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Research paper

Cytokine profile changes in gingival crevicular fluid after placement different brackets types

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ABSTRACT

Objective: The aim of this study was to examine the relationship between bracket design and ratio of five proinflammatory cytokine, in gingival crevicular fluid (GCF), and bacterial adhesion without tooth movement influence.

Design: The sample was comprised of 20 participants, aged 11 to 15 years old (mean age: 13.3 years ± 1.03). A conventional Gemini™ metallic bracket and two self-ligating brackets, In-Ovation®R and SmartClip™, were bonded to the maxillary incisors and canines. GCF was collected using a standard filter paper strip before and 60 days after bonding. The cytokine levels (IL-12, IL-1α, IL-1β, IL-6 and TNF-α) were performed by the LUMINEX assay. The levels of the red and orange bacterial complexes were analyzed by the Checkerboard *DNA-DNA hybridization.* The data of cytokine and bacterial complexes were carried out using the non-parametric tests at 5% of significance level.

Results: Increased cytokine levels were observed. However, only the SmartClip™ group showed a significantly increased level of TNF-α ($p = 0.046$). The SmartClip™ brackets group presented higher levels of red complex bacteria.

Conclusions: The bracket design affected cytokine levels and bacterial adhesion since it was observed that the proinflammatory cytokines released in GCF to the SmartClip™ group showed an increase in the TNF-α levels associated with higher bacterial levels, which possibly represents greater inflammatory potential. Thereby, the bracket design should be considered in patients with risk of periodontal disease and root resorption.

1. Introduction

Nowadays, patients expect treatments that are effective, fast, and that do not promote damage in the teeth and periodontal tissues. In this context, many types of orthodontic brackets are commercially available for clinical use. Self-ligating brackets present some advantages in comparison with conventional brackets, such as reduced treatment time, reduced number of dental appointments, and the effectiveness of treatment (Čelar, Schedlberger, Dörfler, & Bertl, 2013; Fleming & O'Brien, 2013; Harradine, 2013). Regardless of the type, any orthodontic appliance promotes significant changes in the homeostasis of the periodontal tissues (Alfuriji et al., 2014) by the increase of dental plaque and the release of chemical mediators in the gingival sulcus (Jurela et al., 2013; van Gastel, Quirynen, Teughels, Coucke, & Carels, 2008).

Cytokines induce and maintain a chronic inflammatory response in the periodontium. Gingivitis increases blood flow, vascular permeability, and inflammatory cell migration (neutrophils and macrophages) from peripheral blood to the crevicular fluid. Subsequently, T and B-lymphocytes appear at the injury site. Host cells produce and release cytokines such as IL-1α, IL-1β, IL-6, IL-8, TNF-α, and prostaglandins (Marcaccini, Amato, Leão, Gerlach, & Ferreira, 2010; Ziegler et al., 2010). In this way, the literature has empathized the role of the cytokines in orthodontic movement (Andrade, Silva, Silva,

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Teixeira, & Teixeira, 2007; Garlet et al., 2008; Kapoor, Kharbanda, Monga, Miglani, & Kapila, 2014). In pathological conditions, these cytokines regulate bone reabsorption, which could lead to the occurrence of bone or radicular resorption (Belibasakis & Bostanci, 2012; Sims & Gooi, 2008) during orthodontic treatment (Marcaccini, Amato, Leão, Gerlach, & Ferreira, 2010; Viecilli, Katona, Chen, Hartsfield, & Roberts, 2009; Ziegler et al., 2010).

Thus, to evaluate if the bracket design induces the accumulation of bacterial plaque and promotes inflammation of the supporting tissues, our research group carried out an ample study that analyzed the periodontal indexes, bacterial behavior, and gingival crevicular fluid 60 days after bonding different types of orthodontic brackets: conventional metallic (Gemini™) and active (In-Ovation®R) and passive (SmartClip™) self-ligating brackets. Initially, the periodontal parameters and the volume of the gingival crevicular fluid were evaluated, and it was verified that the bracket design influenced the plaque index and fluid volume. In these features, the self-ligating SmartClip™ presented the worst performance (Bergamo et al., 2016). When the bacterial dynamics correlated with periodontal disease were evaluated over 60 days, a distinct contamination pattern was observed for the self-ligating brackets, which showed highest levels of bacterial species involved in periodontal disease (Bergamo et al., 2017).

On a multilevel aspect, the bonding process, as well as the bracket design, may promote changes in gingival and periodontal tissues, even in the absence of orthodontic forces. However, only a few studies have focused on the evaluation of these alterations according to self-ligating brackets.

Therefore, the aim of this study was to evaluate the cytokine levels (IL-12, IL-6, IL1-α, IL-1β and TNF-α) in the gingival crevicular fluid, and the bacterial complex profile *in situ,* before and 60 days after bonding of self-ligating and conventional brackets. The null hypothesis tested was that the bracket design does not affect the cytokine profile, orange and red complexes levels.

