

## Evaluation of Intraoral Vitamin D Toothpaste Formulation with Polysorbate 80 and Dimethyl Sulfoxide

Jaeseok Yoon and Jongbin Lee\*

\*Biomedical Research Division, STEM Science Center 111, Charlotte Place Ste 100, Englewood Cliffs, NJ 07632, USA.

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### ABSTRACT

Booming in the oral care industry and exponentially growing by advanced and functional items, the most popular types of toothpaste have recently become more remarkable in their active roles and functionality. Vitamin D deficiency has been diagnosed in 42 percent of Americans, irrespective of age and region. Regularly taking vitamin D is usually a recommendation of healthcare professionals, but taking the daily doses with remembering would be nearly impossible. In contrast, it is a fantastic idea if teeth brushing could guarantee delivering the daily vitamin requirements. The study aimed to formulate a toothpaste for delivering vitamin D into the bloodstream intraorally to alleviate the widespread vitamin D deficiency. In this study, an emulsion-based toothpaste blended with polysorbate 80 and Dimethyl sulfoxide (DMSO) in addition to the other compositions and then with vitamin D. Traditional testing methods for toothpaste characteristics included abrasiveness, scratchiness, pH, spreadability, cleaning, foaming ability, and antibacterial strength with the vitamin D toothpaste compared with those of another commercial brand toothpaste. An earthworm transport study and transepithelial electrical resistance (TEER) value test was conducted using *L. terrestris* skin to examine the feasibility of vitamin D transport across through the oral cavity. Our results confirmed the high feasibility of transport of vitamin D intraorally; considering those two skin studies with various experimental support with Student t-tests ( $P < 0.05$ ), our vitamin D toothpaste had favorable characteristics with other commercial products for different functionality.

**Keywords:** Drug delivery system, Functional toothpaste, Intraoral delivery, Pharmaceutical transport, Vitamin D delivery

**Abbreviations:** DMSO: Dimethyl Sulfoxide; GI: Gastrointestinal; GMO: Genetically Modified Organism; IOVD: Intraoral Vitamin D; MIC: *Micrococcus Luteus*; RHO: *Rhodococcus Rhodochrous*; SAR: *Sarcina Aurantiaca*; SER: *Serratia Marcescens*; TEER: Transepithelial Electrical Resistance; USDA: United States Department of Agriculture

### INTRODUCTION

Vitamin D falls in the category of the fat-soluble vitamin group. Vitamin D acquired from foods and nutritional supplements is accumulated in the mass of fat for emergencies [1,2]. During the vitamin D circulates in the bloodstream in deficient amounts, acquiring the calcium needed in the body is complicated. Vitamin D assumes an essential role in cell growth and immunity, anti-inflammation in management, and the nerve system operating appropriately [3,4]. Furthermore, the liver and kidneys aid in changing vitamin D to the final active product, 1,25-dihydroxy vitamin D [5]. In practical situations, vitamin D required for a year can be generated in a short period by direct sunshine bathing a couple of times every week during the sunny summer seasons. However, the majority of populations do not have enough vitamin D since their skin cannot absorb it and, further, because they do not stay long enough outdoors [6]. Vitamin D can be acquired from food and dietary supplements, but typical people do not

take enough of it from these sources. The United States Department of Agriculture (USDA)'s 2022 dietary guidelines for Americans alerted the public, reporting that most children and adults do not produce much up to the daily essential vitamin D level [7]. Adequate vitamin D levels are required for strong bones. Even minor vitamin D deficiency can influence bone strength because it forces its physiological mechanism to take calcium from bones to maintain homeostasis [8]. As a result, various bone pathological problems, i.e., rickets, afflict newborns and

**Corresponding author:** Jongbin Lee, Biomedical Research Division, STEM Science Center 111 Charlotte Place Ste 100, Englewood Cliffs, NJ 07632, USA, Tel:(845)242-7422; E-mail: JLee@STEMsc.org

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toddlers and may aggravate them because of weak bone formation. Grown-ups can set in osteomalacia that initiates bone and muscular discomfort; osteoporosis results in bone thinning and bone density loss; fracture risk increases [9,10]. Intraoral delivery was researched first with the concept of aerosol spray that was beneficial for various reasons, including the on-site release of medications in a tiny amount that could be readily delivered by the cheek mucosa and the direct and quick release of the uniquely formulated solutions [11,12]. This fact is well comprehended that the medication encounters numerous damaging obstacles for oral administration via the gastrointestinal (G.I.) tract prior to being absorbed by the epithelial system. A dramatic shift in G.I. pH is brought out from stomach pH 1.0 - 2.0 to pH 7.0 - 7.4 in the intestines and changes in many breakdown enzymes and intestinal environment [13,14]. Contrary to this hostile environment, molecular transport across the intraoral epithelium provides somewhat stable and relaxing physiological conditions. The majority of pharmaceutical scientists on buccal delivery find that the prevailing mechanism might derive from the passive transport through lipid membranes via paracellular and transcellular channels [15]. Sublingual administration involves depositing the drug on the tongue, which then enters the bloodstream via the tongue's ventral surface and the mouth's floor [16]. The pharmaceutical molecules are quickly transferred by the reticulated vein located beneath the oral mucosa. Tablets ingested through the sublingual pathway react quicker compared to those received through the oral route. Furthermore, the drug molecules introduced via sublingual vasculatures are more accumulated than the fraction absorbed via the hepatic first-pass metabolic pathway, i.e., oral absorption [17,18]. The preferential method for pharmaceutical delivery into the oral tissue depends on passive diffusion through the lip membrane layer. Most pharmaceutical molecules are passed through the sublingual route without the first-pass effect [19]. Though sublingual delivery from the intraoral epithelium carries vitamin D straight into the systemic bloodstream, orally ingested vitamin D is transported into the portal circulation across the intestinal tracks which collects it to the liver prior to before distributing it throughout the body. Vitamin D uptake from the G.I. tract is inefficient, with local intestinal breakdown and hepatic metabolism occurring [20]. People with drug digestion issues and those with stomach bypass surgery, particularly Lap-band surgeries, may have trouble absorbing vitamin D through their intestines [21]. Medical professionals have initiated alarming messages to reduce vitamin D deficiency problem, which is far more occurrences than estimated. In this study, two penetration enhancers were adjuvanted to maximize vitamin D delivery using their synergistic effects. The first one was the polysorbate 80, also called another way, tween 80, which was considered to increase the transport of vitamin D through the lipophilic and the hydrophilic molecular principles that may break the lipid layer in the stratum

