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Pulp tissue dissolution capacities of different irrigation agitation techniques in artificial internal resorption cavities

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Purpose
The aim of this in vitro study was to compare the organic tissue dissolution capacities of 3 different irrigation agitation techniques (IATs) in artificial internal root resorption cavities (IRCs).

Materials and Methods
Ninety freshly extracted maxillary human incisors were selected. After decoronation procedure, the roots were split longitudinally, and a standard IRC were prepared in the canals on each half of the roots. Then, the bovine pulp samples (~2,3 mg) were previously weighed and placed into the cavities. The root fragments were reassembled and cemented to create a circular IRC within the canal. Teeth samples were randomly divided into 6 groups (n=15); sodium chlorur (NaCl) and sonic irrigation (SI), sodium hypochlorite (NaOCl) and SI, NaCl and passive ultrasonic irrigation (PUI), NaOCl and PUI, NaCl and laser activated irrigation (LAI), NaOCl and LAI. After that, the teeth were decemented and the tissue samples inside the cavities were weighed again. The percentage of weight loss was calculated and statistically analyzed.

Results
SI has significantly more successful results than PUI and LAI in groups which the irrigant was NaCl. There was also a significant difference between LAI and PUI in groups which the irrigant was NaOCl (Group 6 > Group 4, p=0.003). There was no significant difference between LAI and SI with NaOCl.

Conclusion
Complete dissolution of bovine pulp tissue from IRCs was not achieved by any tested techniques. However, the LAI with NaOCl was more effective than other IATs. In addition, there is no significant difference between the LAI and SI with NaOCl.

Keywords: *Dissolution, internal resorption cavity, tooth, pulp, activation*

Introduction

Internal root resorption (IRR) in permanent teeth is a pathological progressive destruction with hard tissue loss in dental hard tissue that occurs because of clastic activation (1,2). Resorption process begins when the underlying mineralized dentine is exposed to odontoclasts as the outer protective odontoblast layer and predentin of the root canal system are damaged (3). Dental trauma and the inflammation of pulp are the most reported reason for IRR with various other etiologic factors such as tooth decays, periodontal inflammation, excessive heat generation during dental treatment, calcium hydroxide procedures, anachoresis, orthodontic movement of teeth, cracked teeth and idiopathic reasons within normal pulp tissue (4,5).

The resorption will not be progressive without bacterial contamination and cannot be diagnosed with neither clinically nor radiographically. Furthermore, the vital pulp tissue located at apical part to resorption area

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provides blood supply and nutrients for clastic cells. On the other hand, necrotic part of pulp located at coronal portion of root canal system stimulates these clastic cells (4,6). After diagnosis of IRR, whether the teeth can be restorable, root canal treatment (RCT) is indicated to remove all the vital and necrotic pulp tissue, which cause a risk in aspect of stimulation of resorptive cells, and to disinfect and filling root canal system (7).

Although there are advanced endodontic instruments and treatment techniques, there remain areas in the root canal where bacteria and debris cannot be removed due to the complexity of the root canals (4,8). Several irrigation agitation techniques (IATs) such as sonics, ultrasonics, lasers, brushes, and manual dynamic agitation were suggested to access these restricted areas and to increase dissolution capacity of organic tissue (8-10).

Sonic and ultrasonic IATs have been extensively tested and are usually employed in RCT as irrigant agitation devices (11-13). 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 (11). Among these devices, the EndoActivator system (Dentsply Maillefer, Baillagues, Switzerland) is the most studied device with a frequency of 0.166 - 0.3 kHz (14). It is a cordless, battery-powered handpiece with a sonic motor. Passive ultrasonic irrigation (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 (15). An ultrasonic tip is activated in the canal up to the WL and is moved passively in and up and down motion to ensure it does not bind with the root canal walls. 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.

Laser activated irrigation (LAI) is another type of IAT, 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 (16,17). Among several laser devices, Er:YAG and Er,Cr:YSGG lasers are promising as a method for activating the irrigants. Aldeen *et al.* (17) 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 (18). This technique differs from other laser IATs in that only the tip is placed into the pulp chamber, thereby preventing contact with the root canal wall. In a study PIPS was more effective in the removal of apically placed dentinal debris (19). Akçay *et al.* (20) 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 (21) have warned about more apically extruded debris.

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. 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. Thus, IATs are suggested to increase the efficacy of irrigant delivery and improve root canal cleanliness (9-11).

The aim of this *in vitro* study was to compare the organic tissue dissolution capacities of 3 different IATs (sonic, ultrasonic, and laser-activated) in the presence of saline or NaOCl irrigants in artificial simulated IRCs. The null hypothesis tested was that there are no differences in pulp tissue dissolution capacity among the used 3 IATs in the presence of 2 irrigants.

