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Delivery of Vitamins through the Intraoral Cavity with Toothpaste Using Penetration Enhancers

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Dental health is rarely deemed a significant factor for a healthy life despite its considerable impact on the human body. Many presume that regular dental check-ups and daily toothbrushing are sufficient to prevent oral health diseases. However, consuming certain nutrients is also required to attain a healthy oral cavity. Nutrients necessary for preserving dental health, namely calcium, phosphorus, fluorine, and various vitamins, contribute to the development, maintenance, and repair of oral tissues. Nevertheless, it can be challenging to be conscious of taking such nutrients on a daily basis. In this study, aiming to abolish such inconveniences, vitamins essential for dental health were combined in a toothpaste formulation with penetration enhancers (PEs) to achieve intraoral delivery of the vitamins while toothbrushing. Multiple tests were performed to evaluate the characteristics of the intraoral vitamin-delivery toothpaste (IOVT), such as abrasiveness and antibacterial strength tests. The same tests were also conducted on three different commercial toothpaste to draw comparisons with the IOVT. Additionally, to verify the IOVT's penetration capability, a mass transport study, and a transepithelial electric resistance (TEER) value test were conducted using nightcrawlers' skin. The data collected demonstrated congruency between multiple

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characteristics of the IOVT and those of the commercial toothpaste. Our key finding was that the greater TEER values demonstrated the high plausibility of effective intraoral delivery of vitamins, assisted by PEs, as components of the IOVT.

Keywords: Dental health nutrients; epithelial transport; intraoral delivery; oral health vitamins; penetration enhancers.

1. INTRODUCTION

The oral cavity refers to the intraoral space covering an area from the lips to the throat and laterally enclosed by the cheeks; it comprises various soft and hard tissues, including the oral mucosa, teeth, periodontal tissues, and the tongue [1]. Virtually everyone uses the oral cavity every day, as it assists crucial physiologic mechanisms of speaking, eating, digesting, and breathing [2]. Due to its eminently frequent utilization, the oral cavity is perpetually vulnerable to divergent infections. Given that some oral diseases could potentially develop severe systemic diseases, notably diabetes mellitus, cardiovascular disease, and bacterial pneumonia, it is indeed necessary to maintain a healthy oral cavity [3]. Many presume that regular dental check-ups and daily toothbrushing are sufficient to prevent oral health diseases. However, a healthy oral cavity requires the intake of certain nutrients, namely fluoride, potassium, calcium, phosphorus, and vitamins A, B, C, D, E, and K [4].

Nonetheless, many find it challenging to consume nutritional supplements routinely. This study therefore aims to convert the consumption of essential nutrients into a daily routine; integrating the nutrition intake into toothbrushing. Vitamins A, B, C, D, E, and K, among the essential nutrients for dental health, were selected to be intraorally delivered while toothbrushing. The oral cavity benefits differently from each of these vitamins. The objective of the study was to formulate a toothpaste that could intraorally deliver the vitamins required for maintaining tooth strength. The hypothesis was established that the TEER values from the IOVT formulation could be greater, compared to those from other commercial brand toothpaste. The TEER values were considered an indirect indicator of material transport often used in the pharmaceutical industry for verifying their proof of concept.

Vitamin A contributes to the development of oral tissues. Vitamin A promotes bone growth and maintains the oral mucosal tissue by keeping mucous cells from being keratinized [5]. The vitamin also maintains the conditions of salivary glands and teeth [6]. Lack of vitamin A consumption increases the risk of tooth decay, as the vitamin is a prerequisite for the synthesis of glycoproteins [7]. Moreover, vitamin A deficiency reduces the growth of epithelial layers and causes an undergrowth of teeth [8].

Vitamin B has noticeable impacts on oral structures. The abundance of vitamin B in the body discourages dental defects such as cleft lip, alveolar cleft, and cleft palate, which causes splitting of the lip, and upper gum line, and deformities in the palate and neural tube [7]. Insufficient consumption of vitamin B provokes burning sensations in the soft tissues of the oral cavity, due to oral manifestations such as glossitis, glossodynia, recurrent ulcers, cheilitis, dysgeusia, and lingual paresthesia [9]. Vitamin B deficiency further stimulates inflammation and detachment of periodontal fibers [8].

Vitamin C provides numerous benefits for the oral cavity. The vitamin enhances the synthesis of collagen, a protein that provides tooth structure, support, and maintenance [10]. Further, the antioxidant properties of vitamin C support the maintenance of oral tissues and activate the reparative mechanism [7]. A deficiency in vitamin C could interfere with effective wound healing, provoke irregular dentin formation, and cause scurvy, an inflammation of the gingiva [7,8].

Vitamin D contributes to the fortification of hard tissues of the oral cavity, such as dentin, and enamel [11]. Vitamin D enhances the intestine's absorption of calcium, magnesium, and phosphorous, the necessary nutrients for growth and maintenance of teeth [6]. The vitamin also reduces plaque scores when consumed with high calcium and dairy servings [6]. Insufficiency of vitamin D heightens the risk of enamel and dentin hypoplasia, the incomplete development of the hard tissues; vitamin D deficiency also could lead to delayed eruption of teeth [6].