corneum and increase the water content of the proteins in the barrier. The Tween 80's structure is suitable for this type of role and play [22]. Polysorbate 80 is recognized to become a nonionic surfactant in the professions of many pharmaceutical products. They increase the permeable capability of phospholipid bio membranes, creating the transport of low molecular mass compounds. Interaction between bio membranes and polysorbate 80 has been found to be in a 2-4- to 4-times increase in the synthesis rates of phospholipids, suggesting that the polysorbates cause some damage to epidermal membranes [22]. Polysorbate 80 also may create a modification in the physicochemical integrities of bio membranes, particularly an enhancement in the penetration ability of the sarcoplasmic reticulum [23]. The faster penetration of the drug through the dermal tissue might be explained by the presence of polysorbate. The adsorption and admission of therapeutic molecules onto the epithelial surface may bring in thermodynamic activity change to be high as the driving force for molecule transport. Additionally, the molecular effect of penetration agents reduces the hindrance barrier of the stratum corneum. Further, the subtle change in the complicated network of the stratum corneum may set in a weakening condition for the cellular lipid layer of the stratum corneum and finally induce more manageable by the permeation enhancers [24]. The polysorbate effect may be due to skin barrier properties changes and the vehicle stratum corneum partition coefficient. Sarpotdar and Tatz [25] found an enhancement in the transdermal flux of hydrocortisone in the presence of nonionic surfactants. Polysorbate 80 was recognized to enhance the dermal permeability of hydrocortisone and lidocaine. Multiple compounds are claimed to modify the epithelial barrier function and make the penetration of therapeutic agent molecules possible to transport. Among the penetration helpers, urea and its derivatives alkyl sulfoxides, dimethyl sulfoxide (DMSO), surfactants, and oleic acid have been studied [26]. The concentration of DMSO has a proportional relation to the effects of penetration, and it has been suggested that concentrations of approximately 60% in the composition are needed to maintain a promising enhancement [27]. DMSO is also known to be effective not only for hydrophilic but also for hydrophobic chemicals. Even though DMSO's penetration enhancement capability has been researched comprehensively, a detailed mechanism in its molecular mode of action has been unexplored. The mutual interaction of DMSO with biolipids must be understood to further enhance capability and minimize tissue integrity disruption. It has been claimed that DMSO enhances lipid fluidity by opening up the walled barriers of the lipid chains to increase molecular transport [28]. DMSO is also recognized to bind with biolipids and is widely utilized in pharmaceutical formulations for its ability to initiate differentiation and cell fusion [29]. Vitamin D should be taken daily. However, many cannot keep the routine from skipping by simply forgetting. Blending the most conventional ingredients, our

toothpaste was adjuvanted with vitamin D and penetration enhancers such as polysorbate 80 and DMSO. A series of crucial tests were executed to confirm the toothpaste's quality and its transport. The study might contribute to formulating therapeutic toothpaste that can carry pharmaceutical agents into the bloodstream.

## EXPERIMENTAL METHODS

### Materials and Reagents

Made from California Estate Extra Virgin Olive Oil, Olive oil was produced in St. Lauderdale (F.L., USA) and purchased from a supermarket (Stop and Shop, New City, NY). The Emulsifying Wax NF, Non-GMO Premium Quality Polysorbate 60/ Pola wax 80 oz / 5 Pound was obtained at Plant Guru (Plainfield, NJ). Reverse Osmosis filtering water was purchased from Poland Water Supply (Palisades Park, NJ). Other vital ingredients, i.e., maltitol, were bought from Sigma Aldrich (St. Louis, MO). Further, Sodium Bicarbonate from Bulk Supplements ScienceLab.com Inc (Houston, TX), Vitamin D (cholecalciferol-D), Sodium Dodecyl Sulfate, Polysorbate 80 from the Spectrum Chemical Corp (New Brunswick, NJ). Hydrogen peroxide from Wall Green Pharmacy Inc., Thera Breath Oral Care Probiotics Citrus from Amazon.com, and Multiple Food Color (Net 1 Fl Oz 29 mL) from Earth Health, Inc. (Hunt Valley, MD). DMSO was obtained from Sigma Aldrich (St. Louis, MO). Three commercial toothpastes were obtained and used to examine our formulation, comparing their characteristics for our intraoral vitamin D toothpaste. The Colgate Toothpaste Total S.F. was created for antigingivitic, anticavity, and non-sensitivity toothpaste with stannous fluoride. Secondly, the Crest brand toothpaste, formulated for tartar protection (Sensitive paste) with fluoride anticavity ability, was bought from the local marts (CVS, Orangeburg, NY). The third brand was Meich PUSH toothpaste (Broadway Drug Store, NJ).

### Emulsion Formulation

The formulation and testing methods were published previously for other types of penetration enhancers [30]. Glassware and experimental supplies were collected. Two 300 mL beakers were gathered, and the beakers were written as aqueous and oily phases on the surface. One hundred twenty milliliters of distilled water were poured into the aqueous beaker, and 20 mL of olive oil, 30 mL of polysorbate 80, and 45g of emulsifying wax were measured and poured into the oily marked beaker. Then, the beakers were moved onto a stainless-steel container with water with a jacket and heated to a yellowish melting solution at approximately 70 degrees Celsius. After confirming a complete melting in the oily phase container, the contents of the water-phase beaker were combined into a mixing ball crystal glass cup. The ingredients in the crystal glass cup were then brought to an electric stirring of 1500 ~ 2000 rpm,

adjusting its intensity according to the thickness of the mixture for approximately 60 min.

