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Assessing the efficacy of Denture Cleaners with Quantitative Light-Induced Fluorescence (QLF)

Adejumoke Adeola Adeyemi*, Fadi Jarad†, Neil Pender‡ and Susan Mary Higham§

Abstract - *The aim of this study was to evaluate Quantitative Light Induced Fluorescence (QLF) analysis as a means of determining the ability of denture cleaners to remove stain from denture teeth. This study investigated the appearance of acrylic resin denture teeth subjected to QLF conditions in vitro. It aimed to determine if QLF was an appropriate method for detecting stain and its removal on acrylic resin denture teeth in vitro and also to develop a quantitative and reproducible method for assessing the efficacy of denture cleaners using QLF. This study showed that not all acrylic resin denture teeth fluoresced under QLF examination. QLF demonstrated the ability to detect and quantify longitudinal changes in stain removal by the various denture cleaners used in the study.*

KEYWORDS: QLF, Fluorescence, Denture Cleansers, Staining, Stain removal

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INTRODUCTION

Complete dentures are the most common treatment for total loss of teeth in a dental arch[1]. The aesthetics of the denture is of prime importance to the patient. The ultimate objective of aesthetics in dentistry is to create a beautiful smile, with teeth of pleasing inherent proportions to one another, and a pleasing tooth arrangement in harmony with the gingivae, lips and face of the patient[2]. While function is of fundamental importance, the aesthetics and maintenance of the denture are important to the overall health of the patient thus, in contemporary dentistry, the needs of the patients are considered in terms of function and dental appearance[3].

There continues to be a widespread need for dentists to provide excellent complete denture services. An essential component of this service is patient education about denture hygiene[1]. A clean denture that is devoid of stains, deposits and relatively free of micro organisms is an essential part of treatment and management of patients with dentures[4].

Care of dentures and the mucosal tissues of the edentulous mouth can be important to the overall health especially in older patients. Unclean dentures cause or contribute to mucosal disease and / or impairment in eating and may have profound effects on frail elderly persons[1]. Some denture wearers experience difficulty cleaning their dentures and as a consequence many elderly people wear dirty dentures and suffer from halitosis[1,5].

* BDS, MSc (Med Sci)
† BDS, PhD, MFDSRCS (Eng)
‡ BDS, MScD, PhD, FDSRCS(Ed), FDSRCS (Eng), DDOrthRCPS
§ BSc, PhD, CBiol, MIBiol

Denture stomatitis results from unhygienic and dirty dentures and occurs commonly in denture wearers[6]. Bacterial plaque, yeast and associated micro organisms are major factors in the aetiology of denture stomatitis[6,7,8,9,10]. If left to accumulate over a short period of time they can cause mucosal inflammation and halitosis[11]. Denture plaque firmly adheres because the mucopolysaccharide matrix on which it is formed binds to dentures[11].

Olsen[12] stated the futility of eliminating associated micro organisms from the mouth if a contaminated denture repeatedly inoculates the oral tissues. Thus cleansing of the denture is the most effective preventive and curative treatment for the denture stomatitis[13]. The maintenance of denture prosthesis is important for the health of patients and in maintaining an aesthetic, odour-free appliance[1].

Formation of stains and denture debris

Extrinsic stains may be of metallic or non metallic origin. The causes of staining may be compounds that are incorporated into the pellicle and produce a stain as a result of their basic colour or those that lead to staining caused by chemical interaction at the tooth surface[14].

Chromogens derived from dietary sources or habitually placed in the mouth are taken up by pellicle. The colour imparted is determined by the natural colour of the chromogen as seen with tobacco smoking and chewing, consumption of beverages such as tea and coffee, mouth rinses and other medicaments[14].

Mouth rinses containing substances such as chlorhexidine, potassium permanganate and substances containing copper, silver nitrate, stannous fluoride have been associated with staining produced on the tooth surface[14,15].

Surfaces in the oral cavity become covered within 30 minutes in pellicle precipitate[1617,18]. Oral debris from food

particles, mucin, desquamated epithelial cells and micro-organisms adhere readily to the pellicle and this is also favoured by the rough and irregular topography of the denture[1].

The oral deposits that adhere to the denture impair denture aesthetics. Deposits that form on the acrylic resin denture bases and on teeth are assumed to be caused by the same mechanisms and substances that cause deposits on natural teeth of which salivary calculus and tobacco tars are the most common and are difficult to remove[14,19].

