EFFECT OF SILVER DIAMINE FLUORIDE ON THE TREATMENT OF GINGIVITIS IN GERIATRIC PATIENTS

A Dissertation

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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August 2020

Major Subject: Oral Biology

ABSTRACT

Background: Gingivitis in the geriatric population is a growing public health concern. Finding an effective non-invasive approach to prevent and treat gingivitis is crucial and challenging. It was evident in many studies that silver diamine fluoride was effective in arresting dental caries because of its antibacterial activities. Silver diamine fluoride contains silver and fluoride ions. It has been shown that both ions have a role on inhibition of cariogenic biofilms. The potential of this agent to treat gingivitis had never been tested.

Objectives: This study investigated the effectiveness in clinical signs of inflammation following application of 38% silver diamine fluoride (SDF) to the gingiva of healthy geriatric patients with gingivitis. In addition, it determined whether SDF treatment has an effect on dental plaque accumulation after multiple applications.

Materials and Methods: This was a 7-week randomized, controlled, prospective double-blinded in-vivo study. Thirty geriatric participants (≥65-y old) with gingivitis; living in senior-retirement-homes; were identified based on inclusion criteria and randomly allocated to two groups (n=15 each). The experimental group received silver diamine fluoride (38%) applications while the placebo (saline group) received blue-tinted saline solution applications. Solutions were applied once a week for three consecutive weeks. Gingival index (GI) and Plaque index (PI) scores were obtained at baseline before treatment and at three follow up time-points (weeks 3, 5 and 7). Clinical intraoral photographs of teeth and gingivae were used for visual assessment of gingival health status.

Results: Between-groups comparison revealed statistically significant reduction in the mean GI levels ($p<0.05$) using Löe-Silness gingival index and PI mean levels (Silness-Loe plaque index) ($p<0.05$) with visually less dental plaque accumulation and less signs of gingival inflammation (redness, swelling and bleeding) in silver diamine fluoride group compared to placebo group at all follow up time points starting week 3. No silver diamine fluoride-related adverse events were reported or observed. **Conclusion**: These results provide tangible evidence that silver diamine fluoride application is associated with better gingival health. Silver diamine fluoride has the potential to be a new adjunctive, cost effective and noninvasive tool for treating gingivitis.

DEDICATION

To my mother and father without them I would not be the person I am today. They gave me love, support and motivation throughout my life.

To my sisters and brother for their continuous support and love.

To my amazing husband who accepted the challenge to let me live in a different part of the world to achieve my dream. His continuous patience and love made me strong to complete my journey with happiness until the end.

To my first happiness and my little princess, Reema, who always was my smile whenever I was down.

To my family in law for their continuous support.

ACKNOWLEDGEMENTS

I started my PhD journey four and half years ago, in each year, I gained valuable skills and learned things I never think I will learn so fast. Now, I believed that PhD is a unique journey should be documented. From the bottom of my heart, I would like to say big thank you for Dr. Kathy Svoboda and Dr. Amal Noureldin for their consistent support and guidance during this research and without whom I would not have been able to complete this research. The guidance and consistent motivation I have received from Dr. Kathy Svoboda, program director and my committee chair, helped me to achieve my goals. She worked hard with me from the first day in my journey until I made my research plan and put the wonderful committee together. She encouraged me to do what I am passionate about and made my choices without any pressure. She always directed me whenever I got off the road and put me back on the track. Her office was always open for me whenever I needed advice.

Dr. Amal Noureldin, my committee co-chair and mentor for this research, gave me what a student really need to be a clinical researcher. She was the foundation of this research and helped in all the aspects from the beginning until the end. She also participated in the visits to senior homes. She always made me feel confident in my abilities to accomplish my research. It was a dream for me to conduct a clinical research and a challenge I accepted and learned how to face. I learned how to multitask, be an investigator, photographer, coordinator... etc. Dr. Noureldin taught me how to move forward whenever unexpected obstacles were thrown in our way. She handled my

everyday visits to her office sometimes several visit in a day and always welcomed me with her smile.

My sincere thanks to Dr. Helena Tapias for being a part of my committee and an examiner in the clinical project. I am grateful for her help, efforts, and time spent with me recruiting participants from several senior homes. She was available whenever I needed assistance and always made things easier on me. We made more than 150 visits to senior homes last year. In all of them, we shared memories and I learned special skills from her experience with geriatric populations.

I would like to express my sincere gratitude towards Ms. Lisa Mallonee, Dr. Peggy Timothe and Jacqueline Plemons for being a part of my committee and for all their support and guidance throughout the years. Ms. Mallonee was a great help as a reviewer of my first paper. Also special thanks to Ms. Jane Cotter who helped in reviewing my first paper. My sincere thanks to Dr. Peter Buschang for his great advice and help in the statistical analysis. A special thanks to Dr. Larry Bellinger, Dr. Lynne Opperman and the graduate research staff, Kim, Marge, Nancy, Sophie, and everyone for their help throughout the program. And a big thank you to my friends and colleagues Afnan, Amani, Mirali, Mickey, Mohammed, Qian, Rajay, and all graduate students.

I would like to thank all senior homes coordinators for allowing us to recruit and conduct the research in their facilities. Special thanks to the wonderful residents who participated in the research and enabled this research.

Biggest thanks to my family overseas for all the support you have shown me through this research. For my little princess, Reema, sorry for being even grumpier than

normal whilst I wrote this dissertation! And for my husband Turki, thanks for all your support, without which I would not have completed this journey.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a dissertation committee consisting of Dr. Kathy Svoboda of the Department of Biomedical Sciences, Oral biology Program Director and committee chair, Dr. Amal Noureldin of the Department of Public Health Sciences, committee co-chair, Dr. Helena Tapias of the Department of Restorative Sciences, Dr. Jacqueline Plemons of the Department of Periodontics, Ms. Lisa Mallonee of the Caruth School of Dental Hygiene and Dr. Peggy Timothe of the Department of Public Health Sciences.

The recruitment of participants and data collection for Chapter 2 were performed at eight Independent Senior Living centers (Dickinson Place, Notre Dame, Aya Village, Lakeland Hills, Casa Trevino, Nolen grand, Hillside West). Wedad Alshehri wrote the manuscripts and performed the majority of the research requirements, recruiting, collecting data, and interpretation of the data. Dr. Helena Tapias and Amal Noureldin helped in performing the dental examinations for the participants. Part of the data analyzed for Chapter 2 was provided by Dr. Peter Buschang of the Department of Orthodontics.

Funding Sources

Funding for the research was provided by Clinical Research Grant Award-2018 from the Office of Graduate Studies in Texas A&M University College of Dentistry. The financial support of the graduate study for PhD degree was provided by a full scholarship from King Saud University, Saudi Arabia.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

Page

LIST OF TABLES

Page

1. INTRODUCTION- SILVER DIAMINE FLUORIDE AS A NON-INVASIVE METHOD FOR MANAGING DENTAL CARIES (REVIEW)

1.1. Overview

Silver diamine fluoride (SDF) is an inexpensive, antimicrobial liquid that is indicated for the treatment of treating tooth sensitivity. It is also used off label for the prevention and treatment of dental caries. Since SDF approval by the Food and Drug Administration (FDA) in 2014, several clinical trials have investigated its effectiveness, potential side effects and safety in addition to its acceptance by parents whose children are at high risk for dental caries. Due to the high prevalence of dental caries especially in young children, patients with special health care needs and geriatric population, there is a need to move from invasive traditional therapies to noninvasive methods.

A possible barrier to patient acceptance is that SDF causes black staining when applied to carious lesions. Educating both oral health practitioners and patients about its effectiveness, and the esthetic options available after treatment might impact the attitudes and acceptance of SDF. Increasing oral health practitioners' knowledge of SDF including its components, mechanism of actions, indications, procedure of application and potential side effects may positively impact SDF adoption and increase its use in high risk populations. The acceptance of SDF could make a significant change in the preventive management and treatment approaches for dental caries.

