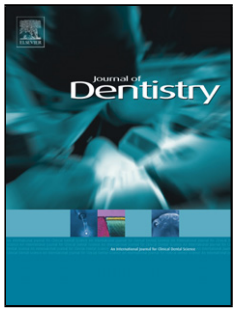

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Disinfection procedures: their efficacy and effect on dimensional accuracy and surface quality of an irreversible hydrocolloid impression material.

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Abstract

Objectives: This study investigated the antibacterial efficacy and effect of 0.55% ortho-phthalaldehyde (Cidex OPA®) and 0.5% sodium hypochlorite (NaOCl) on the dimensional accuracy and surface quality of gypsum casts retrieved from an irreversible hydrocolloid impression material.

Methods: A simulated clinical cast and technique was developed to compare the dimensional accuracy and surface quality changes of the test gypsum casts with controls. Dimensional accuracy measurements were completed between fixed points using a travelling microscope under low angle illumination at a magnification of X3. Surface quality changes of "smooth" and "rough" areas on the cast were evaluated by means of optical profilometry. The efficacy of the disinfection procedures against *Pseudomonas aeruginosa* was evaluated by determining the number of colony forming units (cfu) recovered after disinfection of alginate discs inoculated with $1x10^6$ cfu for defined intervals.

Results: The dimensional accuracy of the gypsum casts was not significantly affected by the disinfection protocols. Neither disinfectant solution nor immersion time had an effect on the surface roughness of the "smooth" area on the cast, however, a significant increase in surface roughness was observed with increasing immersion time for the "rough" surface. Complete elimination of viable *Pseudomonas aeruginosa* cells from alginate discs was obtained after 30 and 120 s immersion in Cidex OPA® and NaOCl, respectively.

Conclusions: Immersion of irreversible hydrocolloid impressions in Cidex OPA® for 30 s was proved to be the most effective disinfection procedure.

Introduction

The increased awareness of infectious diseases and the recognition of the potential for transmission of infectious microorganisms during dental procedures has led to an increased concern for, and attention to, infection control and prevention in dental practice [1]. It has been suggested in the literature that dental impressions which become contaminated with patients' saliva and/or blood can cross-contaminate stone casts poured against them [2]. The subsequent handling of the impressions, following removal from the oral cavity also has potential for microbial transmission [3]. Until 1991, rinsing under running tap water was the recommended procedure for "disinfection" of dental impressions [4]. However, it has been shown that washing impression materials with water alone removes as little as 40% of bacteria, viruses and fungi[5] and therefore is totally inadequate as a means to reduce potential infection risks [5]. Best practice advocates that chemical disinfection of impressions is the most effective means of minimising infection risks [4,6]. A wide variety of disinfectants are commercially available, but specific recommendations about which one to use are primarily based on the disinfection characteristics of individual disinfectants [1]. Chemical disinfectants can be broadly classified into three categories [7]: High-level disinfectants namely, ethylene oxide gas or glutaraldehyde solutions which are able to inactivate spores and all other microbial forms [8]; Intermediate level disinfectants namely, formaldehyde, chlorine compounds, iodophors, and alcohols phenolic compounds which may not inactivate spores but will destroy other microbes, in particular tubercle bacilli [7]; and Low level disinfectants namely, quaternary ammonium compounds, simple phenols, detergents which are unacceptable for disinfection of contaminated impressions [7]. Chemical agents suitable for the

disinfection of dental impressions routinely used in dentistry include: sodium hypochlorite, glutaraldehyde, iodophor, and phenol [8-10]. However, not all impression materials are compatible with all types of disinfectant and the potential for disinfectants to modify the properties of the impression material (surface roughness, and dimensional stability) are a distinct possibility [11]. Controversy exists in the dental literature as to whether the disinfection process causes degradation or distortion of dental impressions and to what extent [8,12].

Important factors to be considered when a disinfection protocol for dental impressions is being considered include the effectiveness of the disinfection procedure, the chemical stability of the disinfecting solution and the influence of the disinfectant procedure on the dimensional stability and surface reproduction of the impression materials and resultant casts [1,13]. In the dental literature, numerous studies investigating different disinfection products, disinfection procedures, contact times, and disinfectant agents suggest that there are no universally recognised disinfection protocols for dental impressions [1,14-15]. Therefore, individual analysis of impression materials is required.