2. Materials and methods

The ethics committee approved the present study (research protocol number #0062.0.138.000-10). Informed consent was obtained from the patients or their parents before the study. This protocol was performed in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

The sample size calculation was performed using the SPSS program SamplePower (IBM software-Statistical Package for the Social Sciences, Inc. Chicago Illinois, USA). The calculation was based on five factors: I) difference between initial and final means; II) dispersion of scores; III) sample loss; IV) alpha value of 0.05; and V) bicaudal analysis. A sample of 20 subjects per group would have 80% power.

Twenty patients referred to the Orthodontic Clinic were included. The subjects were selected according to the following exclusion criteria: history of previous orthodontic treatment, history of antibiotic therapy in the last 3 months, history of systemic medication in use, current smoker, diagnosis of systemic disease, and signs of gingivitis and/or periodontitis. Patients with severe crowding, overjet, and overbite were also excluded.

Standardized hygiene instructions were given to all patients by the same investigator. Patients were provided with a toothbrush (Professional®, Colgate-Palmolive Industry, São Bernardo do Campo, SP, Brazil) and a toothpaste (Oral-B® Pro-Saúde©, 2012 Procter & Gamble of Brazil).

2.1. Bracket bonding and debonding

All the patients received metallic brackets: two self-ligating (In-Ovation®R, Dentsply, GAC and SmartClip™, 3M Unitek, Monrovia, CA, USA) and one conventional bracket, used with elastomeric ligatures (Gemini™, 3M Unitek, Monrovia, CA, USA).

The schematic drawings of the six anterior teeth were designed to distribute the different types of brackets in the previous six teeth selected for bonding. Thus, the different brackets had been listed according to the type of the bracket and the time of debonding. The brackets were numbered from 4 to 6 with the following distribution: the number 4 matched the In-Ovation®R bracket, the number 5 matched the (Gemini™, 3M Unitek, Monrovia, CA, USA). SmartClip™ bracket, and the number 6 matched the Gemini™ bracket, removed 60 days after bonding. This random assignment also ensured that the number of each type of bracket removed, sixty days after bonding, was similar for each anterior tooth analyzed for both the left and the right side. A total of 60 brackets were removed, 60 days after bonding, 20 of each type, distributed similarly among the different dental elements.

The Transbond XT system (3M Unitek, Monrovia, CA, USA) was selected for the bonding. After bonding, a 0.014" orthodontic archwire was inserted passively.

After 60 days, the brackets were debonded and were placed into the coded sterile microtube tubes containing 150 μL of TE (10MmTris-HCl, 1MmEDTA pH 7.6) and mixture in Vortex. The brackets were removed using the sterilized pliers followed by the addition of 100 μL of 0.5 M NaOH and stored at −20° C until the *DNA–DNA checkerboard hybridization* was performed, according to Bergamo et al. (2016).

After this stage, all patients were enrolled in a corrective orthodontic treatment and received new brackets.

2.2. Gingival crevicular fluid collection

At the baseline, before the GCF collection and bracket bonding, the teeth were pumiced, washed, and dried, the areas were isolated with cotton rolls and gently dried. The GCF was collected according to Iwasaki, Haack, Nickel, Reinhardt, and Petro (2001). PerioPaper absorbent strips (PerioPaper®, Oraflow Inc., Plainview, USA) were placed into the sulcus. After keeping the strip in place for 30 s, the absorbed volume was measured with the Periotron® 8000 (Oraflow Inc., Plainview, USA). Strips with blood contamination were discarded. In order to minimize evaporation, the volume was analyzed as fast as possible. Three strips were collected from three sites on the buccal surface (mesial, central, and distal) in each tooth.

The brackets were debonded after 60 days, and the GCF collection was repeated, before debonding. The PerioPaper strips were placed in coded sterile microtubes and stored at −70° C until cytokine analysis.

2.3. Cytokines measurement

Cytokine levels of IL-12, IL-1α, IL-1β, IL-6, and TNF-α were determined in the GCF atT0 and T1 using LUMINEX® assay. A high light sensitivity human cytokine kit (HCYTOMAG-60K-05; Milipore, Bilerica, MA, EUA) was used according to the manufacturer's instructions, using the multiplexing instrument MAGpix™ (MiraiBio, Alameda, CA, USA).

The samples were individually evaluated, and the concentrations were estimated from the standard curve using a five-parameter polynomial equation using Xponent® software (Millipore Corporation, Billerica, MA, USA). The mean concentration of each biomarker was calculated, adjusted to GFC volume, and expressed as pg/mL.

Briefly, a 96-well plate was prewet with washing buffer, which was subsequently discarded, followed by the addition of microsphere magnetic beads coated with monoclonal antibodies against the five different target analytes to the wells. Samples and standards were added to the wells and incubated for two hours under gentle agitation and in darkness. The wells were washed using a magnetic manifold, and a mixture of biotinylated secondary antibodies was added. After incubation for 1 h, streptavidin conjugated to the fluorescent protein RPhycoerythrin was added to the beads and incubated for 30 min. After washing to remove the unbound reagents, sheath fluid was added to the wells, and the beads (minimum of 50 per analyte) were analyzed in the multiplex assay instrument. Samples were diluted with the diluents