1.2. Use of Silver Compounds in Dentistry

In dentistry, silver nitrate ($AgNO_3$), one of the most common silver salts, was first used as early as the 1840s.[1] It was used in the 1800s as an antimicrobial solution because of its antibacterial activity, low toxicity and lack of bacterial resistance.[2] Another silver compound, silver fluoride (AgF) was used in the 1970s by the Western Australia School Dental Service as part of the treatment of disadvantaged young children. Application of silver fluoride was followed by an application of stannous fluoride (SnF_2) to prevent the recurrence of new carious lesions. This approach resulted in 74% of the proximal and 90% of the occlusal surfaces of the existing carious lesions remaining arrested without the need for further operative intervention.[3] Regardless of the success of this approach in controlling caries, there were limited trials investigating this method after 1990's.[4]

In Japan, ammonia (NH_3) was added to AgF which created the silver diamine fluoride (SDF) formulation $AgF(NH_3)_2$. This was first investigated in 1969 by Mizuho Nishino at Osaka University in Japan.[5] As a result of using this compound, dental caries was arrested, and hypersensitivity was reduced due to occlusion of the dentinal tubules by formation of precipitates. Soon after these investigations, SDF was approved as a as a therapeutic cariostatic agent in Japan and was marketed as Saforide (Toyo Seiyaku Kasei Co. Ltd., Osaka, Japan).[6] Since the 1980s, the use of SDF as an alternative dental treatment for the management of dental caries has been available in many countries such as Japan and Australia.[7]

In the United States (U.S.), three of the founders of modern dentistry including: W.D. Miller, G.V. Black and Percy Howe used silver nitrate to arrest carious lesions.[8, 9] A

protocol in the late 1800s was developed by Black in which silver nitrate was applied multiple times to the dental carious lesion until it was arrested.[10] Likewise, Howe was well known for using ammoniacal silver nitrate solution ($AgNH_3NO_3$) for the treatment of dental caries. Hence, this solution was called Howe's solution by many dental professionals in the 1920s.[11] In the 1950s, Howe's solution was used as an antimicrobial product to sterilize infected dentin, disinfect root canals and treatment of deep carious lesions.[12] Early literature demonstrated silver nitrate as an effective agent in arresting initial carious lesions.[13] In the mid-20th century, however, silver nitrate use diminished and was no longer being used.[9]

In 2005, Black's protocol was reintroduced and implemented by Dr. Steven Duffin. The combined application of 25% silver nitrate solution with 5% sodium fluoride varnish was used to treat more than 5000 children at Shoreview Dental in Keizer, Oregon (2005-2011). Using this protocol , the carious lesions in almost all the affected teeth were arrested.[9] In 2014, the US Food and Drug Administration (FDA) cleared SDF as a Class II medical device for treating tooth sensitivity (Figure 1) and it is currently marketed as Advantage Arrest™ (Elevate Oral Care LLC, West Palm Beach, FL).[14] In 2016, "the FDA awarded breakthrough therapy status as a commitment to an application for approval of SDF as a drug to treat severe early childhood caries. The breakthrough therapy designation announcement suggests that SDF may become the first FDA-approved drug for treating dental caries".[15] In 2017, Canada approved SDF use with an indication of anti-caries.[16]

1.3. Mechanism of Silver Diamine Fluoride

SDF is an inexpensive and chemotherapeutic agent. It is a liquid composed of three main components. It contains fluoride (5%) as the active material to promote remineralization and silver (25%) as an active material that works as an antimicrobial. In addition, inactive agents include water (62%) and ammonia (8%).[7] The mechanism of action of SDF for treating dentinal hypersensitivity is similar to that of 5% sodium fluoride (NaF) varnish. SDF forms insoluble precipitates with phosphate or calcium in the dentinal tubules which block nerve impulses. The fluoride ions in SDF bind with hydroxyapatite forming calcium fluoride to occlude the exposed dentinal tubules.[17] Silver ions aid in the formation of sclerotic dentin through the deposition of calcium fluoride and silver phosphates. This hardening of the tissue with the dark brown or black layer is considered a clinical indication of caries arrest (Figure 2).[7]

SDF has bifunctional properties. The silver ions have an antimicrobial and bactericidal capacity through which it can destroy bacterial membranes, denature proteins, and inhibit the replication of DNA. The fluoride ions help to promote remineralization and prevent demineralization by creating fluorapatite.[18] A study by Duangthip et al. in 2015 indicated that silver ion particles could remain unchanged after application and provide a lethal effect on bacteria.[19] The dead bacterial cells act as a reservoir for these silver particles. When silver ions are released from this reservoir, they become bactericidal to immediately surrounding pathogens.[19] SDF is the most concentrated fluoride product available for caries management. The 38% SDF solution contains a higher level of fluoride ions (44,800 ppm) than 5% sodium fluoride varnish (22,600 ppm).[20]

1.4. Procedure of SDF Application

SDF application is considered a noninvasive procedure but there are precautions that should be followed prior to application.[15] The patient should sign a consent form following education about the procedure, indications for use and possible side effects. SDF corrodes metal and glass, so it is recommended to use a plastic dappen dish during application**.** A plastic lined bib should be worn to protect the patient's clothing and as well as protective eyewear due to the high pH of SDF. The gingiva and mucous membranes should be protected by petroleum jelly to avoid potential changes such as pigmentation or irritation. Gross debris should be removed to allow better SDF contact with denatured dentin. Carious dentin excavation is not necessary; however, it may reduce the size of the arrested black lesion.[21] It is essential to dry the tooth with a gentle flow of compressed air (or use cotton rolls/gauze to isolate). SDF is then applied to the dentinal surface. To remove the taste after SDF application, the tongue should be rinsed or wiped.[16]

A review by Horst et al. in 2016 suggested the recommended application time ranges from one to three minutes. [14] A recent review in 2017 by Crystal et al., recommended the ideal application time is one minute.[16] Thus, current literature demonstrates that dental caries arrest and/or prevention rates do not correlate to time of application.[14] There are no limitations to the patient eating, drinking or brushing after SDF application. However, a clinical trial by Zhi et al. recommended the same post-operative instructions that are typical for topical fluoride applications including no drinking or eating for 30 minutes to one hour as it may provide a better result(not verified by clinical studies.[22]

1.5. Indications for using SDF

The incidence of oral disease is relatively greater for young children, medically compromised individuals, and lower-income and rural populations.[23] Indications for using SDF in the clinical setting include populations with high caries risk due to xerostomia, behavioral challenges or complex medical histories and the presence of several carious lesions that will require multiple appointments. Additionally, SDF is indicated for patient populations with limited access to care.[15] SDF is approved for the treatment of dentinal hypersensitivity, but similar to fluoride varnish, SDF is used off-label in the U.S. to arrest tooth decay.[14]

SDF has the potential to reduce Streptococcus mutans (*S. mutans)* counts due to its higher fluoride concentration which interferes with S. *mutans* cell function and reducing acid solubility in addition to the antibacterial effect of silver.[24] In 2017, a systematic review by Crystal et al. concluded that the mechanism of action of SDF is bactericidal to cariogenic bacteria primarily, *S. mutans.* In addition, they found that the growth rate of S. *mutans* was also reduced.[25] Literature reviews by Rosenblatt et al. and Peng et al., demonstrated that SDF has been used more widely than other silver fluoride-based preparations.[4, 7] In Japan, several studies on children claim its effectiveness. The significant findings of Lo et al. indicated the effectiveness of 38% SDF on arresting coronal caries in primary teeth and preventing new coronal caries in primary and secondary teeth.[26] Chu et al. and Zhi et al. conducted studies on pre-school children using 38% concentration of SDF. This concentration level showed a significantly higher number of arrested caries lesions when compared to placebo or no treatment.[21, 22] Sharma et al.

compared two different concentrations of SDF 38% and 12% and concluded that 38% SDF was more effective in arresting dental caries in primary dentitions than 12% SDF.[27]

Clinical trials since 2000 have indicated that SDF application is efficient in arresting caries when applied to occlusal, facial, and lingual surfaces.[14, 22] A systematic review in 2011 by Yeung et al., estimated an 81% likelihood of caries arrest in the primary teeth following treatment with 38% SDF regardless of the application regimen and duration of evaluation.[28] In 2017, a study conducted in Oregon by Clemens et al. documented 100% caries arrest rate after 3 months.[29]

A recent clinical trial suggests beginning treatment with more frequent applications and decreasing the application frequency with time, while maintaining at least annual application and removing the rinse step after SDF treatment.[30] Individuals with high caries risk are treated with a more intensive regimen which includes multiple applications for the first few weeks, followed by semiannual maintenance doses.[15]

In 2010 a study conducted on elderly subjects by Tan et al. concluded that 38% SDF had a greater effect on preventing root caries compared to the other interventions such as OHI, use of 5% NaF or 1% chlorhexidine varnish.[31] A study in 2013 by Zhang et al. on elderly subjects, indicated that the group who received biannual oral hygiene instructions (OHI) and oral health education (OHE) in addition to the SDF application, showed greater effect in arresting root caries. This study was the first to assess the effectiveness of SDF application in arresting root surface caries and as a method to prevent the development of new carious lesions in this population.[32] SDF could potentially improve oral health and reduce the need for emergency care and treatment.[7]

1.6. Side Effects

The main disadvantage of SDF use is the permanent staining of the carious lesion.[14] However, the arrested carious lesion can be restored to improve esthetics. In addition, stains on the margins of composite restorations and crowns can be removed with gentle rubber cup polishing.[33] A review in 2009 reported that SDF may cause gingival/mucosal irritation; this is a temporary effect and heals spontaneously within 48 hours without treatment.[7] Therefore, precautions should be taken to avoid contacting the soft tissues during application.

