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RESEARCH ARTICLE

Application of Calcium Hydroxide with Vehicles Relate to the pH Change, Calcium Ion Diffusion, Roughness, and Frequency of Chemical Compound in Root Canal

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ABSTRACT:

Calcium hydroxide is often used as a root canal medicament. Together with vehicle aqueous, glycerine, polyethylene glycol, and chitosan can increase calcium ion diffusion, pH balance, and anti-bacterial power. To analyze the effect of different vehicles on alkaline pH changes, diffusion of calcium ions, roughness, and frequency of chemical elements in the root canal after applying calcium hydroxide. A total of 100 dental samples in 5 groups of vehicles were examined for pH using a pH meter, calcium ion diffusion using Atomic Absorption Spectrophotometric, and surface roughness of the root canals chemical compounds were analyzed by SEM-EDX. All the vehicles can maintain the balance of pH changes to alkaline in the root canal wall ($p < 0.05$: 0.00). Maximum calcium ion diffusion occurs on day 30, especially in the vehicle Chitosan and Polyethylene glycol ($p < 0.05$: 0.00). Vehicle chitosan and glycerine more strongly reduce root surface wall roughness after calcium hydroxide medicament (20% roughness score). Specifically, vehicle chitosan can increase calcium (21.71%) compared to control and other vehicles in the root canal wall ($p < 0.05$: 0.02). The chitosan vehicles and other vehicles can maintain an alkaline pH balance, diffusion of calcium ions, and decrease in roughness and increase in the quantity of calcium in the root canal wall.

KEYWORDS: Calcium Hydroxide, Ion calcium, pH, root canal, vehicle.

INTRODUCTION:

The success of root canal treatment depends on the principle of debridement, disinfection, and adequate obturation to preserve teeth and provide an environment conducive to the healing process of infection in the periradicular¹. The intracanal medicament is needed in pulp necrosis cases with periapical lesions and loss of periradicular bone to support the elimination of persistent bacteria in the root canal². The root canal medicament most commonly used in dentistry is calcium hydroxide (gold standard) as an endodontic antimicrobial agent to control exudate from the root canal and treat root resorption in external and internal inflammation³.

The use of $\text{Ca}(\text{OH})_2$ as a root canal medicament is expected to result in the dissociation of calcium (Ca^{2+}) and hydroxyl (OH^-) ions into the periradicular tissue, thereby affecting tissue healing and microbial development control⁴. As an anti-bacterial, the release of hydroxyl ions is known to increase the pH to alkalis (12.5), eliminating cell membranes and bacterial protein structures and altering the integrity of the cytoplasmic membrane, which will cause cell destruction⁵. Dissociation of calcium ions (Ca^{2+}) in high concentrations causes an increase in the pyrophosphatase enzyme's role through dentine mineralization to maintain the integrity of the root canal from environmental changes of bacterial infection⁶. The effectiveness of calcium hydroxide as a medicament is primarily determined by the vehicle and the duration of time to control microbes' development⁷. Adding calcium hydroxide to various vehicles boosts its biological

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activity and influences ion dissociation rate into Ca^{2+} and OH^- ions. Types of vehicles used include (1) vehicle aqueous (containing water), (2) vehicle viscous (viscous), and (3) vehicle oily (oil-based)⁸.

Prolonged Calcium hydroxide application can cause weakness in the root canal, leading to an increased risk of root fracture, internal resorption, leaving a residue known as ZOE (Zinc Oxide Eugenol), and resin-based sealer⁹. Therefore, natural materials are currently being developed as alternative materials that are more biocompatible, save costs, are environmentally friendly, and heal faster. One of the new and promising biomaterials applied in dentistry is chitosan. Chitosan and its derivatives have excellent biocompatibility, non-toxic to humans, biodegradability, high bioactivity, selective permeability, polyelectrolytic action, antimicrobial activity, ability to form gels, films, and sponges, absorption, anti-inflammatory, and wound healing¹⁰. Chitosan has high reactivity, which free amino groups influence. This group causes chitosan to act as a substituted amino¹¹.

