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RESEARCH

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The effect of octenidine dihydrochloride on the antibacterial activity of a formulated resin composite: an in vitro study

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Abstract

Background It has been noticed that failure of composite resin restorations can be attributed to either of the two following causes: fracture or secondary caries. For that reason, it is mandatory to formulate a restorative material with antibacterial effect. An octenidine dihydrochloride (OCT) has been assessed as an alternative to antimicrobial material, such as chlorhexidine due to their higher microbicidal effect and less cytotoxicity.

Objective Current study aimed to add different concentrations of octenidine dihydrochloride into experimental flowable resin composite and evaluate its antibacterial activity over different periods of time to provide the manufacturers with more precise information.

Methods A flowable resin composite material mix was formulated. Octenidine dihydrochloride antibacterial material was then added separately to the formulated mix at 1% wt. and 1.5% wt. concentration, respectively. Antibacterial activity was assessed against *Streptococcus mutans* using agar diffusion test and compared to a commercial resin composite.

Results It showed that by increasing the percentage of incorporated octenidine dihydrochloride (1% and 1.5%), respectively, the antibacterial efficacy against the *Streptococcus mutans* increased. Results of this study also showed the time had a significant decrease in the antibacterial effect.

Conclusions It can be concluded that by the incorporation of octenidine dihydrochloride (1% and 1.5%), respectively, the antibacterial efficacy against the *Streptococcus mutans* increased. Time had a significant decrease in the antibacterial effect of OCT.

Keywords Composite, Octenidine dihydrochloride, Antibacterial and agar diffusion test

Background

Dental caries is a common oral disease coming from pathogenic bacteria that causes dental hard tissues damage. The different treatment modalities of dental caries can include removal of decayed dental tissues and restoring them with various types of restorations, such as metal alloys, resin composites, and ceramics (Saiprasert et al. 2023).

The scientific advances in restorative dental materials, have made resin composites one of the most used materials worldwide for different classes of restorations. Mechanical and chemical properties of resin composites

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can be altered to suit a wider range of uses by altering several factors (AlSahafi et al. 2022). Flowable composite resins with improved mechanical and chemical characteristics have been widely used in the clinical practice. These resin composites having less inorganic fillers and a higher percentage of resinous components are high flow materials that aid in preserving the seal of the restoration's margins. However, their low elastic modulus and minimal stress contraction are challenged during stress increase (Harp et al. 2022).

As dental caries is a transmissible infection initiated by cariogenic microorganisms, trials to make an antibacterial restorative material turn out to be an appealing issue in dental biomaterials.

Recently, octenidine dihydrochloride has been used as a mouthwash and as an antiseptic agent to treat wounds and skin burns. Prior research has demonstrated that octenidine dihydrochloride and chlorhexidine were equally effective at combating microorganisms causing dental plaque. Octenidine dihydrochloride has been examined as replacements to chlorhexidine due to their higher antimicrobial effect and less toxic effects (Liang et al. 2020).

This study was conducted to evaluate the antibacterial effect of various concentrations of octenidine dihydrochloride across varying time periods in an experimental flowable resin composite. The null hypothesis is that the addition of octenidine dihydrochloride (OCT) to the experimental composite resin had the capability to increase the antibacterial efficacy against the *Streptococcus mutans*.

Methods

The materials chemical name, description, batch number, and their manufacturers are listed in Table 1.

Streptococcus mutans was provided from the European Culture Collection Center (Belgium, Europe). The Herculite Ultra Flowable, by Kerr, was used as the commercially available flowable resin composite.

Resin matrix preparation

The flowable resin matrix was formulated by the following:

- Blending Bis-phenol A-glycidyl Methacrylate (Bis-GMA) with Tri-ethylene Glycol Di-methacrylate (TEGDMA) in 70:30 ratio by weight % (Niedźwiedź et al. 2023).
- The photo-initiator powder system was prepared by adding camphorquinone powder: ethyl 4-di-methyl-amino-benzoate powder in a ratio 1:1.
- The powder of prepared photo-initiator was gently added to the prepared resin matrix in a dark container.
- The prepared mixture of resin matrix and powder was stirred steadily on a magnetic stirrer by a small magnet for 2 h to secure homogenous mixture and thorough monomers dissolution (Fidalgo-Pereira et al. 2022).

