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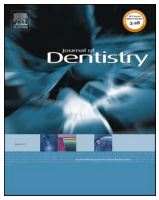

Stain removal, abrasion and anticaries properties of a novel low abrasion dentifrice containing micro-fibrillated cellulose: *in vitro* assessments

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ABSTRACT

Objectives: This laboratory study assessed the performance of a novel fluoride dentifrice containing micro-fibrillated cellulose (MFC) and entrapped silica.
Methods: Removal of extrinsic stains was assessed using the pellicle cleaning ratio (PCR) method, and radioactive dentin abrasivity (RDA) was measured, to calculate a cleaning efficiency index (CEI). Fluoride efficacy was evaluated using widely used remineralization and fluoride uptake methods. The test product (Protegera™) was compared to common dentifrices (Crest - Cavity Protection™ and ProHealth™, Sensodyne Pronamel™, Arm & Hammer™ Advanced Whitening, Crest ProHealth™, and Colgate Optic White™).
Results: The PCR for the MFC dentifrice (141) was comparable to three known marketed stain-removing dentifrices (Arm & Hammer™ Advanced Whitening, Crest ProHealth™, and Colgate Optic White™) but it had a significantly lower RDA (88 ± 6) than 5 other products. This gave it the highest CEI of the tested products (2.0). In a 10-day pH cycling study, the fluoride efficacy of the MFC product was comparable to Sensodyne Pronamel and Crest Cavity Protection. The MFC dentifrice was superior for promoting fluoride uptake into incipient enamel lesions compared to the USP reference dentifrice.
Conclusion: The MFC dentifrice has low abrasion, but despite this, it is highly effective in removing stained pellicle. It also is an efficacious fluoride source when compared to relevant commercially available fluoride dentifrices with high dentin abrasivity.
Clinical significance: The addition of micro-fibrillated cellulose to a fluoride dentifrice gives a low abrasive product that can effectively remove external stains, and serve as an effective fluoride source. This combination of benefits seems well suited to enamel protection and caries prevention.

1. Introduction

Extrinsic stains on teeth develop when the normal salivary pellicle layer (unstained pellicle) takes up stains from the diet, including from chlorhexidine mouthwashes, tea, coffee, red wine, and other colored foods and drinks, to become stained pellicle [1–4]. Extrinsic staining of teeth is unsightly, and this cosmetic problem has driven the development of dentifrices.

The primary purposes of dentifrices are to remove dental plaque biofilm and stained pellicle from teeth, oral appliances, and prostheses [5,6]. To achieve this, current commercial dentifrices include various insoluble abrasive particles, including silica, calcium pyrophosphate, and calcium carbonate [7–9]. The intention is that, during brushing, some of the force applied to the toothbrush will be directed onto abrasive particles located between the bristle ends and the tooth surface. The movement of the abrasive particles horizontally along the tooth surface as they are pushed along by the toothbrush filaments (i.e., without vertical pressure) should then dislodge some stained pellicle and some biofilm. If abrasive particles were "pushed" into the surface by the bristles, that would likely remove more stained pellicle [10,11].

How well this theory applies in practice depends on the interaction of multiple variables, including the function of saliva as both a diluent and

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a lubricant; the rheological properties of the dentifrice as it is diluted by saliva; the presence of more complex topography (enamel pitting, supragingival calculus, restoration contours); the applied geometry of the brush and its bristles, the toothbrushing technique used (angulation, direction, force); and subjective reactions of users to components of the dentifrice, such as flavors and detergents. These factors also influence how much the abrasive particles in dentifrices will abrade dentin [12].

Marketed toothpastes vary in the extent to which they can remove stained pellicles, and in how much abrasive wear they cause to dentin [13]. There is a long-standing belief that increasing the loading of abrasive particles in a dentifrice should increase stain removal, but at the risk of abrasion to dentin and root surfaces [14,15]. Marketed dentifrices vary from, at one extreme, those designed for aggressive extrinsic stain removal, for "whitening," with high RDA values, to those intended for use by patients with cervical dentin hypersensitivity, with low RDA values [16,17]. This inherent trade-off between stain removal and dentin abrasion has characterized dentifrices for many years [18,19].

By changing the fundamental cleaning technology, such trade-offs may be avoided. As an example, the inclusion of micro-fibrillated cellulose (MFC) into a dentifrice increases its ability to remove dental plaque, with improvements of 3- to 4-fold in whole-mouth plaque reductions shown in a recent clinical trial [20]. Including MFC can mitigate problems caused by the saliva acting as a lubricant and a rheology-transforming agent [21]. The silica content of an MFC dentifrice is 7 % by weight, and the silica is a high cleaning silica product (Zeodent® 103, Evonik, Theodore, AL, USA) that is designed to provide superior performance in toothpaste formulations. Entrapped silica abrasive particles within the MFC matrix reach the tooth surface, where they provide the necessary shear force to dislodge biofilm [22]. Based on the same considerations, the action of MFC with entrapped abrasive should enhance stain removal [23].

Hence, the primary aim of this study was to compare the performance of a novel fluoride dentifrice containing MFC with entrapped silica (MFC + F) with conventional dentifrices (Table 1), using well established laboratory assessments for the removal of stained pellicle and dentin abrasivity. The secondary aim was to assess the efficacy of fluoride in the novel dentifrice containing MFC, using laboratory assays for in vitro enamel remineralization and fluoride bioavailability.

