to six times that of bulk alloy compositions. Other alloying elements were released at levels similar to or lower than bulk levels [21]. Wataha et al. [23] tested different gold alloys for element release into cell-culture medium, and found that gold (Au) and palladium (Pd) ions generally did not dissolve into the medium, but that silver (Ag), copper (Cu) and zinc (Zn) ions frequently dissolved. Third, certain environmental conditions around the alloy will affect the release of elements. A reduction in pH will increase elemental release from dental alloys. This effect is especially pronounced for nickel-based alloys. Covington et al. [20] studied the release of nickel and beryllium from base-metal dental casting alloys in acidified saliva at several pH levels. They found that decreased pH increased the levels of nickel and beryllium released. However, Wataha et al. [18] found that exposure of high-noble and noble gold alloys to acidic medium did not alter elemental release from these alloys.

The most dependable method for measuring ion release is probably the atomic spectroscopic tests. Ion release from dental alloys has been evaluated mainly by *in vitro* studies, in which the alloy is subjected to different settings: galvanism [19], electrolyte bath [14], oral proteins [24], different pH levels [18], brushing with toothpaste [25], artificial oral environment capable of reproducing three-dimensional force-movement cycles of human mastication [26].

López-Alías et al. [27] quantified the metallic ions released by various dental alloys subjected to a continuous flow of saliva. They found that nickel-based alloys essentially released nickel and chromium, while the beryllium-containing alloy released beryllium and significantly more nickel. Noble and high-noble alloys were, in general, much more resistant to corrosion and released a very low amount of zinc. In addition, Tai et al. [26] found that nickel and beryllium were released *in vitro* both by dissolution and occlusal wear

from nickel–chromium alloy after a simulated one-year period of mastication.

Wataha et al. [12] found that the mass loss from nickel–chromium alloy after 10-month conditioning period in a biological medium containing serum proteins was about <10 ug/cm^2. Then, Wataha et al. [24] measured elemental release from different alloys with compositions ranging from 0 to 94 at% noble elements after exposure for 1 week to different biological media. More elemental release occurred into the saline-bovine serum albumin (BSA) solution compared to saline alone for all released elements (Ag, Cu, Pd, and Zn) except for nickel. Elemental release was less in the cell-culture medium than in the saline-BSA solution for most elements.

The kinetics of released elements from different precious and non-precious alloys in the polished and polished-cleaned conditions was evaluated so that the effects of cleaning could be determined. Cleaning did not change the pattern of release but did generally significantly increase the quantities of Au, Pd and Ni released, while decreasing the abundance of Ag and Cu [28].

In addition, the effect of tooth brushing on elemental release from different precious (gold alloys) and non-precious (nickel–chromium) alloys after tooth brushing was evaluated. For the major classes of dental alloys, brushing alone caused no significant elemental release during the brushing, and only minor increases after brushing. Brushing with toothpaste caused significant increases in elemental release for all elements of all alloys, but the largest increases were for the nickel-based alloys [25].

At another point of view, there are different studies of different durations for the determination of levels of elemental release. The release of elements from alloys may change significantly with time for some formulations over 80 h [28]. But, by 10 months in a cell culture medium, the release of elements from alloys is higher initially then becomes almost substantially lower than in the initial weeks with reaching a constant rate after less than 100 days of exposure to the medium [4]. According to a previous *in vitro* study done at weakly intervals through 4 weeks, it was found that initial release from single-phase alloys was often significantly higher in the first weeks than in subsequent weeks such as silver, nickel, and zinc, but not for all elements in an alloy (such as copper). Multiple-phase alloys showed steady or increasing release relative to the first week [29].

In spite of all the previous studies, it is difficult to predict the actual ion release of an alloy inside the patient mouth based on *in vitro* studies, since factors such as diet, changes in saliva quantity and quality, oral hygiene, tooth brushing, or the amount and distribution of occlusal forces can influence corrosion to varying degrees [27].

Mostly, *in vivo* ion release studies of dental alloys were done for stainless-steel alloys used for orthodontic appliances. Nickel and iron ions release in saliva from patients receiving treatment with fixed orthodontic appliances were evaluated at three weeks after placement using electro-thermal atomic absorption spectroscopy. No statistical significant difference either in concentrations or in absolute masses of nickel or iron in samples taken without appliances and in those obtained with appliances [30]. Also, no significant differences were found for chromium and nickel salivary concentrations between the appliance-free subjects and orthodontic patients after insertion of the appliances [31].

