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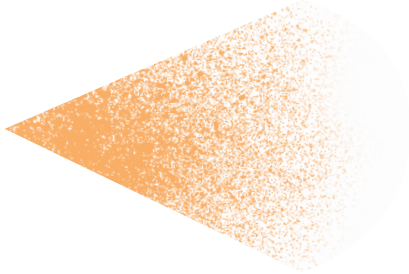

A Microbiologic Investigation Following the Disinfection of Irreversible Hydrocolloid Materials Using the Spray Method

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ABSTRACT Antimicrobial efficacy of three spray disinfectants — 0.525 percent sodium hypochlorite (bleach), deconex and Sanosil — was evaluated on contaminated alginate disks. Disks were sprayed eight to 10 times after rinsing in water for 15 seconds. The samples were then placed into plastic bags containing a sterile moist cotton roll for 10 minutes. The use of 0.525 percent sodium hypochlorite sprayed onto the surface of alginate effectively disinfected 96.6 percent of the samples.

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It has been said that the most biologically contaminated objects to leave the dentist's office for further handling are the dental impressions on their way to the laboratory. Dental impressions get contaminated with microorganisms from a patient's blood and saliva. This makes them noteworthy cross-infection vehicles for hazardous microorganisms. Therefore, the handling of impression materials following their removal from the oral cavity leads to the potential for disease transmission.

Alginate or irreversible hydrocolloid is a common dental impression material in everyday practice. However, these materials, because of their composition, texture and hydrophilic setting mechanisms get easily contaminated with microorganisms present in the oral cavity. Thus, these materials carry significantly higher numbers of bacteria than do elastomeric materials.[1,2] Therefore, establishing a method for disinfecting these materials seems necessary.

FIGURE 1. Precision-machined metal molds.

FIGURE 2. The prepared alginate disks.

Disinfection of impressions, however, can be a challenging task. To be considered efficient, a disinfectant must effectively kill the microorganisms that are transported on the impression without damaging the impression or reducing its accuracy. A wide range of disinfectants are used in everyday practice such as glutaraldehydes, sodium hypochlorite, iodophores, and phenolics. These solutions can be used either in spray form or by immersing the impression in them.[3]

Nonetheless, none of the mentioned solutions or the techniques has become a universally accepted standard. Day by day, the range of disinfectants introduced to the market increases and consequently the number of protocols in use multiplies.[4-6] Unfortunately, appropriate guidelines for impression disinfection are not properly followed.[6]

In the interest of improving compliance, it would appear that storage and transportation of alginate in the moist environment of a sealed bag with the disinfectant sprayed on its surface is a practical alternative. However, studies on disinfection efficiency of irreversible hydrocolloid impressions using various sprays are limited.

This paper is an in vitro evaluation of three different spray disinfectants on irreversible hydrocolloid materials and is designed to provide data regarding the disinfection of dental impressions following removal from the patient's mouth and before entering the dental laboratory to prevent cross-contamination.

Materials and Methods

Three spray disinfectants with different active ingredients were applied in this study. Chloro-Sol Spray (Medtrol, Ill.), deconex Solarsept (Borer chemie, Switzerland), and Sanosil Super 25 (Sanosil Ltd, Feldmeilen, Switzerland) were used in this controlled in vitro study and their antimicrobial efficacy were evaluated on prepared irreversible hydrocolloid disks contaminated with 12 bacterial strains. Chloro-Sol is a sodium hypochlorite solution 0.525 percent; deconex Solarsept is an alcohol-based disinfectant and Sanosil Super 25 is composed of silver and hydrogen peroxide.

Disk Fabrication Method

Regular set alginate (Alginoplast, Heraeus Kulzer) was used in this study. The material was hand mixed according to the manufacturer's instructions, in strict aseptic conditions. For each scoop (8 g) of powder, 19 ml of water was added. Distilled water was used at approximately 23 degrees Celsius. Once the material appeared to be creamy in consistency, the impression material was loaded into prepared metal molds. These molds had an internal diameter of 13 mm and a height of 2 mm (**FIGURE 1**).