2. Methods

2.1. Testing approach

For the primary study aim, the pellicle cleaning ratio (PCR) and the radioactive dentin abrasivity (RDA) were the outcome measures. Under standardized laboratory conditions, the removal of stained pellicle was

Table 1
Commercial dentifrices used in the study.

Code	MFC + F	CCP	CPH	C3DW	AHAW	SDP	COW	TMFF
Name	Protegera™	Crest Cavity Protection™	Crest ProHealth™	Crest 3D-White™	Arm & Hammer™ Advance White™	Sensodyne™ Pronamel™	Colgate™ Optic White™	Tom's of Maine Fluoride-Free™
Manufacturer	Protegera, Madison, WI, USA	Procter & Gamble, Cincinnati, OH, USA	Procter & Gamble, Cincinnati, OH, USA	Procter & Gamble, Cincinnati, OH, USA	Church & Dwight, Trenton, NJ, USA	Haleon, Warren, NJ, USA	Colgate Palmolive, Piscataway, NJ, USA	Colgate Palmolive, Piscataway, NJ, USA
Fluoride ppm	1086 ppm F from 0.24 % NaF	1100 ppm F from 0.243 % NaF	1100 ppm from 0.454 % SnF_2	1100 ppm F from 0.243 % NaF	1086 ppm F from 0.24 % NaF	1130 ppm F from 0.25 % NaF	1000 ppm F from 0.76 % sodium monofluoro-phosphate	Zero
Abrasives	Hydrated silica (7 %)	Hydrated silica	Hydrated silica	Calcium pyrophosphate, Disodium pyrophosphate, Tetrasodium pyrophosphate	Sodium bicarbonate, Tetrasodium pyrophosphate, Hydrated silica	Hydrated silica	Calcium pyrophosphate, Tetrasodium pyrophosphate, Disodium pyrophosphate, Hydrated silica	Calcium carbonate, Hydrated silica
Other ingredients	Water, Glycerin, Microcrystalline cellulose, Micro-fibrillated cellulose, Flavor, Cocamidopropyl betaine, Sodium gluconate, Carbomer, Sodium benzoate, Gantrez™ S-97, Poloxamer 407, Xanthan gum, Sodium hydroxide	Sorbitol, Water, Sodium lauryl sulfate, Trisodium phosphate, Flavor, Cellulose gum, Sodium phosphate, Carbomer, Sodium saccharin, Titanium dioxide, Blue 1 dye.	Sodium hexameta-phosphate, Propylene glycol, PEG-6, water, Zinc lactate, Trisodium phosphate, Flavor, Sodium lauryl sulfate, Sodium gluconate, Carrageenan, Sodium saccharin, Xanthan gum, Mica, Titanium dioxide, Blue 1 dye	Water, Glycerin, Hydrogen peroxide (4 % w/v), Sodium lauryl sulfate, Flavor, Polyacrylate crosspolymer-6, Cetearyl alcohol, Sucralose.	PEG-8, PEG/PPG 116/66 Copolymer, Sodium percarbonate, Sodium saccharin, Flavor, Water, Sodium lauroyl sarcosinate, Sodium lauryl sulfate	Water, Sorbitol, Glycerin, Potassium nitrate, PEG-6, Cocamidopropyl betaine, Aroma, Titanium dioxide, Xanthan gum, Sodium saccharin, Sodium hydroxide, Limonene.	Propylene glycol, PVP, PEG/PPG-116/66 Copolymer, Hydrogen peroxide, Flavor, Sodium lauryl sulfate, Sodium saccharin, Sucralose, BHT.	Glycerin, Water, Xylitol, Flavor, Zinc citrate, Sodium lauryl sulfate, Carrageenan, Sodium bicarbonate
PCR (N = 16)	141.0 ± 28.6 a	91.1 ± 18.2 b	143.4 ± 23.3 a		145.1 ± 32.5 a	43.3 ± 15.2 c	137.4 ± 22.0 a	
RDA (N = 8)	87.9 ± 6.1 b	108* c	166.4 ± 24.7 d	182.9 ± 10.3 d	110* c	34* a	100* c	85* ±4 b
CEI	2.0	1.4	1.6		1.9	0.8	1.9	1.2*

Abbreviations: PCR: pellicle cleaning ratio, RDA: radioactive dentin abrasivity; CEI: Cleaning efficiency index; NaF: sodium fluoride, SnF_2: stannous fluoride. Lower case letters show the statistical comparison between groups in each horizontal row. Data values with the same letter are not significantly different from one another. Values for RDA with asterisks are published values from previous work conducted in the same lab (Ref. 17) and were used to calculate CEI values.

assessed to calculate the PCR. The PCR method was developed at Indiana University by Stookey and colleagues [24]. This approach was used in the present study as it is one of the best-known and most accepted laboratory methods worldwide for assessing the stain removal ability of dentifrice. Moreover, the results of a stained pellicle removal test using dentifrice slurries may be considered to predict clinical findings for extrinsic stain removal with a reasonable degree of confidence [24,25].