Nickel ions release in saliva from patients receiving treatment with fixed orthodontic appliances were investigated at 16 months after placement using electro-thermal atomic absorption spectroscopy. They found that nickel release occurred into the saliva in a situation that may reflect time dependence of its release [32]. Moreover, nickel ion release into cheek mucosa from patients with fixed orthodontic appliances was quantified using the coupled plasma mass spectrometry. Nickel concentrations were 3.4 times higher in patients than in the controls (without orthodontic appliances) [33].

6. Toxicity of dental alloys

6.1. Means of cytotoxicity testing

Current knowledge about biomaterials–tissue interactions has been gained through bioassays *in vitro* and *in vivo*. Taking into account biocompatibility tests available in the general field, cytotoxicity assays are of special concern. *In vitro* studies are mainly performed to evaluate the cytotoxicity. A vast number of different *in vitro* test methods exists which include both quantitative and qualitative methods of acute cytotoxic effect, i.e. cell damage or lysis caused by membrane leakage [34]. However, each test method basically consists of three components: (a) the biological system, (b) the cell/material contact, and (c) the biological endpoint and corresponding recording system.

The biological system used in *in vitro* cytotoxicity tests may be (a) organ cultures, (b) cells in culture or (c) cell organelles. The most widely-used biological

systems for *in vitro* toxicity testing of dental materials are cells in culture. Two types of cells are used; permanent cell lines derived from type-culture collections (L929 or 3T3 mouse fibroblasts) or primary cells derived from gingival or mucosal explants and established in each individual laboratory. Permanent cell lines are well defined and generally available [35].

The cell-material contact may be direct; the cells grow next to, or even on the test material. In *in vitro* tests, direct cell/material contact methods simulate the *in vivo* situation in certain instances [35]. In indirect contact, materials and cells are separated by a barrier [36,37]. Eluates derived from a dental material by storing it for a specific period of time in a liquid, such as the nutrient medium, may be used for toxicity testing instead of the material itself [35].

Besides the description of cell morphology, different biological endpoints can be used as indicators for cell damage: membrane effects, cell activity and proliferation rate. The cell reaction can be described morphologically as is done with the lysis index in the agar overlay test. However, this method is considered to be only qualitative, or at most, semi-quantitative in nature. Furthermore, some dental filling materials contain or produce considerable amounts of ingredients, which if applied to cells in culture; the morphology of the cells will appear to be normal, indicating no cell damage even though the cells are no longer vital [38]. The use of membrane effects, cell activity and proliferation rate have no such drawbacks. Membrane effects can be demonstrated by dye exclusion (trypan blue). The trypan blue exclusion assay can be used to indicate cytotoxicity, where the dead cells take up the blue stain of trypan blue, and the live cells have yellow nuclei [39]. Direct cell counting is easy to perform and can be combined with a vital stain in order to exclude dead cells [40].

6.2. Toxicity related to dental alloys used in fixed prosthodontics

Till now, some manufacturers produce nickel−chromium alloys with increased percentage of nickel in their casting alloys. The resultant compositions possess a number of improved clinical characteristics, but concern has existed regarding biological compatibility.

Stainless-steel alloys, as mentioned before, have an oxide film that serves as a passivating, or protective, coating. When scratched, the surface oxide usually can reform to protect the underlying metal. This protective layer does not form as easily in a solution containing chloride ions. Because saliva contains high levels of chloride ions as the result of the presence of sodium chloride, stainless-steel surfaces can be corroded in the mouth when they are scratched or nicked leading to the availability of some elements that can affect the tissues around alloy [22].

Regarding noble and high noble alloys, few studies showed a trend that copper and silver were the metal elements which induced cytotoxic effects of the respective alloys [41,42].