The aim of the current investigation was to evaluate the effect of two different disinfection solutions (a high level disinfectant: ortho-phthalaldehyde (Cidex OPA®; Johnson & Johnson, East Windsor, NJ, USA) and an intermediate level disinfectant: sodium hypochlorite (NaOCl)) at seven immersion times on 1) the dimensional accuracy and surface quality of gypsum casts retrieved from disinfected irreversible hydrocolloid impressions and 2) the antimicrobial efficacy of the disinfecting solutions used in relation to disinfection immersion time.

Materials and methods

Part I -Evaluation of dimensional accuracy and surface quality

A stainless steel master model made from a maxillary Dentoform mould (Columbia Dentoform Corporation, Long Island City, NY, USA) with edentulous polished surfaces parallel to the horizontal plane (Figure 1) similar to that used previously by Byrne *et al*. [16] was used in the current study. The teeth were marked with 100 µm wide cross lines in the horizontal plane with two indentations on the mesial and distal side of the prepared surfaces for measuring purposes [16]. The edentulous surface on the right side of the master cast was polished to create a "smooth" area and the edentulous surface on left side was roughened using an acrylic polishing bur (Tungsten Carbide Cutter; Edenta AG, Heidelberg, Switzerland) to create a "rough" area.

To prepare the hydrocolloid impressions, one size stock plastic impression trays (O-tray Upper No 3; Dentaurum, Ispringen, Germany) were used with a new tray used for each impression. Alginate adhesive (Pegasus tray adhesive liquid; Pegasus Dental Supplies Ltd, Altrincham, England) was applied to the impression tray in a thin layer 5 mins before the impression was made. The irreversible hydrocolloid impression material (Hydrogum thixotropic; Zhermack SpA, Badia Polesine, Italy; Batch No. A078B) was manipulated in accordance with the manufacturer's recommended powder to liquid mixing ratio of 1 g to 2 mL, respectively. To prepare the Hydrogum impressions, 0.9 g of powder was measured into a mixing bowl using a balance accurate to 1 µm (Sartorius Expert; Sartorius AG, Goettingen, Germany) and 18 mL of distilled water was dispensed on top using a disposable syringe. The powder and

liquid constituents were hand-mixed together using a spatula for 10 s and then mechanically-mixed under vacuum for 20 s using an automated mixer (Alginator II; Cadco, Oxnard, CA, USA). To standardise the impression technique, a positioning device consisting of a base and three parallel guide posts similar to that described previously by Stauffer *et al*. [17] was used (Figure 2). To ensure accurate and reproducible positioning of the trays to the positioning device, two indexes were fabricated using Pattern Resin (GC, Dental Products Corporation, Japan), one was used to accurately open the screw hole on the stock trays and the other to position the stock tray on the metal plate. Before each impression, the master cast was steam cleaned for 10 s. The recommended setting time of Hydrogum was increased from 130 s to 300 s, to compensate for a delayed setting of the material at room temperature (21 ± 2ºC) compared with closed mouth temperature (37ºC) [18]. After setting, each impression was rinsed for 10 s under cold tap water and immersed in 0.55% (v/v) ortho-phthalaldehyde (Cidex OPA®) or 0.5% (w/v) sodium hypochlorite (NaOCl) for 30, 60, 90, 120, 180, 240, and 300 s with the control group undergoing no disinfection immersion procedure. Ten impressions were made for each group under investigation.