The molds were placed on a sterile glass slab and then the impression material was placed into the mold and packed. Alginate was then pressed against a glass slab with balanced pressure. In accordance with the manufacturer's instructions, the material was allowed to set for about three minutes. The disks then were removed from the rings using high-pressure compressed dry air to avoid damaging the surface of the prepared disks during removal. The mentioned steps were carefully applied to all the samples by one of the authors. A total of 792 disks were made (**FIGURE 2**).

Specimen Selection and Collection

A total of 12 bacterial strains were used in this study. Of the microorganisms, six were standard strains obtained from the Persian-type Culture Collection (PTCC), Tehran, Iran. These included *Streptococcus sanguis* (PTCC 1449), *Streptococcus pyogenes* (PTCC 1447), *Streptococcus mutans* (PTCC 1683), *Staphylococcus epidermidis* (PTCC 1114), *Staphylococcus aureus* (PTCC 1112), and *Pseudomonas aeruginosa* (PTCC 1430).

The other six microorganisms were Ghaem Hospital Center clinical isolates. These included *S. epidermidis, S. aureus, Klebsiella pneumoniae, Streptococcus agalactiae,* and *Enterecoccus faecalis.*

All strains were sent to the microbiology laboratory at Ghaem Hospital Center, Mashhad, Iran, and were isolated by morphological and biochemical tests. Each individual strain was cultured on blood agar or MacConkey agar plates (Merck, Darmstadt, Germany) for 24 hours at 37 degrees Celsius. After growth, two or three colonies of that strain were inoculated to test tubes containing a broth medium and a bacterial suspension of 10^8 CFU/ml of that strain was prepared.

Disinfection Treatment and Microbiologic Evaluation

Samples were assigned to three groups according to the disinfection regimen; each group consisted of 240 disks. Sixty samples were made per strain (20 in each group).

TABLE 1

Presence of Microorganisms on the Alginate Disks After Disinfection According to Their Period of Immersion in Bacterial Suspensions

Time	Positive[1]	Negative[2]	Total
	No. (%)	No. (%)	No.
1 min *	60 (16.66)	300 (83.33)	360
4 min **	69 (19.16)	291 (80.83)	360
Total	129 (17.91)	591 (82.08)	720
Result		χ^2= 0.76	
		P= 0.38	

No. = Number of disks
* Disks contaminated with the bacterial suspension of 10^8 Cfu/ml for one minute.
** Disks contaminated with the bacterial suspension of 10^8 Cfu/ml for four minutes.
1. Bacterial growth
2. No bacterial growth

TABLE 2

Antimicrobial Efficiency of Each Individual Disinfectant

Disinfectants†	Active ingredient	Positive[1] No. (%)	Negative[2] No. (%)	Total No.
Deconex Solarsept	Alcohol	71 (29.58)	169 (70.41)	240
Sanosil Super 25	Silver, H_2O_2	50 (20.83)	190 (79.16)	240
Chloro-Sol	NaOCL	8 (3.33)	232 (96.66)	240
Total		129 (17.91)	591 (82.08)	720
	Chi-square test result		χ^2= 58.3 P<0.001	

*All samples were sprayed with the products for 30 seconds and were kept in sealed plastic bags for 10 minutes.
No. = Number of disks
1. Bacterial growth
2. No bacterial growth

From 20 disks used for each bacterium, 10 were immersed in bacterial suspension for one minute and the other 10 were immersed in the suspension for four minutes. In order not to have a decrease in the suspension's concentration, an individual tube was used for each alginate disk (792 tubes for 792 disks).

After contamination, disks were washed with 50 cc of distilled water for 15 seconds. Excess water was removed by a gentle shake. According to the predefined group, the disinfectant was applied on the disks by spraying for 30 seconds (approximately eight to 10 sprays). The samples were then stored in a plastic bag containing a sterile damp cotton roll for 10 minutes.

