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In vitro evaluation of cytotoxicity of fixed functional appliances

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Abstract

Objective The objective of this study was to evaluate the cytotoxicity of fixed functional appliances that are commonly used in the treatment of Class II malocclusion caused by mandibular retrognathia, using the MTT cell viability assays [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (Sigma Chemical Co., Milan, Italy).

Materials and methods The cytotoxicity of five different fixed functional appliances commonly used in orthodontic treatments was assessed. The appliances evaluated included Jasper Jumper (American Orthodontics, Wisconsin, USA), Power Scope (American Orthodontics, Wisconsin, USA), Herbst (American Orthodontics, Wisconsin, USA), Forsus™ Fatigue Resistant Device Kits (3 M, MN, USA), and Twin Force Bite Corrector (Henry Schein Orthodontics, California, USA). Assays were conducted using one appliance from each type, resulting in a total of seven groups. Cytotoxicity testing was performed using the MTT assay on a human gingival fibroblast (HGF) cell line. Data analysis was conducted using the Kruskal–Wallis test with a significance level of $p = 0.05$.

Results All appliances demonstrated cell viability rates exceeding 90%, categorizing them as non-cytotoxic under ISO 10993–5 standards. Herbst exhibited the highest proliferation index (2.62 ± 1.13), while Power Scope (2.05 ± 1.06) and Jasper Jumper (1.90 ± 1.23) showed the lowest indices. Despite these variations, statistical analysis revealed no significant cytotoxic effects when compared to the control group ($p > 0.05$). All appliances were confirmed as biologically safe for cell health.

Conclusion The findings demonstrate that all evaluated appliances exhibit a biocompatible interaction with HGF cells, with no evidence of adverse cytotoxic effects. These results support the safe clinical use of these fixed functional appliances in orthodontic treatment.

Keywords Fixed functional appliances, Cytotoxicity, Metal, Corrosion, Twin force bite corrector, Herbst, Power scope, Jasper jumper, Forsus

Introduction

Fixed functional appliances are commonly used in orthodontics to correct Class II malocclusions associated with mandibular retrognathia by stimulating forward mandibular growth and restricting maxillary growth. These appliances function by transmitting forces through dental arches when the patients closes their mouth, encouraging the forward growth of the mandible [1]. Unlike removable functional appliances, which rely on patient compliance, fixed appliances offer the advantage of continuous use [2].

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Fixed functional appliances are made from a combination of various materials. The metal components of these appliances are typically fabricated from stainless steel due to its strength and corrosion resistance. These properties are crucial for ensuring appliance longevity, considering the chewing forces and orthodontic adjustments they must endure [3]. Nickel-titanium, known for its superelastic properties, is another material used in the springs and active components of certain functional appliances, providing continuous forces upon activation [4]. Another fixed functional appliance, the Jasper Jumper, has soft grey synthetic material encasing the open-coil spring, which tends to deform after approximately three months of use [5, 6].

The typical duration of comprehensive orthodontic treatment with fixed functional appliance is approximately two years [7]. The exact composition of the five fixed functional appliances; Jasper Jumper (American Orthodontics, Wisconsin, USA), Power Scope (American Orthodontics, Wisconsin, USA), Herbst (American Orthodontics, Wisconsin, USA), Forsus™ Fatigue Resistant Device Kits (3 M, MN, USA), and Twin Force Bite Corrector (Henry Schein Orthodontics, California, USA)) commonly used in orthodontics is not fully known.

The compositions of fixed functional appliances differ from one another (Table 1). The composition of the Herbst and Jasper Jumper appliances used in our study is provided by American Orthodontics in their safety sheet. The appliances contain elements such as iron, nickel, chromium, silicon, manganese, molybdenum, titanium, copper, aluminum, niobium, carbon, and tantalum. However, the specific proportions of these elements within the appliances and the exact composition of the gray material covering the metal part of the Jasper Jumper appliance are not clearly specified. Similarly, the composition of the Power Scope [8] appliance produced by American Orthodontics includes elements such as silver, copper, nickel [9], and zinc.