Denture cleaners:

Denture cleaners may be pastes or immersion cleaners. Whilst pastes contain abrasives amongst other constituents to aid in the removal of debris and plaque from the denture, the immersion type of cleaners have no abrasive particles. Immersion cleaners contain cleaning agents such as effervescent peroxide or sodium hypochlorite[11].

Abrasive denture cleaners are thought to produce a suitable rough surface that might influence plaque formation or inhibit its removal[20, 21]. Abrasion of the denture surface would occur during the brushing phase before and after soaking [11]. Immersion cleaners have been found to be effective against Candida *albicans*[22]. They contain fungicidal agents (in particular hypochlorite), which are effective in dissolving mucin and other organic substances, whilst leaving the surface of the denture unchanged and possibly less susceptible to plaque accumulation[23, 24, 25]. The use of immersion cleaners is becoming increasing popular among denture wearers as they are simple to use especially for the elderly or physically challenged persons. They are relatively efficient in their cleansing ability but can lead to damage of the denture base material if incorrectly used [11]. They remove plaque and stains at temperatures of 50°C [26].

Assessing the efficacy of denture cleaners.

Various methods have been reported in literature for testing the efficacy of denture cleaners. These include *in vivo* and *in vitro* methods investigating either stain removal or plaque.

These range from visual inspection of dentures and use of a grid; use of photographic records with quantitative imaging; in *vitro* studies such as profilometry, scanning electron microscopy (SEM) or spectrophotometric readings of colour change[3]. Various microbiological studies have been used *in vivo* and *in vitro* to assess the efficacy of cleaners. These studies have used different plaque quantification methods such as plaque staining[27]; microscopic counting of Candida albicans[22, 28], dry weight measurements; viable count; pH measurements and Adenosine Triphosphate (ATP) analysis[29, 30, 31].

Most of the methods for the assessment and evaluation of denture cleaner efficacy are not quantitative and lack standardisation in methodology which makes direct comparison of results from various studies difficult[13]. The immersion cleaners used in these studies are among the most popular brands marketed in the United Kingdom for use in maintaining clean dentures.

Aims of the study:

The aims of the study were threefold. Firstly to investigate the appearance of acrylic resin denture teeth subjected to Quantitative Light Induced Fluorescence (QLF) in *vitro*. Secondly to determine if QLF was an appropriate method for detecting stain and its removal on acrylic resin denture teeth *in vitro*. Finally to develop a quantitative and reproducible method to assess the efficacy of denture cleaners using QLF.

MATERIALS AND METHODS

Six upper anterior acrylic resin teeth from each manufacturer [Basic 6®, Viviodent DCL®, Enigma®, Senator® and Cosmo HXL®] used in the fabrication of dentures at the Liverpool University Dental Hospital were examined under QLF conditions for fluorescence. Fluorescence is the important phenomenon measured by QLF to detect the differences between stained and unstained denture teeth. Teeth that do not fluoresce cannot be measured to assess these differences. All denture teeth tested except Senator® teeth fluoresced.

A total of forty acrylic resin teeth (Cosmo HXL®) with their labial surfaces devoid of stain, cracks, fractures or other defects were used. Cosmo HXL® teeth were used because they were available in large numbers. Clear acid resistant nail varnish was applied to the labial surfaces leaving an exposed window measuring approximately 12mm². Baseline QLF images were captured for all the teeth.

The teeth were attached to lengths of cotton threaded through plastic tubing to prevent tangling and attached in groups of five through the caps of the immersion pots (Figure 1).

Figure 1. *Pot containing stained teeth*

The teeth were given identification codes. Staining was developed by dipping the specimens in pots containing 30ml of artificial saliva, 30 ml of chlorhexidine mouth wash and 30 ml of tea for 2minutes each with gentle agitation. Artificial saliva was prepared in the Laboratory (methyl–p-hydroxybenzoate, 2.00g/l; sodium carboxymethyl cellulose, 10g/l; KCl, 8.38 mM; $MgCl_2$.6H2O, 0.29MM; $CaCl._2H_2O$, 1.13 mM; K_2 HPO_4, 4.62 mM; KH_2PO_4 2.4 mM; fluoride 0.22 ppm, pH 7.2) and the tea solution was prepared by brewing tea bags (10g/500ml) in boiling water and allowing the infusion to cool over a period of three hours. The teeth were subjected to ten continuous staining cycles until the stain intensity appeared unchanged on visual inspection. Following each cycle the periphery of the windows were cleaned using a cotton swab stick to leave a clear margin around the windows. At the end of each staining cycle images of the teeth were taken using QLF (version 2.00c). The images were then analysed to ensure that there was a uniform stain range across the groups.

