METAL IONS LEACHABLES AND MICROSTRUCTURE OF FAKE ORTHODONTIC BRACES: AN *IN VITRO* STUDY

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by

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LIST OF SYMBOLS

B	Boron
Ba	Barium
Ca	Calcium
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cu	Copper
Fe	Iron
K	Potassium
Li	Lithium
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
Na	Sodium
Ni	Nickel
P	Phosphorus
Pb	Lead
Sb	Antimony
Si	Silicon
Ti	Titanium
V	Vanadium
Zn	Zinic

LIST OF ABBREVIATIONS

AMDI	Advanced Medical and Dental Institute
ARC	Animal Research Complex
AS	Artificial Saliva
AW	Achwire
AW1	Fake Archwire Type 1
AW2	Fake Archwire Type 2
AW3	Fake Archwire Type 3
AW4	Fake Archwire Type 4
AW5	Fake Archwire Type 5
B	Bracket
B 1	Fake Bracket Type 1
B 2	Fake Bracket Type 2
B 3	Fake Bracket Type 3
B 4	Fake Bracket Type 4
B 5	Fake Bracket Type 5
CBCT	Cone-Beam Computed Tomography
CoCr	Cobalt Chromium
CTC	Clinical Trial Complex
CuNiTi	Copper Nickel Titanium
IARC	International Agency for Research on Cancer
ICP-MS	Inductively Couple Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
MOH	Ministry of Health
NiCo	Nickel-Cobalt
NiTi	Nickel-Titanium

OPG	Orthopantomogram
RDI	Recommended Dietary Intake
SBF	Simulated Body Fluid
SEM-EDX	Scanning Electron Microscope Energy Dispersive X-ray Spectroscopy
SPSS	Statistical Package of Social Science
SS	Stainless Steel
TiMb	Titanium Molybdenum
USM	Universiti Sains Malaysia
WHO	World Health Organisation

ION LOGAM TERLARUT- RESAP DAN STRUKTUR MIKRO PENDAKAP ORTODONTIK PALSU: KAJIAN IN VITRO

ABSTRAK

Pendakap palsu merupakan alatan ortodontik buatan yang kini mendapat perhatian serta semakin popular dalam kalangan remaja dan belia di Malaysia. Ia melibatkan pengamal haram yang kualitinya diragui dan standard alat dan bahan yang tidak disterilkan. Oleh itu, kajian ini bertujuan untuk menilai perubahan elemen dan struktur mikro pendakap palsu yang direndam dalam cecair badan simulasi (SBF) dan air liur buatan (AS). Lima pendakap palsu berbeza direndam dalam SBF dan AS pada suhu 37 ˚C, dan satu alat pendakap ortodontik tetap digunakan sebagai kawalan. Perubahan elemen pada pendakap palsu dianalisis menggunakan ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry), dan SEM-EDX (Scanning Electron Microscope Energy Dispersive X-ray Spectroscopy) digunakan untuk menganalisis perubahan morfologi pendakap, yang dinilai pada hari 0, 1, 4, 7, 14, dan 28. Data telah dianalisis menggunakan ANOVA sehala bersama Bonferroni post hoc dengan $P<0.05$ dianggap signifikan secara statistik. Setelah rendaman dalam SBF, ICP-OES menunjukkan ion Na, K, Mg, Ca, Mo, B, Pb, Al, dan P yang terlarut-resap daripada sampel pendakap palsu mempunyai ion yang signifikan berbanding sampel kawalan. Apabila direndam dalam AS, Na, K, Mg, Ca, Cu, Pb, dan Al mempunyai ion terlarut-resap yang signifikan dari sampel pendakap palsu. SEM menunjukkan perubahan morfologi telah diperhatikan pada permukaan pendakap palsu ini berbanding dengan sampel kawalan. Analisis EDX terhadap AW1 dan AW2 menunjukkan elemen seperti Fe, Cr, C, dan Ni, sementara AW4 terdiri daripada elemen seperti Ni, Ti, O, dan Al. B1, B2, B4 mengandungi Fe, Cr, Cu, dan C. Kajian

ini menunjukkan perbezaan ion yang terlarut-resap dari wayar arkus dan braket palsu berbanding dengan kawalan AW dan B. Kesimpulannya, terdapat perubahan pH yang ketara antara wayar arkus dan braket palsu; dan kawalan AW dan B. Ion Na, K, Mg, Ca, Mo, B, Pb, Al, dan P yang terlarut resap ke dalam SBF dari sampel pendakap palsu adalah signifikan berbanding dengan sampel kawalan. Namun, untuk sampel yang direndam dalam AS, kajian ini hanya mengesan sepuluh ion yang terlarut resap dari sampel palsu, di mana ion Na, K, Mg, Ca, Cu, Pb, dan Al dari sampel palsu mempunyai ion yang signifikan berbanding dengan sampel kawalan. Selain itu, dapat disarankan bahawa pendakap palsu dibuat terutamanya dari dua jenis aloi: keluli tahan karat (SS) dan nikel-titanium (NiTi).

