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PULP TISSUE DISSOLUTION CAPACITIES OF DIFFERENT IRRIGATION AGITATION TECHNIQUES

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ABSTRACT

Background and Aim: Different irrigation agitation (IA) techniques have been proposed to improve the efficacy of irrigation solutions. The aim of this study was to compare the organic tissue dissolution capacities of 3 different IA techniques.

Materials and Methods: Fifty bovine pulp tissue samples were prepared and then placed in tubes. Samples were randomly divided into 5 groups (n = 10). The test groups were as follows: *(1)* saline (SS) *(no activation), (2)* sodium hypochlorite (SH) *(no activation), (3)* sonic irrigation (SI), *(4)* passive ultrasonic irrigation (PUI), and *(5)* laser-activated irrigation (LAI). In the SS group; tissue samples were placed in tubes with 1.25 mL of SS. After resting for 6 min, the solution was withdrawn, and fresh SS was added (repeated 5 times). In the SH group, the same method in the SS group was achieved by replacing the SH. Samples in each agitation group waited in SH for 5 min and then 1 min activation with respective agitation device (repeated 5 times). The tissue samples were weighed before and after treatment. The percentage of weight loss was calculated and analyzed.

Results: The test showed statistically significant differences between the activated and the non-activated groups. There was no significant difference between SI and LAI groups ($p= 0.508$), while the difference between these groups except the SH group and PUI group was significant ($p< 0.05$).

Conclusion: IA techniques have been found to be effective in resolving bovine pulp tissue. Among these, SI and LAI were the most effective techniques.

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CLINICAL DENTISTRY AND RESEARCH 2021; 45(3): 127-134 Orijinal Araştırma

FARKLI İRRİGASYON AKTİVASYON TEKNİKLERİNİN PULPA DOKUSUNU ÇÖZME KAPASİTELERİNİN KARŞILAŞTIRILMASI

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ÖZ

Amaç: İrrigasyon solüsyonlarının etkinliğini arttırmak için, farklı irrigasyon aktivasyon (İA) teknikleri önerilmiştir. Bu çalışmanın amacı, 3 farklı irrigasyon aktivasyon tekniğinin organik doku çözünme kapasitelerinin karşılaştırılmasıdır.

Gereç ve Yöntem: Elli adet sığır diş pulpa örnekleri hazırlandı ve ardından tüplere yerleştirildi. Örnekler rastgele 5 deney grubuna (n= 10) ayrıldı. Test grupları; *(1)* salin (SS) *(aktivasyon uygulanmadı), (2)* sodyum hipoklorit (SH) *(aktivasyon uygulanmadı), (3)* sonik irrigasyon (SI), *(4)* pasif ultrasonik irrigasyon (PUI) ve *(5)* lazer-aktive irrigasyon (LAI). SS grubunda; doku örnekleri 1.25 mL SS'li tüplere yerleştirildi. Altı dakikalık bekleme süresinden sonra solüsyon alınıp yerine taze SS ilave edildi (5 defa tekrarlandı). SH grubunda, solüsyon olarak SH yerleştirilerek, SS grubundaki aynı prosedür uygulandı. Her bir aktivasyon grubunda SH 5 dak. tüp içerisinde bekletildi ve ardından 1 dak. aktivasyon uygulandı (5 defa tekrarlandı). Doku örnekleri işlem öncesi ve sonrasında tartıldı. Doku kayıp yüzdesi hesaplandı ve analiz edildi.

Bulgular: Aktive ve aktive edilmeyen grupları arasında istatistiksel olarak anlamlı farklılıklar bulundu. SI ve LAI grupları arasında anlamlı bir fark bulunmazken, bu gruplar ile SH grubu ve PUI grubu arasındaki fark anlamlıydı ($p< 0,05$).

Sonuç: İA tekniklerinin sığır pulpa dokusunu çözmede etkili olduğu bulunmuştur. Bunlar arasında SI ve LAI en etkili tekniklerdir.