Drops of acetic acid were added to ethanol solution in a small beaker to decrease the pH to 3 or 4 by using a pH meter for monitoring (Jenway, 3505, UK). A 3% by wt of tri-methoxy-silane was applied to the adjusted ethanol solution and then blended in a beaker using a magnetic stirrer for one hour (Moharam et al. 2018).

Sintering of the silicon di-oxide nanopowder was done at 1300 °C for 20 min using a sintering electric furnace (TEGRA SPEED, Teknik Dental, Turkey). The sintered particles aggregated as white clusters and were ground

Table 1 Materials used

	Chemical name	Manufacturer	Batch Number
1	Bis-phenol-A glycerolatedimethacrylate (Bis-GMA) ($C_{29}H_{36}O_8$), Mol. Wt: 512.59	Sigma-Aldrich, USA	494,356
2	Tri-ethylene glycol di-methacrylate (TEGDMA)	Sigma-Aldrich, USA	261,548
3	Tri-methoxy-silane ($C_3H_{10}O_3Si$)	Sigma-Aldrich, USA	282,626
4	Silicon di-oxide nanoparticles (spherical) (SiO_2)	Sigma-Aldrich, USA	637,246
5	Ethyl 4-di-methyl-amino-benzoate ($C_{11}H_{15}NO_2$)	Sigma-Aldrich, USA	E24905
6	Camphorquinone ($C_{10}H_{14}O_2$)	Alpha Aesar, USA	124,893
7	Glacial Acetic Acid	Pio-Chem, Egypt	AC0121
8	Alcohol (Ethanol) 70% wt	Pio-Chem, Egypt	Et0012
10	DURACAL (Buffer solution, pH 4.01)	Sigma-Aldrich, USA	242,142
11	Octenidine dihydrochloride	MedChem Express (MCE), USA	23,549

manually by using mortar and pestle. The ground particles were then sieved using a stainless-steel sieve (400 mesh) (Moharam et al. 2022).

Sintered silicone dioxide nanoparticles were added in the formulated silane coupling agent and blended for two hours by stirring with the magnetic stirrer. The prepared solution was centrifuged at temperature of 25 °C for 30 min, with 6000 rpm speed using a centrifugal machine (Laborzentrifugen GmbH Sigma 3-16-KL, Germany). The nanosilica particles were precipitated at the tube bottom after centrifugation. The final precipitate was rinsed off using ethanol, and then mixing was performed in a vortex mixer (Stuart, SA7, UK) at speed 1800 rpm to secure homogenization of the mix. Centrifugation was performed for the mixture for 5 min by the apparatus of centrifugation. This procedure was done for three times, and the extra ethanol was rinsed off (Meshram et al. 2019).

The final precipitate was then left to dry in a Petri dish using an drying furnace (FD 23, 20L, BINDER, Germany). The operating temperature was continually kept for 60 min at 105 °C (Souza et al. 2023). The Petri dish was then placed in a desiccator for 10 min after removal from the furnace and sealed with foil.

The octenidine dihydrochloride particles were added to the previously silanized silica fillers at concentration of 1% wt. and 1.5% wt to prepare group 3 and 4, respectively. These concentrations were chosen according to a pilot study that was observed prior to the current test.

The prepared mixture of salinized silica nanofillers together with the antibacterial particles was added to the formulated resin matrix. The un-cured resin composite was then placed in a dark tinted bottle and kept in a dark place over-night.

Four groups were tested as follows:

Group 1 commercial resin composite.
Group 2 experimental resin composite without octenidine dihydrochloride.
Group 3 experimental resin composite containing octenidine dihydrochloride 1%
Group 4 experimental resin composite containing octenidine dihydrochloride 1.5%

Evaluation of the antibacterial activity using agar disc diffusion test

Resin composite discs specimens were prepared by packing the uncured mix into a sterile Teflon mold with dimensions (5mm X 5mm) (Abou ElReash et al. 2019). Then, in accordance with the manufacturer's instructions, the uncured mix was light cured using a light cure unit with a tip diameter of 10 mm (Premium Plus light cure C02-D, Premium Plus-UK, England) with an irradiance of 2500 mW cm^2 on normal mode for 20 s via each side of the mold. After removing the specimens from the molds, any excess cured resin was removed and cut using silicon carbide paper grit with a size of P800 µm.

