

# Biofilm retention by 3 methods of ligation on orthodontic brackets: A microbiologic and optical coherence tomography analysis

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**Introduction:** The aim of this study was to evaluate biofilm retention around orthodontic brackets related to the method of ligation by using optical coherence tomography (OCT) and microbiologic sampling. **Methods:** Seventy-five plastic central incisors for dentures were divided into 3 groups and used with metal brackets with a 0.022-in slot with elastomeric ligature (n = 25), metal brackets with a 0.022-in slot with steel wire ligature (n = 25), and self-ligating brackets with a 0.022-in slot (n = 25). The samples were submersed in a suspension of *Streptococcus mutans*, genetically engineered to express green fluorescent protein, at 37°C for 72 hours to allow biofilm formation. The samples were then submitted to microbiologic analysis and OCT imaging. **Results:** The microbiologic analysis and the OCT showed significant differences in biofilm formation depending on the ligating method. Brackets ligated with elastomeric rings held more *S mutans* biofilm, and steel wire ligation had less biofilm retention compared with the other brackets. **Conclusions:** This study provided validation that OCT can be used as a potential qualitative marker of total plaque bacteria that can be rapidly and reliably visualized around orthodontic brackets. (Am J Orthod Dentofacial Orthop 2011;140:e193-e198)

Orthodontic treatment with fixed appliances leads to increased biofilm accumulation and elevated levels of cariogenic and periodontal bacteria,<sup>1-3</sup> mainly because orthodontic brackets make good oral hygiene difficult, resulting in plaque accumulation and significantly increased risks for enamel demineralization or periodontal disease.<sup>4</sup>

Fixed orthodontic appliances create new retention areas that are suitable for bacterial colonization and lead to increases in the absolute numbers and the percentages of *Streptococcus mutans* and lactobacilli. Many studies have evaluated the effect of fixed orthodontic appliances on microbial flora and periodontal status<sup>5-8</sup>; however, only a few studies evaluated the method of ligation as an additional factor.<sup>9,10</sup>

Conventionally, to connect the wire to the bracket, 3 methods are used: ligation with steel wires, elastomeric ligatures, and self-ligating brackets. All of these methods have advantages and disadvantages, but, concerning biofilm retention, the literature suggests that elastomeric ligatures increase the retention of dental biofilm compared with the 2 other methods.<sup>4,9</sup>

In this study, we used a noninvasive method to visualize the biofilm formed around the brackets related to the ligating method: optical coherence tomography (OCT). This technique provides a 3-dimensional (3D) way to visualize the biofilm and allows a quantitative analysis of the retention area. For in-vitro studies, it can provide a reliable method for measuring biofilm area and thickness without destruction or alteration of the samples. Typically, the methods for plaque identification in vitro use microbiologic sampling<sup>11</sup> or scanning electron microscopy<sup>12</sup> that are invasive and destructive. In vivo, the procedures include biologic dyes and digital images to visualize and quantify the biofilm; however, this is normally uncomfortable for patients, since the dyes can stain oral tissues or orthodontic appliances. In addition, the dye has difficulty in staining thin biofilms. OCT does not need dyes to identify the biofilm and can provide image enhancement in a clinical environment that is impossible to obtain without a microscope.

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OCT has been developed for cross-sectional imaging in biologic systems. It uses low-coherence interferometry and explores the optical properties of scattering samples to produce images from internal tissue microstructures in a way that is analogous to ultrasound pulse-echo imaging.<sup>13</sup> This technique is a noninvasive, harmless diagnostic method and can depict the microstructure of biologic samples.<sup>14,15</sup> The use of OCT to reveal biofilms on brackets is innovative. Image software can process the data obtained by OCT to quantify the biofilm retention area and identify in a 3D analysis the site of retention.

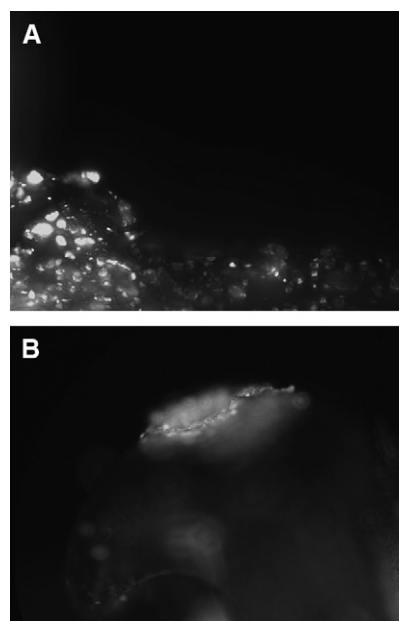
The aims of this study were to evaluate biofilm retention around orthodontic brackets as function of the method of ligation by using OCT and to compare the results with conventional microbiologic sampling.

