Scientific Research Publishing

Advances in Bioscience and Biotechnology, 2023, 14, 1-17
https://www.scirp.org/journal/abb
ISSN Online: 2156-8502
ISSN Print: 2156-8456

Investigating a Vitamin D Delivery Toothpaste Using a Penetration Enhancer Compound

Naree Lee, Jongbin Lee

Medical and Pharmaceutical Divisions, STEM Science Center, Englewood Cliffs, NJ, USA
Email: JLee@STEMsc.org

How to cite this paper: Lee, N. and Lee, J. (2023) Investigating a Vitamin D Delivery Toothpaste Using a Penetration Enhancer Compound. *Advances in Bioscience and Biotechnology*, **14**, 1-17.
https://doi.org/10.4236/abb.2023.141001

Received: November 21, 2022
Accepted: January 10, 2023
Published: January 13, 2023

Abstract

Expanding in the oral care business, being passionately driven by innovative and scientific products, functional toothpaste has recently become more popular for functionality, variety, and efficacy. Many new types of toothpaste are commercially manufactured with diverse fragrances, colors, probiotics, and pharmaceutical ingredients to enhance the functionalities of toothpaste. Our study attempted to create a toothpaste formulation that might facilitate the intraoral delivery of vitamin D3 into the bloodstream. Simply brushing our teeth with toothpaste should be easy to take the essential vitamin regularly. In this study, an emulsion-based toothpaste mixed with an azone compound and sodium dodecyl sulfate as penetration enhancers blended thoroughly with other ingredients and then with vitamin D. Multiple toothpaste characteristic tests were performed, such as abrasiveness, scratchiness, spreadability, pH, foaming, cleaning, and antibacterial strength with our vitamin D toothpaste, and compared with those of other commercial brand toothpaste. To confirm the intraoral delivery of vitamin D through the oral cavity, an earthworm transport study and TEER value test were conducted using L. terrestris skin. Our data demonstrated the high feasibility of intraoral delivery of vitamin D based on those two skin studies with various experimental support; our vitamin D toothpaste had comparable characteristics with other commercial toothpaste for cleaning functionality.

Keywords

Azone Compound, Drug Delivery System, Intraoral Delivery, Penetration Enhancer, Toothpaste, Vitamin D

1. Introduction

Vitamin D is a fat-soluble vitamin. The vitamin D from foods and nutritional supplements accumulated in fat tissue for later use [1] [2]. When the vitamin D

circulating in your bloodstream is deficient, it's impossible to absorb all the calcium required in the body. Vitamin D also involves cell growth and immune function, preventing inflammation in check and your nervous system functioning correctly [3] [4].

Strong sunlight initiates vitamin D production in the skin. And the liver and kidneys facilitate the conversion from vitamin D to its most active form, 1,25-dihydroxy vitamin D [5]. In ideal conditions, all the vitamin D needed for the year can be formed within a few minutes of direct exposure to the sunlight a few times a week during the summer months. But, most populations don't generate the required vitamin D, either because their skin cannot absorb it or because they don't spend enough time outside [6]. Vitamin D is also absorbable from food and dietary supplements, but many need help to get what they need here. According to the USDA's 2022 dietary guidelines for Americans, most children and adults do not succeed in producing the required amount of vitamin D [7].

Adequate vitamin D is essential to make strong bones. Even slight insufficiency in vitamin D may impact bone strength, as it will squeeze the body to obtain calcium from bones to maintain the body's balance [8] [9]. Consequently, several bone health problems, such as rickets, affect infants and children and might worsen the conditions due to improper bone development. Adults may develop osteomalacia, which causes bone and muscle pain, or osteoporosis, which is the process of thinning the bones and losing bone density, increasing the risk of fracture [10] [11].

Intraoral delivery has been studied with some historical background [12] [13]. In 1790, the concept of aerosol appeared in France. The oral spray was efficient for many reasons: the quick release of medicaments in a minimal size, which might be absorbed easily by the buccal mucosa, and a direct and rapid dispersion of a practical solution [14]. It is known that for oral delivery through the gastrointestinal (GI) tract, the drug faces many destructive barriers before being absorbed through the epithelial tissue. There is a significant change in GI pH from pH 1 - 2 in the stomach to 7 - 7.4 in the intestinal tract, unpredictable GI transit, numerous digestive enzymes, and intestinal flora [15].

Compared to this harsh condition, drug absorption via the oral cavity offers relatively consistent and friendly physiological conditions. Most studies of buccal absorption indicate that the predominant mechanism is passive diffusion across lipid membranes via the paracellular or transcellular pathways [16].

In sublingual administration, the drug is deposited on the tongue, reaching directly into the bloodstream through the tongue's ventral surface and the mouth's floor [17]. The drug solutes are rapidly absorbed into the reticulated vein, which lies underneath oral mucosa; then transported through the facial veins, internal jugular vein, and brachiocephalic vein and poured into the systemic circulation. Compared to orally absorbed tables, those absorbed through the sublingual route react faster. Furthermore, the portion absorbed through the sublingual blood vessels is greater than that absorbed through the hepatic

first-pass metabolic process, in other words, oral absorption. [18] [19]. The primary mechanism for absorption of the drug into oral mucosa is via passive diffusion into the lipoidal membrane. The absorption of the drug through the sublingual route is three to ten times greater than the oral route and is only surpassed by hypodermic injection. The small volume of saliva is usually sufficient for these formulations to result in tablet disintegration in the oral cavity [20].

Several countries across the globe have reported a high prevalence of Vitamin D deficiency to the tune of almost 90%. Many countries have started vitamin D fortification foods or recommend using vitamin D supplements [21]. However, several studies have reported differences in the bioavailability of vitamin D supplements in some populations. Decreased bioavailability may be due to altered absorption of vitamin D from the small intestine, or it may be due to altered metabolism of vitamin D in the body. Intestinal malabsorption disorders may cause a decrease in vitamin D absorption due to a decreased ability to absorb lipids [22].

While sublingual absorption takes vitamin D directly into the systemic circulation, like vitamin D from the skin, orally ingested vitamin D, in contrast, is absorbed into the portal circulation from the intestines, which takes it to the liver first before entering into the systemic circulation. Vitamin D absorption from the GI tract is incomplete, and there is local intestinal degradation and hepatic metabolism. The population with digestion problems for taking medications and people with stomach bypass surgery, including those with Lap-band procedures, may have difficulty in the intestinal absorption of vitamin D [23]. The medical association has started with wake-up calls to eliminate vitamin D deficiency which is surprisingly much more common than anticipated.