After the immersion time had elapsed, each impression was rinsed for 10 s under cold tap water and sealed in a plastic bag for 7 mins (at room temperature). All impressions were cast using gypsum (Jade stone type IV; Whip Mix, Louisville, KY, USA; Batch No. 18597) prepared using a powder to liquid mixing ratio of 4.5 g to 1 mL, respectively. Distilled water (15.5 mL) was placed into a vacuum mixing bowl and 70 g of gypsum powder was slowly added and hand-mixed using a spatula for 10 s until the powder was completely wetted by the liquid. The powder and liquid were then vacuum-mixed for 20 s using an automated mixer (Vac-U-Vestor; Whip Mix,

Louisville, KY, USA) and poured into the impression using vibration (Vac-U-Vestor). The casts were allowed to set for 45 mins before separation from the impression and none of the casts were mechanically trimmed. The models were stored at room temperature for 48 h prior to analysis. Cross-arch (A-B), antero-posterior (B-C) and cross-arch antero-posterior (A-C) dimensions (mm) (Figure 3) were measured on each cast using a travelling microscope (Nikon Measuring Microscope MM-40; Inspection Equipment Co. Ltd., Dublin, Ireland) with an accuracy of ± 0.001 mm under low angle illumination at X3 magnification. Ten measurements of A-B, B-C and A-C dimensions were performed for each group under investigation.

To assess the surface quality of the retrieved gypsum casts, the surface roughness of the casts was assessed using an optical profilometer consisting of a non-contact 3 mm range chromatic length aberration gauge (Talysurf CLI 2000; Taylor-Hobson Precision, Leicester, England). Three profilometric traces were performed on each of the polished edentulous surfaces ("smooth" and "rough") at a speed of 200 µm/s with measurements taken every 2 µm intervals and a 2 µm spacing between traces. The roughness (Ra) of each profile (the arithmetic mean deviation of the roughness profile) was determined in accordance with ISO 4287:1997 [19] using a Gaussian filter and a 0.25 mm cut-off.

Statistical analyses (two and three-way analyses of variance (ANOVAs), Tukey's post-hoc tests and regression analyses) were made in software (SPSS 12.0.1; SPSS Inc., Chicago, IL, USA) at a significance value of p=0.05. Individual two-way ANOVAs (disinfectant solution x immersion time) and Tukey's post-hoc tests were conducted for each dimension measured (A-B, B-C and A-C). A three-way ANOVA

(disinfectant solution x immersion time x roughness area) was performed for the Ra data. Tukey's post-hoc tests employed to determine significant differences between groups. Individual regression analyses were conducted where required to check for general trends within the dimensional accuracy and Ra data with increasing immersion time.

Part II -Evaluation of antimicrobial efficacy

Disc-shaped Hydrogum specimens (15 mm diameter, 4 mm height), were prepared (n=10) using the manipulation procedure outlined previously and placed into individual wells of a 24 well cell culture plate. The test organism, *Pseudomonas aeruginosa* 246 wild type environmental isolate which was recovered from a dental chair unit suction system [20] was cultured in Brain Heart Infusion (BHI; Oxoid, Basingstoke, England) broth at 37°C for 15 h in a shaking incubator (Gallenkamp, Leicester, England) at 150 rpm. *Pseudomonas aeruginosa* 246 produces green pigmented colonies typical of the species on BHI agar following 15 h incubation at 37 °C. Using a fresh 15 h BHI agar culture, the bacterial cell density was adjusted to approximately 1 x 10^7 colony forming units (cfu) per mL by dilution in sterile phosphate buffered saline (PBS). An inoculum of 1 x 10^6 in a final volume of 100 µL was applied to discs using a sterile pipette tip fitted to a Gilson P200 laboratory pipette (Gilson, Middleton, WI, USA). Inocula were left to air dry in a Class 2 laminar airflow safety cabinet for 20 min. Inoculated and control discs were completely immersed in disinfectant solution contained in a sterile 90 mm Petri dishes for the desired time, removed with a sterile forceps and rinsed thoroughly with sterile PBS. The disinfection protocol applied was: 1) Immersion in Cidex OPA® for 30, 45 or 60 s; 2) Immersion in NaOCl for 60, 120 or 180 s; or 3) No disinfection (control group).

