A Comparative Evaluation of Smear Layer Removal Ability of EDTA, Maleic Acid, and Glycolic Acid with and without Sonic Activation: An in Vitro Study

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Abstract

Aim: To compare the smear layer removal ability of 17% EDTA , 7% maleic acid (MA), and 5% glycolic acid (GA) with and without sonic activation(SA) from the root canals. **Materials and Methods:** Sixty extracted human single-rooted teeth were divided into six groups (n=10): Group 1 – 17% EDTA; Group 2 – 7% MA; Group 3 – 5% GA; Group 4 – SA + 17% EDTA; Group 5 – SA + 7% MA; Group 6 – SA + 5% GA. Smear layer removal was assessed under scanning electron microscopy and scored using a standardized scale. Statistical analysis was done using Kruskal-Wallis and Dunn's post hoc tests ($p < 0.05$). **Results:** Maleic acid with Sonic activation showed the highest smear layer removal efficacy, followed by maleic acid alone. Sonic activation enhanced the efficacy of all chelating agents significantly ($p < 0.001$) whereas Glycolic acid as an irrigant showed the lowest efficacy. **Conclusion:** Sonic activation significantly improves smear layer removal by chelating agents, especially maleic acid suggesting enhanced clinical outcomes with activated irrigation protocols.

Key words: Sonic Activation, EDTA, Maleic Acid, Glycolic Acid, Smear Layer Removal, Root Canal Irrigation, Chelating Agents, Scanning Electron Microscopy, Endodontic Irrigation Protocols, Dentin Cleanliness, Irrigant Activation Techniques

Introduction

The smear layer is an amorphous layer that occludes the dentinal tubules of the root canal system during the process of cleaning and shaping with hand or rotary instruments. 1

It was first identified by Eick et al. using an electron microprobe under a scanning electron microscope (SEM), revealing particles ranging from 0.5 to 15 µm. 2 Mc Comb and Smith were the first to report the smear layer on instrumented root

canal surfaces. 3 Cameron and Mader et al. later divided the smear layer into a superficial layer (1–2 µm) and a deeper smear plug (up to 40 µm), while Aktenar et al. reported its penetration up to 110 µm with the use of surface-active reagents. 4,5,6 Bacteria have also been found to infiltrate to a depth of more than approximately 500 µm into the deeper regions of the smear layer. 7

Along with bacteria smear layer is composed of various other organic and inorganic components like pulp remnants, odontoblastic processes, dentinal debris, etc. 8 Therefore, removal of the smear layer is recommended. Numerous irrigants have been used to eliminate the smear layer like chelating agents, organic acids (like citric acid, polyacrylic acid), chitosan, etc. 9

However, 17% EDTA (ethylenediaminetetraacetic acid) remains as one of the most widely used chelating agent in smear layer removal. 10 Though effective, EDTA causes denaturation of collagen fibrils, erosion of intertubular and peritubular dentin, causes cytotoxicity if extruded beyond the apical foramen, impairs the adhesive properties of the dentin surface, compromises root canal seal and the industrial-scale production of EDTA utilizes contaminants that are considered harmful pollutants towards aquatic life, leading to calcium imbalance in various organisms. 10,11

Therefore, alternative irrigants are being sought that are comparatively biocompatible and effective in smear layer removal.

Maleic acid (MA) is a potential irrigant that is commonly used as a mild organic acid for acid conditioning in adhesive dentistry. 12 Compared to 17% EDTA, 7% MA is considered superior, particularly in the apical third of the root canals, as demonstrated by previous studies. 13 Other advantages include an increase in the surface roughness of intra-radicular dentin, which enhances the bonding of obturating materials, improves the wettability of sealers, and increases the micro push-out bond strength with comparatively less cytotoxicity and genotoxicity. 13,14,15

On the other hand, glycolic acid (GA) is another prospective irrigant researched. Presently, it is being used in dentistry for enamel and dentin etching during restorative procedures and is widely used in dermatology. 16 When compared to 17% EDTA, 5% GA demonstrated similar efficacy in removing the smear layer with lesser effect on microhardness of the root dentin and fewer adverse biological effects and is known to stimulate fibroblast growth and collagen production. 17

To improve irrigation efficacy, various activation techniques have been studied. Sonic activation using the EndoActivator is one such technique, and studies have shown that sonic activation using the EndoActivator has been shown to

outperform passive ultrasonic irrigation (PUI) and manual dynamic irrigation, particularly in the apical third of canals. 18,19

Given that both 7% maleic acid and 5% glycolic acid demonstrate effective smear layer removal with minimal detrimental effects on dentin, and that sonic activation enhances irrigant performance, the purpose of this study is to compare the efficacy of 17% EDTA, 7% maleic acid, and 5% glycolic acid in smear layer removal from root canal dentin using sonic activation.