Typically, the parental route has given way to oral administration for a long time. But the sublingual route is reasonably a replaceable pathway to eliminate vitamin D deficiency. However, more evidence might be verified from future studies to see whether it is the ideal way to administer vitamin D compared to the conventional ones and justify the notion [24].

The sublingual route for the absorption of vitamin D supplements should be superior to oral ingestion because sublingual absorption takes vitamin D directly into the systemic circulation, just like when vitamin D is naturally synthesized in the skin from exposure to sunlight [25].

In contrast, vitamin D from oral ingestion is absorbed into the portal circulation from the intestines, which takes it to the liver first before entering into the systemic circulation. The hepatic breakdown of vitamin D is another barrier to overcome to reach the bloodstream by the vitamin D molecules. The extreme journey of the vitamin D from the mouth to the GI tracts and the hepatic circulation is called the "first pass," which might be removed by delivering the vitamin D into the intraoral cavity [26].

It is not easy to create a routine, especially if it is required to do something daily. Many have difficulty in remembering what they must do and remembering to take their prescription medications. In this study, in addition to the most

popular ingredients, our toothpaste was added with vitamin D and penetration enhancers such as the azone compound and sodium dodecyl sulfate. And batteries of appropriate tests were carried out to verify the quality of toothpaste and the feasibility of vitamin D delivery. The study might contribute to developing functional toothpaste that can deliver therapeutic agents.

2. Experimental Methods

2.1. Materials and Reagents

Olive oil, specifically Filippo Berio's extra virgin olive oil, was harvested in Italy and acquired from local supermarkets (Hannam, NJ). The Emulsifying Wax NF (lotions/cream thickening agent) was purchased at Plant Guru. Reverse Osmosis water was purchased from Bergenfield Water Depot or Divine Purified Water Company. Other key ingredients such as Maltitol were bought from Nuts.com, Sodium Bicarbonate from ScienceLab.com Inc (Houston, TX), Vitamin D3 (cholecalciferol-D3) from Bulksupplements.com (Henderson, NV), Sodium Dodecyl Sulfate from Carolina Biological Supply Company (Burlington, NC), Polysorbate 80 from the Angels Candle & Soap Supply, hydrogen peroxide from CVS Pharmacy Inc, TheraBreath Oral Care Probiotics Citrus from iHerb.com, and Red Food Color (Net 1 Fl Oz 29 mL) from McCormick & Co., Inc (Hunt Valley, MD).

Third commercial toothpaste brands were bought to compare toothpaste qualities with our formulation. The first toothpaste purchased was the Colgate Toothpaste Total SF for anticavity, antigingivitis, and anti-sensitivity toothpaste with its active ingredient as stannous fluoride 0.454%. The second toothpaste was Crest Toothpaste, manufactured for tartar protection (Regular paste) with fluoride anticavity ability, purchased from the local marts (Walgreens, Fort Lee, NJ). The third brand was Meich PUSH toothpaste (Hannil Pharmaceutical, Korea).

2.2. Emulsion Creation for Formulation Base

Two 200 mL beakers were collected and labeled as aqueous and oil phases. The distilled water of 60 mL was filled into the water-phase beaker while Extra virgin olive oil of 10 mL, 15 mL polysorbate 80, and 22.5 g emulsifying wax were brought into the oil-phase beaker. Then, both beakers were placed into a water bath with a jacket and heated to 65˚C. After observing a complete thawing of contents in the oil-phase beaker, the contents of the water-phase beaker were poured into a round-bottom crystal container.

2.3. Formulating Our Intraoral Vitamin D Toothpaste (VDTP)

The content in the crystal container was then subjected to an electric stirrer of 500 - 1000 RPM, gradually increasing for approximately 60 minutes. While the mixture was stirred, other powdered and granular ingredients were weighed out into a round-bottom glass container in which they were poured and crushed in-

to powder using a plastic deeper. Those ingredients include 2.5 g sodium dodecyl sulfate, 20 g sodium bicarbonate, 3 g Silica, 2.5 g Xanthan gum, and 4 g Vitamin D3. After 60 minutes, the beaker was taken off the stirrer and combined with the contents of the round-bottom glass. After that, 10 mL of glycerin and 10 mL of hydrogen peroxide were added. Then, the well-mixed formulation was divided into two portions, where one portion was added with 1.5 mL of azone.

Table 1 lists the ingredients of our VDTP added after the emulation formulation.

2.4. Our Toothpaste Characteristic Evaluation

2.4.1. Preparation of Testing Stock Solutions

In 80 mL of distilled water, the toothpaste of 2.0 g was placed into a 250 mL beaker. The beaker with the paste sample in water was thoroughly dissolved on a magnetic stirrer. Other stock solutions for comparison groups were prepared with identical methods. Afterward, the beakers were added with magnetic bars and placed on a magnetic stirrer for 30 minutes to ensure uniform suspension. The solutions were used for pH measurement and foaming ability tests. The stock solutions were stored in the cold refrigerator (4˚C) until used.

2.4.2. Evaluation of Abrasive Capability

Each sample of approximately 2.0 cm toothpaste was squeezed onto waxed paper. Each sample was pressed with an index finger along its length to examine the presence of brittle and sharp-edged formations with abrasive particles. The degree of edged contour was closely evaluated and scored on a scale from 0 to 5; 0 for most soft while 5 for most rough.

2.4.3. Petri Dish Scratchy Test

The scratch test was performed as follows; approximately 1.5 g of each toothpaste

Table 1. Scratch Test

Functions	%	Ingredients	Actual Wt.
Humectants	4.88	Glycerin	8.0 mL
Medium	36.59	Water	60.0 mL
Buffers/Salts	3.05	Salts	5.0 g
Malititol	4.27	Binder	7.0 g
Tartar Control	1.83	Silica, Fumed	3.0 g
Abrasives	15.24	Sodium Bicarbonate	25.0 g
Antibacterial	4.88	Hydrogen Peroxide	8
Surfactants	2.74	Sodium Dodecyl Sulfate	4.5 g
Delivery Agent	0.92	Azone	1.5 mL
Vitamin D3	1.22	Vitamin D3	2.0 g
Emulsion Medium	24.39	Emulsifying Wax	40.0 g
TOTAL	100		162 g

was pushed or placed out from the container onto a clean, separate petri dish, and two drops of distilled water were added to each dish. Using a sterilized cotton swab, the toothpaste sample on the petri dish was rubbed back and forth, 2.0 cm, twenty-five times. The toothpaste was then thoroughly removed from the petri dish and carefully dried with a paper towel. The surface of the petri dish was then examined under a digital microscope, illuminated from above, to score the degree of scratches on the surface of the slide. The degree of scratches was then rated on a scale from 0 when no scratches existed, to 5, when significant scratches. The test was repeated five times for the individual toothpaste sample.