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Anahtar Kelimeler: Çözünme, Laser Aktivasyon, Pulpa, Sonik, Ultrasonik

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INTRODUCTION

Root canal treatment (RCT) success is based on removing the pulpal debridement and microorganisms from the root canal system. Because of the complex anatomy of root canals with lateral canals, isthmuses, and deltas, up to 50% of canal walls remain uninstrumented during shaping, resulting in inadequate debridement and it might be responsible for treatment failure.[1] Irrigation play an important role in cleaning and disinfection of the root canals while cleaning the uninstrumented areas of the root canals and removing the debris, smear layer and pulp tissue remnant.[1-3]

Pulp tissue dissolution is a highly desirable property of any irrigant because it potentially enhances root canal cleansing. Therefore, the tissue-dissolving property of irrigants is the most important reason for choosing an endodontic irrigant.[4,5] Sodium hypochlorite (SH) is the most widely preferred irrigant because of its spacious antimicrobial effect, the capacity of dissolving the organic part of the smear layer, and pulp tissue remnants.[2] Thus, different irrigation agitation techniques (IAT) are suggested to increase the efficacy of irrigant delivery and improve root canal cleanliness.[6]

Sonic and ultrasonic IATs have been extensively tested and are usually used in root canal treatment (RCT) as irrigant agitation devices.[7-9] Sonic irrigation (SI) devices produce a hydrodynamic phenomenon through the oscillation of smooth and highly flexible polymer tips at frequencies of 1-10 kHz to induce bacterial elimination and pulpal debridement.[7] Among sonic devices, the EndoActivator system (Dentsply Maillefer, Baillagues, Switzerland) is the most studied device with a frequency of 0.166 - 0.3 kHz.[10,11] It is a cordless, battery-powered handpiece with a sonic motor. Passive ultrasonic activation (PUI) produces microstreaming by utilizing small noncutting files oscillating freely in shaped canals at ultrasonic frequencies (25-30 kHz) and activating irrigants through secondary acoustic microstreaming.[12] An ultrasonic tip is activated in the canal up to the working length and is moved passively in an up-and-down motion to ensure it does not bind with the root canal walls.[13] Due to the metal alloy of ultrasonic tips, there can be undesired deformation in root canal surfaces, if the tips touch the root canal walls.[12,13]

Laser-activated irrigation (LAI) is another type of agitation technique, based on the activation of irrigants by medium-infrared lasers (2780 and 2940 nm), and has recently become a popular option in endodontics. The radiation emitted by the laser is strongly absorbed by water-based solutions and expanded and collapsed vapor bubbles are formed at the fiber tip, which results in cavitation. These changes in the size of collapsed bubbles lead to localized shock waves and a distinct fluid movement. Therefore, secondary cavitation bubbles are triggered with subsequent laser pulses. This results in the generation of acoustic streaming of the irrigant throughout the entire root canal system.[14,15] Among several laser devices, Er:YAG and Er,Cr:YSGG lasers are promising as a method for activating the irrigants. Aldeen et al. (15) reported that using Er:YAG laser was removed significantly more debris than PUI and conventional irrigation. One laser-induced activation is PIPS (photon-induced photoacoustic streaming), performed by a pulsed Er:YAG laser, which uses low pulse energies (10 or 20 mJ) with a short pulse length (50 µs) resulting in high peak powers and efficient cavitation.[16] This technique differs from other laser agitation techniques in that only the tip is placed into the pulp chamber, thereby preventing contact with the root canal wall.[5,17] In a study,[18] PIPS was more effective in the removal of apically placed dentinal debris. Akçay et al.[19] showed that the activation of the irrigant and the creation of the streaming with the Er:YAG laser have positive effects on the irrigant penetration. But, Kustarci and Er[20] have warned about more apically extruded debris.

The aim of this *in vitro* study was to compare the organic tissue dissolution capacities of 3 different IATs (sonic, ultrasonic, and laser-activated). The null hypothesis tested was that there are no differences in pulp tissue dissolution capacity amongst different IATs.

MATERIALS AND METHODS

Pulp tissue preparation

Thirty intact, freshly extracted, young bovine mandibular and maxillary incisors were used in this study. This study was not classified as an animal study because it did not influence the premortal fate of the animals or the slaughtering process in any way. Thus, this protocol was accepted by the institutional ethics committee without any objections. The teeth were extracted within 24 h purchased from a slaughterhouse and immediately placed in glass vials with distilled water and stored at -20 °C until required.

