AN EVALUATION OF PROPERTIES OF DIE STONE AFTER ADDING ANTIMICROBIAL AGENTS

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The concept of universal precaution is advocated because not all patients with infectious diseases are aware of their status nor can they be identified by medical history or physical examination. Therefore it is important to prevent cross contamination during every dental procedure. This had lead to the need for incorporating a disinfectant directly into the type IV diestone. The present study evaluated the effectiveness of disinfectants(phenol, chloramine- T and sodium fluoride) incorporated into die stone against oral microorganisms(Staphylococcus, Lactobacillus and E.coli) and observed the changes in the mechanical properties (dimensional changes and compressive strength) of die stone. Result shows that chloramine-T was the most effective disinfectant and sodium fluoride was least effective. All the disinfectants did not have any significant adverse effect on the mechanical properties of die stone.

The oral environment harbors a large number of microorganisms in the saliva and blood that may contain infectious bacteria and virus like E.coli, Staphylococcus aureus, Hepatitis B virus and HIV etc. These microorganisms can produce diseases like common cold, hepatitis B etc. Others although are not normally pathogenic they may cause opportunistic infections in patients debilitated by age or disease in immunocompromised individuals[1].

Dental impression, denture, crowns and wax occlusal rims all carry microorganisms from mouth and are transferred to the cast.

The concept of universal precaution is advocated because not all patients with infectious diseases are aware of their status nor can they be identified by medical history or physical examination. Therefore it is the primary responsibility of dental office to control infection and to prevent a cycle of cross contamination[2]

The potentially damaging effects of the immersion technique, the difficulty in covering the entire surface of the cast with the spray disinfecting solution, and the inability to assume that every impression presented to the laboratory has been disinfected have led to the need for incorporating a disinfectant directly into the diestone powder or into the liquid used for mixing the die stone

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AIM AND OBJECTIVE

A) To evaluate the effectiveness of antimicrobial agents incorporated into die stone against microorganism.

B) And to assess the dimensional changes and compressive strength of die stone after addition of various antimicrobial agents.

MATERIALS AND METHOD

Standardized materials and equipments for the estimation of the antimicrobial properties of the study samples were used. These materials were:—

1. **The die stone** (Kalabhai, kalrock; kalabhai karson Pvt Ltd; Mumbai)
2. **Three Antimicrobial agents** used were-
   - **Sodium fluoride** (Qualigens fine chemicals; Glaxosmithkline pharmacauticals Ltd; Mumbai)
   - **Phenol** (Qualigens; Qualigens fine chemicals; Glaxosmithkline pharmacauticals Ltd; Mumbai)
   - **Chloramine-T** (Qualigens; Qualigens fine chemicals; Glaxosmithkline pharmacauticals Ltd; Mumbai)
3. **MacConkey agar media** (Himedia Lab Pvt Ltd; Mumbai, India)

4. **Nutrient broth** (Himedia Lab Pvt Ltd; Mumbai, India)
5. **Three microorganism** commonly associated with oral cavity were –
   - **Escherichia coli**
   - **Lactobacillus**
   - **Staphylococcus**
6. **Moulds for making test specimens for dimensional changes[3] and compressive strength[4]** – made at IIT Roorkee as per ADA specification 19 and 25 respectively **Fig (1& 2)**
7. **Computerized Universal testing machine** (Unitek 9450, FIE, India)
8. **Traveling Microscope** (Achsemhohe 11867)

In order to asses the antimicrobial property of die stone, the preparation of nutrient broth, MacConkey media, storage of bacterial strains, preparation of MacConkey plates and test specimens had to be carried out prior to testing.

Cultivation of bacteria

For the Cultivation of E.Coli drain water was collected, for staphylococcus Nasal and throat samples and for Lactobacillus sachet of sporlac powder (UNISANKYO LTD) were taken. They were all mixed separately in nutrient broth and incubated at 37Ú C for 24 hrs. Then a loop full of this sample was transferred to nutrient agar plate and incubated at 37Ú C for 24 hrs. On the next day single colony was transferred from that plate to a fresh agar plate and again incubated at 37Ú C for 24 hrs. Then a smear was prepared from a single colony of this plate and gram staining and suitable biochemical tests were done to confirm the presence of lactobacillus.

