AMMONIUM HEXAFLUOROSILICATE:

A PROSPECTIVE ALTERNATIVE TO SILVER DIAMINE FLUORIDE

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

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According to the 2015 National Health and Nutrition Examination Survey, one in every seven United States children, between the ages two and eight, have untreated dental caries. Arresting dental caries is critical within the primary dentition to prevent further complications in permanent tooth development. Recent research suggests silver diamine fluoride (SDF) is an increasingly popular method for arresting dental caries in pediatric patients. However, SDF precipitates an irreversible black stain due to formation of silver oxide. Ammonium hexafluorosilicate (AHF) is an innovative approach to arresting dental caries and treating dentinal hypersensitivity without the effect of staining due to the use of silica instead of silver. Research has shown that AHF elicits calcium phosphate precipitation and dentinal tubule occlusion. Therefore, AHF could be an effective treatment for dentinal hypersensitivity and arresting dental caries. Cytotoxicity tests have been conducted to evaluate cell cultures enzyme activities, membrane integrity, alteration of cell morphology and cell growth inhibition using concentrations ranging from 0.001%-1%. Concentrations of AHF ranging from 0.1%-1.0% have generated significant loss ($p<0.05$) of human gingival fibroblasts (hGFs); however, lower SDF

concentrations of 0.01%-1.0% promoted similar significant losses of hGFs. Until further research is completed, the cytotoxicity of AHF appears to be acceptable for gingival tissue use at concentrations of 0.001%-0.01%, especially when compared to the cytotoxicity of SDF being acceptable at concentrations of 0%-0.005% on hGFs. In conclusion, *in-vivo* studies are needed to determine the clinical efficacy and long-term effects of AHF as a potential product to treat dental caries and dentinal hypersensitivity without the negative aspects of staining.

DEDICATION

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KEY WORDS

AHF	Ammonium Hexafluorosilicate
CA	Caries Arrest
C	Cytotoxicity
DH	Dentinal Hypersensitivity
SDF	Silver Diamine Fluoride
S	Staining

INTRODUCTION

According to the National Health and Nutrition Examination Survey in 2015, one in every seven US children ages two to eight have untreated dental caries.[1] Arresting dental caries is critical within the primary dentition to prevent further complications in the development of permanent teeth. Fluoride varnish is currently being used as the primary strategy for preventing dental caries; however, it is not able to restore deeper cavitated lesions.[1] These cavitated lesions (also known as dental caries) require mechanical removal by rotary burs and are treated with a restorative material or a crown. These supplies can be an expensive option for some parents, while others may perceive restorative treatment to be unnecessary for deciduous teeth that will eventually be replaced by permanent teeth.

Recent research suggests silver diamine fluoride (SDF) is becoming an increasingly popular method for arresting dental caries in pediatric patients.[2] However, SDF applied to teeth and surrounding tissues precipitates an irreversible black stain due to formation of silver oxide.[1] The adverse aesthetic results of SDF could be a potential deterrent for treatment, especially for anterior teeth.[2] Ammonium hexafluorosilicate (AHF) is an innovative approach to arresting dental caries and treating dentinal hypersensitivity without the staining effect illustrated by SDF.[3] In 2015, Savas et al. demonstrated that by replacing the silver component of SDF with silica, AHF remains comparable in caries arrest while also displaying aesthetically pleasing results.[4]

Research on AHF as an alternative to SDF supports the current National Dental Hygiene Research Agenda priority area Oral Health Care-New Therapies & Prevention Modalities. The purpose of this literature review is to address the ability of AHF to arrest caries, treat dentinal

hypersensitivity, its toxicity on the tissues, and how it compares to SDF. Further research on this topic could encourage dental practitioners to offer AHF as a more aesthetically pleasing option to stop the progression of caries and treat dentinal hypersensitivity.