METAL IONS LEACHABLES AND MICROSTRUCTURE OF FAKE ORTHODONTIC BRACES: AN *IN VITRO* STUDY

ABSTRACT

Fake braces are an artificial orthodontic device that are now gaining attention and becoming increasingly popular among teenagers and youths in Malaysia. It involves illegal practitioners with doubtful quality and standard of tools and materials which have not been sterilised. Therefore, this study aimed to assess the elemental changes and the microstructure of fake braces immersed in simulated body fluid (SBF) and artificial saliva (AS). Five different fake braces were immersed in SBF and AS at 37 ˚C, and one fixed orthodontic appliance was used as control. The elementary changes of fake braces were analysed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), and Scanning Electron Microscope Energy Dispersive X-ray Spectroscopy (SEM-EDX) was used to analyse the morphological changes of the braces, assessed at days 0, 1, 4, 7, 14, and 28. The data have been analysed by using One-way ANOVA with post-hoc Bonferroni with $P<0.05$ considered as statistically significant. After immersion in SBF, ICP-OES revealed Na, K, Mg, Ca, Mo, B, Pb, Al, and P ions released from fake braces samples had significant ions release compared to control samples. When immersed in AS, Na, K, Mg, Ca, Cu, Pb, and Al ions, have significant ions releasing fake samples. SEM revealed morphological changes were observed on the surface of these fake braces compared to control samples. EDX analysis of fake AW1 and AW2 revealed elements such as Fe, Cr, C, and Ni, while AW4 was composed of elements, such as Ni, Ti, O, and Al. B1, B2, B4 were composed of Fe, Cr, Cu, and C. This study showed differences in ions released from fake archwires and brackets compared to the control AW and B. In

conclusion, there were significant pH changes between fake archwires and brackets; and control AW and B. The Na, K, Mg, Ca, Mo, B, Pb, Al, and P ions released into SBF from fake braces samples had significant ions release compared to control samples. However, for samples immersed in the AS, this study detected only ten ions released from fake samples, in which Na, K, Mg, Ca, Cu, Pb, and Al ions from fake samples had significant ions release compared to control samples. In addition, it can be suggested that fake braces were mainly constructed from two types of alloys: stainless steel (SS) and nickel-titanium (NiTi).

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Orthodontic history, beginning with Kingsley, indicates that the use of metal alloys is an essential part of orthodontic therapy (Heravi *et al.*, 2013). Orthodontic care provides malocclusion correction and inharmonious jaw location with the advantage of improved functioning and teeth protection. In Malaysia around 65% of patients seek orthodontic care to have a better dental appearance. While the urge to have a better straight teeth, around 48% Malaysian orthodontic patients to receive orthodontic treatment (Samsonyanová & Broukal, 2014; Abdullah *et al.*, 2001).

While receiving the orthodontic treatment the patients are exposed to a noticeable amount of metal alloys. There is release of metallic ions from orthodontic appliance into oral fluids of patients undergoing fixed orthodontic treatment (Heravi *et al.*, 2013). Thermal, microbiological and aqueous properties of the oral environment are responsible for the metallic ions release from orthodontic appliance. Beside these pH fluctuations in oral cavity due to intake of food, various drinks and mouthwash also facilitate the corrosion of orthodontic appliance and lead to release of metallic ions (Heravi *et al.*, 2013). Many oral diseases, dentition appearance, malocclusion, and the treatment for these disorders on function and aesthetic bases received a broad focus from researchers.

Once the patient is referred to an orthodontist, in order to assess the prognosis and location of teeth orthodontist takes radiographs (OPG) and sometimes lateral cephalograms are adjunct with cone-beam computed tomography (CBCT). Beside these, the orthodontist must obtain the patient's teeth impression for construction of study model. According to recent published report, in Malaysia there is a long waiting list of patients in all government dental clinics (Loke, 2006; Mohd *et al.*, 2020), moreover children under 18 years old and patients in need of multidisciplinary specialist care are accepted in these governmental institutes (P. Then, 2015). Owing to overburden in government dental clinic some patients have to seek treatment in private clinics. Since orthodontic treatment is costly, especially when patients seek treatment in private clinics, the duration of wearing an orthodontic appliance is designated for at least 2-3 years. The high cost and prolonged duration of treatment tend a few individuals to prefer the cheapest and easiest ways to wear braces, such as fake braces.

The usage of fake braces (an artificial orthodontic appliance) among teenagers and young adults is alarming (Haleem *et al.*, 2020). These days tooth surfaces, decorated by various design and colourful orthodontic rubber bands, are considered an accessory, just like earrings or necklaces. Furthermore, fake braces are mostly advertised online, and it can be self-fixed, or an illegal practitioner can do the affixation at the beauty salons, hotels, and even at home. They can be purchased at a much cheaper cost, and there is no follow-up review to monitor the teeth movement and oral hygiene. There are no proper dental examinations or further investigations like radiographs or study models before the affixation of fake braces. This may lead to various complications such as gum bleeding and erosion on teeth surfaces because of the staining of glue. Their teeth may be well aligned as the wearing of fake braces is purely for fashion trends. Alarmingly, some fake braces can affect the teeth arrangements. However, for those cases which involve severe crowding and skeletal pattern discrepancy, wearing fake braces may cause worsening of the facial profile. The content of the material used in these artificial orthodontics fake braces is unknown, and some claimed it might contain lead and cause mouth ulcers intraorally, blood poisoning, and nerve damage (Behrents, 2014).

