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International Journal of Current Research
Vol. 9, Issue, 05, pp.50685-50688, May, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

COMPARATIVE EVALUATION OF CONTACT ANGLE OF CALCIUM HYDROXIDE AND TRIPLE ANTIBIOTIC PASTE USING CHITOSAN AS CARRIER

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ARTICLE INFO

Article History:
Received 15th February, 2017
Received in revised form
16th March, 2017
Accepted 20th April, 2017
Published online 23rd May, 2017

Key words:
Calcium hydroxide,
Triple antibiotic paste,
Chitosan,
Contact Angle.

ABSTRACT

Introduction: Calcium hydroxide and triple antibiotic paste are both popular medicaments in endodontic therapy. Recently chitosan has been suggested as a carrier for these medicaments. This study was conducted with the objective of evaluating and comparing the contact angle, and thereby the wetting ability, of these 2 medicaments when used with chitosan against the surface of radicular dentin.

Materials and Methods: 40 dentin blocks of thickness 2 mm were prepared and divided into 2 groups of 20 each. Controlled volume (0.1 ml) droplets of calcium hydroxide + 2% chitosan were placed onto the blocks in group I, and triple antibiotic paste + 2% chitosan in group II. The contact angle in each case was measured using Dataphysics OCA Easydrop software on a dynamic contact angle analyzer for 10 mins. Results were analyzed using unpaired t test.

Results: Group I showed a mean contact angle of 64.55° and group II showed a mean contact angle of 54.9°. The mean difference between the 2 groups was found to be 9.65° which had high statistical significance ($p<0.001$).

Conclusion: Triple antibiotic paste + 2% chitosan produced significantly better and faster wetting of radicular dentin as compared to calcium hydroxide + 2% chitosan.

Citation: Pratik Kotadia, Sania Singh and Srinidhi Surya Raghavendra. 2017. "Comparative evaluation of contact angle of calcium hydroxide and triple antibiotic paste using chitosan as carrier", *International Journal of Current Research*, 9, (05), 50685-50688.

INTRODUCTION

The primary objectives of root canal therapy include the eradication of bacteria from the root canal system and prevention of the regrowth of any residual microorganisms (Vaghela, 2011). These microorganisms may survive during endodontic procedures due to the various anatomical complexities seen in teeth, making them difficult to access by instrumentation and irrigants (Shaik, 2004). Their subsequent replication between appointments can often make them reach the same levels as at the start of treatment. Thus, the use of intracanalmedicaments between appointments can serve as an important aid in the sterilization of the root canal system (Mallya, 2012). Calcium hydroxide (CH) has been the medicament of choice for several years. It has various biological properties, such as antimicrobial activity, inhibition of tooth resorption, and hard tissue formation, which make it suitable for this purpose (Kim, 2014 and Mohammadi, 2012).

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Another medicament which has been found to be effective, especially in chronic long-term cases, is the triple antibiotic paste (TAP). Itcontains metronidazole, ciprofloxacin and minocycline and has been advocated for use in regenerative procedures for thorough disinfectionof the root canal (Adl, 2012). For optimal effect, these medicaments should eliminate bacterianot only from the main canal, but also from lateral canals, apical deltas and within dentinal tubules. This requires both substantivity of the intracanal medicament, and proper wetting of the radicular dentin (Mallya, 2012). Measurement of contact angle serves as a useful method to assess the wetting behavior of any liquid against a particular surface. This angle is formed at the interface where the liquid meets the solid (Tummala, 2012). While propylene glycol has classically been used as carrier for TAP (Vijayaraghavan, 2012), calcium hydroxide has been used with a variety of vehicles both aqueous (distilled water, anaesthetic solution, etc.) and non-aqueous (glycerin, propylene glycol, etc.) (Mallya, 2012). Chitosan is formed by the partial deacetylation of chitin, which is the second most abundant naturally occurring polysaccharide. It has been used extensively in the pharmaceutical industry due to its biocompatibility and the additional advantage of slow and controlled drug release.[2]For these reasons, it has been recently proposed as a carrier for intracanal medicaments in dentistry (Elsaka, 2012 and Grover, 2014). Studies have been done previously to estimate the wet ability of calcium hydroxide with various vehicles (Shaik, 2014 and Ozcelik, 2000), but no study so far has been carried out to evaluate the contact angle of calcium hydroxide or TAP when used with chitosan as carrier against the surface of radicular dentin. Thus, the purpose of this study was to determine and compare the contact angle of these medicaments using chitosan as a vehicle and thereby estimate their wetting ability. The null hypothesis was that there was no difference in the contact angle of the two groups.