If SDF is accidentally applied to the skin or gum, a brown or white stain may appear that does not cause any harm but cannot be washed off, however it will disappear in one to three weeks. Moreover, a bitter or metallic taste has been reported by patients.[14] Contraindications for SDF use include allergy to silver, mucosal inflammation, and an exposed pulp.[14] SDF could be irritating to the pulp tissues in deep lesions. However, there are no reports of severe pulpal damage or reaction to SDF application. A study by Duangthip et al. concluded that there is very low risk of oral pain, gum swelling and gum bleaching after SDF application. In addition, none of the participants reported any symptoms associated with acute toxicity or systemic illness.[34] To date, there have been no reports of toxicity, death or adverse systemic effects associated with the recommended application of SDF.[16]

1.7. Acceptance and adoption of SDF

The management of dental caries, in children under the age of 3 years, those with severe early childhood caries or patients with special health care needs could be complicated due to cost and risk. The use of sedation and general anesthesia prior to

operative treatment could be costly and a potential risk to life. [35, 36] The clearance of SDF in the U.S. provided an agent for change to noninvasive caries management. Rapid adoption of SDF despite the non-esthetic results indicates that parents prefer it compared to traditional operative dentistry provided under sedation or general anesthesia.[15] A study by Tesoriero et al. in 2016 asked 33 parents to choose between treatment with SDF or white plastic resin fillings. The majority of the parents chose SDF and preferred a black stain over injections, anesthesia, and prolonged treatment time.[37]

Due to the black staining caused by SDF, studies have been conducted to collect feedback and gain insight on the perceptions of the parents of children who had black staining caused by SDF. A study by Chu et al., concluded that satisfaction of the parents (n=120) with their child's appearance after treatment with SDF was not significantly different than it was prior the application.[21] The acceptance of staining of posterior teeth was higher (67.5%) than acceptance of staining on the anterior teeth (29.7%).[38] Although the staining of the anterior teeth is undesirable, most of the parents (60.3%), especially those with young children or with special needs were accepting of the stain to avoid sedation or general anesthesia. Conversely, about one-third of parents did not accept SDF treatment under any circumstance due to the staining of teeth.[38]

A recent study by Nelson et al., surveyed a group of dental hygienists about the use of SDF. Approximately half of the respondents were unfamiliar with SDF. After explaining the rationale for use of SDF, the dental hygienists responded that the advantages of SDF outweighed the disadvantage of permanent black staining of the carious lesion.[39] The topic of SDF is not addressed in most dental schools but more recently it has become a common topic introduced in pediatric dental residency

programs.[40] SDF application is cost effective. One 8mL bottle of Advantage Arrest™ is approximately $174 and it contains 8mL of 38% SDF. It provides approximately 250 drops. This is enough to treat 125 sites. A site is defined as “up to 5 teeth”.[14] Regulations and state practice acts may vary from state to state regarding who can apply SDF.[20]

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2. SILVER DIAMINE FLUORIDE SIGNIFICANTLY DECREASED GINGIVITIS IN GERIATRIC PATIENTS IN THREE WEEKS (CLINICAL TRIAL)

2.1. Introduction

In many countries, life expectancy is 80 years. Worldwide in 2010, the world health organization (WHO) indicated that 524 million people were older than 65 years and the number was predicted to grow to approximately 1.5 billion by 2050. This increase in the life span was associated with challenges. An example of these challenges, was the health problems associated with aging which can be manageable or preventable.[1] Many physical, behavioral, and social changes were associated with aging.[2] A study conducted in Kenya on elderly persons, indicated that 40% suffered from dental problems.[3] The WHO in collaboration with the government of India conducted a study on an elderly population to determine health problems affecting the aging population. They found that 32.6% of the elderly had dental problems as well as other medical problems.[4] Several factors could act as barriers in the utilization of dental services. Examples included cost of treatment, lack of dental insurance, accessibility to dental clinics, education status of patient, fear of dental treatment, and functional and medical status of individual.[5-7] Based on several studies, the main factor that caused elderly patients to avoid dental visits was that they did not recognize or appreciate the need for any dental treatment.[8]

Some elderly patients were more susceptible to oral diseases because they retained their teeth longer and poor oral hygiene was common. The elders who lived in

long-term care facilities usually had poor oral health and experienced more untreated dental problems.[9] Dental caries and periodontal diseases were common in elderly people.[10, 11] The incidence of root caries increased with age based on many epidemiological studies.[12] The frequent use of some commonly prescribed medications among elders may have increased the risk for dry mouth which increased the risk for oral disease.[13] Oral dryness and root surface exposure due to periodontal disease also increased the risk for root caries.[14] Based on the studies by Holm-Pedersen et al., elders were more susceptible to periodontal inflammation.[15]

Periodontal diseases are classified into gingivitis and periodontitis. Page and Schroeder divided the stages of the periodontal diseases; gingivitis and periodontitis into the early lesion, established lesion and advanced lesion with the last representing periodontitis, a destructive phase.[16] In the oral cavity, dental plaque was recognized as a biofilm of microorganisms that accumulated on hard and adjacent soft tissues.[17, 18] Dental plaque was identified as the main causative agent of gingivitis and implicated for a significant role in the initiation of periodontitis.[19] The studies by Holm-Pedersen et al., demonstrated that elders were more susceptible to periodontal inflammation induced by microbes.[15] Periodontal diseases including both gingivitis and periodontitis were considered the most common chronic infections in the elderly population.[20] Furthermore, the duration of exposure to bacterial plaque may have increased the susceptibility to other chronic diseases.[21]

Gingivitis has been defined as localized inflammation of the gums without loss of bone structures supporting the teeth, and the symptoms for gingivitis including swollen

gums with erythema and bleeding with or without brushing. Usually, pain is not associated with gingivitis.[22] Gingivitis could be prevented by good oral hygiene, including adequate tooth brushing and flossing. If gingivitis was left untreated, it may progress to a destructive phase, termed periodontitis. In contrast, periodontitis is characterized by loss of connective tissue attachment and supporting bone which can lead to tooth mobility and tooth loss.[22] The severity and the prevalence of periodontitis increases with age.[23, 24] As proposed in other studies, the increase in chronic diseases with age including cardiovascular, diabetes and Alzheimer's diseases have been associated with periodontal disease.[25-27] Therefore, periodontal disease, should not be underestimated; and the advancement of e treatment and prevention should be a priority in dental and medical fields.[20]

For successful treatment of gingival inflammation, it is crucial to implement effective oral hygiene and plaque control methods. Effective mechanical plaque control (tooth brushing) has been challenging. It was shown that a majority of the adult population did not practice adequate plaque control.[28] Therefore, many studies have been conducted searching for an effective adjunct agent to mechanical plaque control methods.[29, 30] Several agents (toothpastes, mouthwashes, gels and varnishes) have been tested in clinical trials for anti-plaque and anti-gingivitis effects.[22] In addition, mouth rinses have been used widely as an oral hygiene aid to deliver therapeutic agents to gums and teeth.[31]

Chlorhexidine gluconate (CHX) has been considered the gold standard as an effective antimicrobial agent and chemotherapeutic aid to control plaque and gingivitis. It has been available as a mouthwash, topical gel and biodegradable chips.[32] For more than

40 years, CHX was the primary antiplaque agent studied and used in many clinical applications in dentistry.[33] However, the side effects associated with CHX including altered taste sensation, increased calculus formation and teeth staining were undesirable.[31] Various studies reported that 2% CHX used as a topical gel resulted in significant reduction in gingivitis.[34] In addition, CHX mouth rinses provided significantly decreased plaque and gingivitis.[35] CHX mouth rinse was the first choice for patients who could not practice adequate oral hygiene.[36]

Thus, the use of CHX in solution such as Peridex (0.12% CHX) for elderly patients at risk for dental caries and periodontal disease was considered beneficial.[37] A rinse with 0.12% chlorhexidine solution (Peridex) in elderly subjects either daily or weekly for 6 weeks was effective in reducing gingivitis; and improving periodontal conditions (plaque index, gingival index and probing depths) at week 6.[37] Moreover, hexetidine (Oraldene) 0.1% rinse usually reversed gingivitis.[38] Mouth rinses containing essential oils such as Listerine (Listerine Antiseptic) appear to be as effective as chlorhexidine in the treatment of gingivitis.[39] In addition, turmeric gel (Curenext gel manufactured by Abbott Pharmaceuticals) was more accepted because of fewer adverse effects such as dryness and teeth staining.[22]

A study in 2016 by Vangipuram et al., concluded that Aloe vera herbal mouthwash was effective in reducing plaque and gingival indices.[19] The effectiveness of the aforementioned agents in treating gingivitis was highly dependent on patient's compliance which was considered a hurdle in treatment. Therefore, the search for effective agents that could be utilized without solely depending on patient compliance was and still is important. The experimental intervention in the current study was Advantage Arrest™

Silver Diamine Fluoride 38% (SDF). SDF was cleared by the United States Food and Drug Administration (FDA) in 2014; and became commercially available in 2015. SDF has been indicated for the treatment of dentinal hypersensitivity, but used extensively by oral health professionals in the U.S. off-label for the arrest and prevention of dental caries. In October of 2016 the U.S. (FDA) granted "Breakthrough Therapy Designation" to Advantage Arrest™ Silver Diamine Fluoride 38% for the arrest of tooth decay in children and adults.[40]

SDF composed of both silver and fluoride ions. The silver has bactericidal activity against cariogenic bacteria and the silver and fluoride interact to form fluorapatite that promoted remineralization. The bactericidal activity was effective throughout the oral cavity.[41] The silver deposits on the carious lesions created brown-black staining. This stain together with the hardening of the lesion was considered a clinical indication of caries arrest.[41, 42]

A study in 2013 by Zhang et al. involving an elderly population, concluded that the annual application of SDF on root caries was effective in arresting existing root caries and decreased the development of new root carious lesions.[11] This study was the first that used SDF to assess the effectiveness of its application in arresting root surface caries and preventing new lesions in elderly patients.[11] An in vitro study by Suzuki et al, demonstrated that fluoride with silver ion combination produced the antiplaque action.[43] A study by Shah et al., found that after three applications of SDF at 6th and 12th and 18th month, a significant reduction in plaque scores was only found in the 18th month follow up compared to baseline.[44] Moreover, SDF had the potential to reduce *Streptococcus*

mutans due to the high fluoride concentration in combination with the antibacterial effect of silver.[44]

Clinical observation of improvement in gingival condition have been reported in patients who received silver nitrate and fluoride varnish application to treat carious lesions. In many studies, SDF proved to have statistically significant and substantial effect in arresting and preventing caries.[45] However, to date there are no studies that have investigated the effect of silver diamine fluoride on gingivitis. Treatment outcomes may be more successful if results are not as dependent on patient compliance. Thus, the objective of this study was to evaluate the effect of SDF application on gingivitis and dental biofilm in a geriatric population compared to a placebo group. The null hypothesis tested was that there would be no statistically significant improvement in gingivitis before and after treatment using silver diamine fluoride.