The specific material compositions of the Twin Force Bite Corrector and Forsus appliances are not explicitly detailed in the available sources. However, it is common for such orthodontic devices to be constructed from materials like stainless steel and nickel-titanium alloys [8]. For instance, the Twin Force Bite Corrector utilizes superelastic nickel-titanium springs to deliver continuous force for dental corrections [10]. Similarly, the Forsus Fatigue Resistant Device employs a telescoping coaxial spring mechanism, which is typically made from stainless steel and superelastic nickel-titanium coil spring components [9]. These materials are chosen for their durability, flexibility, and biocompatibility, essential for effective orthodontic treatment.

Modern orthodontics incorporates a wide range of appliances and devices to facilitate the required degree of tooth movement. The appliances can include metal, plastic, polymers or ceramic materials. Metals such as stainless steel, cobalt-chromium, nickel-titanium, and β-titanium are utilised individually or in combination to create the biomechanical force system [11]. Extended use of these metallic alloys in different combinations during orthodontic treatment requires a comprehensive biocompatibility evaluation. The oral cavity serves as a corrosion environment, influenced by numerous factors that accelerate the biological degradation of orthodontic appliances [12]. Saliva acts as an medium for electron and ion transmission. Intraoral temperature, pH, enzymatic and microbial activity, and various chemicals in food and beverages all function as conductors contributing to corrosion [13]. Electrochemical corrosion can occur in the oral cavity because saliva acts as a mild electrolyte [14]. The natural variability of metallic alloys, their combination with other materials, surface irregularities, applied forces on appliances, and friction further contribute to corrosion [15]. Consequently, these variables can

Table 1 The composition of the appliances used in the study

Appliance	Material Composition	Notes
Jasper Jumper	Iron, Nickel, Chromium, Silicon, Manganese, Molybdenum, Titanium, Copper, Aluminum, Niobium, Carbon, Tantalum	Provided by American Orthodontics; specific proportions not detailed. Includes a gray material covering which the composition is not specified
Power Scope	Silver, Copper, Nickel, Zinc	Provided by American Orthodontics; specific proportions not detailed
Herbst	Iron, Nickel, Chromium, Silicon, Manganese, Molybdenum, Titanium, Copper, Aluminum, Niobium, Carbon, Tantalum	Provided by American Orthodontics; specific proportions not detailed
Forsus TM Fatigue Resistant Device Kits	Stainless Steel, Superelastic Nickel-Titanium (NiTi)	The content details of the appliances are not disclosed by the manufacturers
Twin Force Bite Corrector	Superelastic Nickel-Titanium (NiTi)	The content details of the appliances are not disclosed by the manufacturers

modulate the rate of corrosion [16, 17]. Bacteria compromise the protective capabilities of metals and their passivation layers, rendering them ineffective against biological corrosion. Additional corrosion contributors include pH fluctuations from ingested foods and beverages, medical conditions like gastroesophageal reflux or hyperacidity, variations in drink temperatures, and manufacturing errors in alloy production and their thermal or mechanical processing. As corrosion progresses, the concentration of metal ions in saliva tends to increase over time [18, 19].

Orthodontic appliances made from stainless steel, cobalt-chromium, and titanium alloys have a passive surface oxide film for corrosion resistance. However, this protective layer is not entirely impervious, as it remains susceptible to disruption by both mechanical and chemical factors. Even when intact, oxide films tend to dissolve slowly despite passivation, and they reform (repassivation) when the metal surface interacts with oxygen in the air or the surrounding environment. Acidic conditions and chloride ions can significantly accelerate this process. Diets high in sodium chloride and acidic carbonated beverages introduce frequent corrosive agents into the oral cavity. Additionally, fluoride-containing products such as toothpaste and mouthwash can contribute to acidic oral conditions. Laboratory studies have shown that in acidic, fluoridated environments, certain metals (particularly titanium) exhibit increased susceptibility to corrosion [20, 21].

Metals are biologically non-degradable, and the accumulation of ions released over time in tissues can result in irreversible toxic effects. Prolonged exposure may also restrict the recovery time required for cellular repair [22]. Contrary to the common assumption that corrosion products released from orthodontic appliances are harmless, this perspective lacks supporting evidence [23, 24]. Recent findings on metal toxicity's cellular and molecular mechanisms raise concerns regarding orthodontic appliances. These concerns arise from their prolonged direct contact with oral tissues and the release of various types and quantities of metal ions due to corrosion [25]. The literature on cancer research and metal toxicology contains numerous reports on the risks associated with various metal ions [25, 26]. Notably, nickel and chromium ions, which are commonly present in most orthodontic alloys, are classified as chemical carcinogens [26].