1.2 Problem statement

Presently the use of fake braces is increasing in Malaysia and e-commerce platforms have been the primary source of these artificial orthodontic appliance. So, the Ministry of Health Malaysia (MOH) has given broad attention to the danger of using fake braces and ways to prevent patients from using them (Rani *et al.*, 2020). The MOH has taken several steps to target the use of these fake braces among the general population. Recently, the assessment of pH changed, ions released, and composition of fake braces has become a matter of great interest among researchers. The efforts around the world are in full swing to study the consequences of the fake braces' usage. Thus, the current study is designed to determine the composition of fake braces and assess the elements present in them. The composition and safety of materials of fixed appliances used in orthodontic clinic were approved by the Medical Device Authority, a statutory body under MOH; while the composition of materials used in fake braces are unknown and its safety is a concern.

1.3 Objectives of the study

1.3.1 General objective

The general objective of the study is to determine the level of leachables metallic ions and evaluate the microstructure of fake orthodontic braces.

1.3.2 Specific objectives

1. To determine the level of metallic ions, present in fake braces apparatus in simulated body fluid (SBF) and artificial saliva (AS) by using Inductively coupled plasma optical emission spectrometry (ICP-OES).
2. To determine the microstructural changes of fake braces apparatus in SBF and AS using by field emission scanning electron microscopy with energy dispersive X-ray analysis (FESEM-EDX)

1.4 Research questions

The questions of this study have been determined as follows:

1. What are the ions released from fake braces into SBF and AS?
2. What is the composition of fake braces?

1.5 Research hypothesis

H_o: There are no significant differences in ions released and metallic composition for fake braces when compared to the fixed orthodontic appliance by using ICP-OES and FESEM-EDX.

H_a: There are significant differences in ions released and metallic composition for fake braces when compared to the fixed orthodontic appliance by using ICP-OES and FESEM-EDX.

1.6 Significant of research

This research will significantly contribute to the literature about the hazardous effects linked with the use of artificial orthodontic appliance. It has been reported that there is high atomic percentage for Nickle (Ni) element in fake braces which may lead to Ni allergy causing gingival inflammation (Syed *et al.*, 2015). This study will provide the significant information about the level of metallic ions that may be released from fake braces apparatus. Moreover, this research is expected to provide the data about the morphological changes of fake braces.

1.7 Thesis outline

This thesis is written in six chapters. Chapter one provides the background of a conventional orthodontic appliance and artificial fixed orthodontic appliance (fake braces), the research problem, the study's objective, the research hypothesis, and the significance of the study.

Chapter two presents the necessary knowledge and a review of the concerned literature regarding conventional orthodontic components, fake braces and its complication, toxicity of orthodontic appliances, and elemental changes of microstructural changes of orthodontic appliances, and change in pH with orthodontic appliances.

Chapter three clarifies the details of samples of fake braces and control, preparation of SBF and AS, and selected equipment used to achieve this study's objectives. The methodology of the research is described in detail.

Chapter four presents the morphological and elemental analysis results in SBF and AS by using ICP-OES and FESEM-EDX. The elemental composition of fake braces and control are presented as weight percentage and atomic percentage.

Chapter five discusses the discussion on findings from the results of ICP-OES and FESEM-EDX.

Chapter six concluded the study along with the provision of final results of ICP-OES and FESEM-EDX and the recommendations for future endeavours.

CHAPTER 2

LITERATURE REVIEW

2.1 Orthodontic treatment modalities

Orthodontics is the branch of dentistry discipline dealing with the growth of the face, development of occlusion, and the prevention and correction of occlusal anomalies (Phulari, 2011). Individuals with malocclusion are treated by specialists (orthodontists) which involve obtaining the patient's background, thorough medical history, and dental examination. The patient who seeks orthodontic treatment usually has a concern regarding their teeth, teeth alignment and occlusal problems.

When orthodontist sees patient, the treatment will consist of a study model, radiographs (2-dimensional or 3-dimensional), and photos of intra and extra oral features. These investigations will be used to understand the severity and an aetiology of the malocclusion, hence planning for optimum treatment options. Patients will be prescribed orthodontic treatment modalities with appliances which are divided into removable and fixed appliances.

2.1.1 Removable appliances

The removable appliance is an orthodontic device that can be removed for cleaning by the patients; designed to apply forces to the teeth by means of springs and screw as an active component and other mechanical components as anchorage and retention (Figure 2.1). Removable orthodontic appliances are easier to design and adjust, cheaper, and less chair time (Al-Moghrabi *et al.*, 2017). A wide range of valuable roles of the removable appliances such as reducing overbite and overjet, as a retainer after fixed appliance (Luther & Nelson-Moon, 2012), treatment of anterior or posterior crossbites (Popat *et al.*, 2010), and as space maintainers in the mixed dentition (Khanna *et al.*, 2015). However, removable orthodontic appliances have been commonly used in orthodontics, either to correct malocclusion problems or for retain treatment results (Tsomos *et al.*, 2014). There are several problems related to the removable appliances; for example, patients may misplace the appliance, the appliance became loose, broken components, and unable to wear. Consequently, patients are required to return to the clinic on a scheduled visit to replace the appliance, tighten the components, remove the broken part or address the trauma. The management should be done as soon as possible to avoid relapse (Popat *et al.*, 2010).

