PRELIMINARY STUDY

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Bioluminometer analysis and scanning electron microscopy analysis on the surfaces of removable orthodontic aligners after using Bitewash: preliminary study

Authors

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INTRODUCTION

For those who use removable orthodontic devices, it is essential that these are clean and free of bacteria before they are worn; correct hygiene actually prevents accumulated bacteria on their surface from forming an infectious vector for the oral cavity, transferring and placing the bacteria in constant, direct contact with the dental surface and gums. For this reason, these devices require appropriate cleaning and disinfection. However, the information that dental practitioners give to patients regarding the hygiene of such removable devices is often incomplete and unclear (Axe, 2015). The problem concerns removable orthodontic aligners, but it could also apply to orthodontic devices for the correction of growth defects in the jaws of patients who are still growing, or patients undergoing gnathological treatment.
In a study conducted on 450 orthodontists, the most frequent recommendation was mechanical cleaning with a toothbrush (99%). Chemical detergents (37%) and treatment with diluted vinegar or citric acid (30%) were mentioned less often (Eichenauer,2011). Overnight storage of the dentures in water with a cleansing tablet significantly reduced the total bacterial count (Duyck 2016) compared with normal manual cleaning methods for complete dentures (Srinivasan, 2010).
Levrini demonstrated that using effervescent tablets of sodium carbonate and sulphate combined with mechanical removal using a brush was the most effective cleaning method for Invisalign clear aligners (Levrini,2016). Levrini (2015) also used SEM to analyse the behaviour of the biofilm after aligners were sanitised in various ways; the study clearly showed that lack of hygiene causes significant build-up of the bacterial biofilm. Brushing with a toothbrush and using cleaning products intended for removable dentures are the preferred methods for cleaning removable orthodontic appliances. [Apostolos,2019]. Dietrich analysed the microbiological colonisation on removable orthodontic devices (Dietrich 1989) after three different procedures. The results showed that the use of a brush alone was not a satisfactory method for decontaminating the device, while treatment with ultrasound was highly effective. It could be inferred, however, that none of these procedures resulted in complete decontamination of the removable devices.
Some studies recorded in the literature show that, from both a microbiological point of view and through SEM analysis, sprays or solutions based on chlorhexidine cause a reduction in bacterial biofilm formation on the surface of removable acrylic resin devices, as this leads to a reduction in total bacterial count on their surface (Peixoto, 2013; Lessa, 2007; Faria, 2013).

AIM

The aim of this study was to evaluate the efficacy in removing the bacterial biofilm (by means of SEM)

and the bacteria concentration (by means of bioluminometer) on Invisalign clear aligners using a new cleaning method (bitewash).

MATERIALS AND METHODS

Fifteen consecutive patients undergoing orthodontic treatment with clear aligners (Invisalign®, Align Technology, Santa Clara, California), aged 18 to 30 years, were enrolled in this study. All patients were informed of the nature of the study, which was conducted on an individual basis, and read and signed a written consent form. The study protocol was conducted in accordance with the 1975 Declaration of Helsinki, revised in 2007. The exclusion criteria included: smoking, patients with fixed crowns/bridges or partial dentures, previous non-surgical periodontal treatment or more than one week of treatment with antibiotics, steroids or non-steroidal anti-inflammatory drugs in the previous 6 months. All subjects were instructed to adopt the following home oral hygiene regimen: use a soft brush with a rolling technique, fluoride toothpaste and dental floss. For the bioluminometer analysis, the aligners were used for one week and then divided into two parts: one for the control group (water) and one for the experimental group following the manufacturer's directions (bitewash). For the SEM analysis, 2 aligners were chosen at random from the two groups and analysed.

SEM Analysis

The study was conducted in the Laboratory of Human Morphology at the University of Insubria. All samples were air dried, reduced to a small size, mounted on standard SEM stubs with carbon-based conducting adhesive and coated in gold using an Emitech K–550 sputter-coater (Emitech Ltd, Ashford, UK) in a controlled argon atmosphere at a pressure of 1 × 10–1 mbar. All observations were carried out using an FEI XL–30 FEG field scanning electron microscope (FEI, Eindhoven, Netherlands) operating at an acceleration voltage of 7kV. All images were obtained directly in digital form as 1424x968 pixel TIFF images, 8bpp in grayscale.

