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Cytotoxic effects of polycarbonate-based orthodontic brackets by activation of mitochondrial apoptotic mechanisms

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ABSTRACT

Objectives. The aim of the study was to evaluate the biological effects of water eluents from polycarbonate based esthetic orthodontic brackets.

Methods. The composite polycarbonate brackets tested were Silkon Plus (SL, fiber-glass-reinforced), Elan ME (EL, ceramic particle-reinforced) and Elegance (EG, fiber-glass-reinforced). An unfilled polyoxymethylene bracket (Brilliant, BR) was used as control. The brackets' composition was analyzed by ATR-FTIR spectrometry. The cytotoxicity and estrogenicity of the eluents obtained after 3 months storage of the brackets in water (37 °C) were investigated in murine fibroblasts (NIH 3T3), breast (MCF-7) and cervical cancer (CCl-2/Hela) cell lines.

Results. SL and EG were based on aromatic-polycarbonate matrix, whereas EL consisted of an aromatic polycarbonate-polyethylene terepthalate copolymer. A significant induction of cell death and a concurrent decrease in cell proliferation was noted in the EG eluent-treated cells. Moreover, EG eluent significantly reduced the levels of the estrogen signaling associated gene pS2, specifically in MCF7 cells, suggesting that cell death induced by this material is associated with downregulation of estrogen signaling pathways. Even though oxidative stress mechanisms were equally activated by all eluents, the EG eluents induced expression of apoptosis inducing factor (AIF) and reduced Bcl-xL protein levels.

Significance. Some polycarbonate-based composite brackets when exposed to water release substances than activate mitochondrial apoptosis.

1. Introduction

Polycarbonate resins have been increasingly used as dental biomaterials due to their biocompatibility, exceptional esthetics and tailored mechanical attributes [1]. An interesting and growing application of polycarbonate resins was the production of esthetic orthodontic brackets. Early attempts to produce orthodontic brackets from unfilled polycarbonates were unsuccessful, due to excessive in-service distortion, discoloration and staining [2–4]. To improve water resistance, new glass-particle or glass-fiber reinforced materials were

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introduced with metallic-strengthened slots, in an attempt to diminish the undesirable features.

Bisphenol-A (BPA) is the main raw material used in the production of the aromatic polycarbonate (ArPCB) matrix of many plastic esthetic brackets. The benzene rings and the quaternary carbon atoms of the BPA structure create a bulk, stiff chain which offers rigidity, strength and less susceptibility to biodegradation in comparison with aliphatic polycarbonates [5]. Moreover, ArPCBs offer temperature and impact resistance, excellent optical properties, large plastic deformations without cracking and easy molding and thermoforming capacity, making this material attractive for component manufacturing.

The widespread use of polycarbonate-based orthodontic brackets has caused concerns on the possible biological and systemic health side-effects of the eluents of these materials released intraorally by physical and chemical processes. Brackets made of ArPCBs have demonstrated sensitivity to water plasticization and water cracking, resulting in water degradation and release of traceable amounts of BPA in the oral cavity of patients and in aqueous environments after long term immersion [6–9]. Cytotoxic responses of plastic bracket eluents in human gingival fibroblasts have been presented so far, leading to reduced viability, plasma membrane damage, DNA fragmentation and increased cell death [10].

BPA and BPA derivatives, increase the levels of reactive oxygen species [9,11] that are known mediators of signaling cascades under physiological conditions. Elevated levels of such compounds can disrupt the cellular redox equilibrium, causing oxidative DNA damage and apoptosis in mammalian cells. BPA specifically, has been previously shown to activate multiple cytotoxic mechanisms and induce DNA damage by activating oxidative stress, p53 [12] and other cell cycle proteins [13], mitochondrial [14] and endoplasmic reticulum proteins [15] and mTOR pathways [16]. The role of BPA in the canonical apoptotic pathways has been poorly examined and there is limited data associating its role in mitochondrial cell death of T cell lines [17] and germ cells after UV irradiation and hydroquinone treatment [18]. At the same time, epidemiological and genetic studies have shown that BPA is an environmental estrogenic compound that can exert proliferative responses and more specifically can induce hormonal-related effects including altered peripubertal mammary gland development in mice [19]; early puberty in females [20] and feminization in males; higher risk for breast cancer in females and prostate cancer in males [21]; induction of calcium influx, which leads to prolactin release and associated behavioral effects [22,23]; development of hyperglycemia and insulin tolerance [24]; elevation of oxidative stress mediators [25] and upregulation of the cAMP response element-binding factor, which inhibits apoptosis [26].

Due to the significant use of polycarbonate particle- and fiber-reinforced esthetic brackets in the orthodontic practice and the implication of their constituents with contrasting biological activities, the present study was designed aiming to investigate possible cytotoxic and estrogenic effects of eluents from three types of polycarbonate brackets on NIH 3T3 fibroblasts, Hela cells and the estrogen receptive MCF7 cell lines. The hypothesis tested was that there are no statistically significant differences in the performance of the brackets.

2. Materials and methods

2.1. Brackets

The brackets used in this study are listed in Table 1. According to the manufacturers' product information sheets, EL, EG and SL are all polycarbonate composite brackets reinforced with filler-particles (EL) or glass-fibers (EG, SL). The polycarbonate-free bracket (BR), composed of unfilled polyoxymethylene, was used as a control, whereas triple distilled water was used as the immersion medium for brackets in all the experiments performed.

2.2. Composition

The molecular composition of the plastic brackets was investigated by micro-attenuated reflection Fourier transform infrared spectroscopy (micro-ATR FTIR). The wings of each bracket were pressed against the diamond reflective element of a micro-ATR accessory (Golden-Gate MKII; Specac) attached to an FTIR spectrometer (Spectrum GX; Perkin-Elmer) and spectra were recorded under the following conditions: 4000–600 cm^{-1} range, 4 cm^{-1} resolution, 40 scans acquisition, 2 mm diameter sampling area, single-reflection diamond ATR element, 2 μm estimated depth of analysis. Spectra were subjected to baseline and ATR correction, employing Spectrum v 5.1 (Perkin-Elmer) software.

2.3. In vitro aging

Five brackets of each brand (upper incisors) were immersed in sterile silicone-sealed glass-beakers with 200 ml of triple-distilled water and kept at 37 °C for 3 months. The brackets were then removed, the eluents were diluted (1:10) and used

Table 1 – The plastic brackets tested.

Product (code)	Composition[a]	Slot type	Manufacturer
Brilliant (BR)	Unfilled polyoxymethylene	Plastic	Forestadent GmbH, Pforzheim, Germany
Elan ME (EL)	Ceramic particle-reinforced polycarbonate	Metallic	GAC, Central Islip, NY, USA
Elegance (EG)	Fiber-glass-reinforced polycarbonate	Metallic	Dentaurum GmbH, Ispringen, Germany
Silkon Plus (SL)	Fiber-glass-reinforced polycarbonate	Plastic	American Orthodontics, Sheboygan, WI, USA

[a] According to manufacturers' data sheet information.