Vitamin E plays a role in inhibiting oral diseases. The vitamin prevents preceding stages of oral cancer and inflammation with its antioxidant properties [12]. Vitamin E is also found to improve periodontal diseases by balancing the redox status and enhancing the healing of wounds [13]. Vitamin E is often employed for the prevention and treatment of oral mucositis, a common condition in patients treated for oral cancer [14].

Vitamin K assists preservation of bone health in the oral cavity. Vitamin K exerts its anabolic effect in promoting osteoblast differentiation and upregulating transcription, which critically supports the mineralization of the oral cavity's hard tissues [15]. Vitamin K also hinders inflammation in the oral cavity, as it regulates immune cells that cause inflammation, and lowers the concentration of inflammatory markers [16].

Among recently developed drug delivery methods, the most prominent includes the use of penetration enhancers (PEs): a mechanism of decreasing the resistance of epithelial tissues to penetrate drug molecules through the skin, directly into the bloodstream [17]. Researchers have been examining numerous sites of drug transportation employing different PEs, in search of a desirable combination to augment drug delivery through epithelial layers. Between the PE and the delivery site, the latter has more significance, as there still exists uncertainty in drug delivery across thick skin [18]. In this regard, the oral mucosa, which is the mucous membrane within the oral cavity limits, makes an ideal site for drug delivery by penetration [19]. Drug molecules could relatively permeate readily through the oral mucosa, as it comprises two layers of epithelial tissues, while the skin generally consists of four to five epithelial layers [19,20]. Withal, due to the rich blood supply that the oral mucosal surfaces receive, drug molecules would enjoy rapid penetration, and avoid degradation from the first-pass hepatic metabolism [21]. As numerous studies have proven the feasibility of micronutrient delivery through the dermal epithelium, intraoral delivery should correspondingly be attainable due to the preceding reasons.

Due to the diversity of PEs, numerous methods exist to permeate drug molecules through the skin. The IOVT accordingly incorporated three PEs, each with distinct mechanisms of penetration. Polypropylene glycol increases the solubility of drug molecules in the stratum corneum, the outermost layer of the skin, by temporarily adjusting the solubility parameter of the skin; polysorbate 80 induces both hydrophobic and hydrophilic characteristics to a drug molecule with its ethylene oxide and a long hydrocarbon chain; sodium dodecyl sulfate increases the fluidity of epidermal lipids, therefore enhancing drug penetration [22,23]. Additionally, research has found that vitamin E enhances the permeability of drugs by the alteration of membrane characteristics, due to the disordering of lipids in the gel phase [24]. Using an emulsion base, the six vitamins and three PEs were combined with general toothpaste compositions to create the IOVT. This study should contribute to developing functional toothpaste with the capability of transporting multiple vitamins across the oral mucosa.

Table 1. Presents the ingredients and compositions used for creating our IOVT toothpaste.

Functions	Ingredients	Added Amount
Emulsifier	Glycerin	8.0 mL
Medium/Solvent	Water	40.0 mL
Emulsifier Oily Phase	Olive Oil	20 mL
Abrasives	Salts	5.0 g
Plaque Remover	Silica, Fumed	3.0 g
Plaque Remover	Sodium Bicarbonate	25.0 g
Whitening/Disinfecting	Hydrogen Peroxide	8 mL
Detergent/Penetration Enhancer		
Penetration Enhancer	Sodium Dodecyl Sulfate	4.5 g
Penetration Enhancer	Polypropylene Glycol	2.5 mL
Emulsifier	Emulsifying Wax	45.0 g
Flavoring Agent	Sorbitol	10 g
Toothpaste Stabilizer	Gua Gum	20 g
Binder	Maltitol	15 g
Removing Dental Caries	Xylitol	5 g
Vitamin	Vitamin A	5.25 g
Vitamin	Vitamin B	15ug
Vitamin	Vitamin C	600mg
Vitamin	Vitamin D3	125ug
Vitamin	Vitamin E	135mg
Vitamin	Vitamin K2	300ug

2. METHODS

2.1 Materials and Chemical Compounds

Olive oil, a product of California Estate Extra Virgin Olive Oil, was purchased from a local supermarket (Stop and Shop, New City, NY). The Emulsifying Wax NF was harvested at Plant Guru (Plainfield, NJ). Reverse Osmosis water was purchased from Poland Water Supply (Palisades Park, NJ). Additional key ingredients, namely Maltitol, were obtained from Sigma Aldrich (St. Louis, MO), Sodium Bicarbonate from ScienceLab.com Inc. (Houston, TX), Sodium Dodecyl Sulfate from Carolina Biological Supply Company (Burlington, NC), Hydrogen Peroxide from CVS Pharmacy Inc, and Polysorbate 80 from the Angels Candle & Soap Supply. All six vitamins were purchased from Bulksupplements.com (Henderson, NV).

Three tubes of commercial toothpaste, Pronamel, Sensodyne, and Colgate, were purchased to draw comparisons with the IOVT. The Pronamel toothpaste was formulated for treating many pathological conditions of the oral cavity such as antigingivitis, with anti-sensitivity characteristics. The Sensodyne Toothpaste was manufactured for tartar protection and anti-sensitive formulation with fluoride anticavity strength. The Colgate toothpaste was fabricated for total whitening. All three tubes of toothpaste were purchased from CVS Phramacy Inc.