For the purposes of comparison, a range of commercial fluoride dentifrices that make advertising claims of stain removal were included in the PCR and RDA assays (Table 1). These products use silica and/or pyrophosphates as abrasives. Dentin abrasion was determined using a toothbrushing machine, and the relative dentin abrasivity (RDA) score was calculated by comparing dentifrice samples with a reference material that had known abrasive qualities [12,26–28]. Past work has shown that the PCR and RDA measures are helpful ways of comparing the performance of novel dentifrices with recognized standard preparations and marketed products [8,29,30]. As a control in the PCR and RDA assays, a version of the MFC dentifrice was included that was free of silica.

The secondary purpose of the present study was to assess the efficacy of fluoride in the MFC + F dentifrice. For this purpose, established laboratory assays were chosen, including assays for fluoride bioavailability, in vitro enamel remineralization using a pH cycling model (the White model) [31–33] and assays for enamel fluoride uptake [34]. These laboratory methods are used widely for assessing novel dentifrice formulations [8,29,35]. For the purposes of comparison, a range of commercial fluoride dentifrices were used, as well as a version of the MFC dentifrice that was free of fluoride.

2.2. Pellicle cleaning ratio

In this part of the study, the PCR was determined for seven products (Table 1), comprising the novel MFC + F dentifrice, five other commercial dentifrices with advertised claims for stain removal (CCP, CPH, AHAW, SDP, and COW), and an ISO/ADA reference material. This work followed the established methodology for determining PCR [24], and was undertaken in compliance with the US Food and Drug Administration Guidelines for Good Laboratory Practice for Nonclinical Laboratory Studies. The ISO/ADA reference material that was also included for comparison was prepared by mixing 10 g of calcium pyrophosphate powder (RDA standard grade, Odontex Inc, Lawrence, KS, USA) into 50 mL of glycerin containing 0.5 % (w/v) carboxymethyl cellulose (CMC-7MF, Hercules Inc, Wilmington, DE, USA).

Specimens were prepared from bovine permanent central incisors to a size of 8 × 8 mm, then embedded into 15 mm square blocks of autopolymerizing methacrylate resin so that only the enamel surface was exposed. The enamel surfaces were then smoothed on a lapidary wheel, polished with flour of pumice and water to allow uniform instrumental color readings, and placed in an ultrasonic cleaner to remove debris. The finished specimens were examined under a dissecting microscope, and any samples with surface imperfections were discarded. To enhance extrinsic stain adherence and accumulation, the enamel was etched lightly using the following protocol: 0.12 M hydrochloric acid for 60 s, followed by saturated sodium carbonate solution for 30 s, and then 1.0 % phytic acid for 60 s.

To generate extrinsic stains, the samples were connected to a rod rotating at 1.5 rpm to alternately immerse them into a trough containing a staining broth, and to air drying, for a total period of 10 days at 37 °C, with the specimens rotating continuously through the staining broth and air. The staining broth contained porcine gastric mucin as a protein source, instant coffee, instant tea, and ferric chloride. The broth was replaced once daily for ten consecutive days. By the end of this time, the enamel surface had developed a visibly dark pellicle stain. The extent of external staining was assessed from a 3 mm diameter circular region in the center of the enamel surface using a portable sphere spectrophotometer with a 3 mm aperture and an internal xenon light source (model CM-26dG, Konica Minolta, Osaka, Japan), to determine the L* (lightness) value of the CIELAB color space scale. The lower this value, the darker the stain. The stained enamel specimens were air-dried at room temperature for 30 mins before color measurements were made by aligning the center of the stained enamel square directly over the targeting aperture of the spectrophotometer. Three color readings were made and averaged for each specimen. All specimens after staining had L* values between 30 and 38. Based on their individual L* values, the specimens were then stratified and distributed into groups of N = 16, so that each group had the same average L* score at baseline.

To assess stained pellicle removal, samples were exposed to the 6 test products and an ISO/ADA reference material using toothbrushes (Oral-B™ Indicator 40 brushes with soft nylon filaments, Procter and Gamble, Cincinnati, OH, USA) in a mechanical V-8 cross-brushing machine (Sabri Dental Enterprises, Downers Grove, IL, USA) at a constant load of 150 g. The load used for pellicle removal test was based on past studies of RDA [12,26].

Before use, the toothbrushes were conditioned by running the brushing machine for 1,000 strokes with deionized water. For testing, slurries were prepared by mixing 25 g of the test dentifrices or reference standard with 40 mL of deionized water. A different portion of the appropriate slurry was used to brush each specimen. The specimens were brushed for 800 double strokes, after which they were rinsed, blotted dry, and scored again for stain using the spectrophotometer.

Differences between the pre- and post-brushing L* values were calculated. Group data was assessed for normality before calculating means, standard deviations and standard errors (SEM). The cleaning ratio for the reference material (an increase in L* score of 13.55, from 34.25 to 47.80) was assigned a reference PCR value of 100.0, and the reduction achieved by the reference material group was divided into 100, to provide a scale on which to place the 6 test materials. The higher the calculated PCR, the greater the amount of stained pellicle removed.