2.2. Materials and Methods

2.2.1. Study Design

The study was a 7-week randomized, controlled, prospective double-blinded clinical trial conducted in eight senior retirement homes in Dallas, Texas, United States. The research protocol was approved by Texas A&M University Human Research Protection Program (HRPP) (ID:2017-0917-CFB). After approval, all invitations and flyers for the study were delivered to the coordinators of eight senior retirement homes and approval letters from these centers were obtained prior to patient recruitment. The trial was registered at ClinicialTrials.gov (identifier no.: NCT03445286). It was conducted from December 2018 to December 2019.

2.2.2. Study Participants

A total of 116 geriatric participants (≥ 65 years old) were screened to determine their eligibility. Out of 116 participants, 48 participants with gingivitis (age range: 65-83 years, 20 males and 28 females) were enrolled in the study following the inclusion criteria. Participants were randomly allocated to two groups: experimental group: receiving silver diamine fluoride (SDF) (n=24) and placebo (saline) group: receiving normal saline (S) (n=24). Forty-eight participants were enrolled in the study and thirty participants (10 males and 20 females) completed the study and their data were analyzed. The use of a CONSORT 2010 flow diagram for the recruitment details of the study participants.[46] The participant flow in this trial is illustrated in the flow diagram (Figure 1). All participants from either SDF group or placebo group were given a prophylaxis by the end of the study if their gingivitis was not treated.

2.2.3. Sample Size

Sample size calculations were not able to be performed since there were no previous studies that investigated the effect of SDF treatment on gingivitis. The sample size used in this study was based on previous studies by Kandwal et al. 2015, Dadkhah et al. 2014, Charles et al. 2004 which investigated the effect of different antimicrobial agents such as chlorhexidine on periodontal health and used a similar methodology.[22, 47, 48] Sample size for each group was determined to be 21. Given the expected dropout rate of up to 5 participants over the seven weeks of the study, the recommended group size was 24 individuals with a total of 48 individuals for the study.

2.2.4. Inclusion and Exclusion Criteria

Inclusion criteria for this study were: (1) participants aged 65 years and older; (2) presence of gingivitis; (3) both sexes (male and female); (4) a minimum of 6 remaining teeth (at least one posterior tooth should be present) and teeth should not be fully covered; (5) teeth with probing depth ≤ 4 mm, gingival index (GI) ≥1 and plaque index (PI) ≥1; (6) participants agreed not to use any mouthwash for the entire study period.

The following were exclusion criteria (1) active chemotherapy or radiotherapy; (2) uncontrolled diabetes (3); teeth with periodontitis (probing depth > 4 mm); (4) presence of any gingival or perioral ulceration or stomatitis; (5) use of antibiotics in the last three months; (5) periodontal therapy in the last 3 months; (6)SDF application in the last 3 months; (7) known allergies to the ingredients in the SDF; (8) smokers; (9) medications that caused gingival overgrowth; (10) unable to give consent (11) participants that were immunocompromised.

2.2.5. Clinical Procedures

Following informed consent, patients received a dental examination to determine their eligibility for participation. At the baseline visit, demographic data, medical history, current medications, and oral health habits were recorded by the dental hygienist. Intraoral photographs and an oral hygiene questionnaire were obtained at the beginning of each visit followed by recording of gingival and plaque index scores by the dental examiner. Treatment intervention with SDF or saline was carried out at the end of the first three sessions. Solutions were applied once a week for three consecutive weeks (Figure 2).

2.2.6. Randomization

A simple randomization was used by assigning a number from one to forty-eight for each participant at the baseline visit (week 1). The ordinal sequence was based on arrival times for the baseline visit. Participants were then allocated to one of the intervention groups with an allocation ratio of 1:1.

2.2.7. Blinding

Both the biostatistician and participants were blinded to the interventions throughout the study. The study coordinator (dental hygienist) was not blinded and knew who received which intervention. The dentist examiner undertaking the clinical dental examinations was not informed of the arm to which the participant had been randomized at baseline visit. Blinding of the examiner was not possible for the remainder of the study due to dark staining that occurred following SDF treatment, which would obviously indicate the likely treatment a participant received.

2.2.8. Treatment Agents (Interventions)

The two materials used as an intervention were: normal saline solution (S) and silver diamine fluoride 38% (SDF) solution which is marketed as Advantage Arrest™ (Elevate Oral Care LLC, West Palm Beach, FL). As described by the manufacturer's, the new blue tinted formula of the SDF Advantage Arrest 10 ml dropper bottle contained 8 ml of SDF; and provided approximately 250 drops (enough to treat 125 sites, with a site defined as up to 5 teeth). One drop (25 µl) treated 4-6 surfaces and contained 9.5 mg silver diamine fluoride. On each visit, 2 drops (50 µl) of either solution was dispensed and applied to dental surfaces of the test teeth.

Blinding of the examiner was further attempted by tinting the saline solution blue and dispensing SDF and saline liquids from similar dropper bottles assigned with codes A and B. Both liquids were dispensed into similar disposable dapping dishes by the dental hygienist who was not involved in the clinical examination.

2.2.9. Clinical Protocol

Basic set-up at the seniors residential centers consisted of a disposable mouth mirror, colored-coded periodontal probe, cotton rolls, gauze, dental bib, protective eyewear, cheek retractors, disposable dappen dish, silver diamine fluoride (SDF) solution, normal saline solution (S), petroleum jelly, micro brush/applicator, headlights, data collection forms and professional digital camera (Canon EOS Rebel T6i DSLR Camera).

Participants were instructed not to brush their teeth for 3 days prior to the day of visits. Solutions were applied once a week for three consecutive weeks. Treatment application was as follows: 1- teeth were cleaned with a dry toothbrush; 2 - the lips and gingival tissues were protected with petroleum jelly; 3 - the selected teeth were dried using gauze; 4 - cotton rolls were used to isolate teeth from the saliva; 5 - the micro brush/applicator was used to place a small amount of SDF or saline on the selected teeth with gingivitis (the material was applied to the facial and lingual/ palatal surfaces of the teeth using a micro brush; 6- teeth were allowed to dry for one minute; 7- excess solution was wiped off the tongue without rinsing and 8- participants were dismissed with instructions to avoid food or drink or rinsing for one hour and were given contact information should they experience any discomfort. They were also instructed not brush for three days prior to each of their future visits.

2.2.10. Primary outcomes

There were two primary outcomes assessed. First, the gingival index (GI) using Löe-Silness index which measured the gingival inflammation surrounding the teeth surfaces (facial, palatal/lingual, mesial). This index use numerical values ranging from 0 to 3 as described in Table 1.[17] Second, the plaque index (PI) using Silness-Loe index which measured the accumulation of the plaque on teeth surfaces (facial, palatal/lingual, mesial). This index use numerical values ranging from 0 to 3 as described in Table 2.[49] GI and PI were assessed at beginning of the study; baseline (week1) and at follow up time-points (week 3, week 5 and week 7). Mean score values for GI and PI scores were calculated for each participant at each examination.

2.2.11. Secondary outcomes

Secondary outcome measures were collected at all four follow up time-points. These included: 1) intraoral photographs and 2) an oral hygiene evaluation questionnaire completed prior to the clinical examination. Each participant underwent a focused questionnaire addressing oral hygiene habits (tooth brushing, use of additional oral hygiene products including mouthwash, dental floss, whitening products) to confirm their compliance in following instructions between visits.

2.2.12. Statistical analysis

All statistical analyses were performed using statistical software (SPSS v 26; IBM Corp., Armonk, NY). Parametric tests were used for the data analysis. Independent t-test was used to compare the changes from baseline at different time points between experimental and placebo groups. To test the changes within each group; one sample t-test was used. The significance level was $p<0.05$.