Fig. 1. Presents the chemical compositions of the IOVT laid out.

2.2 Emulsion Production for Toothpaste Base

The primary step in manufacturing the IOVT was to create an emulsion base, to attain the gel texture of the toothpaste. Two 250 mL beakers were gathered, and each was labeled as aqueous and oil phases. 40 mL of Reverse Osmosis (RO) water was poured into the aqueous phase beaker, and 20 mL of olive oil, 2.5 mL of polysorbate 80, and 45 g of emulsifying wax were poured into the oil-phase beaker. Polysorbate 80 was introduced at this stage due to its properties as an emulsifier, other than its role as a penetration enhancer. Both beakers, containing their respective compositions, were brought into a water bath of a metal container, and heated to approximately 70°C. After the complete melting of the contents in the oil-phase container, the contents of both beakers were put together in a round-bottom crystal container; the container was placed under an electric stirrer and stirred at 1000 - 1500 RPM, gradually increasing the RPM over approximately 1 hour.

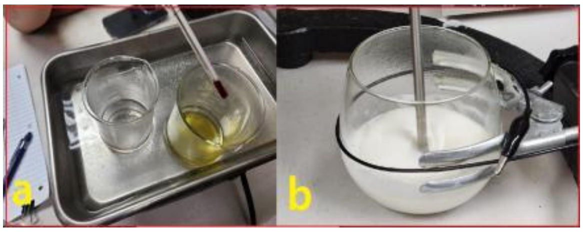

Fig. 2. (a) shows the heating of the aqueous and oil phase beakers; (b) illustrates the stirring of the mixture of contents of the two beakers to produce emulsion for the lotion base

2.3 Formulation for the Intraoral Vitamin Delivery Toothpaste (IOVT)

The remaining chemicals from the list of compositions were measured and weighed into the round-bottom crystal container. Some granular forms of ingredients were crushed into a powder form with a glass spatula. To achieve a homogeneous mixture, the round-bottom crystal container was again brought under the electric stirrer and its contents were stirred at 500 RPM for approximately 30 minutes.

2.4 Toothpaste Quality Evaluation

2.4.1 Stock solution preparation

In a 200 mL beaker, 100 mL of RO water and 5.0 g of the IOVT were added. The beaker with the toothpaste sample in water was placed on an electric magnetic stirrer and stirred for approximately 20 minutes to attain a homogeneous solution. Other stock solutions, each comprising the three commercial toothpaste samples, were prepared with identical procedures for comparison purposes. The stock solutions were stored in a refrigerator, with a temperature ranging from 4 - 6°C, until usage. All stock solutions were consumed within two weeks; when solutions exceeded two weeks from production, new batches were prepared. The solutions were utilized for pH evaluation, foaming capability, and antibacterial strength tests.

2.4.2 Abrasiveness degree evaluation

The IOVT and three commercial toothpaste samples were placed onto waxed paper, in strips with lengths of approximately 2.5 cm. Each sample was pressed down with an index finger to scrutinize the presence of abrasive and sharp-edged particles. Because this evaluation is prone to human error, an effort was made to maintain objectivity in the evaluation of each sample. A comparative scoring system, with a range of 0 to 5, was employed to quantify the results; 0 represented a soft, abrasiveness sample, and 5 represented a rough sample with an abundance of sharp-edged particles.

Fig. 3. Illustrates the four strips of toothpaste, following the evaluation with an index finger

2.4.3 Scratchiness degree evaluation

2.0 g of the IOVT and three commercial toothpaste samples were each placed on sterilized Petri dishes. Using a sterilized cotton ball, the toothpaste samples were rubbed back and forth on their respective Petri dishes; the movement was kept at 5.0 cm in length, with a repetition of 30 times. Because this evaluation is prone to human error, effort was made to keep the control variables alike for the evaluation of each sample. After the procedure was done on all four Petri dishes, the dishes were rinsed with tap water to remove any remaining toothpaste and were left to completely dry. The scratched surfaces of the Petri dishes were observed under a digital microscope. A comparative scoring system, with a range of 0 to 5, was used to quantify the observations; 0 represented no visible scratches, and 5 represented an abundance of scratches, all over the rubbed area.

2.4.4 Spreadability evaluation

2.5 grams of the IOVT and the three commercial toothpaste samples were each placed between two respective microscope slides. A 200-gram standard weight was subsequently placed on top of each toothpaste sample for 20 minutes, allowing the toothpaste samples to expand in length and width. Immediately after removing the standard weight, the diametrical and vertical lengths of each toothpaste sample were measured with a caliper ruler and averaged. The final diameters of each sample were summarized as mean and standard deviation.

Fig. 4. Represents the scratchiness evaluation, rubbing toothpaste samples onto their respective Petri dishes with sterilized cotton balls

Fig. 5. Shows the procedure of the spreadability evaluation; standard weights are placed on top of toothpaste samples between microscope slides

2.4.5 pH Measurement

30 mL of pre-prepared stock solutions of the IOVT and three commercial toothpaste samples were each poured into 150 mL beakers. The Thermo Scientific A111 pH meter was utilized to evaluate the pH level of each stock solution; the pH meter was calibrated to pH levels of 4.0 and 10.0, prior to the procedures of evaluation, as the vendor's user guidelines explained. The respective pH levels of each solution were assessed, as the pH electrode was immersed in each solution at room temperature.

