The fluoride release, abrasion resistance, and cytotoxicity to hGFs of a novel cyanoacrylate-based fluoride varnish compared with conventional fluoride varnish

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The important factors contributing to the effectiveness of fluoride varnish are the amount of fluoride ion release and the retention time of the varnish on the tooth surface. Commercial fluoride varnishes are susceptible to mechanical removal; therefore, patients are informed to avoid eating or performing oral hygiene for at least 12–24 h, which results in patient inconvenience. However, cyanoacrylate-based fluoride varnish would not have these disadvantages. This study compared the daily fluoride ion release, abrasion resistance to brushing, and toxicity to human gingival fibroblasts (hGFs) between a newly-developed cyanoacrylate-based fluoride varnish and conventional fluoride varnish (Duraphat varnish). The results demonstrated that the cyanoacrylate varnish had a significantly higher fluoride release for 9 days after application, higher abrasion resistance to brushing, and slightly less toxicity to hGFs compared with Duraphat varnish. This novel cyanoacrylate varnish could be an alternative fluoride varnish for preventing dental caries.

Keywords: Fluoride varnish, Cyanoacrylate, Fluoride release

INTRODUCTION

Applying fluoride to the tooth surface was introduced in clinical dentistry for dental caries prevention and remineralization in 1940 and was approved by the U.S. Food and Drug Administration in 1994. The first commercial fluoride varnish was Duraphat varnish (5% NaF or 50 mg/mL). Duraphat varnish has been the most widely used and extensively studied fluoride varnish. Several studies demonstrated that Duraphat varnish was an effective topical fluoride treatment for preventing caries1-4. In clinical dentistry, Duraphat varnish is considered to be the fluoride varnish of choice.

Topical fluoride prevents caries due to the fluoride ions released from the varnish that form fluoroapatite in the demineralized tooth structure and CaF₂-like globules on the tooth surface. The CaF₂ globules are stabilized by phosphate-binding protein from saliva and serve as fluoride reservoirs. During cariogenic challenge, acidic pH induces the dissolution of CaF₂ from the globules5. Subsequently, the released fluoride ions promote demineralized tooth structure remineralization5 and the calcium ions neutralize the acid and increase the pH. The remineralization reaction and amount of CaF₂ globules formed are related to the fluoride ion concentration6,7 and the retention time of the topical fluoride on the tooth surface, and remineralization increases over time6,11.

A disadvantage of the commercial fluoride varnishes is that they are susceptible to mechanical removal. To increase the retention time of the varnish on the tooth surface, the mechanical removal of a fluoride varnish should be postponed by refraining from eating hard food for a few hours after application and refraining from tooth brushing for 12–24 h after fluoride varnish application12,13. These protocols cause inconvenience to the patients, which are considered to be the disadvantages of the commercial fluoride varnishes. Incorporating cyanoacrylate into varnish could solve these problems.

Cyanoacrylate polymers are polar, linear molecules. Cyanoacrylate monomers can create strong covalent bonds to high surface energy substances, such as body tissue, skin, wood, leather, metal, glass, and plastic14 and polymerize rapidly in the presence of water, –OH groups, or any weak base on the substance. The cyanoacrylate monomer is a clear liquid with low viscosity (1–3 MPa). Cyanoacrylate can polymerize through 2 mechanisms, free radical and anionic polymerization15. The most common polymerization mechanism of cyanoacrylate monomer is the anionic reaction because it is energetically more favorable and faster than the radical reaction. Poly-cyanoacrylates have 2 degradation mechanisms, enzyme-dependent (esterase, superoxide dismutase, indomethacin, and acetyl-salicylic acid) and hydrolysis in the presence of water, with hydrolytic degradation being the most common16. The products from hydrolytic degradation are formaldehyde and alkyl cyanoacetate that are moderately toxic17. Factors that affect the rate of hydrolysis are temperature, pH, and alkyl chain length. The lower the temperature or pH, or the longer the alkyl chain, the slower the rate of degradation. Common derivatives of cyanoacrylate monomer are alkyl-cyanoacrylates, such as methyl 2-cyanoacrylate (MCA) and ethyl 2-cyanoacrylate (ECA, known as superglue).
which are commonly used in industry and the home. N-butyl cyanoacrylate (n-BCA), octyl cyanoacrylate, and 2-octyl cyanoacrylate are more biocompatible and used in medical and veterinary surgery as tissue adhesives to replace using sutures20-23.