Considering their duration of use in the oral cavity, evaluating the cytotoxicity of these appliances is very important. In our study, we conducted an MTT assay [3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyl tetrazolium bromide] (Sigma Chemical Co., Milan, Italy) to assess their cytotoxicity. The MTT assay is highly favored method. It works by utilizing living cells' ability to convert the tetrazolium salt MTT into insoluble formazan, a process facilitated by mitochondrial enzyme succinate dehydrogenase. In addition to this, there are other assays that focus on staining cells, and methods that assess morphological alterations by microscopic examination [27–29]. The intensity of the color, which reflects the quantity of viable cells, is then quantitatively measured using photometric methods after the formazan is dissolved in alcohol [30].

Today, patients are more informed and proactive about the treatments they undergo. They are curious whether the fluoride release from toothpaste will have an effect, and they inquire whether the ingredients are natural or synthetic [31]. Similarly, they want to know the composition of any large appliances that will be placed in their mouths and are concerned about whether these appliances might cause them harm. Concerns about allergic reactions among orthodontic patients are common. Despite this, adverse effects from orthodontic devices are seldom reported, which is notable given the widespread occurrence of nickel allergies, particularly among women [32, 33]. As orthodontists, conducting cytotoxicity studies like ours helps clarify these concerns by providing crucial information to patients. This research illuminates the potential impacts and safety of orthodontic appliances, aiding in patient education and ensuring informed healthcare decisions.

In light of these findings, the present study aimed to investigate the cytotoxicity of five commonly used fixed functional appliances. The hypothesis of the study is that appliances with different material compositions will show varying levels of cytotoxicity. To our knowledge, this is the first study to assess their biocompatibility by evaluating their effects on cell proliferation and viability.

Materials and method

This study was conducted as part of a PhD thesis with the approval of the Non-Interventional Clinical Research Ethics Committee of Biruni University (Decision No: 2023/85–09). In our study, we investigated the cytotoxicity of five different fixed functional appliances, commonly used in orthodontic treatment, which are composed of both metallic and non-metallic components. The appliances included Jasper Jumper (American Orthodontics, Wisconsin, USA), Power Scope (American Orthodontics, Wisconsin, USA), Herbst (American Orthodontics, Wisconsin, USA), Forsus TM Fatigue Resistant Device Kits (FRD, 3 M, St. Paul, MN, USA), and Twin Force Bite Corrector (Henry Schein Orthodontics, CA, USA). The materials and brands are presented in Table 2. The study utilized of 5 fixed functional appliances samples, with 10 samples in each group was compared with control groups

Table 2 The appliances and their brands used in the study

Appliance	Brand
Jasper Jumper	(American Orthodontics, Wisconsin, USA)
Power Scope	(American Orthodontics, Wisconsin, USA)
Herbst	(American Orthodontics, Wisconsin, USA)
Forsus TM Fatigue Resistant Device Kits	(FRD, 3 M, St. Paul, MN, USA)
Twin Force Bite Corrector	(Henry Schein Orthodontics, CA, USA)

of a human gingival fibroblast (HGF) cell line (PCS-201–018, ATCC; Manassas, Virginia, USA).

Sample size calculation

The sample size was calculated as $n=70$ ($n=10$ per group/cell line). In this research, each of the 5 fixed functional appliances, 1 positive control group and 1 negative control group was tested 10 times per group to investigate variations within independent groups and over time. Prior studies were reviewed to help establish an appropriate sample size. Using the "G. Power-3.1.9.2" software (Heinrich Heine University Düsseldorf, Germany) the required sample size was calculated at a 95% confidence level [34]. The analysis determined that with $p=0.05$ and a standardized effect size of 1.1082, obtained from a similar study [35], the minimum sample size required per group was calculated as 3, ensuring a theoretical power of 95%.