Denture Teeth Cleaning

The forty stained acrylic teeth were randomly assigned to four groups of ten teeth (five per pot). These were immersed into solutions containing the following

Group A: Steradent 3minutes®

Group B: Steradent Active Plus®

Group C: Die Blauen ® (Kukident)

Group D: Deionised Water

Figure 2. *Denture cleaners used in the experiment*

The products are shown in Figure 2. The solutions were prepared at 50°C. and a total of ten immersion cycles were carried out according to manufacturers instruction. The solutions were gently agitated. At the end of each cycle, the teeth were taken out of the solution, gently rinsed in deionised water and allowed to air dry for 30 minutes. A fresh solution was prepared for each cycle. QLF images were taken at the end of each cycle and later analysed for stain reduction (Figure 3).

QLF Images

The QLF camera was mounted in a fixed position over a laboratory jack. A black repositioning device was made and placed on the jack to enable reproducible placement of the teeth on each occasion and helped reduced internal reflections to aid production of sharp clear images. A clinical version of the QLF system (Inspektor™ Pro) used in this study is shown in Figure 4. Each tooth was placed on the repositioning device and an image was taken. Focussing of the QLF image was obtained by fine adjustment of the jack.

Analysis of the QLF image

The lesions captured by the QLF *in vitro* system were analysed quantitatively with QLF software to calculate the percentage of fluorescence loss (delta Q). The system consists of a special camera device connected to a computer to which the QLF software (version 2.00c) was installed. In the visualisation and capturing of tooth images, white light from an arc lamp based on xenon technology was filtered through a blue- transmitting band pass filter with peak intensity of λ= 410nm and a band width of 80nm, to illuminate the tooth with blue-violet light with the aid of a CCD sensor which had a yellow transmitting filter ($\lambda \geq$ 520nm) positioned in front of it to filter out reflected and backscattered light. The fluorescence loss, delta Q was obtained by reconstruction of fluorescence of the sound enamel at the site of the lesion from the fluorescence of the surrounding sound enamel. The decrease in fluorescence was determined by calculating the percentage difference between the actual and reconstructed fluorescence surface.

An analysis patch was placed over the stained area ensuring that the borders of the patch fell on sound or unstained enamel. The image was analysed and the reconstructed stained area checked to ensure that it mimicked the original stained area morphology.

QLF images were stored and analysed by a single examiner using QLF software (version 2.00c). The percentage stain removal was calculated from the results obtained from the QLF analysis.

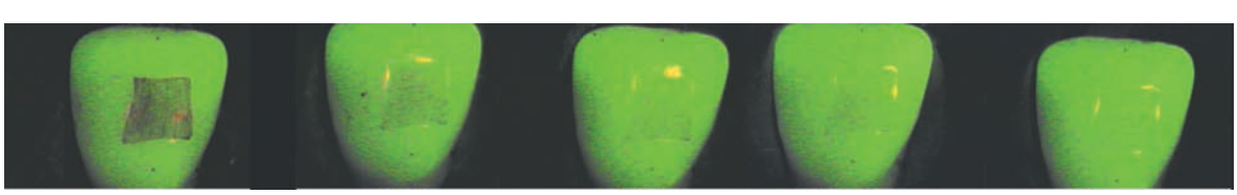

Figure 3. *Example of QLF images obtained at the end of each cleaning application cycle*

Figure 4. *Example of QLF system, Inspektor™ Pro for chair side use (image courtesy of Inspektor Research Systems BV, The Netherlands)*

Statistics

ANOVA and Post Hoc (Tukey) tests were performed to evaluate the statistical significance of all the data. Significance was assumed at the 5% level.

RESULTS

Basic 6®, Viviodent DCL®, Enigma® and Cosmo HXL® teeth all fluoresced when subjected to Quantitative Light-induced fluorescence (QLF). Senator® teeth did not fluoresce under QLF. The measurement of staining intensity and removal by the four groups was measured by QLF and recorded in delta Q values. The mean stain delta Q values and standard deviations of the teeth are shown in table 1. There was no significant difference between the stains formed in groups ($p = 0.23$, single factor ANOVA).

The ability of all three denture cleaners to remove stain when compared with water as detected by QLF was improved significantly ($p<0.001$). Steradent 3 minutes® showed the greatest percentage stain removal efficacy amongst the groups after the first application (43.8%). Die Blauen ® (Kukident) showed 38.8% efficacy, Steradent Active Plus® showed 7.1% and water 1.4% respectively.

