COMPARISON OF CELL VIABILITY, CYTOMORPHOMETRIC AND PERIODONTAL INDEX OF HUMAN ORAL MUCOSAL CELL EXPOSED TO TWO CONVENTIONAL FIXED ORTHODONTIC APPLIANCES

RAMI K. M. ALMASHHARAWI

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by

RAMI K. M. ALMASHHARAWI

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TABLE OF CONTENTS

LIST OF APPENDICES

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

α	Alpha
β	Beta
%	Percentage
µl	Microliter
$µm^2$	Square micrometre
ABO	American Board of Orthodontics
Au	Gold
BOP	Bleeding on probing
CA	Cytoplasmic Area
CAL	Clinical attachment loss
CEJ	Cemento enamel junction
Co	Cobalt
Cr	Chromium
Cu	Copper
DNA	Deoxyribonucleic acid
DSA	Dental surgery assistant
e.g.	Example
et al.	and others
Fe	Iron
GM	Gingival margin
GR	Gingival recession

HUSM	Hospital Universiti Sains Malaysia
MIM	Metal injection moulding
min	Minute
ml	Millilitre
mm	Millimetre
Ni	Nickel
NA	Nuclear Area
Ni-Ti	Nickel-titanium
N/C	Nucleus-Cytoplasmic ratio
PAP	Papanicolaou
PBS	Phosphate buffer saline
Pd	Palladium
pH	Potential hydrogen
PI	Plaque index
PPD	Periodontal pocket depth
Pt	Platinum
rpm	Round per minute
SPSS	Statistical Package for the Social Sciences
T	Metallic group
TC	Ceramic group
Ti	Titanium
USA	United States of America
USM	Universiti Sains Malaysia

PERBANDINGAN KEBOLEHIDUPAN SEL, SITOMORFOMETRIK DAN INDEKS PERIODONTAL SEL MUKOSA MULUT MANUSIA APABILA TERDEDAH KEPADA DUA APLIANS TETAP ORTODONTIK KONVENSIONAL

ABSTRAK

Keadaan persekitaran rongga mulut yang mengkakis merupakan faktor utama yang menjadi kebimbangan semasa penggunaan aplians ortodontik. Hal yang demikian kerana aplians tetap ortodontik diperbuat daripada bahan aloi yang berbeza. Pendedahan tisu lembut pada bahan-bahan ini semasa menggunakan aplians berkenaan boleh menyebabkan beberapa tindak balas kimia akibat daripada degradasi bahan yang berkemungkinan membebaskan beberapa jenis ion tertentu. Tujuan kajian ini adalah untuk menganalisis perubahan kebolehidupan sel dan pengubahan sitomorfometrik pada kawasan nukleus, kawasan sitoplasma, dan nisbah nukleus-sitoplasma pada mukosa bukal pesakit yang masing-masing dirawat menggunakan aplians ortodontik yang diperbuat dari logam atau seramik. Kajian ini juga menilai kesihatan periodontal pesakit semasa menjalani rawatan ortodontik. Dalam kajian ini, seramai 26 pesakit yang merupakan pesakit ortodontik yang mendapatkan rawatan di Klinik Pergigian Hospital Universiti Sains Malaysia telah dipilih. Subjek-subjek tersebut dibahagikan kepada dua kumpulan; satu kumpulan (n=13) menggunakan aplians logam manakala satu kumpulan lagi (n=13) menggunakan aplians seramik. Swab bukal diambil daripada setiap pesakit sebanyak 3 kali iaitu sebelum rawatan dijalankan, 3 bulan selepas dan seterusnya 6 bulan selepas rawatan. Untuk menganalisi tahap kesihatan periodontal pesakit, 4 parameter periodontal