Preparation of MacConkey agar plates

MacConkey agar plates were prepared by pouring serum MacConkey in petridish. The 24hrs fresh culture of bacteria (0.5 ml) from nutrient broth tube was then overlaid on these sterile MacConkey agar plates in front of a gas burner to maintain the sterility and was dried in an incubator at 37°C for 20 minutes.

Preparation of modified formula of the stone-

The modified formula of die stone was prepared by mixing the antibacterial agents in following concentrations

1. Die stone + .1% sodium fluoride (99.9 gm diestone in .1gm of NaF)
2. Die stone + .15% sodium fluoride (99.85 gm diestone in .15gm of NaF)
3. Die stone + .2% sodium fluoride (99.8 gm diestone in .2gm of NaF)
4. Die stone + 3% phenol (97 gm of diestone in 3 gm of phenol)
5. Die stone + 3.5% phenol (96.5 gm of diestone in 3.5 gm of phenol)
6. Die stone + 4% phenol (96 gm of diestone in 4 gm of phenol)
7. Die stone + .2% Chloramine T (99.8 gm of diestone in.2gm Chloramine T)
8. Die stone + .25% Chloramine T (99.75 gm of diestone in.25gm Chloramine T)
9. Die stone + .3% Chloramine T (99.70 gm of diestone in.3gm Chloramine T)

Pellets of filter paper were prepared with a punching machine. They were then dipped in the mix of premeasured volume of water and specially formulated die stone. These pellets in three different percentages were put in a circular manner on the petridish. The control was kept in the centre of the plate. These plates were then placed at room temperature for 24 hours, After 24 hours the inhibition halozones around the pellets were measured with the help of a vernier caliper in two perpendicular directions.

After measurement these pellets were placed on new serum agar plates seated with the 24 hour fresh culture of the same micro-organism. The inhibition halozones were examined likewise after 1 day, 3 days, 1 week, 3 weeks and 5 weeks

Evaluation of mechanical properties

Fifty test specimens of identical dimensions were obtained by using the split cylinder mold for prepar-

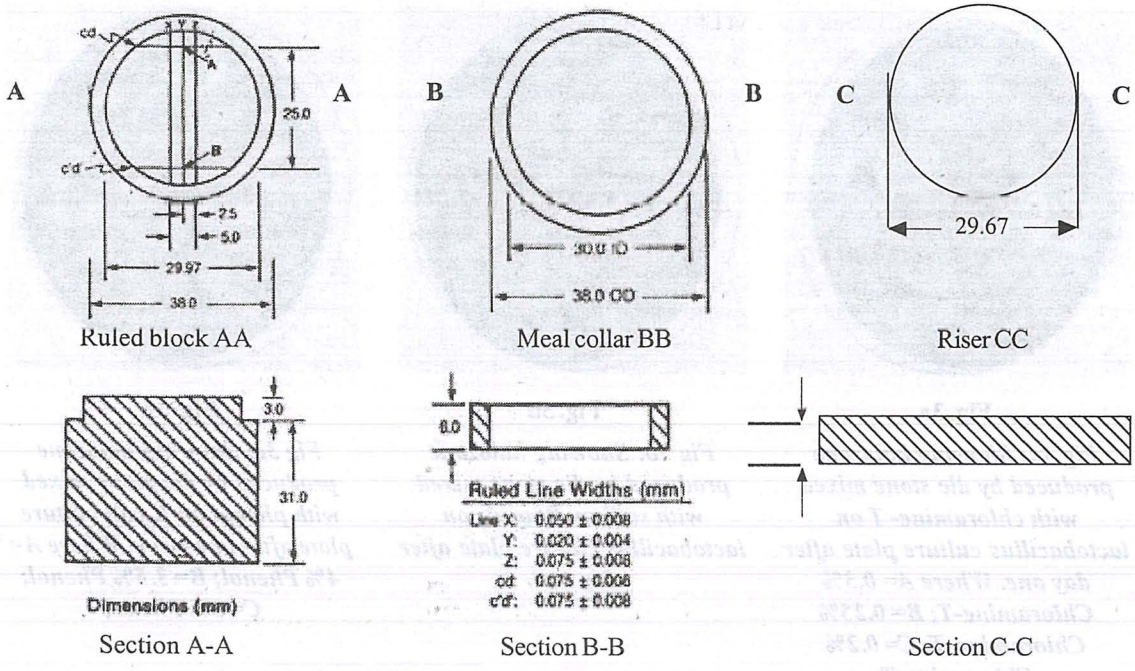


Fig. 1

Fig 1: Mould for making test specimens for dimensional changes.

