Disinfection of irreversible hydrocolloid impression material with chlorinated compounds

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ABSTRACT

Problem: Irreversible hydrocolloid (alginate) impressions are dimensionally unstable and difficult to disinfect.

Purpose: To evaluate the antimicrobial efficacy of a chlorite disinfectant (Presept®) and a new formulation chlorine dioxide based disinfectant (Aseptrol®) on irreversible hydrocolloid (alginate) impression material.

Materials and Methods: Alginate blocks were contaminated with *Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus mutans and Bacillus subtilis* spores. The blocks were placed either in sterile distilled water as control, or in 48-ppm Aseptrol® or Presept® solution containing organic matter. Immersion times were 30 seconds, 1, 1.5, 2, 3 and 5 minutes. The blocks were then placed in sterile 0.5% sodium thiosulphate neutralizer and surviving organisms were harvested and counted using the serial dilution technique followed by culturing on appropriate media. The anti-microbial efficacy of the solution was tested for 37 days.

Results: There was a consistent significant reduction (99.99%) in all tests of vegetative organisms after immersion in the Aseptrol® for 30 seconds, and for spores after 1.5 minutes. It was effective against vegetative organisms for up to 27 days for a 30-second exposure. Presept® significantly reduced (99.99%) *C. albicans, S. aureus and S. mutans* in 30 seconds, *P. aeruginosa* in 60 seconds, but for *B. subtilis* spores took at least 5 minutes. It was effective against vegetative organisms for >37 days for a 30-second exposure.

Conclusion: Within the limits of this study it was found that both compounds effectively disinfected the alginate in the presence of organic material, but that Aseptrol® did so after an immersion time of only 1.5 minutes. This immersion time is less likely to affect the dimensional properties of the impression material.

Clinical implications: The short action time of Aseptrol® may make it ideal for the disinfection of alginate impressions, and it may also find many uses for disinfection and possible sterilisation.

Keywords: disinfection; alginate; chlorinated compounds; sodium dichloroisocyanurate; chlorine dioxide.

INTRODUCTION

Dental impressions are one of the potential transmission pathways for a wide variety of pathogenic microorganisms that colonise or infect the oral cavity and respiratory tract.[1,2,3] Because of the increased need for infection control in dental work places, routine disinfection of impressions has been recommended to protect clinicians, laboratory personnel, and patients.[4,5] However, disinfection of irreversible hydrocolloid (alginate) impressions continues to be problematic because the material is dimensionally unstable and its surface characteristics make it difficult to disinfect.[6] An ideal disinfectant would be one that can kill target organisms quickly without affecting the dimensional stability or surface characteristics of an impression material.

Alginate possesses a retentive potential for microbes with an ability to transfer three to four times more organisms than elastomeric impression materials during impression making[7], even though elastomers remain in contact with oral tissues for longer. Furthermore, microorganisms survive for a variable but longer duration in alginate, with *Candida albicans* surviving longer than *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus mutans*.[7] Another problem is that the high water content of the material may cause dilution of the disinfectant.[8] The majority of studies in the available literature seem to have been involved in investigating the effects of disinfection on the physical characteristics of the impression materials, with sparse bacteriological data about the efficacy of disinfecting contaminated impressions. [9-12]

Some authors have recommended immersion disinfection to ensure adequate contact[13-15] but immersion disinfection using commonly used disinfectants for 10 minutes or more was found to be inadequate for treating alginate impressions, and treatment time can have a significant effect on the quality of the casts.[8]

Currently, sodium hypochlorite solutions are most commonly used to disinfect alginate impressions because of their relatively short exposure time.[16] However, recommended regimens show considerable variations including immersion in 0.525% solution for 10 minutes,[3] 5.25% solution for 5 minutes,[9] 1% for 10 minutes[17] and 0.0125% for 30 seconds.[18] Commercially available sodium hypochlorite (5.25%) is at pH of about 11.7 to 12.5.[19] It has been shown that 0.525% sodium hypochlorite could disinfect alginate most effectively at pH 10 or lower.[10] However, pH levels lower

than 10 cause an increase in chlorine gas release, instability, and increased surface deterioration of alginates.[19]