Materials and Methods

Sixty freshly extracted human mandibular first premolars extracted for orthodontic and periodontal reasons were selected based on inclusion criteria: (1) mandibular premolars with a single root and canal, (2) teeth with closed apices, and (3) teeth of similar canal shape and size and exclusion criteria: (1) morphological defects, (2) previous restorations, (3) cracks or fracture lines, (4) previous endodontic treatment, (5) calcified canals, (6) resorption, and (7) immature apex. Samples were stored in 0.9% NaCl solution. After access cavity preparation with a No. 2 diamond; patency was confirmed using a #10 K-file. All samples were decoronated to a standardized root length of 17 mm and root canal preparation was done using ProTaper rotary files from S1 to F3. Irrigation was done with 2 ml of 3% NaOCl between each file, followed by irrigation with 5 ml of distilled water. Samples were then randomly divided into six groups (n=10): Group 1 – 17% EDTA; Group 2 – 7% Maleic Acid; Group 3 – 5% Glycolic Acid; Group 4 – Sonic Activation + EDTA; Group 5 – Sonic Activation + Maleic Acid; Group 6 – Sonic Activation + Glycolic Acid. Irrigation was performed with 5 ml of the respective solution for 1 minute. Sonic activation was done using the EndoActivator at 10,000 cycles/min for 30 seconds. Canals were finally rinsed with 5 ml distilled water and dried. Deep grooves were made on buccal and lingual surfaces, and teeth were split longitudinally. One half of each tooth was gold-palladium sputter-coated and examined under SEM at 1500X magnification and 15 kV in the apical third (1–2 mm from the apex). Smear layer removal was evaluated using Torabinejad's scoring system: Score 1 – no smear layer (all tubules clean and open), Score 2 – moderate smear layer (surface clean, tubules contain debris), and Score 3 – heavy smear layer (surface and tubules covered). [1,7]

Results

The test results indicated that the mean smear layer removal scores for the glycolic acid group were 2.80 ± 0.42; for the maleic acid group, it was 1.40 ± 0.52; and for the EDTA group, it was 2.40 ± 0.52. Additionally, the mean score for the Sonic

activation combined with glycolic acid group was 2.10 ± 0.57; for the Sonic activation combined with maleic acid group, it was 1.10 ± 0.32; and for the Sonic activation combined with EDTA group, it was 1.80 ± 0.63. The observed differences in the mean Smear Layer Removal Scores across these six groups were statistically significant ($p < 0.001$). (Table 1)

Sonic activation with 7% Maleic Acid (Group 5) showed the highest efficacy (1.10 ± 0.32), making it the most potent irrigant. It was followed by 7% Maleic Acid (Group 2, 1.40 ± 0.52) and Sonic Activation with EDTA (Group 4, 1.80 ± 0.63). Sonic activation improved smear layer removal in Glycolic Acid (Group 6, 2.10 ± 0.57) and EDTA (Group 1, 2.40 ± 0.52) compared to their unactivated forms. 5% Glycolic Acid (Group 3, 2.80 ± 0.42) (Table 1) was the least effective group, further confirming sonic activation enhances irrigant performance.

Discussion

The research on smear layer has been vast. After McComb and Smith identified the existence of smear layer on instrumented root canals in 1975, Mader et al. further explained that the smear layer consists of two distinct parts: a superficial layer that covers the dentin surface and a smear plug that occludes the dentinal tubules. The superficial layer is a thin coating of mineralized tissue approximately 1–2 µm thick. 3,4

Later Ghorbanzadeh et al. noted the smear plug thickness itself to be about 40 µm, with bacterial penetration potentially reaching 500 µm. 7 It is also loosely adherent and can serve as a conduit of leakage between the root filling and the dentinal walls, limiting the penetration of irrigants and medicaments, compromising disinfection and obturation. 1 It further affects bonding in fibre post supported restorations and the root end filling seal in cases of apicoectomy. 11,20 Consequently, smear layer removal is crucial.