dinilai pada masa yang sama; indeks plak (PI), pendarahan semasa pemproban (BOP), kedalaman poket periodontal (PPD), dan kehilangan atakmen klinikal (CAL). Kebolehidupan sel mukosa bukal mulut dinilai dengan penanda *Trypan* biru, diikuti dengan analisis mikroskop cahaya. Untuk sitomorfometri, sel tersebut diwarna menggunakan stain *Papanicolaou*, dan seterusnya dinilai menggunakan perisian ImageJ. Semua data kemudiannya dilakukan analisis statistik. Pada peringkat 3-bulan, kedua-dua kumpulan menunjukkan penurunan yang signifikan di dalam kebolehidupan sel-sel tersebut; logam (56.01±SE1.69, p≤0.05) dan seramik (64.41±SE 1.34, p ≤ 0.05), dibandingkan dengan data dasar. Pemerhatian analisis sitomorfografi sel mukosa bukal pada bulan ke-3 menunjukkan terdapat penurunan NA yang signifikan; logam (45.5±SE 0.94, p ≤0.05) dan seramik (55.2±SE, 0.63,p≤0.05). Nisbah N/C untuk logam ialah (30.1±SE 1.02, p≤0.05) manakala seramik (41.1±SE 0.92, p ≤0.05). Analisis menunjukkan terdapat peningkatan signifikan CA kumpulan logam sebanyak (125.1±SE 1.22, p≤ 0.05) berbanding dengan seramik sebanyak (118.3±SE 1.16, p ≤ 0.05). PI menunjukkan peningkatan signifikan pada peringkat 3-bulan pada logam (1.98±SD0.39, p ≤0.05) dan seramik (1.7±SD0.45 p ≤0.05). BOP juga menunjukkan keputusan yang sama di mana terdapat peningkatan yang signifikan pada peringkat 3-bulan, logam (0.30±SD0.09, p≥0.05) manakala seramik (0.20±SD0.08, p≤0.05). PPD pula tidak menunjukkan perubahan yang signifikan pada peringkat 3-bulan dalam kedua-duanya; logam (1.88±SD0.61, p ≥0.05) dan seramik (1.86±SD0.60, p ≥0.05), yang bersamaan dengan CAL, kumpulan logam (0.20±SD0.08, p ≤ 0.05) dan kumpulan seramik (0.62±SD0.14, p ≥0.05). Bagaimanapun, semua pemboleh ubah dan parameter yang dikaji menunjukkan tiada perubahan ketara berlaku pada peringkat 6-bulan berbanding dengan data dasar. Sebagai kesimpulannya, aplians logam dan seramik ortodontik boleh menyebabkan kesitotoksikan kepada sel mukosa bukal, perubahan kepada morfologi sel dan menjejaskan kesihatan periodontal pada 3-bulan selepas rawatan ortodonik. Perubahan ini lebih signifikan dalam kumpulan metalik. Sementara itu semua perubahan pada 6-bulan menunjukkan tiada perbezaan yang signifikan yang menyatakan bahawa terdapat toleransi sel untuk proses penyembuhan. Kedua-dua aplians logam dan seramik dianggap bioserasi, terutamanya aplians yang diperbuat dari seramik.

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ABSTRACT

The corrosive environment of the oral cavity is a major cause of concern during the use of orthodontic appliances. The reasons are because fixed orthodontic appliances are made from different alloys materials. Exposure of soft tissues to these materials while using the appliances may lead to some chemical reactions due to material degradation which may release certain type of ions. The study aims to analyse the cell viability changes, and cytomorphometric alterations in the nuclear area (NA), cytoplasmic area (CA), and nuclear-cytoplasmic ratio (N/C) of the human buccal mucosa of patients treated with metallic and ceramic orthodontic appliances respectively. The study was also carried out to assess the periodontal health of patients under those orthodontic treatments. In this study, twenty-six subjects who were orthodontic patients attending Dental Clinic at Hospital Universiti Sains Malaysia were recruited. The subjects were divided into two groups; one group was treated with metallic appliances (n=13), while another was treated with ceramic appliances (n=13). The buccal swab was taken from each participant three times, prior to treatment (baseline), at 3-month post-treatment, and then at 6-month post-treatment. To examine the periodontal health of patients, four periodontal parameters were assessed at the same time points; plaque index (PI), bleeding on probing (BOP), periodontal pocket depth (PPD) and clinical attachment loss (CAL). Cell viability of the oral buccal mucosa was evaluated using Trypan blue staining, followed by light microscopy analysis. For cytomorphometry, the cells were stained using Papanicolaou stain, followed by an assessment using ImageJ software. All data were subjected to statistical analysis. At 3-month both metallic (56.01±SE1.69, $p \leq 0.05$) and ceramic (64.41±SE 1.34, $p \leq 0.05$) groups showed a significant decrease in the cellular viability respectively in comparison to the baseline group. Cytomorphometry analysis of the buccal mucosa cells at 3-month showed a significant decrease of NA in both metallic (45.5±SE 0.94, $p \leq 0.05$) and ceramic (55.2±SE 0.63, $p \leq 0.05$) groups. The N/C ratio was (30.1±SE 1.02, $p \leq 0.05$) for metallic, while ceramic was (41.1±SE 0.92, $p \leq 0.05$). The analysis showed that there was an increase in CA of metallic (125.1±SE 1.22, $p \leq 0.05$) in comparison to ceramic (118.3±SE 1.16, $p \leq 0.05$). PI analysis showed a significant increased at 3-month in both metallic (1.98±SD0.39, $p \leq 0.05$) and ceramic groups (1.7±SD0.45, $p \leq 0.05$). Similarly, BOP showed a significant increased at 3-month in both metallic (0.30±SD0.09, $p \leq 0.05$) and ceramic groups (0.20±SD0.08, $p \leq 0.05$). PPD showed no significant difference at 3-month in both metallic (1.88±SD0.61, $p \geq 0.05$) and ceramic group (1.86±SD0.60, $p \geq 0.05$), similar to CAL, in which the metallic group is (1.99 ±SD0.72, $p \geq 0.05$) and the ceramic group is (1.98±SD0.87, $p \geq 0.05$). However, all investigated variables and parameters have no significant difference at 6-month in comparison to the baseline group. Fixed metallic and ceramic orthodontic appliances can induce cytotoxicity to the buccal mucosa cells, changes in cellular morphology and affects periodontal health at 3-month after the orthodontic treatment. These changes were more prominent in the metallic group, while all changes at 6-month showed no significant difference which indicates cells tolerance for healing. Both metallic and ceramic appliances are considered biocompatible. Using ceramic appliances being more advantageous.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

The worldwide prevalence of malocclusion is high, that makes the need of orthodontic treatment high too. Orthodontics is the dental speciality focused on diagnosis and treatment of dental and associated facial irregularities. This branch of dentistry defined by the American Board of Orthodontics (ABO) and later adopted by the American Association of Orthodontists states as:

“Orthodontics is that specific area of the dental profession that has as its responsibility the study and supervision of the growth and development of the dentition and its related anatomical structures from birth to dental maturity, including all preventive and corrective procedures of dental irregularities requiring the repositioning of teeth by functional and mechanical means to establish normal occlusion and pleasing facial contours” (Singh, 2015b).

