Abstract

Objective: A new fluoride varnish (Embrace™) containing xylitol-coated calcium and phosphate (CXP™) claims to release ten times more fluoride over a four-hour period than leading fluoride varnishes. The purpose of this study was to compare the quantity and rate of fluoride release from enamel of a newly marketed fluoride varnish compared to three other fluoride systems.

Study design: Human third molars were cut into sections and Embrace™, Enamel Pro®, Duraphat®, or Vanish™ fluoride varnishes were applied to the enamel surfaces. Specimens were immersed in synthetic saliva which was replaced at tested intervals. The concentration of fluoride in ppm was measured after specified hourly intervals during the first week and then weekly until the limit of detection (LOD). Mean cumulative fluoride release and rate of release were analyzed with a one-way ANOVA/Tukey (α=0.05).

Results: Significant differences existed between groups (p<0.001). Mean cumulative fluoride release is Embrace™>Enamel Pro®>Duraphat®=Vanish™. Rate of fluoride depletion is Embrace™>Duraphat®=Enamel Pro®=Vanish™.

Conclusions: Embrace™ had the greatest initial fluoride release, exceeding ten times the release of a leading fluoride varnish in the first four hours; however, Embrace™ had the highest rate of fluoride depletion and lowest substantivity of all varnishes tested.

Keywords: Varnish; Fluoride; Xylitol-coated calcium and phosphate

Introduction

For the caries process to occur there must be a susceptible host, bacteria, environment, and time. The teeth are in a constant cycle of demineralization and remineralization. When the rate of demineralization exceeds that of remineralization with a sustained drop in pH below 5.5, enamel dissolution occurs and the caries process begins [1]. A challenge to the critical pH may occur as a result of the ingestion of fermentable carbohydrates and retention of cariogenic bacteria in plaque resulting in demineralization of tooth structure [2]. Fluoride concentrated in plaque and saliva shifts the oral cavity demineralization/remineralization equilibrium to the mineralization state [3]. Sound and carious enamel have significantly differing reactivities to fluoride in regards to remineralization. Carious enamel shows high fluoride reactivity thus rapidly acquiring greater amounts of total fluoride. Interestingly and perhaps of even more benefit, fluoride is not readily lost in carious enamel suggesting that the lesion may serve as a fluoride retention source [4].

Drinking-water fluoridation first began in 1945 in four different communities with a caries reduction of 50-70% seen among children [5]. Furthermore, the development of fluoride-containing products including dietary supplements, toothpastes, mouth rinses, and professionally prescribed or applied foams, gels, or varnishes was a result of the noted success of water fluoridation in preventing and controlling caries [6]. Despite this knowledge, the Food and Drug Administration (FDA) Center for Devices and Radiological Health has sanctioned the use of topical fluoride varnishes only as root desensitizing agents and cavity liners at this time [7]. Although widely used in Canada and Europe as anticaries agents since the 1970s, the FDA has not approved topical fluoride varnishes for this purpose. Prior to marketing these varnishes as anticaries agents, companies would be required to submit appropriate clinical trial evidence for review. However, topical fluoride varnishes are applied in professional healthcare settings for their “off-label” use of caries prevention based on professional judgment [8].

Today, since their introduction in Europe, topical fluoride varnishes have become routinely used to remineralize tooth structure in high caries risk populations. These varnishes are either 5% sodium fluoride (2.26% F - or 22,600 ppm) or 1% difluorosilane (0.1% F - or 1000 ppm). More recently, experimental Ti, varnishes of varying percentages have been evaluated indicating an equal protective potential to sodium fluoride (NaF) formulations with greater fluoride release [9,10]. As mentioned previously, fluoride shifts the oral cavity from a state of demineralization to a state of remineralization. When applied to tooth structure as a topical varnish, the fluoride is dissolved in an organic solvent that evaporates in a moist environment. Through a remineralization reaction, calcium fluoride deposited on the tooth’s porous surface is converted to fluorapatite [11]. At neutral pH there is an insoluble reservoir of fluoride on the tooth surface providing substantivity and potentiating anticaries effects. The rate of dissolution.
of the fluoride reservoir increases with a cariogenic challenge and corresponding drop in pH. The solubility constants of calcium and phosphate ions are lowered resulting in fluoride release. Plaque calcium and phosphate ion concentrations are also increased. This mechanism prevents calcium and phosphate dissolution from tooth mineral and/or increases the remineralization rate or reprecipitation of lost minerals [12].