### Intraoral Formulation for Vitamin D Delivery (IOVD)

While the mixture was agitated, other liquids were measured out, and granular chemicals were taken out into a 500 mL beaker. The contents as seen in **Table 1** below were collected and ground manually into powder form using a plastic scoop. Those ingredients were 5.0 g sodium bicarbonate, 4.0 g Silica, and 3 g of Vitamin D. The beaker was brought into the stirrer approximately for sixty minutes and combined with the contents in a round-bottom glass cup.

**Table 1.** The compositions of our IOVD blended in the toothpaste base emulsion.

Ingredients	Functions	Amount	Wt%
DMSO	Delivery Agent	3.5 mL	2.78
Drug	Vitamin D	3.0 g	2.38
Emulsifying Wax	Emulsion Medium	4.5 g	3.57
Glycerin	Humectants	10.0 mL	7.94
Hydrogen Peroxide	Antibacterial	6.0 mL	4.76
Malititol	Binder	6.0 g	4.76
Polysorbate 80	Delivery Agent	5.0 mL	3.97
Salts	Buffers/ Salts	4.0 g	3.17
Silica, Fumed	Tartar Contron	4.0 g	3.17
Sodium Bicarbonate	Abrasives	20.0 g	15.87
Sodium Dodecyl Sulfate	Surfactants	5.0 g	3.97
Water	Medium	55.0 mL	43.65
Total		126.0 g	100 %

After that, 10.0 mL of glycerin and 6.0 mL of hydrogen peroxide were added and mixed. Further, the thoroughly blended final product was divided into two groups, where the first portion was formulated with 3.50 mL DMSO.

### Quality Evaluation of Toothpaste

#### Stock solution preparations

Brought in 100 mL distilled water, the 4.0 g toothpaste was weighed out into a 300 ml container as in **Figure 1**. Subsequently, the container with the toothpaste was utterly dispersed by a stirring bar on an electric magnetic stirrer. The other stock solutions were created with identical procedures for comparison groups. Then, a magnetic bar was

dropped into the containers, which then brought on a magnetic stirrer and stirred for approximately 20 min to attain homogeneous dissolution. The stock solutions were consumed for pH evaluation and foaming capability evaluations. The stock solutions were placed in the cold refrigerator (4°C) prior to being used.



**Figure 1.** The creation of stock solution on the magnetic stirrer.

### **Abrasiveness degree Score evaluation**

Approximately 2.5 cm of toothpaste was obtained from its tube and placed onto a piece of waxed paper. Each stripe of toothpaste was smeared with the middle finger for the length of approximately 5.0 cm to closely observe the edges of rough and sharp-edged portions with soft and runny edge shapes. The degree of the sharp-edged profile was carefully observed and scored from 0 - 5; 0 for smooth edges while 5 for rough grainy edges.

### **Scratchiness degree evaluation**

The scratchiness evaluation was carried out as follows: 2.0 g of each toothpaste was squeezed out from the tube on a sterilized, individualized wrapped petri dish, and 200 ul distilled water was dropped over the sample using an Eppendorf pipette as in **Figure 2**. The toothpaste on the petri dish was stroked back and forth using a sterilized cotton ball, 5.0 centimeters, approximately thirty times. Then, the toothpaste was utterly washed out from the petri dish using

running tap water and thoroughly dried with a paper towel. The scratchy surface created on the petri dish was then carefully observed under a digital microscope to grade the degrees of scratches on the surface. The degree of scratches was then graded on a score, from 0 when no scratches existed to 5 when highly outstanding scratches were over the surface. The evaluation was carried out five times for each toothpaste brand.

### **Spreadability evaluation**

A toothpaste of 2.0g was placed on the electric balance to weigh out by pushing from the tube and distributed on the plexiglass panel. And then, an additional plastic plate was prepared to cover the toothpaste. Then, a 500g standard weight was used to press the toothpaste on the plexiglass plate for 20.0 min as in **Figure 3**. Immediately after removing the weight standard, the diagonal and its vertical lengths were evaluated with a ruler. Its size was measured as mean and standard deviation.



**Figure 2.** Presents the scratchiness test with Petri Dish and sterile cotton swabs on toothpaste samples.



**Figure 3.** Presents the spreadability evaluation of toothpaste on a slide glass with standard weights.

**pH Measurement**

The Thermo Scientific A111 pH meter was calibrated first regarding pH 4.0 and 10.0 according to the vendor's user guidelines explained. A 30 mL toothpaste stock solution was acquired and brought into 150 ml beakers before the pH measurement. After setting up all necessary preparations, the pH was read after the pH electrode was slowly inserted into

the stock solution with the confirmation of room temperature.

**Foam-generating ability evaluation**

Thirty millimeters of the stock solutions of each toothpaste prepared in advance were poured into 200 ml graduated cylinders. The height of the stock solution was marked on the surface. The cylinder was subsequently moved back and

forth 20 times vigorously with its mouth closed with a piece of thin plastic wrap and pressed down with a thumb finger so as not to spill as in **Figure 4**. The graduated cylinder was

then put on a flat, balanced table surface, and the foam's length in the cylinder was evaluated.



**Figure 4.** Presents the pictorial presentation of foaming tests by shaking a 100 mL graduated cylinder filled with toothpaste stock solution.

#### Tooth cleaning ability evaluation

The toothpaste's cleaning ability was examined for the toothpaste's feasibility to rid the red food dye colored on the skin of eggs. The procedure was performed with four eggs boiled in distilled water with red food coloring dye to paint the surface as red for 25 min and left them to cool down at room temperature for 20 min. After that, a mid-line was marked on the surface of the eggshells, dividing it in half

with a permanent marker. To mimic the typical toothbrushing manner, the toothbrush was made wet with water, and removed any surplus water. On one side of each colored egg, 25 reciprocating toothbrush movements of 6.0 cm in length were repeated for each of the toothpastes of the comparative groups as in **Figure 5**. The magnitude of the region at which color was seen to fade out was visually outlined and estimated for its shortest and longest length as centimeters to quantify the area of each cleaned-out contour.