Orthodontists can choose between two types of orthodontic appliance system either fixed or removable for treating most of the patients according to each patient’s need, whereas the removable appliances can do some things better than fixed appliances, and variants within fixed appliance systems do some things better than removable (Proffit *et al.*, 2013).

For the fixed type of orthodontic appliances, the technologies have brought a lot of modification in existing appliance systems such as new bands, wires, elastic and brackets.

As well as new methods for malocclusion correction, for instance, clear aligners. To correct malocclusion in most cases, a patient required using these fixed appliances for over a year or more. (Proffit *et al.*, 2013). These fixed orthodontic appliances are made from alloys that are composed of wide arrays of metallic, ceramic, and polymeric materials. Also, these materials have a combination of various percentages (Brantley, 2001). Most metallic orthodontic appliances that normally used during treatment procedure are made from alloys containing nickel (Ni), titanium (Ti), chromium (Cr), cobalt (Co) and iron (Fe) (Brantley *et al.*, 2001). Among them, Ni and Cr have generated great concern. Orthodontic metallic appliances in an average contain 8–50% Ni and 17–22% Cr, which may lead to increase their intrinsic toxicity (Mikulewicz and Chojnacka, 2010; Mikulewicz *et al.*, 2014). However, most of these metallic ions considered as essential elements. When the remaining of these elements are localised, that may increase the deposits of them in specific areas which may produce a toxic reaction. Since these materials would be inside the intraoral environment for a longer duration, the gradual release of their ions is becoming an important biosafety issue of orthodontic treatment (Martín-Cameán *et al.*, 2015).

For ceramic materials of orthodontic appliances, they are a form of glass, and similar to the glass, the ceramic appliances have a brittle tendency. Currently, ceramics are produced from alumina either as single-crystal or polycrystalline units or made of a monocrystalline ceramic material (Brantley *et al.*, 2001). Some previous studies reported that the ceramic brackets showed chemically inert behaviour on the oral fluids (de Andrade Vitral *et al.*, 2010a; de Andrade Vitral *et al.*, 2010b). Whereas, some authors demonstrated that polycrystalline and polycarbonate brackets showed some different ranges of toxic effects (Retamoso *et al.*, 2012; Suzuki *et al.*, 2000).

Developing and selecting biocompatible materials have been one of the major challenges in dentistry (Jorge *et al.*, 2004). Toxic, inflammatory, allergic or mutagenic reactions are the possible biological responses to these materials. Thus oral condition is considered as the main reflection parameters for evaluating the biological response and the potential damage to cells and tissues related to the use of such materials (Kao *et al.*, 2007; Pithon *et al.*, 2009).

It is a usual expectation that irregular teeth retained more plaque than straight teeth. Treatment with fixed orthodontic devices (such as brackets and bands) creates numerous plaque accumulation sites which disturbed oral hygiene procedures and gradually leading to the development of periodontitis, gingivitis, white spot lesions or caries (Bollen *et al.*, 2008; Liu *et al.*, 2011). It was observed that the treatment with fixed orthodontic appliances might enhance the gingival tissue inflammatory reaction. The presence of new retentive places around the fixed appliances components increases the dental plaque accumulation thus increase the inflammatory response (Alexander, 1991). The dental plaque microbes recognised as the main etiologic factor of dental caries and periodontal disease developments (Baka *et al.*, 2013). Where the treatment with fixed orthodontic appliances may affect the equilibrium of oral microflora and increase bacteria retention and stimulates the growth of a subgingival plaque (Gomes *et al.*, 2007; Petti *et al.*, 1997). The other problem reported to occur is the risk of root resorption due to periodontal complications. Thus, keeping good periodontal health should be considered as one of the success measures in the orthodontic treatment (Dannan, 2010).

On the other hand, the interest for oral exfoliative cytology as a diagnostic and prognostic methodology and monitoring patient's oral tissue has re-emerged recently. However, generally, the cytology analysis depend mainly on the cytologist judgement rather than the cell parameters measurement (Patel *et al.*, 2011). To minimise the false-negative results, some authors, have suggested the use of quantitative techniques, based on the evaluation of parameters, such as nuclear area (NA), cytoplasmic area (CA), and nucleus-to-cytoplasmic area ratio (N/C) (Cowpe *et al.*, 1988; Ogden *et al.*, 1997). This would increase the ability of exfoliative cytology for detecting disorders of oral tissue. Where this technique considered objective, precise, non-invasive and reproducible (Patel *et al.*, 2011). CA is defined as the cell substance between the cell membrane and the nucleus, containing the cytosol, organelles, cytoskeleton and various particles. While NA defined as a region containing the cell's genetic information in eukaryotic cells that is enclosed by the nuclear envelope and contains the chromosomes (Pierce Benjamin, 2005). In eukaryotic cells, the cytoplasm includes all the material inside the cell and outside of the nucleus, such as endoplasmic reticulum, mitochondria and the nucleus (Pierce Benjamin, 2005). N/C ratio is the ratio of the volume of the nucleus to the volume of cytoplasm. Fairly constant for a particular cell type and usually increased in malignant neoplasms, the N/C ratio indicates the maturity of a cell, because as a cell matures the size of its nucleus generally decreases (Turgeon, 2012).