A minimum interval of 14 days has been suggested to evaluate calcium hydroxide's effectiveness as a root canal medicament due to the association with an inflammatory time ranging from 10-14 days when microorganisms disinfect the root canals¹². The changes in pH and the diffusion of calcium ion in root canals after placement of calcium hydroxide with chitosan as a vehicle compared to vehicle viscous such as glycerine and polyethylene glycol in different periods have not been reported. The stability of the environment (alkaline) pH and calcium ions diffusion are registered as a counterweight to root canal treatment. The purpose of this study was to analyze the effect of calcium hydroxide as an intracanal medicament with different vehicles on changes in pH and the diffusion of calcium ions, as well as changes in dentine conditions of the root canal surface by analyzing the roughness and frequency of chemical elements.

MATERIAL AND METHODS:

This research has passed ethics No 235/TGL/KAEPK FK USU-HAM/2019 RSUP from the Faculty of Medicine, University of North Sumatra, Medan-Indonesia. One hundred samples of coronectomy teeth, cleaning and shaping root canals filled with calcium hydroxide a, divided into four vehicle groups; vehicle aqueous (viopaste), glycerine, polyethylene glycol,

Decoronectomy, cleaning, and shaping:

Treatment techniques refer to Johnson's research (2009)¹³. A total of 100 mandibular premolar teeth that have been extracted for orthodontic purposes are manually cleaned using an ultrasonic scaler. All teeth'

crowns are cut (coronectomy) on the cemento-enamel junction using a diamond disc bur under the air-water spray. The root canals were prepared using a crown down technique using the rotary I Race instrument from file size: R1 (# 15/06); R2 (# 25/04), and R3 (#30/04), and the working length is 1 mm from the apical foramen. For each file change, a total of 2 ml of 5.25% sodium hypochlorite solution was used as a root canal irrigation material using a one-side vented 30-gauge syringe. The needle is placed 1 to 2 mm from the working area's length in each root canal. Final irrigation was carried out with 2 mL of 17% EDTA solution (Merk, Darmstadt, Germany) for 1 min, and the root canals were rinsed with 5 mL of saline water to remove EDTA deposits.

Vehicle Preparation and Placement of intracanal medicaments:

The treatment of vehicle preparation and placement of intra-canal medicaments follows the principle of Nalawade (2016)¹⁴. The first step is modeling the acid condition in the root canal as an inflation simulation using HCL pH 4 for 30 min. Furthermore, 4 grams of calcium hydroxide were dissolved with vehicle glycerine, polyethylene glycol 400, and chitosan, each as much as 6 ml with a magnetic stirrer at a speed of 800 rpm to form a paste. Before dissolving with calcium hydroxide, specific treatment with vehicle chitosan, 1 gram Chitosan powder was dissolved in 500 mL of 1% acetic acid, stirred until homogeneous with a magnetic stirrer 2 hours. Commercial calcium hydroxide (Viopaste) 2.0-gram plant preparations in syringes are equipped with a disposable tip, endo-stop, tip cap, and a measured pH. Teeth, randomly divided into five groups of 20 teeth each for placement of intra-canal medicament material dissolved with vehicles aqueous, glycerine, Polyethylene glycol, chitosan, control group. Assessment of pH changes, diffusion of calcium ions, the roughness of the surface on root canal dentin, frequency of chemical elements measured after 48 hours, 7, 14, 21, and 30 days.

Measurement of pH solution:

pH measurements on the sample immersion solution were carried out with a pH meter (Mettler Toledo, Columbus, Ohio, USA) after being deposited in 48 h, seven days, 14 days, 25 days, and 30 days. Three repetitions are performed for optimal results. Every pH check was calibrated to normal pH using HCl and NaOH

Diffusion of Ion Calcium Assay:

The evaluation of ion calcium diffusion is used to Spectrophotometric atomic absorption. After finishing the medicament placement, each tooth sample was coated with nail polish, except the third apical area to cover the lateral canal in the root's coronal and middle third. All samples were stored/soaked separately in a