A cyanoacrylate-based fluoride varnish developed by incorporating cyanoacrylate into varnish would not have the disadvantages of the commercial fluoride varnishes because cyanoacrylate polymers immediately set on contact with moisture, form covalent bonds to the tooth surface, polymerize into strong polymers, and progressively flake off within 5–10 days21). These properties would result in a fluoride varnish that is easy to use, be less inconvenient for the patient, and have a longer retention time on the tooth surface to promote maximum efficiency. Therefore, the aim of this study was to compare the fluoride ion release, resistance to tooth brushing, and the cytotoxicity to human gingival fibroblasts (hGFs) of a novel cyanoacrylate fluoride varnish and Duraphat varnish. The null hypotheses were that there was no significance difference (1) in the daily fluoride ion release and (2) in the number of brushing strokes that the varnish can withstand mechanical removal from toothbrushing between the novel cyanoacrylate based fluoride varnish and Duraphat varnish and (3) the novel cyanoacrylate based fluoride varnish is not toxic to hGFs.

MATERIALS AND METHODS

Duraphat varnish (5% NaF, Colgate-Palmolive, Canton, MA, USA) was used as the control. The cyanoacrylate fluoride varnish was prepared by mixing 35 mg/mL fumed silica (Cab-O-Sil® M-5P, Cabot, Billerica, MA, USA), 50 mg/mL Sodium fluoride (NaF, particle size <45 µm, EMPROVE®, Merck, Darmstadt, Germany) together (this powder part can be pre-mixed), with 0.2 mL/mL Vegetable oil (King® Rice Bran oil, Thai Edible Oil, Nakhon Ratchasima, Thailand), 0.05 mL/mL Sodium lauryl sulfate (SLS, Sulfopon® 1630, BASF, Ludwigshafen, Germany), and 0.35 mL/mL acetone into the powder part and mix for 40 s, then adding 0.4 mL/mL n-butyl cyanoacrylate (Vetbond® tissue adhesive, 3M, St. Paul, MN, USA, 99% by weight n-butyl cyanoacrylate, <1% by weight Hydroquinone, and 0.01% by weight blue dye) and continue mixing for 20 s. The ingredients were mixed in a closed vessel on a stirrer at room temperature. The total mixing time was 1 min.

Viscosity test

The varnish viscosity was measured using a viscometer (HAAKE™ MARS 60™ Rheometer, Thermo Fisher Scientific, Karlsruhe, Germany). Parallel plates (35 mm diameter) were used as the measuring apparatus. One milliliter of each fluoride varnish formulation was gently placed on the lower plate surface to avoid air bubbles. The upper plate was connected to the rotor. The space between the upper and lower plate was 1 mm. The tests were performed in 2 modes. The first mode was the viscosity test of the varnishes by increasing the shear rate from 0.1–150 1/s and 30 data points were collected. The second mode was the viscosity test of the varnish over time when the shear rate was constant at 10 1/s and 100 data points were collected. The tests were performed at 37°C.

In vitro fluoride release test

Seven samples for each fluoride varnish group were made by loading Duraphat varnish and the cyanoacrylate fluoride varnish in an 8 mm diameter and 1 mm deep polyvinyl siloxane mold. Each sample was immersed in 3 mL artificial saliva (3.90 mmol Na3PO4, 4.29 mmol NaCl, 17.98 mmol KCl, 1.10 mmol CaCl2, 0.08 mmol MgCl2, 0.50 mmol H2SO4, 3.27 mmol NaHCO3, and distilled water, at pH 7.2) in a plastic container (polystyrene, PS) and kept in an incubator (Memmert®, 100-800, Memmert, Schwabach, Germany) at 37°C for 14 days. Each day, the samples were removed from the artificial saliva immersion solution and rinsed with deionized water for 30 s, and dried with blotting paper, then placed in a new plastic container with 3 mL artificial saliva. The released fluoride ion concentration in the immersion solution was measured from Day 1–14. 300 µL TISAB III solution (Sigma-Aldrich®, Merck, St. Louis, MO, USA) was added to the immersion solution and stirred on a stirrer for 30 s. A fluoride ion selective electrode (Orion®, 9609BNWP, Thermo Fisher Scientific, Waltham, MA, USA) was placed in the solution for 2 min and the amount of released fluoride (ppm) was measured using an electrochemistry meter (Orion®, VERSASTAR, Thermo Fisher Scientific) and the data were recorded. The electrode and meter were calibrated before each use.