Sample preparation

The appliances were sterilized following the manufacturers' recommendations and in accordance with ISO 10993–5 standards using autoclave sterilization [29, 30, 35, 36]. ISO 10993–5:2009 describes test methods for assessing the in vitro cytotoxicity of medical devices. These methods involve incubating cultured cells in direct or diffusion contact with a device and/or its extracts. The methods are designed to determine the in vitro biological response of mammalian cells using appropriate biological parameters. [30]. The samples were incubated in saline for 8 weeks and maintained at 37 °C under stable conditions. Following ISO recommendations, the sample weights were adjusted to a dilution ratio of 0.1 g/ml by dividing the weights by the dilution volumes. This means for every milliliter of solvent used, 0.1 g of the sample is required. To get this ratio, the total weight of the sample is divided by the dilution volume. For example, if you have 1 g of a sample and you need a 0.1 g/ml concentration, you would need to dissolve this in 10 ml of the solvent. The samples were preserved at − 20 °C until they were analyzed [30]. A negative control group, consisting of saline without any fixed functional appliance material, was cultured under the same conditions.

Preparation of cell cultures

Processes of dissolving gingival cell suspensions

To assess the cytotoxicity of the fixed functional appliances, a human gingival fibroblast (HGF) cell line (PCS-201–018, ATCC; Manassas, Virginia, USA) was utilized. Cells stored in 1 ml medium (95%) + DMSO (Dimethyl sulfoxide) (5%) were used for each cryo-tube (1.8 ml). During the dissolution of the cells, the cells were removed from −80 °C and kept in a water bath at 37 °C for ~ 1 min. To remove the DMSO used in the freezing phase, 5 ml of DMSO-free Minimal Essential Medium (DMEM) (Biochrom KG, Berlin, Germany) was added, and the sample was centrifuged at 1500 rpm for 5 min. The supernatant was poured, 1 ml medium was added to it, and homogenization was achieved by pipetting. In order to determine the number of live and healthy cells, the cells were stained with Trypan blue (1:1), and cell counts were performed. Passages were made according to the number of cells, and they were performed in 25 flasks.

Cell culture

The old medium on the cells was removed and the cells were washed with Phosphate-Buffered Saline (PBS). Trypsin/EDTA solution was added and incubated for 3 min. A new medium was added to the trypsin and centrifuged and cell counts were performed on the toma slide. According to the number of cells, the passage of the cells was carried out in 6 well plates for 0.5×106 cells into 2 ml combination medium. Cell confluence controls were performed under an inverted microscope at 48-h intervals.

Material incubation with media

The materials intended to be applied to the cells were added to each well on 12 well plates and incubated for 1 week with the relevant materials. After the procedure, the media were filtered with a 0.22 µm filter and prepared for MTT experiments. When 96 Well is sown in plates and 80% confluence was achieved the existing media of the cells were removed.

Passage series were created and cells were multiplied up to passage 4 for experiments, and cells were planted on 96 well plates with standard medium for MTT analysis. This is how the 96 well plate plan was made. 20 K cells were placed in the control and sample weller, and only DMEM was placed in the blank. Samples were numbered from DMEMs with both materials individually and left for a week.

3-(4,5-Dimethylthiazol-2-yl)−2,5-diphenyltetrazoliumbromide assay (MTT Assay)

Following an incubation period of 1 week, the MTT was performed. A volume of 10 µl MTT solution was added to each well, and the plates were incubated at 37 °C in a CO_2 incubator for 4 h. To dissolve the formazan crystals produced by viable cells, 100 µl of sodium dodecyl sulfate solution was added to each well and left in the CO_2 incubator for an additional 16–18 h.

After this period, the optical densities of the samples were measured at a wavelength of 540 nm using an ELISA reader (SPECTROstar® Nano, Germany). Cells cultured in DMEM medium without exposure to fixed functional appliance served as the negative control, representing 100% cell viability. This reference was used to determine the cytotoxicity levels.

Cell proliferation was measured using the MTT assay, which is based on the metabolic activity of cells. A higher proliferation index indicates that the cells are active and healthy, while a lower index suggests potential cytotoxicity. High proliferation index: Indicates that the cells are metabolically active, suggesting that the materials (appliances) in the environment have low or no toxic effects. Low proliferation index: Indicates reduced cell growth and metabolic activity, which may suggest that the material exhibits toxic effects.

