Clinical and Experimental Health Sciences

Evaluation of the Effects of Different Irrigation Solutions on MTA and Dentin Microhardness

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ABSTRACT

Objective: The objective of this study was to compare the dissolution effects of different chemical solutions, which are commonly used as root canal irrigants, on partially or fully set mineral trioxide aggregate (MTA). Furthermore, the impact of these solutions on dentin microhardness was also assessed.

Methods: In this study, a total of 80 extracted single-rooted human teeth were utilized. The roots of the teeth were bisected transversely into slices of 6 mm in length. MTA (NeoPUTTY, Avalon Biomed, USA) was applied in layers of 4 mm in thickness within the cavities, sealed with moist cotton, and stored at 37°C under 100% humidity for 24 hours and 21 days. Forty samples were tested at the 24-hour mark, and the remaining 40 samples were tested at the 21-day mark. The samples were randomly allocated to one of five experimental groups: 17% EDTA, 5.25% NaOCl, 2% CHX, 40% citric acid, and saline (control). The Vickers microhardness test was employed to ascertain the hardness values of the MTA and dentin surfaces that had been exposed to the chemical solutions for a period of 10 minutes. Statistical analyses were performed using MedCalc® v19.7.2 (MedCalc Software Ltd, Belgium), with a significance level set at 0.05.

Results: It was observed that the application of EDTA, NaOCl, CHX, and citric acid solutions resulted in statistically significant reductions in the microhardness of both NeoPUTTY MTA (respectively p= .012; p= .012, p= .010; p= .012) and dentin (respectively p= .011; p= .012; p= .012; p= .012). The citric acid group exhibited the most pronounced reduction of MTA (respectively 52.2±1.6; – 39±0.9). In contrast, no statistically significant change in microhardness was observed in the control group treated with saline (for dentin p= .311; for MTA p= .415). The impact of the solutions on MTA at 21 days was found to be less pronounced than that observed at 1 day (p= .012).

Conclusion: The results of this study indicate that the application of citric acid solutions to MTA results in a statistically significant reduction in microhardness. The highest concentration of citric acid was observed to be more effective than the other solutions in the dissolution of MTA. However, these solutions were also found to significantly reduce the microhardness of dentin. It would be advisable to select solutions that facilitate the removal of MTA without damaging the dentin tissue.

Keywords: Citric acid, dentin microhardness, MTA, retreatment.

1. INTRODUCTION

Mineral trioxide aggregate (MTA) is a biocompatible and biologically active material that has gained recognition for its superior sealing ability and structural integrity in endodontic treatments (1). The high compressive strength of MTA, once its setting process is complete, plays a significant role in preserving structural integrity post-treatment. However, this same property presents a significant challenge during retreatment procedures, as it renders the removal of the material more difficult (2,3). Studies have reported that the compressive strength of MTA reaches 40.0 MPa after 24 hours, and the compressive strength of MTA increases to 67.3 MPa after 21 days in the presence of moisture (4). This high level of durability, particularly in root canals, renders the mechanical removal of MTA nearly impossible, necessitating the use of solvents during retreatment (3).

The solubility of MTA has been reported to be very low or non-existent in numerous studies (5-7). However, some research indicates that low pH can affect MTA's hardness, tensile strength, and push-out bond strength by creating pores and voids (8-10). Furthermore, researchers have noted that low pH reduces the microhardness of MTA, making the

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material, which has hardened and is ready for treatment easier to remove. In order to address this issue, a number of solutions with acidic, alkaline, and neutral pH have been tested (11). These include carbonic acid, hydrochloric acid, chlorhexidine (CHX), sodium hypochlorite (NaoCl), ethylenediaminetetraacetic acid (EDTA), and citric acid (CA). It has been demonstrated in the literature that chelating agents have the potential to compromise the integrity of MTA during retreatment (12,13).