Glutaraldehyde solutions are less suitable for routine disinfection of alginates because more than 20 minutes immersion in a 2% solution is required to inactivate mycobacterium organisms and at least 3 hours to inactivate spores.[20] These concentrations and exposure times will clearly have negative effects on alginate impressions.[8,13]

Chlorine dioxide (ClO_2) is a well known disinfectant with a proven fast antimicrobial effect on a broad spectrum of organisms including vegetative bacteria and spores,[3,21] mycobacteria species,[20,22] viruses[23,24] and fungi.[25] It has been used in large-scale municipal and industrial water purification and wastewater treatment systems.[26] An aqueous solution of ClO_2 is unstable, the concentration rapidly decreases, and it is highly oxidative and potentially corrosive.[27] Recent technologies, however, have resulted in formulations of ClO_2 that incorporate stabilising agents and anti-corrosion compounds making them suitable for small-scale applications. A formulation of a base solution and an activator which, when mixed, yielded a solution of about 0.1% ClO_2 with a 14-day shelf life has been used as a chemical sterilant for fibre-optic endoscopes.[27]

The aim of this study was to evaluate the antimicrobial efficacy of a new controlled and sustained release formulation of chlorine dioxide (Aseptrol®, Engelhard Corp, Iselin, USA) and to compare it with Sodium dichloroisocyanurate based Presept® (Johnson & Johnson Medical, Ascot, England) on alginate impression material artificially contaminated with five different organisms, in the presence of organic material.

MATERIALS AND METHODS

Preparation of Sample Blocks

Irreversible hydrocolloid impression material (Blueprint® Cremix – Regular Set: Dentsply-Detrey GmbH, Konstanz, Germany) was proportioned and mixed aseptically using sterile distilled water according to the manufacturer's recommendations. A cylindrical mould made by bisecting a 20ml disposable plastic syringe was used to produce cylindrical impression blocks with standard lengths. To ensure accuracy of the dimensions of the impression blocks, 10 blocks were measured and their mean length (24.77 cm, SD 0.06) and mean weight (10.18 cm, SD 0.04) conformed to a 99% level of accuracy. The same brand and batch of impression material was used throughout to make the blocks, and proportioning and mixing was conducted by one person throughout the experiment.

Test Organisms

The organisms used were *Candida albicans* ATCC 90028, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus mutans* NCTC 1044, and B*acillus subtilis* (ATCC 15244) spores.

Preparation of inoculum

Each test organism was grown on the appropriate medium to obtain a primary culture for preparation of inoculum suspensions: *C. albicans* was grown on Sabouraud dextrose agar medium at 37°C for 48 hours. *S. aureus* and *P. aeruginosa* were grown on tryptone soy agar media and incubated at 37°C for 24 hours. *S. mutans* was grown on blood agar incubated at 37°C for 48 hours under carbon dioxide. To prepare the inoculum for vegetative organisms, the resultant growth was harvested using a sterile wire loop and suspended in 20 ml sterile distilled water. Mixing by gentle shaking was performed to obtain a homogenous organism suspension. The optical density of this inoculum was adjusted to 0.2.

B. subtilis was grown on tryptone soy agar with 2 mg/l magnesium sulphate to enhance sporulation and the culture was incubated at 37°C for 7 days.[28,29] The Schaefer and Fulton method for staining spores[30] was used to confirm >90% spore production. The culture was suspended in 20ml of sterile distilled water and placed in a water-bath at 70°C for 20 minutes in order to kill the vegetative cells. The optical density of the resultant spore suspension was adjusted to 0.2 and used as an inoculum.

Disinfectant

Aseptrol® in tablet form was used to make the chlorine dioxide solution. A 48-ppm solution of ClO_2 was made by dissolving one 500g tablet of Aseptrol® in 10 litres of tap water according to the manufacturer's instructions. Presept® solution was prepared by dissolving 7 tablets (2.5g) into a litre of tap water according to the manufacturer's instructions for decontamination of blood spillage. The mixtures were left for 20 minutes to dissolve followed by gentle mixing with a glass rod.