Brushing test

This test measured the number of brushing strokes that the varnish could resist being mechanically removed by tooth brushing. Prior studies found that 20 brushing strokes (10 strokes/area for tooth brushing with tooth brushing being performed twice a day) are equal to the effective daily number of brushing strokes to brush a specific tooth area usually recommended by dentists24,25). The average human brushing force is 1.6±0.3 N26).

Forty human lower incisors and premolars with smooth and non-carious enamel on the buccal surface of the teeth were obtained from patients’ treatment planned for tooth extraction at the Department of Oral Surgery. The method was approved by The Human Research Ethics Committee of the Faculty of Dentistry, Chulalongkorn University (HREC-DCU 2021-057). The samples were cut below the cemento-enamel junction to reduce the tooth size and embedded in acrylic in a 12×18×6 mm epoxy mold. To control the position of the teeth in the acrylic, the flat buccal surfaces of the teeth were attached to a glass slab with 2-sided thin adhesive tape, and the glass slab was placed on the top of the mold that was filled with acrylic to 2/3 of the mold’s depth. After the acrylic was set, the samples were removed and the surfaces of the teeth were cleaned with acetone. The samples were immersed in 37°C artificial saliva
for 24 h. After immersion, the samples were blown dry with oil-free air for 30 s. The samples were randomly divided into 8 groups (n=5), 4 groups were applied with Duraphat varnish (D group) and the other 4 groups were applied with cyanoacrylate fluoride varnish (C group). The area and thickness of the applied varnish were controlled using adhesive tape (100 µm thick) with a 2 mm diameter hole that was attached on tooth surface. The fluoride varnish was applied to the tooth surface and was covered with a glass slide with the constant pressure 0.5 kg for 30 s. The glass slide was taken off, the excess varnish was removed with a micro-brush, and the adhesive tape was removed. The samples were immediately immersed in 37°C artificial saliva for 4 h before performing the brushing test (per the Duraphat varnish manufacturer’s recommendation). The brushing test details are described in Table 1.

The samples were examined using a stereomicroscope (SZ 61, Olympus, Tokyo, Japan) at 30× magnification to capture the images of the applied varnish before testing. The brushing test was performed at room temperature using a V-8 cross brushing machine (SABRI Dental Enterprises, Downers Grove, IL, USA) at 90 strokes/min and a 1.6 N brushing force with soft bristle toothbrushes in the dentifrice slurry (ISO 11609:1995) 40 mL/specimen at a ratio of 25 mg toothpaste/40 mL deionized water (Colgate® Great regular flavor, 1,450 ppm fluoride, Colgate-Palmolive, Chonburi, Thailand). After the brushing test, the sample’s image was captured using a light stereomicroscope at 30× magnification. The percent area loss of varnish was calculated using the ImageJ program. The distance between the samples and the microscope lens before and after the brushing test was fixed at 10.7 cm to control the accuracy of the surface area measurement for each sample.

**Cytotoxicity test**

In this study, hGFs obtained from the gingival tissues of 3 healthy donors were used to evaluate the cytotoxicity of the Duraphat and cyanoacrylate fluoride varnishes. The donors provided informed consent before undergoing the gingivectomy procedure. The method was approved by The Human Research Ethics Committee of the Faculty of Dentistry, Chulalongkorn University (HREC-DCU 2021-057).

The gingival tissues were cut into 2–3 mm pieces and placed on 35-mm culture dishes (SPL Life Sciences, Gyeonggi-do, Korea) and 500 µL complete medium (Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% antibiotic-antimycotic solution (Gibco™, ThermoFisher Scientific)) was added. The gingival tissue cultures were performed at 37°C in an incubator in a humidified 5% CO₂ atmosphere and the culture medium was changed every 2 days until the hGFs reached 95% confluence and 4th–5th passage hGFs were used in the experiments. The cytotoxicity of the fluoride varnishes test was performed using the indirect contact test (ISO 10993-5).

**Indirect contact test**

Eight millimeters diameter and 1.5 mm high Duraphat varnish and cyanoacrylate fluoride varnish samples were prepared. The samples were decontaminated using UV light for 30 min before being immersed in the extraction medium. The varnish extraction medium (complete medium) was prepared following ISO 10993-12. The fluoride varnishes samples were immersed in complete medium at ratio of 0.1 g/mL and incubated at 37°C in a humidified 5% CO₂ atmosphere for 24 h. The extraction medium was diluted 1:2 and 1:10 to generate 3 extraction medium groups, undiluted, diluted 1:2, and diluted 1:10. The hGFs (1.0×10⁴ cells/well) were cultured in 96-well plates (SPL Life Sciences) at 37°C in a humidified 5% CO₂ atmosphere for 24 h. The culture medium in each well was removed, and the hGFs were cultured in 100 µL extraction medium, and the cell viability was determined after incubating the cells at 37°C for 24, 48, and 72 h. The MTT (3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assay was performed to evaluate cell viability. At the end of each culture period, the culture medium in each well was removed and 50 µL MTT reagent (1 mg/mL PBS) was added and incubated at 37°C for 4 h. After the incubation period, the solution in each well was removed and 100 µL dimethyl sulfoxide (DMSO) (AMRESCO, Solon, OH, USA) solution was added to dissolve the precipitated formazan crystals. The optical density (OD) was measured at 570 nm (EPOCH, BioTek Instrument, Winooski, VT, USA). The percentage cell viability was calculated using the following equation:

\[
\text{Percentage cell viability} = \left( \frac{\text{experimental group's OD}}{\text{control group's OD}} \right) \times 100
\]

Untreated hGFs, Triton X-100, and DMSO served as the positive control, negative control, and blank group respectively. Each group was evaluated in triplicate and

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**Table 1** Sample groups and brushing test details

<table>
<thead>
<tr>
<th>Group</th>
<th>brushing test details</th>
<th>Brushing strokes</th>
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<tbody>
<tr>
<td>C1, D1</td>
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<td>20</td>
</tr>
<tr>
<td>C2, D2</td>
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</tr>
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<td>C3, D3</td>
<td></td>
<td>400</td>
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<tr>
<td>C4, D4</td>
<td></td>
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used $n=3$ for experimental groups at each time-point observation.

**Statistical analysis**
Statistical analysis was performed using the IBM SPSS Statistic 28 program. The homogeneity of variances and normal distribution of the *in vitro* fluoride release data and percent area varnish loss from brushing were determined using the Shapiro-Wilk’s test. The group means of fluoride release and percent area loss of varnish were compared using Multivariate Analysis of Variance (MANOVA). Significance was determined at $p<0.05$.

## RESULTS

**Viscosity test**
The viscosity test results are seen in Figs. 1 and 2. The results are demonstrated as the sum of the forces arising from the intermolecular bond strength against the force of the testing machine. In Fig. 1, the shear rate was increased from 0.1–150 1/s. The results revealed that Duraphat varnish had a higher initial viscosity compared with the cyanoacrylate fluoride varnish (Duraphat varnish 92,371.86 mPa, cyanoacrylate varnish 9,151.53 mPa). Both varnishes had shear thinning properties (pseudoplastic) where the viscosity decreased as the shear rate increased. The cyanoacrylate varnish demonstrated shear thinning just prior to the cyanoacrylate setting reaction (period A). When the setting reaction took place, the viscosity of the cyanoacrylate varnish began to increase (period B) because the cyanoacrylate polymerization caused resistance against the force from the testing machine. Until the time point that polymerization ceased, the force from the testing machine was greater (period C), resulting in decreased viscosity.

Figure 2 illustrates the viscosity of both varnishes over time at a shear rate of 10 1/s. The results indicated that at a 10 1/s shear rate, the viscosity of the Duraphat varnish remained almost constant over time (mean...
viscosity of 1,560.396 mPa) and that the Duraphat varnish had a higher viscosity compared with the cyanoacrylate varnish until the cyanoacrylate setting reaction occurred. In contrast, the viscosity of the cyanoacrylate varnish increased over time until polymerization ceased, at that point, the viscosity started to decrease. The viscosity curve of the cyanoacrylate varnish can be divided into 4 time periods. Period 1 (0–333 s), the increase in viscosity was small (from 158.649–939.345 mPa). Period 2 (333–576 s), the steep slope of the curve indicated a dramatic increase in the viscosity of the cyanoacrylate varnish (from 939.345–39,376.27 mPa) because the high rate of polymerization produced a high resistance force against that of the testing machine. Period 3 (576–774 s), the change in the slope of the curve fluctuated due to the cyanoacrylate varnish becoming solid and the testing machine concurrently exerted force against the cyanoacrylate polymer formation until the bond between the polymerized monomers was destroyed. These mechanisms alternated, causing the direction of the curve to fluctuate. At the end of this period, the cyanoacrylate varnish reached its maximum viscosity (46,425.64 mPa) and its polymerization reaction ceased. Period 4 (774–900 s), the curve demonstrated a marked decrease in the viscosity of the cyanoacrylate varnish. This period was the result of the force from the testing machine destroying the bonds between the polycyanoacrylate molecules.

