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Antimicrobial Activity of Irreversible Hydrocolloid Impression against Oral Microorganisms

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ABSTRACT

The oral cavity has a complex microbiota and they could easily caused infection in some undesirable condition or transferred to other person via oral contact. Moreover, it is really important thing in dentistry manipulation and treatment. So, the microbiota and pathogenic oral microorganisms should be controlled by using impression materials. For achieve to the suitable position of controlling infection, our survey focused on alginate, an irrevocable hydrocolloids. Four solutions were containing nano-silver colloid 1000 ppm and 500 ppm, chlorhexidine solution %2, and sterile distilled water were affected on seven microorganisms. The widest zone of inhibition (ZOI) was seen around *staphylococcus aureus* (mean range was 30 mm) in which the solutant was 1000 ppm nanocid. Our investigation support that use of silver ion or metallic silver as well as silver nanoparticles can be exploited in medicine and dentistry.
KEYWORDS: Nano-silver, Oral Microorganisms, Staphylococcus aureus.

1. INTRODUCTION

The spread of infection through dentistry, because of direct contact with saliva, blood and infection of mouth could be transferred easily from non-observance of immunity factors (Wang, 2007; Kugel, 2000). Using impression materials could be carried out concerning its widespread application in dentistry especially in the field of pediatric dentistry, orthodontics and prosthodontics (Rentzia, 2011; Junevicius, 2004). All impression materials are capable of spreading infection to the laboratory, and indirectly to the personnel and other patients in the other offices, so they should be disinfected with effective disinfecting materials before use (Jennings, 1991). Amongst the impression materials, alginate is one of the most frequently used and also between the most critiqued in terms of its disinfection process. Alginate impressions because of their structure, texture and hydrophilic setting mechanisms are more susceptible to infection than some other impression materials (Casemiro, 2007).

American Dental Association (ADA) to limit cross-contamination during dental clinical and laboratory procedures such as impression disinfection has issued guidelines to attain the top method of disinfecting the dental impressions (Blair, 1996). Based on these guidelines, investigators have suggested many approaches of disinfection for irreversible hydrocolloid impression material. Amongst them, spray and immersion are the two most extensively used methods in clinical practice. Though, these conventional strategies present numerous limitations (Wang, 2007). These procedures should be sufficient and exerts no adverse effect on physical properties such as surface details, dimensional accuracy, elasticity and demolition of the surface of impression. Compatibility, the period of immersion or exposure, concentration and composition of disinfectant are some variables which could affect dental impressions and must be attended (Nassar, 2011). Hence, certain disinfectants have been investigated for disinfecting the irreversible hydrocolloid dental impressions (Semensato, 2009; Rai, 2009).

Silver first gained controlling approval for usage as an antimicrobial agent in the early 20th century, but its use reduced with the introduction of antibiotics in the 1940s. Currently, though, topical silver has gained popularity once again, chiefly in the management of open wounds (Demling, 2001; Chopra, 2007). Moyer showed that 0.5% silver nitrate has an antibacterial effect on *E.coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, while it doesn't cause any disruption in the proliferation of the epidermis (Moyer, 1965). Diverse forms of nanomaterials have come up but silver nanoparticles have showed to be most effective as it has good antimicrobial efficiency against bacteria, viruses and other eukaryotic microorganisms (Retchkiman-Schabes, 2006; Gong, 2007). Nowadays, Nanosilver is widely used in medical products. They due to antibacterial properties and low toxicity to mammalian cells are the most commonly used materials compared to other nanoparticles (Rentzia, 2011).

To reach to the suitable position of controlling infection a great deal of work has been done over impression materials, and our purpose in this research was irrevocable hydrocolloids like Alginate.