EDTA, a calcium chelator that is the most effective and widely accepted in smear layer removal, with the application time of 1 to 5 minutes. 10,21 But, due to its limitations, it is essential to explore alternative final irrigants that are biologically compatible, exhibit lower cytotoxicity, and doesn't disrupt the hydration process of MTA and effectively remove the smear layer from the root canal walls without causing dentin erosion and collagen denaturation, thereby contributing to improved clinical outcomes. 10,22

In this study Sonic Activation with 7% maleic acid (Group 5) showed the highest efficacy (1.10 ± 0.32), making it the most potent irrigant. It was followed by 7% maleic acid (Group 2, 1.40 ± 0.52) and Sonic Activation with EDTA (Group 4, 1.80 ± 0.63). This could be attributed to the lower surface tension (0.06345 N/m) of

7% maleic acid compared to 17% EDTA (0.0783 N/m), which may enhance its ability to penetrate the dentinal tubules. 1,13 Also, efficacy of EDTA diminishes over time due to a gradual reduction in pH following calcium ion exchange with dentin. In contrast, Maleic acid's strongly acidic nature allows for a more rapid and efficient demineralization effect. Also, the reduced decalcification of EDTA in the apical portion of the root canal could be due to the low concentration of noncollagenous proteins (NCPs) where dentin is often sclerosed. 1,13,25 Since, maleic acid has lower surface tension and higher demineralizing capability over a shorter time period compared to EDTA, it suggests that maleic acid may be more suitable irrigant for smear layer removal in the apical third. 1 Sonic activation improved smear layer removal in glycolic acid (Group 6, 2.10 ± 0.57) and EDTA (Group 1, 2.40 ± 0.52) compared to their unactivated forms. This can be explained by a study done by Khalap et al. in which sonic activation with the EndoActivator outperformed passive ultrasonic irrigation (PUI) and manual dynamic agitation in the apical third, yielding significantly lower smear layer and debris scores in teeth prepared with ProTaper NEXT. 18 While in another study done by Thapak et al., Er:YAG lasers showed the best overall results compared to EndoActivator. However, EndoActivator remains superior to manual methods for apical smear layer removal, making it a valuable adjunct in clinical protocols. 19 5% glycolic acid (Group 3, 2.80 ± 0.42) was the least effective group. However, in endodontics, GA is regarded as an effective agent for smear layer removal and has shown potential to enhance the microhardness of both dentin and enamel. Studies have indicated that GA and EDTA exhibit similar smear layer removal capability and cause comparable levels of dentin erosion. 17 In a study done by Barcellos et al. energy-dispersive spectroscopy (EDS) analysis revealed that all tested irrigants led to a reduction in the apatite/collagen ratio, and GA particularly at lower pH, maintains the chemical and mechanical properties of dentin without increasing erosion, offering a promising alternative to EDTA for smear layer removal. It's also less cytotoxic and biocompatible than EDTA.10 In another study done by Yanik et al., GA improved the pushout bond strength of fibre posts when used as a final irrigant compared to that of EDTA. 23 Based on these findings, GA emerges as a viable and potentially superior alternative to EDTA for smear layer removal, offering similar efficacy with the added benefits of enhanced biocompatibility and dentin preservation.

In conclusion, MA demonstrates enhanced smear layer removal over EDTA and GA, especially in the challenging apical third. GA offers comparable efficacy to EDTA with added biocompatibility advantages. Incorporating activation

techniques like sonic agitation can further improve smear layer removal and clinical outcomes in endodontics.

While maleic acid (MA) has demonstrated superior efficacy in smear layer removal compared to other chelating agents, its clinical adoption has been limited. This limitation may be attributed to concerns regarding its stability and shelf life, which are crucial factors for ensuring the efficacy and safety of dental irrigants. 24

Therefore, the stability and shelf life of maleic acid remain important considerations that warrant further investigation.

Conclusion

Within the limitations of this in vitro study, it can be concluded that 7% maleic acid with sonic activation is the most effective final irrigant for smear layer removal in the apical third of the root canal system.

Further research should focus on validating these findings clinically and developing stable formulations to enhance shelf life and stability of MA-based irrigants, along with the incorporation of suitable preservatives or stabilizing agents enabling broader use of maleic acid in endodontic practice.