Afterward, the disks were placed onto test tubes containing nutrient broth media for 30 seconds. The tubes were incubated for 24 hours in 37 degrees Celsius, and were plated on soybean casein digest agar plates. These plates were incubated for 24 hours at 37 degrees Celsius for determination of the minimum inhibitory concentrations (MICs) using the macrodilution method.

In the control group, which consisted of 72 disks (six per strain), samples were immersed in bacterial suspension and neither water nor disinfectant was applied on these disks.

Data Analysis

For describing the data, distribution charts and tables were used. For analyzing the data, Chi-square test and logistic regression were applied. In all the stages of evaluation, the P value less than 0.05 was considered significant. All the statistical analysis was done Via SPSS V. 11.5 software (SPSS, Chicago, Ill.).

Results

TABLE 1 summarizes the data regarding the duration of time in which disks were immersed in the bacterial solution. Half of the impressions were immersed in individual microbial suspensions of 10^8 cfu/ml for one minute; the other half was immersed for four minutes. Both groups showed similar results regarding the bacterial growth after disinfection. (P= 0.38, χ^2 =0.76).

The data regarding the antimicrobial efficacy of each individual disinfectant is summarized in **TABLE 2**. Results showed that only eight out of 240 irreversible hydrocolloid disks (3.3 percent) sprayed with 0.525 percent hypochlorite showed bacterial growth. In other words, 96.66 percent of these samples were effectively disinfected. Sanosil killed microorganisms on 79.16 percent of the disks, and the corresponding result was 70.41 percent for deconex (**TABLE 2**). According to the mentioned table, after disinfection, sodium hypochlorite sprayed disks showed the least bacterial growths when compared to the other two products and the Chi-square test showed a statistically significant difference (P <0. 001, χ^2 =58.3).

TABLE 3 summarizes the growth of challenged microorganisms following disinfection with different sprays. Results showed that 0.525 percent sodium hypochlorite spray (Chloro-Sol) had the best antimicrobial activity. None of the strains indicated resistance

TABLE 3

Growth of Challenged Bacteria on Irreversible Hydrocolloid Disks Following Disinfection Treatments

Strains	Deconex Solarsept	Sanosil Super 25	Chloro-Sol	Overall evaluation (n=60)	
	Bacterial growth No.(%)	Bacterial growth No.(%)	Bacterial growth No.(%)	Bacterial growth No.(%)	%
Staphylococcus epidermidis	1 (5)	2 (10)	0 (0)	3	5
Streptococcus pyogenes	2 (10)	2 (10)	2 (10)	6	10
Streptococcus mutans	3 (15)	1 (5)	1 (5)	5	8.3
Pseudomonas aeruginosa	20 (100)	18 (90)	1 (5)	39	65
Streptococcus sanguis	0 (0)	0 (0)	0 (0)	0	0.0
Staphylococcus aureus	2 (10)	1 (5)	1 (5)	4	6.6
*Staphylococcus epidermidis**	2 (10)	1 (5)	0 (0)	3	5
*Enterecoccus faecalis**	0 (0)	1 (5)	0 (0)	1	1.6
*Streptococcus agalactiae**	0 (0)	1 (5)	0 (0)	1	1.6
*Staphylococcus aureus**	2 (10)	3 (15)	1 (5)	6	10
*Pseudomonas aeruginosa**	19 (95)	3 (15)	1 (5)	23	38.3
*Klebsiella pneumoniae**	20 (100)	17 (85)	1 (5)	38	63.3
Total	71	50	8	129	17.9

*Clinically isolated strains
** Sixty disks were used for each individual strain.

against this agent whereas *P. aeruginosa* (both standard and clinically isolated strains) and *K. pneumoniae* were resistant against disinfection by deconex (TABLE 3). Sanosil Super 25 was ineffectual against *K. pneumoniae* and standard strains of *P. aeruginosa*. The growth of the more resistant strains has been summarized in TABLE 4 and compared to other strains.