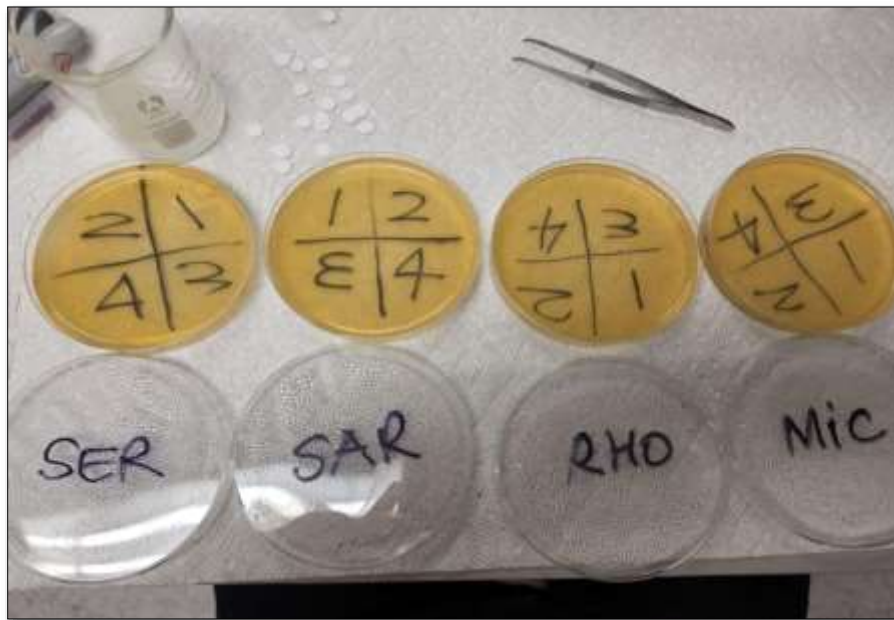


**Figure 5.** The toothpaste cleaning test with boiled eggs and toothbrush on their surfaces.

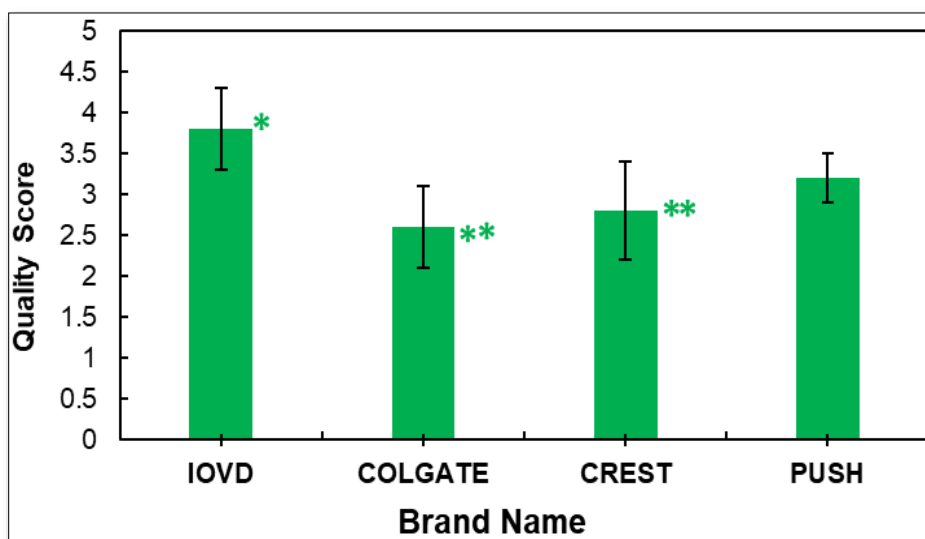
**Antibacterial strength evaluation with the ring of inhibition**

A set of bacterial cultures were obtained from Carolina Biological Supply. The strains were identified with the Gram-stain method, since it was the first step in defining a bacterial family. First, the pigmented bacteria strains were *Micrococcus luteus* (MIC), as yellow as MIC, *Rhodococcus rhodochrous* (RHO), as pink as RHO, *Sarcina aurantiaca* (SAR), as orange-yellow as SAR, and *Serratia marcescens* (SER) D1, as red as SER for the differentiating methods of procedures as in **Figure 6**. Four Petri dishes were divided

into four quarters: 1, 2, 3, and 4, and labeled accordingly. The bacteria were inoculated onto the Petri dishes already coated with nutrient agar with an inoculation wire loop. And the stock solutions of the four kinds of toothpaste were soaked into the circular paper disks that were prepared in advance. The disk was placed with the toothpaste applied on the four areas as soaked and written: 1 - IOVD, 2 - Colgate, 3 - Crest, and 4 - Push toothpaste, as found in **Figure 7** below. The bacterial culturing procedures were performed in a Heratum Incubator (Thermo Scientific, Waltham, MA) for 72 h.



**Figure 6.** The Petri dish’s bottom surface was marked by color lines drawn with a permanent marker into four areas and brought into an incubator. The bacteria with paper discs were immersed in the toothpaste solutions.



**Figure 7.** The mean abrasive ability was significantly greater than that of the Colgate and Crest groups (n=4). The score with one asterisk was statistically different from that with two asterisks (P<0.05).

### Indirect skin transport evaluation with *Lumbricus terrestris*

Sixteen *L. terrestris* were grouped into four groups in 250 ml beakers with labels on the surface after measuring their body weight. Subsequently, 3.0 gms toothpaste was applied around their skin. Their body was weighed again immediately after 1.0 h. The body weight difference was evaluated and reported as weight change%.