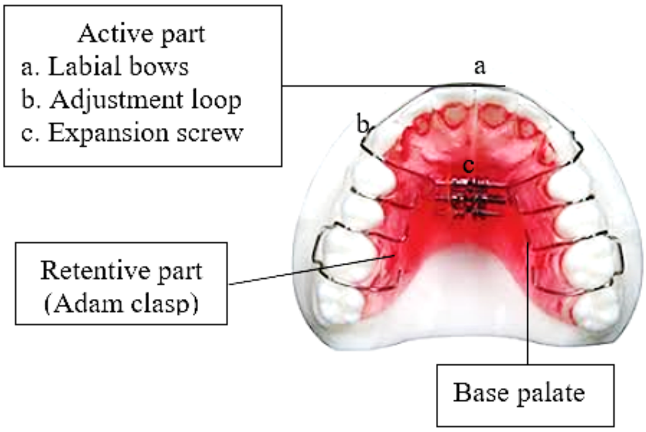

Figure 2.1 Components of removable orthodontic appliance (Retrieved from http://pfddental.com/removable-orthodontic-appliance.html/ Data accessed May.2020)

2.1.2 Fixed appliances

Fixed orthodontic appliance is a device or equipment attached to the teeth that cannot be removed by patients and can causing all types of tooth movement; because of this most patients and orthodontists are frequently preferred fixed orthodontic appliance. However, breakages of the fixed appliances are much more severe than removable appliances (Al-Moghrabi *et al.*, 2017; Tomblyn *et al.*, 2016). Many problems related to a fixed appliance such as debonded bracket, lost or losing band, archwire slipped or fractured, and trauma. Current guidelines recommended that no more than 5% of patient visits under successful orthodontic care will be scheduled. This constitutes one additional visit per typical course of orthodontic treatment that requires twenty visits to complete (Popat *et al.*, 2010). The clinic applied several steps to the affixing appliances process. Firstly, the brackets are attached to each tooth in proportion to the teeth axis. The archwire will then be placed onto the brackets according to the tooth movement requirement and secured by positioning elastomeric modules around the brackets. Each visit replaces the elastomeric rings while archwires are changed in consonance with the orthodontic treatment stages.

2.2 Fixed appliances apparatus

Fixed appliance apparatus consists of passive components such as brackets, bands, molar tube, elastic modules, ligature wire and active components such as archwire and springs (Figure 2.2). The fixed appliances can be fabricated from different alloys such as stainless steel, cobalt chromium, nickel titanium, beta titanium and composite plastics (Mikulewicz *et al.*, 2017; Arango *et al.*, 2013).

Figure 2.2 Components of fixed orthodontic appliance (Retrieved from http://www.encikshino.com/masalah-serta-rawatan-maloklusi-dan-gigi-berlapis/fixed-appliances-braces-atau-aplians-tetap/ data access May 2020)

2.2.1 Conventional orthodontic bracket

The force required for orthodontic tooth movement is transmitted from the active components to teeth through the bracket. There were many types of brackets in the market nowadays, such as metallic, ceramic and plastic brackets. Conventional orthodontic bracket (Figure2.3a) is made of metallic and it can be further divided based on its composition. Commonly, metallic brackets are made from stainless steel, non-nickel, or low-nickel stainless steel brackets and titanium brackets. The nickel element of conventional stainless steel has genotoxic effects and may induce certain allergic reactions to patients. Hence, non-nickel or low nickel stainless steels are alternatives to conventional stainless steel (Narayan, 2018). Ceramic brackets (Figure 2.3b) have greater strength, greater deformation resistance, improved colour stability and are usually favoured for aesthetic purpose. Due to their improved cosmetic quality, ceramic brackets are more flexible and have higher bonding forced than metal and plastic brackets, which may cause bracket breakage and harm to enamel during debonding (Sarp & Gülsoy, 2011).