SECTION I

CARIES ARREST CAPABILITIES AND DENTINAL HYPERSENSITIVITY

Objective 1

Several *in-vitro* studies have shown that AHF could be effective in arresting dental caries and treating dentinal hypersensitivity.[1,3-8] A 2013 study conducted by Hosoya et al. examined the effects of AHF versus SDF on 20 extracted maxillary primary canines.[3] The researchers etched 20 extracted non-carious primary teeth with 35% phosphoric acid for six minutes.[3] The teeth were then immersed in a solution of either AHF, artificial saliva or a combination of the two.[3] Following scanning electron microscopy and x-ray dispersive spectrometry, the authors concluded that AHF application remineralizes enamel, increases the uptake of fluoride, and results in a significantly higher calcium to phosphate ratio.[3] The researches also reported that following AHF application, there was an increase in hydroxyapatite formation, which resulted in a more acid resistant tooth surface.[3] Additionally, they found that AHF application did not significantly affect the structure and elemental content of artificially demineralized primary tooth enamel ($p>0.05$).[3] The authors reported that immediately after AHF was applied to the extracted teeth, the dentinal tubules were completely occluded with a silica-calcium phosphate precipitate to a depth of 20 microns.[3] Hosoya et. al. suggested further studies need to be conducted to determine the stability of AHF as a caries arresting product within the oral cavity.

In 2008, Suge et al. examined how AHF elicits calcium phosphate precipitation and occlusion of the dentinal tubules.[5] The enamel from the extracted tooth was removed with a high-speed rotary handpiece and immersed for three minutes in a 0.5 mol/L

ethylenediaminetetraacetic acid (EDTA) to remove the smear layer and open the dentinal tubules.[5] Suge et al. found that the opened dentinal tubules were completely occluded by a formation of a silica-calcium phosphate complex precipitate, regardless of the concentration of AHF used.[5] The authors repeated the 2008 experiment in 2010 in order to determine if a specific concentration of AHF was more effective at treating dentinal hypersensitivity.[6] They found that a range between 1000 and 9000 ppm of AHF was optimal for occluding dentinal tubules, based on clinical safety and efficacy.[6] The 2010 study continued to further support the clinical application of AHF; however, *in-vivo* studies are needed to determine the long term desensitizing effects.[5,6]

An additional promising technology for the management of dental caries is the use of lasers in combination with AHF and SDF. The erbium-doped yttrium aluminum garnet laser (Er:YAG) laser has been found to be efficient in ablating hard tissue and transforming hydroxyapatite into fluoridated hydroxyapatite.[7] A 2016 study by Kucukyilmaz et al. reported that other studies also suggested the uptake of fluorides in enamel and root surfaces increased post-laser treatment.[7] Kucukyilmaz et al. evaluated the effect of Er:YAG laser irradiation combined with SDF and AHF on sound and caries-affected dentin.[7] Ninety-six extracted molars were used to determine the effects of combining the Er:YAG laser with AHF and SDF.[7] The authors concluded that calcium and phosphate content was significantly higher after applying AHF, as opposed to SDF, to caries affected dentin ($p<0.05$).[7] These results are similar to the Hosoya et al. 2013 study, which found that, upon AHF application, a silica-calcium-phosphate precipitate occluded tubules 20 microns deeper within the dentin surface.[3] In addition, AHF was more effective in arresting dental caries when used in combination with the laser compared to SDF ($p<0.05$).[7]

A 2018 study by Suge et al. evaluated the acid resistance of AHF in combination with four antibacterial products: chlorhexidine (CHX), Cetylpyridinium chloride (CPC), isopropyl methylphenol (IPMP) and epigallocatechin gallate (EGCG).[8] Silver naturally has a higher antibacterial effect than other elements.[8] Therefore, the authors combined the four antibacterial solutions with AHF in order to match the antibacterial effect of SDF.[8] Brain heart infusion (BHI) broth inoculated with *streptococcus mutans* was combined with AHF and the four antibacterial solutions to create the culture media.[8] Hydroxyapatite pellets served as the control to evaluate the acid resistance and depth of demineralization upon exposure to AHF and each antibacterial solution.[8] The authors found that the combination of AHF with CPC, IPMP and EGCC created an acidic pH while CHX created a near-neutral environment.[8] The addition of CPC to AHF was the most effective in occluding dentinal tubules by inducing the formation of a calcium phosphate precipitate from artificial saliva which created the highest antibacterial activity ($p<0.05$).[8] Although there is a need for studies to determine the clinical efficacy of AHF, this study suggests the use of AHF as a promising non-invasive treatment modality for arresting dental caries.