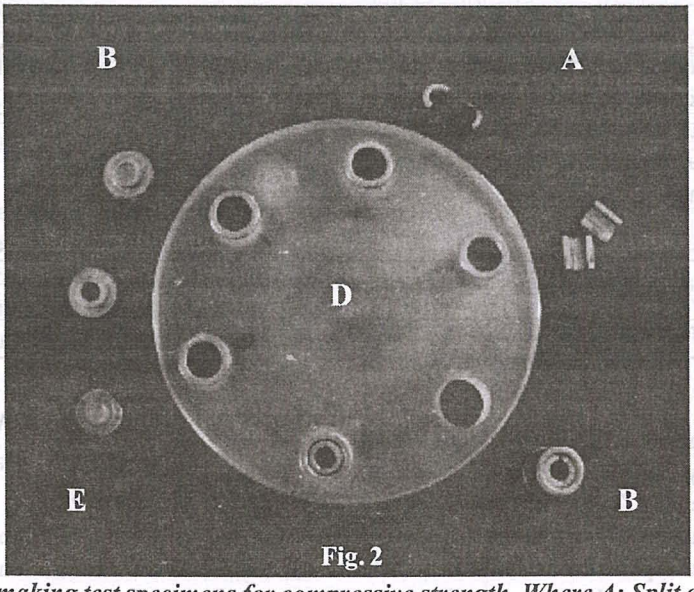


Fig. 2

Fig 2: Mould for making test specimens for compressive strength. Where A: Split cylinder; B: Outer cylinder; C: Clamp ring; D: Base; E: Plastic plugs.

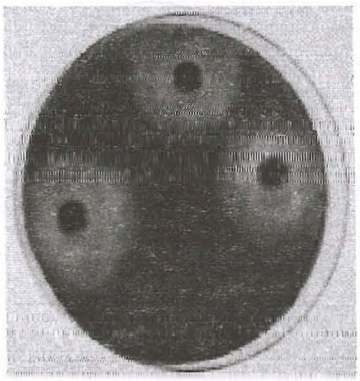


Fig. 3a

Fig 3a: Showing halozone produced by die stone mixed with chloramine- T on lactobacillus culture plate after day one. Where A= 0.3% Chloramine-T; B= 0.25% Chloramine-T; C= 0.2% Chloramine-T.

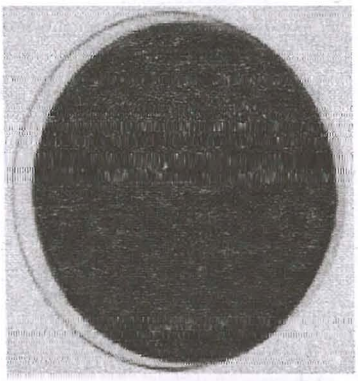


Fig. 3b

Fig 3b: Showing halozone produced by die stone mixed with sodium fluoride on lactobacillus culture plate after day one.

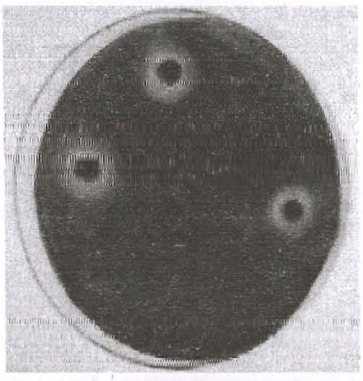


Fig. 3c

Fig 3c: Showing halozone produced by die stone mixed with phenol on E.coli culture plate after one week. Where A= 4% Phenol; B= 3.5% Phenol; C= 3% Phenol.

ing specimens for all the study samples. The compressive strength was evaluated on Universal Testing Machine.

Fifty test specimens of identical dimensions were obtained by using the same mold for preparing specimens for all the study samples. The dimensional changes were evaluated under traveling microscope at 30 x magnifications.

RESULTS

I. DIMENSIONAL CHANGE

Table 1 Shows the mean of observed dimensions (linear) for different antimicrobial agents at different concentrations.