Topical fluoride varnishes are most commonly composed of a natural resin carrier containing fluoride ions and an organo-phosphoric-acid adhesion-promoting agent and may include one or more solvents such as ethanol. The natural resins include shellac, mastic, rosins and/or colophonium. Shellac and mastic provide a flexible, permeable hard surface that prevents rapid dissolution of the varnish in saliva while colophonium is included as a flow enhancer. Manufacturers may also add flavoring agents, sweeteners (e.g., sodium saccharin), and solubilizing agents. Different topical fluoride varnish manufacturers also may include proprietary additives in their formulations such as tri-calcium phosphate (TCP), amorphous calcium phosphate (ACP), and xylitol-coated calcium phosphate (CXP®). These manufacturers have various claims associated with their formulations and their superior clinical efficacies.

Duraphat® (Colgate Palmolive, New York, NY), the first commercially available fluoride varnish, is the most extensively studied and widely used topical fluoride varnish on the market today [13]. It contains either a 2.26% fluoride ion or 5% NaF ion and leaves a yellow film on teeth post-application that must be removed by brushing. Duraphat® is used in more than 40 countries with a reported 30% caries reduction according to Petersson et al [14,15]. Colgate Palmolive does not list any proprietary additives in Duraphat®.

Vanish™ (3M ESPE, St. Paul, MN), the number one selling topical fluoride varnish in the US (according to 3M ESPE), employs 5% NaF and TCP as its proprietary additive ingredient. The manufacturer states that TCP, unlike other calcium phosphate additives, is able to achieve more acid-resistant mineral nucleation through the addition of low levels of functionalized TCP. The increased contact time reportedly allows for the increased efficacy of the fluoride varnish [16]; however independent studies have refuted these claims [17].

Enamel Pro® Varnish (Premier Dental, Plymouth Meeting, PA) includes ACP as an active ingredient with the manufacturer’s claim that it is able to deliver up to four times more fluoride than the leading varnish, suggesting a higher clinical efficacy [18]. In conjunction with 5% NaF, ACP is stated to help reduce dentinal sensitivity [19]. Studies involving Enamel Pro Varnish showed a reduction in hydraulic conductance by 73% and semi-permanent occlusion of dentinal tubules with fluoroapatite supporting their claim [20,21].

In laboratory studies, amorphous calcium phosphate-casein phosphopeptide (ACP-CPP) was shown to remineralize enamel subsurface lesions [22]. ACP-CPP (also known as Recaldent) is a milk-protein-derived product. Casein phosphopeptides (CPP) contain multi phosphoseryl sequences with the capability of stabilizing calcium phosphate in nanocomplexes in ACP solutions as well as binding CPP multi phosphoseryl sequences with the capability of stabilizing calcium milk-protein-derived product. Casein phosphopeptides (CPP) contain subsurface lesions [22]. ACP-CPP (also known as Recaldent) is a conductance by 73% and semi-permanent occlusion of dentinal tubules and TCP as its proprietary additive ingredient. The manufacturer states that TCP, unlike other calcium phosphate additives, is able to achieve more acid-resistant mineral nucleation through the addition of low levels of functionalized TCP. The increased contact time reportedly allows for the increased efficacy of the fluoride varnish [16]; however independent studies have refuted these claims [17].

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Castillo and Milgrom supported the use of topical fluoride varnishes specifically looking at two different application protocols using Duraphat® [24]. The study showed the cumulative release of fluoride was higher and the rate of release was slower in three applications in one week as opposed to one application. Fluoride was available for a longer period with the three application method, resulting in better caries-reduction rates.

Shen and Auto-Gold assessed fluoride concentration uniformity and fluoride release from Duraphat®, CavityShield™ (3M ESPE) and Duraflor® (Medicom, Montreal, Canada) [25]. The study revealed fluoride concentration to be more uniform in Duraphat® and CavityShield™ than in Duraflor®. The most consistent fluoride release was found in Duraphor®. Differences in resin carriers and additives were found to have a significant effect on fluoride release.