At the final application, Steradent 3 minutes ® showed 85.6 % stain removal efficacy, while Die Blauen ® (Kukident) showed 82.4% efficacy, Steradent Active Plus® showed 70% and water 6.1% respectively.

Trend lines showing the cleaning potential of the various cleaning groups are shown in Figure 5. There was no significant difference in the cleaning ability of the denture cleaners at the tenth application.

DISCUSSION

Whilst an enormous amount of work has been done on the evaluation of denture cleaners, there are still some concerns over efficacy, caused by non standardised methods and conflicting results [7]. Various suggestions have been made to over come this, such as a standard method of evaluation closely related to the clinical use of the cleaners.

Table 1. *Mean delta Q (%.mm²) values and standard deviation of acrylic resin teeth obtained at the end of staining (phase 1).*

Group	*Mean ΔQ (%.mm²)*	*SD*
Group A	965.79	278.15
GroupB	1005.37	120.88
Group C	826.35	216.60
Group D	1033.34	295.12

QLF detect stains in a similar way to early demineralisation [18,33]. Its ability to detect stain formation and removal in bovine teeth is documented [34]. QLF is an optical method that uses the natural fluorescence of teeth to distinguish between sound enamel and caries or stains. Its application for visualising stains on acrylic teeth has not been documented in literature. The results of this study showed that Basic 6®, Viviodent DCL®, Enigma® and Cosmo HXL® teeth fluoresced under QLF while Senator® teeth did not. It is interesting to note that not all acrylic resin teeth fluoresced when subjected to QLF. This might be explained by the various methods of manufacturing these teeth and different intrinsic optical properties. There is the need to further investigate the effect that type of pigments, properties of materials, method of manufacturing and shade have on the fluorescing properties of acrylic resin teeth.

Although there was no significant difference in the overall cleaning ability between the three commercial denture cleaners QLF analysis results showed the various cleaning trend pattern of the different denture cleaners used. Steradent 3 minutes® and Die Blauen (Kukident)® showed an initial rapid cleaning trend which slowed down with further applications of the cleaning solution. Steradent Active Plus® had a linear pattern of stain removal. All the denture cleaners cleaned better than water which had a minimal cleaning effect.

The cleaning ability of the cleaners at the final application was not significantly different. The clinical implication of this is that continuous use of the denture cleaners used in this study may help in achieving clean dentures. The greater the percentage of removal of stains by the cleaner the better. It is difficult to speculate if a 100% stain removal is achievable by the cleaners or if 85.6% removal is clinically better than 82.4% removal because factors such as patient compliance, dexterity and the patient's oral ability to cope with dirty dentures all play a role. However, the

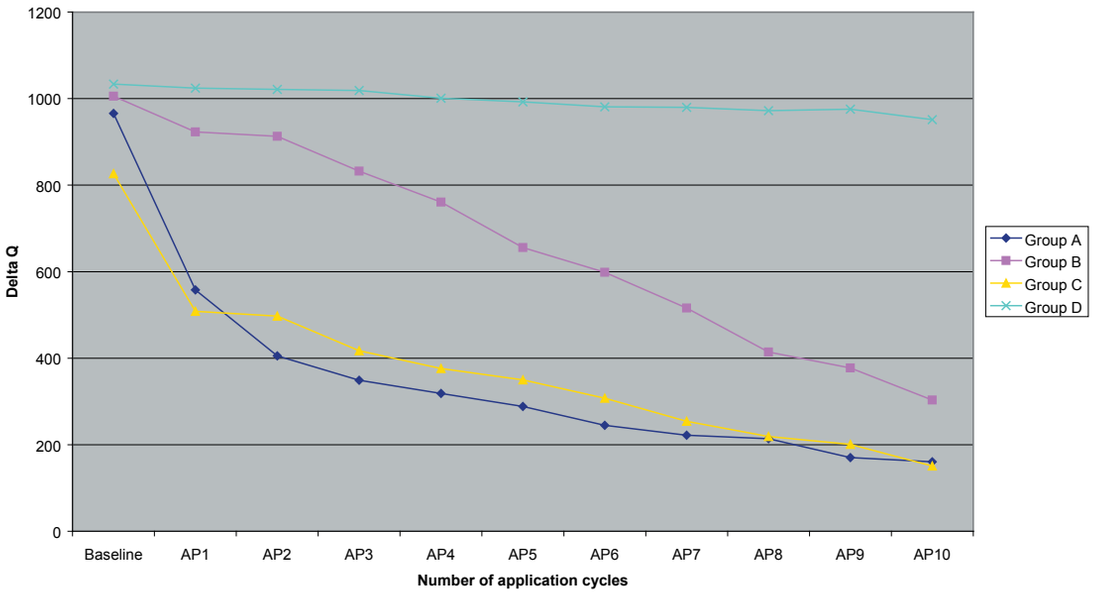

Figure 5. *Showing the cleaning trends of Steradent 3minutes® (Group A) Steradent Active Plus ® (Group B) Die Blauen ® (Kukident) (Group C) and Water (Group D) respectively.*