A range of irrigating solutions have been examined for their capacity to dissolve MTA, with each solution exhibiting distinct chemical characteristics that influence both MTA and dentin. EDTA, a well-known chelating agent, is predominantly employed for the removal of the smear layer; however, it has also been documented to soften MTA by chelating calcium ions (14). NaOCl is an oxidizing agent with significant tissue dissolution capacity, although its effect on MTA solubility remains contentious (15). CHX is an antimicrobial agent with limited demineralizing properties (16), whereas CA is a potent acid that has demonstrated efficacy in dissolving calcium-based materials (17). The selection of irrigant is of paramount importance, as these solutions have the potential to influence not only the solubility of MTA but also the microhardness of root canal dentin, thereby potentially compromising its structural integrity. Consequently, a thorough investigation into the impact of these solutions on both MTA and dentin is imperative. Despite the fact that a number of studies have been conducted on the impact of different irrigants on MTA solubility, there remains a paucity of systematic investigations that evaluate and compare their effectiveness under uniform experimental conditions.

It is anticipated that an optimal solution for the removal of MTA will result in a reduction in its microhardness. It is important to note, however, that the use of acidic and chemical agents as root canal irrigants may result in alterations to the microhardness of both radicular and coronal dentin. In this context, it is of great importance to conduct a comprehensive investigation into the effects of various irrigation solutions on both MTA and root canal dentin (18). The objective of this study is to compare and contrast the effects of different chemicals, when used as root canal irrigants, on the dissolution of partially or fully set MTA. Furthermore, the influence of these solutions on dentin microhardness was assessed. The null hypothesis (H_0) of the study posits that the utilization of high-concentration citric acid does not yield superior efficacy in the dissolution of MTA in comparison to alternative solutions.

2. METHODS

The study was conducted in accordance with the ethical standards of the 2008 Declaration of Helsinki and approved by the Ethics Committee of the Institute of Health Sciences at Marmara University (approval number: 24.02.2022-2022/46). The study comprised a total of eighty single-rooted human teeth with straight canals that had been extracted for reasons related to the treatment of the patient. The sample size was determined based on the methodology of previous similar studies. In the existing literature, comparable in vitro studies have commonly utilized 10 samples per group. Accordingly, the present study adopted a similar approach to ensure methodological consistency with prior research (19-22). Teeth exhibiting root fractures, cracks, resorption, calcifications, or anatomical irregularities were excluded from the study. The teeth were disinfected by immersion in a solution of 0.5% NaOCl (Microvem, Altun Medical, Turkey) for a period of 48 hours, after which they were preserved in saline solution (23).

The crowns of the teeth were sectioned using a diamond disc (Tizkavan, Tehran, Iran). Subsequently, transverse sections of 6 mm in height were obtained from the roots using a diamond disc (Buehler Diamond Cut-Off Wheels 114243; Buehler, Lake Bluff, IL) attached to an electric saw (IsoMet Low Speed Saw; Buehler). The 80 samples were then embedded in groups of four in Bakelite moulds (Figure 1). The mechanical preparation was conducted under water cooling via the sequential utilization of Gates Glidden drills (Maillefer, Switzerland) numbered 2, 3, 4 and 5, respectively. Following the use of each instrument, irrigation with 5.25% NaOCl (Microvem, Altun Medical, Turkey) was conducted. The final cavity diameter was standardized at 1.3 mm, which corresponds to the diameter of the last instrument used (Gates Glidden #5). The measurements were verified using a digital caliper with an accuracy of 0.01 mm to ensure consistency across all samples. Specifically, NeoPUTTY (Avalon Biomed, USA) is a premixed, ready-to-use bioceramic material that does not require additional liquid for activation. The material was dispensed directly from its container and placed into the prepared cavities using a sterile spatula. Following placement, it was incrementally compacted to a final thickness of 4 mm using a suitable hand plugger (Dentsply, Switzerland) to ensure uniform adaptation to the cavity walls and minimize void formation. A layer of moist cotton was then placed on top of the MTA. All canals were sealed with a temporary restoration material (Coltosol, Ariadent, Tehran, Iran) and stored at 37°C with 100% humidity. 40 samples were stored for 24 hours, and the remaining 40 for 21 days.