Analysis of variance (ANOVA) was done which revealed statistically significant differences among the groups. Post-hoc tests (Tukey HSD) was applied in order to determine exactly where the differences took place. To further explore the data, test for homogeneity was conducted in order to see which groups could be put together in terms of dimensional change. The results show that in each subset any antimicrobial could be used with equal efficacy as far as dimensional changes were concerned.

II : COMPRESSIVE STRENGTH

Table 2 shows the mean of observed compressive strength (MPa) for different antimicrobial groups at different concentrations.

ANOVA was done which revealed no statistically significant difference in mean compressive strength among the various groups under study. Applying post-hoc tests showed that none of the differences revealed a statistically significant difference in mean compressive strength.

III: ANTIMICROBIAL EFFICACY

Table 3 Shows Size of Halozones in different groups at different days.

On day 1 - In Phenol group, minimum halozone was seen for 3% concentration for *S. aureus* and *E. coli* (halozone size = 23 mm) and maximum halozone size was seen at concentration 3.5% and 4% both for

Table 1: Mean Dimensions in different groups along with analytical data

	N	Mean	SD	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Control	5	25.10	0.32	24.71	25.50	24.77	25.63
Chloramine-T 0.2%	5	24.12	0.10	23.99	24.25	23.94	24.20
Chloramine-T 0.25%	5	24.52	0.25	24.21	24.84	24.26	24.89
Chloramine-T 0.3%	5	25.62	0.26	25.30	25.94	25.22	25.85
Sodium Fluoride 0.1%	5	**24.08**	0.24	23.78	24.39	23.73	24.35
Sodium Fluoride 0.15%	5	25.07	0.52	24.42	25.72	24.17	25.55
Sodium Fluoride 0.2%	5	25.19	0.16	24.99	25.38	24.96	25.39
Phenol 2.5%	5	23.79	0.33	23.38	24.20	23.38	24.14
Phenol 3%	5	24.10	0.33	23.70	24.51	23.74	24.62
Phenol 3.5%	5	**25.68**	0.23	25.40	25.97	25.33	25.95

Entries in bold show minimum and maximum values

Table 2: Mean compressive strength in different groups along with analytical data.

	N	Mean	SD	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Control	5	647.00	34.30	15.34	604.41	587	689.59
Chloramine-T 0.2%	5	684.00	67.58	30.22	600.09	588	767.91
Chloramine-T 0.25%	5	677.40	108.09	48.34	543.18	498	811.62
Chloramine-T 0.3%	5	763.20	42.34	18.94	710.63	706	815.77
Sodium Fluoride 0.1%	5	644.60	80.67	36.08	544.43	585	744.77
Sodium Fluoride 0.15%	5	659.80	89.10	39.85	549.17	588	770.43
Sodium Fluoride 0.2%	5	**607.60**	64.27	28.74	527.80	538	687.40
Phenol 2.5%	5	**727.00**	97.44	43.58	606.01	580	847.99
Phenol 3%	5	638.20	85.08	38.05	532.56	533	743.84
Phenol 3.5%	5	685.80	92.51	41.37	570.94	600	800.66

Entries in bold show minimum and maximum values

Lactobacillus sp. In Chloramine group, minimum halozone was seen at 0.2% concentration (halozone size=28 mm) whereas maximum size was seen at 0.25% and 0.3% concentrations (halozone = 31 mm) for *Lactobacillus sp.* In sodium fluoride group, no halozone was seen at any concentration for any of the bacterial strains under study.

On Day 3- In Phenol group, minimum halozone was seen for 3% concentration for *S. aureus* and *E. coli* (halozone size = 20 mm) and maximum halozone size was seen at concentration 3.5% and 4% both for *Lactobacillus sp.* (halozone – 24 mm). In Chloramine group, minimum halozone was seen at 0.2% concentration (halozone size=22 mm) for *E. coli* whereas maximum size was seen at all the three concentrations *viz.* 0.2%, 0.25% and 0.3% (halozone = 28 mm) for *S. aureus* and for *Lactobacillus sp.* at 0.3% concentration. In sodium fluoride group, no halozone was seen at any concentration for any of the bacterial strains under study.

Table 3: Size of Halozones in different groups.