2.3. Results

2.3.1. Baseline Data

Thirty geriatric subjects participated and completed the study. Ten were males and twenty were females between 65 and 83 years of age. Their mean age was 70 years. Mean gingival and plaque index scores were recorded for both groups (Table 3). The placebo (saline) group had lower gingival index (GI) and plaque index (PI) scores at the beginning of the study which were statistically significant (baseline or W_0). By the third week following treatment, the SDF treatment group had lower scores with both measures than the saline group.

2.3.2. Effect of Treatments on Gingival Index

Between-groups comparison: There were statistically significant differences between the SDF treatment and placebo groups at all time points (Figure 3). There was a significant reduction in the mean GI scores using Löe-Silness gingival index ($p<0.05$) in the SDF treatment group (-0.93±0.37) in week 3 (W_3) compared to the placebo group (-0.067±0.21) (Table 4), The placebo (saline) group had no reduction in GI scores throughout the examination period (7 weeks) (Table 4, Figure 3). The significant reduction in GI scores in the SDF treatment group continued until week 7 (W_7) (-1.280 ±0.305) but not in the saline group (0.004 ±0.187).

Within SDF Group, GI scores (Table 5) showed statistically significant reduction in the mean values ($p<0.05$) within 3 weeks (W_3) (-0.93±0.37) compared to baseline scores (W_0) (1.89±0.39). The GI scores continued to decrease significantly until week 7 (W_7) (-1.82 ±0.31) in the SDF treatment group (Figure 3).

Within placebo (saline) Group, in comaprsion to basleine scores (W_0) (1.45±0.25), the GI scores were not reduced during the study and stayed close to the same; week 3 (W_3), week 5 (W_5) and week 7 (W_7) respectively (p=0.236, 0.475, 0.937) (Figure 3). In contrast, there was a tendency of increased in GI scores in the saline group, which indicated poorer oral hygiene and health (Table 6).

2.3.3. Effect of Treatments on Plaque Index

Between-groups comparison, similar to the gingival index, there was a statistically significant reduction in plaque index (PI) scores using Silness-Loe plaque index ($p<0.05$) starting at week 3 (W_3) and it decreased until week 7 (W_7) in the SDF treatment group compared to the placebo (saline) group (Table 4, Figure 4).

Within SDF group, the PI mean scores were reduced ($p<0.05$) at all time points compared to the baseline score (W_0) (1.89±0.44) (Table 5, Figure 4). The PI scores significantly decreased ($p<0.05$) until week 7 (W_7) (-1.281±0.539) (Table 5).

Within placebo (saline) group, in contrast, the PI mean scores were almost the same at the timepoints studies at week 3 (W_3) and week 5 (W_5) respectively (p=0.275, 0.132) compared to its baseline scores (W_0) (1.28±0.37) (Table 6). In addition, there was significant increase in plaque index scores at week 7 (p=0.023).

2.3.4. Clinical Digital Photographic Aseesement

Intraoral photographs of teeth and gingivae were taken for both groups before and after the application of the treatment (SDF or saline) to observe the changes in the gingival condition. In the SDF treatment group, the gingival tissues had decreased redness, swelling and bleeding by week 3 (W_3) (Figures. 5-13) compared to the placebo (saline) group images that did not show clinical improvement (Figures. 14 and 15). Less

plaque biofilm accumulation was also visually observed in the SDF group compared to placebo group at all time points.

None of the participants reported any discomfort, pain or burning sensation after the application in both SDF treatment and placebo groups. In SDF treatment group, some of the subjects reported the metallic taste after application which went away in one day.

2.3.5. Oral Hygiene Assessment

The two groups did not differ with regard to oral hygiene practices. All patients were compliant in following hygiene instructions.

2.4. Discussion

Previous clinical studies on SDF mainly focused on its effectiveness in arresting dental caries. This is the first clinical trial investigating the efficacy of SDF on the treatment of gingivitis in geriatric participants. We hypothesized that 38% SDF would have no significant effect on treating gingivitis. This hypothesis was not accepted since the main finding was that the SDF-treated group had significantly better gingival health status than the saline placebo group in terms of gingival inflammation and plaque levels. Our finding of overall improvement in gingival condition was consistent with a clinical observation from a published case-report of a 14-year old boy with an autoimmune systemic condition who suffered from rampant caries. In this case report, the dental team used 38% SDF treatment at baseline visit, two weeks later, then after four weeks. They reported significant improvement in the overall oral hygiene, arrested carious lesions, less hypersensitivity and a significant improvement of the gingival condition.[50] However, their finding of improvement of the condition of the gingiva was based on observation only as they did not report quantitative data assessing improvement.

In this study, there was a significant reduction in the mean GI scores ($p<0.05$) in the SDF-treated group compared to placebo, which started on week 3 and continued to decrease significantly to week 7. These results were consistent with the visual evidence from the intraoral photographs that clearly showed the gingival tissues with decreased erythema, swelling and bleeding by the third week. One way to explain these clinical findings can be explained by the well documented antibacterial activities of the 38% SDF applied topically to the participants' teeth. We predict that SDF may be involved in shaping the composition of the resident oral microbiota, and therefore potentially influence oral health status. SDF contains silver and fluoride ions that have been proven to have a role in the inhibition of cariogenic biofilms (mainly on *Streptococcus mutans* and lactobacilli).[44, 51-53] Zhao et al, reported that silver ions acted directly against bacteria in lesions by breaking down membranes, denaturing proteins and inhibiting DNA replication.[54]

Previous studies have demonstrated the tendency of 38% SDF to affect the ability of colonizing bacteria to form a pathogenic biofilm.[43, 44, 55, 56] This could be the explanation for the significant reduction in PI scores and the visual reduction in the biomass of dental plaque seen in the SDF-treated group compared to the placebo saline group. This finding agreed with results from Shah et al study, in 2013 that reported a significant reduction in PI in the 38% SDF-treated group in a 18th month follow up examination compared to baseline. Interestingly, no significant reduction in PI levels were found in the 6th month or 12th month follow up examinations.[44] These findings might be attributed to their use of a different SDF product (Saforide-J. Morita company, Japan).

In the present study, we did not instruct the participants to modify their oral hygiene habits nor did they change their daily routine during this 7-weeks of the_study. Our recruited cohort were geriatric patients with a mean age of 70. The recorded baseline oral hygiene practices showed that the majority used toothbrushing as the only mechanical plaque control aid once daily. They had no regular dental or dental hygiene visits within six months prior to recruitment. On their baseline visit, the recorded PI and GI mean scores reflected their poor oral hygiene status (Table 3). We demonstrated that three applications of 38% SDF was able to induce drastic improvement in the gingival condition without requiring the patient's compliance. This improvement was comparable to studies that tested 0.2% chlorohexidine mouthwash and 2% chlorhexidine gel[19, 57, 58], which reported significant GI and PI improvement but with stringent measures to maximize compliance of participants with using the mouthwash or with patients receiving professional prophylaxis before the beginning of the study.[59]

Another recently published randomized clinical trial in 2020 by Levine et al. investigated a foaming dental gel composed of cetylpridinium chloride (CPC), hydrogen peroxide, sodium bicarbonate and antioxidants. They utilized two delivery methods: mouthpiece and enhancing light, or twice daily brushing. They reported a significant reduction on GI at day 42 of the study.[60] In our opinion, the patient's compliance when using home oral care products has to be at its best to achieve these results, which would be very challenging in geriatric patients.

The ability of 38% SDF to induce gingival improvement was quantitatively (statistically significant difference in GI and PI) and clinically evident in three weeks. This

result was consistent with the findings from Siddeshappa et al. study in 2018, which compared the efficacy of herbal mouthwash and chlorine dioxide mouthwash. In their study, professional prophylaxis was completed before the intervention which may have boosted and masked the real efficacy of the mouthwash.[61] Magaz et al. in 2018 reported similar results in 21 days but with daily use of the new toothpaste and mouthwash formula (containing chlorhexidine, dexpanthenol, allantoin and bioadhesive excipient).[57] Notably, most of the reported gingivitis-treatment agents were significantly effective by the seventh week of continuous usage of the study agent (~40 days).[62-65] A recent Cochrane systematic review in 2017 indicated that the use of chlorhexidine rinse showed a (-0.21) reduction in GI.[66] In comparison, the SDF-treated group in the present study had a greater reduction in mean GI score by (-1.28) at day 49 compared to the baseline.

The longevity and sustainability of 38% SDF post treatment was demonstrated in the present study through the continuous significant reduction in the GI and PI mean scores as well as the visual clinical improvement in the gingival condition over one month (week 7) following the last received treatment on week 3. This extended effect of 38%SDF could be explained by silver ions being able to penetrate the interprismatic space and bind to either the protein scaffolding or to hydroxyapatite crystals in normal unaffected enamel.[67] Silver ions could act as a reservoir that could be released over time. The presence of silver atoms in this environment may influence and suppress the cascade of events that eventually lead to dental diseases, such as caries and gingivitis; in a substantive manner.