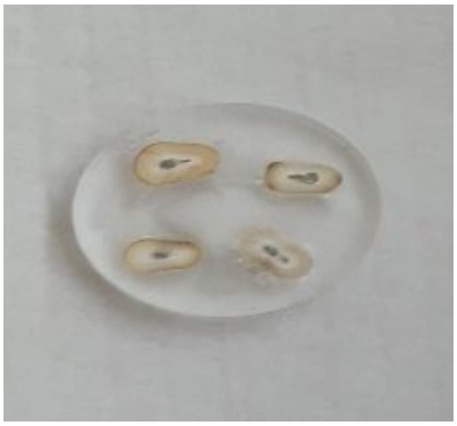

Figure 1. *Samples embedded in bakelite.*

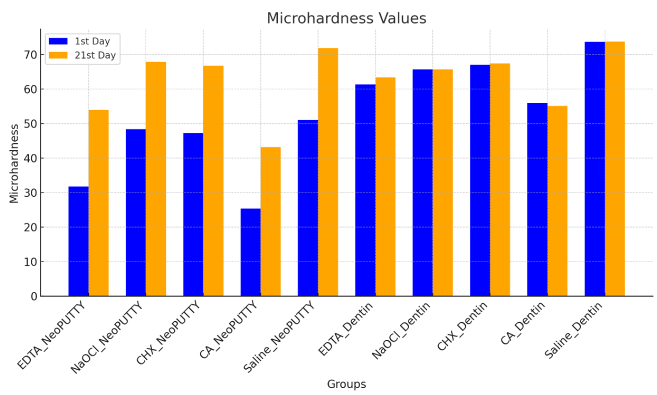

Figure 2. *Comparison of the effects of all solutions on MTA and dentin microhardness on day 1 and day 21.*

A total of 40 samples were subjected to a Vickers microhardness (Buehler Micromet 3 Micro Hardness Tester, Buhler, USA) testing procedure, utilizing a square-based pyramidal diamond indenter to assess the hardness of the MTA and dentin surfaces 24 hours following the initial setup. The indentations were made at a minimum of three similarly positioned points using a 100 g load and a dwell time of five seconds. The Vickers microhardness values were displayed and recorded from the digital readout of the microhardness tester.

Subsequently, the samples were randomly divided into five experimental groups (n = 8) and exposed to the designated solution for 10 minutes, with continuous application (3 drops/5 minutes) (Table 1). Subsequently, the samples were rinsed with distilled water for a period of one minute. Once the samples had been allowed to dry, the Vickers microhardness test was repeated, and the resulting measurements were recorded.

Table 1. *Experimental groups and details of the materials used.*

Experimental Groups	Solution	Manufacturer / Country
Group 1	17% Ethylenediaminetetraacetic Acid Solution (EDTA)	SAVER, Produits Dentaires, Switzerland
Group 2	5.25% Sodium Hypochlorite Solution (NaOCl)	Microvem, Altun Medical, Turkey
Group 3	2% Chlorhexidine Solution (CHX)	Microvem, Altun Medical, Turkey
Group 4	40% Citric Acid Solution (CA)	Prepared in laboratory: 109.58 g citric acid dissolved in water to a final volume of 250 mL
Group 5 (Control)	Saline Solution	House Brand, USA

The aforementioned procedures were then repeated for the remaining 40 samples after a 21-day period of NeoPUTTY MTA (Avalon Biomed, USA) setting. The execution of all experimental procedures was conducted by a single operator. The data were subjected to evaluation, and the mean microhardness values for all groups were determined both before and after chemical exposure.

2.1. Statistical Analysis

The analyses were conducted using MedCalc® Statistical Software, version 19.7.2 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021). Descriptive statistics, including mean, standard deviation, minimum, median, and maximum, were employed to characterize continuous variables. The normality of distribution for continuous variables was evaluated through the implementation of the Shapiro-Wilk test. In instances where two dependent variables did not follow a normal distribution, the Wilcoxon signed-rank test was employed for comparison. In instances where comparisons were to be made between more than two independent groups whose distributions were not normal, the Kruskal-Wallis test was employed. In the event of a statistically significant result, post hoc pairwise comparisons were conducted using the Bonferroni-corrected Mann-Whitney U test. A significance level of 0.05 was employed.

