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# Effect of maleic acid and ethylenediaminetetraacetic acid on the dissolution of human pulp tissue – an *in vitro* study

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## Abstract

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**Aim** To compare *in vitro*, the tissue-dissolution capacity of 7% maleic acid (MA), 17% ethylenediaminetetraacetic acid (EDTA), 2.5% sodium hypochlorite (NaOCl) (positive control) and 0.9% saline (NaCl) (negative control) on human pulp tissue.

**Methodology** Forty pieces of human pulp tissue, each weighing 0.026 g, were divided randomly into four groups ($n$ = 10): (i) 7% MA solution, (ii) 17% EDTA solution, (iii) 2.5% NaOCl solution and (iv) 0.9% NaCl solution. The pulp tissue was placed in beakers containing the test solutions and then placed on a vibrator. Pulp tissue from the four experimental solutions was blotted dry and weighed after 30, 60, 90 and 120 min. The percentage of weight loss was calculated, and the data were statistically analysed using Kruskal–Wallis and Mann–Whitney *U*-test.

**Results** At all time intervals, 2.5% NaOCl dissolved pulp tissue significantly more than the other solutions ($P < 0.001$). There was no significant difference in the pulp-dissolution capacity between 7% MA and 17% EDTA at any of the time intervals. NaCl (0.9%) did not have any effect on pulp tissue.

**Conclusion** Seven percentage of MA and 17% EDTA had minimal tissue-dissolution capacity when compared to NaOCl.

**Keywords:** ethylenediaminetetraacetic acid, maleic acid, pulp dissolution, sodium hypochlorite.

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## Introduction

The success of root canal treatment relies on thorough chemomechanical procedures (Stewart 1955). Because of the complex anatomy of root canals, approximately 40–50% of the surface of root canal walls remains uninstrumented during preparation, which may result in insufficient debridement (Peters *et al.* 2001). The persistence of necrotic pulp tissue remnants would provide a source of nutrition for surviving bacteria (Love 2001). Therefore, irrigation with a tissue-dissolving antimicrobial solution is a prerequisite for effective root canal treatment, although it could not guarantee the absence of viable microbes in the root canal system (Byström & Sundqvist 1983). Dissolution of pulpal tissue is a highly desirable property in any irrigating solution because it enhances both cleansing of the root canal and removal of necrotic tissue (Koskinen *et al.* 1980, Moorer & Wesselink 1982). Sodium hypochlorite is one of the most commonly used root canal irrigants, based on its antibacterial, tissue-dissolving and lubricating properties of intracanal walls during instrumentation (Barbakow *et al.* 1995). Several studies have demonstrated its tissue-dissolving capability at various concentrations (Hand *et al.* 1978, The 1979, Koskinen *et al.* 1980, Moorer &

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Wesselink 1982, Hasselgren *et al.* 1988, Naenni *et al.* 2004).

Mechanical instrumentation of the root canal produces a smear layer that covers the walls and enters the dentinal tubules (McComb & Smith 1975). Smear layer consists of inorganic dentine debris as well as organic materials such as pulp tissue, odontoblastic processes, necrotic debris, microorganisms and their metabolic products (Torabinejad *et al.* 2002). Various chelating agents including ethylenediaminetetraacetic acid (EDTA), citric acid and MTAD, which is a mixture of tetracycline isomer (doxycycline), an acid (citric acid) and a detergent (Tween 80) (Biopure; Dentsply Tulsa Dental, Tulsa, OK, USA), have been used for the removal of smear layer (Calt & Serper 2000, Scelza *et al.* 2000, Torabinejad *et al.* 2003). As there is no single solution that has the ability to dissolve organic tissues and to remove the smear layer, the combined application of EDTA and NaOCl is commonly used for the effective removal of the smear layer (Torabinejad *et al.* 2003, Zehnder 2006). However, studies have shown that EDTA has limited capacity to dissolve soft tissue (Beltz *et al.* 2003, Grawehr *et al.* 2003). Maleic acid is a mild organic acid used as an acid conditioner in adhesive dentistry (Wieczkowski *et al.* 1992). Recently, 7% maleic acid has been found to be more effective than 17% EDTA in removing the smear layer from canal walls (Ballal *et al.* 2009). To date, there are no studies reported on the human pulp tissue–dissolving effect of maleic acid. Hence, the purpose of present study was to evaluate the tissue-dissolution effect of 7% maleic acid and compare it with 17% EDTA and 2.5% NaOCl on human pulp tissue.