Materials and Methods

Bovine 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. The teeth were extracted within 24 h after purchasing the bovine jaws 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. All pulp tissue were irrigated with distilled water to remove excess blood and blood clot. During the experiment, pulp tissues were only manipulated by using cotton pliers to avoid errors. All testing procedures were performed at room temperature.

Root canal preparation

A total of 90 single-rooted maxillary human incisors free of resorption, restoration, immature apex, cracks or fractures were selected. The crowns were removed with a sectioning saw (Isomet; Buehler, Düsseldorf, Germany) under water cooling to standardize root length as 15±1 mm. Apical patency was achieved by inserting a size 15 K-file (Dentsply Sirona) into the root canals until the tip was visible at the apical foramen. This length was reduced by 0.5 mm to determine the WL.

ProTaper Next rotary NiTi files (X1-X5) (Dentsply Sirona) were used for the root canal instrumentation according to the recommendation provided by the manufacturer, to the full WL. The canals were irrigated with 5 mL of 2.5% NaOCl using 30-G side vented needle attached to a plastic syringe (Canal Clean; Biodent, Paju, South Korea) placed 1 mm short of the WL. Final irrigation was done with 5 mL of 17% EDTA for 1 min and 10 mL of distilled water. Then, the canals were dried with paper points (ProTaper Next X5; Dentsply Sirona).

Preparation of samples

The grooves on the buccal and lingual root surfaces were created with the diamond disc and the teeth were split longitudinally in the buccolingual direction with the help of a hammer and chisel. Under magnification, the cavities with a diameter of 1.6 mm and a depth of 0.8 mm, at 6 mm beyond from the apex in both separated root fragments, were created with diamond round burs (FD.D. 801; Gmund/Tegernsee, Germany). The cavity surfaces were etched with 37% phosphoric acid (Dia-Etch; DiaDent, Seoul, Korea) for 30 s, then flushed with distilled water and dried with blotting paper. The diameters of the created cavities were checked with a digital caliper.

Bovine pulp tissue samples were prepared by using a scalpel. They were placed in a way to completely fill the cavities and then removed. Samples were weighed 3 times on a precision balance (XB 220A; Kunz Precisa, Zofingen, Switzerland) with an accuracy of 0.0001 gr, and the average of the 3 measurements was calculated. In this way, it was tried to prepare samples with standard weights (~ 2.3 mg each). Bovine pulps were placed again into the IRCs and the fragments of teeth were reassembled with the help of glue (Pattex Super Glue, Henkel, Duesseldorf, Germany).

The teeth were divided into 6 groups (n=15) randomly according to the types of agitation and placed in alginate mold before irrigation agitation protocol. The test groups were as follows: saline with SI (NaCl + SI) group, sodium hypochlorite with SI (NaOCl + SI) group, saline with PUI (NaCl + PUI) group, sodium hypochlorite with PUI (NaOCl + PUI) group, saline with LAI (NaCl + LAI) group, and sodium hypochlorite with LAI (NaOCl + LAI) group. *Group 1 (NaCl + SI):* The root canals were irrigated with 5 mL 0.9% NaCl (I.E. Ulagay Drug Industry, Istanbul, Turkey). The EndoActivator (Dentsply Maillefer) device was used for sonic agitation. It was performed using the EndoActivator handpiece set at 10.000 cycles per min with a medium polymer tip (#25/.04). The tip of the EndoActivator was placed at the IRCs and activation was made for 1 min. This process was repeated 2 times. *Group 2 (NaOCl + SI):* 5 mL 2.5% NaOCl was used as an irrigant. The same activation procedures that were previously described for Group 1 were applied. *Group 3 (NaCl+PUI):* The root canals were irrigated with 5 mL 0.9% NaCl. A noncutting #25 file (Irrisafe; Satelec Acteon, Merignac, France) driven by an ultrasonic device (Satalec P5 Newtron XS; Satelec Acteon) was used for ultrasonic activation in root canals for 3×20 sec at 50% power for 1 min. The tip was immersed in root canal containing irrigant throughout the IRC. This process was repeated 2 times. *Group 4 (NaOCl + PUI):* 5 mL 2.5% NaOCl was used as an irrigant. The same activation procedures that were previously described for Group 3 were applied. *Group 5 (NaCl + LAI):* 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. After the root canals were irrigated with 5 mL 0.9% NaCl, Er,Cr:YSGG laser system was used for activation for 1 min. This process was repeated 2 times. *Group 6 (NaOCl + LAI):* 5 mL 2.5% NaOCl was used as an irrigant. The same activation procedures that were previously described for Group 5 were applied.