In the present study, assessment of oral mucosal cells viability exposed to two types of orthodontic appliances (metallic and ceramic respectively) was conducted by collecting oral mucosal epithelium from the same patient before and after applying of appliances. The cells obtained were also subjected to cytomorphometric analysis. In addition, observation and assessment of the periodontal health before and after applying the orthodontic appliances were also conducted.

1.2 Gap statement

To the best of our knowledge, there is a limited number of studies reported on fixed orthodontic treatment on the soft tissue of oral cavity, for 3 until 6 months duration on the same patient. All previous *in vivo* studies (Angelieri *et al.*, 2011; Hafez *et al.*, 2011) were investigated using one assessment procedure such as cytotoxicity only or periodontal assessment only. On top of that, most of the previous approaches were done *in vitro* (Martín-Cameán *et al.*, 2015; Mikulewicz *et al.*, 2014). Therefore, there is a need to investigate using more than one assessment procedure *in vivo*.

1.3 Justification of study

There is a controversy in the literature about the biocompatibility of orthodontic appliance materials. The reason is that there is a widely different in the usage of commercially manufactured fixed orthodontic appliances in different countries. Besides, there is a lack of understanding of ions which are released from these appliances intraorally and their effect on oral mucosal cells and periodontal index. Therefore, some studies reported that appliances are biocompatible and safe for use, and on the other hand some studies reported that the appliances need to be studied further to ensure its biosafety (Hafez *et al.*, 2011).

Most of the approaches for studying these effects are *in vitro* studies. The clinical situation that happens in the intraoral environment is more complex than compared to the controlled experimental *in vitro* environment. In this study, we would want to understand the effect of metallic and ceramic appliances orthodontic materials on intraoral mucosa and periodontal health that at 3-month and 6-month timelines. No such previous *in vivo* investigation was done using the brands that we used in our study, which normally used in USM Orthodontic Specialist Clinic.

1.4 Objective

1.4.1 General Objective

To investigate the oral mucosal cell viability, its cytomorphology and periodontal health of patients exposed to metallic and ceramic orthodontic appliances, respectively, with that of prior to treatment (baseline).

1.4.2 Specific Objectives

1) To assess the cell viability of human epithelial buccal mucosal cells before (at 0-month; baseline) and after (at 3- and 6-month) exposing to metallic and ceramic fixed orthodontic appliances.
2) To assess the cytomorphometric parameters of epithelial buccal mucosal cells before (at 0-month; baseline) and after (at 3- and 6-month) exposing to metallic and ceramic fixed orthodontic appliances.
3) To investigate the periodontal health before (at 0-month; baseline) and after (at 3- and 6-month) placement of metallic and ceramic orthodontic appliances.

1.5 Research questions

a. Do orthodontic metallic or ceramic appliances have cytotoxicity effect on oral mucosa and cause cell morphology changes?

b. Do orthodontic metallic or ceramic appliances affect periodontal health?

1.6 Research hypothesis

a. There is no significant effect on cell viability and morphological changes on oral mucosa with that of prior to treatment (baseline) and after exposing to metallic and ceramic appliances.

b. There is a periodontal health difference between patients with the metallic and ceramic orthodontic appliances with that of prior to treatment (baseline).

CHAPTER 2

LITERATURE REVIEW

2.1 Orthodontic in dentistry

Orthodontics is the dentistry branch concerning the development of the occlusion, dentition, facial growth, and the diagnosis as well as treatment of occlusal abnormalities. The malocclusion treatment is introduced by Edward Hartley Angle over 100 years ago. Since then, numerous methods have been described for the efficient orthodontic tooth movement (Proffit, 2013). The main objective of orthodontic treatment is to improve jaw and dental function, as well as dentofacial aesthetics, and thus enhancing the patient quality life. This is achieved by obtaining optimal occlusal and proximal contact of teeth within the framework of normal function and physiologic adaptation, acceptable dentofacial aesthetics, self-image and reasonable stability (Graber *et al.*, 2016).

Orthodontic complications can be a consequence of genetic or environmental factors. This requires that the diagnosis is made thoroughly before starting treatment. Proper diagnosis involves case history, clinical examination, specific radiographs, facial photographs and study models, where proper decisions for the treatment procedure could be made. Treatment period usually depends on the severity of the orthodontic problem and the age of the patient, which may take from 6 to 30 months (Kapoor and Singh, 2015a).