Several properties are related to ceramic brackets such as, increasing visibility and recently the ceramic brackets have shown a higher improved mechanical characteristic, much more aesthetics and more compliant with the intraoral environment. On other hand, the plastic brackets (Figure 2.3c) have significant deformation and discolouration, with a low toughness in their usages in orthodontic areas. Alternatively, ceramic brackets demonstrate greater toughness and rigidity, but have demonstrated a higher rate of fracturing attributable to fragility and ageing in the oral system.

a b c

Figure 2.3 Conventional orthodontic bracket (Retrieved from https://baistra.en.made-in-china.com/product/bymnOXvKLPcL/China-Top-Supplier-Modern-Mini-Roth-018-Metal-Orthodontic-Bracket-Braces.html , https://usortho.com/shop/product/promise-x-ploy-sapphire-plus-clear-bracket-system/ , https://www.dentalcompare.com/Orthodontic-Supplies/4731-Plastic-Brackets/ data access May 2020)

2.2.2 Conventional orthodontic archwire

In orthodontic clinics, common archwires used are nickel titanium, stainless steel, cobalt chrome and beta titanium (Pakshir *et al.*, 2013; Kuhta *et al.*, 2009). The composition of these archwires is known in which each component is specifically incorporated for its purpose. For instance, nickel titanium (NiTi) archwire is characterised by high degree of flexibility and low force delivery over a long ranges that can be aligned during the first stage of treatment (Bellini *et al.*, 2016). Stainless steel (SS) alloy consists of iron (Fe), chromium (Cr) and nickel (Ni), that have a wide elasticity modulus and which are normally and resistant to the deformation (Mantripragada *et al.*, 2013; Castro *et al.*, 2015). This characteristic of SS archwire making it ideal as working archwires (Castro *et al.*, 2015). Gil *et al.*, (2012) described a significant reduction in Ni ion release from heated NiTi archwires in artificial saliva compared to original archwires. They found a reduction in Ni content in the matrix of these archwires after thermal treatment because of Ni4Ti3 precipitation of the surface material. The concentration of ions released in these two types of archwires in the artificial saliva increased very fast initially, but later the concentration became saturated. Copper (Cu) has been added to NiTi archwire for the potential clinical advantage of lower stress hysteresis. Furlan *et al*., (2018) used 10 CuNiTi archwires from every commercial brand. The main finding was the immersion of the NiTi Memory Wire and the Flexy CuNiTi archwires in both neutral and acid media solutions presented significantly higher mean Ni concentration values than the other archwires. Immersion of Tanzo CuNiTi and Flexy CuNiTi archwires in neutral solution released a higher amount of Cu compared to other groups. Only Damon Optimal-Force CuNiTi presented no experimental segment with detectable Cu concentration when placed in an acidic solution. However, Cr release could not be quantified.

Furthermore, different analytical methods were adopted for assessing metal ion leaching, which resulted in different sensitivity and detection limits making similar results comparison difficult (Mikulewicz & Chojnacka, 2011). Milheiro *et al.*, (2012) used five other multi stranded wires from different alloys where four of them were SS and one Ni-free by inductively coupled plasma mass spectrometry (ICP-MS) and all wires released considerable amounts of Ni ions. Spalj *et al.*, (2012) reported that SS archwire had the most biocompatibility while NiTi had the lowest when using six types of orthodontic archwires (SS, NiTi, CuNiTi, rhodium coated NiTi, CoCr blue elgiloy and Timb).

2.2.3 Activation modules

Activation modules are used with a fixed orthodontic appliance, including springers coil, expansion screw and separators. In addition to brackets and wires, these components consist of auxiliary parts that provide the required forces to align the teeth and jaws. Different types of springs are used for both inside one arch and between the upper and lower arches. Some components such as expansion screw and Herbst appliance are welded and soldered to bands that have been cemented on the teeth; other components are connected to the existing appliance and held by frictional forces, whereby the patient can remove some portions of the appliance, for example, lip bumper for brushing or eating. Quadhelix and auxiliary extension known as the jockey arch are common devices used for adult maxillary growth. The jockey arch appliance is simple and inexpensive to create, and it can be built into a fixed appliance. This is constructed from 0.040- to 0.050-inch SS wire and placed in the headgear tubes of the first maxillary molar bands (de Araújo Gurgel *et al.*, 2017). The interproximal separation between molars and premolars is necessary for operation with a fixed orthodontic appliance to provide adequate space for the bands that support the appliance (Sandhu *et al.*, 2013). The elastomeric power chains (EPC) were popular in the last few years due to their elastic properties, ease of operation, lack of patient cooperation, low expenses, fairly hygienic and irritation free design due to their smooth coating. EPCs have mostly been used in comparison to other orthodontic retraction materials (Kanuru *et al.*, 2014).

2.3 Metal based apparatus in fixed appliances

Traditionally, the fixed appliance includes bands, brackets and archwire manufactured from SS, NiCo and NiTi alloy, containing various percentages of Ni, Cr and Fe. Fixed orthodontic appliances from similar kinds of alloys (SS and NiTi alloys) produced by different companies have different alloy compositions due to various production technologies and electrolytic coatings (Espinar *et al.*, 2011; Faccioni *et al.*, 2003). Most metals and alloys are well known to be corrosion prone. Corrosion is defined as electrochemical reactions on the metallic surface leading to reduce material properties (Popoola *et al.*, 2014). Orthodontic appliances corrosion in the oral environment focus on two principal interests; internal corrosive factors assessed by the metal composition and structure, and external factors that rely on biological surroundings (e.g., pH value, media composition, strain) (Kuhta *et al.*, 2009). The corrosion produces, if created, may be absorbed into the human body and lead to systemic or localised effects. The corrosion occurs from loss of metal ions into solution or progressive dissolution of a surface layer, usually a sulphide or an oxide (Chaturvedi & Upadhayay, 2010).