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References:

1. Ballal NV, Kandian S, Mala K, Bhat KS, Acharya S. Comparison of the efficacy of maleic acid and ethylenediaminetetraacetic acid in smear layer removal from instrumented human root canal: a scanning electron microscopic study. J Endod 2009;35:1573–6.

2. Eick JD, Wilko RA, Anderson CH, Sorensen SE. Scanning electron microscopy of cut tooth surfaces and identification of debris by use of the electron microprobe. J Dent Res 1970;49:1359–68.
3. McComb D, Smith DC. A preliminary scanning electron microscopic study of root canals after endodontic procedures. J Endod 1975;1:238–42.
4. Cameron JA. The use of ultrasonics in the removal of the smear layer: a scanning electron microscope study. J Endod 1983;9:289–92.
5. Mader CL, Baumgartner JC, Peters DD. Scanning electron microscopic investigation of the smeared layer on root canal walls. J Endod 1984;10:477–83.
6. Aktener BO, Cengiz T, Piskin B. The penetration of smear material into dentinal tubules during instrumentation with surface-active reagents: a scanning electron microscopic study. J Endod 1989;15:588–90.
7. Zancan RF, Di Maio A, Tomson PL, Duarte MAH, Camilleri J. The presence of smear layer affects the antimicrobial action of root canal sealers. Int Endod J 2021;54:1369–82.
8. Kuruvilla A, Jaganath BM, Krishnegowda SC, Ramachandra PK, Johns DA, Abraham A. A comparative evaluation of smear layer removal by using EDTA, etidronic acid, and maleic acid as root canal irrigants: an in vitro scanning electron microscopic study. J Conserv Dent 2015;18:247–51.
9. Ballal NV, Sona M, Tay FR. Effects of smear layer removal agents on the physical properties and microstructure of mineral trioxide aggregate cement. J Dent 2017;66:32–6.
10. Barcellos DPDC, Farina AP, Barcellos R, Souza MA, Borba M, Bedran-Russo AK et al. Effect of a new irrigant solution containing glycolic acid on smear layer removal and chemical/mechanical properties of dentin. Sci Rep 2020;10:7313.
11. Fan F, Ibrahim M, Dai P, Mao Y, He B, Wu G et al. Effect of maleic acid on the bond strength of fibre posts to root dentine. Eur J Oral Sci 2017;125:396–402.
12. Mankeliya S, Singhal RK, Gupta A, Jaiswal N, Pathak VK, Kushwah A. A comparative evaluation of smear layer removal by using four different irrigation solutions like root canal irrigants: an in vitro SEM study. J Contemp Dent Pract 2021;22:527–31.
13. Ballal NV, Kundabala M, Bhat KS. Comparative evaluation of smear layer removal by using EDTA, EGTA, and maleic acid: an SEM study. J Conserv Dent 2013;16:362–6.

14. Rao S, Ballal NV, Narkedamalli RK. Efficacy of SmearOFF, maleic acid, and ethylenediaminetetraacetic acid combined with sodium hypochlorite in removal of smear layer from curved root canals: in vitro study. Saudi Endod J 2021;11:221–7.
15. Topbaş C, Çınar Ş, Altan B, Şirin DA, Fildişi MA. The effects of different root canal irrigation protocols and artificial aging procedures on the bond strength between dentin and hybrid ceramic posts. BMC Oral Health 2022;22:590.
16. Souza MA, Bischoff KF, Ricci R, Bischoff LF, Reuter E, Gomes NDS et al. Glycolic acid and ultrasonic activation: effects on smear layer removal, dentin penetration, dentin structure and bond strength of the root dentin filling material. J Clin Exp Dent 2024;16:e1269–77.
17. Gottumukkala SNVS, Lakshmi MR, Penmetsa GS, Ramesh KSV, Pasupuleti MK, Bypalli V. Scanning electron microscopic analysis of 5% glycolic acid as a novel alternative to 17% ethylenediaminetetraacetic acid in root biomodification: an in vitro study. J Indian Soc Periodontol 2024;28:79–83.
18. Khalap ND, Kokate S, Hegde V. Ultrasonic versus sonic activation of the final irrigant in root canals instrumented with rotary/reciprocating files: an in vitro scanning electron microscopy analysis. J Conserv Dent 2016;19:368–72.
19. Thapak G, Arya A, Grewal MS, Arora A. A comparative evaluation of smear layer removal using Erbium:YAG laser-activated irrigation, sonic irrigation, and manual dynamic irrigation: a scanning electron microscope study. J Lasers Med Sci 2021;12:e22.
20. Bhanja J, Agrawal S, Mathur R, Sharma D, Gazal S, Awadesh AR. To compare and evaluate microcracks and smear layer after root end preparation by laser, ultrasonic tip, and conventional bur in single-rooted teeth: scanning electron microscope study. J Conserv Dent Endod 2025;28:16–20.
21. Ortigara GA, Prado M, Lopes RT, Dos Santos BC, Gusman H. Micro-computed tomographic evaluation of smear layer and accumulated hard tissue debris removal. J Conserv Dent 2020;23:249–53.
22. Ballal NV, Sona M, Tay FR. Effects of smear layer removal agents on the physical properties and microstructure of mineral trioxide aggregate cement. J Dent 2017;66:32–6.
23. Souza MA, Bischoff KF, Ricci R, Bischoff LF, Reuter E, Gomes NDS et al. Glycolic acid and ultrasonic activation: effects on smear layer removal,