Table 2. *Comparison of the effectiveness of different solutions on MTA microhardness on day1.*

	Day 1/ NeoPUTTY				p value
	Before		After		
	Mean±SD	Med(min-max)	Mean±SD	Med(min-max)	
EDTA	55±1.7	54.7 (53 – 58.4)	31.7±0.8	31.6 (30.6 – 33.1)	**.012**
NaOCl	53.8±0.6	53.7 (53 – 54.8)	48.4±0.4	48.3 (47.8-49)	**.012**
CHX	53.4±0.8	53.7 (52.2 – 54.5)	47.2±0.3	47.2 (46.8 – 47.8)	**.012**
CA	53±1.1	53.1 (51.5 – 54.5)	25.4±0.9	25.4 (24.3 – 26.8)	**.012**
Saline	54±0.9	53.8 (52.8 – 55.4)	51.1±0.5	51.1 (50.4 – 51.6)	.224

Table 3. *Comparison of the effectiveness of different solutions on MTA microhardness on day21.*

	Day 21/ NeoPUTTY				p value
	Before		After		
	Mean±SD	Med(min-max)	Mean±SD	Med(min-max)	
EDTA	71.3±1.4	71.9 (69.3 – 72.7)	54±0.6	53.9 (53.2-55)	**.012**
NaOCl	72±1.2	72.3 (69.6 – 73.7)	67.9±1	67.9 (66.6 – 69.4)	**.012**
CHX	71.7±0.9	71.9 (70.2 – 72.6)	66.8±0.6	66.9 (66-67.6)	**.010**
CA	70.8±1	70.7 (69.5 – 72.4)	43.2±0.7	43.1 (42.1 – 44.6)	**.012**
Saline	73.4±0.6	73.4 (72.6 – 74.4)	71.9±0.6	71.9 (71-72.8)	.415

3. RESULTS

The effects of various solutions on the microhardness of NeoPUTTY and dentin at days 1 and 21 are presented in Tables 2–5. The findings demonstrated that EDTA, NaOCl, CHX, and CA solutions resulted in statistically significant reductions in the microhardness of both NeoPUTTY and dentin at both time points (day 1 for dentin respectively p= .012; p= .012; p= .012; p= .012; day 21 for dentin respectively p= .011; p= .012; p= .012; p= .012) (Fig. 2). The most pronounced reduction on microhardness of MTA (respectively 52.2±1.6; – 39±0.9) was observed in the CA group. In the control group treated with saline solution, no statistically significant change in microhardness values was observed (for dentin p= .311; for MTA p= .415). Furthermore, the impact of the solutions on MTA at 21 days was found to be less pronounced than that observed at 1 day (p= .012). As demonstrated in Table 6, post hoc pairwise comparisons revealed significant differences between the following groups on both days: differences between the CA-NaOCl (day 1 p= .001; day 21 p= .011), CA-CHX(day 1 p= .028; day 21 p= .011), CA-Saline (day 1 p< .001; day 21 p< .001), EDTA-NaOCl (day 1 p= .013), EDTA-CHX(day 1 p= .014), and EDTA-Saline (day 1 p< .001; day 21 p< .001) groups. Although CA caused a greater reduction in microhardness than EDTA for both NeoPUTTY MTA and dentin, this difference was not statistically significant (p= 1.00).

Table 4. *Comparison of the effectiveness of different solutions on dentin microhardness on day1.*

	Day 1/ Dentin				p value
	Before		After		
	Mean±SD	Med(min-max)	Mean±SD	Med(min-max)	
EDTA	74.1±0.9	74.1 (72.9 – 75.4)	61.4±0.8	61.4 (60.4 – 62.6)	.012
NaOCl	73.6±1.1	73.6 (72.2 – 75.2)	66±0.9	65.7 (65.1 – 67.4)	.012
CHX	73.9±0.9	73.8 (72.5 – 75.2)	67.1±0.8	67 (66.1-68.2)	.012
CA	73.6±0.9	73.6 (72.4 – 74.8)	56±1.2	55.8 (54.5 – 57.8)	.012
Saline	74±1	73.9 (72.7 – 75.4)	73.5±0.8	73.7 (72.4 – 74.9)	.216

Table 5. *Comparison of the effectiveness of different solutions on dentin microhardness on day21.*