2.4 Polymer based apparatus in fixed appliances

The increased aesthetic demands of orthodontic patients has led to produce various aesthetic materials, such as brackets. The ceramic and plastic brackets are the two principal types of aesthetic brackets (Walton *et al.*, 2010). A patent for a new plastic bracket of polyoxymethylene was issued in 1997 (Foerster, 1997). Plastic brackets water sorption may have a plasticising effect, this may reduce the polymer structure's properties in a wet environment. During clinical usage, some limitations of plastic brackets has been found due to their decreased hardness and discolouration, disputing their effectiveness. It can be limited in colour changes (Faltermeier *et al.*, 2007), morphological changes and structural or hardness variations (Gkantidis *et al.*, 2012). Plastic brackets are mainly made of unfilled polycarbonate and were used in orthodontic therapy in the early 70s and aesthetics had significantly improved (Ali *et al.*, 2012).

2.5 Ceramic based apparatus in fixed appliances

Ceramic brackets have proved to be an economically viable alternative in the field of aesthetic orthodontics. In addition, ceramic brackets show satisfactory bonding strength with enamel and in some cases higher than that provided by metal brackets (Guarita *et al.*, 2015). There were two types of ceramic brackets on the market; polycrystalline and monocrystalline alumina (single crystalline) (Reddy *et al.*, 2013). Ceramic brackets are stronger, more resistant to deformation, better colour stability and are often favored for cosmetic reasons (Sarp & Gülsoy, 2011). During the treatment with ceramic brackets, excessive wear of enamel surfaces on opposing teeth was observed (Krauss *et al.*, 2010).

2.6 Fake braces

2.6.1 Definition of fake braces

In south east Asia, fake orthodontic braces are currently considered a fashion icon. Fake as a general term is defined as not true, not real or not genuine or artificial (Hakami *et al.*, 2020). In terms of dentistry, fake braces are those braces that are often sold online or available at a much cheaper rate from uncertified sellers. They are often self-fixed or installed by an unqualified person. There is no proper dental examination or further investigation such as radiographs or study model taken before fake braces were affixed (Sorooshian & Kamarozaman, 2018). Without careful dental professional supervision, this action may lead to caries and bleeding gum (Behrents, 2014), worsening the facial profile. Severe effects of fake braces have been reported where two teenagers living in Thailand lost their lives from infection after wearing these braces (Wahab *et al.*, 2019).

2.6.2 Type of apparatus in fake braces

The archwires may range from plain ligature wire to twisted multi ligature wire, figure eight, NiTi wires supported by ring positioning or elastomeric chain (E chain) of different colours and patterns. Bracket selection may vary from metal to ceramic, with or without hook, big or small and so on. The patients may also show a picture from the internet or a picture of fake braces in a friend's mouth and demand that their brackets, attachments or plastic chain be placed in a similar pattern (Rai, 2015). Most suppliers typically hold a wide variety of modules and elastic chains to satisfy the clients' pattern and colour demand. They are mostly every possible colour and theme from flowers, power O, Mickey mouse to Hello Kitty (Rai, 2015). Such braces are typically applied primarily but not completely to the maxillary arch. Two types of fake braces are commonly available; active and passive. These fake braces are mounted in the mouth using etching and bonding techniques by unprofessional individuals where active fake braces were bonded to the teeth. Although the passive type of fake braces is not attached to the teeth, fake braces will easily wear independently. This means that it just fits for a limited period (Nasir *et al.*, 2018).

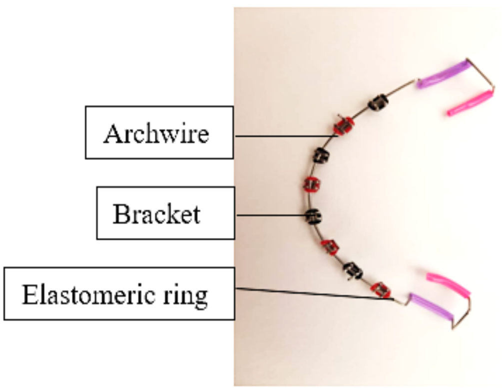

Figure 2.4 Fake braces apparatus

2.6.3 Issues related to fake braces

In the first report on fake braces in 2006, Greg Kyriakakis published an article regarding teens in Thailand who wear a fake braces, the braces price and the way to sell them in markets (Kyriakakis, 2005). Almost ten years later in 2015 Rai, (2015) introduced the term fake braces, analysed the components and risks brought by the fake braces. Nasir *et al.*, (2017) investigated the surface texture of nine as received brackets (passive fake braces and active fake braces) by using high resolution scanning electron microscopy (SEM), and the researchers found the surface texture and morphology of passive fake braces and active fake braces were different when compared to standard brackets. Subsequently in 2018, the author investigated metal composition, bracket design and surface texture of nine brackets consisting of passive fake braces and active fake braces by using high resolution SEM. They found that all fake braces did not contain any harmful elements. In another study, the author used three fake braces and three standard appliances to investigate the release of ions when the samples were immersed in artificial saliva at pH 4.9 and pH 7.8 by using ICP-MS to analyse the amount of metallic ions released on days 1, 14 and 28. Their results showed that fake braces released a higher concentration of metal ions as compared to standard braces (Nasir, 2019). The release of metal ions from orthodontic brackets is influenced by time and pH (Macedo de Menezes & Cardoso Abdo Quintão, 2010). Kamaruzaman *et al.*, (2019) explored the awareness, causes and use of illegal orthodontic services among adolescents in Manjung district, Perak using self-reported questionnaires and found that despite the adequate understanding of orthodontic services, there is still a low level of risk perception from fake braces. Wahab *et al.*, (2019) investigated the level of fake braces awareness among 170 students between the ages of 19 to 25 years old from a university using self-reported questionnaires.