2.3. Dentin abrasion

Relative dentin abrasion (RDA) testing was undertaken following the American Dental Association recommended procedure for determination of dentifrice abrasivity as detailed in section 5.2 and Annex A of ISO 11609:2017 (Dentifrices - Requirements, test methods, and marking) and the corresponding ANSI/ADA Standard No. 130, as described previously [17,26]. This radiotracer method was used since it is more reliable and robust than the alternative surface profilometry method, and is better for differentiating between products [36].

RDA was determined for five dentifrices: CPH, C3DW, and two versions of the MFC dentifrice. One had the normal loading (7 %) of high cleaning silica. The other version had zero silica, and was included to assess the impact of the base vehicle with MFC on dentin abrasion.

Human root dentin specimens from permanent teeth (N = 8) were placed in a neutron flux under the controlled conditions outlined in ISO 11609. During irradiation, a part of the phosphorus (P^{31}) in hydroxyapatite is converted into radioactive P^{32}, while some of the calcium, by neutron capture, also changes to radioactive isotopes [12]. The dentin specimens were then mounted in polymethylmethacrylate resin to fit into the same V-8 cross-brushing machine used for the PCR assessment. The same toothbrush type (Oral B Indicator 40) and load (150 g) were used throughout the RDA component of the study.

The specimens were first brushed for a 1500-stroke precondition run using a slurry of 10 g of the same ISO/ADA reference material as used previously (10 g calcium pyrophosphate in 50 mL glycerin with 0.5 % carboxymethyl cellulose) for 1500 strokes. Following this, a sandwich design was used so that a slurry of each test dentifrice (25 g in 40 mL of deionized water) was flanked by the ISO/ADA reference material.

To assess the radioactivity of the dentin removed from the surface, 1.0 mL samples of the post-brushing slurry were taken, weighed to the nearest 10 mg, and added to 4.5 mL of a liquid scintillation cocktail. After thorough mixing, radioactivity was determined using a

scintillation counter. After correcting for background radiation, the net counts per minute (CPM) were then divided by the measured weight of the sample to determine the CPM per gram of slurry. For comparison, the CPM/g of the two flanking samples using the ISO/ADA reference material was calculated, and the average was used to assign a relative value based on a ratio, setting the ISO/ADA reference at 100. After checking for normality, the means, standard deviations and standard errors were calculated from the collated data sets, with N = 8 replicates for each test dentifrice.

2.4. Cleaning efficiency index

The Cleaning Efficiency Index (CEI) was calculated according to the equation: CEI = (RDA + PCR – 50) ÷ RDA, as described previously [25]. This index emphasizes the importance of effective stain removal properties and low dentin abrasion, and is based on clinical data that indicates a PCR value of at least 50 is needed to provide acceptable extrinsic stain removal [25]. As the CEI was calculated from the mean RDA value and mean PCR value, there was no standard deviation.

2.5. In vitro enamel remineralization and fluoride bioavailability

These evaluations explored the efficacy of fluoride dentifrices in promoting enamel fluoride uptake, and in promoting the remineralization of incipient enamel lesions, under dynamic conditions simulating in vivo caries formation, using a pH cycling model (White model) [31–33]. Briefly, the caries-like lesions produced by this approach have a lesion depth of 70 μm and a dense surface mineral zone of 15 μm thick.

Specimens of bovine enamel (3 mm in diameter) were prepared from extracted sound bovine maxillary central incisors by cutting perpendicular to the labial surface with a hollow-core diamond drill bit. This was performed underwater to prevent overheating of the specimens. The enamel specimens were then embedded in the end of a Plexiglass® rod using poly-methyl methacrylate resin. The excess acrylic resin was cut away, exposing the enamel surface. The samples were ground (using 600 grit wet/dry paper) and then polished to a high luster using gamma alumina.

The baseline microhardness of the sound enamel of the specimens was determined using a Vickers micro-indentation hardness tester (model AM247AT, Leco, St. Joseph, MI, USA) at a load of 200 g for 15 s. Four indentations were made on the surface of each specimen, and the average microhardness (Vicker's hardness number, VHN) was determined. These baseline values ranged from 300–390 VHN.

Artificial incipient carious lesions were formed in the enamel specimens by immersing them for 33 h in a demineralization solution (0.1 M lactic acid, 0.2 % Carbopol C907, 50 % saturated with hydroxyapatite, pH 5.0). This created lesions of approximately 70 μm in depth. A second (post-demineralization) measurement of surface microhardness was then undertaken. The target VHN for lesions required for acceptance into this part of the study was 25-45 VHN. Based on these values, specimens were allocated into seven groups (N = 18 in each) and, from there, into two subgroups of N = 9 for each of the seven products to be tested. The included products were as follows: MFC + F (1086 ppm fluoride from 0.24 % NaF), CCP (1100 ppm fluoride from 0.243 % NaF), CPH (1100 ppm fluoride from 0.454 % SnF_2), SDP (1130 ppm fluoride from 0.25 % NaF), TMFF (negative control), and two fluoride-free versions of the MFC dentifrice (as vehicle controls).

During treatment, the specimens were immersed in dentifrice slurries to simulate daily brushing. The slurries were prepared by adding 5.0 g of dentifrice to 10.0 g of deionized (DI) water (1:3 w/w dilution) in a beaker with a magnetic stirrer. Two fresh slurries of each test sample were prepared just before each treatment period and each slurry was used to treat N = 9 specimens per subgroup.