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MATERIAL AND METHODS

This study was designed based on four experimental groups. Alginate impression was prepared in sterile condition by using alginate powder which comes in the package covered with aluminum foil combined solutions exist in three experimental groups and a control sample according to the manufacturer's constructions describing the amount of powder, water, mixing and setting time. These four solutions were containing: nano-silver colloid (Nanocid) 1000 ppm and 500 ppm, chlorhexidine solution %2, and sterile distilled water. The powder of irreversible hydrocolloid was chosen from GC Leuver, Belgium. Nano-silver colloid (Nanocid) used in this experimental study was obtained from Nano Nasb Pars Company, Iran. The microorganisms were obtained from American Type Culture Collection (ATCC), the USA. They included seven bacterial strains indicating mouth and teeth disease 1) *E.coli*- ATCC 25922 with specialized nutrient agar, 2) *Pseudomonas aeruginosa*- ATCC 15442 with specialized nutrient agar, 3) *Streptococcus mutans*- ATCC 35668 with specialized blood agar, 4) *Staphylococcus aureus*- ATCC 6536 with specialized tripticas soy agar, 5) *Lactobacillus acidophilus*- ATCC 4356 with specialized MRS, 6) *Candida albicans*- ATCC 10231 with specialized YMA, 7) *Actinomyces viscosus*- ATCC 19249 with specialized BHI. Blood agar, tripticas soy agar, yeast mold agar and nutrient agar were obtained from Merck Company, Germany.

The instruments used in this stage were prepared by disinfecting solutions. After mixing Alginate, the prepared product transferred to the sterile penetrated plate to make alginate 7 millimeters disks. Immediately after preparation of disks they were carried to the prepared plates. This time was considered as starting time (zero) and as time of origin for tests calculation.

The necessary laboratory equipments for recognition of antimicrobial effectiveness were similar to the standard experiments of microbiology tests. To prepare the culture dishes 100 ml of suspension (0.5 of McFarland standard) was transferred by sampler (micropipette) to sterile plate containing of specific media. Then it was spread evenly on the surface of plate by using pure plate method. After drying of suspension on the surface of plate, prepared samples in sterile conditions placed on the surface of plate in specified intervals.

Incubation of plates carried out for 24 to 48 hours in 35-37°C. A plate related to the bacterial strains should be incubated for 48 hours in an anaerobic jar. After passed time, zone of inhibition was checked on the scale of millimeter and strains were compared depending on the size of the zone of inhibition.

Quantitative method used and Zone of Inhibition (ZOI) was observed, in case of positive result. Data was gathered and analyzed by SPSS software. Repeated measure was utilized to find out the significance of difference between the measurements. For all the tests, a P value of less than 0.05 was used for statistical significance.

RESULTS

As it was expected, there was no zone of inhibition around each sample in the control group in which water was used as solutant so as to prepare the alginate. All the other groups illustrated a range of ZOI from 5 to 32 millimeters around samples depending on the group and micro organism. The widest ZOI was seen around *staphylococcus aureus* (mean range was 30 mm) in the first group in which the solutant was 1000 ppm nanocid. In this group the lowest mean effect was observed on *pseudomonas aeruginosa*, *lactobacillus acidophilus*, and *actinomyces viscosus*, almost 11 mm. ZOI around *staphylococcus aureus* was significantly more than all other micro organisms (p=.000), when other had no significant differences among them. (Table 1)

In the second group which nanocid 500 ppm nano-silver was used as solution, showed the similar result with group one in a less diameter while the widest range was around *staphylococcus aureus* 23.33 mm ZOI and the narrowest ZOI was seen around *lactobacillus acidophilus*, *actinomyces viscosus* and *pseudomonas aeruginosa* with almost 7mm. In this group *staphylococcus aureus* had significantly wider ZOI in comparing with all other micro organisms ($p \leq .001$). Other microorganisms had no significant differences among them too. (Table 1)

In the third group which 2% chlorhexidine was solution in mixed alginate impression, the results were almost similar to second group in a little smaller size of ZOI, except for *streptococcus mutans* and *candida albicans* which had wider range. In this group *staphylococcus aureus* had significantly wider ZOI in compare of each other microorganism ($p \leq .023$). After that, *streptococcus mutans* had significantly wider ZOI in compare with all rest five microorganism ($p \leq .001$). (Table 1)

Next, the effect of antibacterial activity of three groups were analyzed and compared within groups. The analyzing statistics showed the significant more ability of disinfection in the first group than all other three groups. The second and third group had no significant differences totally but in individually analysis for each microorganism it was seen that the third group had more effect on Streptococcus and Candida albicans (18.00 mm and 11.33 mm ZOI in the third group in compare with group two which were 9.17 mm and 8.50 mm in respectively showed in Table1). All experimental groups had significantly more effect in compare with control group (p=.000).