Streptococcus mutans, a facultatively anaerobic, gram-positive bacterial strain, was used in this investigation. Giza, Egypt's Food Technology Research Institute, donated the *Streptococcus mutans*. *Streptococcus mutans* has the American Type Culture Collection (ATCC) number 35668.

In order to achieve a high growth concentration of roughly 106 CFU/mL, the antibacterial test was carried out in a sterilized brain–heart infusion (B-H-I) utilizing inoculation loop broth that was incubated for 24 h at 37 °C under anaerobic conditions (Hernandes et al. 2014).

Colonies were selected the day before the test and grown in 5 ml broth medium at 35–37 °C for 16–18 h. One hundred microliters of the diluted culture was distributed uniformly across the media plate. With a diameter of 5 mm and a depth of 3 mm, a total of 16 bacterial wells were produced and divided into 16 plates containing bacterial strains.

After polymerization, specimens of resin composite discs were inserted into the bacterial wells using a pair of tweezers that had been flame-sterilized. In order to facilitate the growth of bacterial cultures, plates were incubated for 24 h at 37 °C in an incubator (Fisher Scientific isotemp incubator model 655d, USA).

Positive results were scored when a zone of inhibition was observed around the specimens. The inhibitory zone was measured by the shortest distance in (mm) from the specimen outer margin to the microbial growth initial point. The measurements were taken at 30, 60, and 90 days.

Results

At 30 days, the highest mean inhibition zones were recorded in groups 3 and 4, while no inhibition zones were recorded in groups 1 and 2. Among the four tested groups, ANOVA test showed a significant difference (Table 2).

At 60 and 90 days, the highest mean inhibition zones were recorded in group 3, followed by group 4, while no inhibition zones were recorded in groups 1 and 2. Among the four tested groups, ANOVA test showed a significant difference (Table 2). There was no significant difference between tested groups 3and 4 using Tukey's post hoc test (Table 3).

Table 2 Descriptive statistics of antibacterial effect against *Streptococcus mutans*

Streptococcus mutans		Mean	Std. dev.	Std. error	Min	Max	*F*	*P*
30days	Group1	0.00[b]	0.00	0.00	0.00	0.00		
	Group2	0.00[b]	0.00	0.00	0.00	0.00	81.8	0.00*
	Group3	2.08[a]	0.64	0.23	1.00	3.00		
	Group4	2.08[a]	0.39	0.14	1.60	2.70		
60days	Group1	0.00[b]	0.00	0.00	0.00	0.00	100.76	0.00*
	Group2	0.00[b]	0.00	0.00	0.00	0.00		
	Group3	1.70[a]	0.35	0.13	1.00	2.00		
	Group4	1.65[a]	0.41	0.15	1.00	2.30		
90days	Group1	0.00[c]	0.00	0.00	0.00	0.00	72.67	0.00*
	Group2	0.00[c]	0.00	0.00	0.00	0.00		
	Group3	1.35[a]	0.30	0.11	1.00	1.80		
	Group4	0.61[b]	0.30	0.11	0.20	1.00		

Significance $p \leq 0.05$, significant = *, non-significant = ns, superscript letters different = significantly different

Comparison at different times within the same group

Groups 1 and 2 showed no inhibition zones throughout the study and recorded a constant value (0) as shown in (Table 4).

In Group 3 the mean inhibition zones measurements increased significantly at 30 days and then decreased significantly at 60 and 90 days. The time effect on group 3 was significant, as shown in (Table 4).

In Group 4 the mean inhibition zone measurements increased significantly at 30 days; afterward, time decreased significantly at 60 days, followed by another significant decrease at 90 days. The time effect on group 4 was significant (Table 4).

Discussion

Secondary caries can be described as a lesion occurring at the margins of an existing restoration, which is caused mainly by *Streptococcus mutans* bacteria.

A simple method to provide materials for dental restorations with bactericidal activity, is by adding an antibacterial additive to the material. These additives can subsequently be liberated in an aqueous environment, to eliminate the damaging bacteria.