2.4.4. Determination of Spreadability

Toothpaste of 1.50 g was weighed out by squeezing from the container and placed on the center of a plexiglass slide. Subsequently, another plexiglass plate was prepared to cover the sample. Then, a 500 g weight standard was used to press the sample on top of the plexiglass plate for 15.0 minutes. After releasing the weight pressure, the longest horizontal and vertical distances were measured. And its diameter was reported as mean and average, as seen in the figure.

2.4.5. pH Evaluation

The Hana Ion pH meter was calibrated first with reference solutions of pH 4.0, 7.0, and 10.0 as described in the manufacturer's manual. Before the measurement, 25 mL of each pre-prepared toothpaste solution was ready and filled into clean 100 ml beakers. After establishing all necessary preparation, the pH was measured for each sample solution at room temperature.

2.4.6. Foaming Capability Determination

The 25 ml of each pre-prepared stock solution for each toothpaste was filled into individual 100 ml graduated cylinders. The cylinder was then shaken 30 times with its top closed with a piece of parafilm to prevent spillage. The graduated cylinder was then placed on the flat balanced level on the ground, and the foam's height in the cylinder was measured and recorded.

2.4.7. Cleaning Capability

The cleaning capability of toothpaste was evaluated through the toothpaste's ability to remove red food coloring stained from the surface of an eggshell. It was prepared by four eggs being first boiled in water for staining with red food coloring--for approximately 20 minutes and cooled at room temperature for another 30 minutes. Using a permanent marker, a diagonal line was drawn along the length of an eggshell dividing it in half. To simulate the regular brushing method, the toothbrush was moistened with water and shaken to remove any excess water. On one side of each colored egg, 15 back-and-forth toothbrush strokes of 4.0 cm in length were made with each of the four types of toothpaste. The magnitude of the area where color was seen to be removed was visually defined and measured for its vertical and horizontal diameter (cm) to calculate the area of each elliptical pattern.

2.4.8. Antibacterial Test of Ring of Inhibition

Four strains of bacterial tube cultures were used. The strains were identified by the Gram-stain method, which is used as the first step in identifying a bacterium. The pigmented bacteria strains were Micrococcus luteus (yellow) as MIC, Rhodococcus rhodochrous (pink) as RHO, Sarcina aurantiaca (orange-yellow) as SAR, and Serratia marcescens D1 (red) as SER for experimental labeling. Four Petri dishes were labeled into 4 sections 1, 2, 3, and 4. Using the inoculation wire ring, the bacteria were inoculated into the Petri dishes that were already coated with nutrient agar. And the stock solutions of 4 different kinds of toothpaste were soaked on the circular paper disk. Subsequently, Place the disk with the applied toothpaste on four sections labeled, 1—VDTP, 2—Colgate, 3—Crest, and 4—Push toothpaste, as seen in **Figure 1**. The bacterial culture was cultured in an Heratum Incubator (Thermo Scientific, Waltham, MA) for 48 hours.

2.4.9. Skin Transport Test Using Lumbricus Terrestris Skin

Sixteen L. terrestris were divided into four groups and placed into 200 ml beakers with appropriate labels on the glass surface after weighing their body weight. Subsequently, 2.0 grams of each toothpaste was rubbed around their body. After 30 minutes, their body weight was measured again. The body weight difference was calculated and converted into % weight change.

2.4.10. Evaluation of TEER Values

In pharmaceutical areas, the drug delivery system uses the TEER values to examine the transport of many natural and synthetic elements, such as leukocytes, molecules, and particles across cellular barriers in the body, while opening tight cell-junction, which may be transient and relatively innocuous or more prolonged [27]. This pathway can be assessed by measuring the transepithelial electrical resistance (TEER) and passive paracellular diffusion of molecules, whereby decreased resistance to an electrical current or increased paracellular leakage of an inert compound into the basolateral space indicate the opening of cell junctions, respectively. At first, a stock solution was prepared with the toothpaste of

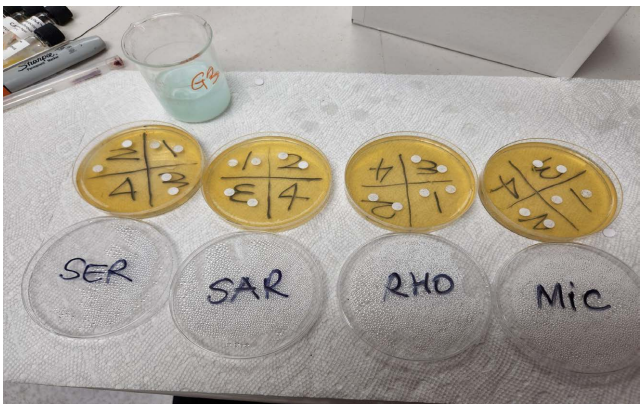

Figure 1. The bottom surface of the bacteria-inoculated Petri dish was divided, and lines were drawn into four regions and incubated the bacteria with paper discs soaked with each toothpaste solution accordingly.

10.0 g mixed with 80.0 mL of distilled water to make a 12.5% solution sample for each toothpaste. The enamel-coated copper wires created two electrodes for positive and negative poles. And the coated enamel layer was stripped with a cutting-edge knife. Both ends were attached with a drop of soldering lead to facilitate their insertion into the L. terrestris. Data I-245 Data Acquisition System was used to obtain the decreasing resistance profiles between the skin using toothpaste solutions. A 9 Volt battery was used for priming the electrical potential during the study. After finishing the preparation procedures of toothpaste solutions and turning on the data acquisition system, an L. terrestris was cleaned and weighed on an electrical balance and placed into a container holding 10% ethyl alcohol as an anesthetic. Once confirmed the depth of anesthesia was approximately 6 minutes, the L. terrestris was moved into the test tube filled with the testing toothpaste solution up to 70% height of the test tube (18 × 150 mm, borosilicate culturing tube). The positive electrode was carefully inserted into the animal up to the medium length between the clitellum and anus. The other end was connected to the data acquisition system and the positive button of a 9V battery.