Recently, Pulpdent Corporation (Watertown, MA) introduced Embrace™ Varnish (5% sodium fluoride with CXP®). The manufacturer claims Embrace™ Varnish has ten times more fluoride release over a 4-hour period than the leading varnish brand (i.e., Vanish). The incorporation of CXP™ (xylitol-coated calcium and phosphate) purportedly drives the sustained time-released properties of this varnish. Saliva dissolves the xylitol coating of the permeable resin matrix allowing calcium and phosphate ions to react continuously with the fluoride ions to form fluoroapatite on teeth [26].

Manufacturers’ claims, formulations with specific active ingredients, popularity, familiarity, and newly marketed products all play a role in a clinician’s choice of topical fluoride varnish. Ultimately, the choice of a specific topical fluoride varnish may also depend on patient feedback regarding taste and color as well as price. For both remineralization and caries prevention, clinicians desire a topical fluoride varnish that possesses the highest cumulative and sustainable fluoride release profile. Many patients are seen bi-annually by clinicians for routine examinations where low levels of fluoride release over many months are most beneficial. If continual cumulative and sustainable effects are achieved with periodic topical fluoride applications, the cycle of restorative treatment and possible premature tooth loss may be significantly reduced in populations at risk for caries. Salivary fluoride levels following the application of fluoride varnish are influenced by the initial fluoride concentration applied, time since exposure, delivery method, fluoride retention, and fluoride clearance from the oral cavity. Previous research has shown that the greatest release of fluoride occurs in the first three weeks and then tapers [24,27].

The purpose of this in vitro study was to evaluate the substantivity of a new topical fluoride varnish, Embrace™ Varnish (5% NaF) with CXP®, when compared to the quantity and rate of fluoride release from enamel of currently marketed topical fluoride varnishes. The null hypotheses tested were that there would not be a difference in the cumulative fluoride release or the rate of fluoride depletion between the various fluoride varnishes.

**Methods and Materials**

The study protocol was approved by the Institutional Review Board at Wilford Hall Ambulatory Surgical Center, JBSA-Lackland, Texas. Fifty extracted human third molars with sound enamel free of caries, demineralization, and enamel defects were chosen for this study and stored in 0.5% Chloramine-T until use. Extracted teeth were randomly collected from patients exposed to various levels of systemic and topical fluoridation and were used within three months of extraction. Using a diamond-tipped disk, two 4×4 mm sections were cut from each tooth using the middle third portion of the crown on the buccal and lingual
surfaces. Only one buccal and lingual specimen was sectioned from each tooth. There were 5 groups with 20 specimens, each consisting of 10 buccal enamel surfaces and 10 lingual enamel surfaces. After sectioning, specimens were stored in vials labeled as buccal or lingual. Specimens were rinsed with water to remove debris then dried with cotton gauze. Nail lacquer was applied to all dentin surfaces leaving only the enamel surface exposed and specimens were again stored in Chloramine-T until protocol initiation. Specimens were dried with cotton gauze prior to varnish application.

Specimens were weighed using an analytical balance (GH-252 semi-micro balance, A&D Weighing, San Jose, CA), the balance was tared, and then 3 mg of each of Duraphat®, Embrace™, Enamel Pro®, or Vanish™ topical fluoride varnishes were applied by pipette to exposed enamel surfaces using a new pipette per application. One group served as a control and did not receive fluoride varnish. Each specimen's weight was recorded after adding varnish.

A housing assembly was designed (Figure 1) to prevent specimens from adhering to the sides of the dilution vials with possible loss of fluoride and to facilitate specimen transfer when changing saliva. The housing assembly components (Oatey Plumber’s Putty, Charlotte, NC; Shoe Goo® flexible adhesive-sealant, Eugene, OR; caps from 1.5 ml Simport microcentrifuge tubes and lids from 10 ml polyethylene Simport Cryovials, Belloq, QC, Canada) were tested using a fluoride combination ion-selective electrode (Accumat, Fisher Scientific, Pittsburgh, PA) and an ion analyzer (AR50 meter, Accumet, Fisher Scientific) prior to initiation of the protocol to confirm the absence of extraneous release of fluoride. Measurements were made in 20 ml polystyrene dilution vials (Evergreen Scientific, Los Angeles, CA).