The cyclic treatment regimen consisted of the following daily routine for ten days: 8.00 AM: a soak in the test dentifrice (120 s), followed by a running deionized water rinse, then into artificial saliva, 10.00AM: a 4 h soak in the lactic acid lesion-forming solution, followed by a running deionized water rinse, then into artificial saliva, 4.00 PM: a second soak in the test dentifrice (120 s followed by a running deionized water rinse), and then overnight into artificial saliva. The artificial saliva comprised 20 mM HEPES buffer with 1.45 mM calcium chloride dihydrate, 5.4 mM potassium dihydrogen orthophosphate, and 130 mM potassium chloride (pH 7.0) [37]. The lactic acid solution exposure was conducted under static conditions, while the other directions were under agitation conditions (350 rpm).

Following ten days of treatment, the average specimen surface microhardness was determined from four indentations. The difference between the surface microhardness following the treatment regimen and initial lesion surface microhardness was calculated (Δ VHN), and the percent surface microhardness recovery (%SMHR) was determined, as follows: % SMHR = [(D1 − R)/(D1 − B)] × 100, where B = surface microhardness (VHN) of sound enamel specimen at baseline; D1 = VHN after pre-treatment in vitro demineralization; and R = VHN after the 10-day in vitro treatment regimen.

In addition, at the end of the 10-day treatment regimen, the fluoride concentration of each enamel specimen was determined using a microdrill biopsy technique to a depth of 100 μm [38]. The diameter of the drill hole was determined by microscopic examination. The enamel powder from the drill hole was collected, and dissolved into a mixture comprising 20 μL of $HClO_4$, 40 μL citrate/EDTA buffer, and 40 μL deionized water. The fluoride concentration was then measured using a fluoride ion-selective electrode (ISE) by interpolation from a standard curve. The enamel fluoride concentration of each specimen was expressed in μg F/cm^3, factoring in the dilution factor and drilling volume. Data were expressed as the mean Δ VHN and %SMHR for each dentifrice for N = 18 replicates.

2.6. Soluble available fluoride

Soluble available fluoride concentrations were determined using method 29 in the FDA Monograph for three independent lots of freshly made MFC + F, with samples tested in triplicate. A 1:100 dilution of each sample into deionized water was prepared (0.25 g into 25 mL), and the solution mixed thoroughly for 5 min, before being centrifuged for 10 min at 10,000 rpm. To assess the fluoride level, a 1.0 mL aliquot of the supernatant was added to 1.0 mL of total ionic strength adjustment buffer (TISAB II), and the fluoride concentration measured using a fluoride ion selective electrode, as described in the ANSI/ADA Standard No. 116 Oral Rinses or ISO 16408 Dentistry – Oral hygiene products – Oral rinses. The measurement approach employed a standard curve (using fluoride standards at 1, 10, 100 and 475 ppm F).

The same set of samples from three different lots were used to determine 1-minute fluoride release. The same approach was used, but this time the baseline samples were 4.00 ± 0.10 g, and were added to 12.0 mL deionized water (1:4 dilution), with immediate homogenizing using a non-aerating stirrer for exactly 60 s, followed immediately by centrifugation for 10 min, before assessment of fluoride concentration. The test was designed on the ADA requirement that at least 80 % of the labeled fluoride concentration is released within 1 min of homogenization at a 1:4 dilution.

2.7. Fluoride uptake into incipient enamel lesions

In a variation of the above methods, a further experiment was conducted using methods for in situ fluoride uptake from fluoride dentifrices by carious enamel [39], as documented in Procedure 40 in the FDA Monograph 21 for dentifrices (Part 355 Anticaries Drug Products for Over-the-Counter Human Use). In brief, lesions were formed in enamel using a solution of 0.1 M lactic acid in 0.2 % Carbopol 907 that was 50 % saturated with hydroxyapatite, at a pH of 5.0. Supernatants of selected dentifrices were used to treat incipient enamel lesions, rather than a slurry, and then the fluoride level in the treated lesion was

determined.

Slurries were made by combining 9 g of dentifrice with 27 g of deionized water. The products tested were MFC + F, a positive control (the United States Pharmacopeia (USP) reference dentifrice with NaF (1100 ppm fluoride)), and a negative control dentifrice with no fluoride (TMFF). After thorough mixing for 5 min, the slurries were centrifuged at 10,000 rpm for 10 min. The specimens with incipient enamel lesions (N = 12 per group) were then immersed into 25 mL of their assigned supernatant with constant stirring (350 rpm) for 30 min. Samples were then washed thoroughly to remove traces of the treatment supernatants. Fluoride levels were determined following immersion of samples into 0.5 mL of 1M $HClO_4$ for 15 s, before and after treatment with the supernatants.

2.8. Enamel solubility reduction (ESR)

The final part of the study explored the effect of test dentifrices on the promotion of enamel resistance to demineralization from 0.1 M lactate buffer, using method 33 in the FDA Monograph. The seven test products comprised MFC + F, three commercial fluoride dentifrices (CCP, CPH, and SDP), two formulations of the MFC dentifrice with no fluoride, and a fluoride-free dentifrice (TMFF).