In contrast, the negative electrode was placed into the testing solution of 25 mL and connected to the system and negative button of the battery. Data were recorded while maintaining the real-time display mode. After the experiment, the data was retrieved using the Data waveform browser (Akron, OH) and exported into MS Excel, where it was analyzed as scheduled.

2.5. Data Analysis

All the data were reported as mean and standard deviation. Some observational data were scored into a numerical numbering system, facilitating quantitative comparison between groups. A student t-test was performed when required. The slopes of the TEER values were calculated in the worksheet of Microsoft Excel with the regression analysis functions.

3. Results and Discussion

3.1. Formulation Development

Our vitamin D toothpaste was created starting from an emulsion formulated with distilled water and olive oil, as described above. The formula was then finalized with a thorough mixture of toothpaste ingredients with manual mixing tools. SDS and granular ingredients were crushed in the jar completely with a plastic spatula and blended for approximately 20 minutes for a homogenous mixture. The toothpaste did not have any flavoring agents, or artificial dye added. To enhance the consumer's preference for toothpaste, adding a tint of mint flavor or green coloring could be an improvement. Azone (1-dodecylazacycloheptan-2-one) is a hybrid of two potent permeation enhancers, namely pyrrolidone and decylmethylsulfoxide, but lacks the aprotic sulfoxide group (Williams and Barry, 2004). To increase Vitamin D3 absorption, azone [28] and sodium

dodecyl sulfate [29] was employed as chemical penetration enhancers. Other common toothpaste ingredients, such as humectants, abrasives, and binders, were blended in sequential order. For creating a high-quality toothpaste, its wetness and hardness were closely managed by adding an adequate amount for each ingredient, starting from the volume of distilled water added into the oil phase for the emulsion base.

3.2. Evaluation of Abrasive Capability

Tooth abrasion is a leading dental point in the common population. The main degrading quality of this is toothpaste abrasives. Hence, measurement and standardization of toothpaste are required. Understanding abrasiveness in toothpaste is essential. A group of scientists used a radiometric method for an abrasiveness evaluation in samples of silica and calcium carbonate used as an abrasive in a dentifrice to help in a thoughtful choice of materials by dentifrice producers [30]. Some people prefer feeling a small number of abrasive particles inside the toothpaste. All the other three kinds of toothpaste did not or had almost no abrasive particles, while our toothpaste had a small number of particles that were felt with fingers. As shown in **Figure 2**, our vitamin D toothpaste (VDTP) had a greater mean abrasive capability score compared to that of Colgate and Crest and no significant difference with the Push brand ($P < 0.05$).

3.3. Petri Dish Scratchy Test

A scratchy test was performed using a Petri dish. This quality test was conducted to examine if the toothpaste could efficiently remove the plaque on the surface of the teeth. If the scratch was not satisfactory enough, the teeth might still have a high possibility of leaving any pieces of food particles. But if the scratch power was too strong, the surface enamel might be eroded quickly. So an optimal strength of scratch might be crucial when formulating the toothpaste. According to the petri dish scratchy test, VDTP was shown to be compatible with other commercial products ($P < 0.05$), as seen in **Figure 3**.

3.4. Spreadability Determination

The spreadability test was executed to investigate how large an equal pressure could form the diameter of the spread of the toothpaste sample. The longest spread diameter could be an essential factor to consider because the greater spread diameter could increase the absorption of vitamin D. As seen below in **Figure 4**, our VDTP toothpaste had the greatest mean diagonal spreadability.

3.5. Determination of pH

Our teeth' enamel initiates demineralizing when exposed to a pH of about 5.5. Therefore, the pH of toothpaste should be significant and considered carefully. Using toothpaste with fluoride has been a good choice for enamel health. Daily use of toothpaste with incompatible pH could erode the tooth's surface. It has

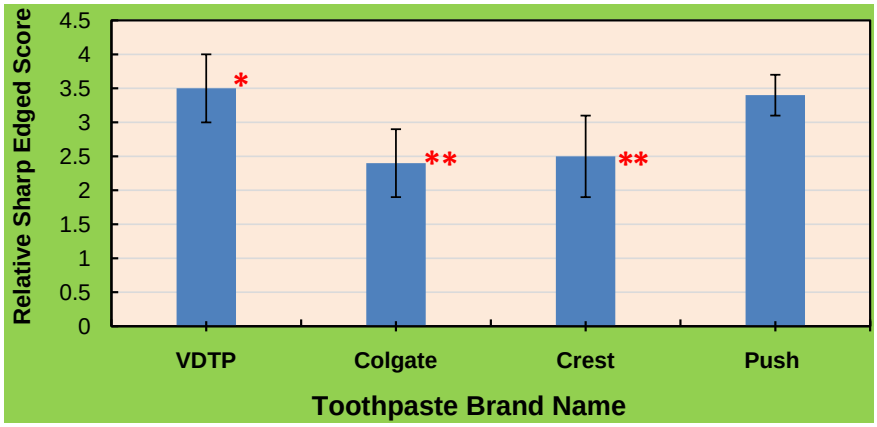

Figure 2. The mean abrasive capability is significantly higher than that of the Colgate and Crest groups (n = 4). The score with one asterisk was significantly different from that with two asterisks.

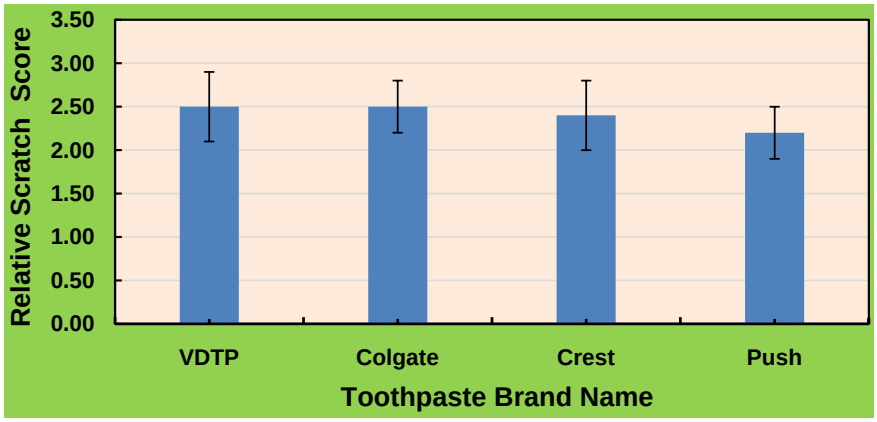

Figure 3. The mean scratchy test scores that were comparable to the groups of other commercial products (n = 4).