2.1.1 Orthodontic appliances

The contemporary orthodontic treatment utilised either fixed or removable appliances. Orthodontic appliances have evolved steadily, and nowadays intraoral fixed or removable orthodontic appliance is the integral part of orthodontic treatment in clinical dentistry. The technological advances have brought improvements in existing appliance systems. The improved technology has greatly increased the productivity of orthodontists (Proffit *et al.*, 2013).

2.1.1(a) Fixed orthodontic appliances

Fixed orthodontic appliances are defined as the devices with attachments which fixed on to the tooth surface. The forces are exerted via these attachments using archwires and or other auxiliaries. (Singh, 2015a). The use of the fixed appliance in orthodontics is referred to directly as the guides to move the teeth to the occlusion line (Proffit, 2013). Thus, designing of devices should be able to control and produce of three-dimensional movement of teeth. This movement will allow the teeth to be at the normal alignment and enhances the occlusion condition. Normal alignment and occlusion condition are the main objectives in designing the devices (Proffit, 2013).

The control of treatment with fixed orthodontic appliances depends solely on the clinician rather than the patient. Unlike removable orthodontic appliances which greatly depend on the patient. Thus, the outcome achieved with fixed appliances is much better in comparison to the removable appliances. Also, fixed orthodontic appliances can produce teeth movement in the three planes of space.

Fixed orthodontic appliances have two main categories, active components and passive components. The active and passive appliances depend on the ability of forces generated by the component, as well the kind of attachment provided to the other auxiliaries and or to the teeth (Figure 2.1) (Singh, 2015a).

The active components consist of separators, elastics, archwires, springs, and elastomerics. While the passive components consist of brackets, bands, accessories, molar tube and ligature wires. There are certain indicators that the use of fixed orthodontic appliances can be applied such as multiple tooth movements, correction of rotation, active closure of spaces, intrusion or extrusion of teeth, and bodily tooth movement. However, contraindication of fixed orthodontic appliances should be carried out if the patient is poorly motivated, poor dental health, lack of special operator skills, and the malocclusion are beyond the scope of the fixed appliance (Singh, 2015a).

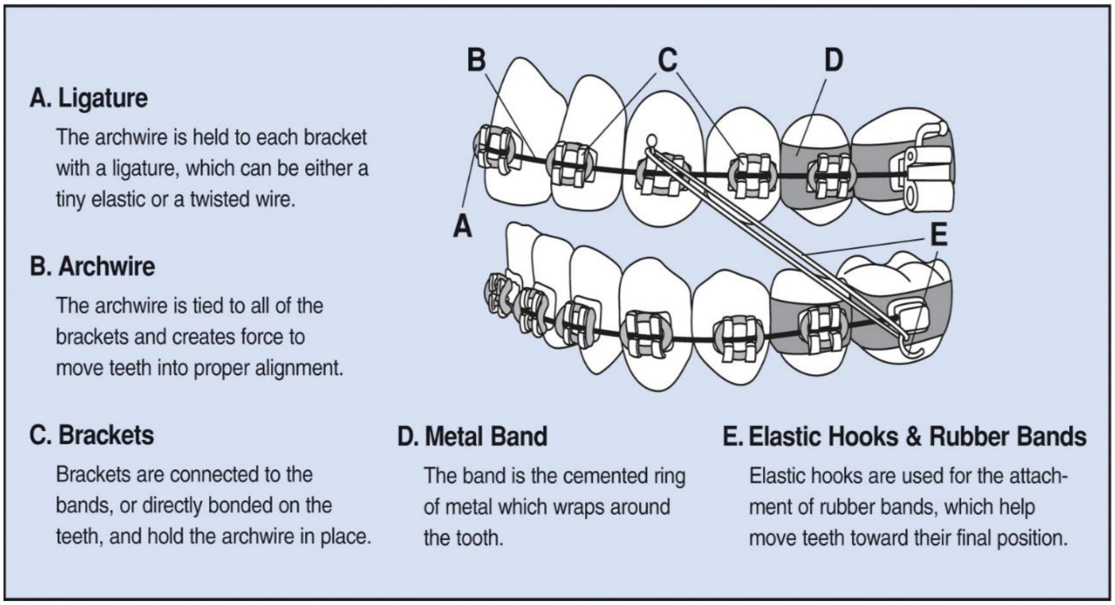

Figure 2.1: Fixed orthodontic appliances. The illustration showed different components types and functions of active and passive fixed orthodontic appliances (http://couserorthodontics.com/dental-dictionary/).

2.1.1(b) Removable orthodontic appliances

Removable orthodontic appliances are the appliances that can be removed and inserted in the mouth by patients. It is defined as a device through which an optimal orthodontic force is delivered to a tooth or a group of teeth in a predetermined direction (Graber and Neumann, 1984; Vijayalakshmi, 2008). Removable appliances are clinically successful treatment in contemporary orthodontic practices (Kharbanda, 2013). However, the clinical result of fixed orthodontic technique lead to an increase in its demand and frequently use by the orthodontist in comparison to the removable appliances. One of the reason is that fixed appliances can generate complex tooth movement, while, removable appliances are not able to produce the three planes of space movements (Proffit *et al.*, 2013).