In vitro fluoride release test

The in vitro fluoride release test results comparing the mean fluoride release (ppm) between the cyanoacrylate and Duraphat varnishes from day 1–14 are presented in Table 2 and Fig. 3. The Shapiro-Wilk test indicated that the in vitro fluoride ion release data had a normal distribution. The MANOVA of the day 1–14 fluoride release results between the cyanoacrylate and Duraphat varnishes revealed a significant difference in fluoride release between the cyanoacrylate and Duraphat varnishes ($p<0.05$, Wilk’s lambda=0.000.

<table>
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<tr>
<th>Varnish</th>
<th>Day</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tr>
<td>Cyanoacrylate</td>
<td></td>
<td>218.53±5.52</td>
<td>121.30±7.74</td>
<td>110.60±9.78</td>
<td>101.87±7.06</td>
<td>78.85±5.88</td>
<td>57.87±5.11</td>
<td>48.57±6.47</td>
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<td>Duraphat</td>
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<td>15.31±2.61</td>
<td>4.28±1.91</td>
<td>4.04±1.87</td>
<td>3.85±1.76</td>
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<th>11</th>
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<th>13</th>
<th>14</th>
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<td>19.85±2.95</td>
<td>5.19±2.33</td>
<td>0.39±0.21</td>
<td>0.30±0.15</td>
<td>0.26±0.16</td>
<td>0.05±0.02</td>
<td>0.07±0.02</td>
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<tr>
<td>Duraphat</td>
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<td>3.39±1.60</td>
<td>2.54±1.14</td>
<td>4.61±2.36</td>
<td>3.35±1.50</td>
<td>3.49±1.84</td>
<td>3.48±1.81</td>
<td>2.76±1.12</td>
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</table>

**Fig. 3** The average fluoride release (ppm) of the novel cyanoacrylate and Duraphat varnish.
The results demonstrated that the fluoride release from the cyanoacrylate varnish was higher compared with the Duraphat varnish from day 1–9 and then very little fluoride was released after day 9 of immersion. In contrast, although Duraphat varnish released less fluoride, it released fluoride for a longer time compared with the cyanoacrylate varnish. Duraphat varnish released fluoride through day 14 and could be expected to continue to release fluoride over more time.

**Brushing test**

The stereomicroscopic images before and after the brushing test are shown in Figs. 4 and 5. The brushing test results demonstrated that none of the Duraphat varnish groups (20, 200, 400, or 600 brushing strokes) had any residual Duraphat varnish on the tooth surface after brushing. In contrast, residual cyanoacrylate varnish was found on the tooth surface in all cyanoacrylate groups after brushing. The percent area loss of the cyanoacrylate varnish on the tooth surface after brushing increased in a stroke-dependent manner, i.e. the greater the number of brushing strokes, the larger the percent area loss of varnish after brushing (Table 3).

The results of the brushing test indicated that the cyanoacrylate varnish had a significantly better abrasion resistance compared with Duraphat varnish. The Duraphat varnish was easily removed by abrasion as demonstrated by no residual Duraphat varnish being present on the tooth surface in all groups after brushing, including the 20 brushing stroke group, which is considered a small number of brushing strokes and equivalent to one day of brushing\(^{24,25}\).

**Table 3** Percent area loss of the novel cyanoacrylate varnish samples using ImageJ program

<table>
<thead>
<tr>
<th>Strokes</th>
<th>Sample</th>
<th>1</th>
<th>2</th>
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<td>7.26</td>
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<td>400</td>
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<td>79.98</td>
<td>74.68</td>
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<td>72.42</td>
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<td>4.43</td>
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<tr>
<td>600</td>
<td></td>
<td>100</td>
<td>92.37</td>
<td>93.51</td>
<td>100</td>
<td>100</td>
<td>97.18</td>
<td>3.89</td>
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The current commercial fluoride varnishes are used to enhance hGF proliferation. The percent cell viability of the higher dilution medium of each varnish was higher than 100% due to toxicity to the hGFs only at 24 h and not toxic to the hGFs at 48 and 72 h (percent cell viability 24 h=52.67±0.58%, 48 h=112.33±1.15%, and 72 h=91.67±0.58%). When the extraction medium of each varnish was diluted 1:10, the percent cell viability 24 h=24.67±3.51%, 48 h=4.67±0.58%, and 72 h=1.67±0.58%). In contrast, the cyanoacrylate varnish extraction medium diluted 1:2 was toxic to the hGFs only at 24 h and not toxic to the hGFs at 48 and 72 h (percent cell viability 24 h=5.267±0.58%, 48 h=11.23±1.15%, and 72 h=91.67±0.58%). When the extraction medium of each varnish was diluted 1:10, no toxicity to the hGFs was observed. Moreover, at 48 and 72 h, the percent cell viability of the higher dilution groups of both varnishes was higher than 100% due to hGF proliferation.

