Chemical interaction of 7% MA with 2% CHX and 5% NaOCl – an in vitro study.

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Abstract: Successful root canal treatment requires the complete elimination of microbial flora from root canal system. Irrigating solutions play vital role in destruction of microbes. However, interaction of various irrigating solution can be proved to be detrimental to the outcome of root canal therapy.

The Purpose of this study were: (1) to evaluate the interaction between 7% maleic acid (MA) and 2% chlorhexidine gluconate solution (CHX) and to find out the availability of individual irrigant and (2) to determine the free available chlorine content when 7% MA was mixed with 5% sodium hypochlorite (NaOCl) solution.

Results: It was observed that more than 90% free MA and CHX were available when MA was combined with CHX. It was also observed that there was no precipitate formation when 7% MA was mixed with 2% CHX. Available chlorine content decreased significantly in the MA/NaOCl mixture.

Key Words: Irrigation, Maleic acid, Chlorhexidine gluconate, Sodium hypochlorite.

I. Introduction

Mechanical instrumentation of the root canal leaves around 40%–50% of the root canal walls untouched because of the complexity of the root canal system and also produces smear layer that covers the dentinal tubules. And therefore Chemical irrigants are required along with mechanical instrumentation to produce optimal results of the root canal therapy. (1, 2) Various types of irrigants have been used during the root canal preparation to minimize the residual debris, necrotic tissue, bacteria, as well as the smear layer formed by the mechanical instrumentation of the root canal system.

The most common irrigant used during root canal preparation is sodium hypochlorite (NaOCl). It is an effective tissue solvent and has excellent antimicrobial property. Chlorhexidine gluconate (CHX) is a broad-spectrum antimicrobial agent that has been advocated for root canal disinfection (3). Prolonged exposure of the root dentin to CHX might result in residual antimicrobial activity of the dentin surface.

Maleic acid (MA) is a mild organic acid used as an acid conditioner in adhesive dentistry (4). Studies have shown that 7% MA was significantly better than 17% EDTA in removing smear layer from the apical third of the root canal system(5).

Also, 7% MA was found to be less cytotoxic when compared with 17% EDTA (6).

This study compares the effectiveness of Chlorhexidine if used together with maleic acid and available free chlorine of sodium hypochlorite when mixed with Maleic acid.

II. Materials and Methods


Standard Solutions of CHX and MA: Concentrations of 25, 50, 75, 100, and 150 mg/mL in deionized water were prepared from the 2% solution of CHX. Concentrations of 50, 100, 150, 200, and 250 mg/mL in deionized water were prepared from 7% solution of MA.

Sample Solution. Five milliliters of 2% CHX and 5 mL of 7% MA were mixed in a 100-mL volumetric flask, kept for 30 minutes, and made up the volume with deionized water. Five milliliters of this solution was again diluted to 100 mL in another volumetric flask to get concentrations of 50 mg/mL of CHX and 175 mg/mL of MA. Mobile Phase. A variable mixture of solution A and B was prepared.

Solution A was prepared as a solution of 27.6 g of monobasic sodium phosphate and 10 mL of triethylamine in about 1.5 L of water. The pH was adjusted to 3.0 with phosphoric acid, and the solution was diluted with water to 2000 mL, mixed, and filtered through a Millipore filter. Solution B was acetonitrile.
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Quantification of MA and CHX.
A high-performance liquid chromatography (HPLC) method was used. Detection was done by using an ultraviolet detector at 239 nm, where both CHX and MA showed good response. The column was maintained at a constant temperature of 40 degree C. The flow rate was 1.5 mL per minute.

Evaluation for Precipitate Formation.
Five milliliters of CHX was added drop-wise to 5 mL of MA to simulate the clinical condition, and both the solutions were vigorously mixed in a beaker with magnetic stirrer for half an hour. For each combination, the test was repeated 3 times to access the reproducibility of the results.