2.4.6 Foam-generating ability evaluation

30 mL of pre-prepared stock solutions of the IOVT and three commercial toothpaste were poured into 100 mL graduated cylinders. Each

cylinder was subsequently shaken 20 times with its mouth closed with parafilm and pressed down with a finger to avoid spillage. Because this evaluation is prone to human error, effort was made to keep the control variables alike for the evaluation of each sample. As foam was produced, the graduated cylinder was placed on a flat, balanced table surface, and the foam's height in the cylinder was measured with a ruler and averaged.

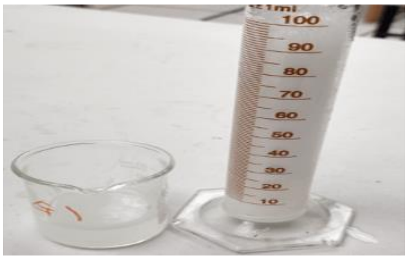

Fig. 6. Illustrates the procedure of the foaming ability test with stock solutions of the four toothpaste samples and 100 mL graduated cylinders

2.4.7 Cleaning capability evaluation

Four eggs were boiled in water for 15 minutes with red food coloring to dye the surfaces and left at room temperature for 20 minutes. A vertical line was drawn on each egg with a permanent marker, dividing the surface area in half. To imitate the conditions of toothbrushing, the IOVT, and three commercial toothpaste samples were applied to a wet toothbrush, in which any surplus water was removed beforehand. With the vertical line in consideration, half of each dyed egg surfaces were brushed with the toothbrush and respective toothpaste samples. 20 back-and-forth movements were made, with 5.0 cm in the length of the motion. Because this evaluation is prone to human error, effort was made to keep the control variables alike for the evaluation of each sample. The regions of the egg surfaces at which color was seen to be faded out, were visually outlined and estimated their most extended vertical and horizontal length in centimeters, to quantify the area of each cleaned-out contour.

2.4.8 Antibacterial strength evaluation with ring of inhibition

Four strains of bacterial culture tubes were obtained from Carolina Biological Supply. The Gram-stain method identified each strain of the bacterial family. The *Micrococcus luteus* (MIC) was pigmented yellow, *Rhodococcus rhodochrous* (RHO) in pink, *Sarcina aurantiaca* (SAR) in orange-yellow, and *Serratia marcescens* D1 (SER) in red. All four bacterial strains are commonly found within the human oral cavity. Four agar-coated Petri dishes were divided into four sections, 1, 2, 3, and 4, and labeled accordingly. The four bacterial strains were each inoculated on respective Petri dishes with an inoculation wire loop. Each pre-prepared toothpaste solution, of the IOVT and three commercial toothpaste, soaked four paper disks, and the disks soaked in the same solution were each put on four different Petri dishes with different bacterial strains. The disks soaked in the IOVT solution were placed in section 1 of each Petri dish, Pronamel disks in section 2, Sensodyne in 3, and Colgate in 4, as found in Fig. 8. The bacterial strains were incubated in a Heratum Incubator (Thermo Scientific, MA) for 72 hours.

Fig. 7. Presents the procedure of the cleaning capability evaluation with boiled eggs, in which toothpastes were brushed on their surfaces with respective toothpaste samples

Fig. 8. Represents the procedure of antibacterial strength evaluation, with 4 paper disks soaked in each toothpaste solution - 16 in total - placed on each Petri dish, inoculated with different bacterial strains

2.4.9 Mass transport study with nightcrawlers

Four nightcrawlers were gathered, and the body weight of each nightcrawler was measured; to keep track of the nightcrawlers, each nightcrawler was put in a labeled plastic container. 2.0 grams of the IOVT and three commercial toothpaste samples were each applied to the skin of a nightcrawler. After 60 minutes, all toothpaste samples applied on the nightcrawlers' skins were rinsed off, and the body weight of each nightcrawler was again measured. The weight difference of the nightcrawlers before the application of toothpaste samples and after rinsing the toothpaste off was compared. A second evaluation was conducted to eliminate potential errors, with identical procedures. The weight difference was calculated and reported as a body weight percentage change.

Fig. 9. Shows the procedure of the mass transport study; four nightcrawlers, each inside respective containers, in which toothpaste samples were applied on their skin

2.4.10 Evaluation of Transepithelial Electrical Resistance (TEER) values

Two 30-centimeter enamel-coated 8.0 gauge copper wires created positive and negative electrodes after the coated enamel layer was removed with an electrical wire stripper. Both tips of the wire formed a ball shape with soldering, to soften their insertion into the body of a nightcrawler through its mouth. The pre-prepared solution of the IOVT was poured into an 18 × 150 mm borosilicate culturing tube, up to 70% of the tube's height. After the preparation phase was complete, a nightcrawler was washed and brought into a plastic container filled with 10% ethyl alcohol for anesthetization of the nightcrawler; after 10 minutes, the positive electrode was cautiously pushed into the mouth of the animal, up to the medium length near the clitellum. The other end of the positive electrode was circuited to the positive button of a 5V battery to enhance electrical potential, and the Data I-245 Data Acquisition System to evaluate the TEER rate. On the other hand, one end of the negative electrode was also wired to the data acquisition system and the negative tip of the 5-volt battery. The positive electrode with the nightcrawler on it, and the negative electrode were simultaneously introduced into the borosilicate culturing tube containing the IOVT solution for TEER value evaluation, as seen in Fig. 10. Data were automatically saved while monitoring the real-time display mode, and the data was played back with the Data waveform browser (Akron, OH). All data were exported into Microsoft Excel, where it was plotted and analyzed with trendline functionality as planned. This procedure was identically repeated three times, each incorporating different solutions of the three commercial toothpaste for comparison.