### Evaluation of transepithelial electrical (TEER) values

Many drug delivery groups in the pharmaceutical company usually evaluate the TEER parameters to measure the movement of natural and synthetic elements of interest. The opening of tight junctions might be momentarily and almost instantaneously without adverse effects under the presence of penetration enhancers [31]. This transport can be evaluated by monitoring the transepithelial electrical resistance (TEER) that relates to passive paracellular transport of molecules, whereby reduced resistance to an electrical current increases paracellular transport of an inert compound into the opposite side, indicating the opening of cell junctions. For the study, a dissolved solution was prepared with the toothpaste of 60.0 g mixed with 200 mL of purified water with a reverse osmosis (R.O.) system to create a 20% solution for each toothpaste. The 30-centimeter enamel-coated 10.0-gauge copper wires created two electrodes for positive and negative poles after the coated insulation layer was removed with a typical electrical stripper plier. Both tips of the wire formed a ball shape with soldering, leading to softening their insertion into the *L. terrestris* through its mouth. Data I-245 Data Acquisition System (Dataq Instrument, Ohio) was employed to acquire the reducing resistance across the epithelial layer by the effect of toothpaste solutions. A 9.0 V dry battery was wired during the study to upgrade the electrical potential. Post-completing the procedures in toothpaste solutions and initiating the data acquisition, the earthworm was washed, and its body weight weighed on an electrical balance and brought into a plastic beaker filled with 5% ethyl alcohol for anesthetization. After confirming the unconsciousness, about 8 min, it was taken into the test tube and poured with the toothpaste solution up to 80% of the height of the test tube, 18x150 mm. Subsequently, the positive electrode was cautiously pushed into the mouth of the animal approximately up to the middle length posterior to the clitellum. The other tip of the positive electrode was wired to the matrix and P.C. computer with the positive button of a 9V battery. On the other hand, the negative electrode was just placed into the testing solution of 30 ml wired to the system and the negative tip of the battery. Data were automatically saved while monitoring the data acquisition display mode. The data was played back using the waveform browser software (Akron, OH) after the experiment and transported into M.S. Excel. The data was summarized,

graphed, and analyzed in M.S. Excel with trendline functionality as planned in advance.

### Data analysis and summary

Mean and standard deviation were the summarized methods first. Some qualitative data were graded into a numeric conversion, supporting quantitative comparison between groups. A student t-test was carried out if needed ( $P < 0.05$ ). The TEER slopes were estimated in the spreadsheet in M.S. Excel with the regression analysis.

## RESULTS AND DISCUSSIONS

### Toothpaste Creation Procedures

The toothpaste for intraoral vitamin D delivery was prepared from an emulsion base preparation created with distilled water, olive oil and emulsifying wax. The formulation was completed after thoroughly blending toothpaste ingredients manually. Any granular compositions were homogeneously broken down to powdery in a crystal jar with a large plastic spatula and mixed for 30 min to be like a form of sticky dough. The toothpaste did not have any coloring or flavoring chemicals supported. To increase the user's attraction to toothpaste, decorating a trace of mint fragrance or green dye could be an additional decoration. Other classical toothpaste ingredients, i.e., abrasives, humectants, and binders, were mixed sequentially. To formulate a user-friendly toothpaste, its viscosity and texture were controlled by balancing the optimal amount for each composition.

### Abrasive Ability Examination

Tooth abrasion is a primary toothpaste interest for general consumers. The key degrading characteristic of toothpaste is its abrasiveness. Therefore, trust evaluation and comparison with commercial brand toothpaste are essential. Comprehending the abrasiveness of toothpaste is a must. Some scientists employed a radiometric method for an abrasiveness measurement in silica and calcium carbonate samples for an abrasive in a dentifrice to help in a fantastic choice of materials by dentifrice manufacturers [32]. Patients sometimes like the rough feeling of abrasive particles from toothpaste. **Figure 7** shows that all three other toothpastes did not find abrasive findings. However, our toothpaste presented the feeling of particles that were proven with middle fingers. Our vitamin D toothpaste (IOVD) showed a more significant mean abrasive ability score, in contrast to that of other commercial products, and no statistical difference with the Push toothpaste ( $P < 0.05$ ), as seen in **Figure 7**.

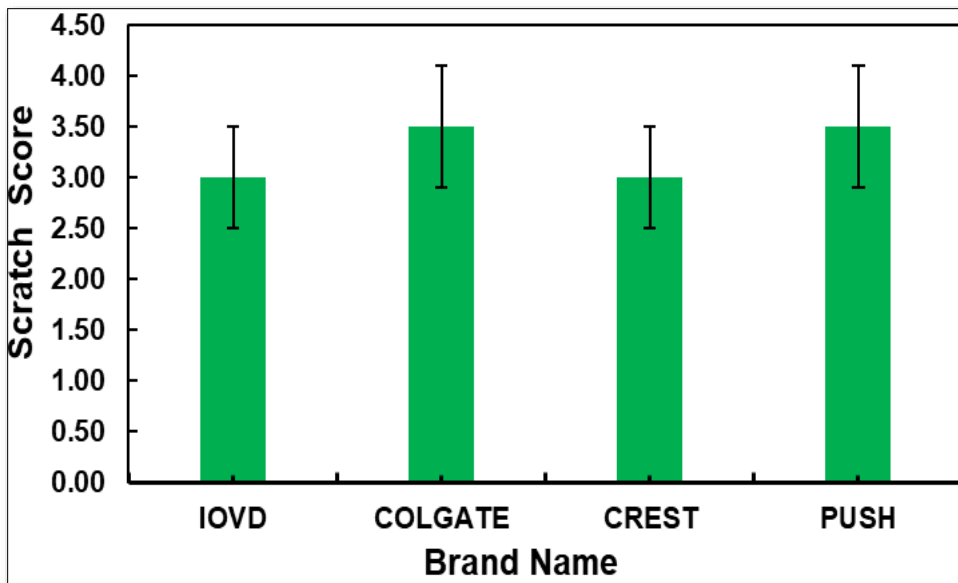
### Petri Dish Scratchy Evaluation

A scratchy test was carried out with a Petri dish. This quality evaluation investigated for the toothpaste to clear off the plaque deposits effectively from the tooth's surface. If the scratch was not visually recognizable enough, the toothpaste should still have high feasibility, not removing some food



debris. However, when the scratchy ability is too excessive, the tooth's surface enamel might be worn out easily. An optimal scratchy ability might be examined when creating

any new toothpaste. The IOVD was demonstrated to be compatible with other commercial products based on our petri dish scratchy evaluation ( $P < 0.05$ ), as given in **Figure 8**.