Bovine incisors were decoronated at cemento-enamel junction by using diamond fissure burs and the pulp tissues were removed carefully (Figure 1). All pulps were washed with distilled water to remove excess blood and then blotted

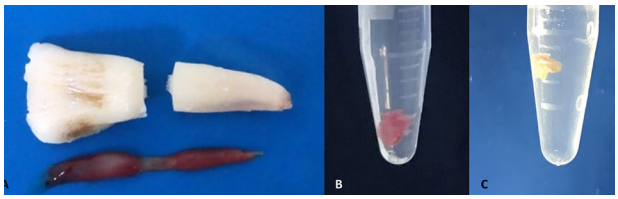


Figure 1. (A) Bovine mandibular incisor tooth and pulp tissue, (B) Pulp tissue before activation, (C) Sample of a pulp tissue after activation.

dry. Fifty samples were adjusted by weighing on an analytical balance (XB 220A; Kunz Precisa, Zofingen, Switzerland) and each sample weight (initial weight) was approximately 25 mg with a no. 15 surgical blade. The measurement was repeated 3 times for each sample and the average was recorded. During the experiment, pulp tissues were only interfered with with cotton pliers to avoid errors. All testing procedures were performed at room temperature.

Test design

After the initial weights were recorded, samples were randomly divided into 2 control and 3 experimental groups (*n*= 10 in each group) and individually placed into 2 mL Eppendorf safe-lock tubes. The test groups were as follows: *(1)* saline solution (SS) group, *(2)* sodium hypochlorite (SH) solution group, *(3)* sonic irrigation (Sİ) group, *(4)* passive ultrasonic irrigation (PUI) group, and *(5)* laser-activated irrigation (LAI) group.

SS group (negative control, no activation): 1.25 mL isotonic SS (0.9% NaCl) (I.E. Ulagay Drug Industry, Istanbul, Turkey) was added to Eppendorf tubes containing tissue samples. The samples were exposed to SS for 6 min. After this period, solutions were removed in each tube with a 30 G syringe, and a 1.25 mL fresh solution was added to each tube. This protocol was repeated 5 times for each tube. Consequently, the total solution exposure time was 30 min with a total volume of 6.25 mL irrigant for each sample.

SH group (positive control, no activation): 1.25 mL 5.25% SH (Wizard, Rehber Kimya, Istanbul, Turkey) was added to Eppendorf tubes containing tissue samples. After, the same procedures were applied like the SS group.

SI group: The Endoactivator (Dentsply Maillefer) device was used for sonic agitation of 5.25% SH. It was performed using the Endoactivator handpiece set at 10.000 cycles per min with a medium polymer tip (#25/.04). The tip was immersed in tubes containing irrigant solution throughout their working length. 1.25 mL 5.25% SH was added to Eppendorf tubes containing tissue samples. All samples were exposed to SH for 5 min (resting time), with agitation times of 1 min after resting. After this period, the solution was removed in each tube with a 30 G dental syringe and 1.25 mL fresh SH was added. This protocol was repeated 5 times for each tube. Consequently, the total solution exposure time was 30 min with a total volume of 6.25 mL irrigant for each sample.

The same procedures (solution, time, and volume) were identical to the PUI and LAI groups.

PUI Group: A noncutting #25 file (Irrisafe; Satelec Acteon, Merignac, France) driven by an ultrasonic device (Satalec P5 Newtron XS; Satelec Acteon) was used for ultrasonic agitation of 5.25% SH in the tube for 3×20 sec at 50% power. The tip was immersed in tubes containing irrigant solution throughout their working length.

LAI group: The Er,Cr:YSGG laser system (2780 nm wavelength) (Waterlase iPlus; Biolase Technology, Irvine, CA, USA) was used at a panel setting of 0.5 W at 20 Hz (25 mJ/pulse) without air or/water spray. The pulses were focused using a fiber tip (RFPT5) with a diameter of 580 µm and a length of 14 mm.

Then, tissue samples were removed from the solutions, washed with distilled water to remove dissolved or suspended tissue remnants, blotted dry, and weighed again. All samples were weighed by a single operator without knowing which group the sample belonged to. The measurement was repeated 3 times for each sample and the average was recorded.

Statistical analysis

The difference in weights of the tissue sample, before and after exposure to the test solution, was divided by the original tissue weight and multiplied by 100 to obtain the percentage of tissue weight loss. After investigating the normality (Kolmogorov–Smirnov test) and the homogeneity of variance (Levene's test), the data were statistically evaluated using ANOVA to determine any statistical differences among the groups. Post-hoc pairwise multiple comparisons were conducted using Tukey's test or Dunnett T3 test according to the statistical conditions. Statistical significance was assumed at $p< 0.05$. All calculations were performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

Mean and standard deviation values of tissue weight before and after irrigation and percentage of weight reduction

were shown in Table 1. Comparison of percentage reduction of tissue weight and p values were indicated in Table 2. No pulp tissue dissolution was observed in the negative control group. Although the positive control group dissolved more tissue than the negative control group, the difference was not statistically significant (p= 0.45). The percentage reduction of pulp tissue was shown in Figure 2. The test showed statistically significant differences between the control and the experimental groups (p< 0.05). There was no significant difference between SI and LAI groups (p= 0.508), while there is a significant difference between SI and PUI groups (p< 0.01). A significant difference was also shown between LAI and PUI groups (p< 0.01). The organic tissue dissolution capacity of SI and LAI groups was indicated higher than the other groups.