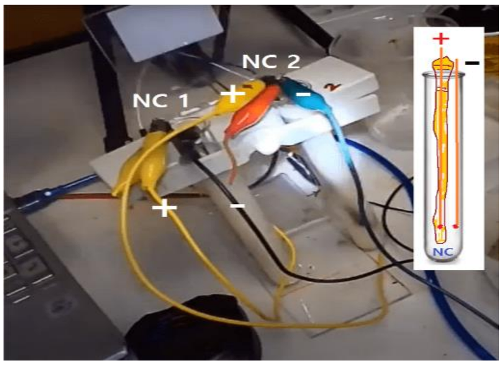

Fig. 10. Illustrates the procedure of the TEER value evaluation; as depicted in the diagram, the change in resistance of the epithelial tissue of a nightcrawler due to the toothpaste solutions was evaluated

2.5 Data Summary and Analysis

All data were summarized as mean and standard deviation. Some data were represented with a comparative scoring system, supporting the quantitative comparison between groups. A student t-test was carried out if needed. The TEER slopes were calculated in the worksheet in Microsoft Excel with the regression analysis.

3. RESULTS AND DISCUSSION

3.1 Toothpaste Creation Procedures

The IOVT was produced with an emulsion base, a mixture of distilled water and olive oil. The procedure was completed when a homogeneous toothpaste mixture of the compositions was attained, with a manual mixing instrument. The toothpaste formulation was stirred with an electric stirrer for approximately 30 minutes to create a sticky dough texture. No coloring nor flavoring were added to the IOVT. A trace of mint fragrance or green coloring could be incorporated to enhance the user's attraction to the IOVT. To formulate a user-friendly toothpaste, the IOVT's viscosity and texture were controlled in detail, by balancing the aqueous and oil phase of the emulsion base.

3.2 Microscopic Observations

Although all toothpaste follows a general formulation, most products are unique in their composition, eventually leading to a distinctive microstructure. A toothpaste generally consists of a dense suspension of abrasive substances, flavoring agents, and therapeutic ingredients in a background liquid of humectants, exhibiting complex rheological behaviors. The four toothpaste samples were observed under a microscope to examine their morphological properties; Fig. 11 depicts the microscopic view of the four samples. There were no significant visual differences among the samples. Though not visible in Fig. 11 (A), a few 10 to 20-um emulsion spheres were discovered from the IOVT.

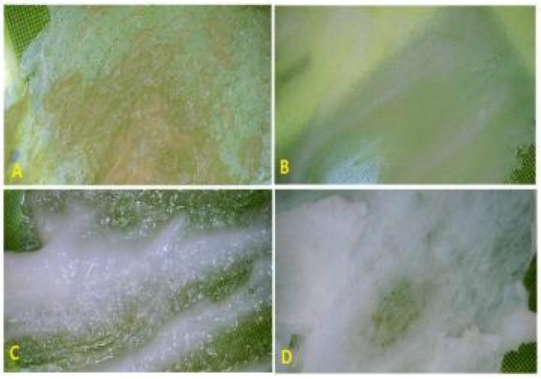

Fig. 11. Presents the microscopic images of the toothpaste samples captured with a digital microscope. Black metric mesh (10 um) was placed under the slide to estimate particle sizes. (A) represents the IOVT, (B) represents Pronamel, (C) represents Sensodyne, and (D) represents Colgate

3.3 Interpretation of the Abrasiveness Evaluation Data

Abrasiveness is a key characteristic of toothpaste, as the abrasive particles assist in the removal of dental plaques while toothbrushing. An adequate degree of abrasiveness is required in toothpaste, as excessive abrasive particles could potentially cause tooth abrasion, and insufficiency in abrasives would not be able to effectively remove food particles while toothbrushing. The IOVT demonstrated a significantly higher mean abrasiveness score than that of Pronamel and Sensodyne; there was no statistical difference with the Colgate toothpaste ($P < 0.05$), as seen in Fig. 12.