Test Procedure

For each experiment, 20 ml of organism inoculum (of approx. 10^8 orgs/ml) was freshly prepared. Five alginate blocks were prepared, placed in a sterile container and covered with sterile gauzes soaked in sterile distilled water to protect the material from drying and shrinkage. Each impression block was artificially contaminated by immersion into 20 ml suspension of inoculum for two minutes. This time corresponds with the setting time of alginate in the patient's mouth as recommended by the manufacturer. After contamination, the control block was removed from the inoculum suspension and placed in 20 ml sterile distilled water. The bacterial count on the control block was determined using the serial dilution technique and by culturing onto appropriate media. This control count of bacteria was taken as the count of challenged organisms.

Each of the contaminated test blocks was placed in 20 ml of Aseptrol® or Presept® disinfectant to which 20µl of skim milk as an organic material had been added to test the efficacy of the disinfectant in the presence of organic material. The presence of organic materials is reported to have a negative effect on some disinfectants particularly chlorine releasing agents.[31] Organic material consists of proteins and other debris that interfere chemically with the antimicrobial activity of disinfectants,[32] and its physical presence decreases the contact between the disinfectant and the organisms. The use of skim milk to simulate organic material is documented,[33] and the amount used in this study was considered sufficient to demonstrate the effect of organic material without affecting the dilution of the disinfectant.

Test blocks were exposed to the disinfectant for 30 seconds, 1, 1½ , 2, 3 and 5 minutes' duration after which each block was removed from the disinfectant. The blocks were gently shaken to remove excess disinfectant and placed into sterile jars containing 20 ml of sterile solution of 0.5% sodium thiosulphate as a

Table 1. Time taken to kill the test organisms by Aseptrol and Presept

Organism	Disinfectant	Mean number of challenged organisms (cfu /Block)	Percentage kill Mean ± SD (n=5)					
			30 Sec	1 Min	1½ Min	2 Min	3 Min	5 Min
C. albicans ATCC 90028	Aseptrol	2.2×10^5	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00
	Presept	2.2×10^5	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00
S. aureus ATCC 29213	Aseptrol	1.3×10^6	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00
	Presept	4.2×10^6	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00
P. aeruginosa ATCC 27853	Aseptrol	1.2×10^6	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00
	Presept	1.7×10^6	**99.98 ± 0.03**	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00
S. mutans NCTC 1044	Aseptrol	5.8×10^6	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00
	Presept	2.2×10^6	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00
B. subtilis spores ATCC 15244	Aseptrol	8.2×10^5	**99.24 ± 0.31**	**99.87 ± 0.08**	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00	99.99 ± 0.00
	Presept	1.0×10^7	**73.5 ± 12.4**	**91.05 ± 3.61**	**96.24 ± 4.97**	**99.45 ± 0.41**	**99.92 ± 0.06**	**99.99 ± 0.01**

Table 2. Antimicrobial shelf-life of Aseptrol and Presept

Organisms	Disinfectant	Effectiveness (days)	
		Time taken to kill	
		30 seconds	60 seconds
S. aureus	Aseptrol	**27**	**27**
	Presept	>37	>37
S. mutans	Aseptrol	**35**	>37
	Presept	>37	>37
P. aeruginosa	Aseptrol	**35**	>37
	Presept	>37	>37
C. albicans	Aseptrol	**30**	>37
	Presept	>37	>37

neutralizer to stop any killing effect. The samples were shaken to gently dislodge surviving organisms from the blocks. Sterile forceps were used each time to handle and transfer the blocks during the experiment. Five tests were performed for each exposure time on every organism to ensure the reproducibility of the results.

The bacterial count in the 0.5% sodium thiosulphate solution was determined using the serial dilution technique and culturing onto appropriate media. Percentage kill was calculated using the number of organisms challenged from the control block.