Generally, metals may be biologically active in one or all of three chemically distinct states: (1) the pure metal as an ingot or dust (many metals are vastly more reactive as dust [for example, nickel becomes flammable]), (2) organo-metallic and metallic salt compounds, and (3) alloys. However, not all chemical states of a certain metal appear to be equally hazardous. Non-precious metal use may conceivably expose dental personnel to a metal in all three of these chemical states. Pure metal vapor evolved during casting may undergo chemical reactions and/or condense as dust. Organo-metallic and metallic salts form during corrosion, both in the mouth and during casting. The alloyed metal may exist as both an ingot (in the mouth) and as dust (in the laboratory) [43].

The first step to approach dental alloy toxicity is to analyze the toxic potential of metal ions; e.g. in cell culture systems. Data from such experiments are dependent upon the cell culture conditions chosen; e.g. the cell line, cell culture medium, incubation time [44]. Fourteen metal ions which leach from dental casting alloys were screened for cytotoxicity by the use of four different cell lines (Balb/c3T3, L929, ROS17/2.8 and WI-38). Succinic dehydrogenase activity was used to monitor cytotoxic response. It was found that the cell lines responded differently to most of the studied metallic elements including nickel [45].

Hornez et al. [46] use both epithelial cells and fibroblasts to detect the *in vitro* cytotoxicity of different precious and semi-precious alloys using colony forming cell viability methods. *In vitro* cell viability tests showed that gold (Au), palladium (Pd), platinum (Pt) and indium (In) ions have no cytotoxic effect; Chromium (Cr), copper (Cu) and silver (Ag) ions were toxic; nickel (Ni), zinc (Zn) and cobalt (Co) ions were highly toxic. Also, Wataha et al. [42] studied the correlation between cytotoxicity and the elements released by noble gold alloys and found that Ag and Cu appeared to be the primary cause of cytotoxicity with these alloys.

Single-salt solutions were also investigated for the determination of cytotoxic alterations due to an

individual metal. Wataha et al. [23] evaluated *in vitro* the cytotoxicity of nine metal cations common in dental casting alloys using Balb/c3T3 fibroblasts. Concentrations causing 50% toxicity compared to controls (TC50's) and reversibility of these effects were determined. Their results revealed that all metal cations tested (Ag^{1+}, Au^{4+}, Cd^{2+}, Cu^{2+}, Ga^{3+}, Ni^{2+}, Pd^{2+}, and Zn^{2+}) exhibited toxicity, and the rank for potency of metal cation toxicity (most toxic to least toxic) was: Cd^{2+}, Ag^{1+}, Zn^{2+}, Cu^{2+}, Ga^{3+}, Au^{4+}, Ni^{2+}, Pd^{2+}. All toxic effects were reversible at concentrations near the TC50 concentrations.

In addition, Schmalz et al. [41] compared the cytotoxic potency of medium alloy extracts and identical salt solutions. They detected Ag, Cu, Ni, and Zn elements in extracts of dental alloys by inductively coupled plasma mass spectroscopy (ICP-MS). The amount of elements that caused 50% cell death was slightly lower in corresponding salt solutions prepared from chloride than in extracts. Therefore, cytotoxicity of medium extracts consistently proved to be slightly less than that of the corresponding salt solutions. On the contrary, Messer and Lucas [47] evaluated the response of cultured human gingival fibroblasts to both nickel–chromium alloy discs and salt solutions of ions (Be^{+2}, Cr^{+6}, Cr^{+3}, Ni^{+2}, Mo^{+6}) which were released from nickel–chromium alloy. Viability and alterations in metabolic activity were determined by staining the cells with trypan blue. They found that the combination of ions released from nickel–chromium alloy discs caused cellular alterations at concentrations significantly less (ppm range) when compared to the salt solutions of the individual ions (ppm range).

Al-Hiyasat et al. [48] investigated the cytotoxicity of four different nickel–chromium alloys discs by the di-Methyl Thiazol diphenyl-Tetrazolium (MTT) method. The results showed the difference in the cytotoxicity of alloys which was markedly affected by their composition. Nickel–chromium alloys containing high amounts of copper (12.3%) showed the highest cytotoxicity, while the others were within a much smaller range of toxicity but the higher concentration of chromium and molybdenum, the lower the cytotoxicity were present.