Analysis by logistic regression showed that significant difference exists between the antimicrobial efficiency of disinfectants (TABLE 5). Antimicrobial efficiency of Chloro-Sol was 125 times more than deconex (OR=125) and 20 times more than Sanosil. Analysis by logistic regression also showed that resistance of *K. pneumoniae* against disinfection was 3.7 times more than other strains all together. This result was 4.3 for *P. aeruginos*a (standard strain).

According to FIGURE 1 the overall resistance of the standard and clinically isolated strains against disinfection was the same (P= 0.14, χ^2 =2.1).

Discussion

During practice, dentists are exposed to microorganisms harmful to health. The source of these harmful microorganisms is, in most cases, the patient.[7] A fundamental route of dentists' exposure to these pathogens is through saliva. Risk factors spread via the saliva include a vast range of microorganisms. According to Miller and Cottone, a saliva droplet contains 50,000 bacteria belonging to 25 genera; many of which are potential pathogens.[8,9] These pathogens can be easily spread through impression materials, especially irreversible hydrocolloids, which harbor microorganisms more than any other impression material.

From the point of view of the dentist's occupational hazards, common carriership of potentially pathogenic bacteria is an epidemiologically unfavorable phenomenon. Surveys show that the frequency of *S. pyogenes* carriership in the nasopharynx amounts to 10 percent in the general population, of *S. pneumoniae* — 20-33 percent and *S. aureus* 30 percent.[7] Pathogenic bacteria can easily penetrate from the nasopharynx to the oral cavity, contaminate the impressions, and create a threat to both the dentist and the dental technician.[7,10-12]

It has been estimated that more than a million dental impressions are made each week in the United States. Impressions are made of the teeth and soft tissues regularly by dentists for the fabrication of crowns, bridges, dentures, orthodontic appliances, and many other dental devices. If contaminated, beside the direct threat to the dentist, the transmission of microorganisms from impressions to dental laboratory technicians seems likely. The disinfection protocol is an essential precaution for preventing cross-infection and protecting laboratory personnel. The American Dental Association, the Centers for Disease Control and Prevention, and the Occupational

TABLE 4

Growth of Resistant Strains

Strain †	Deconex Solarsept No. (%)	Sanosil Super 25 No. (%)	Chloro-Sol No. (%)
**Klebsiella pneumoniae*	20 (100)	17 (85)	1 (5)
**Pseudomonas aeruginosa*	19 (95)	3 (15)	1 (5)
Pseudomonas aeruginosa	20 (100)	18 (90)	1 (5)
Other strains[1]	12 (60)	11 (55)	5 (25)
Total	71 (88.7)	49 (61.2)	8 (10)

1. *S. sanguis, S. epidermidis*, S. pyogenes, S. aureus*, S. mutans, S. epidermidis, S. aureus, S. agalactiae,* and *E. faecalis*
* Clinically isolated strains
† Twenty strains per group

TABLE 5

Coefficient of Binary Logistic Regression Regarding the Products' Efficacy and Bacterial Resistance

Variable	ß	P-value	Odds ratio	CI (95%)
Deconex Solarsept	4.8	<0.001	125	(39.9-394)
Sanosil Super 25	2.0	<0.00	20.4	(7.9-52.9)
Chloro-Sol	0	—	—	—
**Klebsiella pneumoniae*	1.3	0.01	3.7	(1.3-10.8)
**Pseudomonas aeruginosa*	-0.58	0.229	0.55	(0.21-1.4)
Pseudomonas aeruginosa	1.47	0.007	4.3	(1.5-12.7)
Other strains[1]	0	—	—	—

CI: Confidence interval
1. *S. sanguis, S. epidermidis*, S. pyogenes, S. aureus*, S. mutans, S. epidermidis, S. aureus, S. agalactiae,* and *E. faecalis*
* Clinically isolated strains

Safety and Health Administration state that every impression be disinfected after removing from the patient's mouth and before entering the dental laboratory in order to prevent cross-infection.[11,12]

