Original Article

The corrosion products of proprietary and generic orthodontic fixed lingual retainers and their in-vitro cytotoxicity

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ABSTRACT

Objective: To assess the corrosion products and cytotoxicity of generic and proprietary fixed lingual retainers (FLRs).

Materials and Methods: Seven FLRs were investigated. Wires were submersed in solution for 34 days, at 37°C, under constant agitation. A proportion of this solution was analyzed to determine the concentration of metallic ions leaching off the wires. The remainder was diluted to 5%, 10% and 20% followed by exposure to human gingival fibroblasts and analysis of cytotoxicity of the wires.

Results: Three wires (Dentaflex, Universal, and AZDent) released excessive concentrations of lead, two wires (MeshMark and Orthoflex) released excessive concentrations of nickel, and one wire (Universal) released excessive concentrations of molybdenum into solution. No statistically significant difference was found between the wires analyzed ($P = .24$). Slight cytotoxicity was noted in only one wire (Dentaflex) at a 20% dilution of eluent. This was also the wire which released the highest concentration of lead into solution. All other wires, at all concentrations, were deemed noncytotoxic, but five samples overall were deemed statistically significant ($P < .0024$). A statistically significant difference existed between wires ($P = .013$) and concentrations analyzed ($P < .001$).

Conclusions: Metals were released in differing quantities from all wires, with some elemental concentrations measuring more than that deemed acceptable in drinking water in Australia. A trend toward increased cell viability across samples was found with only one demonstrating cytotoxicity. There was no indication that generic FLRs were more or less biocompatible than their proprietary counterparts. (*Angle Orthod*. 2024;94:664–671.)

KEY WORDS: Corrosion; Cytotoxicity; Fixed lingual retainer; Retainers; Orthodontics

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INTRODUCTION

Relapse is the return of the dentition toward its preorthodontic position. Retainers are used to minimize this change.[1] Fixed lingual retainers (FLRs) are commonly used in orthodontics for the prevention of relapse. Their use is becoming more popular, with lifelong wear being recommended by many clinicians.[2] The retention phase constitutes the longest phase of orthodontic treatment.

Biocompatibility of medical devices is of primary importance to clinicians. Therefore, FLRs typically undergo rigorous testing prior to their use. However, availability of products online means that illegally imported products can bypass these tests. Metallic appliances are known to corrode in the mouth, and release of ions has been observed in vitro and in vivo.[3] These ions can result in adverse reactions to the patient, both locally and systemically.[4] The main elements which have previously undergone investigation in this context are nickel and chromium.[5] These and many other ions have been detected in small concentrations leaching from orthodontic appliances including FLRs.[6,7] Fluoride intake, agitation conditions, exposure to high temperatures, and pH variations have been found to influence the metal release from metallic appliances.[8,9] The release of ions is generally not proportional to the overall content of that metal in the wire.[10]

Although authors of some studies have shown evidence of cytotoxicity and mutagenicity,[11,12] the concentrations have generally been deemed to fall below the toxic threshold. Despite this, subtoxic concentrations can still initiate reactions. Associations have been highlighted between leached ions and allergic reactions, asthma, eczema, and local inflammatory reactions.[4] Nickel-containing orthodontic appliances have the propensity to cause swelling, gingival hyperplasia, angular cheilitis, labial desquamation, and burning sensations of the oral mucosa.[13] This inflammatory response is defined as a type-IV hypersensitivity reaction. Adverse reactions are estimated to occur in 1 in every 100 patients, with most of these (85%) presenting in the form of contact dermatitis associated with extraoral headgear components.[14]

The aims of this study were to assess the cytotoxicity of generic and proprietary FLRs and to analyze the concentration of corrosion products leaching from these wires into solution.