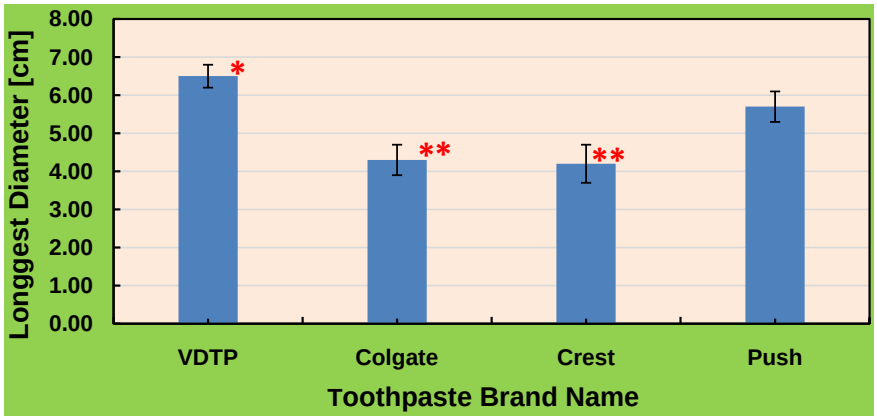

Figure 4. The mean spreadability area in VDTP was significant compared to that of the groups from (n = 4). The diameters with one asterisk were significantly different from those with two asterisks.

been found that pH levels should range from an acidic 3.76 to a primary, safer side of 9.68 [31]. The pH of our VDTP toothpaste was as basic as pH 7.34, which

might be considered a favorable and safe pH for protecting the enamel layer with no adverse effects on the intraoral epithelial tissues in Figure 5.

3.6. Determination of Foaming Ability

Many types of toothpaste are designed to enhance the medical and hygienic effects using the foaming ability. Further, toothpaste could produce a pleasant feeling in the mouth during toothbrushing. Foam formation plays a vital role in these sensations. Investigating the foamability and consistency of the foam is a meaningful procedure in the formulation of toothpaste. Figure 6 presents that the mean foam height of VDTP was only significantly greater than that of Push brand, while no statistical difference in foaming ability was observed from that of Colgate and Crest.

3.7. Cleaning Ability Evaluation

Toothpaste should be designed to clean teeth effectively and safely for long-term health. These relatively simple conditions should be kept realistic because most

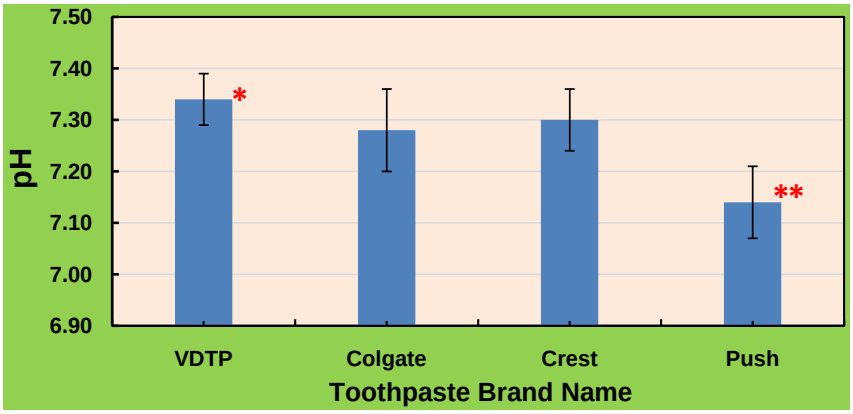

Figure 5. The mean pH of VDTP was statistically more significant than that of Push brand toothpaste (n = 4). The pH with one asterisk was significantly different from that with two asterisks ($P < 0.05$).

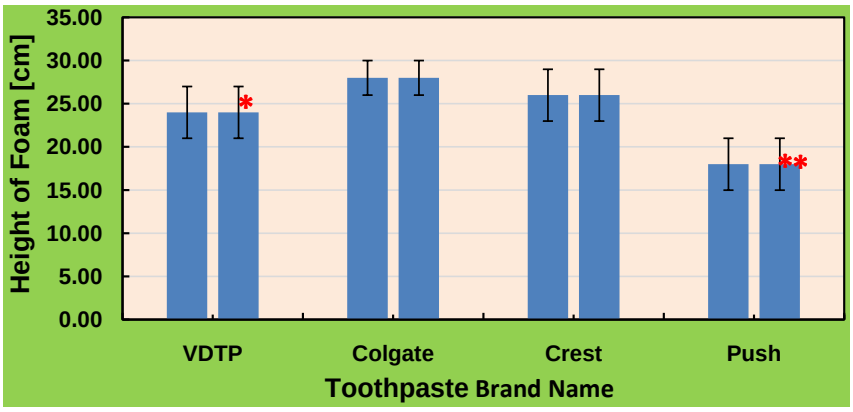

Figure 6. The mean foam height of VDTP was only significantly greater than that of the Push brand (n = 4). The foam height with one asterisk was substantially different from that with two asterisks ($P < 0.05$).

consumers desire to maintain their teeth without unsightly stains and potentially harmful bacterial plaque. Though multiple methods of estimating cleanliness are published [32], the degree of cleaning ability should be mutually supportive to some degree. While evaluating the cleaning ability because the egg was dyed only on the surface in red food coloring pigment, it had a limitation in determining the cleaning ability of each toothpaste with a clear-cut assessment. If the food coloring material was changed from red to blue or black, it might be easier to measure the cleaning ability. However, there was definite differentiation that some lineation of the exposed area by the force of brushing on the dyed egg was scientifically reasonable. In this study, as seen in **Figure 7**, the cleaning ability examined by the light of exposed areas shows that our formulation VDTP was comparable to that of Colgate and Crest and significantly greater than that of the Push brand.