### MATERIAL AND METHODS

Seventy-five plastic central incisors for dentures (Biotone, Dentsply, Rio de Janeiro, Brazil) were used. The samples were divided into 3 groups: conventional metal brackets with a 0.022-in slot with elastomeric ligature (n = 25), the same type of brackets with steel wires ligature (n = 25), and 25 self-ligating brackets with a 0.022-in slot for the straight-arch technique (respectively, Ovation and in-Ovation brackets; Dentsply/GAC, Bohemia, NY). All brackets used were maxillary central incisor brackets, bonded with composite bonding material (TPH, Dentsply). The samples were disinfected before the experiment by immersion in 1 mL of 70% ethanol for 30 minutes.

Genetically modified bacteria, *S mutans* (ATCC 25175), to express the green fluorescent protein were aerobically grown in brain-heart infusion broth at 37°C with shaking (150 rpm) for 48 hours, until they formed a stationary growth phase suspension. Genetically modified fluorescent bacteria were used to easily confirm, by image analysis, the formation of biofilms and the identification of the main site of biofilm retention on the brackets.

Bacterial cells were suspended in broth nutrient medium of brain-heart infusion to achieve a cell concentration of 10<sup>9</sup> colony-forming units per milliliter (confirmed by photospectroscopy at  $\lambda = 540$  nm). One-milliliter aliquots were added to a petri dish with approximately 20 mL of brain-heart infusion broth with the samples inside. The petri dish was then sealed, kept upright, and incubated aerobically for 72 hours at 37°C with shaking (100 rpm) to allow biofilm formation. The brain-heart infusion broth was changed each 24 hours, and the presence of a microbial biofilm rather than planktonic bacteria was confirmed by the failure of irrigation with saline solution to significantly diminish



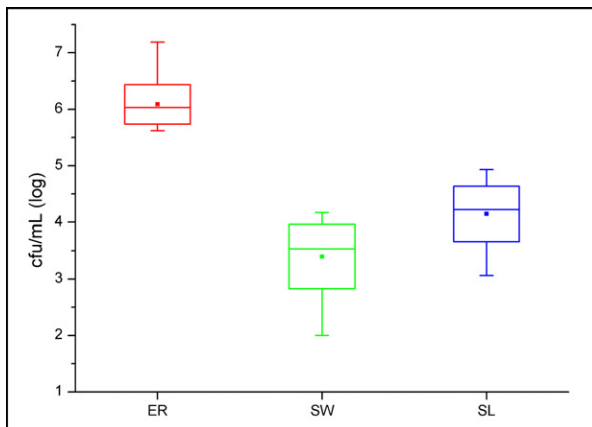
**Fig 1. A**, Three-dimensional reconstruction of a confocal sequence of *S mutans* biofilm fluorescent images at the interface of the bracket and the artificial teeth (note the intense fluorescence of the biofilm); **B**, fluorescent image of *S. mutans* biofilm over the bracket in a buccal view over the bracket wing after irrigation with 10 mL of saline solution, indicating the adherence of the 3-day-old biofilm.

the fluorescence signal analyzed at a wavelength of 520 nm by fluorescence microscopy (Fig 1).

The configuration used in the OCT system was a fiber Michelson interferometer (OCP930SR; Thorlabs, Newton, NJ) that uses a superluminescent diode at 930 nm with 2 mW of optical power in the sample arm. The system can produce up to 8 frames per second with 2000 × 512 pixels with axial and lateral resolutions in air of 6.0  $\mu$ m. The system has a hand probe implemented with optical fiber, making data collection easier.