SECTION II

TOXICITY ON GINGIVAL TISSUES

Objective 2

The cytotoxicity of AHF and any potential harmful clinical effects has yet to be determined.[9] Cytotoxicity tests involve the evaluation of cell cultures on their enzyme activities, membrane integrity, alteration of cell morphology, and determination of cell growth inhibition.[9] The research in this area is limited and only one study has explored whether AHF is cytotoxic to the tissues in the oral cavity.

In 2013, Song et al. used a 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazoli-umbromide (MTT) assay, mitochondrial membrane integrity examination (MMP test) through 5,5,6,6-tetrachloro-1,1,3,3-tetraethylbenzimidazolyl-carbocyanine iodide (JC-1) staining and viewed the intracellular Glutathione (GSH) levels to determine if AHF was cytotoxic to the cells.[9] Song et al. examined the different dosages and durations of AHF on human gingival fibroblasts (hGFs) and determined their outcomes.[9] The AHF concentrations used in this study were 0.001%, 0.01%, 0.1% and 1% and were applied to the different cell cultures at one, five, ten, and thirty minutes.[9] Song et al. used an MTT assay to determine the cell viability of the hGFs after AHF was applied.[9] The authors found that treatment with AHF affected cell viability in both a dose and time dependent manner.[9] The MTT assay showed that there was a significantly lower number of viable cells when the hGFs were treated with concentrations of AHF at 0.01% (for ten minutes), 0.1% (ten and thirty minutes) and 1% (for five, ten, and thirty minutes), when compared to their respective control groups ($p<0.05$).[9] The human gingival

fibroblasts exposed for one minute with all the different AHF concentrations showed no significant differences in cell viability ($p>0.05$).[9]

In 2013, Song et al. also examined the effects of AHF to the mitochondrial membranes using an MMP assay and the GSH depletion to verify the underlying mechanism of the AHF cytotoxicity.[9] The MMP assays used cells that were seeded in 6-well plates and cultured until the mitochondria in the cells attached to the plates and demonstrated a change in MMP concentration.[9] A decrease in MMP is responsible for executing the apoptosis cascade in numerous cell cycles, which can ultimately lead to further cell death.[9] The results showed that longer exposure times of ten and thirty minutes, as well as higher AHF concentrations of 0.1% and 1%, demonstrated a larger reduction in the MMPs, but not enough to be considered a significant decrease in these mitochondrial proteins ($p>0.05$).[9]

GSH levels were determined by using 6-well plates that were cultured the same as the MMPs and treated with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB).[9] When the plates turned yellow, this meant the solution was reacting with levels of GSH.[9] Cellular GSH is a thiol that, when reduced, can lead to oxidative stress, cell viability inhibition, apoptosis, and tissue inflammation.[9] GSH depletion levels increased when applied with higher concentrations of 0.1% and 1% AHF and with longer durations of ten and thirty minutes.[9] Even the cells with 0.001% AHF at thirty minutes and the 0.1% concentration at one, five, and ten minutes showed GSH depletion levels when compared to the control group, which had no concentration of AHF applied ($p<0.05$).[9] Song et al. findings suggest that GSH levels may be more sensitive to AHF application, as opposed to the other results from the MTT and MMP assays.[9]

Although the findings reported by Song et al. showed cytotoxicity at different concentrations, the results also demonstrated that when AHF was placed for a shorter duration

(one to five minutes) and a smaller concentration (0.001%-0.01%) they were not significantly damaging to the tissues ($p>0.05$).[9] Song et al. also reported that a concentration of 0.01% AHF placed on the tissues for less than five minutes showed no significant effects on the relative growth rate of the hGFs ($p>0.05$).[9] This is important when determining whether AHF will be applied in future clinical studies because partially cytotoxic materials should not be placed or left on the tissues for longer than five minutes.