3.8. Antibacterial Test

In this study, four disks soaked in four different types of toothpaste—VDTP (1), Colgate (2), Crest (3), and Push (4)—were placed on top of the agar layer that was previously inoculated with four types of bacteria as described in the method section. After 48 hours, the diameter of the ring of inhibition at four sites was measured and averaged for unbiased estimation. **Figure 8** presents a picture of a colony-established Petri dish after 48 hours. As seen in the group of MIC and RHO strains, the antibacterial strength of VDTP could be confirmed compared to other types of toothpaste. **Figure 9** shows our summarized data on the ring diameter of inhibition. It was found that VDTP has the largest radius, which demonstrated that VDT might have the ability to eliminate harmful bacteria inside the mouth.

3.9. Mass Transport Study with Lumbricus Terrestris Skin

It is a type of universal law that any particles or objects move from high concentration to low concentration, which is the underlying mechanism of osmotic pressure.

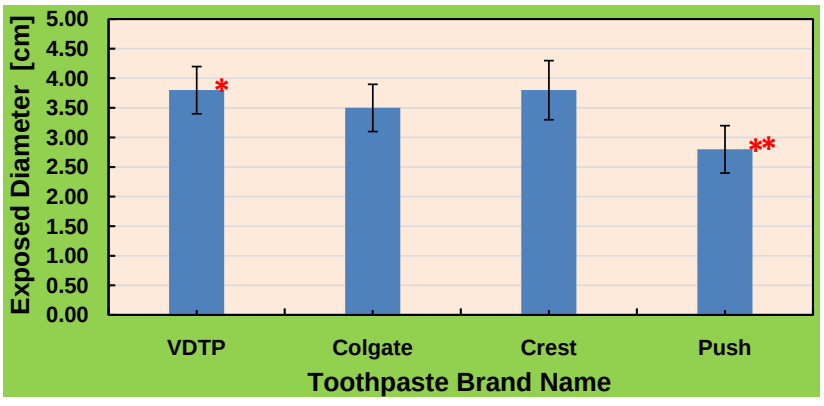

Figure 7. The mean cleaning ability was significant compared to that of the Push brand (n = 4). The diameter with one asterisk was significantly different from that with two asterisks ($P < 0.05$).

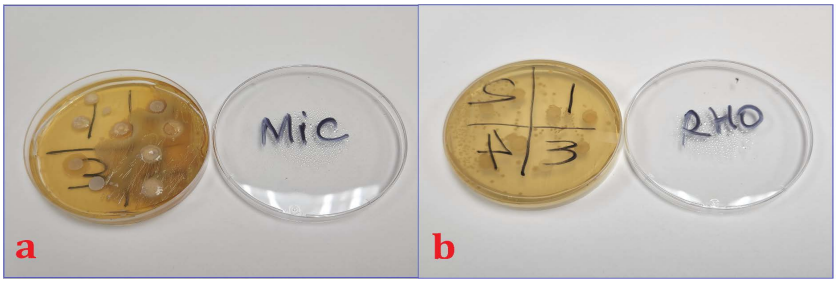

Figure 8. The bacteria growth pattern after 48 hours of incubation under 37˚C.

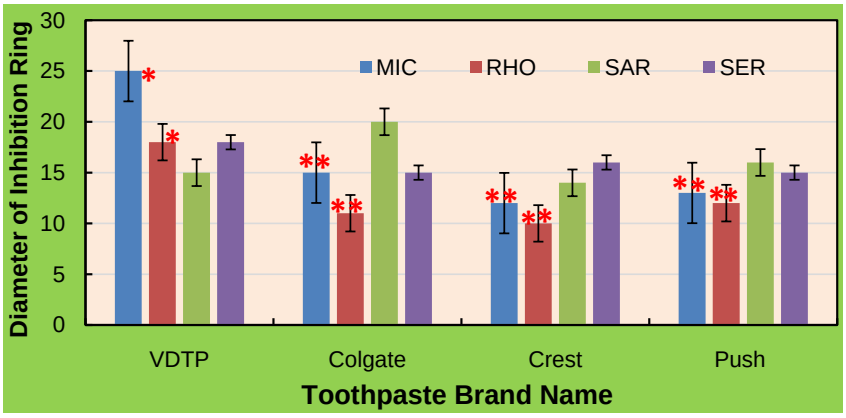

Figure 9. The diameter of the inhibition ring from four strains of bacteria of the 48-hour incubation study (n = 4). The diameter with one asterisk was significantly different from that with two asterisks ($P < 0.05$).

When a high concentration of particles is placed outside the L. terrestris skin, some osmotic pressure must be established, and materials inside the skin should be sucked out. If there existed any transport capability increased with penetration enhancers such as azone and SDS. Sixteen L. terrestris were divided into four groups and placed into 200 ml beakers with appropriate labels on the glass surface after weighing their body weight. Subsequently, 2.0 grams of each toothpaste sample was rubbed around their body and left at room temperature. After 30 minutes, their body weight was measured again. The body weight difference was calculated and converted into % weight change, as presented in **Figure 10**.

3.10. Mass Transport Study with TEER Values across L. Terrestris Skin

Transepithelial electrical resistance (TEER) is a widely accepted quantitative technique to measure the integrity of tight junctions in cell culture models of endothelial and epithelial monolayers for drug delivery studies [33]. TEER values' fundamental principles might differ from the technique used here in this study. However, the application to the skin of L. terrestris could be applicable since the L. terrestris skin is also epithelial tissue, similar to other animals. When testing the delivery capability in a preliminary study, it was confirmed that the slope of the decreasing TEER value from a regression line was inversely related to the concentration of penetration enhancers. **Figure 11** shows that the TEER

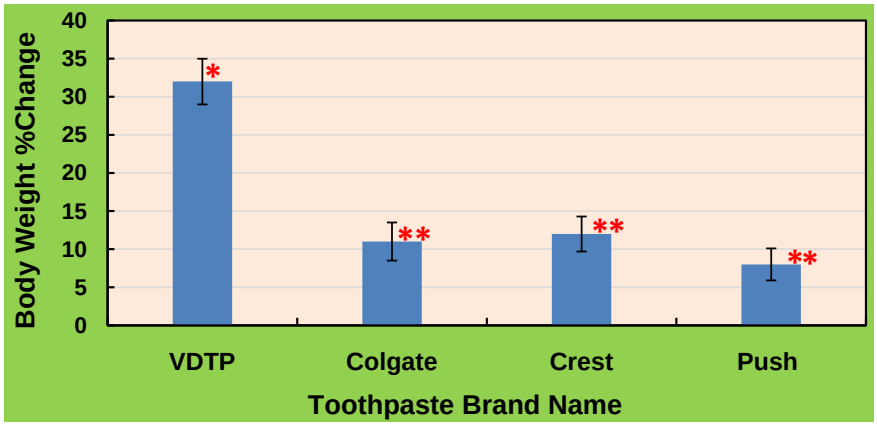

Figure 10. The body weight %change measured 30 minutes after the skin coating with toothpaste. The weight %change with one asterisk significantly differed from that with two asterisks ($P < 0.05$).