In Persson et al. study, 0.12% chlorhexidine mouth wash (Peridex) did not show a similar sustained effect. It was reported that 0.12% chlorhexidine mouth wash (Peridex);

which was used either daily or weekly by geriatric patients under supervision for 6 weeks; caused significant improvement in 6 weeks ($p < 0.001$). However, the improvements did not last another 6 weeks after the final rinse.[37]

In this study, the 38% SDF did not entirely show incomplete remission of gingivitis, similar to what have been shown with other agents. The application protocol chosen in this study; 3 consecutive applications, may have accounted for the observed incomplete remission. It is worthy to mention that a six month follow up has been scheduled to document the changes in the gingival condition of the current study's geriatric participants. More clinical trials are needed to determine if the rate of remission and prevention of gingivitis in elders will increase with more frequent or altered application protocol of the SDF solution.

One limitation of this study was the sample size. Elder recruitment was a challenging task and this study would likely have shown a greater significant effect with a larger sample size. Another limitation was the inability of having the dental examiner blinded through the course of the study due to the dark discoloration from SDF, which indicated the likely treatment received. Also, the study used a placebo and did not include an active control arm such as chlorhexidine.

Our study was aimed to help find an optimal tool/agent for treating and preventing gingivitis in elderly patients without depending on their personal oral hygiene compliance. Treating gingivitis is a prerequisite for progression to periodontitis leading to attachment loss, bone loss, tooth mobility, tooth loss and poor quality of life for such a vulnerable population. SDF has become increasingly popular because of its anticariogenic properties

and its non-invasive treatment protocol. This simple non-invasive treatment could help patients who have difficulties in maintaining a proper oral hygiene habits due to aging and/or illness. Investigating such agents as the cornerstone for managing and preventing oral diseases and the systemic complications associated with poor oral hygiene and the lack of compliance.

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3. CONCLUSIONS

The first conclusion based on the literature review (section 1): is the need to conduct more randomized clinical trials to investigate alternative uses of SDF in comparison with other available fluoride products. Potential adverse effects merit further investigation. Moreover, the topic of SDF needs to be addressed in all dental and dental hygiene educational programs. Another important aspect pertains to the regulations regarding who can apply the SDF depending on individual state regulations. It would be prudent for all health care professionals to be trained in SDF application as an alternative solution for individuals who cannot afford regular preventive care; or have limited access to dental care. Further research is warranted to investigate the impact of SDF on the amount of biofilm accumulation, microbial counts, and gingivitis in varied population.

A second conclusion identified from the clinical study (section 2): is that 38% SDF treatment was associated with better gingival health status with no patient compliance needed. In addition, 38% SDF significantly decreased gingival index and plaque index scores in 3 weeks. Moreover, 38% SDF has the potential to cause a sustained and continuous significant effect on gingivitis for at least one-month post treatment. Finally, the data from this double blinded clinical trial provided tangible evidence that SDF application has the potential to be a new adjunctive, cost effective and noninvasive tool for treating gingivitis.

Future direction: While direct comparisons with the present study were not valid because of different protocols followed and different outcome measures assessed, the findings regarding the effect of SDF on gingival tissues were of interest. In this study, gingival health improvement was determined by traditional clinical parameters. It would be very interesting if microbiome sequencing could be utilized to investigate the microbiological shift while treating gingivitis with 38% SDF. This may reveal the mechanisms underlying the outcome data from the present study. Future studies are needed to include a larger sample size in addition to compare the efficacy of SDF to other antimicrobial agent on gingivitis.

APPENDIX A
LIST OF TABLES

Table 1 - Chapter 2: Gingival index (GI) numeric values

GI	Numeric Descriptor
Score 0	Gingiva of normal texture and color, no bleeding
Score 1	Mild inflammation: slight change in color and slight edema but no bleeding on probing
Score 2	Moderate inflammation: redness, edema and glazing, bleeding on probing
Score 3	Severe inflammation: marked redness and edema, ulceration with tendency to spontaneous bleeding

Table 2 - Chapter 2: Plaque index (PI) numeric values

PI	Numeric Descriptor
Score 0	No plaque
Score 1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth, which cannot be seen with the naked eye. But only by using disclosing solution or by using probe
Score 2	Moderate accumulation of deposits within the gingival pocket, on the gingival margin and/ or adjacent tooth surface, which can be seen with the naked eye
Score 3	Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin

Table 3 - Chapter 2: Gingival and plaque index mean scores in SDF and placebo (saline) groups at different timepoints

	SDF Group		Saline Group	
Time Point	**Gingival Index** Mean± SD	**Plaque Index** Mean± SD	**Gingival Index** Mean± SD	**Plaque Index** Mean± SD
W_0	1.89±0.39	1.89±0.44	1.45±0.25	1.28± 0.37
W_3	0.97± 0.52	0.67±0.53	1.38± 0.36	1.25± 0.43
W_5	0.70± 0.46	0.66±0.40	1.42± 0.33	1.34± 0.44
W_7	0.62± 0.34	0.78± 0.37	1.45± 0.31	1.30± 0.39

W_0: baseline (week 1), **W_3**: week 3, **W_5**: week 5, **W_7**: week 7, SD: Standard deviation *Significant decrease in in gingival index and plaque index

Table 4 - Chapter 2: Comparison of gingival and plaque index mean scores changes from baseline between SDF group and placebo/saline group at different time points

	Gingival Index (GI)				Plaque Index (PI)			
	SDF	**Saline**			**SDF**	**Saline**		
Time Point	Mean± SD	Mean± SD	t-test	***P*-value**	Mean± SD	Mean± SD	t-test	***P*-value**
$W_0 – W_3$	-0.93± 0.37	-0.07± 0.21	-7.84	**0.001***	-0.93± 0.52	0.099±0.87	-6.455	**0.001***
$W_0 – W_5$	-1.19± 0.38	-0.33± 0.17	-10.82	**0.001***	-1.19± 0.63	0.13± 0.32	-7.260	**0.001***
$W_0 – W_7$	-1.28± 0.31	0.004± 0.19	-13.91	**0.001***	-1.28± 0.54	0.17± 0.26	-9.402	**0.001***

W_0: baseline (week 1), **W_3**: week 3, **W_5**: week 5, **W_7**: week 7, SD: Standard deviation,
*Significant decrease in in gingival index and plaque index

Table 5 - Chapter 2: Changes in gingival index and plaque index mean scores within SDF Group

	SDF Group Gingival Index (GI)			SDF Group Plaque Index (PI)		
Time Point	**Mean (SD)**	**t-test**	***P*-value**	**Mean (SD)**	**t-test**	***P*-value**
$W_0 - W_3$	-0.93± 0.37	-9.65	0.001*	-0.93± 0.52	-6.96	0.001*
$W_0 - W_5$	-1.19± 0.38	-12.14	0.001*	-1.19± 0.63	-7.33	0.001*
$W_0 - W_7$	-1.28± 0.31	-16.28	0.001*	-1.28± 0.54	-9.20	0.001*

W_0: baseline (week 1), **W_3**: week 3, **W_5**: week 5, **W_7**: week 7, SD: Standard deviation
*Significant decrease in in gingival index and plaque index

Table 6 - Chapter 2: Changes in gingival index and plaque index mean scores within placebo (saline) Group

	Saline Group Gingival Index (GI)			Saline Group Plaque Index (PI)		
Time Point	**Mean (SD)**	**t-test**	***P*-value**	**Mean (SD)**	**t-test**	***P*-value**
$W_0 - W_3$	-0.07± 0.21	-1.24	0.236**	0.099± 0.87	1.14	0.275**
$W_0 - W_5$	-0.33± 0.17	-0.08	0.475**	0.13± 0.32	1.60	0.132**
$W_0 - W_7$	0.004± 0.19	0.08	0.937**	0.17± 0.26	2.55	0.023*

W_0: baseline (week 1), **W_3**: week 3, **W_5**: week 5, **W_7**: week 7, SD: Standard deviation,
*Significant increase in plaque index ** No significant change in plaque index

APPENDIX B
LIST OF FIGURES

Figure 1 - Chapter 1: Silver diamine fluoride (SDF) marketed as Advantage Arrest

Figure 2 - Chapter 1: These images of the patient were taken before the application of silver diamine fluoride (SDF) and six weeks after the application