In all groups, total agitation time was 2 min, and all teeth in each group were irrigated with 5 mL of NaCl as final irrigant to prevent the prolonged effect of irrigant. After that, the teeth were separated again and the pulp residues remaining in the IRCs were removed and dried with cotton pellets. The average weight of pulp tissue was calculated by measuring 3 times on a precision balance.

Statistical analysis

The difference in weights of the tissue sample, before and after exposure to the test irrigant, was divided by the original tissue weight and multiplied by 100 to obtain the percentage of tissue weight loss. The normality was assessed by Kolmogorov-Smirnov test and all the groups showed normal distribution. The data were therefore analyzed statistically using One-way analysis of variance and Tukey's HSD post hoc tests. Statistical significance was assumed at $p<0.05$. All calculations were performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA).

Results

Mean value of tissue weight before and after irrigation was shown in Table 1. Comparison of percentage reduction of tissue weight and p values were indicated in Table 2. SI has significantly more successful results than PUI and LAI in the groups in which the irrigant was NaCl (Group 1 > Group 3= Group 5). There was also a significant difference between LAI and PUI in the groups in which the irrigant was NaOCl (Group 6 > Group 4, p=0.003). On the other hand, the tissue weight changes of the NaOCl groups using SI and PUI techniques were not significantly different from each other.

Table 1: *Measurements of tissue weight before and after irrigation agitation protocols*

Groups	n	Tissue weight before irrigation Mean ± SD (*10-3*)	Tissue weight after irrigation Mean ± SD (*10-3*)
Group 1 (NaCl + SI)	*15*	**2.2±0.40**	0.8±0.52
Group 2 (NaOCl + SI)	*15*	2.1±0.23	0.7±0.51
Group 3 (NaCl + PUI)	*15*	2.2±0.33	2.0±1.83
Group 4 (NaOCl + PUI)	*15*	2.6±0.32	1.4±0.58
Group 5 (NaCl + LAI)	*15*	2.4±0.26	1.6±0.24
Group 6 (NaOCl + LAI)	*15*	2.7±0.84	0.7±0.66

*SD: Standard deviation

Table 2: *Pairwise comparisons, means and standard deviations of % reduction in tissue weight values*

	Groups	n	Mean ± SD (%)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Reduction in tissue weight	**Group 1 (NaCl + SI)**	*15*	61.5±23.54	-	0.998	**0.002**	0.247	0.006	0.483
	Group 2 (NaOCl + SI)	*15*	65.0±25.8	0.998	-	<0.0001	0.096	**0.001**	0.753
	Group 3 (NaCl + PUI)	*15*	30.8±16.89	**0.002**	**<0.0001**	-	0.469	1	**<0.0001**
	Group 4 (NaOCl + PUI)	*15*	44.6±21.21	0.247	0.096	0.469	-	0.66	**0.003**
	Group 5 (NaCl + LAI)	*15*	32.9±13.79	0.006	**0.001**	1	0.66	-	**<0.0001**
	Group 6 (NaOCl + LAI)	*15*	76.0±21.87	0.483	0.753	**<0.0001**	**0.003**	**<0.0001**	-

**SD: Standard deviation*

Discussion

The remaining organic tissue inside the root canal space and IRCs can cause the growth of surviving microorganisms and affect the success of RCT (22). NaOCl is the most common irrigant used in the RCTs with its tissue dissolving capacity and its high surface tension, which increase the penetration to dentin tubules and therefore its antibacterial efficacy (23). In this study, 2.5% NaOCl and 0.9% NaCl (as a control) irrigants were selected as an irrigants and maxillary central incisors with simulated IRCs were used, because IRR is most frequently seen in these teeth (24). Because of its similarity to human pulp, fresh bovine pulp was preferred to simulate the organic tissue remnants (25). De Gregorio *et al.* (26) stated that the apical preparation size and taper effect the volume and exchange of irrigant at the WL. According to these findings, the apical enlargement was completed with a suitable size and taper in this study.

Frequency of activation, amount of tissue in relation to amount of irrigant in the root canal system and surface area of tissue that was available were the dependent factors for the tissue dissolution (27). The effect of different IATs on dissolution capacity of pulp tissue in artificial IRCs were the aim of this study. The results demonstrated statistical differences among the different tested protocols. Therefore, the null hypothesis was rejected.