Their results showed that many of the respondents reported that fake braces were cheaper and were stylishly advanced and only 35% of respondents were aware of fake braces dangerous side effects. The study concluded that these students were aware of oral hygiene and oral therapies but only a small number were conscious regarding the side effects of wearing fake braces.

2.7 *In vitro* cytotoxicity studies related to fixed braces appliances

There have been several articles concerning *in vitro* studies. Such experiences were carried out to determine concentrations of metallic ions released from orthodontic appliances. Brackets are fixed to the teeth during orthodontic treatment and will last until it is time to debond (removing the bracket from the teeth), while archwires are changed (monthly or yearly) according to the demands of tooth movement. The subsection below describes the types of ions leached, the concentration of ions releases from these appliances, and the methodology used to determine these ions.

2.7.1 Types of materials used in fixed braces appliances

Many dental materials for orthodontics clinical used are considered as medical devices in the biomedical field and depend on the biocompatibility of these materials. Thus, the cytotoxicity assessments of the materials using a wide collection of tests from different models are of great interest (Velasco-Ortega *et al.*, 2010). Fixed orthodontic appliance are mainly constructed from two types of alloys: SS which release Cu, Cr, Fe, Ni ions and NiTi alloys (usually wires) which release Ni and Ti.

2.7.2 Type and duration of incubation medium

Artificial saliva (AS) is commonly used in *in vitro* studies to simulate oral conditions. Modification of Meyer's solution in distilled water with a pH adjusted and controlled with NaOH solution is also used to monitor corrosion activity and contain chloride concentration similar to natural saliva (Alves *et al.*, 2016). Mikulewicz, Wołowiec, Janeczek, *et al.*, (2014a) set up a specially designed thermostatic glass reactor, where the artificial saliva was flowing at the rate of 0.5 mL/min, reflecting the flow of saliva in humans. Several studies were measuring the release of metallic ions in artificial saliva with different pH to simulate changing conditions in the oral cavity. Average pH in the oral cavity usually ranges from pH 6.75 to 7.4 but more acidic condition (pH 3.5 to 4.2) mimics the presence of dental plaque on the teeth. Saline solution (Hank's Balanced Salt Solution) and neutral or acid solutions were also a medium of choice to simulate oral condition.

Two studies use different mouthwashes containing fluoride and bleaching agent as media composition to compare metal ions release from orthodontic devices (Danaei *et al.*, 2011; Mirhashemi *et al.*, 2018). Mirhashemi *et al.*, (2018) found a high release of Ni and Cr ions in all mouthwashes at each time point from all types of archwires. The highest release of Ni ions was found in Listerine followed by Listerine Advance White, Oral B 3D White Luxe and the least in Oral B. Additionally, the release of Cr from SS wires was similar to Ni. However, Cr release did not follow a similar trend observed in NiTi wires. Danaei *et al.*, (2011) reported the release of Cr, Cu, Fe, Mn and Ni from SS orthodontic brackets after treatment with three different types of mouthwashes (Oral B, Chlorhexidine, *Persica spp.*). They exhibited a reduction in the risk of white-spot lesions around the brackets. The highest amount of ion released occurred in the presence of chlorhexidine mouthwash after 45 days of immersion and suggested to prevent chlorhexidine mouthwash in patients who have sensitivities.

Kuhta *et al.*, (2009) investigated the release of Cu, Ni, Fe, Ti, Cr, and Zn ions released from three types of orthodontic archwires (SS, NiTi and thermo NiTi) after 1, 7, 14, and 28 days of immersion in artificial saliva with different pH 6.75±0.15 and 3.5±0.15. The main findings showed that the appliance released measurable amounts of the ions and the change in pH had a very strong effect on the ions released where heavy ions release was observed after a week of immersion. In another study, the release of Ni ions was investigated on three types of orthodontic brackets (new conventional SS, recycled SS, and Ni free brackets). Sfondrini *et al.*, (2010) immersed brackets in artificial saliva with different pH of 4.2, 6.5 and 7.6 and at a different duration of 15 minutes, 1 hour, 24, 48 and 120 hours. Their results showed that recycled SS brackets released the most Ni ions and the highest ions release was observed in an acidic conditions of pH 4.2 and the least at pH 6.5 and 7.6. Suárez *et al.*, (2010) investigated six different types of lingual orthodontic archwires from the same manufacturer which were constructed from SS, NiTi and copper nickel–titanium (CuNiTi) after 7, 14 and 30 days of immersion in saline solution (Hank's Balanced Salt Solution). Interestingly, the study showed that SS archwires released the highest amount of Ni, followed by NiTi and CuNiTi archwires, despite SS has only 8% of Ni on its composition. In comparison, both NiTi and CuNiTi have 50% Ni content.