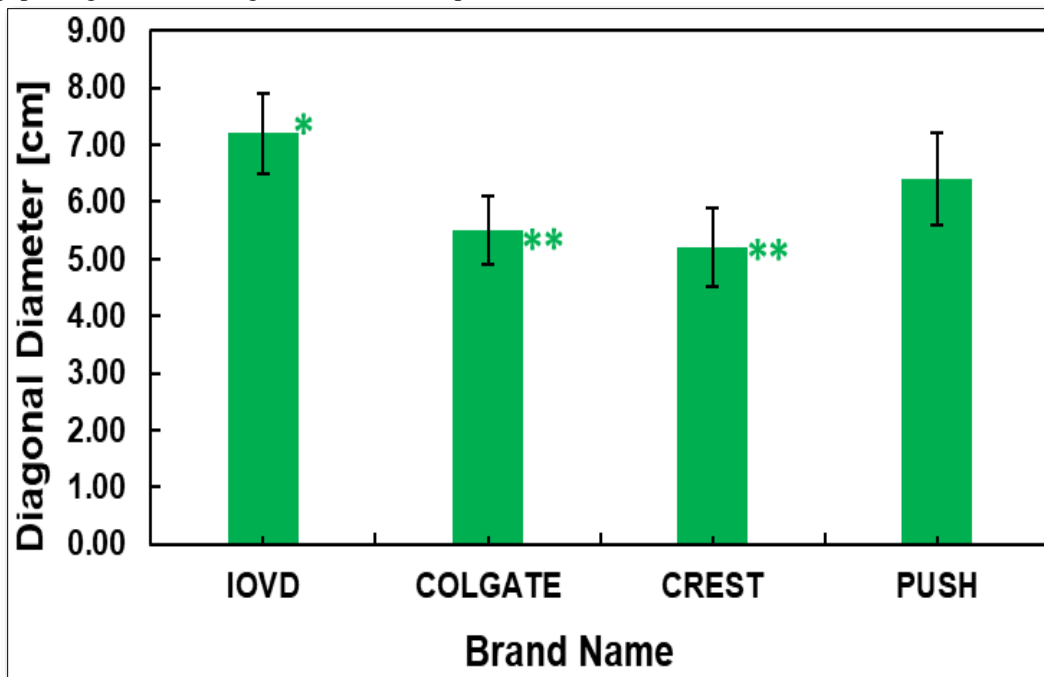


**Figure 8.** Presents the mean scratchy evaluation scores that were not statistically different among the groups (n=4).

**Spreadability Evaluation**

The spreadable ability evaluation was performed to examine how large the diagonal length of the spread toothpaste could be created by placing identical weights over the toothpaste.

The diagonal diameter of the spread might be a significant indicator to estimate because the larger spread could enhance vitamin D transport. Our IOVD toothpaste showed the largest mean diagonal spread ability, as in **Figure 9**.

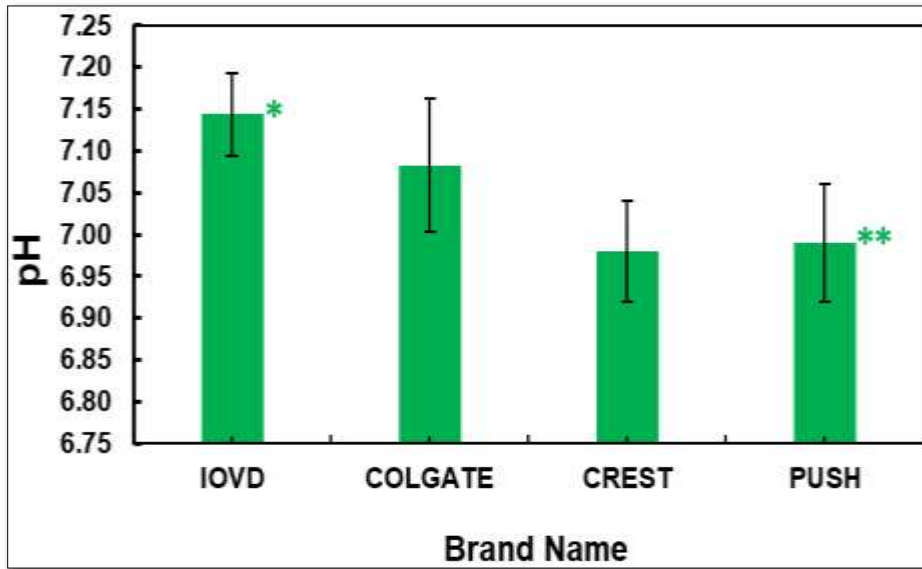


**Figure 9.** Demonstrates that the mean spread ability of IOVD was significantly more extensive than that of the groups from (n=4). The diameters with one asterisk were substantially more significant than those with two asterisks.

**Toothpaste pH Evaluation**

Our teeth' enamel triggers the loss of minerals when the teeth are left at a pH level lower than approximately 5.0. Therefore, the toothpaste pH should be carefully evaluated and controlled accordingly. Using toothpaste with fluoride should be an excellent choice for enamel protection. Daily

and long-term use of toothpaste with low pH might melt down the tooth surface. It has been recommended that the pH might be from an acidic 5.76 to a basic, safer side of 9.68 [33]. Given in **Figure 10**, our IOVD toothpaste pH was as basic as pH 7.14, which is a healthy and safe pH for protecting the enamel layer without harmful effects on the epithelial mucosa of oral cavity.