Duplicate disinfected discs were immersed aseptically in 5 mL of sterile BHI broth in separate 50 mL Falcon tubes (Becton Dickinson, Oxford, England) and vortexed thoroughly for 1 min, after which time 1 mL aliquots were removed in 1.5 mL Eppendorf Safelock tubes and centrifuged at 3000 x g in a bench top microfuge for 5 min to recover bacterial cells. Following centrifugation, pellets were resuspended in 0.1 mL PBS supplemented with 0.5% (w/v) sodium thiosulphate to neutralise residual disinfectant and plated directly on fresh BHI agar medium and incubated at 37 °C for 15 h in a static incubator (Gallenkamp, Leicester, England). Following incubation, plates were examined and the number of green bacterial colonies present, if any, were counted and recorded. The number of colonies was then multiplied by 5 to obtain the total number of bacterial cfu per mL recovered from the disinfected discs.

Results

Dimensional accuracy

The mean dimensions and associated standard deviations measured between points A-B, B-C and A-C on the retrieved gypsum casts are shown in Table 1. The individual two-way ANOVAs (disinfectant solution x immersion time) showed that there was no significant effect of disinfectant solution on the dimensions measured between points A-B ($p=0.912$), B-C ($p=0.056$) and A-C ($p=0.844$). In addition, no significant effect of immersion time was shown for the dimensions measured between points A-B ($p=0.417$) and A-C ($p=0.593$), however, there was a significant effect for the dimensions measured between points B-C ($p=0.029$). Regardless of the immersion time, the mean dimensions measured between points A-B, B-C and C-A for the groups immersed in Cidex OPA® and NaOCl solutions did not differ significantly ($p>0.250$) compared with the control group.

Surface quality (roughness)

The mean Ra values of the "smooth" and "rough" surfaces on the retrieved gypsum casts for the control group and the groups immersed in Cidex OPA® and NaOCl for immersion times ranging from 30 to 300 s are shown graphically in Figure 4. The three-way ANOVA of disinfectant solution x immersion time x roughness area for the Ra data showed that the disinfectant solution did not have a significant effect ($p=0.087$), however there was a significant effect of immersion time ($p=0.006$) on the Ra data. As a result, the Ra data for each roughness area ("smooth" and "rough") was pooled and individual regression analyses were conducted to check for significant trends within the Ra data with increasing immersion time. There was no significant

effect of immersion time on the Ra data for the “smooth” surface ($p=0.551$), however, a statistically significant increase in Ra ($p<0.0001$) was observed with increasing immersion time for the “rough” surface.

Microbiological evaluation

The results showed that no bacterial growth was recovered from the irreversible hydrocolloid discs immersed in Cidex OPA® for at least 30 s or in NaOCl for at least 120 s. Furthermore, the results confirmed the asepsis of the technique with no bacterial growth recovered from the control PBS inoculum discs as well as before contamination with the inoculum. No bacterial growth was observed on the negative control agar plates used, indicating an aseptic experimental environment. The positive results observed with the positive control demonstrated the validity of the assay method. The cfu per mL for the test, control, and negative control specimens are presented in Table 2.

Discussion

Increased emphasis continues to be placed on infection control and prevention in the dental surgery because of the potential threat presented by a range of overt and opportunistic microbial pathogens. A wide variety of disinfecting solutions are available in the dental market, but a universally recognised disinfection protocol for dental impressions is lacking because of the varying response of each brand of impression material and gypsum product to different disinfection procedures [11]. A number of studies [3,21-24] have investigated a wide variety of brands of irreversible hydrocolloid impression materials, disinfecting solutions, and dental stones using a variety of different disinfection protocols, which has led to inconclusive results reported in the dental literature regarding the most efficient protocol for disinfection of dental impressions. The present study was undertaken to evaluate the disinfection protocol applied in the Dublin Dental University Hospital for the brands of irreversible hydrocolloid impression and dental stone currently used.

NaOCl is one of the original and most widely used disinfectants [25]. The literature shows that it is effective against a broad spectrum of micro-organisms including Human Immunodeficiency Virus [26-27], hepatitis B virus [28] as well as numerous other bacterial species [3,21-24] and their spores [29], viruses [5,22] and fungi [23]. Cidex OPA®, a relatively new high level disinfectant, was introduced to the market as a safer alternative to glutaraldehyde even though there was little evidence at that time to support such claims [30]. A limited number of studies [31-32] have shown favourable results for Cidex OPA® solution as a viable alternative to glutaraldehyde

for high level disinfection of endoscopes. However, to date no studies have evaluated the effect of Cidex OPA® on the physical properties of dental impression materials.