MATERIALS AND METHODS

Metallic Components Leaching From Wires

Seven stainless steel FLRs (four proprietary and three generic) were analyzed (Table 1). All wires were kept in their original packaging until the beginning of the experiment. A 1 g mass of each wire was cut, weighed, and sterilized at 134°C for 30 minutes. Based on the exact weights of the wires, high glucose, phenol red-free Dulbecco's Modified Eagle Medium (DMEM) supplemented with an antibiotic solution containing 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B (Gibco, Life Technologies, Grand Island, NY) was added to achieve a wire to DMEM concentration of 0.2 g/mL (20% w/v solution) as per the protocol defined by the International Standards Organization (ISO).[15] Samples were placed on an agitating table for 34 days at 37.4°C and 5% carbon dioxide. Here, 3 mL of solution was then microwave acid digested and analyzed using inductively coupled plasma mass spectrometry (Nexion 300X, Perkin Elmer, Houston, Tx) and inductively coupled plasma optical emission spectroscopy. A control sample consisting of DMEM only was analyzed and later subtracted from the readings of the test samples.

Table 1. Samples Selected

	Wire	Supplier
Generic		
1	AZDENT	eBay
2	Universal	eBay
3	MeshMark	eBay
Proprietary		
1	Dentaflex	Dentaurum Australia
2	Remanium	Dentaurum Australia
3	Bond A Braid	Orthodontic Supplies Australia
4	Ortho Flextech	Orthodontic Supplies Australia

Cytotoxicity of Human Gingival Fibroblasts When Exposed to Eluent

Human gingival fibroblasts (HGFs), American Type Culture Collection (PCS-201-018), were serial passaged until Passage 5 and full confluency was achieved. Cells were seeded at a density of 14,000 cells and 30% confluency in a treated 96-well microplate (Sigma-Aldrich, Darmstadt, Germany).

Eluent was received in its 20% concentration and was diluted to 10% and 5% (v/v) in DMEM/antibiotics/Fetal Bovine Serum (FBS) 10% under sterile conditions.

Here, 100 μl of each sample eluent was pipetted into its corresponding well in triplicate. Additional wells were seeded for control purposes. Three wells contained cells exposed to DMEM/antibiotics and served as negative control. Three wells contained cells exposed to DMEM/antibiotics/FBS10% and served as a positive control. Three wells contained no cells but were treated with a DMEM/antibiotics/FBS10% and acted as the blank. After 24 hours of incubation, 20 μL of MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] assay (Promega, Cell Titer 96® AQueous One Solution Assay, Beijing, China)

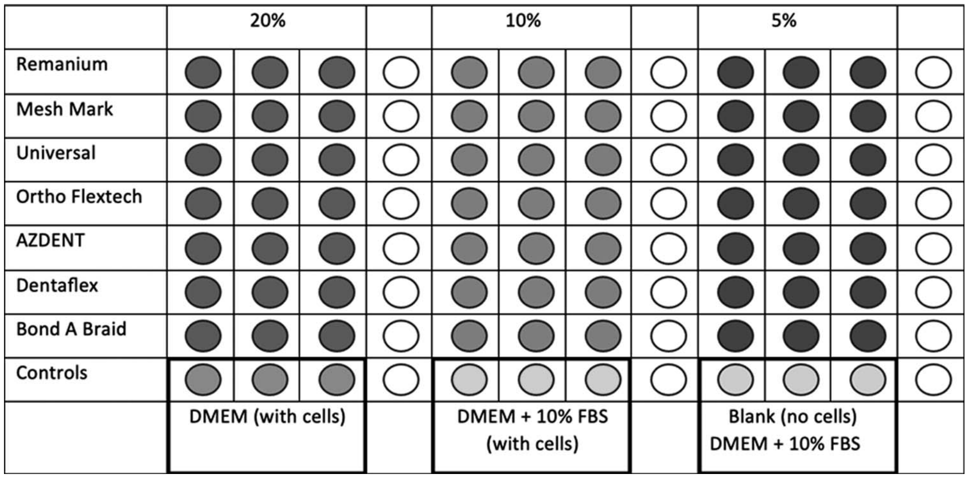

Figure 1. 96-well plate design.

was added to each well (Figure 1), and the microplate was incubated for a further 2.5 hours, at which point a color change was evident. Optical density of the wells was measured at 490 nm using a Spectra Max i3 and SoftMax Pro6.5 software (Molecular Devices San Jose, CA).