Further tests were performed to establish the antimicrobial shelf-life of both the disinfectants. Disinfectant solutions were prepared as above, and 20ml of disinfectant solution was mixed with 20µl of inoculum containing approximately 10^6/ml of test organisms. Every 30 and 60 seconds, 50µl of the mixture was removed, mixed with 50µl of 0.5% sodium thiosulphate solution and spread onto appropriate culture plates. *S. aureus* and *P. aeruginosa* were grown on tryptone soy agar media and incubated at 37°C for 24 hours. *S. mutans* was grown on blood agar incubated at 37°C for 48 hours under carbon dioxide and *C. albicans* was grown on Sabouraud dextrose agar. This procedure was repeated every alternate day for two weeks and thereafter every day for 37 days using the same prepared disinfectant solution. Culture plates were examined for any growth.

RESULTS

A 99.99% reduction in the number of organisms exposed to the disinfectant was considered significant to evaluate the efficacy. In Aseptrol®, vegetative organisms required 30 seconds immersion to achieve 99.99% reduction, and bacterial spores required 1½ minutes to achieve this level (Table 1). With Presept®, *C. albicans, S. aureus* and *S. mutans* required 30 seconds immersion whilst *P. aeruginosa* required 60 seconds and bacterial spores required more than 5 minutes to achieve 99.99% reduction.

The prepared solution of Aseptrol® was effective for 27 days (Table 2) whereas Presept® was active for >37 days. Although some bacteria were not killed after 30-35 days at 30 seconds exposure by Aseptrol®, 60 seconds exposure was sufficient to eliminate them.

DISCUSSION

The poor dimensional stability of alginate limits the length of exposure time, and its physical properties have negative effects on the efficacy of many disinfectants. Hence, there is a need for a disinfectant that can significantly reduce organisms on alginate impressions within a short duration without affecting dimensional stability or surface characteristics.

To be effective a disinfectant must produce consistently high percentage kill levels on every test, and not only a high average value that includes some low values.[10] For every test organism, five tests were performed using every exposure time to ensure consistency, and all tests were in the presence of organic material. The consistency of both disinfectants in achieving the required reduction of challenged organisms was demonstrated, but in different times: Presept® required twice the time to kill *P. aeruginosa* than Aseptrol®, but more importantly, Presept® required more than 5 minutes to kill spores. Aseptrol® was effective against spores in only 90 seconds.

Sporicidal activity is necessary for the high-level disinfection required to decontaminate instruments that enter sterile body cavities.[34] A medium-level disinfectant, with bactericidal as well as mycobactericidal activity is recommended for dental impressions.[35] It is generally believed that bacterial spores are more resistant to disinfection than mycobacteria,[34] and that HIV and hepatitis B and

C viruses are readily inactivated by disinfectants.[20,36] Moreover, previous studies have reported the efficacy of chlorine dioxide on mycobacteria.[22,37] Although the mycobactericidal and virucidal activity of Aseptrol® were not directly tested in our study, its sporicidal activity indicates that it can be categorized as a high-level disinfectant. It has received USA Environmental Protection Agency registration for use against hospital pathogens, including tuberculosis.

Antifungal activity in disinfectants is required less often than bactericidal or virucidal activity.[34] Impression disinfection targets oral microorganisms, so the effect against *C. albicans* is important because it is common in the oral environment and is an opportunistic pathogen in immune suppressed individuals. The results show that Aseptrol® is effective against *C. albicans* in this study, which confirms published *in vivo* results.[38]

Most disinfectants are diluted to recommended concentrations before use, but only for a limited time. Both disinfectants displayed an acceptably long shelf life which is a necessary economical advantage.

The most important clinical aspect, though, is that a short exposure time is essential, because of the dangers of dimensional change when dealing with alginate impressions, and here Aseptrol® displayed a distinct advantage. However, this study did not test the effect of Aseptrol® on the surface quality of the subsequent casts, and this requires further investigation.

CONCLUSION

Aseptrol®, a new sustained-release formulation of chlorine dioxide, in a 48 ppm solution, effectively disinfected contaminated alginate impression material in the presence of organic material using immersion for 1½ minutes, whereas Presept® required at least 5 minutes for the equivalent level of disinfection. With respect to shelf life, the Aseptrol® solution was effective against vegetative forms of bacteria for up to 27 days at 30 seconds' exposure whereas Presept® was effective for up to >37 days.

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