sealed plastic tube containing 20 mL of bidistilled water and stored in an incubator in 100% humidity and 37°C. In this media, pH and diffusion of calcium ions were recorded at different analysis times, namely 48 hours, 7.14, 21, and 30 days. Then, it analyzed the solution in a tube for the presence and quantification of calcium ion extrusion using Atomic Absorption Spectrophotometric (AAS) according to the manufacturer's instructions for each sample. Next, the sample solution is poured into a beaker glass, and 20 ml of concentrated nitric acid is added. Next, the solution is heated using an electric heater until the solution is almost dry (± 10 ml). The distillation of aqua is added to the 15 ml limit, and the solution is stored in a measuring flask. Before measuring the calcium ion, 5 mL of standard calcium solution of 5 mL was added, and distilled water was added to the limit mark. Next, the calibration curve for standard solution Ca ($y = 0.05259x + 0.00490$; $R^2 = 0.99927$) was determined with calibration concentrations of 2000, 4000, 6000, 8000, and 10,000 (ppm). Then, calcium measurement begins with AAS at a wavelength (λ) 422.70 nm. Results are recorded separately according to measurement day after 48 hours, 7th day, 14th day, 21st day, and 30th day.

Measurement of Roughness and Chemical Elements:

SEM-EDX (Scanning Electron Microscopy-Energy Dispersive X-Ray Spectroscopy) was used to evaluate of roughness and composition of chemical elements of the root canal. The treatment begins by cutting the tooth's crown longitudinally from the cemento-enamel junction to the apex end. The sample is cleaned to remove impurities and obtain a good surface structure of the sample, then polishing. After that, each sample was examined using SEM-EDX (Hitachi TM 3000, Japan). The sample is put into a vacuum in SEM and magnifies 1200x, 1500x, and 2000x. In the 1500x magnification, the image will be divided into nine observation areas, assessed using the double-blind assessment method (two times) carried out by two different people. Measuring the level of cleanliness of the root canals of the smear layer in the observation of nine areas was determined using a smear layer score on the root canal wall according to Torabinejad (2003) as reported by Virdee (2018)¹⁵. Score 1 (no smear layer), score 2 (moderate smear layer), score 3 (heavy smear layer). EDX analysis of the composition of the root canal wall elements remaining after placement of the calcium hydroxide medicament with different vehicles was carried out to compare each ingredient's effect on the element to the control group.

Statistical Analysis:

Calcium ion diffusion data and pH changes were analyzed by One Way ANOVA, then the chemical composition data from the root canal wall were analyzed by Independent Test t-test. Both of these tests used significance limits $p < 0.05$.

RESULTS AND DISCUSSION:

Figure 1 shows the profile of the smear layer on the root canal wall induced by different vehicles. Each vehicle influences changes in roughness at different levels. Vehicle *Chitosan* shows a better reduction in roughness after placing calcium hydroxide medicaments. Quantitatively, data on changes in roughness were assessed by two comparator observers to obtain roughness scores.

Figure 2 shows that the highest percentage of aqueous roughness (viopaste) score is 39% compared to other vehicles. The lowest percentage scores are vehicle glycerine and chitosan (which score 20% each) and polyethylene glycol, which shows 21%. It means that these three vehicles can reduce or maintain the surface roughness of the root canal wall. In other words, these materials are competent in increasing adhesives in endodontic treatment.

Table 1 shows a change in the pH of the immersion solution of dental samples given various vehicles. In general, all vehicles significantly affect pH by changing the pH to alkaline and balanced. This change in pH is expected in the endodontic treatment process to avoid pathogenic bacteria in the teeth' root canals. Based on these results, all vehicles can act as a control against environmental changes. One of these actions is maintaining the pH balance to a lighter alkaline pH. One Way ANOVA analysis shows significant differences ($p < 0.05; 0.00$). Table 2 explains the diffusion of calcium ions which reaches their maximum values on the 30th day.

Table 2 explains the diffusion of calcium ions which reaches their maximum values on the 30th day. The Polyethylene glycol group/vehicle had a strong effect, which vehicle chitosan then followed compared to the viopaste and glycerine group ($p < 0.05; 0.00$). Whereas the control group (no vehicle) showed the diffusion value of calcium ions decreased gradually at each time interval of measurement, the value of calcium ion diffusion decreased gradually at each measurement time interval.

Table 3 shows some chemical elements found on the surface of the root canal wall. Vehicle *Chitosan* has a significant role in increasing calcium (21.71%) compared to control and other vehicles. Based on the Independent t-test, it is significantly different ($p = 0.02 < 0.05$). Vehicle Viopaste, glycerine, and polyethylene glycol decreased compared to controls (without calcium hydroxide and vehicle medicaments). All three showed no significant difference to the control group ($p > 0.05$).