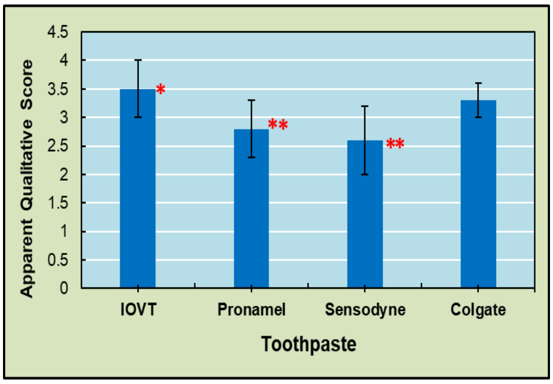

Fig. 12. Demonstrates a significantly greater mean abrasiveness score of the IOVT than that of the Pronamel and Sensodyne groups (n = 4). The score with one asterisk was statistically different from that with two asterisks (P < 0.05)

3.4 Interpretation of the Scratchiness Degree Evaluation Data

Alike the abrasiveness evaluation, the scratchiness degree evaluation was conducted to examine the abrasive particles within the toothpaste. If the scratch is not visually perceivable, the toothpaste would have high feasibility for leaving some food particles unremoved. An excessive degree of scratchiness would cause the enamel to wear out. An optimal magnitude of scratchiness is required in formulating any toothpaste. The IOVT was compatible with commercial toothpaste according to the scratchiness evaluation ($P < 0.05$), as given in Fig. 13.

3.5 Interpretation of the Spreadability Evaluation Data

The spreadability evaluation represents the viscosity of toothpaste; although it could differ in some products, there is a range of toothpaste viscosity that the general consumers are familiar with. Further, spreadability represents the capability of toothpaste to reach into the regions of the oral cavity while toothbrushing. A toothpaste with high spreadability would supposedly be able to reach more surfaces than one with a lower spreadability; vitamin absorption could be consequently enhanced with high spreadability, due to the larger area that the toothpaste would cover. Despite the absence of a statistical difference, the IOVT demonstrated the largest mean spreadability, as shown in Fig. 14.

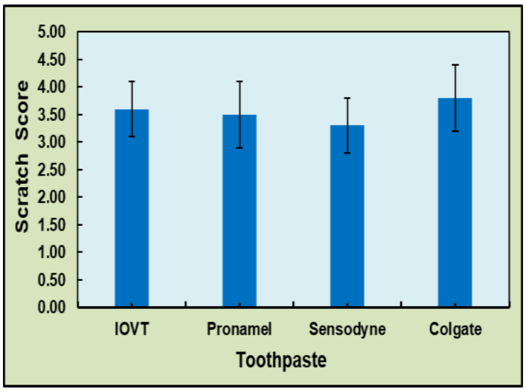

Fig. 13. Displays no statistical difference in mean scratchiness evaluation scores among the groups (n = 4)

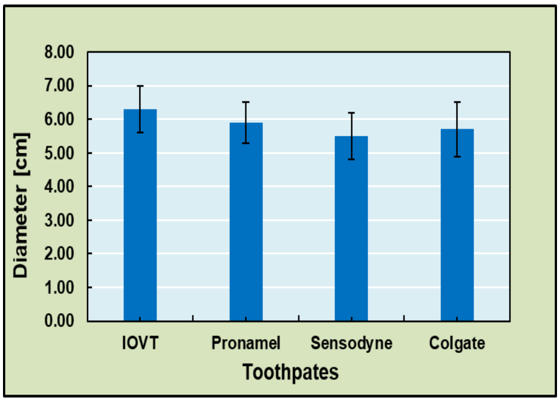

Fig. 14. Exhibits no significant difference in mean spreadability among groups, though the IOVT exhibited the greatest diameter (n = 4)

3.6 Interpretation of the pH Level Evaluation Data

An adequate pH level is a prerequisite for commercial toothpaste. Daily and long-term usage of toothpaste with a low pH level could trigger demineralization of enamel and dentin; commercial toothpaste, therefore, generally ranges from 7 to 10 in pH level [25]. As indicated in Fig. 15, the mean pH level was as basic as pH 7.14, which is within the range to safeguard the enamel layer with minimal adverse effects.

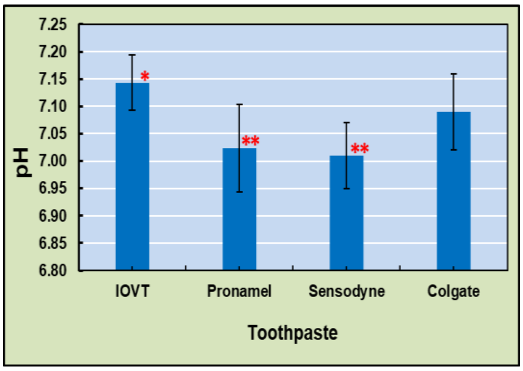

Fig. 15. Illustrates a statistically higher mean pH level of the IOVT than those of Pronamel and Sensodyne (n = 4). The pH level with one asterisk was significantly different compared to two asterisks (P < 0.05)

3.7 Interpretation of the Foaming Ability Evaluation Data

The foaming ability of toothpaste, similar to spreadability, ensures the capability of the toothpaste formulation to reach all regions of the oral cavity, including those that are difficult to access. The foam thus ensures the even distribution of active ingredients during toothbrushing. Additionally, the foam generated could create a pleasant sensation in the mouth while toothbrushing. Fig. 16 below presents a significantly lower mean foam height of the IOVT compared to Colgate, as IOVT had a 23% lower height. There were no significant differences in mean foam height between the IOVT and Pronamel and Sensodyne groups.