Analysis of Optical Densities

A mean of the three blank readings was calculated and subtracted from all the other readings to correct for the optical density of the MTS reagent. The formula used for determination of cell viability was that described by Ahrari et al.[16]:

$$([\text{Optical density of test group}/\ \text{optical density of positive control}] \times 100).$$

A value more than 90% signified no cytotoxicity, 60–90% signified slight cytotoxicity, 30–59% signified moderate cytotoxicity, and $<30\%$ signified severe cytotoxicity.

Statistical Analysis

The statistical analysis of the cytotoxicity data was conducted using a general linear model (analysis of variance [ANOVA]) for the adjusted %cytotoxicity. Wire (seven materials) and concentration (5%, 10%, and 20%) were fixed factors, and a full factorial model with interaction was fitted. To further examine the statistical significance of differences from 100% in each group, one sample *t*-test comparisons were carried out with Bonferroni adjustment for multiple testing ($P = .0024$ level of significance). Dunnett's test was employed to analyze the data against positive controls. A further two-way ANOVA was used to analyze the statistical significance of the composition of the eluent.

RESULTS

Metallic Components Leaching from Wires

The elements eluted from the FLRs are tabulated in Table 2. Overall, no statistically significant difference was found among the wires analyzed ($P = .24$); however, the findings were interesting. Sulfur and zinc were the most abundant elements, while cadmium and palladium were the least eluted elements. Lead was noted in the eluent of all wires with Universal, AZDent, and Dentaflex measuring the highest concentrations. Copper release was highest in MeshMark, Universal, and Orthoflex and was 3 to 5 times less in all other wires. Iron was a stable component of the wires with only two of the proprietary wires demonstrating iron leaching. Similarly, chromium was considered stable, as it leached from only Remanium, MeshMark, and Orthoflex in small quantities. Arsenic leached from all wires, with Remanium measuring the highest and BondABraid measuring the lowest. Nickel leached from all wires except Remanium. The highest concentration of nickel leached from MeshMark, which was five times higher than any other value. Three of the proprietary brands—Remanium, BondABraid, and Dentaflex—leached the smallest concentrations of nickel into solution. Molybdenum release was an exceptionally high leaching product of the Universal wire, which measured 10 times more than the next highest wire, Orthoflex.

Cytotoxicity of HGFs when Exposed to Eluent

The viability for 5%, 10%, and 20% concentration of each wire was calculated.[16] Means of the triplicates are tabulated with their corresponding percentage standard deviations (Table 3). The mean viabilities are illustrated graphically in Figure 2, in which the positive control has a value of 100%. A pipetting error occurred with one

Table 2. Concentration of Eluted Elements in Solution

	Unit	Detection Limit	Generic		
			AZ Dent	MeshMark	Universal
Sulfur (S)	p.p.m	0.5	1.2	1.1	1.3
Zinc (Zn)	p.p.m	0.000159	1.0589	1.6429	1.2029
Iron (Fe)	p.p.b	0.388	—	—	—
Copper (Cu)	p.p.b	0.049	43.31	154.66	176.52
Nickel (Ni)	p.p.b	0.041	13.09	118.93	3.68
Manganese (Mn)	p.p.b	0.010	1.49	6.34	5.20
Lead (Pb)	p.p.b	0.002	18.72	5.61	19.68
Molybdenum (Mo)	p.p.b	0.107	9.82	19.57	530.37
Selenium (Se)	p.p.b	0.071	0.93	5.20	6.12
Arsenic (As)	p.p.b	0.008	1.36	4.78	3.88
Strontium (Sr)	p.p.b	0.0009	3.02	2.63	2.65
Chromium (Cr)	p.p.b	0.010	—	0.16	—
Cadmium (Cd)	p.p.b	0.027	0.06	0.74	0.72
Palladium (Pd)	p.p.b	0.001	0.13	0.82	0.19

sample of MeshMark 10%; therefore, this value was excluded, and means were calculated from the remaining two samples. Table 3 also illustrates the wire-concentration combinations with statistical significance ($P = .0024$). When the concentrations were combined, ANOVA revealed statistically significant differences among the tested wires ($P = .013$). In addition, when the wires were combined as one group, ANOVA displayed high statistical significance among the concentrations of eluent ($P < .001$), where significance was $P < .05$.