Different studies have shown that nickel–chromium alloys may elicit adverse tissue and cellular reactions. Elements released from these alloys may subsequently interfere with many biochemical and enzymatic cellular reactions, resulting in necrosis. They may also influence the synthesis of special cellular products which themselves are involved in a clinical reaction (inflammation), or which protect the cell from damage (detoxification) [8]. Nickel–chromium alloys and their corrosion products did not affect morphology or viabilities of the cultured cells, but did decrease cellular proliferation [21]. Messer and Lucas [49] evaluated the metabolic and morphological response (cell viability, DNA-/RNA-/protein synthesis, membrane integrity) of cultured human gingival fibroblasts to salt solutions of ions which may be released from nickel–chromium alloys. Chromium (Cr^{+6}) and beryllium (Be^{+2}) were the most toxic cations; nickel (Ni^{+2}) was moderately toxic, while chromium (Cr^{+3}) and molybdenum (Mo^{+6}) were the least toxic ions.

Furthermore, direct contact experiments with solid alloy specimens placed together with the cells in the culture wells at the same time have been performed. In these studies, effects due to released metal ions and to surface parameters were investigated as well. However, contradictory results were reported. Craig and Hanks [50] evaluated the cytotoxicity of a series of different dental alloys including gold alloys and nickel–chromium alloys using succinic dehydrogenase histo-chemical reaction in fibroblast cell culture. They found that gold alloys and nickel–chromium alloys were biocompatible. Craig and Hanks [51] also investigated the cytotoxicity of a series of 29 experimental alloys and six pure metals succinic dehydrogenase histo-chemistry in fibroblasts. Of the pure metals, Au and Pd were the least cytotoxic, followed by Ag, then Ni, and finally Cu. Single-phase alloys with moderately high Cu and without high Pd and Au concentrations had high cytotoxicity, as did multiphase alloys, even when they were high in Au and Ag.

When, Wataha et al. [23] evaluated nickel-containing alloy cytotoxicity using Balb/c3T3 fibroblasts and MTT-formazan production tests, they found that nickel was only moderately toxic (small, but statistically significant). Later, Bumgardner and Lucas [21] found that nickel was highly cytotoxic in primary human gingival fibroblasts as evidenced by a prominent decrease in cellular proliferation.

Craig and Hanks [51] evaluated the cytotoxicity of a stainless-steel alloy (69% Fe, 18.5% Cr, 11% Ni) with a cell culture technique and succinic dehydrogenase histo-chemistry. The width of any ring of inhibition and optical density of histo-chemically stained cells were determined. They found that stainless-steel alloy was highly biocompatible with a 0.0–0.1 mm width ring of inhibition of cells around the alloy.

In addition to affecting adjacent oral soft tissues, ions released from metallic restoration may also have an adverse effect on nearby alveolar bone. Osteoblastic differentiation of human bone marrow cells was

influenced, *in vitro*, by Fe^{3+}, Cr^{3+}, and Ni^{2+}, depending upon the state of differentiation of the cultured cells due to the effect of corrosion products of stainless-steel alloy [52].

Anyway, one important condition that limits the relevance of all these previous *in vitro* tests is the duration of the exposure of the material to the cell cultures. Most "direct contact" *in vitro* tests, which place the material directly adjacent to cells, are less than 168 h long because of the various limitations involved in culturing cells for longer periods of time. These limitations include microbial contamination, loss of potency or nutrition of the medium, or cell overgrowth. The relatively short contact times are not relevant to materials such as dental alloys, which are present in the mouth for years [12,54]. One alternative to direct contact testing is indirect contact testing. In this strategy, the tested material is cultured with cell culture medium (but no cells) for a specific length of time, and then the medium is transferred to the cells for toxicity testing in a second step. Using the indirect strategy, it is possible to "age" the material in a biological medium and change the extracted medium several times, testing its toxicity on cell cultures periodically. This strategy has been used to test the cytotoxicity of a variety of dental alloys. Nelson et al. [7] evaluated the cytotoxicity of different Au–Ag–Cu–Pd alloys with Balb/c fibroblasts after short-term conditioning in either saline, cell-culture medium, or a saline/bovine serum albumin solution for 72 h. Cell viability was assessed by succinic dehydrogenase activity, and compared to unconditioned alloys. Their results revealed that the alloys were cytotoxic initially and then became less cytotoxic after preconditioning. In another study, Nelson et al. [5] evaluated the cytotoxicity of nickel-chromium alloys after more prolonged conditioning (168 h). They found that alloy toxicity varied with the conditioning solution. The saline/BSA conditioning solution reduced the cytotoxicity of the alloys compared with unconditioned alloy cytotoxicity. The other conditioning solutions were not as uniform in their acceleration effect: some were increasing toxicity (saline Ag–Pd), others decreasing it (saline Au–Ag–Cu).