For tridimensional image reconstruction, a precise displacement of the sample in the y-axis (orthogonal to the image acquisition by the OCT system) during the image acquisition was done by a computer-controlled linear translation stage (T25-XYZ, Thorlabs) with a minimum step of 0.05  $\mu$ m. The 3D images were reconstructed by using VGStudioMax (version 1.2; Volume Graphics, Heidelberg, Germany) and analyzed with the software Image J (National Institutes of Health, Bethesda, Md). For the analysis, all areas of light scattering on each image, showing the presence of biofilm, were selected, and the software analyzed the values in square millimeters.

For the microbiologic analysis, after 72 hours, each plastic tooth was washed by immersion in phosphate-buffered saline solution to remove the brain-heart



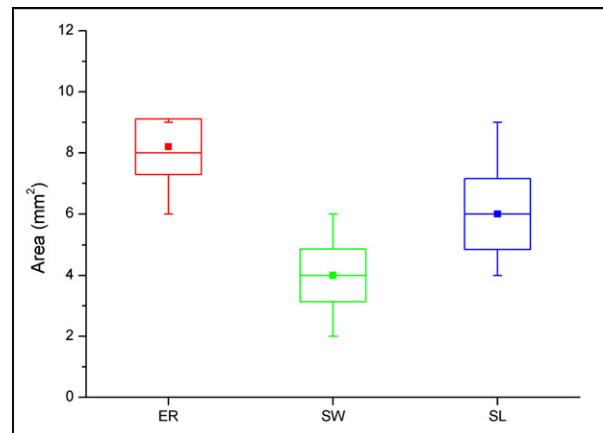
**Fig 2.** Microbiologic analysis of biofilm retention on different brackets. *Squares* represent mean values; boxes represent standard deviations. *Bars* indicate ranges, and the *horizontal lines* in the boxes correspond to the medians. Steel wire (SW) ligation showed less biofilm retention than self-ligating (SL) brackets and elastomeric rings (ER) ( $P < 0.05$ ).

infusion broth and placed in a microcentrifuge tube with 1 mL of phosphate-buffered saline solution, that was subsequently sealed and vortexed for 1 minute and, after that, challenged with a low-output ultrasonication to unbind the biofilm.<sup>16</sup> One hundred-microliter aliquots were added to the wells of a 96-well plate for serial dilution and streaking on square brain-heart infusion agar plates. The plates were incubated by using standard aerobic conditions at 37°C for 24 hours so that the colony-forming units could be counted according to the method of Jett et al.<sup>17</sup> After we counted the colony-forming units, we computed the mean values from samples in each group.

For comparison and validation of the image area analysis, the data from both experiments were compared.

### Statistical analysis

Medians and means for bacterial counts and biofilm areas with the corresponding standard deviations were calculated. The mean bacterial counts (in colony-forming units per milliliter) and the biofilm area determination (in square millimeters) from teeth with steel wire ligation, self-ligating brackets, and elastomeric rings were tested for significant differences by using analysis of variance (ANOVA) followed by Tukey tests.  $P < 0.05$  was considered statistically significant. Statistical comparisons between means were performed with software (Origin, version 8.5; OriginLab, Northampton, Mass).

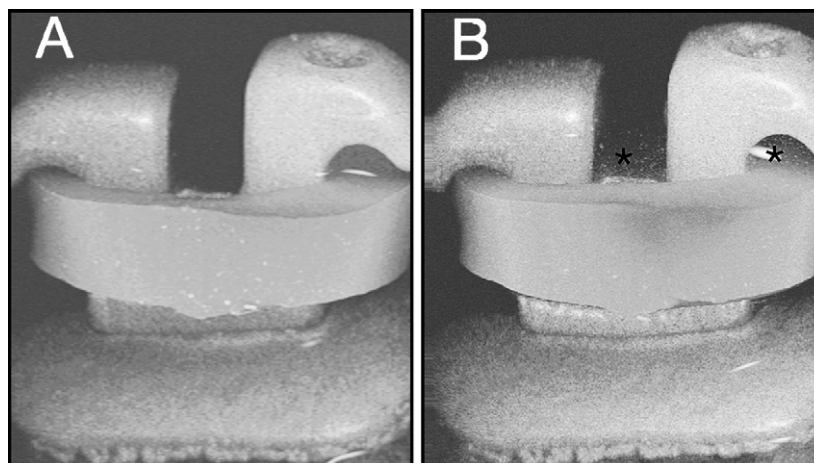


**Fig 3.** Quantification of biofilm area on different brackets based on OCT images analyzed with the Image J software. *Squares* represent mean values; boxes represent standard deviations. *Bars* indicate ranges, and the *horizontal lines* in the boxes correspond to the medians. Steel wire (SW) ligation showed less biofilm area retention than self-ligating (SL) brackets and elastomeric rings (ER) ( $P < 0.05$ ).