In the present study, the two-way ANOVAs of the dimensional accuracy data for the retrieved gypsum casts showed no significant difference between the two disinfectant solutions investigated. For the dimensions measured between points B-C a statistically significant effect of immersion time was found (P=0.029), however, this was not considered to be clinically significant since the greatest mean deviation from the control group was 0.061 mm after 60 s immersion in Cidex OPA® and 0.042 mm after 120 s immersion in NaOCl over a distance of 20.387 mm. Initially it was expected that exposure of irreversible hydrocolloid impression material to disinfecting solutions would adversely affect the dimensional accuracy due to the hydrophilic nature of the material and the phenomenon of imbibition. The dimensional stability observed in this study may be attributed to initial syneresis which causes contraction of the impression material, counteracted by imbibition during disinfection and/or linear expansion of the dental stone during setting [10]. The results reported in the present study with respect to the effect of NaOCl on alginate impressions, are in agreement with other publications [9-10,33-36] where the same conclusions were drawn. However, dimensional changes following disinfection of irreversible hydrocolloid impression using a range of concentrations of NaOCl have been reported in the literature [12,21,37]. Rueggeberg *et al.* [21] examined the effects of alginate disinfection using a 0.525% NaOCl spray or impression immersion in 0.525% NaOCl solution for 10 mins. The results showed that immersion disinfection created dimensional distortion of the resultant casts. Nevertheless, direct comparison of results is not possible due to the variety of materials tested, the disinfection protocols applied, and the measuring

techniques used in each study. This emphasizes the need for compatible studies to ensure that the most appropriate disinfection protocol is used for each given impression material.

Surface quality of gypsum casts retrieved from irreversible hydrocolloid impression materials has been proposed as an indicator of the compatibility of the impression material and dental stone [38]. The results of this study demonstrated that the Ra of gypsum casts were increased after immersion of Hydrogum impressions in Cidex OPA® for 60 s or in NaOCl for 300 s. This was particularly true for the "rough" surface but it is unclear whether the increased Ra observed would have a clinically significant effect on the surface quality of the casts. In addition, for the "smooth" surface, it is not known if its quality remained the same, or if a clinically significant distortion occurred whilst maintaining the smoothness of the surface. It may be that the "smooth" surface was rapidly degraded by the disinfecting solutions but remained smooth as the concentration of the solution on the surface was more equal than would be found on an irregular surface. Immersion in Cidex OPA® increased the "rough" Ra at an early stage (90 s) which may indicate a chemical reaction of the solution with the impression material or the dental stone. However, due to the fact that this effect of Cidex OPA® was observed only for the rough surface, it may be possible that an additional chemical reaction occurred between the dental stone and the residual disinfecting solution which remained in the irregularities of the rough surface even after rinsing with water. Additionally, the low water solubility of Cidex OPA® could also explain the early stage (90 s) increase in Ra observed on the gypsum casts. This may be due to the fact that 10 s rinsing was not adequate to remove the dose of Cidex OPA® retained on the porous impression surface resulting in surface distortion.

Further laboratory research is required to explain the mechanism and the chemical reaction between Cidex OPA® and both alginate and dental stone materials.