However, the indirect contact strategy had some disadvantages. The medium must be changed on the cells. This procedure can itself kill a percentage of the cells. Furthermore, the indirect system was not a dynamic system that allowed the material and cells to interact over time [54]. A second alternative to traditional direct contact testing is to first condition the material in a biological medium, then use a direct contact test to evaluate the cytotoxicity of the material, not the conditioning medium. This modified direct test allows the material and cells to dynamically interact, limits the practical problems of the indirect tests, and allows the material to be aged to give more relevant information [12]. Therefore, Wataha et al. [12] tested *in vitro* the cytotoxicity of different dental casting alloys, including gold alloys and nickel–chromium alloys, indirectly in cell culture of mouse fibroblasts after polishing (initial) or after 10 months of conditioning in a biological medium containing serum proteins. Their results showed that alloys with little initial cytotoxicity showed no 10-month cytotoxicity and alloys with significant initial toxicity showed significantly less toxicity at 10 months, which indicated that short-term cytotoxicity tests may not accurately measure the long-term cytotoxicity.

Whatever is the material used for fixed prosthodontic appliance, it is nevertheless difficult to predict the clinical behavior of an alloy from *in vitro* studies, since factors such as changes in the quantity and quality of saliva, diet, oral hygiene, polishing of the alloy, amount and distribution of occlusal forces, or brushing with toothpaste, can all influence corrosion to varying degrees [27]. From a biocompatibility standpoint, the corrosion of an alloy indicates that some of the elements are available to affect the tissues around it [9].

Therefore, a study was performed to quantitatively assess the element release from gold alloy crowns into saliva of fixed prosthodontic patients. A significant increased release of zinc and copper ions from gold crowns into saliva was evident after three months of clinical service. However, these amounts of released elements were not sufficient to cause visible signs of gingival inflammation in patients [54].

Finally, although the release of elements from dental fixed prosthodontic materials is well established *in vitro* and *in vivo*, the local biologic effects of these released elements are still a topic of intense debate. The central question in this debate is whether the levels of elements that are released are sufficient to affect or alter the viability of the tissues around the fixed prosthodontic materials. Unfortunately, insufficient evidence exists to definitively answer this question.

7. Conclusions

Substances are released from dental alloys into the surrounding tissues; mainly nickel, zinc, and copper. Some alloys such as nickel–chromium alloy have shown to be cytotoxic *in vitro*. Also, elements released

from gold alloy showed *in vitro* cytotoxic effect. Therefore, clinicians should give up assuming that gold alloy is completely inert and biocompatible with oral tissues. The clinical relevance of these findings remains unclear. Further *in vitro* studies, as well as controlled clinical trials, are needed due to possible exceptions.

References

[1] Wataha JC. Alloys for prosthodontic restorations. J Prosthet Dent 2002;87:351−63.

[2] Wataha JC, Messer RL. Casting alloys. Dent Clin North Am 2004;48:499−512.

[3] Williams DF. On the mechanisms of biocompatibility. Biomaterials 2008;29:2941−53.

[4] Wataha JC, Lockwood PE. Release of elements from dental casting alloys into cell-culture medium over 10 months. Dent Mater 1998;14:158−63.

[5] Nelson SK, Wataha JC, Lockwood PE. Accelerated toxicity testing of casting alloys and reduction of intraoral release of elements. J Prosthet Dent 1999 (a);81:715−20.

[6] Wataha JC. Principles of biocompatibility for dental practitioners. J Prosthet Dent 2001;86:203−9.

[7] Nelson SK, Wataha JC, Neme AL, Cibirka RM, Lockwood PE. Cytotoxicity of dental casting alloys pretreated with biologic solutions. J Prosthet Dent 1999 (b);81:591−6.

[8] Schmalz G, Garhammer P. Biological interactions of dental cast alloys with oral tissues. Dent Mater 2002;18:396−406.