Effect of MA on Available Chlorine in NaOCl Solution.
The 5% NaOCl solution was obtained from Prime Dental products, 7% MA solution was prepared by dissolving pure MA (Merck) in deionized water. Aqueous mixtures were prepared at different ratios with MA and NaOCl. Available chlorine (HOCl + OCl) contents in these mixtures were determined by the standard iodine/thiosulfate titration method.

III. Results
Interaction between MA and CHX Solution
Precipitate Formation.
On combining 2% CHX and 7% MA solution, there was no precipitate formation observed. However, when 20% CHX was mixed with 7% MA, precipitate was formed.

Statistics and results:

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<th>Chlorhexidine Gluconate (100)</th>
<th>Maleic Acid (200)</th>
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<td>Area %</td>
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Sample Solution (50 microgm/ml of Chlorhexidine with 175 micro gm/ml of Maleic Acid)

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Retention Time</th>
<th>Height</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
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<td>100</td>
</tr>
<tr>
<td>Maleic Acid</td>
<td>30 minutes</td>
<td>3193924</td>
<td>100</td>
</tr>
</tbody>
</table>

Results:
On combining 2% CHX and 7% MA solution, there was no precipitate formation observed. The amount of Chlorhexidine gluconate available after 30 minutes was found to be 41.60 microgm/ml. The amount of Maleic Acid available after 30 minutes was found to be 159.60 microgm/ml.

Estimation of Amount of CHX and MA after Mixing
On combining 2% chlorhexidine and 7 % Maleic acid, there was no precipitate formation observed. The amount of Chlorhexidine after 30 minutes was found to be 41.60 microgram/ml. The amount of maleic acid after 30 minutes was found to be 159.60 microgram/ml.

Available Chlorine in NaOCl Solution.
5% aqueous NaOCl solution was assigned to contain 100% available chlorine. The combination of NaOCl and MA caused a decrease in the amount of available chlorine. The reduction in the available chlorine was very evident when the combination ratios were 2:1 and 1:1. The NaOCl and MA ratio of 1:1 demonstrated almost a complete loss of available chlorine in NaOCl solution.

IV. Discussion.

Success of Endodontic therapy depends upon mechanical instrumentation and also the chemical irrigation performed in the root canals.

Earlier studies have demonstrated the interactions between irrigating solutions like EDTA/CHX, EDTA/NaOCl, and NaOCl/CHX and their associated problems (7-10). When EDTA and CHX were combined, there was formation of a precipitate (7).

Similarly, when NaOCl and CHX were combined, a precipitate was formed that is known as parachloroaniline (PCA). PCA can further degrade into 1-chloro-4-nitrobenzene, which is also known to be a carcinogen. Bui et al. demonstrated that this precipitate obliterated the dentinal tubules and might affect the seal of the root canal system. It is well-established that no single irrigant can completely eliminate all the organic and inorganic matter and impart a substantive residual antibacterial property to the root canal dentin. A suggested protocol for irrigating the root canal system before obturation consists of irrigation with EDTA and NaOCl to remove the inorganic and organic parts of smear layer and irrigation with CHX gluconate to impart substantive antibacterial activity (12). When these irrigants are used in combination, their chemical interactions have to be taken into consideration.

When Chlorhexidine and maleic acid solution are used together, there is negligible loss of available individual irrigating solution indicating that these two solutions can be used together. However, the combination of 7% maleic acid and 5% sodium hypochlorite significantly reduced the available free chlorine. However, when 20% CHX was mixed with 7% MA, precipitate was formed. (11)

Limitations of the study:
1) It is an in vitro study
2) Effect of Chemicals interaction is not being evaluated in clinical situations.
3) Only effect of maleic acid on available free chlorine is evaluated. However, effect of sodium hypochlorite on the efficiency of maleic acid is not evaluated.

V. Conclusion
1. The interaction study demonstrated that the reduction in the availability of CHX when it was mixed with MA was very marginal.
2. There was no precipitate formation on combining 7% MA with 2% CHX.
3. When 5% NaOCl was mixed with 7% MA, it lost available chlorine.

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