The result of qualitative experiments of Zone of Inhibition (ZOI) around seven micro organisms in four experimental and control groups (based on mm)

Microorganism	Groups							
	1000ppm Nano silver		500ppm Nano silver		2% chlorhexidine		Water	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
E.coli	13.33	1.37	9.17	.75	9.17	.75	.00	.00
Pseudomonas aeruginosa	10.67	1.03	7.83	.75	6.33	.52	.00	.00
Streptococcus mutans	12.67	1.51	9.17	.75	18.00	.63	.00	.00
Staphylococcus aureus	30.17	1.94	23.33	1.51	22.50	1.38	.00	.00
Lactobacillus acidophilus	11.00	1.55	7.33	.82	7.00	.89	.00	.00
Candida albicans	13.17	.98	8.50	1.05	11.33	.82	.00	.00
Actinomyces viscosus	11.00	1.55	7.33	.82	6.67	1.21	.00	.00

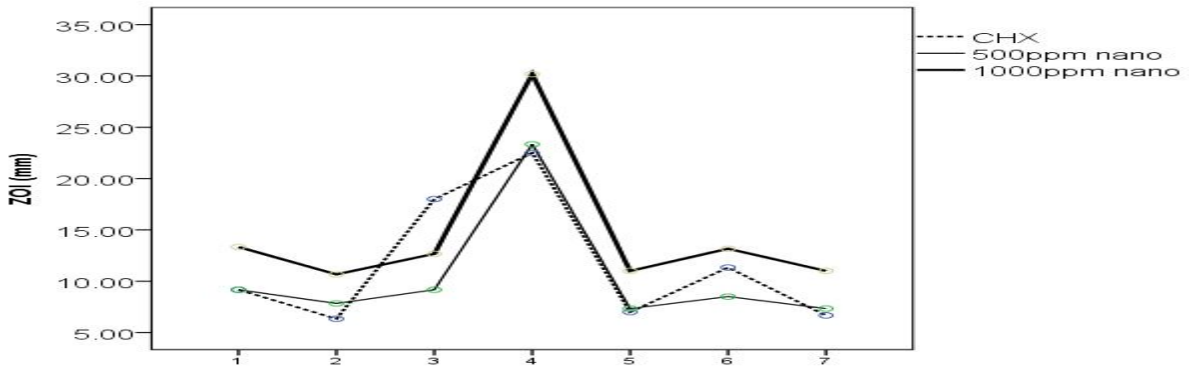

Figure 1- Micro organisms noted on the horizontal dimension: 1) *E. coli* 2) *Pseudomonas aeruginosa* 3) *Streptococcus mutans* 4) *Staphylococcus aureus* 5) *Lactobacillus acidophilus* 6) *Candida albicans* 7) *Actinomyces viscosus*

Table 2- Micro organisms noted on the first culumn: 1) *E. coli* 2) *Pseudomonas aeruginosa* 3) *Streptococcus mutans* 4) *Staphylococcus aureus* 5) *Lactobacillus acidophilus* 6) *Candida albicans* 7) *Actinomyces viscosus*

		Groups					
		1000ppm Nano silver		500ppm Nano silver		2% chlorhexidine	
(I) Micro organism 1	(J) Micro organism 2	Mean Difference (I-J)	Sig.a	Mean Difference (I-J)	Sig.a	Mean Difference (I-J)	Sig.a
1	2	2.667	.099	1.333	.196	2.833	.005
	3	.667	.996	.000	.	-8.833	.000
	4	-16.833	.000	-14.167	.000	-13.333	.000
	5	2.333	.864	1.833	.454	2.167	.018
	6	.167	1.000	.667	.982	-2.167	.197
	7	2.333	.447	1.833	.342	2.500	.083
2	1	-2.667	.099	-1.333	.196	-2.833	.005
	3	-2.000	.129	-1.333	.196	-11.667	.000
	4	-19.500	.000	-15.500	.000	-16.167	.000
	5	-.333	1.000	.500	1.000	-.667	.895
	6	-2.500	.189	-.667	.895	-5.000	.004
	7	-.333	1.000	.500	1.000	-.333	1.000
3	1	-.667	.996	.000	.	8.833	.000
	2	2.000	.129	1.333	.196	11.667	.000
	4	-17.500	.000	-14.167	.000	-4.500	.023
	5	1.667	.990	1.833	.454	11.000	.000
	6	-.500	1.000	.667	.982	6.667	.001
	7	1.667	.903	1.833	.342	11.333	.000
4	1	16.833	.000	14.167	.000	13.333	.000
	2	19.500	.000	15.500	.000	16.167	.000
	3	17.500	.000	14.167	.000	4.500	.023
	5	19.167	.000	16.000	.000	15.500	.000
	6	17.000	.000	14.833	.001	11.167	.000
	7	19.167	.000	16.000	.000	15.833	.000
5	1	-2.333	.864	-1.833	.454	-2.167	.018
	2	.333	1.000	-.500	1.000	.667	.895
	3	-1.667	.990	-1.833	.454	-11.000	.000
	4	-19.167	.000	-16.000	.000	-15.500	.000
	6	-2.167	.442	-1.167	.842	-4.333	.012
	7	.000	1.000	.000	1.000	.333	1.000
6	1	-.167	1.000	-.667	.982	2.167	.197
	2	2.500	.189	.667	.895	5.000	.004
	3	.500	1.000	-.667	.982	-6.667	.001
	4	-17.000	.000	-14.833	.001	-11.167	.000
	5	2.167	.442	1.167	.842	4.333	.012
	7	2.167	.745	1.167	.973	4.667	.034
7	1	-2.333	.447	-1.833	.342	-2.500	.083
	2	.333	1.000	-.500	1.000	.333	1.000
	3	-1.667	.903	-1.833	.342	-11.333	.000
	4	-19.167	.000	-16.000	.000	-15.833	.000
	5	.000	1.000	.000	1.000	-.333	1.000
	6	-2.167	.745	-1.167	.973	-4.667	.034