The cell proliferation percentages for each test material were calculated using the optical density values obtained from the reader and the formula provided by Vande Vannet et al. [29, 37]

The calculation was performed as follows:

$$\text{Cell Viability } (\%) = (\textit{Optical Density of Test Group} / \textit{Optical Density of Cellular Control Group}) \times 100$$

To assess cytotoxicity levels, the classification defined by Ahrari et al. [38] was applied:

- More than 90% cell viability: No cytotoxicity
- 60% to 90% cell viability: Mild cytotoxicity
- 30% to 59% cell viability: Moderate cytotoxicity
- Less than 30% cell viability: Severe cytotoxicity

Statistical analysis

In this study, descriptive statistics of the data (number, percentage, mean, standard deviation, median, minimum, and maximum) were provided. The assumption of normality was tested using the Shapiro–Wilk test. For comparisons among three or more independent groups that do not meet the assumption of normality, the Kruskal–Wallis test was used. Post hoc Bonferroni-adjusted tests were conducted to identify the group or groups responsible for the observed differences. The analysis determined that with $p = 0.05$ and a standardized effect size of 1.1082, obtained from a similar study [35], the minimum sample size required per group was calculated as 3, ensuring a theoretical power of 95%. All analyses were performed using IBM SPSS version 25(IBM, Chicago, IL,USA).

Results

The cell viability rates of all appliances were comparatively analyzed against the control group. According to the results of Dunnett's multiple comparison test (Post Hoc), none of the appliances demonstrated a statistically significant cytotoxic effect compared to the control group ($p > 0.05$). The distributions and comparisons of the measurements across the study groups are presented in Table 3.

The assumption of normality was tested using the Shapiro–Wilk test. For comparisons among three or more independent groups that do not meet the assumption of normality, the Kruskal–Wallis test was used.

None of the appliances demonstrated a statistically significant cytotoxic effect compared to the control group ($p > 0.05$). However, the analysis revealed statistically significant differences in measurements among the study groups ($p < 0.05$). According to the Bonferroni post-hoc tests, statistically significant differences were identified between the Blank group and the control, Power Scope, Jasper Jumper, Herbst, Forsus, and Twin Force groups ($p = 0.011$, $p = 0.008$, $p = 0.003$, $p < 0.001$, $p < 0.001$, and $p < 0.001$). The measurements (Fig. 1) for the control, Power Scope, Jasper Jumper, Herbst, Forcus, and Twin Force groups were higher than those of the Blank group.

Although such differences in proliferation values were observed, statistical analyses revealed no significant differences between the control group and the study samples ($p > 0.05$). This indicates that all appliances used in the study can be classified as non-cytotoxic.

Table 3 The distributions and comparisons of the measurements across the study groups (*$p < 0.05$)

	Min.-Max.**	Mean ± SD***	p	Post Hoc
Blank[a]	0.05–0.16	0.06 ± 0.03(0.05)	< 0.001*	b > a
Control[b]	2.07–3.88	2.66 ± 0,72(2.29)		
Power Scope[b]	0.47–3.37	2.05 ± 1.06(2.20)		
Jasper Jumper[b]	0.49–3.91	1.90 ± 1.23(1.91)		
Herbst[b]	0.84–3.6	2.62 ± 1.13(3.19)		
Forsus[b]	1.3–3.83	2.48 ± 0.98(2.53)		
Twin Force[b]	0.72–3.75	2.33 ± 1.21(3.03)		