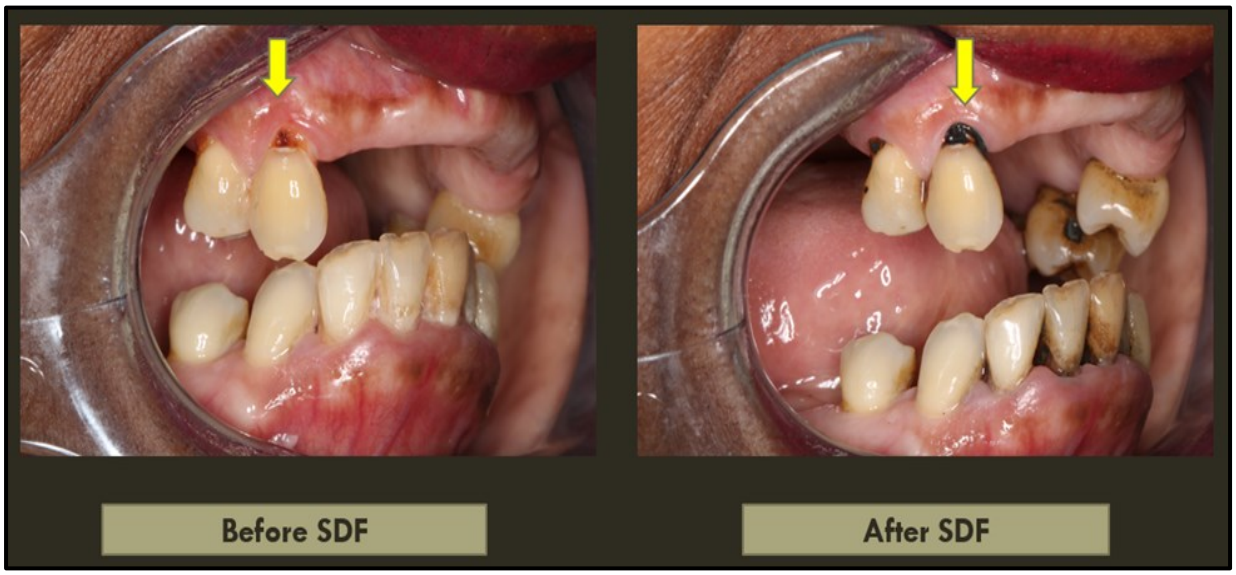

Figure 1 - Chapter 2: Flow diagram of geriatric participants receiving either SDF or placebo treatment

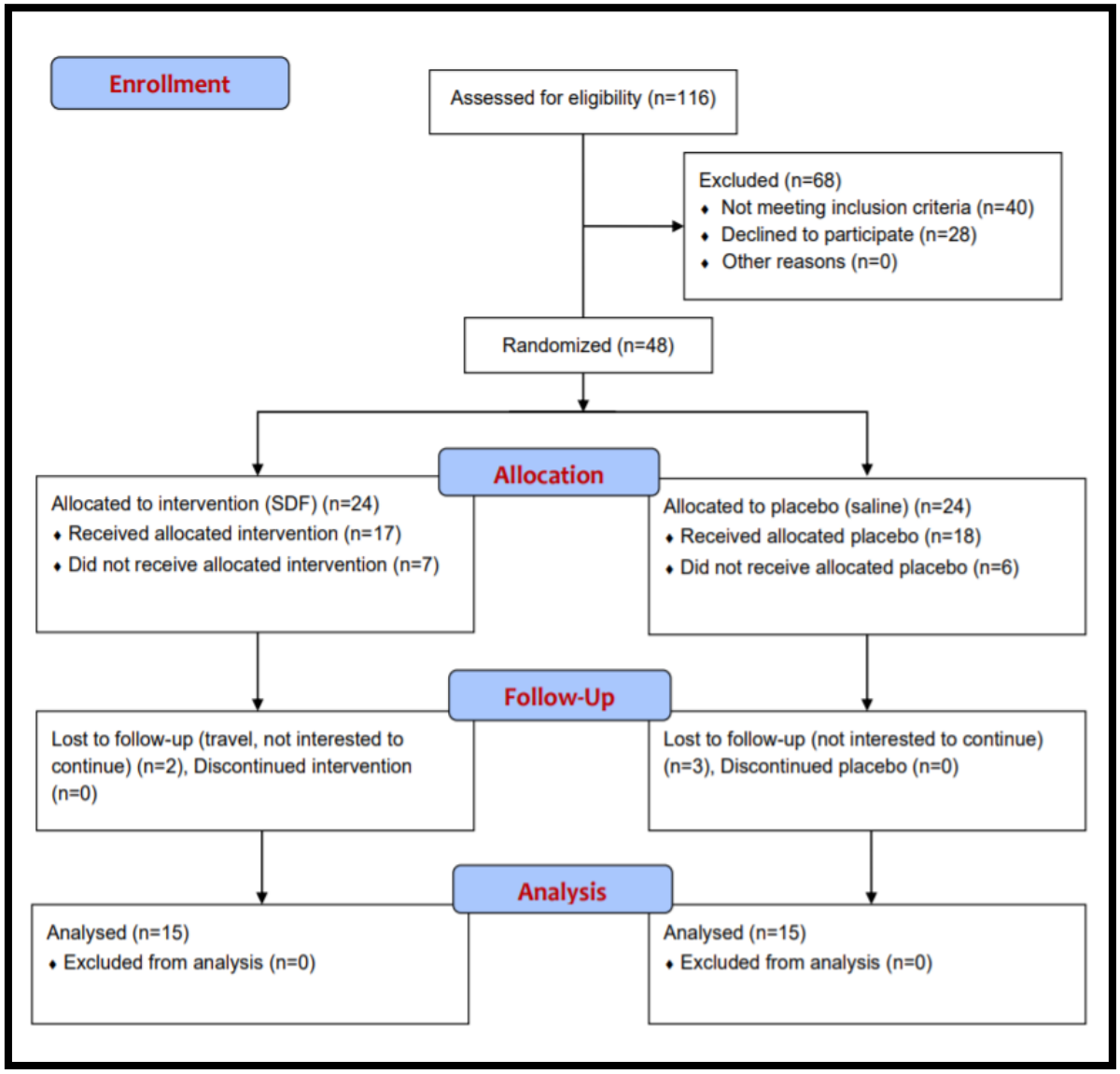

Figure 2 - Chapter 2: Trial Schema

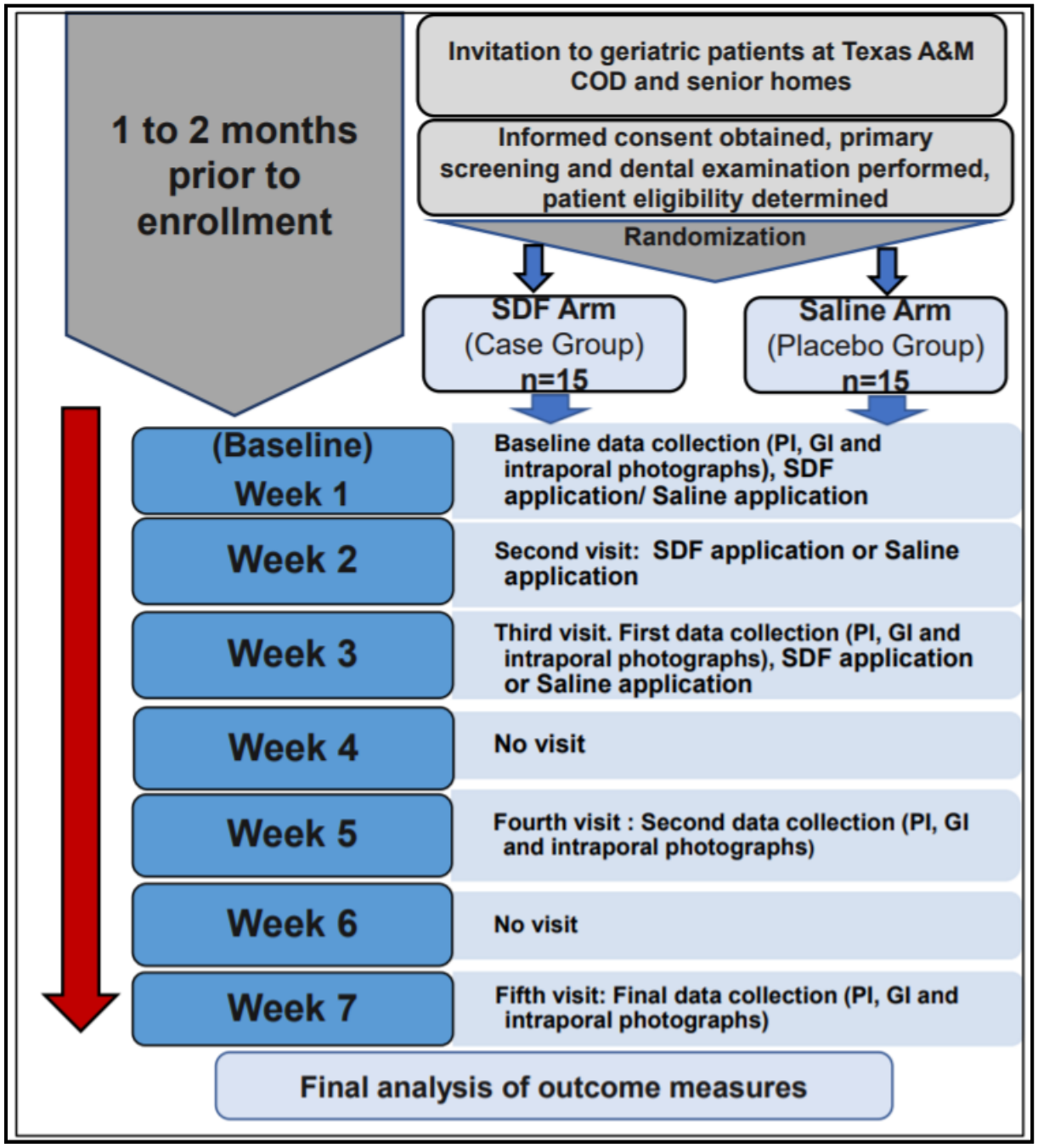

Figure 3 - Chapter 2: Changes in gingival Index (GI) between SDF group and placebo (saline) group at different time points