When analyzing the statistical significance between wires when the factor of concentrations was grouped together, the only wire combination with statistically significant variations in cytotoxicity was Universal with Dentaflex ($P = .023$). When comparing concentrations with the factor of wires grouped together, 5% with 10% was statistically significantly different ($P = .002$) as well as 5% with 20% ($P < .001$).

In most cases, higher viability was observed in the cells which had been exposed to the eluent than the controls. The highest viability was noted in Universal 5% at 132%. Of interest, 5% solutions had a general trend toward higher viability than the 20% solutions. The 20% solutions had a mean viability of 102%, the 10% readings had a mean of 103%, and the 5% readings had mean of 119%. The lowest mean viability was 88% in Dentaflex 20% which indicated slight cytotoxicity.[16]

DISCUSSION

A wide variety of metal concentrations leached off the FLRs assessed. Worldwide safety thresholds for exposure to elements has not been definitively established. For example, the tolerable upper intake level for adults in the United States and Canada is 1 mg/day,[17] but no available tolerable upper intake level for chromium exists. However, an adequate intake has been defined which serves as a guide to the recommended intake, which is 20–35 μg/day for adults.[18] Since definitive literature regarding the recommended daily allowances of elements is scarce, reference to the tolerable thresholds of elements in drinking water is commonly referred to as a benchmark. Release of lead is of particular concern to human health due to the association with kidney damage and interference with erythropoiesis.[19] The maximum amount of lead acceptable in drinking water is 10 ppb. However, three wires leached concentrations of lead which exceeded this. Dentaflex, a proprietary wire, leached the most lead (27.28 ppb), and two generic wires, Universal and AZDent, also exceeded this value (19.68 and 18.72 ppb, respectively).

Data available are insufficient to set a maximum safe threshold for presence of iron and zinc in water. However, the taste threshold has been cited as 300 ppb for iron and 3000 ppb for zinc. In the current study, all leached iron and zinc concentrations fell below this threshold, with the maximum being 43 ppb in Remanium for iron, and 1826.9 ppb in Orthoflex for zinc.

Chromium is commonly present in stainless steel because it confers corrosion resistance. Chromium leached from Remanium, MeshMark, and Orthoflex in small quantities. The highest release was from Orthoflex at 4.14 ppb, which was more than 10 times below the acceptable drinking water concentration of 50 ppb.[19]

Nickel provides ductility, strength, and toughness to orthodontic materials. However, it can be released from metallic appliances during corrosion and has associated biocompatibility concerns including nephrotoxicity, dermatitis, lung, sinus, and nasal cancer. Nickel was evident in the eluent from all wires except for Remanium, in which the content was comparable with the control value. The highest amount of nickel

Table 2. Extended

	Unit	Proprietary			
		Remanium	Orthoflex	Dentaflex	BondABraid
Sulfur (S)	p.p.m	0.5	1.4	0.6	1.0
Zinc (Zn)	p.p.m	0.000159	1.8269	1.4709	0.8759
Iron (Fe)	p.p.b	0.388	—	41	—
Copper (Cu)	p.p.b	0.049	115.28	44.89	49.03
Nickel (Ni)	p.p.b	0.041	22.38	1.16	1.45
Manganese (Mn)	p.p.b	0.010	47.65	15.58	8.20
Lead (Pb)	p.p.b	0.002	8.68	27.28	6.34
Molybdenum (Mo)	p.p.b	0.107	45.37	2.85	1.65
Selenium (Se)	p.p.b	0.071	2.31	0.11	—
Arsenic (As)	p.p.b	0.008	2.55	0.85	0.52
Strontium (Sr)	p.p.b	0.0009	2.07	1.71	1.46
Chromium (Cr)	p.p.b	0.010	4.14	—	—
Cadmium (Cd)	p.p.b	0.027	0.42	0.26	0.08
Palladium (Pd)	p.p.b	0.001	0.67	0.19	0.09

leached from MeshMark (118.93 ppb), which was five times higher than any other value. Orthoflex leached the second highest amount of nickel (22.38 ppb) into solution. Both wires exceeded the concentration permissible in drinking water of 20 ppb.[19] Therefore, the use of MeshMark and Orthoflex should be carefully considered, especially in those with known nickel allergy, at least until further studies have been carried out to validate the results. Three of the proprietary brands, Remanium, BondABraid, and Dentaflex, leached the smallest concentrations of nickel into solution.