Specimens were immersed in 20 ml of artificial saliva at room temperature using the artificial saliva formula as described by Lata et al. It is composed of Na₃PO₄, 3.90 mM; NaCl, 4.29 mM; KCl, 17.98 mM; CaCl₂, 1.10 mM; MgCl₂, 0.08 mM; NaHCO₃, 3.27 mM and distilled water; and titrated with H₂SO₄ to pH 7.2 [28]. At the specified testing intervals, specimens were transferred to 20 ml of fresh artificial saliva in new dilution vials. The preceding 20 ml of artificial saliva was vortex mixed and fluoride analysis was conducted using a 10 ml aliquot of the saliva combined with 10 ml of total ionic strength adjusting buffer (TISAB II, Fisher Scientific).

The fluoride electrode and ion analyzer were used to measure fluoride concentration in the specimens. Validation of precision and accuracy was performed on four different days using a fluoride solution of known concentration. The validated limit of detection was 0.02 ppm fluoride for this assay system. A styrofoam spacer was placed on the magnetic stirrer surface to prevent heat from reaching the sample fluoride and to facilitate specimen transfer when changing saliva. Measurements were made in 20 ml polystyrene dilution vials (Evergreen Scientific, Los Angeles, CA).

The fluoride electrode was calibrated every 2 hours with TISAB II solution and fluoride standards at 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.5, 1.0 and 5.0 ppm. At the beginning of each timed-interval fluoride analysis, 0.1, 0.5, 1.0 and 5.0 ppm control (standard) samples were run with each group of the test specimens. Fluoride concentration was measured at 1, 4, 8, 12, 24, and 48 hours, then daily for the remainder of the first week, and weekly thereafter until the limit of detection. Specimens were run against a standard curve. The Accumet AR50 meter computed calculations internally with results reported in ppm.

A one-way analysis of variance (ANOVA) and Tukey’s post hoc tests (α=0.05) were used to analyze the mean cumulative fluoride release until the limit of detection and the rate of release at four hours among the varnishes tested.

**Results**

The cumulative fluoride release until the limit of detection was reached is displayed graphically in Figure 2. Table 1 illustrates the significant differences between groups (p<0.001) as determined by the one-way ANOVA statistical analysis for both mean cumulative fluoride release in ppm until the limit of detection and the rate of fluoride depletion in ppm/hr over the first four hours. None of the negative control groups exceeded the 0.02 ppm limit of detection for any measured time interval.

Embrace™ exhibited a significantly greater mean cumulative fluoride release among the varnishes tested.
release of all groups tested (2.870 ppm) but also the lowest substantivity with levels dropping below the limit of detection at two days. Enamel Pro® had a significantly lower mean cumulative fluoride release (1.507 ppm) than Embrace™ with levels dropping below the limit of detection at four days. Duraphat® and Vanish™ had a significantly lower mean cumulative fluoride release (1.028 ppm and 0.780 ppm respectively) than Embrace™ and Enamel Pro® but were not significantly different from each other. Duraphat® fluoride levels were below the limit of detection at three weeks while Vanish™ had the highest substantivity with levels dropping below the limit of detection after five weeks.

In the first four hours, Embrace™ had the greatest rate of initial fluoride release, delivering more than ten times the mean cumulative amount of fluoride measured in ppm compared to both Duraphat® and Vanish™ and six times greater when compared to Enamel Pro®. The mean cumulative fluoride release in ppm at four hours was 2.677 for Embrace™, 0.439 for Enamel Pro®, 0.209 for Vanish™ and 0.180 for Duraphat® as shown in Figure 3. For both Duraphat® and Vanish™, the rate of mean cumulative fluoride release plateaued at one week until dropping below the limit of detection as shown in Figure 2.

Figure 4 illustrates the rate of fluoride depletion of the groups tested over the first twenty-four hours. The greatest rates of fluoride depletion occurred in the first four hours for all of the groups tested. As shown in Table 1, the rate of fluoride depletion of Embrace™ was significantly greater than the other three varnishes tested, which were not significantly different from each other. The rate of fluoride depletion began to plateau after eight hours until the limit of detection for all groups tested except Enamel Pro® which did so after twelve hours.