More recently, an *in-vitro* study by Fancher et al. in 2019 investigated the effects of SDF on hGFs and hydroxyapatite (HA) discs using 38% SDF.[10] The product was diluted with saline to generate different concentrations of 0%, 0.001%, 0.002%, 0.005%, 0.01%, 0.02%, 0.05%, 0.1%, 0.2%, 0.5% and 1%, that were all placed on separate plates containing hGFs for two minutes to mimic a brief exposure.[10] The plates were then rinsed with saline for up to 93 days in order to determine the lasting effects of the cytotoxicity on the tissues and HA discs.[10] The results showed that SDF was cytotoxic to the hGFs at concentrations of 0.01% - 1% ($p<0.05$), and retained cytotoxicity when bound to the HA discs for an additional 63 days after application.[10] Compared to SDF, a higher concentration of 0.1% AHF is needed to cause a significant loss of hGFs ($p<0.05$).[9] Until further research is done to determine the best concentration of AHF to arrest caries and stop dentinal hypersensitivity, the cytotoxicity of AHF at concentrations of 0.001%-0.01% are considered acceptable for intraoral use.

SECTION III

COMPARING AND CONTRASTING AHF TO SDF

Objective 3

The most notable difference of AHF compared to SDF is the lack of staining after application to the teeth.[3] Over time, SDF turns the tooth a dark brown/black color, which could result in lower acceptance and satisfaction among patients, parents, and dental professionals.[1,2] However, *in-vitro* studies have shown that treating a carious lesion with AHF does not produce staining due to replacing the silver with silica.[4-8] In 2019, Magno et al. found that staining from SDF was not a concern among parents of pediatric patients.[2] Instead, dental professionals assumed the parents would complain about staining aspect.[2] Crystal et al. in 2017 reported approximately 92% of 74 pediatric dentistry program directors assumed that the staining of SDF would be a concern among parents.[11] The authors then surveyed 120 parents of children with previous caries to determine whether aesthetics affected their decision for treatment.[11] Tooth location played an important factor in parental acceptance with significantly lower acceptance for anterior teeth (10.2%) than for posterior teeth (21.7%) ($p<0.001$).[11] The findings from this study suggests that poor aesthetics can impact acceptance of a new dental technique or restorative material.

An additional aspect to consider for arresting dental caries is the invasiveness of the procedure. SDF and AHF are both non-invasive treatments that are applied topically and do not require the mechanical removal of enamel.[1,3] In 2017, Crystal et al. also found that more than 90% parents who were hesitant to use SDF (due to its staining effects) would accept SDF treatment if it meant their children would not have to undergo sedation or general anesthesia.[11]

Patients with a disability or those who exhibit dental anxiety, may benefit from a less invasive treatment such as SDF or AHF.[1,3] Once the Food and Drug Administration (FDA) has determined whether AHF is safe to use intraorally, dental professionals will have another treatment option for arresting dental caries.

A 2019 study conducted by Saleh et al. examined the public's knowledge of certain chemicals and whether that information provoked them to fear products with chemicals.[12] Saleh et. al. found that peoples' fear of chemicals is largely fueled by negative associations from the stigmatized term "chemical substances".[12] When consumers express concerns about the chemicals contained in products, they may choose to avoid using the product.[12] While greater knowledge of chemicals has been associated with lower levels of chemophobia, it could be difficult to overcome the negative stigma associated with the term.[12] Negative stigmas, could potentially persuade patients and/or parents to avoid using AHF and/or SDF due to the chemical compounds that are presented in both names. According to Saleh et al., prior studies have shown that informing consumers with the benefits associated with a given product can improve people's level of acceptance and/or promote a positive perception of the product.[12] Another approach to reduce people's fear of chemicals is to stress the implications of alternatives to the chemical substance being used.[12] Although AHF and SDF are products with chemical names, their non-invasiveness provide a different process of arresting dental caries compared to the standard.