Microbiological analysis using bioluminometer

A microbiological analysis was performed using the Bioluminometer System Sure II Plus (RG strumenti, Parma, Italy) with the SuperSnap kit (RG strumenti, Parma, Italy) according
to the manufacturer's instructions. The sample was collected by passing the SuperSnap kit over the aligners; a circular movement was performed on the molars, while on the other parts of the aligners a simple scraping was carried out. The bioluminometer measures the light generated and displays the results in Relative Light Units (RLU), providing the necessary information on the level of contamination detected. The higher the RLU value, the greater the amount of ATP present in the sample, and therefore the dirtier the surface. Since the bioluminometer is a novelty in the dental field, a cross-examination was previously conducted to validate the use of this instrument by comparison with the traditional diagnostic method and validated through a count of colony forming units (CFU) on LB agar plate to determine the total bacterial count (Levrini, 2016).

Sanitising product

Bitewash is a stable, balanced mixture of potassium peroxide compounds that contains an anionic surfactant, organic acids and an inorganic activation system. The following procedure was used: 2.5 grammes of Bitewash was dissolved in a container with 100 ml water and allowed to stand for 10 minutes until an opaque white solution was obtained, immersing the previously rinsed device for 10 minutes.

RESULTS

Microbiological analysis using bioluminometer

The analysis showed a bacterial concentration of 8,747 RLU (relative light units) in the control group using water, and 1,107 RLU in the experimental group, which amounts to a difference between the control and experimental groups of -7,640 (t-student p value < 0.0001) (fig. 1-3).

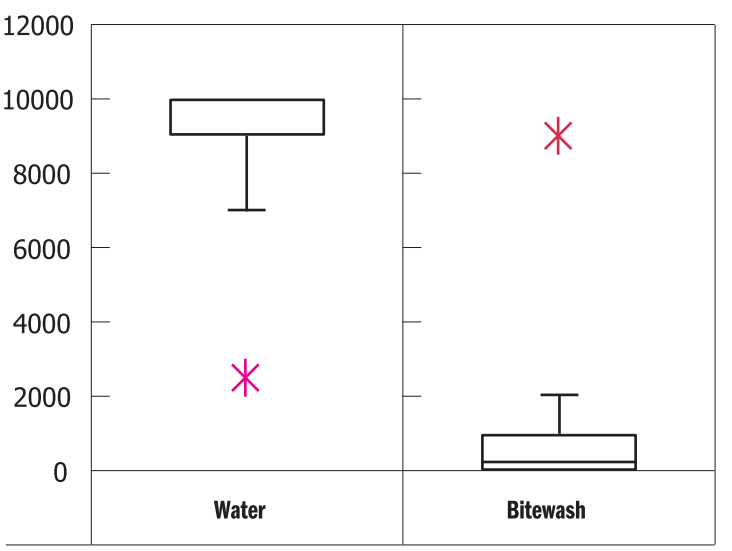
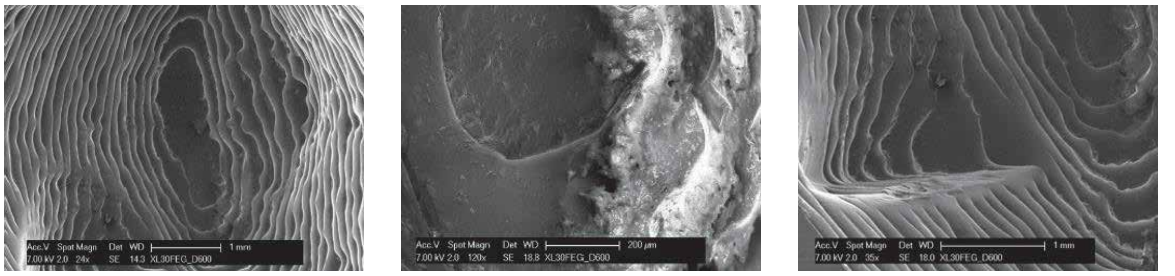


Fig. 1 Box plot comparing the bioluminometer analysis, concentration value expressed in RLU (relative light units) for the control (water) and cleaning method (bitewash).

Fig. 2 SEM images at different magnifications to highlight the presence of microscopic deposits in the experimental group.

Fig. 3 SEM images at different magnifications to highlight the disappearance of microscopic deposits in the experimental group.



CONCLUSION

The results of the qualitative analysis (SEM analysis) and quantitative analysis (bacteria concentration by means of bioluminometer) show that the sanitising product used in the study is effective in removing the bacterial biofilm on clear orthodontic aligners ●

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