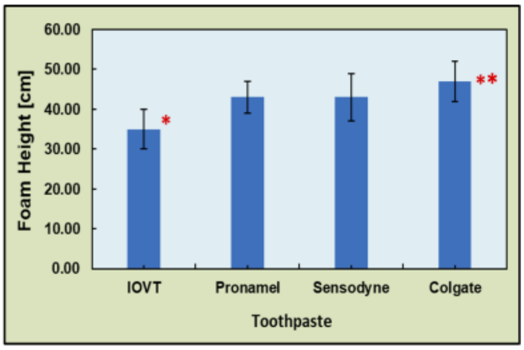

Fig. 16. Presents a significantly lower mean foam height of IOVT, compared to that of Colgate (n = 4). The foam height with one asterisk significantly differed from that with two asterisks (P < 0.05)

3.8 Interpretation of the Cleaning Capability Evaluation Data

For lasting oral health, toothpaste should be formulated to clean the teeth safely and effectively. These fundamental but essential provisions must be kept practical, as the maintenance of teeth without unappealing stains and harmful bacterial growth are the main concerns of most consumers. In evaluating the cleaning capability of toothpaste samples, there were limitations in addressing the results in a definite appraisal, due to the already somewhat light color dyed on the eggs. However, a scientifically reasonable distinction could be found in areas where the dyed color was fully removed. The diameters of the perfectly cleaned-out area of each egg were measured and averaged, avoiding bias in each evaluation. The IOVT exhibited compatibility with commercial toothpaste products in cleaning capability, as seen in Fig. 17.

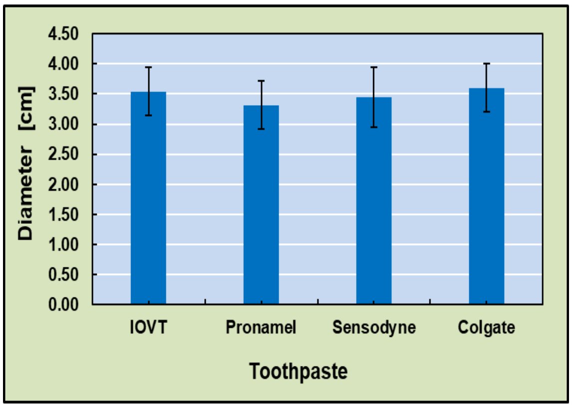

Fig. 17. Demonstrates no statistical difference in the mean diameter of the colorless area among the groups (n = 4)

3.9 Interpretation of the Antibacterial Strength Evaluation Data

Antibacterial strength is one of the key qualities of all toothpaste, as toothpaste should regulate harmful bacterial growth within the oral cavity. Each toothpaste solution soaked four paper disks, and the four disks were each placed on four Petri dishes coated with agar, each inoculated with different bacterial strains as mentioned in the Methods section. The diameters of each ring of inhibition were measured after 72 hours of incubation and summarized in the mean value to minimize errors. The IOVT exhibited the feasibility of eliminating harmful bacteria inside the oral cavity, as it had the largest mean inhibition ring diameter in bacterial strains of MIC, RHO, and SER, as plotted in Fig. 18.

As a result, the antibacterial strength of IOVT was confirmed compared to other types of toothpaste, as seen in the MIC and RHO strains group. Fig. 18 plotted the summarized data on the ring diameter of inhibition. It was estimated that IOVT had the largest radius among the groups, which showed that IOVT might be able to remove harmful bacteria inside the mouth.

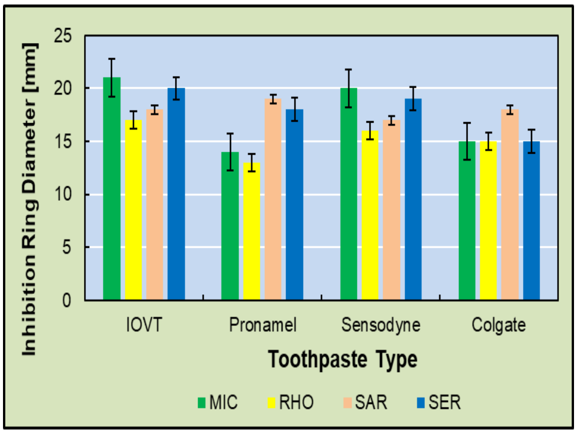

Fig. 18. Displays the mean inhibition ring diameters of paper disks, each soaked in toothpaste solutions. The IOVT demonstrated the largest mean inhibition ring diameter for three out of four bacterial strains after a 72-hour incubation (n = 6)

3.10 Interpretation of the Mass Transport Study Data

It is a universal law that any particles or objects move from high to low concentration, which is the underlying osmotic pressure mechanism. Assuming that a high concentration of drug molecules was created outside a nightcrawler's skin due to the application of toothpaste, the drug molecules of the toothpaste should disperse into the nightcrawler's skin. The PEs of the IOVT would presumably facilitate the diffusion of drug molecules. Each toothpaste sample was applied on two nightcrawlers, and the difference in a nightcrawler's weight before the application of toothpaste and after washing off the excess toothpaste was calculated as a body weight percent change and averaged between the data of two nightcrawlers. The IOVT exhibited a significant dominance, compared to the three toothpaste samples, in dispersing drug molecules through the epithelial tissues of nightcrawlers, as seen in Fig. 19.