Group	S.aureus			E. coli			Lactobacillus		
Day	Day 1	Day3	Week 1	Day 1	Day3	Week 1	Day 1	Day3	Week 1
Phenol 3%	23	20	-	23	22	-	24	20	9
Phenol 3.5%	24	21	8	24	23	11	28	24	11
Phenol 4%	24	22	12	24	23	12	28	24	12
Chloramine-T 0.2%	28	28	12	30	22	12	30	25	12
Chloramine T 0.25%	29	28	13	30	24	13	31	27	17
Chloramine-T 0.3%	30	28	14	30	24	14	31	28	17
Sodium fluoride .1%	–	-	-	-	-	-	-	-	-
Sodium fluoride 0.15%	–	-	-	-	-	-	-	-	-
Sodium fluoride 0.2%	–	-	-	-	-	-	-	-	-

Table 4: Mean Halozones (in mm) (irrespective of strain) (mean±SD)

	Day 1	Day 3	Week 1	Week 3	Week 5
Phenol 3%	23.33± 0.58	20.67± 1.15	3.00± 5.20	–	–
Phenol 3.5%	25.33± 2.31	22.67± 1.53	10.00± 1.73	–	–
Phenol 4%	25.33± 2.31	23.00± 1.00	12.00± 0.00	–	–
Chloramine-T 0.2%	29.33± 1.15	25.00± 3.00	12.00± 0.00	–	–
Chloramine-T 0.25%	30.00± 1.00	26.33± 2.08	14.33± 2.31	–	–
Chloramine-T 0.3%	30.33± 0.58	26.67± 2.31	15.00± 1.73	–	–
Sodium fluoride .1%	–	–	–	–	–
Sodium fluoride 0.15%	–	–	–	–	–
Sodium fluoride 0.2%	–	–	–	–	–

At 1 Week - In Phenol group, no halozones were seen at 3% concentration for *S. aureus* and *E. coli*. The maximum size of halozone was 12 mm for all the three strains at 4% concentration. In Chloramine group, minimum halozone was seen at 0.2% concentration (halozone size–12 mm) for all the three strains under study whereas maximum size was seen at 0.25% and 0.3% concentration (halozone = 17 mm) for *Lactobacillus sp.* In sodium fluoride group, no halozone was seen at any concentration for any of the bacterial strains under study.

- On week 3 and 5, in none of the groups any halozone could be seen.

Table 4: Shows mean halozones (in mm) (irrespective of strain) (mean±SD)

Among phenol and chloramines groups for observations upto week 1, maximum mean halo zones were seen in Chloramine-T 0.3% group (30.33±0.58 mm) at day 1 while minimum were seen in Phenol 3% at week 1 (3.00±5.20 mm). In sodium fluoride group, no halo zones were seen at any time interval. In other groups too, no halo zones were seen after week 1.

Analysis of Variance (ANOVA) test was done which revealed Statistically significant differences among different groups at Day 1, Day 3, Day 5 and Week 1. As the differences were significant, post-hoc tests were conducted to pinpoint the actual differences.

- Post Hoc Tests (Tukey HSD) (Day 1) shows that there was a statistically significant difference between Chloramine (all concentrations) and Phenol with Chloramine showing larger halo zones as compared to phenol. Both chloramines and phenol (at all concentrations) showed significantly larger halo zones as compared to sodium fluoride (all concentrations).
- Post Hoc Tests (Tukey HSD) (Day 3) shows that the results were similar to those obtained at day 1, however, the differences between Chloramine and Phenol started to diminish.
- Post Hoc Tests (Tukey HSD) (week 1) shows that the results were similar to those obtained at day 1, however, the differences between Chloramine and Phenol started to diminish at higher concentrations of phenol.

At week 3 and week 5, no differences were seen among groups.

DISCUSSION

Saliva contaminated dental impressions serve as a source of infectious microorganisms to dental personnel who handle the cast made from them. Leung and. Schonfeld (1983) [5] recovered the microorganisms from the stone cast which shows that dental casts may be a medium of cross- contamination between patients and dental personnel. Therefore a number of systems had been proposed which aimed to disinfect impressions satisfactorily and efficiently. Most of these systems rely on either spraying or immersing the contaminated impressions in disinfectants [S. Pamela Herrera, Virginia A. Merchant (1986), Matyas J, Caputo AA and Lucatorto FM (1990), Hidenobu Tomita et al (1990), Mitchell A. Stern, Glen H. Johnson, L. Brian Toolson (1991), Bass RA, Plummer KD, Anderson EF (1992), Maria del Pillar Rios et al (1996), Martin N, Martin MV, Jedynakiwicz NM (2007)][6,7,8,9,10,11,12]. But these systems has following disadvantages-

Immersing the cast in unsaturated solution could lead to poor strength, poor dimensional stability, metal corrosion & leaching of the surfactant which is used to make addition silicone impression material hydrophilic. This could result in a less wettable impression and resulting gypsum cast with more voids.