Different antibacterial agents such as triclosan, benzalkonium chloride, silver particles, antibacterial prepolymerized resin fillers, and chlorhexidine have been introduced in both commercial and experimental dental resin composites (Ezzeldin et al. 2019).

However, studies showed that incorporating antibacterial additives directly into commercial resin composites showed minimal antibacterial activity after curing, as the amount leached out was close to the minimal inhibitory concentration needed for bacterial inhibition. Consequently, to obtain an antibacterial composite resin, the additive might be either dissolved in the monomers or mixed with the inorganic fillers. Therefore, this study was aimed to add a recent antimicrobial particles (octenidine dihydrochloride) to an experimental resin composite (Chum et al. 2019).

Octenidine dihydrochloride (OCT) is an antibacterial material currently utilized as a mouth wash and for managing skin burns. OCT is a member of the bipyridines, having two cationic functional parts for each molecule, and displaying wide anti-microbial effect on different types of bacteria, fungi, and many viral types (Uzer Celik et al. 2016).

The agar diffusion test was used to assess the antibacterial effect in this study, as it is a broadly applied in vitro way for evaluation of the antibacterial effect of dental restorative materials. The benefit of this way is that the anti-microbial out turn of drugs can be discovered by stimulating the microbial segregates with anti-microbial prepared discs with no modification in the biochemical drug characteristics. It also gives chance for direct comparisons of restorative materials against the tested microorganisms, demonstrating the material which has the more capability to remove bacteria in the local microenvironment (Esenlik et al. 2016).

In the agar diffusion method, the growth inhibition zones are related to the toxic effect of the material applied against the bacteria besides the diffusion ability of the material in the culture media (Stencel et al. 2018).

Results of the current study revealed that by incorporation of octenidine dihydrochloride (1% and 1.5%), respectively, the antibacterial efficacy against *Streptococcus mutans* increased; this was in accordance with (Uzer Celik et al. 2016) who evaluated the effect of several antimicrobial agents in vitro such as (CHX) chlorhexidine di-gluconate, (PHBM) polyhexamethylene biguanide, and (OCT) octenidine dihydrochloride on pathogenic

Table 3 Tukey's post hoc test at 30, 60 and 90 days

Groups (I)		Groups (J)	(I-J)Mean difference	Std. error	Sig.
30 days	Group1	Group2	0.000	0.187	1.000
		Group3	−2.07500−*	0.187	0.000
		Group4	−2.07500−*	0.187	0.000
	Group2	Group1	0.000	0.187	1.000
		Group3	−2.07500−*	0.187	0.000
		Group4	−2.07500−*	0.187	0.000
	Group3	Group1	2.07500*	0.187	0.000
		Group2	2.07500*	0.187	0.000
		Group4	0.000	0.187	1.000
	Group4	Group1	2.07500*	0.187	0.000
		Group2	2.07500*	0.187	0.000
		Group3	0.000	0.187	1.000
60 days	Group1	Group2	0.000	0.136	1.000
		Group3	−1.70000−*	0.136	0.000
		Group4	−1.65000−*	0.136	0.000
	Group2	Group1	0.000	0.136	1.000
		Group3	−1.70000−*	0.136	0.000
		Group4	−1.65000−*	0.136	0.000
	Group3	Group1	1.70000*	0.136	0.000
		Group2	1.70000*	0.136	0.000
		Group4	0.050	0.136	0.983
	Group4	Group1	1.65000*	0.136	0.000
		Group2	1.65000*	0.136	0.000
		Group3	−0.050	0.136	0.983
90 days	Group1	Group2	0.000	0.106	1.000
		Group3	−1.35000−*	0.106	0.000
		Group4	−0.61250−*	0.106	0.000
	Group2	Group1	0.000	0.106	1.000
		Group3	−1.35000−*	0.106	0.000
		Group4	−0.61250−*	0.106	0.000
	Group3	Group1	1.35000*	0.106	0.000
		Group2	1.35000*	0.106	0.000
		Group4	0.73750*	0.106	0.000
	Group4	Group1	0.61250*	0.106	0.000
		Group2	0.61250*	0.106	0.000
		Group3	−0.73750−*	0.106	0.000