** Minimum–Maximum, ***Standart Deviation

[a] blank group, [b]control and appliances groups

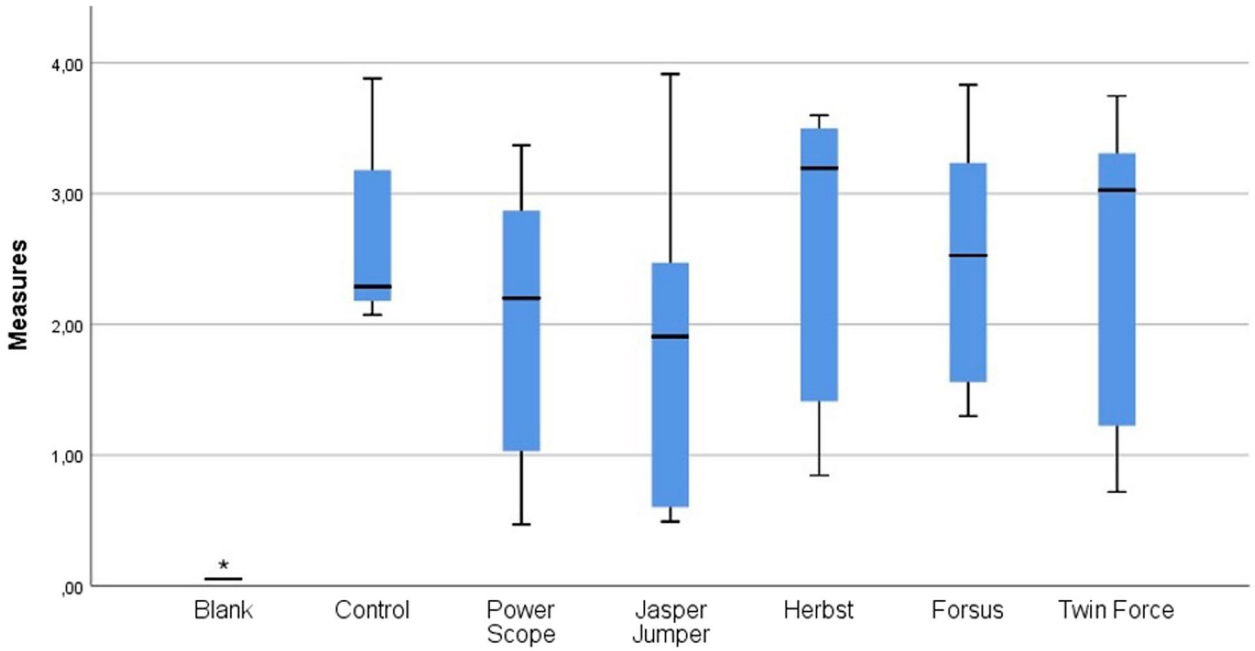

Fig. 1 The box plot shows the distribution of assay measurements across the study groups

The cell proliferation index graph for the cytotoxicity evaluation of fixed functional appliances (Fig. 2) illustrates the cell proliferation rates (mean ± standard deviation) for all appliance groups, including the control group. The findings demonstrate no significant cytotoxic effect compared to the control group, with all groups exhibiting similar viability values.

Herbst demonstrated the highest proliferation index (2.62 ± 1.13), suggesting that this appliance provides a more conducive environment for cellular proliferation.

Forsus (2.48 ± 0.98) and Twin Force Bite Corrector (2.33 ± 1.21) exhibited proliferation indices relatively close to the control group, though marginally reduced.

Power Scope (2.05 ± 1.06) and Jasper Jumper (1.90 ± 1.23) displayed the lowest proliferation indices, placing them at the lower end of the ranking.

Based on the cell viability (%) calculations, viability rates were above 90%. All appliance groups fell into this category, indicating that none of the appliances exhibited clinically significant cytotoxic effects.

All orthodontic appliances were classified as non-cytotoxic according to standard cellular viability criteria. Although there were differences in the cell proliferation indices of the appliances, statistical analysis revealed no significant cytotoxic effects compared to the control group ($p > 0.05$).While no significant increase in proliferation was observed compared to the control group, the distribution of results across all groups was homogeneous.

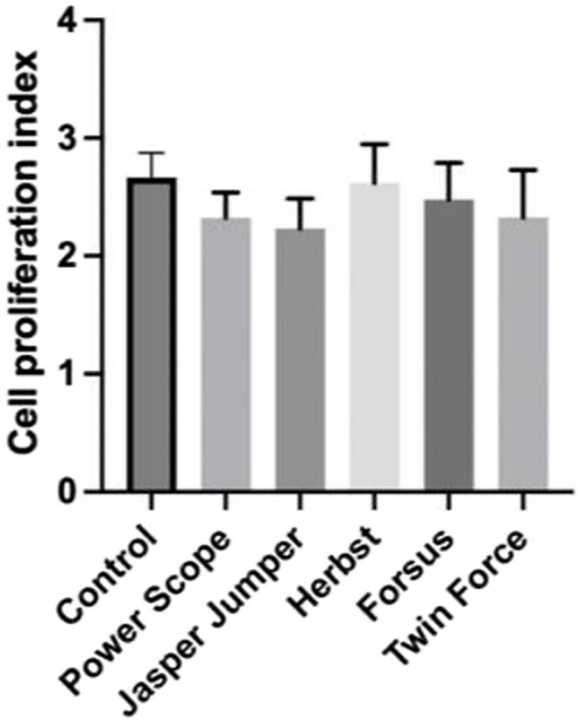

Fig. 2 Cell proliferation index graph for fixed functional appliances used in the study

According to the results, all appliances demonstrated cell viability rates above 90%. Based on the classification by Ahrari et al. [38], the appliances were found to meet international standards (ISO 10993–5).