CONCLUSION

Research has shown that dental practitioners are hesitant to offer SDF as a treatment option due to the perception that patients would be unsatisfied with the aesthetic results.[1,2] *In-vitro* studies support the use of AHF as a promising non-invasive treatment modality for dentinal hypersensitivity and arresting dental caries while having less cytotoxic effects on human gingival tissues than SDF.[1,3-10] Research regarding AHF has been conducted for over the last ten years but is limited to only *in-vitro* studies and one clinical trial.[13] Furthermore, longitudinal studies are needed to determine the clinical efficacy of AHF. Once AHF is approved by the FDA for clinical use, practitioners could choose to use this product as a treatment for arresting dental caries and treating dentinal hypersensitivity without the negative aspects of staining.

REFERENCES

1. Sarvas E, Karp JM. Silver Diamine Fluoride Arrests Untreated Dental Caries but Has Drawbacks. American Academy of Pediatrics News and Journals Gateway Website. https://www.aappublications.org/news/2016/08/05/SilverDiamine080516. Published 2016. Accessed October 3, 2019.

2. Magno MB, Silva LP, Ferreira DM, et al. Aesthetic perception, acceptability and satisfaction in the treatment of caries lesion with silver diamine fluoride: A scoping review. *Int J Pediatr Dent.* 2019;29(3):257-266.

3. Hosoya Y, Tadokoro K, Otani H, et al. Effect of ammonium hexafluorosilicate application for arresting caries treatment on demineralized primary tooth enamel. *J Oral Sci*. 2013;55(2):115-121.

4. Savas S, Kucukyilmaz E, Celik EU, et al. Effects of different antibacterial agents on enamel in a biofilm caries model. *J Oral Sci.* 2015;57(4):367-372.

5. Suge T, Kawasaki A, Ishikawa K, et al. Ammonium hexafluorosilicate elicits calcium phosphate precipitation and shows continuous dentin tubules occlusion. *Dent Mater*. 2008;24(2):192-198.

6. Suge T, Kawasaki A, Ishikawa K, et al. Effects of ammonium hexafluorosilicate concentration on dentin tubule occlusion and composition of the precipitate. *Dent Mater*. 2010;26(1):29-34.

7. Kucukyilmaz E, Savas S, Akcay M, et al. Effect of silver diamine fluoride and ammonium hexafluorosilicate applications with and without Er:YAG laser irradiation on the microtensile bond strength in sound and caries-affected dentin. *Lasers Surg Med*. 2016;48(1):62-69.

8. Suge T, Shibata S, Ishikawa K, et al. Fluoride activity of antibacterial ammonium hexafluorosilicate solution for the prevention of dentin caries. *Am J Dent.* 2018;31(2):103-106.

9. Song DX, Zheng LW, Shen SM, et al. Cytotoxicity of ammonium hexafluorosilicate on human gingival fibroblasts. *Toxicol in Vitro*. 2013;27(8):2149-55.

10. Fancher ME, Fournier S, Townsend J, et al. Cytotoxic effects of silver diamine fluoride. *Am J Dent*. 2019;32(3):152-156.

11. Crystal YO, Janal MN, Hamilton DS, et al. Parental perceptions and acceptance of silver diamine fluoride staining. *J Am Dent Assoc*. 2017;148(7):510-518.

12. Saleh R, Bearth A, Siegrist M. "Chemophobia" today: consumers' knowledge and perceptions of chemicals. *Risk Anal.* 2019;39(10):2117-2349.