Sound human third molar teeth were placed in a disc of red boxing wax so that only the enamel surfaces were exposed. Twelve sets of three teeth each were prepared. All specimens were cleaned and polished with a flour of pumice slurry and a rag wheel to remove any deposits or stains.

For deprotection, before every use cycle, any residual anti-solubility protection afforded by the previous treatment was eliminated by etching the teeth in 0.1 M lactate buffer two periods of 60 mins each, with constant agitation, at room temperature. After deprotection, the samples were rinsed thoroughly with deionized water. A pre-treatment etch was undertaken using 0.1 M lactate buffer at 37 °C in an incubator with constant agitation of the deprotected tooth samples for 15 mins. The used buffer samples were retained for phosphate analysis.

The treatment regimen involved exposing samples a slurry of the test dentifrices (9.0 g added to 27.0 g pooled human saliva) for 5 mins. A second acid exposure was then performed using the same method as the pre-treatment etch, and once again the used lactate buffer solutions were retained for phosphorus analysis. The tooth sets were deprotected, and the procedure repeated so that each of the 7 dentifrices was treated and assayed on each tooth set. A Latin square treatment design was utilized for this purpose.

Phosphate levels in pre- and post-treatment solutions were analyzed using a Klett-Summerson photoelectric colorimeter (Bel-Art Products, Wayne, NJ, USA), with inorganic phosphate concentrations being interpolated from an 8-point standard curve. Mean values were calculated for N = 12 experimental runs.

2.9. Statistical analyses

Data sets for PCR, RDA, Δ VHN, %SMHR, fluoride uptake and ESR were assessed for normality, and then analyzed using one-way analysis of variance, with Student-Newman-Keuls or Tukey-Kramer post-hoc tests, using SigmaPlot version 13 and version 14.5 software (Graffiti, Palo Alto, CA, USA).

For the PCR data, differences between the pre- and post-brushing L* values were used to determine the PCR value by reference to the ISO/ADA reference material. Differences in the PCR values for seven products (N = 16 replicates for each) were then compared using ANOVA followed by post-hoc tests. A similar approach was taken for RDA data (with N = 8 replicates for each). For fluoride uptake into incipient enamel lesions, across the 7 groups, differences were assessed using ANOVA and then post-hoc tests (with N = 18 samples per group).

Likewise, for surface microhardness reduction, for the same 7 groups, differences were assessed using ANOVA and then post-hoc tests (with N = 18 samples per group). For enamel solubility reduction, the percent of enamel solubility reduction was computed from the difference between pre- and post-acid exposure solutions, divided by the amount of phosphate in the pre-solution and multiplied by 100. Differences between the 7 treatment groups were assessed using ANOVA and then by post-hoc tests (with N = 12 replicates for each).

3. Results

All data are presented as mean ± standard deviations.

3.1. PCR results

The seven experimental groups were well-matched at baseline, with no significant differences between them. Across all baseline samples, baseline L* values ranged from 30.8 to 37.7, while group means ranged from 34.2 to 34.3. All groups at baseline had comparable standard deviations (1.6–1.8).

All seven groups showed a statistically significant increase in L* from baseline due to the 800 brushing cycles ($P < 0.001$ for each using the unpaired T-test), indicating a reduction in extrinsic stains on the enamel surface. The mean L* scores after brushing were significantly different from the ISO/ADA control for all groups except CCP ($P < 0.01$), with SDP giving a smaller change than the ISO/ADA reference ($P < 0.001$). Based on the calculated PCR data, the six products could be ranked into three groups based on performance (Table 1), when compared to the ISO/ADA control, which had a PCR of 100.0 ± 22.6. Those within the first group (AHAW, CPH, MFC + F, COW) were the strongest performing, did not exhibit statistically significant differences in stained pellicle cleaning efficacy from one another ($P > 0.673$), exceeded the ISO/ADA reference material, and were significantly more effective than CCP and SDP ($P < 0.001$). While CCP was not significantly different from the ISO/ADA control, it was superior to SDP, which was the lowest-performing product.

3.2. RDA results

RDA data are shown in Table 1. The RDA of the MFC toothpaste with no silica was 10 ± 1. This was 10-fold lower than the ISO/ADA reference (which had an RDA of 100). The RDA for the MFC + F dentifrice with 7 % silica was 88 ± 6, which was below the ISO/ADA reference material, and significantly different from the comparison dentifrices.

Overall, all five tested products gave RDA values within the acceptable limit for dentifrice dentin abrasivity as defined by ISO and ANSI/ADA standards (i.e. RDA ≤ 250). There was a significant difference between the tested commercial dentifrices ($P < 0.001$, Table 1). The two dentifrices from the same manufacturer (CPH and C3DW) had higher RDA values, but these did not differ from one another ($P = 0.071$).

3.3. CEI results

The calculated CEI for the MFC + F dentifrice was 2.03. This was higher than other products (Table 1).

3.4. In vitro enamel remineralization and fluoride bioavailability

After the initial demineralization treatment for 33 hours, all seven experimental groups had comparable enamel microhardness (mean VHN 37.9 ± 5.4), with no significant differences between groups. Data for the 10-day pH cycling study are summarized in Table 2. The three fluoride-free dentifrices showed only a small improvement in surface microhardness, and enamel fluoride levels were in the range of 200 ppm.