DISCUSSION

In this study, the organic tissue dissolution capacity was examined comparing the 3 different (sonic, ultrasonic, and laser) types of IATs. All activation techniques were found successful in terms of tissue dissolution. When the tissue dissolving capacities were compared, the success order was SI, LAI, and PUI groups, respectively. SI and LAI groups were shown a higher tissue dissolution effect than the other groups. Therefore, the null hypothesis that there are no differences in pulp tissue dissolution capacity amongst different IATs can be rejected.

Bovine pulp tissue was used for ethical reasons and because of the unavailability of human teeth in this study. It is also difficult to obtain standardized human pulp tissue samples.[5] Bovine pulp tissue is regarded as comparable to human pulp tissue despite some minor differences from other animal tissues[21] and easier standardization of the surface area of its samples. It has been used in previous studies[4,5,21-23] to evaluate the dissolution capacities of endodontic irrigants.

PUI operates with acoustic streaming and cavitation effect.[11] Several studies[11,16,24] showed favorable results for PUI compared to SI. It was reported that PUI was more effective than syringe needle irrigation for removing pulpal tissue remnants and dentin debris, bacterial reduction, and removal of smear layer. However, there are some limitations of PUI. In a condition when the tip of the file is made a contact with root canal walls, the movement of the instrument can be restricted and the effect of activation can be reduced, especially in curved canals. Therefore, the root canal wall can be damaged as the file was made of metal alloy, although the file has a non-cutting tip.[25,26] Therefore, the use of PUI is only recommended as final irrigation.[26]

Compared with the other studies in the literature, better results were expected for the ultrasonic system. This result may be related to the limitations of our study. The experiment was performed in Eppendorf tubes *in vitro* and that excess moisture in the pulp was removed with gauze, which made it difficult to standardization. Conde et al.[27] found no significant differences in the comparison of different ultrasonic and sonic devices used in porcine palatal mucosa dissolution within artificial grooves in the root canals of the incisor. This discrepancy in the results may be explained by using different irrigation protocols. However, in a study by Galler et al.[28] comparing the penetration of irrigation solutions to dentin tubules, similar success rates as our study were obtained.

In studies comparing sonic devices with ultrasonic devices, the reasons that sonic devices are found to be less successful were generally explained with the lower power of sonic devices than ultrasonic devices. However, sonic devices have some advantages over ultrasonic ones: the oscillating points are made of a plastic-like material, it does not stop when in contact with the root canal wall, and it is not able to deform the root canal.[29] According to the results of this study, SI was found significantly more successful than PUI. This result can be explained by the fact that the ultrasonic tip could be in contact with the Eppendorf tube during the experiment so the effect of activation may be reduced.

It has been reported that LAI has a bactericidal effect,[30] improving the elimination of the dentin smear layer,[31] and the elimination of remnants from the apical part of the root canal system.[32] To our knowledge, there is no study in the literature evaluating the organic tissue dissolution of LAI. In this study, Er,Cr:YSGG pulsed laser (Waterlase iPlus; Biolase Technology, Irvine, CA, USA) was used at a wavelength of 2780 nm. Blanken et al.[14] showed that a turbulent flow and the subsequent formation of vapor bubbles in the liquid during the activation with Er,Cr:YSGG (2780 nm). The bubbles expand during the pulse and then produce pressure waves that displace first at supersonic speed (shock waves) and then at sonic speed (acoustic waves). This creates shear forces along the root canal.[32] LAI showed a higher capacity of dissolving organic tissue in this study which is similar to the study of Betancourt et al.[33] They demonstrated that Er,Cr:YSGG LAI proved to be more effective than PUI in enhancing the antimicrobial activity of 0.5% NaOCl against 10-day-old intracanal *E. faecalis* biofilms.

Table 1. Mean and standard deviation values of tissue weight before and after irrigation and percentage of weight reduction.