## RESULTS

Biofilm was detected on all orthodontic brackets by fluorescence and OCT images. The addition of 1 mL of a suspension containing  $10^9$  cells of *S mutans* into the petri dish with brain-heart infusion broth and the samples followed by 3 days of incubation at 37°C in aerobic conditions reliably and reproducibly produced fluorescent biofilms (Fig 1, A). Irrigation with 10 mL of saline solution did not affect significantly the fluorescence signal when analyzed by fluorescence microscopy. Figure 1, A, shows the fluorescent image that confirms the biofilm presence on the brackets, and Figure 1, B, shows a bracket with an elastomeric ring after the biofilm challenge.

The microbiologic analysis showed a significant difference on biofilm formation depending on the ligating method. Brackets ligated with elastomeric rings collected more *S mutans* biofilm than did self-ligating brackets and steel wire ligation. Figure 2 gives the data obtained from the biofilms removed from each type of ligation. The initial bacterial burden did vary between individual brackets. For elastomeric rings, the mean value was 6.09 log colony-forming units per milliliter (range, 7.19-5.62). Self-ligating brackets had less biofilm compared with elastomeric rings, an average of 4.14 log colony-forming units per milliliter (range, 4.93-3.09). However, steel wire brackets showed still less biofilm, with a mean value of colony-forming units per milliliter of 3.4 log (range, 4.17-2).



**Fig 4.** OCT images from elastomeric ring **A**, before and **B**, after 3 days of incubation with *S mutans*. The asterisks indicate the biofilm. Note the light scattering from the *S mutans* biofilm.

Figure 3 shows the area of biofilm on an OCT image measured by Image J software. The area measured confirms that elastomeric rings retain more biofilm than self-ligating brackets and steel wire ligation, showing good correlation between the methods.

By using image amplification and 3D reconstruction, we can observe the main sites of biofilm retention of each ligating method. As shown in Figure 4, A and B, for elastomeric ligating brackets, the site of biofilm retention is around the elastomeric ring and in the slot; for self-ligating brackets, it is mainly in the slot and under the clip. The steel wire ligating bracket had the least biofilm retention. Both Figures 4 and 5 show the OCT images from brackets that were more representative of each group, and the asterisks identify the main sites of biofilm retention. As seen in the image, the area of light scattering from the biofilm is greater on the elastomeric ligating brackets compared with the self-ligating and steel wire ligating brackets.

## DISCUSSION

The aim of this study was to evaluate biofilm retention around orthodontic brackets as a function of the method of ligation by using images from OCT and microbiologic sampling. OCT is a noninvasive and real-time biofilm study method, facilitating bacterial analysis without altering the sample. Current methods for in-vitro analysis use microbiologic sampling or scanning electron microscopy, which are invasive and destructive methods.

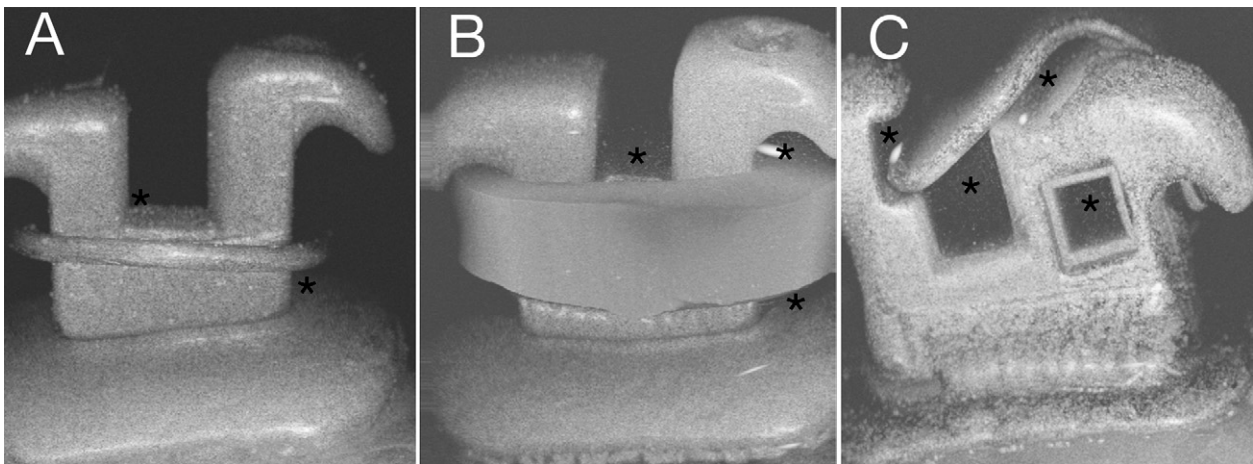
Biofilms were detected on all orthodontic brackets independent of ligation method by microbiologic analysis and OCT images, and confirmed by fluorescence microscopy. These data could indicate good correlations