Removable orthodontic appliances components are designed and constructed according to the planned tooth movement. Besides, the objectives of treatment, tooth eruption and morphologic characteristics, the age of patients and their psychological findings should be considered. The removable orthodontic appliances are constructed of three main components (Figure 2.2) (Kapoor and Singh, 2015b) which are force or active components which consist of elastics, screws, or springs, fixation or retentive components which include clasps, and base plate or framework components (made from acrylic whether heat cured or cold cured). There is a list of indication when the used of removable orthodontic appliances are considered to be used such as for growth modification during mixed dentition, cleft palate and its syndrome associated, limited (tipping) tooth movements (arch expansion individual tooth malocclusion position), retention following orthodontic treatment, adjunct to fixed orthodontic appliances and interference with abnormal orofacial habits. The contraindication of usage of removable orthodontic appliances includes complex malocclusions, special cases requiring (multiple rotations, controlled space closure or bodily movement of teeth), and open bite or severe deep bite (Kapoor and Singh, 2015b; Vijayalakshmi, 2008).

Figure 2.2: Removable orthodontic appliances. Different forms and sizes of removable orthodontic devices that have been formed according to the treatment goal.

2.1.2 Fixed orthodontic appliances materials

The fixed orthodontic appliances are made from alloys that are composed of wide arrays of metallic, ceramic, and polymeric materials. These materials have a combination of various percentages. Most orthodontic appliances which routinely used during treatment are made from alloys that contain cobalt (Co), chromium (Cr), iron (Fe), nickel (Ni), titanium (Ti), monocrystalline, and polycrystalline materials (Brantley *et al.*, 2001). Metallic orthodontic appliances contain in average about 8–50% Ni and 17–22% Cr which lead to having concerns due to their toxicity effects on the oral health (Mikulewicz *et al.*, 2014). The other metallic ions are essential elements and the increase deposits of them at localised regions may lead to producing a toxic reaction. Since these materials, while remaining in the intraoral environment for a longer duration starts to the gradual release of their ion intraorally which consider as an important matter in the biosafety of orthodontic treatment (Martín-Cameán *et al.*, 2015).

With an improvement of technology and esthetic requirement of the public, orthodontic appliances systems have been developed (Willems and Carels, 2000). For example, for engaging the archwires, the steel ligatures are replaced by elastomeric ligatures which are available in different colours according to the patient selection. Ceramic brackets produced to bring a clear and alternative esthetical option than metallic brackets (Russell, 2005). However, these developments in the fixed appliances system also have its complication like discolouration, breakage and decrease the bonding strength to the teeth, which may lead to decrease the efficiency of treatment and increase the cost to the provider and patient (Djeu *et al.*, 2005).

2.1.2(a) Metallic fixed orthodontic appliances materials

Orthodontic appliances have different components. Brackets and archwire appliances are considered as the most important components related to the present study because all participants were provided with these devices. The bracket is defined as a device that projects horizontally to support auxiliaries and is open on one side usually in the vertical or horizontal (Singh, 2015c). The archwires are the wires engaged in brackets to generate forces which can induce tooth movements.

Metallic brackets are constructed from a different range of stainless steel alloys. Current developments in the technologies, such as metal injection moulding (MIM) and laser modifications, as well the presence of new materials has led to the production of new brackets made from titanium alloys, cobalt chromium alloys, and gold alloys (Eliades and Brantley, 2016; Zinelis *et al.*, 2013). Different stainless alloys were used for the production of brackets components such as 303, 304, 316, and the most widespread 17-4 PH (Eliades *et al.*, 2003; Iijima *et al.*, 2017). The 17-4 PH stainless steel alloy produced a greater mechanical property than the 303 and 316 austenite stainless steels, but this alloys may exhibit better tooth movement control. The low resistance to corrosion of the 304 and 17-4 PH stainless steels in the chloride solutions has been reported (Oh *et al.*, 2005). The nickel-free stainless steel has been used for brackets fabrication and presents higher hardness with less corrosion than the conventional stainless steels alloys (Platt *et al.*, 1997). However, the soldering process of stainless steel brackets components (base and wings) mostly depended on alloy's elemental composition; most stainless steels can be soldered using different alloys such as silver, nickel, copper and gold alloys (Brockhurst and Pham, 1989; Iijima *et al.*, 2017).

The metallic archwire materials are classified according to the material composition which includes; gold, stainless steel, chrome-cobalt and nickel-titanium (Singh, 2015c). The gold alloys reflected good biocompatibility and stability into the oral condition. The main disadvantages of gold were the high coast with low yield strength. Chrome cobalt archwires supplied in more formable and softer state which allow increasing its strength. However, the need of soldering with silver or other material as well the need for heat treating during uses together with high elasticity modules, lead to some disadvantages while using (Kusy, 1997; Singh, 2015c).

The most commonly used is the austenitic stainless steel archwire. The stainless steel archwire contains chromium and nickel content in different averages, and its most important advantage is its resistance to corrosion (Brantley, 2001). It is commercially offered to have different values in the yield and elasticity strength but depends on the changes of the parameters during production procedures (Sekhar Kotha *et al.*, 2014). The resistance of corrosion of stainless steel generally is acceptable. However, the release of chromium and nickel in few volumes may induce some adverse reaction like hypersensitivity (House *et al.*, 2008). The bracket-wire friction of stainless steel wires have the advantages in producing of a lower amount in comparing with other wires types (Krishnan and Kumar, 2004). Developing in the stainless steel manufacturing lead to improving the archwires mechanical properties containing lower content of nickel with higher resistance to corrosion (Oh *et al.*, 2004; Sekhar Kotha *et al.*, 2014).