Nevertheless, the Ni released from all archwires is below the levels known to cause cell damage. Bhaskar & Reddy, (2010) studied on comparison of SS archwire, space maintainers and orthodontic bands. The report showed the highest quantities of Cr and Ni ions released from orthodontic bands at the end of the seventh day. However, there were no significant differences in ion released when different orthodontic band materials were used.

The advantage of *in vitro* study is that the measurement of metal ion release can be assessed as frequently and early as possible; by minutes and hourly, the changes can be plotted accurately. The longest duration of immersion was noted at 45 days (Danaei *et al.*, 2011). Orthodontic patients usually have review appointments every 4-6 weeks; hence it is quite challenging to attain results regarding changes in metal ion release if this were done *in vivo*.

The control and test samples, media composition, methods of assessment for measuring metal ions, duration of data collection and main results were included and summarised in Table 2.1.

Table 2.1 *In vitro* studies on the release of metals from orthodontic appliances

Study (year)	Experimental samples	Control	Media composition	Assessment methods	Days of data collection	Main results
Kuhta *et al.*, (2009)	Three types of archwires: stainless steel (SS), nickel titanium (NiTi), thermo NiTi.		Artificial saliva of different pH values (6.75±0.15 and 3.5±0.15) in polyethylene bottles at 37˚C	High-resolution mass spectrophotometer (HR-ICP/MS)	1, 7, 14 and 28 days	Appliances released measurable quantities of Cu, Ni, Fe, Ti , Cr, Zn. pH changes affect ion release. Ion release depend on wire composition but was not proportional to metal content in wire
Bhaskar & Reddy (2010)	Stainless steel wire, Bands and space maintainers		Artificial saliva at 37 ˚C	Atomic absorption spectrometry	1,7,14,21, and 28 days	The highest of Ni and Cr released was on 7th day, and later the rate of release diminished
Sfondrini *et al.*, (2010)	1080brackets, divided into 3 groups: -360new conventional		Artificial saliva at pH 4.2, 6.5, 7.6	- Flame atomic absorption spectrophotometer	15 min, one hour, one, two,	-Release of Ni was higher for recycled brackets, whereas

Study (year)	Experimental samples	Control	Media composition	Assessment methods	Days of data collection	Main results
	Victory stainless steel brackets - 360 Victory stainless steel brackets - 360 Sprint nickel-free brackets			- Inductively coupled plasma atomic emission spectrometer.	and five days	the lowest release of Ni was from Ni-free brackets - This result could be described by the high temperatures that use to heat steel during recycling - A chromium carbide precipitate is formed under high temperatures, and that precipitate is susceptible to intergranular corrosion,
Suárez *et al*., (2010)	6 different types of lingual orthodontic archwires from the same manufacturer -Stainless-steel (SS) -nickel titanium (NiTi) - copper nickel–titanium (CuNiTi)	Empty vial	Saline solution (Hank's Balanced Salt Solution)	Atomic absorption spectrometry	7, 14 and 30 days	SS archwires (8% Ni) released the highest amount of Ni compared to NiTi and NiTiCu archwires (which both have 50% Ni content).
Danaei *et al*., (2011)	160 stainless steel brackets - divided randomly into 4 equal groups	Distilled deionized water	-Oral B - Chlorhexidine - Persica mouth wash	Inductively coupled plasma- optical emission spectroscopy	45 days	Chlorhexidine has highest level of metals release compared with the

Study (year)	Experimental samples	Control	Media composition	Assessment methods	Days of data collection	Main results
						other 2 mouthwashes
Liu *et al.*, (2011)	2 types of commercial NiTi archwires and half of the NiTi wires were exposed to continuous bending stress	Unstressed wires	Artificial saliva at pH 2 and 5.3	Inductively coupled plasma- mass spectrometry	1,3,7, and 14 days	Ni ion release increased by bending stress compared with unstressed specimens in artificial saliva
Espinar *et al.*, (2011)	NiTi archwires treated by oxidation to get Ni-free surfaces	Untreated archwires	Artificial saliva at pH 7.4, 37ºC, for 30 days	Graphite furnace atomic absorption spectrometry	1, 5 h, and at 1,2,5, and 15 days	Initially, the concentration of ion Ni increased very sharply and later reached a saturation level. The film of Titanium oxide reduced Ni release
Gil *et al.*, (2012)	NiTi archwires heated at 500 and 600 ˚C	Untreated archwires	Artificial saliva at pH 7.4 and 37ºC	Graphite furnace atomic absorption spectrometry	1, 5 h, and at 1, 2, 5, and 15 days	Initially, the Ni ion releases increased very fast and after reached a saturation level, The release of Ni was higher in