among biofilm image areas and counts of colony-forming units.

*S mutans* was selected for this study because it is an acidogenic and aciduric bacterium that is considered to be the primary organism responsible for enamel caries.<sup>18</sup> Furthermore, many studies have demonstrated increased retention of *S mutans* and lactobacilli in saliva and dental plaque during orthodontic treatment.<sup>5,7,8</sup> The expression of a fluorescent protein by *S mutans* facilitated the biofilm visualization, avoiding the use of others methods that could interfere with the biofilm structure.

Despite recent advances in orthodontic materials, there has been no decrease in the prevalence of enamel demineralization near the bracket-tooth junction; orthodontic materials create a favorable substratum for biofilm formation. The formation of dental plaque might exacerbate preexisting periodontal diseases and cause enamel decalcification, affecting some patients undergoing orthodontic treatment.<sup>19</sup>

Concerning the method of ligation, Forsberg et al<sup>9</sup> studied the effect of biofilm retention around brackets ligated with steel ligatures and elastomeric ties in patients. They found that brackets attached to archwires with elastomeric rings had more bacteria than those ligated with steel wires. They recommended avoiding the use of elastomeric ligatures in patients with poor oral hygiene because elastomeric ligation rings can significantly increase microbial accumulation on tooth surfaces adjacent to brackets, leading to predisposition for dental caries and gingivitis. Additionally, Pellegrini et al<sup>4</sup> showed in an in-vivo study that, in most cases, teeth bonded with self-ligating attachments had fewer bacteria in plaque than did teeth bonded with elastomeric brackets after 1 to 5 weeks of bonding.



**Fig 5.** OCT images from the brackets: **A**, steel wire ligation on Ovation bracket; **B**, elastomeric ring on Ovation bracket; **C**, self-ligating In-Ovation bracket. *Asterisks* indicate the main sites of biofilm retention on each bracket.

Based on the results of Forsberg et al<sup>9</sup> who found reduced bacterial retention around brackets ligated with steel ligatures compared with elastomeric rings, and Pellegrini et al,<sup>4</sup> who showed greater biofilm on brackets with elastomeric ties as opposed to self-ligating appliances, we compared the 3 methods of ligating with respect to biofilm retention. In contrast, Tukkahraman et al<sup>10</sup> found no significant differences in the numbers of microorganisms from teeth ligated with either elastomeric rings or steel ligature wires. Also, Pandis et al<sup>20</sup> established that the levels of *S mutans* in whole saliva of orthodontically treated patients did not seem to be significantly different between conventional and self-ligating brackets.

Our results from the microbiologic analysis and the OCT images clearly indicated that around steel wire ligatures there was less biofilm compared with self-ligating brackets and elastomeric ligating brackets. The images showed that elastomeric and self-ligating brackets have more microbial shelters, resulting in enhanced accumulation of plaque compared with brackets with steel ligature wires. Pellegrini et al<sup>4</sup> hypothesized that the complete absence of a ligature in a self-ligating mechanism would presumably be equally as hygienic as, if not better than, a stainless steel ligature. However, the image in Figure 5, C, show that, because of the clip and other retention areas on self-ligating brackets, they retain significantly more biofilm than steel wire ligatures. For self-ligating brackets, on the other hand, the main sites of biofilm retention will be filled with the archwire, decreasing the space for retention.

This study has also provided validation that OCT images can be used as a potential qualitative and quantitative marker of total plaque bacteria that can be rapidly and reliably visualized around orthodontic brackets.

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