**DISCUSSION**

The current commercial fluoride varnishes are susceptible to mechanical removal; therefore, patients are informed to avoid mechanically removing the varnish for at least 12–24 h for the fluoride varnish to be most effective. These protocols lead to inconvenience for the patients. The present study developed a novel cyanoacrylate-based fluoride varnish to solve these problems based on the properties of cyanoacrylate, i.e. it immediately sets when contacting moisture, strongly bonds to the tooth surface, and progressively flakes off over 5–10 days, thus, patients can almost immediately eat, drink, and brush their teeth, which is more convenient.

Because there was no previous study that incorporated cyanoacrylate in fluoride varnish, the cyanoacrylate varnish’s formula, the compositions and mixing guideline were obtained from our pilot study using a trial-and-error method to achieve the acceptable cyanoacrylate varnish properties. The results of our pilot study demonstrated that the cyanoacrylate fluoride varnish had a mixing time of 1 min and a working time of 7–8 min. More importantly, the cyanoacrylate fluoride varnish solidified at the surface immediately when in contact with water, developed a rubbery consistency in 30 s, and completely set within 1 min. The cyanoacrylate fluoride varnish was formulated using n-butyl cyanoacrylate as the active ingredient in the varnish rather than 2-octyl cyanoacrylate, because the glass transition temperature (Tg) of n-butyl cyanoacrylate is appropriate for human body temperature. The Tg of n-butyl cyanoacrylate is 130°C, while that of 2-octyl cyanoacrylate polymer is 10°C. Therefore, at the 37°C human body temperature, the n-butyl cyanoacrylate polymer is in the glass phase, while the 2-octyl cyanoacrylate polymer is in the plastic phase. Thus, n-butyl has greater strength to withstand mechanical removal, such as tooth brushing, food, and tongue and cheek scrubbing in the oral environment. To control the type and amount of fluoride in the varnish to be equal to that of Duraphat, 50 mg/mL NaF powder was used. NaF is a basic salt that can initiate the polymerization of the cyanoacrylate monomers. Our pilot study revealed that NaF powder gave a longer working time compared with a NaF solution that has water as the solvent, which can cause cyanoacrylate to have a higher polymerization rate that is more exothermic. Small particle size NaF was used to promote the equal distribution of NaF in the varnish. Because water and ethanol can initiate cyanoacrylate polymerization, acetone was used as the solvent for the cyanoacrylate fluoride varnish. Acetone does not initiate cyanoacrylate polymerization and acetone is a biocompatible solvent that can dissolve NaF and cyanoacrylate, however, the solubility of NaF in acetone is lower compared with water and ethanol. Thus, most of the NaF particles were suspended in the varnish. Fumed silica was added to adjust the viscosity and increase the shear thinning property of the cyanoacrylate fluoride varnish and reduce internal stress and polymerization shrinkage, which cause debonding. The increased viscosity of the varnish also reduced the precipitation of the NaF particles after mixing. However, the unequal distribution of NaF in the varnish was still an issue. A small amount of SLS was added to the varnish as a surfactant that promoted the equal distribution of NaF in the varnish and also increased the working time of the varnish. Vegetable oil was added to control the fluoride release rate from the cyanoacrylate fluoride varnish by being the water repellent in the varnish film, resulting in a decreased dissolution NaF rate.

The results of the viscosity test demonstrated that the cyanoacrylate fluoride varnish had a lower viscosity compared with the Duraphat varnish, which may be an advantage by being easier to apply, and also had a thinner varnish film thickness than Duraphat varnish, which may result in better esthetics and patient acceptance. Typically, the low viscosity of a varnish makes it easier to rinse off with water or saliva. However, this will not occur with the cyanoacrylate fluoride varnish, because cyanoacrylate solidifies immediately when in contact with water, thus the varnish can remain on the tooth surface. In addition, because the cyanoacrylate monomer is a clear liquid, it is easy to adjust the color of the varnish.
varnish to match the natural tooth color.