3.11 Interpretation of the TEER Value Data

In pharmaceutical sciences, transepithelial electrical resistance (TEER) value evaluations are often utilized to examine the movement of biochemical compounds and intermediates across cellular layers. The TEER value is evaluated by monitoring a decrease in electrical resistance of epithelial tissues, largely influenced by the increased paracellular diffusion of molecules, indicating the loosening of cell junctions. The methods of TEER value evaluation in this study may differ from conventional procedures, as uniform membrane samples are customarily employed. Nonetheless, this study's application of nightcrawlers for the TEER value evaluation should be reasonable, as there is mutuality in that the skin of a nightcrawler is composed of epithelial tissues, analogous to the skin of other animals. As the data were assessed, it was observed that the slope of the TEER potential linear regression line was inversely proportional to the concentration of PEs. The TEER rate was evaluated by calculating the average change of volts over 5 minutes. The IOVT demonstrated eminence, contrasted to the three commercial toothpaste samples, in decreasing the electrical resistance of epithelial tissue of a nightcrawler, as seen in Fig. 20. The data suggests a high possibility for the IOVT to have delivered vitamins into the nightcrawler's body. An advanced level of TEER value study would be required in the future to confirm the results of this study.

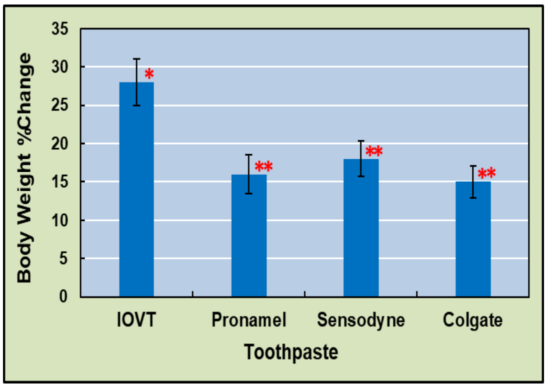

Fig. 19. Displays a significant difference between the IOVT and the three commercial toothpaste samples in body weight percent change, measured 60 minutes after the application of toothpaste samples. The weight change percent with one asterisk shows a statistical difference from two asterisks (P < 0.05)

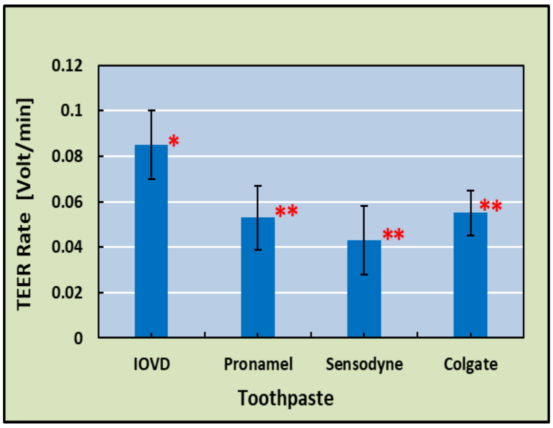

Fig. 20. Illustrates a significantly greater electric potential change of nightcrawlers' epithelial tissues, in which the IOVT was applied, compared to those, in which the three commercial toothpaste samples were applied, in TEER rate. The TEER values with one asterisk are statistically different from those with two asterisks (P < 0.05)

4. DISCUSSION

Multiple tests evaluating the general characteristics of toothpaste were conducted; abrasiveness, scratchiness, spreadability, foaming ability, pH level, cleaning capability, and antibacterial strength of the IOVT were evaluated, and compared with those of three commercial toothpaste formulations. In a nutshell, the IOVT exhibited compatibility with commercial toothpaste products in most evaluations.

A mass transport study and a TEER value evaluation were conducted to verify the ability of the IOVT to permeate drug molecules through the epithelial tissue. In comparison with the three commercial toothpaste samples, the IOVT demonstrated eminence in its ability to disperse drug molecules through and decrease the resistance of epithelial tissues.

Most evaluations encompassed sources of human error, namely the procedures of the scratchiness degree and the cleaning capability evaluations, and the quantifying processes of most data. Some characteristic evaluations overly depended upon human perception, such as the abrasiveness degree evaluation. Further study must employ methods of evaluation that are more scientifically reasonable.

The existence of areas where the IOVT could improve is undoubted. It would be ideal for the

IOVT to range within that of commercial toothpaste samples for the general toothpaste quality evaluations, rather than being on the extreme ends of the spectrum. Future studies should have as an objective to neutralize the IOVT, for a better resemblance of the IOVT with commercial toothpaste.

5. CONCLUSION

Various vitamins are recognized to be essential for one to attain a healthy oral cavity. This study attempted to create a toothpaste formulation that would facilitate the intraoral delivery of vitamins, through the oral epithelium, into the bloodstream. Taking vitamin tablets regularly is not easy but could be made convenient by brushing the teeth using the IOVT.

This study has verified the high feasibility of the IOVT to penetrate vitamins through epithelial tissues, alongside certifying its general toothpaste characteristics, comparable with those of commercial toothpaste. The IOVT should contribute to making advancements in the study of intraoral drug delivery and the general use of penetration enhancers.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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