	Day 21/ Dentin				p value
	Before		After		
	Mean±SD	Med(min-max)	Mean±SD	Med(min-max)	
EDTA	71.3±1.4	71.9 (69.3 – 72.7)	54±0.6	53.9 (53.2-55)	.012
NaOCl	72±1.2	72.3 (69.6 – 73.7)	67.9±1	67.9 (66.6 – 69.4)	.012
CHX	71.7±0.9	71.9 (70.2 – 72.6)	66.8±0.6	66.9 (66-67.6)	.010
CA	70.8±1	70.7 (69.5 – 72.4)	43.2±0.7	43.1 (42.1 – 44.6)	.012
Saline	73.4±0.6	73.4 (72.6 – 74.4)	71.9±0.6	71.9 (71-72.8)	.415

Table 6. *Post Hoc comparison of groups for microhardness changes.*

	Day 1 (p value)	Day 21 (p value)
CA-EDTA	1.000	1.00
CA-NaOCl	.001	.011
CA-CHX	.028	.011
CA – Saline	<.001	<.001
EDTA-NAOCL	.013	.187
EDTA-CHX	.014	.570
EDTA-Saline	<.001	.001
NaOCl-CHX	.392	1.00
NaOCl-Saline	.104	1.00
CHX-Saline	.131	.401

4. DISCUSSION

The original tricalcium silicate-based products, such as “mineral trioxide aggregate” (MTA), have a number of disadvantages, including difficulty of use, long setting times (>2 hours), a tendency for wash-out during setting, poor adhesion to dentin, relatively high solubility before setting, and the potential for tooth discoloration over time (24,25). In 2010, pre-mixed tricalcium silicate-based cements were introduced with the aim of eliminating the issue of inconsistent consistency that arises during the powder-liquid mixing of traditional tricalcium silicate-based hydraulic cements and to improve ease of application (26,27). NeoPUTTY (NuSmile, Houston, TX, USA), used in our study, is a newly formulated pre-mixed paste version. The product is a bioactive tricalcium silicate-based paste, designed by the manufacturer to overcome the issue of short shelf life associated with similar materials.

The ability of a solution to penetrate an MTA is contingent upon a number of factors, including the concentration of the solution, the exposure time, the type of MTA, and the thickness of the MTA. It has been demonstrated that the application of MTA in a minimum thickness of 4 mm enhances the material’s resistance (28). Consequently, this study employed a 4 mm thickness of MTA. Furthermore, it has been documented that MTA attains its maximum compressive strength and complete chemical setting within a period of 21 days (3). Consequently, to guarantee the full setting of MTA, the acid exposure of the samples was conducted after a 21-day interval.

It has been proposed that reducing the microhardness of MTA may facilitate easier removal compared to fully set, treatment-ready MTA (29). However, no gold standard exists in the literature for evaluating the removability of MTA. Accordingly, the present study employed the microhardness measurement method, which has been utilized in previous research (3,23).

In the present study, three solutions were selected for evaluation: NaOCl, EDTA, and CHX. These solutions are commonly employed as final irrigation agents in endodontic treatments. Prior research has demonstrated that EDTA and NaOCl result in a reduction in the microhardness of both partially and fully set MTA (30,31). Additionally, CHX has been shown to significantly impact the microhardness of partially set MTA (11,16). Citric acid, with a pH of 2.15, is a highly prevalent chelating agent in endodontics. A review of the literature reveals that studies have evaluated the use of citric acid in concentrations ranging from 5% to 50% (32). In the study by Bayraktar et al. (33), it was emphasized that as the acidic strength of the solution increases, there is a greater reduction in MTA microhardness. Consequently, the decision was taken to use 40% citric acid in the present study.

Previous studies have demonstrated that NaOCl and CHX solutions can reduce the microhardness of partially set MTA. In the aforementioned studies, the researchers observed that these solutions did not result in a notable difference in the microhardness of fully set MTA at 21 days (3,11,16). However, in the present study, it was found that NaOCl and CHX solutions significantly reduced the microhardness of NeoPUTTY at both days 1 and 21. This discrepancy may be attributed to the fact that NeoPUTTY is a material that completes its setting process at a slower rate compared to the tricalcium silicates that have been used in previous studies.