Within the fluoride dentifrices, the overall ranking for recovery of surface microhardness, from best to worst, was SDP = CCP ($P = 0.239$), followed by MFC + F, then CPH. The differences between MFC + F and the two identical controls without NaF were significant ($P < 0.001$). The

Table 2
In vitro enamel remineralization, fluoride bioavailability, and enamel solubility reduction.

Dentifrice	% SMHR	Enamel fluoride	% ESR
TMFF (negative control)	2.1 ± 2.1[a]	197 ± 42[a]	-1.1 ± 4.8[a]
MFC fluoride-free (A)	18.6 ± 7.2[b]	204 ± 85[a]	0.8 ± 3.5[a]
MFC fluoride-free (B)	14.6 ± 5.1[b]	236 ± 115[a]	1.8 ± 3.8[a]
MFC + F	54.2 ± 6.4[c]	3360 ± 670[c]	23.6 ± 3.8[d]
CCP	58.0 ± 5.1[d]	3186 ± 615[c]	18.0 ± 6.6[c]
CPH	11.1 ± 3.8[b]	2340 ± 555[b]	26.1 ± 6.2[d]
SDP	60.0 ± 4.2[d]	3544 ± 645[c]	12.9 ± 3.8[b]

All data are means and standard deviations for N = 18 samples per group (N = 12 for ESR). Enamel fluoride concentrations following a 10-day pH cycling regimen are expressed in μg F/cm^3. Lower case letters show the statistical comparison between groups in each vertical column. Data values with the same letter are not significantly different from one another. ppm F = parts per million fluoride. % SMHR = percent surface microhardness recovery for incipient enamel lesions over 10 days. % ESR = percent enamel solubility reduction. MFC fluoride-free (A) and (B) refer to two different batches of the vehicle control with no fluoride.

lowest 2 performing products for surface microhardness recovery were CPH with stannous fluoride, and the fluoride-free product TMFF.

Data for enamel fluoride concentrations at the end of the 10-day in vitro pH cycling study are also shown in Table 2. MFC + F, SDP and CCP all performed equally well in promoting fluoride uptake into incipient enamel lesions. All of these were superior to the remaining four products ($P < 0.001$). The next best-performing product was CPH. As with % SMHR, the differences between the MFC + F dentifrice and the two identical vehicle controls without NaF were significant ($P < 0.001$).

3.5. Soluble available fluoride

For the three batches of MFC + F dentifrice, the mean values for soluble available fluoride in ppm (with SD) after 5 min mixing were 1076.2 (±3.7), 1079.4 (±3.9), and 1081.8 (±2.6). The matched values for 1 min mixing were 953.3 (±11.2), 964.1 (±14.1), and 961.9 (±9.7). The amount in these compositions was 1086 ppm F from 0.24 % NaF.

3.6. Fluoride uptake into incipient enamel lesions

The three treatment groups in this experiment were not significantly different at baseline, with enamel fluoride concentrations of 44-51 ppm. Based on promoting fluoride uptake into incipient enamel lesions (change in ppm fluoride ±SD for N = 12), the three tested products were ranked in effectiveness as follows: MFC + F (2,746 ±290 ppm) > Positive control USP reference dentifrice (1,316 ± 228 ppm) > Negative control (TMFF, 20 ± 13 ppm). The MFC + F dentifrice was significantly more effective than the positive and negative controls ($P < 0.001$).

3.7. Enamel solubility reduction

Data for %ESR are shown in Table 2. Of the 7 tested products, the two strongest performing products were CPH containing 0.454 % stannous fluoride (1100 ppm F) and MFC + F, which were not significantly different from one another (26.1 ± 6.2 and 23.6 ± 3.8, $P = 0.204$). Both were superior to the next ranked products, which were CCP and SDP. The three fluoride-free controls did not significantly reduce the solubility of enamel to a lactic acid challenge in the ESR assay.

4. Discussion

The results of this study show that the novel MFC + F dentifrice (Protegera®) is highly effective at removing stained pellicle, whilst having relatively low dentin abrasiveness when compared to marketed dentifrices. At the same time, the MFC + F dentifrice is effective for delivering fluoride for enamel remineralization. Taking these qualities together, the MFC dentifrice challenges the generally accepted view that effective removal of external stains necessitates having a high content of abrasives, which pose an inherent risk of dentin abrasion.

The reason for the surprisingly good performance of the MFC + F dentifrice is that its mode of action involves more than simple abrasion of stained pellicles by abrasive particles that are trapped momentarily between the toothbrush bristles and the stained pellicle. The MFC particles themselves have cleaning actions, and they also entrap silica particles to move these across the surface. They also collect extrinsic stains that have been dislodged from the surface [22].

The MFC + F dentifrice used in this study contained 7 % by weight of a high cleaning silica product designed to provide superior performance in dentifrices. The silica particles have a median particle size of 10 μm. The manufacturer of the silica (Evonik) recommends using this particular material (Zeodent® 103) in dentifrices at a loading of 15–20 % by weight, to achieve an expected PCR of 100 and an RDA of 195. In the MFC dentifrice used in this study, a Zeodent 103 loading of only 7 % by weight was used. This achieved a PCR of 141 and an RDA of 88. This indicates that the MFC particles greatly enhance the performance of the silica particles in removing stained pellicle.