The results of the daily fluoride release experiment indicated that there was a significance difference in the daily fluoride ion release between the cyanoacrylate fluoride varnish and Duraphat varnish, thus the first null hypothesis was rejected. With the same volume of fluoride varnish, the cyanoacrylate fluoride varnish released significantly more fluoride ions compared with the Duraphat varnish for 9 days of immersion and then very little fluoride was released. In contrast, the Duraphat varnish had a fluoride release pattern characterized by releasing small amounts of fluoride over a longer period of time. This pattern was caused by the hydrophobicity of the rosin (colophony) in Duraphat, which makes it difficult for water to penetrate and dissolve the fluoride from the Duraphat varnish. This finding is consistent with that of Castillo et al.\textsuperscript{30}, who reported that Duraphat varnish released fluoride for up to 28 weeks. Although the polycyanoacrylate polymer in the cyanoacrylate fluoride varnish is classified as a hydrophobic polymer, the porosity of the cyanoacrylate polymer and the hydrophilicity of the fumed silica, which was added to the varnish to adjust its viscosity, promotes water to penetrate and dissolve NaF from the cyanoacrylate varnish, and also leave empty spaces that could allow water to move in and dissolve the inner NaF particles. This mechanism allowed the cyanoacrylate varnish to release higher amounts of fluoride ions and very little fluoride was released after 9 days of immersion.

The fluoride concentration in saliva is an important factor in the degree of remineralization and CaF\textsubscript{2} globule formation. Several studies reported that the effectiveness of fluoride in remineralization and caries prevention was directly related to the fluoride ion concentration\textsuperscript{30-46}. Fluoride is a very reactive element, less than 0.1 ppm fluoride is sufficient for fluorapatite formation on the tooth surface\textsuperscript{47} and when the fluoride concentration in plaque is more than 10 ppm, it can interfere with the activity of enolase, an enzyme that is important in carbohydrate fermentation by bacteria\textsuperscript{48}. Moreover, CaF\textsubscript{2} globules can only precipitate when the concentration of fluoride in the plaque and saliva exceeds 100 ppm\textsuperscript{45,47}. The higher the fluoride concentration, the more CaF\textsubscript{2} is formed\textsuperscript{46,48,49}. The most importantly, CaF\textsubscript{2} globules can persist on the tooth surface for weeks or months\textsuperscript{50,51} and dissolve when the pH drops\textsuperscript{7,52}, which creates a mechanism to prevent dental caries. Thus, applying fluoride varnish only two to three times a year can result in caries reduction.

The brushing test indicated that the number of brushing strokes that the cyanoacrylate fluoride varnish could withstand mechanical removal was significantly higher compared with the Duraphat varnish. Based on these results, the second null hypothesis was rejected. The cyanoacrylate fluoride varnish demonstrated a significantly better abrasion resistance than that of the Duraphat varnish. The cyanoacrylate fluoride varnish withstood up to 600 brushing strokes. In contrast, there was no residual varnish on the tooth surface in any Duraphat varnish brushing number group, including the 20 brushing strokes group, which is equivalent to 1 day of brushing. The cyanoacrylate monomers can form covalent bonds, creating strong adhesion to the hydroxyapatite and collagen fibers on the tooth surface and polymerizes into a polymer that exhibits abrasion resistance. In contrast, the Duraphat varnish, which is obtained by dissolving colophony with alcohol, is sticky. Duraphat varnish adheres to the tooth surface by Van der Waals forces and solidifies by alcohol evaporation\textsuperscript{53}, thus, Duraphat has a lower abrasion resistance compared with the cyanoacrylate fluoride varnish.

The brushing test results suggest it is likely that the cyanoacrylate fluoride varnish could survive the mechanical forces that occur in the oral cavity, including eating, drinking, brushing and oral soft tissue scrubbing, longer than the Duraphat varnish. In addition, in the brushing test, smooth enamel surfaces were used as the varnish bonding sites, which were very difficult for the materials to adhere to. In clinical practice, varnish is applied to prevent dental caries on all areas of the teeth and the fluoride ions released from the varnish are always rinsed out and diluted by water and saliva. Thus, if the varnish can adhere to the most challenging surface of the teeth to bond; it can bond to all other tooth surfaces. However, the retention time of the cyanoacrylate varnish and Duraphat varnish on the tooth surface in oral cavity require further investigation.

The remineralization reaction and amount of CaF\textsubscript{2} globules formed are related to the fluoride ion concentration\textsuperscript{6,7} and the retention time of the topical fluoride on the tooth surface, and remineralization increases over time\textsuperscript{8-11}. The fluoride release and brushing test results suggest that during the first 1–9 days after varnish application, there is a high possibility that the cyanoacrylate fluoride varnish could promote a higher degree of remineralization and a higher amount of CaF\textsubscript{2} globule formation on the tooth surface that can serve as fluoride ion reservoirs and play an important role in caries prevention. However, the effect of the novel cyanoacrylate fluoride varnish in caries prevention and remineralization require further investigation.