## Materials and methods

Ethical clearance was obtained from the ethical committee of Manipal University, Manipal, Karnataka, India. Human pulp tissue was collected from 25 teeth, which were extracted for orthodontic reasons or which were impacted. After the extraction, periodontal tissues were removed with a brush and the teeth placed in distilled water containing 0.2% sodium azide and stored at 5 °C until required. Teeth were then carefully split into two using a diamond bur (Diatec, Coltene AG, Switzerland) and a microchisel without entering the pulp chamber and root canals. Pulp tissue was then removed entirely using an excavator. The pulp tissue was then rinsed thoroughly with distilled water to remove excess blood and was placed in the refrigerator for 1 week. Thereafter, the pulps were sectioned with a surgical blade into forty pieces each weighing 0.026 g. Before weighing, each piece of the pulp tissue was blotted on a filter paper and dried and then weighed using a precision balance in an air-tight container (AT 261; Mettler, Greifensee, Switzerland). Pulp tissues were then divided randomly into four experimental groups ($n = 10$):

1. MA group: Pulp specimens were placed in 5 mL of 7% MA solution (KMC Pharmacy, Karnataka, India).
2. EDTA group: Pulp specimens were placed in 5 mL of 17% EDTA solution (Merck, Darmstadt, Germany).
3. NaOCl group (positive control): Pulp specimens were placed in 5 mL of 2.5% NaOCl solution (KMC Pharmacy, Karnataka, India).
4. NaCl group (negative control): Pulp specimens were placed in 5 mL of 0.9% NaCl solution.

Pulp specimens in each group are placed in individual beakers containing the test solution, and all the beakers were placed on a vibrator to agitate the test solution. The pulp tissue was weighed after 30, 60, 90 and 120 min. Prior to weighing, all the specimens were washed with distilled water to remove precipitate, blotted on a filter paper, dried and then weighed. All the samples were weighed by a single investigator who was unaware of the test solutions. The difference in weight of the tissue sample before and after exposure to the test solution was divided by the original weight and multiplied by 100 to obtain the percentage of tissue weight loss or gain. The data were statistically analysed using Kruskal–Wallis and Mann–Whitney *U*-test.

## Results

The median percentage of tissue dissolved by the four experimental solutions is shown in Table 1. All the solutions except 0.9% NaCl exhibited an ability to dissolve human pulp tissue. The 2.5% NaOCl solution at all the time intervals was associated with significantly ($P < 0.001$) more pulp tissue dissolution compared to 7% MA and 17% EDTA solution. At 120 min, pulp tissue was completely dissolved by 2.5% NaOCl solution. There was no statistically significant difference between 7% MA and 17% EDTA solution at all the time intervals tested ($P < 0.05$).

## Discussion

This study assessed the pulp tissue-dissolution capacity of 7% maleic acid and compared it with that of 17% EDTA and revealed that 2.5% NaOCl had the maximum tissue-dissolution capacity compared to 7%

**Table 1** Median percentage of human pulp tissue–dissolution observed amongst experimental groups at various time intervals

| Test solutions | Initial | 30 min | 60 min | 90 min | 120 min |
|---|---|---|---|---|---|
| 7% Maleic acid | 0.023 (0.017–0.028) | 0.025 (0.023–0.028) | 0.023 (0.022–0.024) | 0.021 (0.019–0.023) | 0.019 (0.017–0.02) |
| 17% EDTA | 0.022 (0.016–0.026) | 0.024 (0.019–0.025) | 0.022 (0.018–0.023) | 0.02 (0.017–0.023) | 0.018 (0.016–0.02) |
| 2.5% NaOCl | 0.021 (0.014–0.029) | 0.017* (0.015–0.02) | 0.007* (0.004–0.01) | 0.002* (0.001–0.023) | – |
| 0.9% Saline | 0.023 (0.017–0.028) | 0.022 (0.017–0.025) | 0.023 (0.017–0.028) | 0.023 (0.017–0.028) | 0.022 (0.017–0.025) |

*$P < 0.001$ very highly significant.

maleic acid and 17% EDTA. This finding is in agreement with various other studies that have reported NaOCl to be an effective pulp tissue–dissolving agent (Hand *et al.* 1978, The 1979, Hasselgren *et al.* 1988, Sirtes *et al.* 2005, Cobankara *et al.* 2010). The tissue-dissolution capacity of NaOCl is a direct function of their freely available chlorine in solution (Zehnder *et al.* 2002). Both 17% EDTA and 7% maleic acid dissolved pulp tissue to some extent, but there was no significant difference between the solutions. This finding is in agreement with previous studies that have shown that EDTA has little capacity to dissolve soft tissue (Beltz *et al.* 2003, Grawehr *et al.* 2003).

The pH of irrigating solution might have an effect on tissue dissolution. Higher pH levels resulted in greater tissue dissolution. In addition, it has also been suggested that as pH is lowered, more time is needed for a solution to dissolve the tissue (Christensen *et al.* 2008). This might be a reason why maleic acid was not able to dissolve pulp tissue because the pH of maleic acid is low (1.05).

Moorer & Wesselink (1982) reported that tissue dissolution was dependent on three factors: frequency of agitation, amount of organic matter in relation to amount of irrigation solution and amount of available surface area of the tissue. The present study was standardized by using constant mechanical agitation (vibrator) throughout the test period to simulate fluid movement during root canal instrumentation. Furthermore, the same volume of irrigant was used for all the tissue samples, and all the pulp samples used were of similar weight (0.026 g).

## Conclusion

Overall, 7% MA and 17% EDTA exhibited minimal human pulp tissue–dissolution capacity when compared to that of 2.5% NaOCl.

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