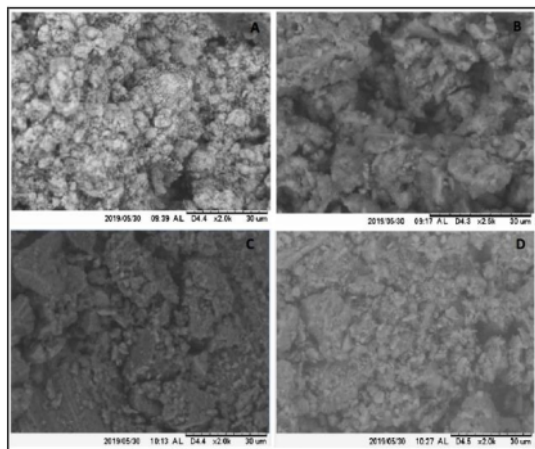


Fig.1: SEM images of root canal wall smear layers using various vehicles. (A) Aqueous (Viopaste), (B) Glycerin, (C) Polyethylene glycol, and (D) Chitosan. Chitosan has a roughness that is relatively the same as *glycerine*. Magnification-2000 x

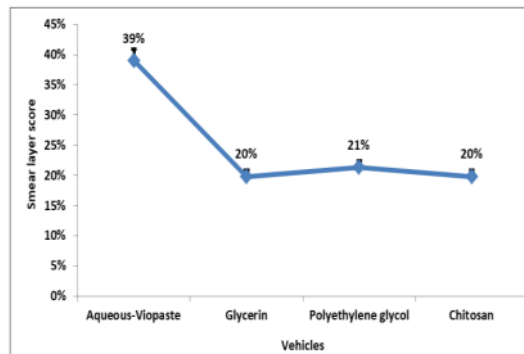


Fig.2: Surface roughness score of root canal wall surfaces induced by different vehicles after calcium ion diffusion. Chitosan and glycerine show a more effective vehicle to maintain calcium on the root canal wall surface with the lowest roughness score.

Bar (roughness score); error bar (error bars with percentage)

Table 1. Changes in the pH of the immersion solution of dental samples with different root canal vehicle medicaments

Vehicles	pH Basic (0 h)	48 h		7 d		14 d		21 d		30 d	
		Mean	SDV	Mean	SDV	Mean	SDV	Mean	SDV	Mean	SDV
Aqueous (Viopaste)	12.3	7.49	0.16	9.07	0.01	7.67	0.07	7.76	0.04	7.80	0.05
Glycerin	6.2	7.70	0.02	8.57	0.02	7.64	0.08	7.77	0.10	8.45	0.08
Polyethylene glycol	5.6	8.00	0.08	8.65	0.02	8.24	0.14	7.92	0.03	9.05	0.02
Chitosan	2.5	7.98	0.03	9.00	0.10	8.39	0.02	8.31	0.09	8.64	0.01
Control	7.0	6.71	0.00	6.69	0.00	6.67	0.00	6.65	0.00	6.62	0.00
ANOVA (F)		159.21		1567.59		301.17		329.27		10124.54	
p		0.00		0.00		0.00		0.00		0.00	
		0.000									

Table 2. The value of calcium ion diffusion (ppm) from a third of dental apicals with different vehicles

Vehicles	Diffusion of calcium ion (ppm)									
	48 h		7 d		14 d		21 d		30 d	
	Mean	SDV	Mean	SDV	Mean	SDV	Mean	SDV	Mean	SDV
Aqueous (Viopaste)	0.42	0.005	0.69	0.009	0.92	0.015	0.97	0.01	1.15	0.005
Glycerine	0.45	0.005	0.86	0.017	0.62	0.009	2.14	0.10	3.11	0.009
Polyethylene glycol	0.35	0.020	2.17	0.012	1.30	0.018	3.59	0.14	6.09	0.053
Chitosan	0.74	0.009	1.24	0.020	1.72	0.040	2.76	0.02	5.49	0.118
Control	0.16	0.005	0.15	0.005	0.14	0.008	0.12	0.00	0.10	0.005
ANOVA (F)	1432.33		11113.85		3182.89		1109.01		7990.70	
p	0.00		0.00		0.00		0.00		0.00	
	0.000									