In reality, disinfection protocols can be neglected. The lack of knowledge regarding the risks posed by the microorganisms, time-consuming disinfection procedures, and the possible destruction of the surface detail of impressions by disinfectants is considered the underlying reasons for negligence.[5,6] A nationwide survey, by Kugel et al. in United States, designed to determine how well dental laboratory personnel are communicating with dentists regarding the disinfection of impressions, revealed that 45 percent of the dental laboratory personnel receive inadequate instruction about appropriate disinfection techniques for various impression materials. Most of the respondents stated that they were not sure if the impressions they received had been disinfected. According to this study, many respondents stated that they use solutions not specifically recommended by the ADA or the impression manufacturer.[6]

Three generations of disinfectants were used and their antimicrobial efficiency was tested in the present study. Bleach was represented by Chloro-Sol, alcohol-based disinfectants were represented by deconex, and peroxide-based disinfectants were represented by Sanosil. All three sprays are commercially available and are commonly used in dental clinics and offices.

One of the least expensive but effective disinfectants is sodium hypochlorite, which is commonly known as bleach. Hypochlorite is listed among the ADA's acceptable disinfectants for dental impressions.[13] Results from some studies have indicated that this substance possesses promising effects as a disinfectant in the immersion technique.[14-16] However, the duration in which the dental impressions are immersed in the solution is of importance. Ideally, that time should be the shortest possible, which still disinfects the impression in order to save the dentist's professional time and to avoid any possible etching or destruction of the surface detail of the impression.

In this regard, studies by Rueggeberg et al. and Westerholm et al. have also shown that impressions can be effectively disinfected by spraying hypochlorite and placing them in a sealed plastic bag.[16,17] In the study conducted by Westerholm et al., 0.525 percent sodium hypochlorite spray was able to affect a 4-log10 (99.99 percent) reduction against *S. aureus*.[16] Rueggeberg et al. reported that the antimicrobial effects of 0.5 percent hypochlorite spray treatment were similar to those of immersion treatment.[17] Similarly, 0.525 percent sodium hypochlorite spray applied in the present study showed to be the most effective disinfectant; 96.6 percent of the samples were effectively disinfected (TABLE 2). Thus, the authors' result corroborates with what Rueggeberg et al. and Westerholm et al. reported. Another interesting observation was the

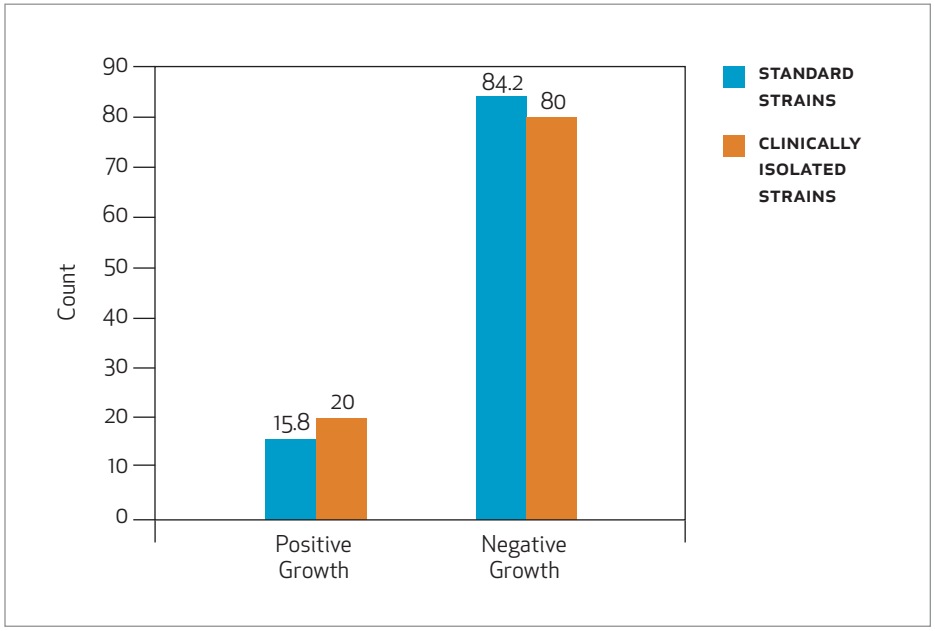

FIGURE 1. Overall resistance of the standard and clinically isolated strains against disinfection.

broad spectrum that sodium hypochlorite possesses as it could kill all gram-positive and gram-negative organisms (**TABLE 3**).