Arsenic has known hazards to humans, and 0.01 ppm is the maximum concentration deemed acceptable in drinking water in Australia.[19] In this study, arsenic leached from all wires to different extents, with Remanium measuring the highest of the group at 5.86 ppb and BondABraid the lowest (0.52 ppb). However, both still fell below that which was acceptable in drinking water of 0.01 ppm. This is consoling since excessive arsenic intake is related to the development of cancer of the skin, lungs, bladder, and kidney.[19]

All wires leached concentrations of cadmium and manganese below that acceptably present in drinking water (2 ppb and 500 ppb, respectively).[19]

Copper release was highest in MeshMark, Universal, and Orthoflex and was 3 to 5 times less in all other wires. Even though copper is known to be easily released from metals (labile),[20] concentrations of leached copper were at least 10 times less than the concentration of 2000 ppb, which is deemed acceptable in drinking water.[19]

The molybdenum released from Universal (530 ppb) was more than 10 times that which was deemed acceptable for drinking water (50 ppb). All other wires fell below the safe threshold for water. Fortunately, authors of studies have shown that significant bioaccumulation of molybdenum in the body does not appear, with approximately 90% of ingested molybdenum being released from the body in urine. Studies are ongoing to investigate any health concerns

Table 3. Mean of Triplicate Ratio of Optical Density of Test Samples to Optical Density of Positive Control with % Standard Deviation

	20%		10%		5%		
	Mean (%)	SD (%)	Mean (%)	SD (%)	Mean (%)	SD (%)	Average (%)
Generic							
AZ Dent	99	21.5	107	8.4	115	11.0	107
MeshMark	101	15.9	96	12.0	127	20.6	114
Universal	120*	9.0	106	5.3	132	24.8	119
Proprietary							
Remanium	114*	9.5	101	1.7	98	5.7	104
Orthoflex	100	2.0	117*	12.3	130*	6.5	116
Dentaflex	88*[a]	3.0	96	15.0	112	18.5	99
BondABraid	93*	2.6	101	2.0	111	9.8	102
Average	102		103		119		108

* Statistically significant, $P < .0024$.
[a] Cytotoxic.

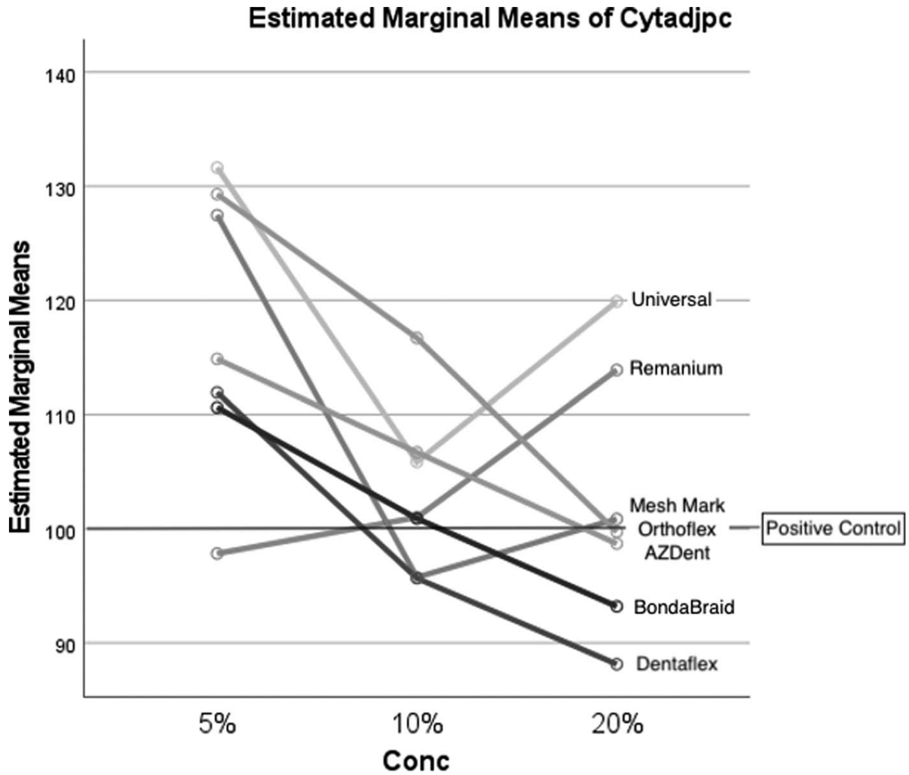

Figure 2. Mean cell viability relative to positive control.