The study conducted by Butt et al. (11) demonstrated that CA significantly reduced the microhardness of both partially and fully set MTA when compared to the CHX and NaOCl groups. These findings are in accordance with our results. The present study demonstrated that CA significantly reduced the microhardness of NeoPUTTY in comparison to the NaOCl, CHX, and saline groups at both day 1 and day 21. A comparison of the CA and EDTA groups revealed that CA caused a numerically greater reduction in microhardness; however, the difference was not statistically significant. In light of these findings, the $\boldsymbol{H_0}$ of this study, which stated that “High-concentration citric acid does not yield superior efficacy in the dissolution of MTA in comparison to alternative solutions”, was rejected.

In assessing the efficacy of solutions employed to facilitate the removal of MTA, it is imperative to consider their impact on dentin tissue. While numerous high-concentration solutions have been demonstrated to effectively dissolve MTA (12,13), it is of paramount importance to ascertain that the selected acid concentration does not precipitate adverse effects on the root canal walls and periodontium. A reduction in dentin microhardness results in a weakening of the mechanical resistance of dentin, rendering it more susceptible to wear, fracture, and caries development. Furthermore, weakened dentin may have a detrimental impact on the success of restorative procedures, as the bonding capacity of restorative materials may be reduced, increasing the risk of post-treatment complications. Consequently, the utilization of biocompatible and safe solutions that do not damage dentin tissue while facilitating the removal of MTA is of paramount importance.

Previous studies have indicated that 2% CHX has a minimal impact on dentin microhardness (11,18). However, the present study has demonstrated that CHX can significantly reduce dentin microhardness. This discrepancy may be attributed to differences in the duration of application. A number of studies have evaluated the effect of NaOCl and EDTA on root canal dentin microhardness. These have consistently demonstrated that both solutions cause a significant reduction in dentin microhardness (11,18,34). This finding is in accordance with the results of the present study, in which all solutions except saline were found to have a reducing effect on dentin microhardness.

It has been demonstrated that EDTA causes demineralization of dentin by dissolving the inorganic components on the dentin surface, leading to a marked reduction in dentin microhardness (35,36). In a study by Cruz-Filho et al. (37), which evaluated the effects of EDTA and 10% citric acid solutions on dentin microhardness, the researchers found that both solutions significantly reduced microhardness, but there was no statistically significant difference between them. Similarly, Eldeniz et al. (36) evaluated the effectiveness of EDTA and 19% citric acid solutions, and the researchers reported a significantly greater reduction in dentin microhardness with citric acid. In the present study, although not statistically significant, the 40% citric acid solution demonstrated a numerically greater reduction in dentin microhardness than EDTA. When all results are considered collectively, it can be inferred that higher concentrations of citric acid led to a greater reduction in dentin microhardness.

The objective of this study was to address a significant shortcoming in the field of endodontic retreatment procedures by examining the impact of various chemical solutions on the dissolution of MTA and the microhardness of dentin. By focusing on the effects of high-concentration citric acid, this study contributes to the existing literature on the removability of MTA. However, it is important to consider the limitations of the study, despite the significant findings. Firstly, it should be noted that the study was conducted under laboratory conditions, which do not fully simulate the biological and dynamic factors present in a clinical environment. Secondly, only specific concentrations and solutions were tested, and the effects of different concentrations and exposure durations were not explored. Furthermore, the biological variability of the human teeth used could lead to individual differences in the results. Finally, in clinical practice, solutions may be applied with varying exposure times and methods, meaning that the results of this study may not fully reflect all clinical scenarios. It is recommended that future studies concentrate on evaluating the effects of varying concentrations, protracted exposure durations, and a range of application techniques under conditions that are more clinically relevant. This would allow for improved simulation of the in vivo environment and greater generalizability of the findings.

5. CONCLUSION

The objective of this study was to evaluate the impact of various chemical solutions on the dissolution of partially and fully set MTA and dentin microhardness. The findings suggest that the high-concentration citric acid solution was the most effective in reducing the microhardness of MTA. However, it was also observed that citric acid significantly reduced dentin microhardness. Furthermore, commonly used endodontic irrigation solutions, including NaOCl, EDTA, and CHX, were found to cause reductions in the microhardness of both MTA and dentin. In light of these findings, solvents aimed at increasing the removability of MTA should also be carefully selected in clinical practice, considering their potential damage to dentin tissue.

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Design of the study: GB, HSO,
Acquisition of data for the study: GB, HSO
Analysis of data for the study: IO, GB, HSO
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