In the groups where NaOCl were used as irrigant, there was no significant difference between PUI and SI groups, although PUI has higher frequencies than SI. This could be explained with attenuation of activation forces because of the tip of PUI touching the root canal walls. Conde *et al.* (10) found similar results and they explained these results with plateau effect where decrease of tissue dissolution occurs when using PUI. In another study, activation with XP-Endo Finisher has higher capacity of removal of simulated organic tissue from artificial IRCs compared to ultrasonics in straight root canals. It was explained that the mechanical removal of the organic tissues by XP-Endo Finisher could have increased the tissue weight loss (28).

LAI with erbium lasers has been proven to be more effective than PUI in the removal of debris and apical smear layer but there is no study evaluating the removal of organic tissue before (29,30,31). In recent study LAI techniques have had more successful results than manual-dynamic irrigation in removing debris from simulated root canal irregularities (32). LAI has significantly more successful results than PUI in this study. These results were consistent with another study in which laser-activation was shown to be more effective for the removal of calcium hydroxide from mesial roots of mandibular molars than PUI (33). The generation of heat increase and the improved irrigant flow dynamics created by laser devices may cause enhancement of tissue dissolution capacity during LAI.

There are several limitations of this type of experiment model (34). The main limitation is that the pulp tissue was packed into the IRC area and therefore lacked any physical attachment to root canal dentin. Besides that, the presence of the same dentin in all the cases can cause a bias on buffering effect. However, this method allowed the use of the same dentin with the same anatomy of the IRC for all groups, reducing the risk of bias in anatomical differences and simulating well the results for this type of complexity. To conduct a study with multiple teeth, it would be necessary a great number of teeth with different anatomies, which would then undermine the standardized comparison of the IATs. Besides, in clinical conditions, some considerable dissolution of organic pulp tissue had already occurred during cleaning and instrumentation procedure. However, in this study, agitated irrigants were directly applied to organic tissue located in IRCs which were simulated to assess the dissolution capacity of the NaOCl agitated with the various techniques. This could be another limitation of this study.

Conclusion

Complete dissolution of bovine pulp tissue from IRCs was not achieved by any tested techniques. However, the LAI with NaOCl was more effective than other IATs. In addition, there is no significant difference between the LAI and SI with NaOCl.

Türkçe özet: *Farklı irrigasyon aktivasyon tekniklerinin simüle internal rezorpsiyon kavitelerinde pulpa dokusunu çözme kapasitelerinin karşılaştırılması. Amaç: Bu in vitro çalışmada, simüle internal rezorpsiyon kavitelerinde (İRK) 3 farklı irrigasyon aktivasyon tekniğinin (İAT'ler) organik doku çözme kapasitelerini karşılaştırmak amaçlanmıştır. Gereç ve Yöntem: Bu çalışma için 90 adet yeni çekilmiş insan maksiller kesici dişi seçildi. Dekoronasyon işleminden sonra kökler bukkolingual yönde uzunlamasına 2 parçaya bölündü. Her bir parçada kök kanalı üzerinde standart bir İRK hazırlandı. Sonra, sığır diş pulpası örnekleri (~ 2,3 mg) tartıldı ve rezorpsiyon kavitelerine yerleştirildi. Kök parçaları yeniden birleştirilerek yapıştırıldı ve dişler rastgele olarak 6 gruba ayrıldı (n=15); sodyum klorur (NaCl) ve sonik irrigasyon (SI), sodyum hipoklorit (NaOCl) ve SI, NaCl ve pasif ultrasonik irrigasyon (PUI), NaOCl ve PUI,NaCl ve lazerle aktifleştirilmiş irrigasyon (LAI), NaOCl ve LAI. Aktivasyon sonrası dişler ayrılarak kavitelerin içindeki doku örnekleri çıkarılıp tartıldı. İlk ve son ölçümler arasındaki fark hesaplandı ve analiz edildi. Bulgular: İrrigasyon solüsyonunun NaCl olduğu gruplarda SI, PUI*

ve LAI'dan anlamlı derecede daha başarılı sonuçlara sahipti. İrrigasyon solüsyonunun NaOCl olduğu gruplarda ise, LAI ve PUI arasında anlamlı fark vardı (Grup 6 > Grup 4, p=0.003). NaOCl ile LAI ve SI arasında anlamlı fark yoktu. Sonuç: Sığır pulpa dokusunun IRK'lerden tamamen çözünmesi, test edilen herhangi bir teknik ile sağlanamadı. Ancak, NaOCl ile LAI, simüle edilmiş İRK'de sığır pulpa dokusunun çözünme kapasitesinde diğer İAT'lerden daha etkiliydi. İlave olarak, NaOCl ile LAI ve SI arasında anlamlı bir fark yoktu. Anahtar kelimeler: Çözünme, internal rezorpsiyon kavitesi, diş, pulpa, aktivasyon.

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