The cytotoxicity test results demonstrated that both varnish’s undiluted extraction mediums were toxic to hGFs. When using 1:2 diluted extraction medium, the cyanoacrylate fluoride varnish was toxic to the hGFs only at 24 h, while at 48 and 72 h, the cyanoacrylate fluoride varnish was not toxic to hGFs. In contrast, the 1:2 diluted Duraphat varnish extraction medium was toxic to the hGFs at all observation times (24, 48, and 72 h). When the extraction medium was diluted 1:10, neither varnish was toxic to the hGFs. Hoang-Dao et al.\textsuperscript{53} also reported that undiluted Duraphat varnish extraction medium was toxic to hGFs and the toxicity significantly decreased when diluted 1:2. The results of the cytotoxicity test indicate the cyanoacrylate fluoride varnish was slightly less toxic to the hGFs compared with the Duraphat varnish. However, although in vitro studies have shown that the undiluted and diluted 1:2 Duraphat varnish extraction medium was toxic to hGFs, clinically,
Duraphat varnish is considered the fluoride varnish of choice and is the most widely used fluoride varnish since 1980 with few incidences of serious pathology to the patient. This may be due to the dynamics of the oral environment, where substances released from the varnish are constantly rinsed out and diluted by water and saliva, thereby minimizing the potential toxicity to the gingival tissue. Thus, the third null hypothesis was not rejected; the cyanoacrylate based fluoride varnish is not toxic to gingival fibroblasts.

The results of this study demonstrated that the cyanoacrylate fluoride varnish released higher amounts of fluoride ion compared with the Duraphat varnish for 9 days after application, solidified immediately when in contact with water, and was more resistant to abrasion. These properties could improve patient comfort. However, the disadvantages of the cyanoacrylate fluoride varnish are that it has a limited working time of 7–8 min and contains highly volatile acetone, which is difficult and complicated for storing and mixing.

Limitations and future studies

The present study used the *in vitro* fluoride release test to compare the fluoride release of the cyanoacrylate fluoride varnish and Duraphat varnish. The experiment was based on the principle that greater fluoride release promotes greater remineralization and increased CaF$_2$ formation. However, clinically, the oral conditions are dynamic. The effect of the novel cyanoacrylate fluoride varnish in caries prevention and remineralization requires further investigation. Moreover, fluoride ions released from the varnish are always rinsed away and diluted by water and saliva that may allow the varnish to release more fluoride ions due to the effect of concentration on the diffusion of the substance compared with the *in vitro* cumulative fluoride release test in this study.

The results of the brushing test demonstrated that the cyanoacrylate fluoride varnish withstood up to 600 brushing strokes, which is equivalent to 30 days of brushing. However, when used clinically there are many uncontrolled and individual factors, such as the tooth brushing technique, eating, drinking, talking, food type, food pH, food composition, and temperature, that can reduce the retention time of the cyanoacrylate varnish on the tooth surface. Moreover, no study has determined how long the varnish remains on the tooth surface after application *in vitro*. This may be because simulating the oral cavity is difficult and there are many uncontrolled and individual factors to consider, that can affect the retention time of varnish on tooth surface. Therefore, the brushing test in this study was solely to compare the varnish's abrasion resistance and the actual retention time of the cyanoacrylate varnish and Duraphat varnish in the oral environment requires further investigation.

For the percent area loss measurement, the ImageJ program measured the area loss of varnish from the captured image and it can measure the changes that occur only when the full thickness of the varnish is removed. It cannot measure the amount of varnish loss when the varnish is only partially removed. However, the aim of the brushing test was to compare the number of brushing strokes that the varnish could resist being mechanically removed by tooth brushing between the novel cyanoacrylate based fluoride varnish and Duraphat varnish.

In addition, the antimicrobial effects on gram positive organism, dental plaque accumulation, and fluoride recharge ability of cyanoacrylate fluoride varnish are important topics and should be evaluated in future studies.

CONCLUSION

Based on the results of this study, the novel cyanoacrylate fluoride varnish polymerizes immediately when exposed to water or moisture, releases higher amounts of fluoride, but for a shorter period, has higher abrasion resistance, and is slightly less toxic to the cell hGFs compared with Duraphat varnish. This novel cyanoacrylate fluoride varnish has the potential to be a new alternative fluoride varnish as a topical fluoride treatment that is easy to use and convenient for patients.

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