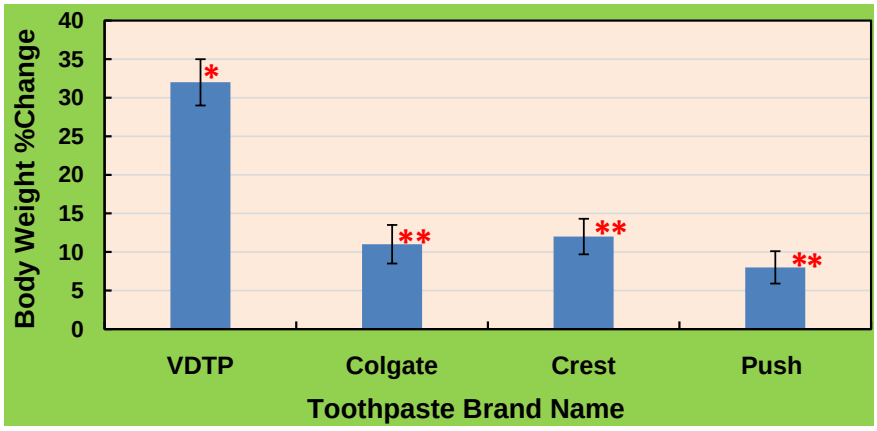

Figure 11. The voltage change rate across the L. terrestris skin as TEER values. The TEER values with one asterisk differed significantly from those with two asterisks ($P < 0.05$).

slope was significantly greater in the group of VDTP than in those from other groups. The data demonstrated that the VDTP toothpaste had high feasibility of delivering the vitamin into the body of L. terrestris. The feasibility of human tissue should be tested for later study.

4. Conclusions

Functional toothpaste has become more popular recently, compared to the traditional roles of removing dietary debris inhibiting bacterial growth and preserving whiter teeth color. Our study attempted to create a toothpaste formulation that might facilitate the intraoral delivery of vitamin D into the bloodstream to treat the widespread deficiency in 42% of the American population today. Taking vitamin D tablets regularly is not easy, but it might be possible by simply brushing our teeth using intraoral toothpaste.

We did various toothpaste characteristic tests such as abrasiveness, scratchiness, spreadability, pH, foaming, cleaning, and antibacterial strength with our vitamin D toothpaste compared with those of other commercial brand tooth-

paste. An earthworm skin test and TEER measurement were conducted using L. terrestris skin to confirm the intraoral delivery of vitamin D through the oral cavity. As a result, our data demonstrated the high feasibility of intraoral delivery of the vitamin. Further, the study supported that our vitamin D toothpaste had comparable characteristics with those from other commercial kinds of toothpaste.

Conflicts of Interest

All authors of submitting the manuscript to the journal disclosed any and all conflicts of interest with the publication of the manuscript in the manuscript.

References

[1] Ross, A.C., Taylor, C.L., Yaktine, A.L., *et al.* (2011) Dietary Reference Intakes for Calcium and Vitamin D. National Academies Press, Washington DC.

[2] Szymczak-Pajor, I., Miazek, K., Selmi, A., Balcerczyk, A. and Śliwińska, A. (2022) The Action of Vitamin D in Adipose Tissue: Is There the Link between Vitamin D Deficiency and Adipost Tissue-Related Metabolic Disorders? *International Journal of Molecular Sciences*, **23**, Article No. 956. https://doi.org/10.3390/ijms23020956

[3] Prietl, B., Treiber, G., Pieber, T.R. and Amrein, K. (2013) Vitamin D and Immune Function. *Nutrients*, **5**, 2502-2521. https://doi.org/10.3390/nu5072502

[4] Martens, P.-J., Gysemans, C., Verstuyf, A. and Mathieu, C. (2020) Vitamin D's Effect on Immune Function. *Nutrients*, **12**, Article No. 1248. https://doi.org/10.3390/nu12051248

[5] Bikle, D.D. (2000) Vitamin D: Production, Metabolism and Mechanisms of Action. In: Feingold, K.R., Anawalt, B., Boyce, A., *et al.*, Eds., *Endotext* [*Internet*], MDText.com, Inc., South Dartmouth.

[6] Nair, R. and Meseeh, A. (2012) Vitamin D: The "Sunshine" Vitamin. *Journal of Pharmacology and Pharmacotherapy*, **3**, 118-126.

[7] U.S. Department of Agriculture and U.S. Department of Health and Human Services (2020) Dietary Guidelines for Americans. 9th Edition, USDA, Washington DC, 2020-2025.

[8] Laird, E., Ward, M., McSorley, E., Strain, J.J. and Wallace, J. (2010) Vitamin D and Bone Health: Potential Mechanisms. *Nutrients*, **2**, 693-724. https://doi.org/10.3390/nu2070693

[9] Kubala, J. and Ware, M. (2022) What Are the Health Benefits of Vitamin D? Medical News Today. https://www.medicalnewstoday.com/articles/161618#dosage

[10] NHS (2021) Overview: Rickets and Osteomalacia. NHS. https://www.nhs.uk/conditions/rickets-and-osteomalacia/

[11] Office of the Surgeon General (US) (2004) Bone Health and Osteoporosis: A Report of the Surgeon General. Office of the Surgeon General, Rockville. https://www.ncbi.nlm.nih.gov/books/NBK45506/

[12] Boron, J.B., Rogers, W.A. and Fisk, A.D. (2013) Everyday Memory Strategies for Medication Adherence. *Geriatric Nurses*, **34**, 395-401. https://doi.org/10.1016/j.gerinurse.2013.05.010

[13] Homayun, B., Lin, X. and Choi, H.J. (2019) Challenges and Recent Progress in Oral Drug Delivery Systems for Biopharmaceuticals. *Pharmaceutics*, **11**, Article No. 129.

https://doi.org/10.3390/pharmaceutics11030129

[14] Hua, S. (2019) Advances in Nanoparticulate Drug Delivery Approaches for Sublingual and Buccal Administration. *Frontier of Pharmacology*, **5**, Article 1328. https://doi.org/10.3389/fphar.2019.01328

[15] Fallingborg, J. (1999) Intraluminal pH of the Human Gastrointestinal Tract. *Danish Medicine of Bulletin*, **46**, 183-196.