**Discussion**

The results of this study demonstrated that the newly marketed fluoride varnish, Embrace™ had a significantly different fluoride release profile compared with the other varnishes tested. The first null hypothesis was rejected. Embrace™ released significantly greater cumulative amounts of fluoride after application than Enamel Pro®, Duraphat®, and Vanish™. Pulpdent claims that Embrace™ varnish with CXP™ releases ten times more fluoride over a four hour period than the leading fluoride varnish. Results of this study showed a mean cumulative fluoride release of 2.677 ppm by Embrace™ at four hours. This level of fluoride release was approximately six, thirteen, and fifteen times higher than that of Enamel Pro®, Vanish™, and Duraphat™ respectively at four hours supporting the manufacturers claim (Table 1). It can be speculated the addition of CXP™ aids in better initial fluoride release with Embrace; however, the manufacturer’s claim that it aids in sustained high fluoride release was not demonstrated or controlled for in this study. These findings would suggest that the clinically relevant time to wait to resume tooth brushing and food consumption is at least four hours after the application of Embrace™. Enamel Pro® had the second highest cumulative fluoride release. Enamel Pro® is similar in formulation to traditional fluoride varnishes but with the addition of ACP. Both Duraphat® and Vanish™ released similar cumulative amounts of fluoride, although Duraphat® reached its limit of detection in a shorter period of time. The second null hypothesis was also rejected. The rate of fluoride depletion into the artificial saliva was significantly higher for Embrace™ than Duraphat®, Enamel Pro®, and Vanish™. Embrace™ exhibited the lowest substantivity with fluoride levels dropping below the limit of detection after only two days followed by Enamel Pro® with only four days.

Similar results were found in a previous study by Jablonowski et al. [29], examining the amount and rate of fluoride release of two recently developed fluoride varnishes, Enamel Pro® and Vanish™ XT (3M ESPE) in comparison to two traditional fluoride varnishes, Duraphat® and Vanish™. Embrace™ with CXP had not yet been marketed. Their study revealed that the greatest cumulative fluoride release was seen with Enamel Pro® followed by Duraphat® and Vanish™ which were not significantly different from each other, and finally by Vanish™ XT. Vanish™ XT had the lowest fluoride release of all the varnishes evaluated, but the slowest rate of release. Vanish™ XT is a resin-modified glass ionomer which may potentially be recharged with additional fluoride exposure. Enamel Pro® had the greatest rate of fluoride depletion from 1 week to the limit of detection followed by Vanish™, Duraphat® and Vanish™ XT. A more recent study by Ritwik et al. also found differences in fluoride release profiles between the varnishes tested [30]. PreviDent® (Colgate), Enamel Pro®, and Vanish™ had plateaus in their rate of fluoride release after four hours. In the first 8 hours, Enamel Pro® Varnish had the highest fluoride release. Vanish™ XT had no significant change in rate of fluoride release at any time. The results of these studies suggest that fluoride...
release may be dependent on the type of resin carrier or additives used by different manufacturers.

During the testing process, differences were noted in the viscosity and ease of application among the varnishes. It was noted that Embrace™ was highly viscous. Vanish™ was also highly viscous, but became sticky and flaky when dried. The claim by the manufacturer that the addition of low levels of functionalized TCP in Vanish™ allows for greater contact time and clinical efficacy [16] may correlate to the sticky nature of the varnish as well as the highest substantivity as found in this study. Enamel Pro™ was neither viscous nor sticky upon opening but became more viscous after 1-2 minutes, and Duraphat™ was mildly viscous.

In vitro studies suggest that even low salivary fluoride levels can inhibit demineralization and bolster remineralization [31]. After fluoride varnish application, salivary fluoride levels represent the fluoride available for caries prevention. Based on in vitro studies, fluoride levels exceeding 0.03 ppm in saliva and plaque result in caries prevention which is near the limit of detection of 0.02 ppm [3]. The fluoride levels measured in this experimental model have no exact clinical implication, but the relevant differences in release profiles between groups may be significant. In the clinical setting, a reduction in fluoride levels would be more rapid due to the effects of saliva on fluoride retention and oral function including chewing, swallowing, dietary acidic challenges, brushing, flossing, and tongue movement. Also, human saliva is constantly changing in regard to temperature, pH, and protein content. Aside from the long-term and geographical impracticalities of collecting teeth from people exposed to the same levels of systemic and topical fluoride; there are also other variables from individual to individual in use of fluoridated dentifrices and mouth rinses, and professional placement of fluoride varnish. This study used fresh artificial saliva at the various time intervals for analysis to simulate more normal oral conditions/clinical scenarios where frequent salivary exchange exists but also to prevent mold and bacterial growth. Specimens were stored on a laboratory rotator and incubator at intraoral temperature which promoted homogenous dispersion of fluoride ions in the artificial saliva throughout the protocol. Without the use of a rotator, supersaturation of fluoride ions would occur in the saliva around teeth causing inaccurate fluoride readings. Specimens were included for analysis only until the limit of detection (0.02 ppm). A control set of specimens without fluoride was run at the same intervals to verify the fluoride probe was running accurately. Data were normalized and time units were made equivalent.