Sanosil is a commercially available disinfectant which is made of silver and hydrogen peroxide. Sanosil's virucidal effect had been tested by Mahnel and Schmidt on drinking water.[18] It has also shown to be effective for control of dental chair unit waterline biofilm.[19] Results from the authors' study indicated that this product was almost effective against all bacterial strains on impression disks except *P. aeruginosa* and *K. pneumoniae* (**TABLE 3**). This might have been due to the method of disinfection (spray). According to **TABLE 5**, deconex is ranked as the third effective disinfectant after Sanosil, with 70.4 percent of the samples effectively decontaminated by this product. Compared to both disinfectants, sodium hypochlorite showed superior activity (P<0.001). It was 125 times more effective than deconex.

A somewhat similar result was reported by Yilmaz et al. in 2005.[20] They studied the effects of disinfectants on denture-lining materials contaminated with different microorganisms. For all microorganisms, soaking in 2 percent sodium hypochlorite reduced the number of microorganisms significantly compared to soaking in 5 percent solution of deconex.

Strains assessed in the current study were sporadically used in some other studies.[21,22] However, the wide range of bacteria used in this study made it possible to scrutinize the efficiency of the disinfectants against each potentially harmful strain. Patient-derived dental impressions are contaminated with numerous microbes, including *streptococci* (100 percent), *staphylococci* (65.4 percent), and *P. aeruginosa*, (7.7 percent), all which are known pathogens responsible for nosocomial and life-threatening infection in the immunocompromised host.[7]

A potential threat to dentists are also some of the gram-negative bacteria, which commonly inhabit the oral cavity and may also come from biofilms occurring inside dental unit waterlines. The most harmful include *P. aeruginosa* and *K. pneumoniae,* which are highly infectious and cause respiratory and urinary tract infections and meningitis. Both *P. aeruginosa* and *K. pneumoniae* were eliminated by hypochlorite in the present study, but deconex and Sanosil were partly ineffective. However, each of the disinfectants were effective in the bacterial elimination of *S. aureus* and *E. faecalis*, which are considered hazardous bacterial strains.[7,9]

According to **TABLE 3**, some weak strains showed sporadic instances of growth. This might be explained by the fact that irreversible hydrocolloid materials are complex carbohydrates that imbibe water. If pathogens are also imbibed into alginate (get trapped subsurface within the material) they would be less exposed to the disinfectant. This indicates that penetration of the disinfectant to such substances might be of importance.

According to **FIGURE 1**, 360 disks were contaminated with standard strains and another 360 were contaminated with clinically isolated strains. Regardless of the each disinfection treatment, when evaluating the resistance of the standard and clinically isolated strains against disinfection, it was demonstrated that disinfection could equally kill the two groups (P= 0.14, χ^2=2.1). Elimination of the clinical isolates, which are often considered more resistant than standard strains, might possibly indicate the application of these products in hospitals settings. However, this result needs further research.

Conclusion

Irreversible hydrocolloid (alginate) can be disinfected by spraying and placing in a sealed plastic bag for the manufacturer's recommended time. The data from this study suggests that 0.525 percent sodium hypochlorite spray is an effective disinfectant agent if the impression is washed under tab water, sprayed for 30 seconds and is put in a sealed plastic bag containing a damp cotton roll for 10 minutes. Due to its effective antimicrobial effect, impression spraying with hypochlorite following

removal from the patient's mouth is strongly advocated by the data from the present study. By disinfecting the impression properly, the safety of both the dentist and the laboratory technician would be guaranteed. ■■■■

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