associated with increased intake of molybdenum, but at present, such evidence is lacking.[19]

In the cytotoxicity investigation, Dentaflex at 20% concentration was the only wire that showed slight cytotoxicity with a reading of 88% viability in comparison with the positive control. Interestingly, Dentaflex also had the highest amount of lead leached into its eluent. This may be one of the factors contributing to the higher cytotoxicity in Dentaflex.

In contrast, the generic branded Universal wire at 5% demonstrated the highest viability at 132%. This was followed by a proprietary wire, Orthoflex, and the other two generic wires (MeshMark and AZDent). This suggested that even generic wires, which were not Therapeutic Goods Administration (TGA) approved, seemed biocompatible. Further research is warranted to verify the exact degree of cytotoxicity in humans and to clarify cause-and-effect relationships.

A general trend was found toward higher viability in the cells which had been exposed to the eluent than the positive control cells. This was also described in previous papers in which authors reported on increased mitochondrial activity because of exposure to the metallic components.[21–23]

Terpiłowska et al.[22] investigated the effects of nickel and iron on the viability of HGFs and discovered that, at certain concentrations, cell viability was stimulated. As the concentration of iron chloride and nickel chloride increased further to 1000 μM and above, the cell viability started to decrease. It was postulated that, when cells are exposed to iron chloride at a concentration of 200 μM and nickel chloride at a concentration of 1000 μM, a protective effect of iron chloride would be found. Iron is known to contribute to the enzymatic activity of mitochondrial succinate dehydrogenase via the formation of iron-sulfur clusters. It was proposed that, at low doses, iron encourages stimulation of this enzyme and thus protects cells from nickel toxicity. Since the MTS assay assesses mitochondrial activity, this could contribute to readings which demonstrate a viability more than the control samples.

In a follow up study, the same authors[21] reported on the antagonistic effects of certain metals which exist in combination. For example, chromium was seen to protect from nickel and molybdenum toxicity. Długosz et al.[24] showed that chromium at low concentrations statistically significantly increased superoxide dismutase activity, which is one of the most important enzymes involved in antioxidant activity. These factors were suggested as an explanation for the increased viability observed.

The concentrations of leached elements detected in this study were obtained from a 0.2 g/mL wire to solution concentration as per the ISO guidelines.[15] These guidelines serve to facilitate a standardized approach to such investigations. However, it cannot be assumed that this correlates clinically to how much ionic release would occur in vivo. The leachable amount may be affected by the length, surface area, and design of the wire; dietary factors; oral hygiene practices; and pH.[3] It is oversimplified to predict that a heavier wire would release more ions than a wire of less mass. Even though this study showed that one wire released more of an element than another in the 0.2 g/mL solution, many other wires and environmental related variables affecting leaching must be considered.

FLRs are expected to stay in situ for many years. The impact of long-term degradation of materials intraorally and the bioaccumulation of corrosion products in the body over time are unknown. In vitro studies are not a perfect reflection of the in vivo environment, and this study is no exception. The ideal investigation would involve a large-scale in vivo trial analyzing the release of leaching products into the saliva as well as at distant bodily sites. Long-term in vivo cytotoxicity investigations involving analysis of bodily tissues both locally and systemically could help to establish the true safety profile of FLRs. No such studies are currently available.

CONCLUSIONS

- Elemental concentrations in the eluent were higher than that deemed acceptable in drinking water in Australia for several elements, including nickel, lead, and molybdenum.
- A trend toward an increase in cell viability following exposure of HGFs to eluents appeared.
- Slight cytotoxicity was noted in one wire. All other wires were noncytotoxic.
- There was no indication that generic FLRs were more or less biocompatible than their proprietary counterparts.
- Further long-term investigations are necessary to establish a full biosafety profile for FLRs.

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