longitudinal assessment of the cleaning abilities of cleaners will help clinicians assess and monitor the hygiene and the suitability of a particular type of denture cleaner on the dentures worn by their patients. This longitudinal feature of QLF will also help clinicians in decision making as to the effect of the cleaning technique used by patients. Most of the methods for the assessment and evaluation of denture cleaner efficacy as summarised by Abelson [13] in a review on denture plaque and cleaners were not quantitative and lacked standardisation in methodology. This made direct comparisons of results from various studies difficult.

The potential for QLF to serve as a quantitative method for assessing stain removal on denture teeth seems promising as most methods for assessing efficacy of denture cleaners and product testing could be at best described as semi quantitative [26].

Few quantitative methods exist which can be used for product testing or assessment of efficacy of stains removal e.g. spectrophotometers which measure colour from the reflected spectrum and are expensive to purchase. The relative large measurement area and the tooth curvature are the biggest limitation for such instruments, making chair side use difficult [35].

Stains on dentures are thought to be caused by tea, tobacco together with blood or food residues. Brown stains that remain in the crevices can be a result of iron phosphate present in calculus on the denture and can be difficult to remove [21]. QLF images of these dentures can be taken and viewed at chair side so that patients can better appreciate stains on the denture and the effect a particular cleaning method can be assessed. Images can be archived and longitudinal monitoring of effectiveness can be assessed.

A draw back in the use of the *in vitro* QLF system in this study to quantify stain, is the need to compare the stained area to the surrounding unstained area as a reference point, hence the creation of a window with clear varnish. Thus the direct application of the system *in vivo* to quantify the degree of stain as a numerical output is not possible. The further development of the system to quantify generalised areas of stain without the need to use surrounding unstained areas as a point of reference is needed and is currently being investigated.

As some denture wearers experience difficulty in cleaning their dentures satisfactorily and may wear dirty dentures[37,38,38]. QLF may be clinically applicable to aid the visualisation of stains and as a useful educational and motivational tool in denture hygiene instruction.

While most studies assess efficacy of denture cleaners by either plaque or stain removal, QLF can detect dental plaque, stain development and removal via (red and green) fluorescence measurements [34,40,40,]. Though the primary aim of this study was not to look at QLF's ability to assess denture plaque removal, this study indicated that there is a potential with the improvement of the present system, for QLF to be able to quantitatively assess stain development and removal in addition to denture plaque *in vivo* looking at whole tooth surfaces. Whilst this study focused on stain on denture teeth only, there is the possibility of assessing the effect of denture cleaners on the whole denture using QLF. The removal of plaque and stains on fitting surfaces and denture bases may be assessed effectively in future.

In this study QLF has demonstrated the ability to detect and quantify changes in stain removal by the various denture cleaners. The methodology used was simple, to use and reproducible. The use of QLF as a method for assessing the efficacy of denture cleaners has been demonstrated. There is a potential for its use as a quantitative assessment tool in denture cleaner product testing *in vitro*.

ACKNOWLEDGMENT

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MANUFACTURERS' DETAILS

- Basic 6® (Heraeus Kulzer, Germany)
- Viviodent DCL® (Ivoclar Vivadent AG, Liechtenstein)
- Enigma® (Schottlander, Herts. U.K)
- Senator® (Wright Health Group LTD, Scotland)
- Cosmo HXL®, (Dentsply Ltd, Surrey. U.K)
- Chlorhexidine mouthwash (Adams Healthcare, England)
- Clear acid resistant nail varnish (Maxfactor, Proctor and Gamble, Ltd, UK)
- QLF (version 2.00c; Inspektor Research Systems BV, NL)
- Steradent 3minutes®, Steradent Active Plus®, Die Blauen ® (Kukident) were manufactured by Reckitt Benckiser (UK) Limited

ADDRESS FOR CORRESPONDENCE

A.A.Adeyemi, The University of Liverpool, School of Dental Sciences, Edwards Building, Daulby Street, Liverpool L69 3GN. E-mail: A.A.Adeyemi@liv.ac.uk

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