The spray technique for disinfecting the surface of stone casts can produce aerosol which are potentially damaging to health, eliminate the surface detail and unable the solution to completely cover and maintain contact with all surfaces of the cast for the required amount of time. It also prevents the application of the solution to the undercut areas and interproximal surfaces, depending on the angle of the spray dispenser. Moreover the spray cannot completely saturate the porous stone cast.

An alternative or additional approach has to be followed for cast disinfection with the aim to decontaminate the cast produced from the impression by incorporating a disinfecting chemical into the gypsum.

Chloramine- T closely resembles the hypochlorite. 0.25% chloramine T whether mixed with the gypsum powder or sprayed on impressions did not adversely affect the dimensional change, of types III, IV, and V gypsum casts (Daniel Hall et al (2004)[13]. Several researchers have reported that addition of small concentration (0.2%- 0.25%) of chloramine-T was effective against gram positive and gram negative bacteria's [Sarah L Rolland, Thomas E. Carrick, Angus W Walls, John F. Mccabe (2007), Kurt Fuursted, Anja Hjort and Lizette Knudsen (1997)][14,15]

Several researchers have reported that NaF mouthrinses reduced the number of viable bacterial count [Tinanoff N, Klock B, Camosci DA et all (1983), Jukka H. Meurman (1987)][16,17].

Phenol did not significantly affect the dimensional accuracy of casts with either the immersion or spray techniques [Matyas J, Caputo AA and Lucatorto FM (1990), Kuang Tan et al (1993)][7,18]. Moreover [Mitchell A. Stern, Glen H. Johnson, L. Brian Toolson (1991)][19] proved that applications of spray disinfectant phenol (2%) increased the compressive strength of type IV stone by 18 %.

As compared to any other antimicrobial agent used in this study, chloramine-T produced the biggest inhibitory halozones (ranged till 30mm diameter) thereby demonstrating the maximum antimicrobial activity throughout the test time period. However sodium fluoride did not show any antimicrobial activity against any microorganism at any concentration right from day one.

Chloramine-T showed maximum activity against lactobacillus at day one. There was mixed activity of the disinfectant at day three and day five. However there was marked increase in activity against lactobacillus at 1 week. Phenol also showed maximum activity against lactobacillus at day one. There was mixed activity of the disinfectant at day three and day five. However at one week 3% phenol did not show any activity against S.mutans and E.coli

At 3rd and 5th week there was no activity of any disinfectant against any bacteria.

There were no statistically significant differences in mean compressive strength among the various groups under study. However Mitchell A. Stern et al in (1991) [19] found that applications of spray disinfectant phenol increased the compressive strength of type IV stone by 18 %. Regarding dimensional changes any antimicrobial could be used with equal efficacy as far as dimensional changes were concerned. Matyas J, Caputo AA and Lucatorto FM (1990)[7] and Kuang Tan et al (1993)[18] Daniel Hall et al (2004) [13]also proved the same

CONCLUSIONS

1. Addition of even minimum concentration (0.2%) chloramine- T increased the antimicrobial activity of the die stone to the maximum
2. Sodium fluoride when mixed with die stone even in maximum concentration (0.2%) did not show any antimicrobial activity against any bacteria under study
3. Both phenol and chloramine-T were more effective against lactobacillus as compared to E.coli and staphylococcus.
4. Both phenol and chloramine-T were effective only till one week. After which no effect of disinfectant was seen
5. Increasing order of effectivity of disinfectants- Sodium fluoride (any conc.) < Phenol 3% ≃ Phenol 3.5% < Phenol 4% < Chloramine 0.2% ≃ Chloramine 0.25% ≃ Chloramine 0.3%
6. None of the disinfectants had any effect on the mechanical properties of die stone in the concentration used in the study.

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