dentin penetration, dentin structure and bond strength of the root dentin filling material. J Clin Exp Dent 2024;16:e1269–77.

24. Huang Y, Sheng B, Wang ZH, Liu QZ, Yuan RX, Xiao DX et al. Deciphering the degradation/chlorination mechanisms of maleic acid in the Fe(II)/peroxymonosulfate process: an often-overlooked effect of chloride. Water Res 2018;145:453–63.
25. Jaiswal S, Patil V, Kumar KS, Ratnakar P, Rairam S, Tripathi S. Comparative analysis of smear layer removal by conventional endodontic irrigants with a newly experimented irrigant—fumaric acid: a scanning electron microscopic study. J Conserv Dent 2018;21:419–23.

Table 1

Table 1 - Comparison of mean Smear Layer Removal Scores b/w 3 groups using Kruskal Wallis Test

Groups	N	Mean	SD	Min	Max	p-value
Glycolic Acid	10	2.80	0.42	2	3	<0.001*
Maleic Acid	10	1.40	0.52	1	2	
EDTA	10	2.40	0.52	2	3	
Sonic Activation + Glycolic Acid	10	2.10	0.57	1	3	
Sonic Activation + Maleic Acid	10	1.10	0.32	1	2	
Sonic Activation + EDTA	10	1.80	0.63	1	3	