Nickel-titanium (Ni-Ti) archwires are characterised by shape and thermal memory with high flexibility, super elasticity and limited formability. Ni-Ti archwire has a high capacity for energy storage greater than stainless stain wires when the same amount of bending activation occurred (Brantley, 2001). The super elasticity of Ni-Ti wires produces a wide-ranging of activation and deflection by low forces delivering, which considered as the most important advantage of this wires in addition to their resistance to corrosion (Huang *et al.*, 2003; Sekhar Kotha *et al.*, 2014). Ni-Ti wires cannot be welded or also fused, and expensive cost in addition to the low formability make it has some disadvantage. The bracket-wire friction amount of Ni-Ti wires is higher if compared with stainless steel wires (Singh, 2015c).

In general, the main advantages of the metallic appliances are their strength and stability in the oral cavity, affordability and the variety of options. While the bad appearance of the metallic appliances and their irritation influence on the gum and other oral tissue in addition to the patient's hypersensitivity that may occur considered main disadvantages of this appliance (Singh, 2015a).

2.1.2(b) Ceramic fixed orthodontic appliances materials

The public demand for esthetic makes the ceramic brackets widely used in orthodontic treatment. Ceramic brackets that commercially available are produced from polycrystalline or monocrystalline alumina materials. The most important advantage of ceramic brackets that their translucency or milky-white appearance, which give an excellent esthetic. However, the main disadvantages of these brackets are the brittle characteristic which makes brackets fractures caused by archwires forces. Additionally, enamel fracture that may be occurred with debonding process, and the bond failure to the tooth surface can happen (Santin *et al.*, 2015; Viazis *et al.*, 1993). The ceramic brackets showed better biocompatibility and mechanical properties with minimal water absorption during treatments period compared with other brackets. Single-crystal alumina brackets have more transparency which presents more esthetic. Also, it has more strength than polycrystalline alumina brackets. While the polycrystalline brackets show lower toughness fractures due to the deficiency in the presence of internal grain boundaries (Iijima *et al.*, 2017).

The esthetic archwires have grown accompaniment rapidly with esthetic brackets to complement each other (Haryani and Ranabhatt, 2016). Esthetic archwire materials are mainly a composite of two materials and can be classified into two groups; ceramic-polymer composite and metallic-polymer composite (Elayyan *et al.*, 2010; Kusy, 1998). The ceramic-polymer composite esthetic archwires made from glass fibres spindles inserted in a polymeric matrix which fiber reinforced composites. This manufactured process named photopultrusion. The problem of these wires is susceptibility for intraoral breakage where consider as brittle wires (Haryani and Ranabhatt, 2016; Kusy, 1997). The self-reinforced polymer polyphenylene thermoplastic archwires which were introduced by Burstone *et al.* (2011). It showed better flexibility comparable to NiTi without suffering from stress relaxation (Burstone *et al.*, 2011).

The coated esthetic wires have a core of a metallic wire covered with inorganic materials or by the tooth-coloured polymer (Kim *et al.*, 2014; Zegan *et al.*, 2012). The coating benefited in hiding of the underlining alloy and gives the esthetic appearance for the wires. However, the coating process can affect the corrosion and friction properties, and the mechanical durability of the archwires. Thus, previous studies found that the archwire damaged may occur due to mastication and enzymes activation (Haryani and Ranabhatt, 2016; Kusy, 1997). In general is advantages of ceramic appliances versus metallic appliances are their esthetic appearance, and it has less irritating behaviour into the oral cavity. The disadvantages of ceramic versus metallic appliances are that they have more friction properties with higher tendency to fracture and causing enamel damage (Singh, 2015c).

2.2 Cell toxicity and biocompatibility in dentistry

Recent dental appliances are made from three materials groups; metals, ceramics and resins. Since these appliances remain in contact with the oral cavity tissues for a long period of duration, they are considered as medical devices and should be part of biomaterials group (Yaneva-Deliverska *et al.*, 2015). These types of biomaterials are mostly non-inert which means there is an interaction between these materials and biological environment.

The American Dental Association recognised the general biocompatibility groups as the following; high noble alloys (noble metal content of ≥ 60%: gold (Au), platinum (Pt), palladium (Pd) and with ≥40% gold), noble alloys (≥ 25% Au, Pt, Pd) and predominantly base metal alloys (< 25% Au). Titanium (Ti) (alloys) (≥ 85% Ti) are also included due to their excellent biocompatibility and placed between the high noble and noble alloys (Affairs, 2003). The main advantage of noble metals that the highly resistant to oxidation and corrosion, which it is not required for alloying elements. Chromium (Cr), as an example, is requiring alloys (which is based on cobalt, nickel or iron) for layer formation of chromium oxide to introduce the alloy passivation. This interaction may induce side effects known as adverse reactions on the patient health. Understanding the degree of these effects will help in the control the safety and biocompatibility of the materials towards the patient (Schmalz and Arenholt-Bindslev, 2009b).