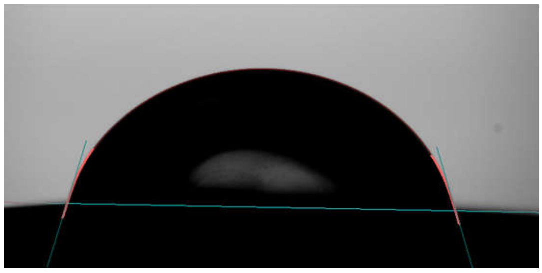
MATERIALS AND METHODS

Twenty non carious extracted human mandibular molars were selected and stored in normal saline. The teeth were decoronated at cementoenamel junction, and distal and mesial roots were separated. Distal root was longitudinally split to expose root canal surface. Two dentin blocks of 2-mm thickness were prepared from each distal root with the use of a diamond disk under running water. Polishing sandpaper of grit 180 was used to reduce the surface roughness of dentin blocks and to create a smooth surface. The blocks were placed in an ultrasonic bath containing distilled water for 5 minutes to remove any extra-organic components. After this, the dentin specimens were dried in a vacuum dessicator for 5 minutes. The samples were then carried directly from the dessicator and placed into the FTA200 dynamic contact angle analyzer (First Ten Angstroms, Inc. Portsmouth, Virginia). 2% chitosan was prepared by adding 0.2 mg of chitosan powder (Analab Fine Chemicals, Mumbai) to 10 ml of 2% acetic acid solution with continuous stirring. The solution was allowed to rest at room temperature overnight to achieve complete homogeneity. The TAP powder was prepared by grinding Ciprofloxacin 500mg, Metronidazole 400mg and Minocycline 100mg tablets in a mortar and pestle and then mixing them in a 1:1:1 ratio. Commercially available CH powder was used (Deepti Dental Products, Mumbai, India).

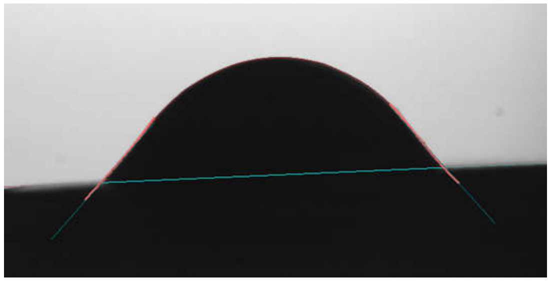
Forty dentin blocks were randomly divided into two groups of 20 each (n=20):

Group I: To the prepared base of 2% chitosan, calcium hydroxide (1% w/v) was added and mixed with magnetic stirrer.
Group II: To the prepared base of 2% chitosan, TAP powder was added manually and mixed on a glass slab with a mixing spatula.

For each group, 20 dentin specimens were positioned on a flat glass surface in the dynamic contact angle analyzer. Controlled volume droplets (0.1 mL) of each intracanal medicament were placed onto the dentin blocks. For group I, this was done by means of a micropipette. For group II, a 1ml insulin syringe was modified and used as a droplet carrier. This difference between the two groups was necessary on account of their handling characteristics and differences in viscosity which did not permit the medicament to be used with the micropipette. After positioning of the droplets on dynamic contact angle analyzer, a video of each specimen was recorded with continuous photography (contra lighted) for 10 minutes. Readings were recorded for both groups. The experiments were performed under controlled conditions of temperature and relative humidity. The temperature was kept constant to within 1°C with the aid of a thermostat. Images of the droplets of each intracanal medicament were digitalized and contact angle measurements were made by the Easydrop OCA Dataphysics software using the Young's equation which is based on the tangent of the angle between the droplet and the solid surface.



(a)



(b)

Image 1. Representative image of (a) Group I and (b) Group II as viewed on Dataphysics OCA Easydrop Software

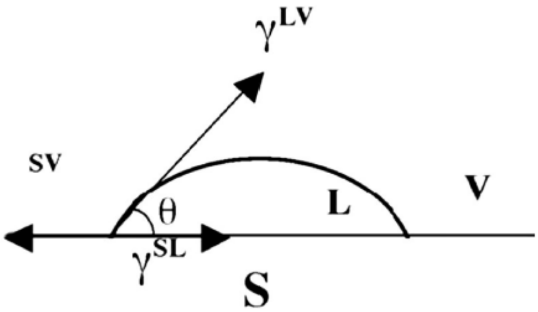


Image 2. Schematic diagram of wettability calculation using Young's equation

RESULTS

The contact angle was calculated according to the Young's equation which can be expressed as:

$\gamma LV\cos\theta = \gamma SV - \gamma SL$, where,

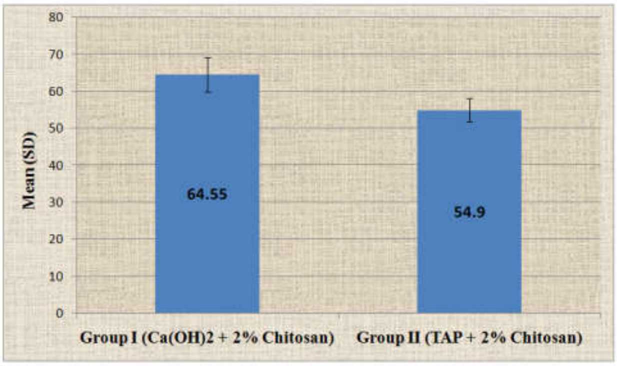
γSL represents the surface tension between the solid and the liquid, γSV and γLV are the surface tension of the solid and the surface tension of the liquid in equilibrium with the air, respectively, and θ is the contact angle (Lopes, 2015). The readings obtained at the end of 10 minutes for both groups were compared using the unpaired t test. The level of significance was set at $p<0.05$. From Tables 1 and 2, it can be seen that TAP + chitosan showed a lower contact angle which equates to better wetting of root dentin as compared to CH + chitosan. The mean difference between the 2 groups was calculated to be 9.650 and this difference was highly significant ($p<0.001$).

Table 1. Comparison of contact angle of group I (Ca(OH)2 + 2% Chitosan) and group II (TAP + 2% Chitosan) using unpaired t test

Group	No of samples	Maximum Value	Minimum Value	Mean (SD)
Group I ($Ca(OH)_2$ + 2% Chitosan)	20	70.06	60.20	64.55 (4.6)
Group II (TAP + 2% Chitosan)	20	60.41	50.10	54.90 (3.2)
Mean difference	-	-	-	9.650
P value	-	-	-	<0.001**

($p< 0.05$ - Significant*, $p < 0.001$ - Highly significant**)

Table 2. Comparison of mean of the two groups



DISCUSSION

The primary rationale behind the use of intracanal medicaments is their ability to kill the bacteria present in the recesses of the root canal system that cannot be readily accessed during cleaning and shaping (Kawashima, 2009). So as to achieve this objective, it is important for the medicament to flow adequately over the surface of radicular dentin. The ability of a liquid to flow or spread over any solid surface can be estimated by calculating its contact angle with that particular surface. The contact angle measurement serves as a useful indicator for the wettability of the liquid being tested. Lower contact angle values (<90°) indicate that the liquid is able to adequately wet the solid, whereas higher values (>90°) are indicative of poor wetting characteristics (Kontakiotis, 2007). This parameter is especially important in the cases of medicaments such as CH and TAP, which do not vaporize and thus require close contact with the dentinal surface to be optimally effective (Mallya, 2012). Changes in temperature and humidity affect the surface tension coefficient of liquids.[14]In this study, the entire procedure was carried out under controlled environmental conditions. The use of dynamic contact analyzer in this study was in accordance to the study conducted by Evangelos *et al*. (Newmann, 1974). It allowed us to record the contact angle not just at the time of placement of the drop, but also follow the changes (if any) in this angle over a period of 10 minutes. Both the medicaments tested in this study showed initial and final contact angle values <90°, implying that they were both capable of adequately wetting the canal walls. CH remains one of the most popular intracanal medicaments, and was thus used in this study with a new carrier, chitosan. Since chitosan contains copolymers of glucosamine and N□acetyl glucosamine (Majeti, 2006), it can be considered as one of the non-aqueous vehicles for CH. Our study incorporated chitosan into the CH powder by means of a magnetic stirrer. This methodology was similar to that used by Grover *et al* (Grover, 2014). The use of the dynamic contact angle analyzer revealed that all samples with CH + chitosan showed a continuous decrease in contact angle from 0 to 10 minutes. The readings obtained after 10 minutes were found to be comparable to those recorded by Mallya *et al*. for other non-aqueous vehicles used with CH, particularly propylene glycol (Mally, 2012). Simon *et al*. had suggested propylene glycol as the best carrier for use with CH (Simon, 1995). But it must be noted that chitosan, besides itself being a non-aqueous material, also offers the additional advantage of intrinsic antimicrobial activity as demonstrated by Ballal *et al*. (Ballal, 2009).

TAP, in contrast to CH, demonstrated a static contact angle, with no change seen between the initial and final readings. However the readings themselves were found to be lower than those recorded for CH, which indicated not only better but faster wetting of the dentinal walls. These lower values of TAP in comparison to CH could be attributed to its decreased particle size in comparison to CH, which enables it to take up more amount of the solvent and thereby show superior flow. While it is known that TAP is traditionally used with propylene glycol as carrier, no studies on its wetting abilities were available for comparison. This indicates the need for further research on the wetting characteristics of TAP with different carriers. In addition, contact angle is dependent on a number of factors such as surface roughness, homogeneity and presence of contamination. While these were standardized in our study, *in vivo* conditions might present considerable variations.

Conclusion

It can be concluded that TAP + 2% chitosan produces significantly better and faster wetting of the root canal walls as compared to CH + 2% chitosan. Furthermore, both groups showed satisfactory wetting characteristics and thus chitosan can be considered as an alternative carrier for these medicaments.

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