The antibacterial efficacy of Cidex OPA® and NaOCl solutions against *Pseudomonas aeruginosa* inoculated irreversible hydrocolloid discs was also evaluated. The Association of Official Analytical Chemists [39] detailed recommended test organisms, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella cholearusuis*, which may be used to classify a disinfectant as bactericidal, tuberculocidal, or sporicidal. The ADA recommendation [40] suggests use of at least a medium level disinfectant for dental impressions, which indicates that the disinfecting solution must be bactericidal and tuberculocidal. Both disinfecting solutions used in the present study satisfy these requirements; NaOCl is classified as a intermediate level disinfectant, while Cidex OPA® is used as a high level disinfectant [31-32,41]. It has been suggested that for a disinfecting solution to be effective it must produce consistently high kill levels on every impression and not just a high average that includes some low values [42]. The results of this study confirmed the aseptic environment under which the experiment was conducted and proved that both test disinfecting solutions were effective against *Pseudomonas aeruginosa*. Complete elimination of viable bacteria from the sample was observed after immersion of inoculated irreversible hydrocolloid discs in Cidex OPA® for 30 s and in NaOCl solution for 120 s. However, comparison of these findings with those of similar studies would not reinforce our conclusions due to the variability of the materials and methods applied. Additionally, the efficacy of a disinfecting solution is not necessarily the same for all impressions depending on the texture and thickness of the impression material. For irreversible hydrocolloid impression material a uniform

cross-sectional thickness of 4-6 mm was proposed by Rudd and Morrow [43] to provide maximum accuracy. In this study the alginate discs used to test the antibacterial efficacy of Cidex OPA® and NaOCl solutions were of a uniform thickness of 4 mm. However, definitive conclusions do not exist in the literature as to whether organisms are present in the body of irreversible hydrocolloid impressions and the extent to which disinfectant solutions can penetrate impressions [44]. Some authors considered the chemical composition of some irreversible hydrocolloid impression materials as having a significant role in inhibiting the efficacy of certain disinfectants [3].

Taylor *et al*. [10] reported that brands of irreversible hydrocolloid impression material which showed the least penetration of disinfecting solution demonstrated superior surface reproduction. However, impression materials that do not absorb disinfecting solutions may not be adequately disinfected if micro-organisms become entrapped within the material when the impression is taken. Individual responses of dental impression materials to immersion protocols for a particular disinfecting agent may explain in part the conflicting reports in the dental literature [35]. In this study, the negative findings presented for NaOCl solution could be attributed to either a reaction between the hypochlorite absorbed into the impression and the dental stone or a direct effect of the hypochlorite on the alginate in relation to surface quality. NaOCl visibly smoothened the surface of the impressions, and a film of disinfectant could be felt on the material even after rinsing with water. In addition, the surface of the retrieved stone discs was extremely smooth and easily abraded, lacking any reproduction of fine detail. These observations are in agreement with a study conducted by Blair *et al*. [15], where surveyed dental laboratories reported softened surfaces on the casts

retrieved from some, but not all, alginate materials following immersion in glutaraldehyde, NaOCl, and sodium dichloroisocyanuratemade [15].

Conclusions

Based on the findings of this study it can be concluded that:

1. The dimensional accuracy of Hydrogum irreversible hydrocolloid impression material was not affected by immersion in either Cidex OPA® or NaOCl solutions for up to 300 s.

2. The disinfecting solution or immersion time did not have a significant effect in terms of Ra on smooth areas on the impressions. However the immersion time significantly increased the distortion of irregular areas of the impressions for both disinfection solutions. Changes were observed after 30 and 300 s immersion in Cidex OPA® and NaOCl solutions, respectively.

3. The complete elimination of *Pseudomonas aeruginosa* colonies was obtained after 30 s immersion in Cidex OPA® and 120 s in NaOCl solution.

4. Based on both effectiveness of disinfection and effects on the resultant gypsum casts the best results in this study were obtained after immersion in Cidex OPA® for 30 s. NaOCl was effective only after longer immersion times.

5. Further research is needed to evaluate the effect of Cidex OPA® on the physical properties of irreversible hydrocolloid impression material and individual analysis of impression materials is required to determine the effect of any given disinfection protocol.