[9] Wataha JC. Biocompatibility of dental casting alloys: a review. J Prosthet Dent 2000;83:223−34.

[10] Lygre H. Prosthodontic biomaterials and adverse reactions: a critical review of the clinical and research literature. Acta Odontol Scand 2002;60:1−9.

[11] Council ADA. Biocompatibility of dental alloys. J Am Dent Assoc 2002;133:758−9.

[12] Wataha JC, Lockwood PE, Nelson SK, Bouillaguet S. Long-term cytotoxicity of dental casting alloys. Int J Prosthodont 1999 (c);12:242−8.

[13] Lucas LC, Lemons JE. Biodegradation of restorative metallic systems. Adv Dent Res 1992;6:32−7.

[14] Sun D, Monaghan P, Brantley WA, Johnston WM. Potentiodynamic polarization study of the *in vitro* corrosion behavior of 3 high-palladium alloys and a gold-palladium alloy in 5 media. J Prosthet Dent 2002;87:86−93.

[15] Johansson BI, Lemons JE, Hao SQ. Corrosion of dental copper, nickel, and gold alloys in artificial saliva and saline solutions. Dent Mater 1989;5:324−8.

[16] Mezger PR, Stols ALH, Vrijhoef MMA, Greener EH. Metallurgical aspects and corrosion behavior of yellow low-gold alloys. Dent Mater 1989;5:350−4.

[17] Corso PP, German RM, Simmons HD. Corrosion evaluation of gold-based dental alloys. J Dent Res 1985;64(5):854−9.

[18] Wataha JC, Lockwood PE, Khajotia SS, Turner R. Effect of pH on elemental release from dental casting alloys. J Prosthet Dent 1998;80:691−8.

[19] Taher NM, Al Jabab AS. Galvanic corrosion behavior of implant suprastructure dental alloys. Dent Mater 2003;19:54−9.

[20] Covington JC, McBride MA, Slagle WF, Disney AL. Quantization of nickel and beryllium leakage from base metal casting alloys. J Prosthet Dent 1985;54:127−36.

[21] Bumgardner JD, Lucas LC. Cellular response to metallic ions released from nickel-chromium dental alloys. J Dent Res 1995;74:1521−7.

[22] Ferrance JL. Metal alloys for orthodontic, prosthodontics and pediatric dentistry. In: Materials in dentistry: principles and applications. Lippincott Company; 1995. p. 281−2.

[23] Wataha JC, Hanks CT, Craig RG. The *in vitro* effects of metal cations on eukaryotic cell metabolism. J Biomed Mater Res 1991 (a);25:1133−49.

[24] Wataha JC, Nelson SK, Lockwood PE. Elemental release from dental casting alloys into biological media with and without protein. Dent Mater 2001;17:409−14.

[25] Wataha JC, Lockwood PE, Mettenburg D, Bouillaguet S. Tooth brushing causes elemental release from dental casting alloys over extended intervals. J Biomed Mater Res Part B: Appl Biomater 2003;65:180−5.

[26] Tai Y, Long RD, Goodkind RJ, Gouglas WHD. Leaching of nickel, chromium and beryllium ions from base metal alloy in an artificial oral environment. J Prosthet Dent 1992;68:692−7.

[27] López-Alías JF, Martinez-Gomis J, Anglada JM, Peraire M. Ion release from dental casting alloys as assessed by a continuous flow system: nutritional and toxicological implications. Dent Mater 2006;6:836−41.

[28] Wataha JC, Craig RG, Hanks CT. The effects of cleaning on the kinetics of *in vitro* metal release from dental casting alloys. J Dent Res 1992;71:1417−22.

[29] Wataha JC, Lockwood PE, Nelson SK. Initial versus subsequent release of elements from dental casting alloys. J Oral Rehab 1999 (a);26:798−803.

[30] Gjerdet NR, Erichsen ES, Remlo HF, Evjen G. Nickel and iron in saliva with patients with fixed orthodontic appliances. Acta Odontol Scand 1991;49:73−8.

[31] Kocadereli I, Ataç A, Kale S, Özer D. Salivary nickel and chromium in patients with fixed orthodontic appliances. Angle Orthod 2000;70(6):431−4.