DISCUSSION

It has been showing a growing anxiety toward the spread of pathogenic microorganisms to dental professionals and patients in the procedures of manufacturing of dental prostheses, orthodontic appliances and other devices used in rehabilitation treatments (Casemiro, 2007). If pathogenic microorganisms on dental impressions and temporary prostheses making devices are not cleansed, direct transmission of infection can occur from patient to dentist, dental laboratory technician or vice versa (Junevicius, 2004). Research shows that alginate impressions are one of the most important carriers in the chain of infection (Watkinson, 1988; Ramer, 1993). Alginate impressions because of their composition, texture and hydrophilic setting mechanisms get simply contaminated with microorganisms present in the oral cavity (Casemiro, 2007).

Today, it is indisputably significant to develop measures to break cross-infection chains, for instance impression disinfection procedures. In terms of irreversible hydrocolloids, disinfection can be performed by numerous methods, and all present advantages and limitations (Powell, 1990).

Disinfection of impressions and works leaving the dental laboratories have become essential and numerous guidelines have been suggested (Vejdani, 2006; Oderinu, 2008). The most common technique for disinfection is spraying the disinfecting agents on alginate impressions, but some studies have shown that these impressions can be disinfected by immersion method as well (Vejdani, 2006). Among the impression materials, irreversible hydrocolloids are among the most commonly used and also among the most criticized in terms of its disinfection process (Casemiro, 2007).

However, spraying that is performed with reduced contact time may restrict the effectiveness of disinfection, particularly for the porous hydrophilic hydrocolloids, where microorganisms can penetrate through the body and survive in the impression (Sofou,2002). It was determined that the incorporation of disinfectants into the hydrocolloid powder or mixing water provides an effective means of additional decontamination, without leading to adverse effects considering stability and accuracy (Jones, 1990; Flanagan, 1998).

Glutaraldehydes, iodophores and phenols are used for disinfection the impressions. Health effects that may occur as a result of exposure to glutaraldehyde include throat and lung irritation, asthma and difficulty breathing, contact and/or allergic dermatitis, nasal irritation, sneezing, wheezing, burning eyes and conjunctivitis. Iodophores are rapidly inactivated in the presence of organic matter. Iodine is corrosive and staining. Phenol is toxic, corrosive and skin irritant. Chlorhexidine is inactivated by anionic soaps. Chloroxylenol is inactivated by hard water (Sridhar, 2008; Rutala, 2008).

The current investigation supports that use of silver ion or metallic silver as well as silver nanoparticles can be exploited in medicine for burn treatment, dental materials, coating stainless steel materials, textile fabrics, water treatment, sunscreen lotions, etc. and possess low toxicity to human cells, high thermal stability and low volatility. Nanotechnology is gaining marvelous motivation in the present century due to its capability of modulating metals into their Nano size, which intensely changes the chemical, physical and optical properties of metals (Duran, 2007; Rai, 2009).

Nanotechnology has been used for medical applications in several forms, including dental practice with the development of silver nanoparticles (Ag NPs) as a useful tool (García-Contreras, 2011). In this research the effect of disinfecting alginate impressions was investigated by nano-silver.

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