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Tables

		Measured dimensions		
Disinfectant	**Immersion time (s)**	**A-B (mm)**	**B-C (mm)**	**C-A (mm)**
Control Group		46.31 (0.03)	20.39 (0.03)	48.14 (0.09)
Cidex OPA®	**30**	46.28 (0.09)	20.35 (0.03)	48.12 (0.28)
	60	46.32 (0.07)	20.45 (0.09)	48.14 (0.10)
	90	46.34 (0.03)	20.39 (0.03)	48.12 (0.07)
	120	46.32 (0.02)	20.38 (0.04)	48.12 (0.23)
	180	46.33 (0.03)	20.41 (0.02)	48.08 (0.05)
	240	46.32 (0.09)	20.36 (0.08)	48.07 (0.15)
	300	46.34 (0.02)	20.39 (0.05)	48.04 (0.04)
NaOCl	**30**	46.31 (0.05)	20.39 (0.04)	48.07 (0.06)
	60	46.33 (0.05)	20.38 (0.03)	48.12 (0.06)
	90	46.33 (0.05)	20.40 (0.04)	48.13 (0.05)
	120	46.35 (0.05)	20.43 (0.03)	48.09 (0.03)
	180	46.34 (0.07)	20.41 (0.02)	48.13 (0.10)
	240	46.36 (0.02)	20.41 (0.06)	48.10 (0.05)
	300	46.32 (0.04)	20.42 (0.03)	48.14 (0.05)

Table 1 The mean measurements of the cross-arch (A-B), antero-posterior (B-C) and cross-arch antero-posterior (A-C) dimensions for the control group and the groups exposed to Cidex OPA® and NaOCl for immersions times ranging from 30 to 300 s (standard deviations are shown in parenthesis).

Test disc	**Recovered bacterial density (cfu/mL)**	
	Sample 1	**Sample 2**
Blank disc (no inoculum)	Nil	Nil
Blank disc + PBS inoculum	Nil	Nil
Disc + inoculum 1 x 10^6 cfu	12,000	10,500
Disc + inoculum 60 s NaOCl	1,500	500
Disc + inoculum 120 s NaOCl	Nil	Nil
Disc + inoculum 180 s NaOCl	Nil	Nil
Disc + inoculum 60 s Cidex OPA®	Nil	Nil
Disc + inoculum 120 s Cidex OPA®	Nil	Nil
Disc + inoculum 180 s Cidex OPA®	Nil	Nil
Agar plate (no inoculum)	Nil	Nil

Table 2 The cfu per mL for the test, control, and negative control specimens.

Figure Captions

Figure 1 - The stainless steel master model cast from a maxillary Dentoform mould with edentulous polished surfaces parallel to the horizontal plane.

Figure 2 - Positioning device with a base and three parallel guide posts to standardise the impression making technique.

Figure 3 - A schematic representation showing the measurements A-B, B-C and C-A performed on the test casts.

Figure 4 - The mean Ra values of the "smooth" and "rough" surfaces on the retrieved gypsum casts for the control group (0 s) and the groups immersed in Cidex OPA® and NaOCl for immersion times ranging from 30 to 300 s (error bars indicating standard deviations are omitted for clarity).