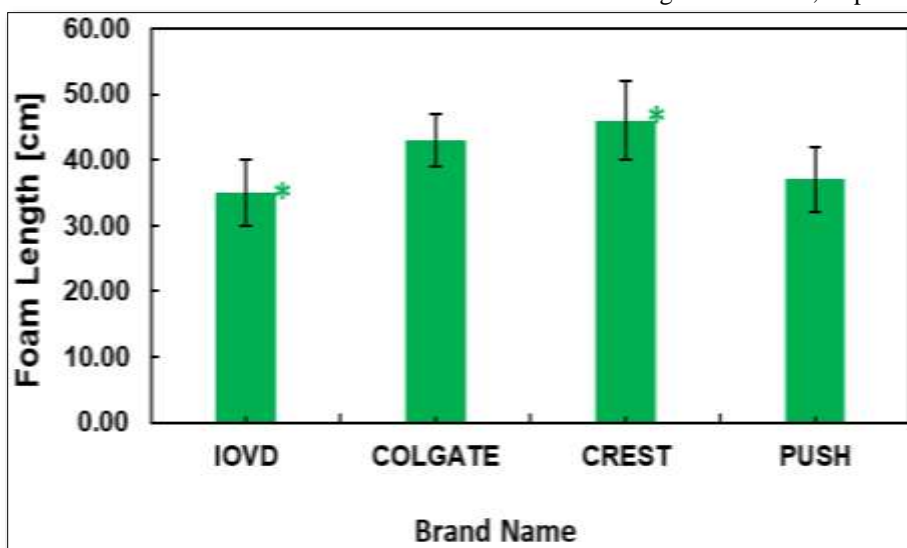


**Figure 10.** Illustrates that the mean pH of IOVD was statistically greater than that of Push brand toothpaste (n=4). The pH with one asterisk was statistically significant compared to that with two asterisks (P<0.05).

**Foaming Ability Evaluation**

Various toothpaste is formulated to increase the hygiene of dental systems using the foaming capability. Toothpaste could generate a soothing feeling in the mouth while brushing. Foam generation enhances its function with better

sensations. Evaluating the foaming ability and foam consistency are essential procedures in manufacturing toothpaste below demonstrates that the mean foam length of IOVD was only statistically greater than that of the Push brand. No statistical difference in foaming ability existed from that of Colgate and Crest, as plotted in **Figure 11**.

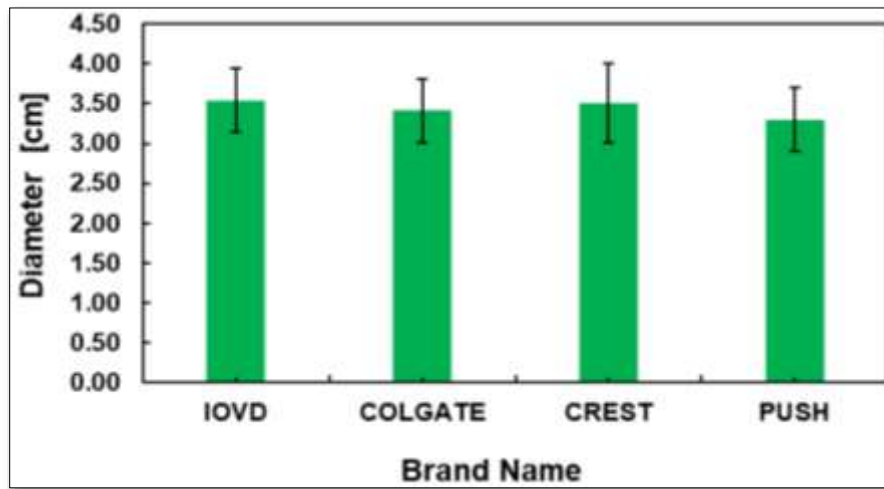


**Figure 11.** Illustrates the mean foam length of IOVD that was statistically larger than that of the Push brand (n=4). The foam height with one asterisk significantly differed from that with two asterisks (P<0.05).

### Cleaning Capability Examination

Toothpaste should be formulated to remove plaques of the teeth more efficiently and effectively for oral health protection from decay. The fundamental but essential characteristics should be kept alive in their functions because most consumers ask the requirement to hold their teeth with stainless and potentially no harmful bacterial growth. Even though other procedures for monitoring cleanliness are documented [34], the cleaning ability should

be verified for any reason. While measuring the cleaning ability with the eggs colored, it appeared difficult to evaluate the cleaning ability of the toothpaste with an apparent definition. Still not for sure, there were improved differentiations that some definition of the discolored region by brushing activities on the surface of the dyed egg was scientifically reasonable. As seen below in **Figure 12**, the cleaning capability presented by the contour of the exposed regions demonstrated that our toothpaste IOVD was similar to that of other commercial products.

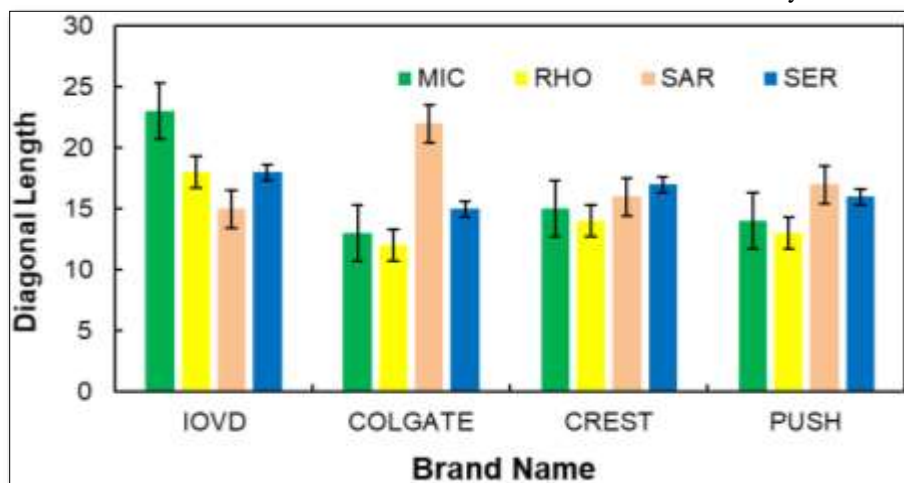


**Figure 12.** The mean diameter that was not significantly different compared to that of the other brands of toothpaste (n=4).