Discussion

The release of metal ions during orthodontic treatment has become a critical factor in assessing the biosafety of orthodontic appliances [39]. The majority of appliances commonly used in orthodontic treatment are composed of alloys containing cobalt (Co), chromium (Cr), iron (Fe), nickel (Ni), and titanium (Ti). Among these, Ni and Cr have become a major concern because of their intrinsic toxicity and the high levels found in metal-based orthodontic appliances, which generally contain 8–50% Ni and 17–22% Cr [40]. Ni and Cr, specifically, have been linked to hypersensitivity reactions, as well as cytotoxic and genotoxic effects [41]. Wataha et al. emphasizes that alloy corrosion is a critical determinant of biocompatibility, as the release of alloy constituents can result in adverse biological effects, including toxicity, hypersensitivity reactions, mutagenicity, and carcinogenicity. The corrosion process facilitates the release of free ions, which can have detrimental effects on surrounding tissues [42].Metals are biologically non-degradable but prolonged ion release can result in irreversible toxic effects due to accumulation in tissues. Moreover, increased exposure can limit the recovery time necessary for cellular repair [22, 43]. The cytotoxicity of a material can be assessed through both in vitro and in vivo methods. In vitro evaluation is conducted using cell culture techniques, which can involve colorimetric, luminometric, or enzymatic assays [44]. Cytotoxicity tests provide initial insights into a chemical's ability to interfere with cellular functions and potentially lead to cell death, making it important to identify the mechanisms behind such effects. While in vitro results can suggest possible in vivo outcomes, a material's cytotoxic behavior in lab settings does not automatically imply toxicity in clinical use. Differences between controlled lab conditions and the complex conditions of the oral environment can significantly influence these results [19, 28, 36]. However, not observing a cytotoxic effect does assure a positive clinical response [45]. Controlled laboratory conditions ensure the reliability of findings from in-vitro studies. The temperature of 37 °C used in this research aligns with the recommendations of the International Organization for Standardization (ISO) which is the most commonly utilized temperature in studies investigating cytotoxicity assays [30, 46]. MTT analysis is a highly sensitive and quantitative colorimetric assay that operates on the principle of reducing yellow tetrazolium MTT salt to dark purple formazan, a reaction catalyzed by the succinate dehydrogenase enzyme present in mitochondria. This reduction occurs exclusively in metabolically active cells, making the assay a reliable method for assessing cell viability. The MTT assay is valued for its rapid and precise results, enabling the detection of even minimal levels of toxicity [47].In our study, a MTT assay was conducted to assess cell viability. This method, widely used in orthodontics, is straightforward, reliable, and reproducible, measuring mitochondrial dehydrogenase activity to determine cytotoxicity [29, 36, 48].

In our study, during the MTT analysis, the fluid in which the appliances were kept for two months was used to culture the cells for one week. The cells were then allowed to proliferate, and samples were subsequently collected after 1 week. The norm ISO 10993–5 does not specify a precise time for sample collection [30]. Different incubation periods have been used in various studies. In their study on the cytotoxicity of dental materials, Diemer et al. [49] established a 1 week incubation period.The study conducted by Ortiz et al. [50], the fibroblasts cultured were maintained for 1 week using minimum essential medium to assess their viability. In their cytotoxicity research, Whishney et al. [51] set the incubation duration as 1 week. House et.al. studied the cytotoxicity of brackets and bands, samples were collected on the 3rd and 14th days [24]. Çifçi Ozkan et.al. examined the cytotoxicity of clear aligners; samples were taken 72 h later [29]. In another study, the cytotoxicity of esthetic, metallic, and nickel-free orthodontic brackets was examined. In that study, samples were collected after 24 h, and an MTT analysis was performed [52]. In another study examining the cytotoxicity of orthodontic bonding adhesives, samples were collected on the 1st, 3rd, 5th, and 7th days [38]. Based on these studies, an incubation period of one week was chosen. Utilizing extended incubation times in different experiments could reveal more insights.