Table 3: Percentage of chemical elements on the root canal wall surface after placement of calcium hydroxide medicaments with different vehicles

Vehicles	N	Chemical elements (Weight %)									
		Carbon		Oxygen		Fluor		Phosphor		Calcium	
		Mean	SDV	Mean	SDV	Mean	SDV	Mean	SDV	Mean	SDV
Aqueous (viopaste)	4	74.83	2.79	9.40	0.61	0.54	1.08	4.10	0.39	13.92	2.67
Glycerin	4	45.49	3.18	32.52	8.41	1.62	1.87	6.56	2.07	14.81	4.71
Polyethylene glycol	4	49.11	9.39	27.61	9.04	1.43	1.02	6.54	0.15	16.95	1.09
Chitosan	4	43.08	9.98	31.37	11.9	0.00	0.00	5.13	1.91	21.71	1.17
Control	4	44.77	0.20	31.38	1.60	1.85	0.08	7.96	1.33	16.65	0.50

This study investigates calcium hydroxide's role by employing various solvents (vehicles) to assess the impact of root canal changes based on pH, calcium ion diffusion, root canal wall roughness, and the frequency of chemical elements, primarily calcium. In this study, besides using vehicle aqueous, glycerine, polyethylene glycol, *chitosan* is also used as a substitute for other synthetic vehicles as root canal medicament, particularly maintaining a balance of alkaline pH, increasing diffusion of calcium ions, and decreasing surface roughness of root canal walls. Table 1 shows that calcium hydroxide administration using different vehicles in the root canal changes the pH at each time interval. In other words, time affects the change in the pH environment of the root canal wall. This change is due to the interaction between calcium hydroxide and vehicle, thereby changing the environment to a balanced alkaline pH.

Carlatto (2016) reports that alkaline pH changes are highly desirable in the endodontic treatment process to avoid calcium degradation and increased infection in the root canal¹⁶. Then, Dalmi (2017) reinforced that alkaline pH can inhibit the metabolism, growth, and division of bacterial cells¹⁷. The release of hydroxyl ions from calcium hydroxide causes an increase in pH to become alkaline, destroying ionic bonds to cause damage (denaturation) of virulent bacterial proteins¹⁸. Also, hydroxyl ions can interfere with DNA replication and bacterial protein synthesis¹⁹.

The positive impact on the pH balance of the root canal, according to Jhajharia (2015), besides eliminating bacteria, also suppresses the growth in the dentine tubules, isthmus, and root canal ramification²⁰. Based on the research findings in Table 1, it can be assumed that all vehicles, including *chitosan*, can control the pH balance of the root canal environment in the endodontic treatment process. Mainly, *chitosan* shows the proper biocompatibility and biodegradability while at the same time having the function of controlling the development of pathogenic bacteria in the root canals of teeth²¹.

Calcium hydroxide with an aqueous vehicle will rapidly release Ca^{2+} and OH^- ions. This nature of the rapid release is desirable in situations of short-term disinfection²². Grover (2014) reported that the distilled water group showed a rapid increase in pH from 6.5 to 11.8 at 24 hours, followed by a gradual decline over 15 days to pH 7.8 in 30 days²³. It is in line with the study results, as shown in Table 1. Changes in pH occur due to dissociation and diffusion of hydroxyl ions in the dentine, affected by vehicle hydro solubility levels, differences in viscosity, acid-base characteristics, and dentin permeability²⁴. In this study, after placing calcium hydroxide medicaments with vehicle *chitosan*, the pH

value increased starting at 48 hours. The pH value in vehicle *chitosan* shows a controlled value. Although the pH decrease occurred on the 21st day, it remained higher than the other group's pH, which reduced the pH value. The binding of amino and hydroxyl groups causes *chitosan* to have a high chemical reactivity and contributes to the polyelectrolyte cation properties that act as amino replacement²⁵.

Table 2 shows that the diffusion value of calcium ions in a third of dental apical with different vehicles reached the maximum value on the 30th day. The strong effect was shown by Vehicle polyethylene glycol, then followed by vehicle *chitosan* compared to other vehicles. Calcium ions play a role in periapical healing and repair. Calcium ions act by cleaning carbon dioxide used by bacteria for anaerobic respiration in cell migration, differentiation, and mineralization²⁶.