[32] Fors R, Persson M. Nickel in dental plaque and saliva in patients with and without orthodontic appliances. Eur J Orthod 2006;28:292−7.

[33] Faccioni F, Franceschetti P, Cerpelloni M, Fracasso ME. *In vitro* study on metal release from fixed orthodontic appliances and DNA damage in oral mucosa cells. Am J Orthod Dentofacial Orthop 2003;124:687−94.

[34] Tomakidi P, Koke U, Kern R, Erdinger L, Krüger H, Kohl A, et al. Assessment of acute cyto- and genotoxicity of corrosion eluates obtained from orthodontic materials using monolayer cultures of immortalized human gingival keratinocytes. J Orofacial Orthopedics 2000;61:2−19.

[35] Schmalz G. Use of cell cultures for toxicity testing of dental materials-advantages and limitations. J Dent 1994;2:6−11.

[36] Sutow EJ, Foong WC, Zakariasen KL, Hall GC, Jones DW. Corrosion and cytotoxicity evaluation of thermafil endodontic obturator carriers. J Endo 1999;25(8):562−6.

[37] Donohue VE, McDonald F, Evans R. *In vitro* cytotoxicity testing of neodymium-iron-boron magnets. J Appl Biomater 1995;6:69−74.

[38] Schmalz G, Netuschil L. A modification of the cell culture agar diffusion test using fluoresceindiacetate staining. J Biomed Mater Res 1985;19:653−61.

[39] Ribeiro DA, Marques MEA, Salvadori DMF. Biocompatibility of glass-ionomer cements using mouse lymphoma cells *in vitro*. J Oral Rehab 2006;33:912−7.

[40] Key JE, Rabemtulla FG, Eleazer PD. Cytotoxicity of a new root canal filling material on human gingival fibroblasts. J Endo 2006;32:756−8.

[41] Schmalz G, Langer H, Schweikl H. Cytotoxicity of dental alloy extracts and corresponding metal salt solutions. J Dent Res 1998;77:1772−8.
[42] Wataha JC, Malcolm CT, Hanks CT. Correlation between cytotoxicity and the elements released by dental casting alloys. Int J Prosthodont 1995;8:9−14.
[43] Kelly JR, Rose TC. Nonprecious alloys for use in fixed prosthodontics: a literature review. J Prosthet Dent 1983;49(3):363−70.
[44] Schmalz G. Concepts in biocompatibility testing of dental restorative materials. Clin Oral Invest 1997;1:154−62.
[45] Wataha JC, Hanks CT, Sun Z. Effect of cell line on *in vitro* metal ion cytotoxicity. Dent Mater 1994;10(3):156−61.
[46] Hornez JC, Lefèvre A, Joly D, Hildebrand HF. Multiple parameter cytotoxicity index on dental alloys and pure metals. Biomol Eng 2002;19:103−17.
[47] Messer RLW, Lucas LC. Cytotoxic evaluation of ions released from nickel-chromium dental alloys. J Dent Res 1996;75:255 (Abst).
[48] Al-Hiyasat AS, Darmani H, Bashabsheh OM. Cytotoxicity of dental casting alloys after conditioning in distilled water. Int J Prosthodont 2003;16:597−601.
[49] Messer RLW, Lucas LC. Evaluation of metabolic activities as biocompatibility tools: a study of individual ions' effects on fibroblasts. Dent Mater 1999;15:1−6.
[50] Craig RG, Hanks CT. Reaction of fibroblasts to various dental casting alloys. J Oral Pathol 1988;17:341−7.
[51] Craig RG, Hanks CT. Cytotoxicity of experimental casting alloys evaluated by cell culture tests. J Dent Res 1990;69(8):1539−42.
[52] Morais S, Sousa P, Fernandes MH, Carvalho GS, de Bruijin JD, van Blitterswijk CA. Decreased consumption of Ca and P during *in vitro* biomineralization and biologically induced deposition of Ni and Cr in presence of stainless steel corrosion products. J Biomed Mater Res 1998;42(2):199−212.
[54] Elshahawy W, Ajlouni R, James W, Abdellatif H, Watanabe I. Elemental ion release from fixed restorative materials into patient saliva. J Oral Rehab 2013;40(5):381−8.