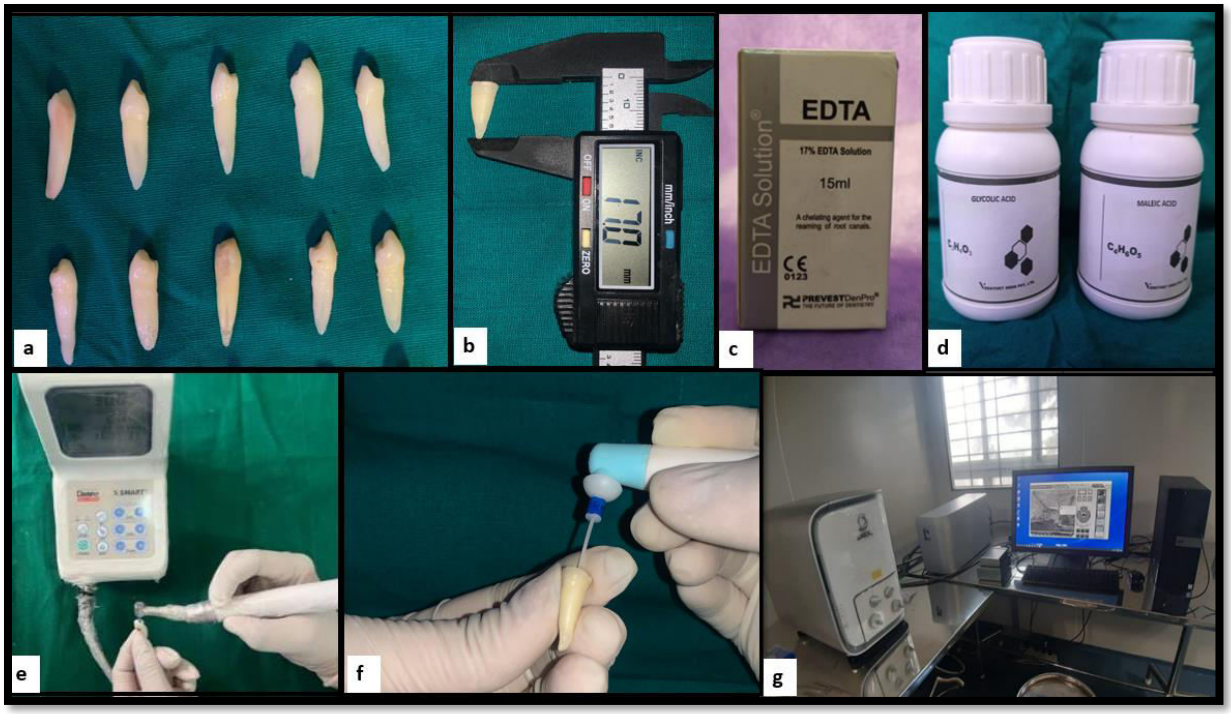

Figure A

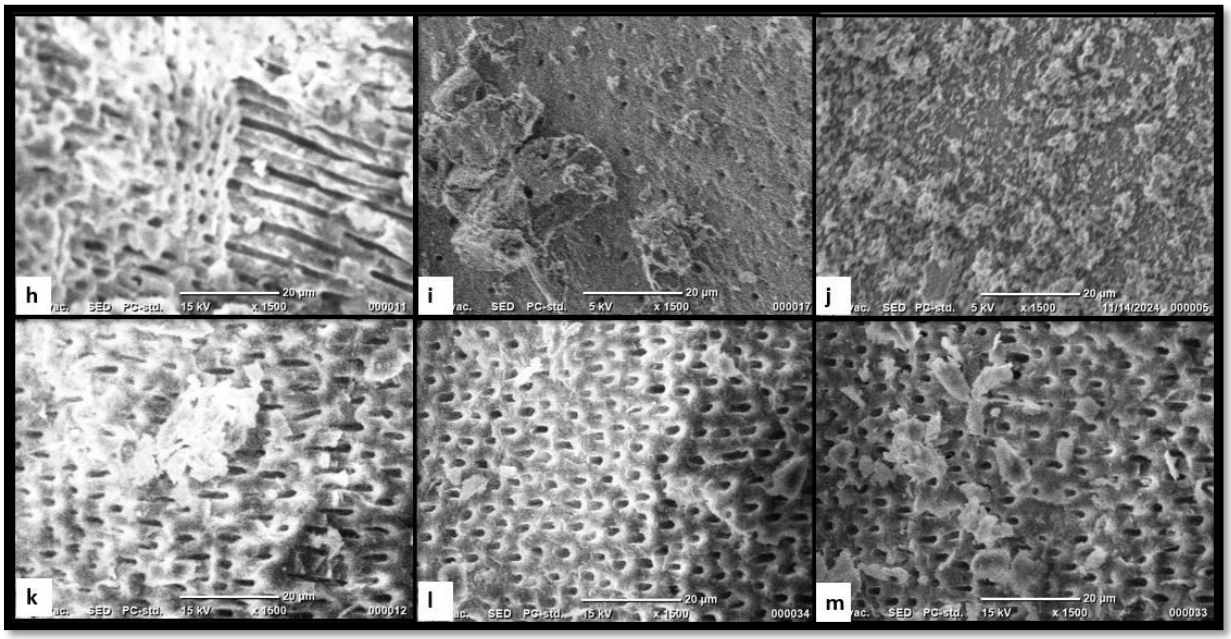

Figure B

Figure1: (a) Samples per group(n= 10), (b) Length post decoronation, (c) 17% EDTA, (d) 5% Glycolic acid and 7% Maleic acid, (e) Biomechanical preparation, (f) Sonic activation with EndoActivator, (g) Scanning electron microscope

Figure2: SEM images of conventional needle irrigation groups: (h) 17% EDTA , (i) 7% Maleic acid, (j) 5% Glycolic acid and with sonic activation groups (k) 17% EDTA , (l) 7% Maleic acid, (m) 5% Glycolic acid