[16] Markovic, M., Zur, M., Garsiani, S., Porat, D., Cvijić, S., Amidon, G.L. and Dahan, A. (2022) The Role of Paracellular Transport in the Intestinal Absorption and Biopharmaceutical Characterization of Minoxidil. *Pharmaceutictics*, **14**, Article No. 1360. https://doi.org/10.3390/pharmaceutics14071360

[17] Narang, N. and Sharma, J. (2011) Sublingual Mucosa as a Route for Systemic Drug Delivery. *International Journal of Pharmacy and Pharmaceutical Sciences*, **3**, 18-22.

[18] Al-Madhagi, W., Albarakani, A.A., Alhag, A.K., Saeed, Z.A., Noman, N.M. and Mohamed, K. (2017) Formulation and Evaluation of New Glimepiride Sublingual Tablets. *Journal of Pharmacology*, **2017**, Article ID: 3690473. https://doi.org/10.1155/2017/3690473

[19] Dhangar, R.S.R., Patil, S.T. and Pawar, S.P. (2017) Sublingual: A Route for Systemic Drug Delivery System. *International Journal of Pharma and Chemical Research*, **3**, 301-306.

[20] Prajapati, S.T., Patel, P.B. and Patel, C.N. (2012) Formulation and Evaluation of Sublingual Tablets Containing Sumatriptan Succinate. *International Journal of Pharmaceutical Investigation*, **2**, 162-168. https://doi.org/10.4103/2230-973X.104400

[21] Amrein, K., Scherkl, M., Hoffmann, M., Neuwersch-Sommeregger, S., Köstenberger, M., Berisha, A.T., Martucci, G., Pilz, S. and Malle, O. (2020) Vitamin D Deficiency 2.0: An Update on the Current Status Worldwide. *European Journal of Clinical Nutrition*, **74**, 1498-1513. https://doi.org/10.1038/s41430-020-0558-y

[22] Han, Z., Margulies, S.L., Kurian, D. and Elliott, M.S. (2016) Vitamin D Deficiencies in Patients with Disorders of the Digestive System: Current Knowledge and Practical Considerarations. In: Parrish, C.R., Ed., *Nutrition Issues in Gastroenterology, Series No.* 153, Practical Gastroenterology, New York, 36-43.

[23] Lupoli, R., Lembo, E., Saldalamacchia, G., Avola, C.K., Angrisani, L. and Capaldo, B. (2017) Bariatric Surgery and long-Term Nutritional Issues. *World Journal of Diabetes*, **8**, 464-474. https://doi.org/10.4239/wjd.v8.i11.464

[24] Pilz, S., Zittermann, A., Trummer, C., Theiler-Schwetz, V., Lerchbaum, E., Keppel, M.H., Grübler, M.R., März, W. and Pandis, M. (2019) Vitamin D Testing and Treatment: A Narrative Review of Current Evidence. *Endocrine Connections*, **8**, R27-R43. https://doi.org/10.1530/EC-18-0432

[25] Šimoliūnas, E., Rinkūnaitė, I., Bukelskienė, Ž. and Bukelskienė, V. (2019) Bioavailability of Different Vitamin D Oral Supplements in Laboratory Animal Model. *Medicina*, **55**, Article No. 265. https://doi.org/10.3390/medicina55060265

[26] Rémond, D., Shahar, D.R., Gille, D., Pinto, P., Kachal, J., Peyron, M.-A., Dos Santos, C.N., Walther, B., Bordoni, A., Dupont, D., Tomás-Cobos, L. and Vergères, G. (2015) Understanding the Gastrointestinal Tract of the Elderly to Develop Dietary Solutions That Prevent Malnutrition. *Oncotarget*, **6**, 13858-13898. https://doi.org/10.18632/oncotarget.4030

[27] Ghaffarian, R. and Muro, S. (2013) Models and Methods to Evaluate Transport of Drug Delivery Systems across Cellular Barriers. *Journal of Visual Experiment*, **17**, e50638. https://doi.org/10.3791/50638

[28] Sugibayashi, K., Nakayama, S., Seki, T., Hosoya, K. and Morimoto, Y. (1992) Mechanism of Skin Penetration-Enhancing Effect by Laurocapram. *Journal of Pharmaceutical Sciences*, **81**, 58-64. https://doi.org/10.1002/jps.2600810112

[29] Dahlgren, D., Sjoblom, M., Hederland, M. and Lennernas, H. (2020) The *in Vivo* Effect of Transcellular Permeation Enhancers on the Intestinal Permeability of Two Peptide Drugs Enalaprilat and Hexarelin. *Pharmaceutics*, **12**, Article No. 99. https://doi.org/10.3390/pharmaceutics12020099

[30] Vranić, E., Lačević, A., Mehmedagić, A. and Uzunović, A. (2004) Formulation Ingredients for Toothpastes and Mouthwashes. *Bosnian Journal of Basic Medical Sciences*, **4**, 51-58. https://doi.org/10.17305/bjbms.2004.3362

[31] Gavic, L., Gorseta, K., Borzabadi-Farahani, A., Tadin, A. and Glavina, D. (2018) Influence of Toothpaste pH on Its Capacity to Prevent Enamel Demineralization. *Contemporary Clinical Dentistry*, **9**, 554-550. https://doi.org/10.4103/ccd.ccd_667_18

[32] Renvert, S., Noack, M.J., Lequart, C., Roldan, S. and Laine, M.L. (2020) The Underestimated Problem of Intra-Oral Halitosis in Dental Practice: An Expert Consensus Review. *Clinical Cosmetic Investigation Dentistry*, **12**, 251-262. https://doi.org/10.2147/CCIDE.S253765

[33] Srinivasan, B., Kolli, A.R., Esch, H.E., Hasan Erbil Abaci, H.E., Shuler, M.L. and Hickman, J.J. (2015) TEER Measurement Techniques for in Vitro Barrier Model Systems. *Journal of Laboratory Automation*, **20**, 107-126. https://doi.org/10.1177/2211068214561025