Figure 1

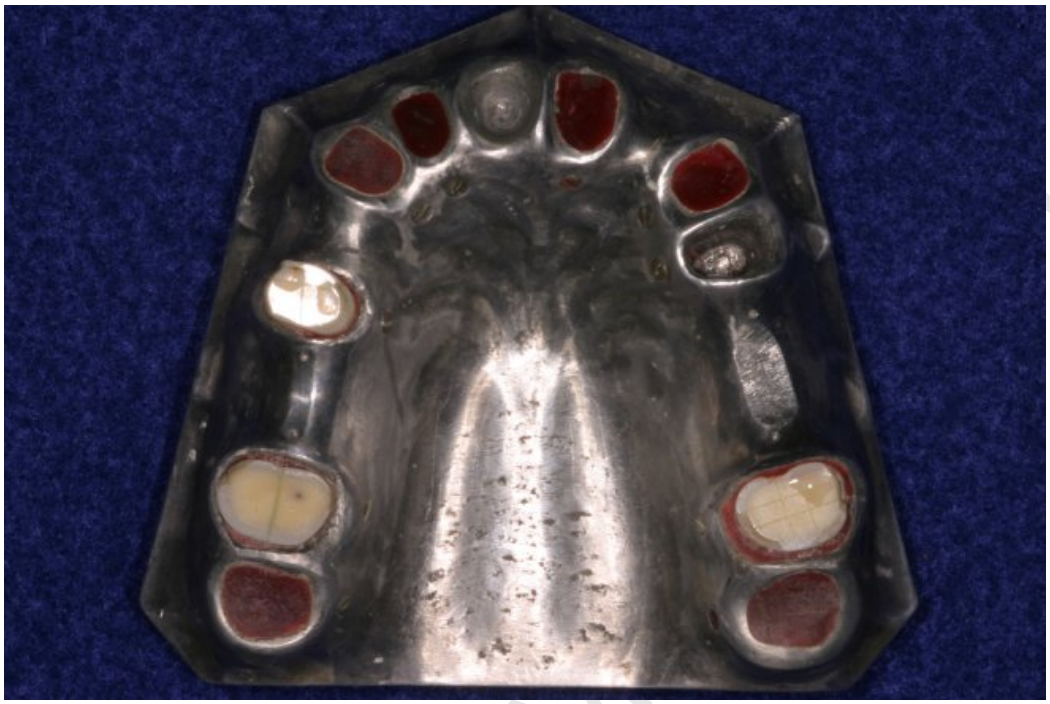

Figure 2

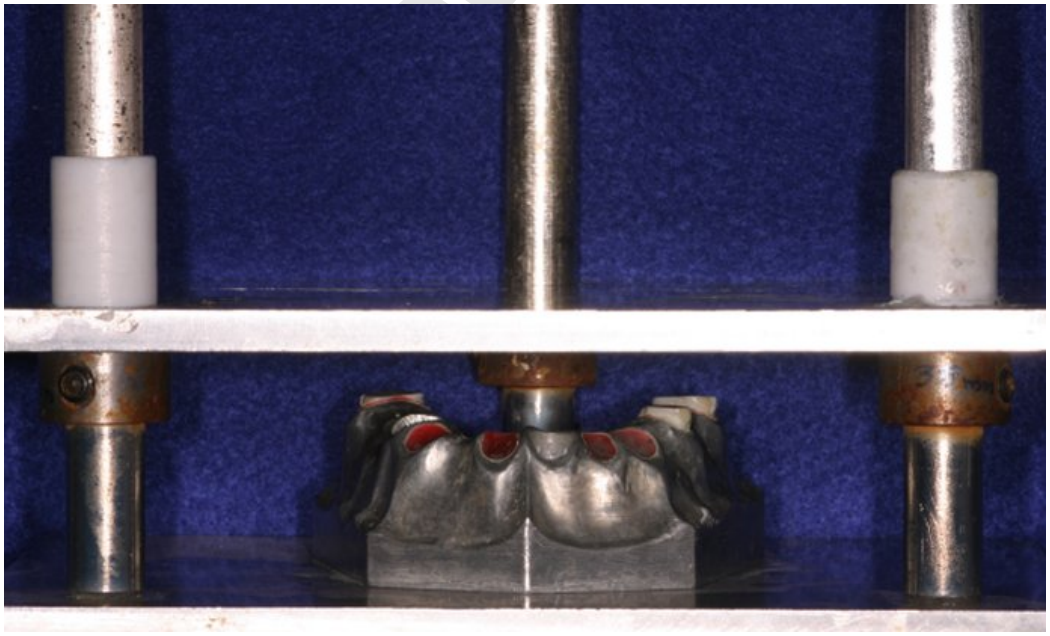

Figure 3

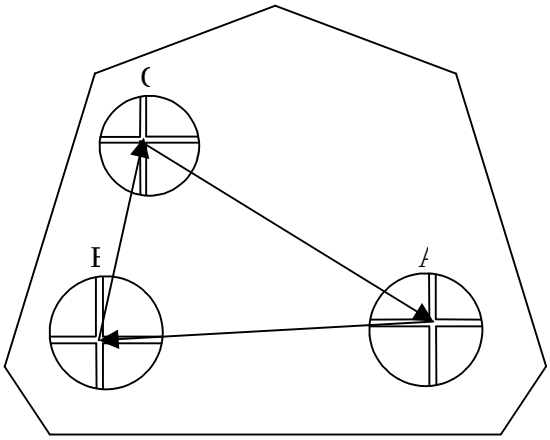

Figure 4