Significance level $p \leq 0.05$, *significant

Table 4 Effect of time on antibacterial effect against *Streptococcus mutans* within the same group

	0-days	30-days	60-days	90-days	*p*
Group 1	0	0	0	0	–
Group 2	0	0	0	0	–
Group 3	$0^{d} \pm 0$	$2.08^{b} \pm 0.64$	$1.7^{c} \pm 0.35$	$1.35^{c} \pm 0.30$	0.00*
Group 4	$0^{e} \pm 0$	$2.08^{b} \pm 0.39$	$1.65^{c} \pm 0.41$	$0.61^{d} \pm 0.30$	0.00*

microorganisms by adding their (M-I-C) minimum inhibitory concentration and (M-B-C) minimum bactericidal concentration. They stated that OCT had an effective antimicrobial effect against the tested bacteria. This can be explained by the fact that octenidine dihydrochloride interferes with cellular walls and membranes which means that it exhibits both fungicidal and bactericidal outcomes. It also interacts with cell wall polysaccharides of microorganisms, bombards the enzymatic structures nearby, terminates cell biological function, and results in cytoplasmic membrane leakage.

Furthermore, OCT appears to be highly effective by means of prolonged antiadhesive activity on bacteria, which might be the cause of its antibacterial activity. This was supported by Uzer Celik et al. (2016) and Stencel et al. (2018).

Additionally, the stated results of this research can be explained by the fact that OCT is a member of the bipyridines, having two cationic functional parts for each molecule, and displaying wide antimicrobial properties on different microorganisms (Malhotra et al. 2016).

Results showed that time had a significant decrease in the antibacterial effect of OCT. In group 4, the antibacterial activity was 2.08 at 30 days. At 60 days the antibacterial activity was 1.6, while at 90 days it was 0.61. Similarly, in group 3, at 30 days the antibacterial activity was 2.08; then, it decreased to 1.7 at 60 days and further decreased to 1.35 at 90 days.

The decline in the antibacterial effect by time can be due to a drop in the rate of release or a drop in the amount of residual material. This was agreed with (Joon et al. 2020).

The component release seems larger at first and then decreases by time; the reduction in the release rate shows that the outer layer constituents are dissolved or washed-out inside the water. Moreover, the constituents inside the resin are discharged with difficulty since the resin constituents restrain such movements. Commonly, the curative agents of dental biomaterials when discharged display a reduction in the rate of release. The water inside the mouth penetrates the resin matrix; the agent in the adhesive dissolves and scatters in smaller concentrations. By time, the agent is released and separated from a deeper layer of the matrix, which means that diffusion time to the exterior environment increases, while the release rate decreases (Joon et al. 2020).

Within the constraints of this study, octenidine dihydrochloride was found to be beneficial when added to flowable resin composites. Additional researches are suggested to demonstrate its impact on hardness, radiopacity, color stability, and working time. Research on the antibacterial effect of octenidine dihydrochloride on a larger variety of cariogenic bacterial species both

in vitro and in vivo is also advised. To be more precise, in order to replicate the release of these antimicrobials in the oral cavity, future studies should design their experiments more carefully, considering teeth and restorations as substrates for biofilm formation under a dynamic and constant flow of saliva.

Conclusions

The addition of octenidine dihydrochloride (OCT) to the experimental composite resin had the capability to increase the antibacterial efficacy against the *Streptococcus mutans*. Incorporation of OCT with 1% and 1.5% showed the same significant antibacterial effect at 30 and 60 days. However, at 90 days the 1% (OCT) showed better results than the 1.5%. These results were radically higher upon comparison with the commercially available resin composite. Time had a significant effect on reduction of the OCT antibacterial effect.

Abbreviations

OCT	Octenidine dihydrochloride
Bis-GMA	Bis-phenol-A glycerolatedimethacrylate
TEGDMA	Tri-ethylene glycol di-methacrylate

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MM completed the practical work, gathered the information, and made significant contributions to the manuscript's composition. TS wrote the text, completed the statistical analysis, and produced the final edit. The final writing, editing, plagiarism check, and submission were completed by HNS. Before submitting, each author has read and approved the final manuscript.

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