### Antibacterial Strength Evaluation

The four disks wetted in the different types of toothpaste were brought on the surface of the agar layer that was inoculated in advance with four strains of bacteria, as mentioned in the Method section. The diameters of the inhibition ring at the divided sections were measured after 72 h of incubation and summarized as the mean value for

minimizing any errors in the measurement. The data showed that the antibacterial strength of IOVD was confirmed compared to other brands of toothpaste, as seen in the MIC and RHO strains group. **Figure 13** plotted our summarized data on the diameter of the inhibition ring. It was estimated that IOVD has the largest radius among the groups, which showed that IOVD could be able to remove unhealthy bacteria inside the oral cavity.

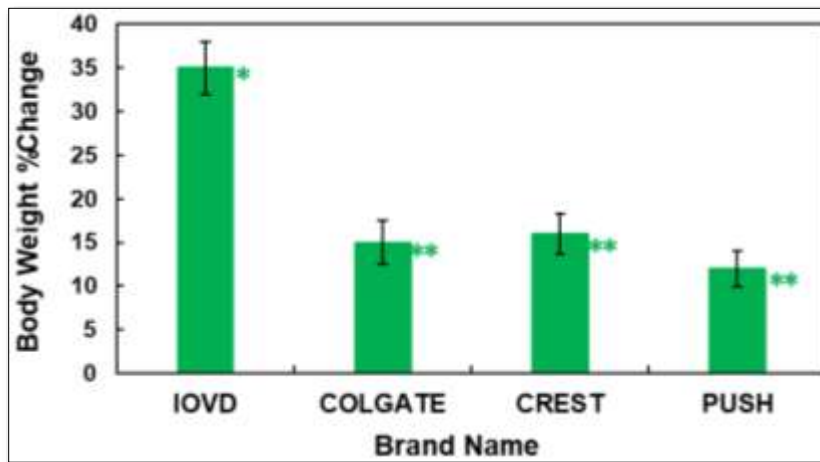


**Figure 13.** The inhibition ring's diameter from four strains of bacteria after 72 h incubation (n=4). The diameter with one asterisk significantly differed from that with two asterisks (P<0.05).

### Mass Transport Study with Earthworm's Skin

It might be called a type of universal law that any object moves from high to low concentrations, which is the principal expression of Fick's diffusion law. When a concentration gradient of particles is created across the skin, the osmotic pressure must be exerted, and materials inside the skin should be pulled out. If the osmotic pressure is established, penetration enhancers such as polysorbate 80

and DMSO should increase any transport capability. Sixteen earthworms were grouped into four containers and brought into 300 ml containers with corresponding marks on the glass surface after weighing their body. Finally, 2.0 gms of each toothpaste sample was applied around their skin and held at room temperature for 30 min. Then, their body weight was measured repeatedly. The change in body weight was evaluated and its unit was calculated to be weight change%, as seen in **Figure 14**.

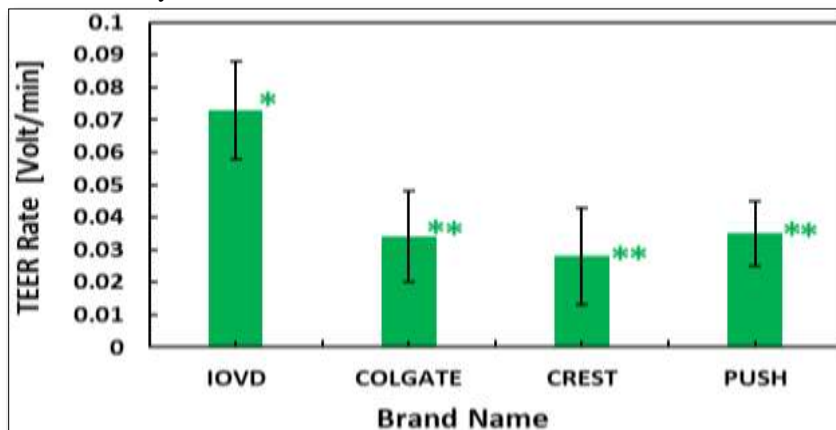


**Figure 14.** Illustrates the body weight %change measured 30 min after the skin was rubbed with the samples. The weight % change with asterisks significantly differed from the two asterisks ( $P < 0.05$ ).

### Material Transport Estimation with TEER Values Across the Skin

Transepithelial electrical resistance (TEER) is a widely recognized indirect quantitative method to evaluate transport through tight junctions in cell culture of epithelial monolayers for drug transport research [35]. TEER values' underlying mechanisms might not be exactly the same as this study's method. The application to the earthworm's skin could be appropriate because the *L. terrestris* skin is epithelial tissue that is not entirely different from other

animals. Our preliminary study for assessing transport capability found that the slope of the TEER value from a regression line had an inverse relationship to the concentration of penetration enhancers. **Figure 15** demonstrated that the TEER slope was significantly greater in the IOVD group than in other groups. The data demonstrated that the IOVD toothpaste had a high feasibility of delivering the vitamin into the body of *L. terrestris*. Study limitations were placed on the fact that no human study was performed.



**Figure 15.** Illustrates the change in TEER Rate across the skin. The TEER rates with asterisks show a significant difference from those with two asterisks ( $P < 0.05$ ).

## CONCLUSION

Therapeutic toothpaste with new concepts has developed; a vitamin D deliverable toothpaste has been formulated with DMSO and polysorbate 80 penetration enhancers. The purpose of our study was to formulate a toothpaste that could support the vitamin D transport into the bloodstream using blood vessels in the oral cavity to alleviate the inadequate intake of vitamin D. In conclusion, our formulation was comparable, or better, in various toothpaste characteristic tests compared with commercial toothpaste. TEER measurement confirmed the feasibility of vitamin D transport through oral cavity administration using a toothpaste formulation. Vitamin D should be delivered into the bloodstream by daily brushing our teeth. However, more studies should be executed to clarify various aspects of practical applications.

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