Additional studies should examine uptake of fluoride in enamel after fluoride varnish application. As tooth structure experiences fluoride reservoir dissolution with drops in pH, fluoride varnish performance at different pH may be of interest [12]. There are studies to indicate that some fluoride-containing products show an increase in release with a decrease in pH while others show a decrease in release with a decrease in pH [32,33]. Intuitively, a topical fluoride varnish with high initial fluoride release would allow for greater amounts of fluoride available for enamel uptake [30]; however, some studies have shown fluoride efficacy cannot be measured by the product’s fluoride release rate [34].

Larsen, et al. concluded the critical pH is between 5.5 and 4.5 when studying the effects of acidic beverages on enamel [1]. As the saliva becomes supersaturated with respect to fluoroapatite and unsaturated with respect to hydroxyapatite, a carious lesion develops as the result of demineralization. Dawes refuted the critical pH as a fixed value but rather that enamel dissolution is inversely proportional to both plaque fluid and salivary concentrations of calcium and phosphate [34]. Furthermore, dental enamel composition slightly varies from individual to individual in respect to the presence of impurities such as carbonate and fluoride. These impurities influence the solubility of enamel. Some authors have suggested that individual ions such as calcium or phosphate may be supersaturated in saliva and plaque fluid [35]. Dawes refuted this theory stating that while saliva and plaque fluid may be supersaturated in respect to tooth enamel, individual ions cannot be supersaturated [34]. The inference that the proprietary carrier CXP™ (xylitol-coated calcium and phosphate) in Embrace™ varnish would allow for a shift to supersaturation of concentrations of calcium or phosphate ions and thus a state of precipitation and remineralization needs further investigation.

Should a supersaturation of calcium and phosphate ions be possible, the clinical implications could theoretically be useful when using a varnish with a fluoride release profile such as Embrace™. Supersaturation of ions may allow for greater fluoride uptake due to the shear amount of fluoride release alone and may be beneficial for patients with a diet consisting of frequent consumption of fermentable carbohydrates and repeated acid challenges [36]. While multiple studies using randomized controlled trials have reported the efficacy of topical varnish application using various recommendations for application intervals, [37-41] Marthaler states it is both higher frequency of application and higher concentration that allow for greater clinical efficacy of topical fluoride [42]. One could speculate that due to such low substantivity, in order for Embrace™ to be as efficacious as the other varnishes tested, it would require a higher frequency of professional application, which may not be practical or cost-effective for some patients.

Based on the results of this in vitro study, all of the products evaluated provide an excellent approach to caries prevention, but varnish selection should be based on each individual patient’s clinical presentation and provider preference. Both Embrace™ with CXP™ and Enamel Pro™ with ACP had greater mean cumulative fluoride release but with low substantivity and may be indicated for patients with high caries risk requiring more frequent follow-up or reapplication. Both Vanish™ and Duraphat™ had lower cumulative fluoride release but relatively high substantivity and may be indicated for lower risk patients with white spot and incipient lesions that require less frequent recalls.

Conclusion

In summary, Embrace™ had the greatest initial fluoride release, exceeding ten times that rate of a leading fluoride varnish (i.e., Vanish™) in a four hour period. However, Embrace™ had the highest rate of fluoride depletion and the lowest substantivity of all varnishes tested. Vanish™ and Duraphat™ had the lowest initial fluoride release; however, they had greater substantivity than Embrace™.

Disclosure

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Conflict of Interest

The views expressed in this article are those of the authors and do not reflect the official policy of the United States Air Force, the Department of Defense, or the United States Government. The authors do not have any financial interest in the companies whose materials are discussed in this article.
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