Many studies have examined the cytotoxicity of materials used in orthodontics throughout history.Previous cytotoxicity and ion releasing researchs have revealed that stainless steel orthodontic bands release iron and nickel at concentrations sufficient to induce cytotoxic and genotoxic effects [28]. Park and Shearer [53] reported the average daily release of nickel and chromium ions in vitro, while Barrett et al. [54] examined the corrosion rates of orthodontic appliances and compared stainless steel and NiTi archwires. Silver, commonly found in orthodontic materials, has been identified as a cytotoxic material [28, 42, 55, 56]. Certain studies have demonstrated that silver solder causes severe cytotoxicity by inhibiting cell proliferation, growth, and development [28, 55, 56]. In our study, we examined the Power Scope appliance, which is specified in its catalog to contain zinc, silver, and copper. It demonstrates lower proliferation

values compared to the Herbst appliance which does not contain silver.

Stainless steel is one of the components used in constructing the appliances. Studies have reported that stainless steel does not impair cellular proliferation and may be regarded as a non-cytotoxic material [35, 55]. The corrosion resistance of stainless steel is primarily attributed to its chromium content, which enables the formation of a protective oxide layer [57]. Based on the findings of our study, the Herbst appliance demonstrated the highest cell proliferation values, suggesting that it can be considered one of the most reliable orthodontic appliances. However, further studies are required to confirm these releases. Jasper Jumper has a soft gray synthetic material which the composition of the gray coating is unspecified, encasing the open-coil spring that tends to deform after approximately three months of use [5, 6]. The Jasper Jumper showed lower cell proliferation values compared to other appliances. This might be attributed to the gray material coating it.

While the study's findings suggest that the appliances are safe, there are several limitations to the experimental design. Both the properties of the material and the characteristics of the host play a role in determining biocompatibility, and thus both aspects must be thoroughly evaluated [58]. Standardizing the methods used for in-vitro evaluations of the cytotoxicity of orthodontic materials would enhance our understanding of this area [59]. A significant constraint of this research is its in vitro setup, which might not accurately reflect the full spectrum of in vivo biological interactions and responses. Future studies should aim to replicate these findings in longer-term clinical settings and expand the range of materials and conditions studied to cover the full spectrum of clinical scenarios encountered in orthodontic practices.

Conclusıon

According to the results of our study, the Herbst appliance demonstrated the highest proliferation index, indicating that it provides the most favorable environment for cellular growth. This suggests a high level of biocompatibility. The Forsus and Twin Force Bite Corrector appliances showed results similar to the control group, indicating that they can be safely used. Although the Jasper Jumper and Power Scope appliances exhibited relatively lower proliferation values, they were classified as non-cytotoxic and are suitable for clinical use. Although variations were observed in the cell proliferation indices among the tested appliances, statistical analysis demonstrated no significant cytotoxic effects compared to the control group ($p > 0.05$). Furthermore, no significant increase in proliferation was detected, and the homogeneous distribution of results across all groups supports the rejection of the study's hypothesis.

From a clinical perspective, the results are reassuring for both practitioners and patients concerned about the potential adverse effects of long-term exposure to orthodontic appliances. Specifically, the study highlights the importance of choosing appliances made from materials that minimize the risk of allergic reactions and toxicity, a concern particularly relevant given the increasing prevalence of metal sensitivities, such as nickel allergies. Moreover, the research emphasizes the need for orthodontic professionals to stay informed about the materials in the devices they use. Providing patients with detailed information about the safety and composition of these materials can enhance patient trust and compliance, which are important for the success of orthodontic treatments.

Abbreviations

HGF	Human Gingival Fibroblast
DMSO	Dimethyl Sulfoxide
DMEM	DMSO-free Minimal Essential Medium

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Not applicable.

Authors' contributions

NO collected the data and AB did the research. AB and HG wrote the paper together. All authors read and approved the final manuscript.

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Data availability

The data underlying this article cannot be shared publicly due to ethical concerns.The data will be shared on reasonable request to the corresponding author.

Declarations

Ethics approval and consent to participate

This study was conducted as part of a PhD thesis with the approval of the Non-Interventional Clinical Research Ethics Committee of Biruni University (Decision No: 2023/85–09).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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