Tissue weight				
Groups	n	Before irrigation Mean+SD (10^{-4})	After irrigation Mean+SD (10^{-4})	Reduction of Tissue Weight (%)
SS	10	25.69 ± 2.16	22.76 ± 2.66	11.29 ± 7.99
SH	10	24.74 ± 1.59	19.48 ± 2.90	20.87 ± 13.19
SI	10	25.68 ± 1.70	0.85 ± 0.57	96.68 ± 2,31
PUI	10	27.04 ± 2.22	2.,26 ± 3.24	25.25 ± 8.37
LAI	10	26.08 ± 2.53	2.81 ± 3.31	88.5 ± 13.46

*SS group:negative control, no activation; SH group: positive control, sodium hypochloride (NaOCl) and no activation; SI group:NaOCl and sonic activation; PUI group: NaOCl and passive ultrasonic activation; LAI group: NaOCl and laser activated irrigation.

Table 2. Comparison of percentage reduction of tissue weight and p values.

Groups	n	Reduction of Tissue Weight (%)	SS	SH	SI	PUI	LAI
SS	15	11,29 ± 7,99	-	.450	≤0.001	.012	≤0.001
SH	15	20,87 ± 13,19	.450	-	≤0.001	.985	≤0.001
SI	15	96,68 ± 2,31	≤0.001	≤0.001	-	≤0.001	.508
PUI	15	25,25 ± 8,37	.012	.985	≤0.001	-	≤0.001
LAI	15	88,5 ± 13,46	≤0.001	.20	.508	≤0.001	-

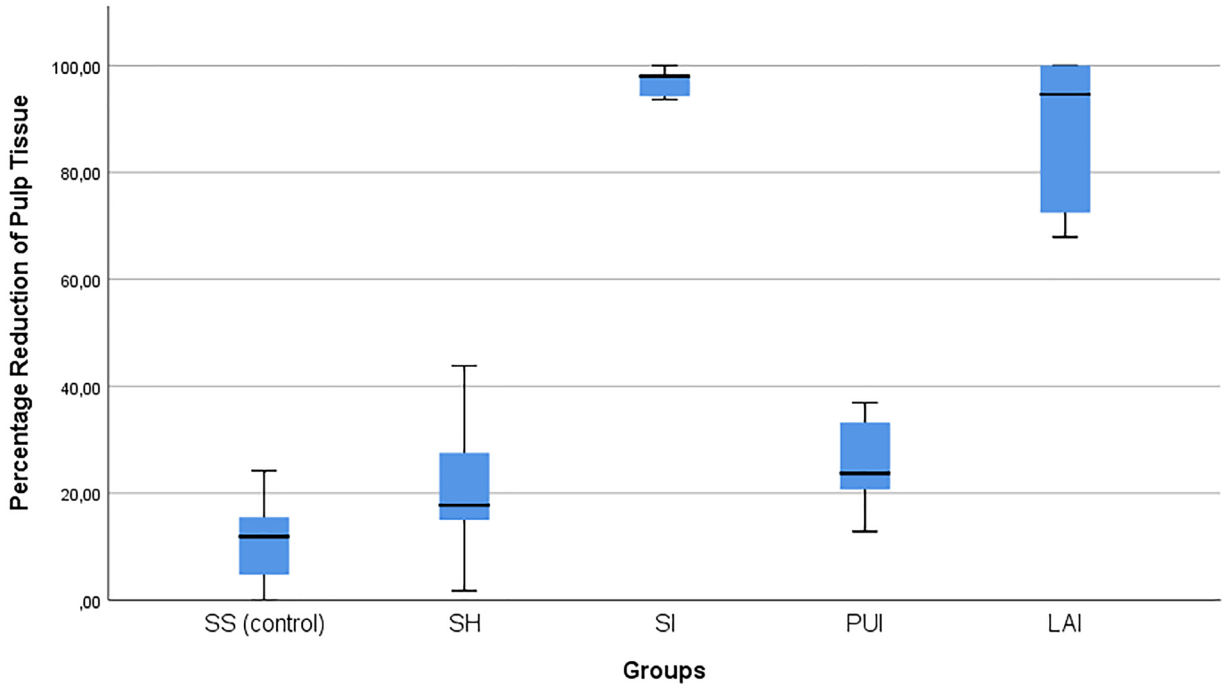


Figure 2. Simple Boxplot of percentage reduction of the pulp tissue.

CONCLUSION

All the activated irrigation techniques used in this study have been found to be more successful than conventional needle irrigation, in terms of improving the capacity of organic tissue dissolution during the endodontic treatment. Among these activation techniques, SI and LAI were the most effective techniques in resolving bovine pulp tissue.

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