Pranab research results (2017), the calcium hydroxide group with vehicle viscous (glycerine) showed the highest calcium ion release occurring among all groups. The calcium hydroxide group with vehicle aquadest showed a high initial calcium ion release based on time intervals^{27,28}. It is in line with this research which shows that the highest quantity of calcium ion diffusion occurred on the 30th-day measurement in the polyethylene glycol group with a value of 6.09ppm, followed by *chitosan* with a value of 5.49ppm, glycerin with a value of 3.11 ppm and aqueous with a value of 1.16 ppm. Chemically, the number of ethylene oxide groups along the chain/group of polyethylene glycol allows polyethylene glycol to form complex bonds with metal cations, including calcium ions from calcium hydroxide²⁹. As a vehicle was viscous, polyethylene glycol has a greater viscosity. Thus, the diffusion speed is longer when compared to vehicle aqueous³⁰. Vehicle viscous is a water-soluble substance with high molecular weight and low diffusion coefficient with a slower working effect³¹. Calcium hydroxide and viscous vehicle release the Ca^{2+} and OH^- ions more slowly for long periods, creating an alkaline condition that is not conducive to bacterial survival⁷.

The pattern of calcium ion release with biphasic vehicle *chitosan* is characterized by a high initial release followed by a slow release over a long period²³. The release of calcium ions showed a continuous increase until it reached its optimal value on the 30th day, 5.49 ppm. *Chitosan* has an ion exchange effect and chelating properties to form complex *chitosan* with metal ions³².

Fig 1 shows the smear layer profile on the root canal wall induced by different vehicles with different quantities (Fig 2). Based on the two pictures, it is clear that each vehicle has a different effect on roughness

changes. Vehicle chitosan and glycerine have a better effect on decreasing roughness after placing calcium hydroxide medicaments. Arslan (2012) reports that calcium hydroxide can close the dentinal tubules and reduce dentin permeability. Thus a leak test using a dye shows the penetration of paint through the root canal wall³³. Based on this study's results, it can be explained that calcium hydroxide reacts well with chitosan and glycerine in preventing leakage of the root canal wall.

Da Silva (2011) reports that teeth that have been given calcium hydroxide medicament show significantly more leakage of the apex area, when not thoroughly cleaned from the root canal, the remaining calcium hydroxide can affect the filling of the root canal will increase the formation of the smear layer³⁴, as shown in Fig 2, where vehicle aqueous (viopaste) which is calcium hydroxide causes higher roughness compared to other vehicles, thus affecting the frequency of the chemical composition of the root canal wall. In this study, the calcium hydroxide group with vehicle aqueous (viopaste) showed the formation of a thick smear layer that covered the root surfaces and dentinal tubules. Vehicle aqueous provides the most significant release effect of dissolved oxygen compared to vehicle glycerin, polyethylene glycol, and chitosan due to the low viscosity of viopaste, which affects ionic dissociation and the low solubility of calcium hydroxide in water³⁵.

Table 3 shows many chemical elements found on the surface of the root canal wall. Chitosan has a very significant role in increasing calcium (21.71%). Besides acting as an anti-bacterial agent, chitosan is also known to have the potential as a remineralization agent. The inhibitory power of chitosan with glycerine solvent is more effective against *Fusobacterium nucleatum*. Meanwhile, vehicle aqueous (viopaste), glycerine, and polyethylene glycol decreased calcium. The results shown in Table 2 align with this fact, where chitosan strongly influences diffusion of calcium ions. The chitosan's role as a substituted amino controls the release of calcium ions from the root canal into the sample immersion solution³⁶. These calcium ions released gradually cause differences in the quantity of remaining calcium ions, which are more numerous in the root canal wall, as reported in Table 2 and Table 3.

CONCLUSION:

Chitosan vehicles are more suitable than vehicle aqueous-vehicle, glycerine, and polyethylene glycol to maintain alkaline pH balance and control the increase in the diffusion of calcium ions and potentially reduce roughness increasing the quantity of calcium in the root canal wall.

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CONFLICT OF INTEREST:

We declare no conflict of interest

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