The current laboratory data add to results from randomized clinical trials that show superior plaque removal for the MFC dentifrice [20]. Logically, better removal of the dental plaque biofilm should contribute to preventing dental caries and improving gingival health, while better removal of stained pellicle should give cosmetic benefits. While the 3-dimensional cellulose network in the MFC + F dentifrice is responsible for both actions, it is not inherently abrasive. This is shown by the MFC + F dentifrice without silica having an RDA of only 10.

Due to the array of colored foods and beverages in a modern diet, and the influence of tobacco and other lifestyle factors on extrinsic stains, many individuals in the community will likely require some degree of abrasive action to prevent the accumulation of extrinsic stains on their teeth. Thus, the challenge lies in finding the least abrasive approach in a dentifrice to achieve this goal [40–42]. Some modern dentifrices contain a high loading of abrasive particles, giving them a high RDA [17]. On the other hand, including MFC in a dentifrice with a low dose of high-cleaning silica can give effective removal of stained pellicle removal, without a high risk of dentin abrasion, to achieve a high cleaning efficiency index.

While PCR and RDA assessments are well-known laboratory assessment parameters with clinical correlates, the ultimate performance measure of a dentifrice is seen when it is used in the clinical setting. Past work has shown that the MFC + F dentifrice has superior dental plaque removal performance compared to CCP [20]. Long term studies are needed to assess the relative performance of the MFC + F dentifrice for reducing the formation of extrinsic stains, in individuals whose diet and lifestyle habits encourage the deposition of stained material onto tooth surfaces, appliances and prostheses.

The fundamental concept in the dentifrice is that the network structure of the MFC particles enhances the rheological properties of the dentifrice. The particles intimately contact the tooth surface, with the force applied by the bristles of the brush. The motion of the toothbrush back and forth (or other motions) propels the MFC + F dentifrice and creates optimal shear forces, which remove both stained pellicle and biofilm together. As well, the MFC particles entrap silica, which enhances its performance in cleaning, but without causing surface damage.

It is noteworthy that the cleaning mechanism of the MFC + F dentifrice is associated with enhanced fluoride uptake. This could be because the surface is now free of deposits that could act as barriers to diffusion. The absence of any such layers would facilitate the diffusion of fluoride ions into incipient enamel lesions, thus enhancing their remineralization.

In terms of protecting enamel, in vitro assessments of fluoride bioavailability at 1 and 5 minutes, fluoride uptake into lesions, increased surface microhardness of treated incipient enamel lesions, and enamel

solubility reductions all show that the MFC + F dentifrice with 1086 ppm fluoride performed similarly to other marketed fluoride dentifrices. It was superior to fluoride-free products, and to those with stannous fluoride. In the 10-day pH cycling study, the MFC + F dentifrice was comparable to other NaF dentifrices, while in the ESR assay, it was superior to other dentifrices with NaF with similar levels of fluoride, and no different from the stannous fluoride dentifrice. This is an unexpected result, and indicates that delivery and efficacy of fluoride is greater than expected, in terms of the protection afforded to the enamel.

When combined with the high CEI and low RDA, these positive results for fluoride efficacy indicate that using MFC in a dentifrice can enhance multiple aspects of its performance. Future work should explore the ability of MFC + F dentifrices to prevent enamel demineralization and impede caries progression in the clinical setting. This would provide a more comprehensive view of the dynamic process of dental caries (both preventing demineralization and promoting remineralization) that could benefit from enhanced plaque removal and high fluoride effectiveness.

5. Conclusions

Under the conditions tested, these laboratory data indicate that a sodium fluoride dentifrice with micro-fibrillated cellulose and entrapped silica can be highly effective at removing extrinsic stains, while at the same time having a low dentin abrasivity, and strong performance for fluoride uptake, promoting the demineralization of incipient enamel carious lesions, and reducing enamel solubility.

CRediT authorship contribution statement

Mohamed E. Labib: Conceptualization, Funding acquisition, Project administration, Methodology, Writing – review & editing. **Antonio Perazzo:** Conceptualization, Resources, Writing – review & editing. **James L. Manganaro:** Resources, Writing – review & editing. **Yacoob Tabani:** Writing – review & editing, Resources. **Carmine J. Durham:** Conceptualization, Funding acquisition, Writing – review & editing. **Bruce R. Schemehorn:** Project administration, Methodology, Validation, Data curation. **Heath C. McClure:** Methodology, Validation, Formal analysis, Data curation, Writing – review & editing. **Laurence J. Walsh:** Conceptualization, Formal analysis, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Mohamed E. Labib, Antonio Perazzo, James L. Manganaro and Yacoob Tabani are employees of NovaFlux, Inc. and consultants to Protegera, Inc.

Carmine Durham is the CEO of Protegera, Inc.

Bruce R. Schemehorn and Heath C. McClure are employees of Therametric Technologies, Inc.

Laurence J Walsh is a consultant to Protegera, Inc.

The authors declare no other competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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