The term biocompatibility is defined as the response of a host organism to the presence of potentially inert biomaterials (Es-Souni *et al.*, 2005). The study of biocompatibility is aimed to investigating the cell toxicity (cytotoxicity) as well as cytological alteration affected the host exposed to the materials after a long period. Cytotoxicity refers to the degree to which a substance has specific destructive action on certain cells. Toxic combinations can cause cell damage or death; via the loss of adhesion and viability

(Schmalz and Arenholt-Bindslev, 2009a). Thus, the host response is considered as an ideal measurement of biocompatibility (Es-Souni *et al.*, 2005). The other concept to understand regarding biocompatibility is that it is an interaction at the material-tissue interface, which affected both the host and the material. The materials may respond to the host environment by degradation, chemical alteration, corrosion or via other interaction. Other factors like ageing, systemic and local host environment factor can also influence the interaction with the materials (Williams, 2008). Another concept is that the reactions at the material-tissue interface. The reaction is a normal function of the tissue where the interface is created, but the result of the reaction differ based on the types of tissues, whether it is skin, bone or tooth pulp (Anderson, 2001). The reaction may include cytotoxicity, acute toxicity or chronic toxicity, sensitisation or irritation (Thyssen and Menné, 2010).

Since biomaterials are considered as foreign bodies, the biocompatibility research should aim to learn about the biological response towards the foreign bodies. Certain types of materials modification involve the addition of peptide sequences to encourage native protein or cell interactions, while some materials are modified to provide a three-dimensional structure to encourage matrix formation. Eventually, the modification of those materials is a process to control the degradation of the materials over time as it will improve the tissues biocompatibility response (Ratner and Bryant, 2004).

Different *in vivo* and *in vitro* studies conducted to assess the cytotoxicity of orthodontic appliances using different methodologies. Most of these approaches assess the ion released (Ni, Cr, Co, Fe, Ti, Mo) from fixed orthodontic appliances using buccal epithelial cells or another biological medium such as blood, hair or saliva, during a period of time, range from few days to the several months. The general findings are there is increasing concentration level of Ni and Cr in the saliva after treatment of fixed appliances (Downarowicz and Mikulewicz, 2017; Martín-Cameán *et al.*, 2015). In the present study, the cell viability of the buccal mucosa evaluated before and during treatment with fixed orthodontic appliances. Since it is important to prevent the cytotoxicity reaction to maintain the vitality of tissues, thus, dental appliances need to be carefully screened before clinically used (Murray *et al.*, 2007).

The oral cavity has many factors that may develop biodegradation corrosion of orthodontic appliances. Previous studies have demonstrated that the saliva can act as a continuous erosion medium also intermediate for emission of electro-galvanic currents during corrosion and ion released from orthodontic appliances (Matos de Souza and Macedo de Menezes, 2008; Petoumenou *et al.*, 2009). Additionally, the microbial and enzymatic activity with the variation of the temperature and pH level as well as the chemicals of food and drinks introduce into the oral cavity, altogether is considered as corrosion conductors. The nature of the micro surface metal alloys and its interaction with other alloys of orthodontic appliances, all these factors add in the corrosion process (Eliades and Bourauel, 2005; Hafez *et al.*, 2011). In the end, manipulation and clinical use of orthodontic appliances might interfere with the materials properties of these appliances which may influence their biocompatibility. Therefore, due to the possible toxic effect that may occur, it is best that they should be assessed.

2.2.1 *In vitro* cytotoxicity studies of materials used in orthodontic treatment

In 2000, a study was conducted by Tomakidi *et al*. to assess the effect of metal release from different orthodontic appliance containing nickel, nickel free and titanium materials. They demonstrated lack in the cell membrane damage present at the period range between 1 to 14 days (Tomakidi *et al.*, 2000). This result approved by another study done which reported that the non-metallic and metallic materials have similar cytotoxicity, and concluded that these materials are considered non-cytotoxic (Mockers *et al.*, 2002). One study has assessed the effect of nine different archwires on the cell viability where the materials are made of stainless steel, nickel-titanium, beta-titanium, and coated nickel-titanium, and negative results have been reported (Toledo *et al.*, 2012). In contrast, another study which assessed the cellular viability of orthodontic brackets (metallic, nickel free, polycarbonate, monocrystalline and polycrystalline material) where the appliances showed cytotoxicity effects (Retamoso *et al.*, 2012). Another study assessed the effects of stainless steel brackets coated with different phases of photocatalytic titanium oxide and the one coated with the anatase phase of titanium oxide has minor cytotoxic effects (Baby *et al.*, 2017). The polycarbonate orthodontic brackets, however, were found not to be cytotoxic (Pithon *et al.*, 2009; Tanimoto *et al.*, 2015).

A study using artificial saliva of four different orthodontic metal brackets reported that although the brackets have good biocompatibility, but different cells types and components exhibit different cellular reactions after exposure to metal brackets (Jacoby *et al.*, 2017; Kao *et al.*, 2007). Another study assessed the artificial saliva showed the archwires formed by solder connection on a nickel-titanium alloy and stainless-steel wire