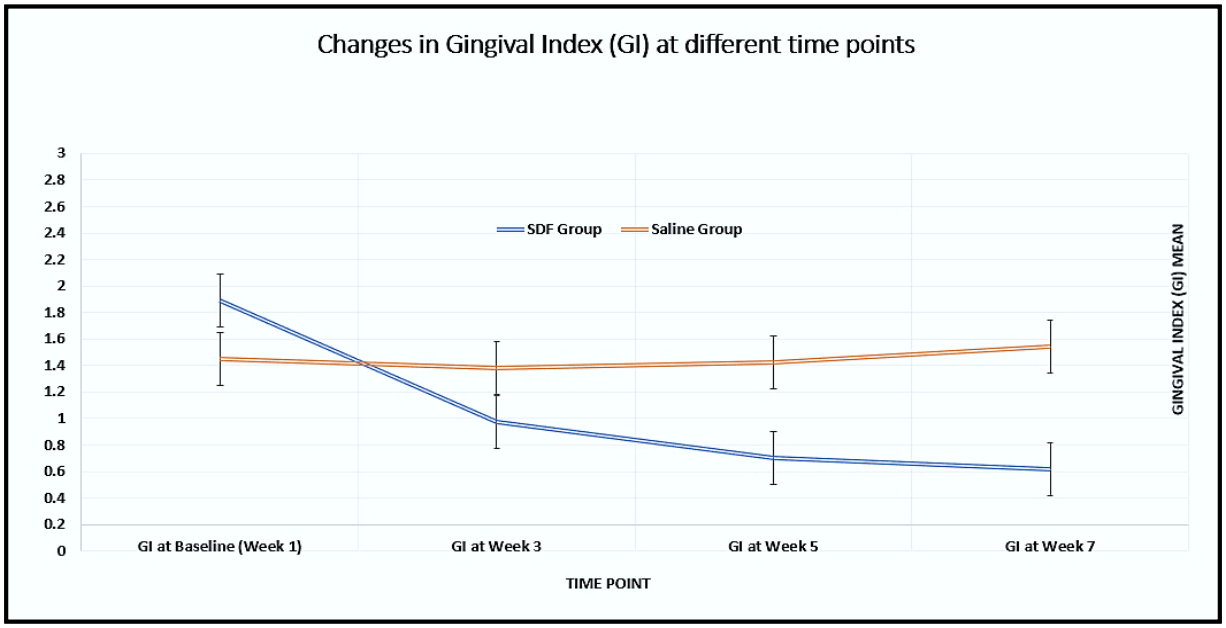

Figure 4 - Chapter 2: Changes in plaque Index (PI) between SDF group and placebo (saline) group at different time points

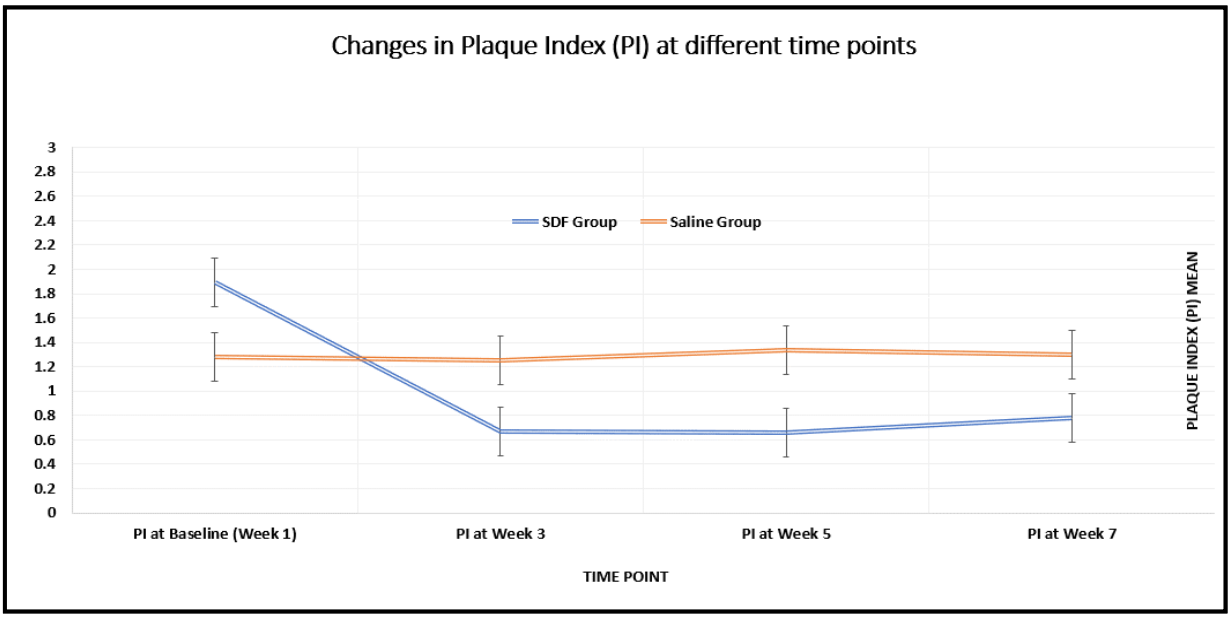

Figure 5 - Chapter 2: Photographic assessment of SDF case before and after SDF application at different time points

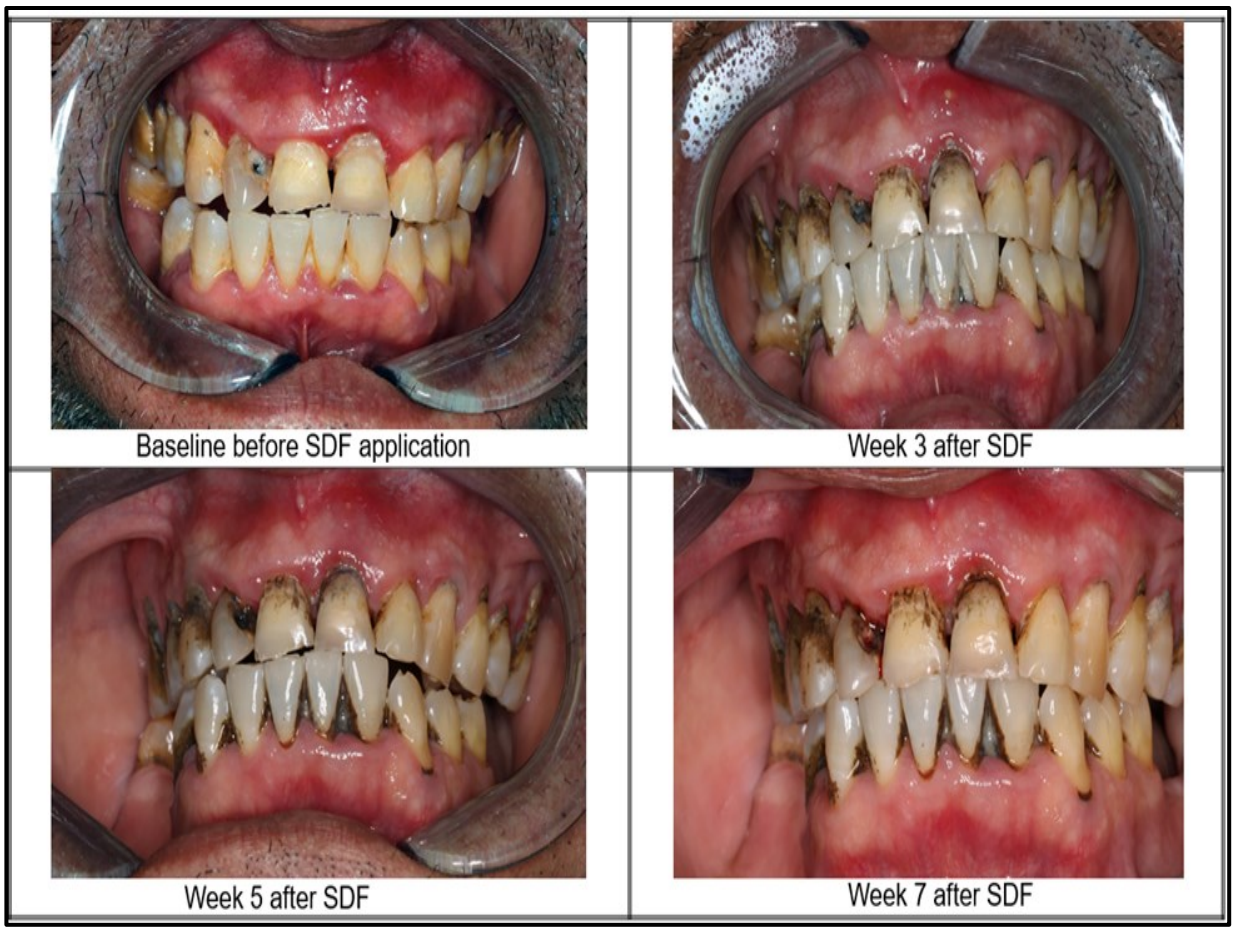

Figure 6 - Chapter 2: Photographic assessment of SDF case before and after SDF application at different time points

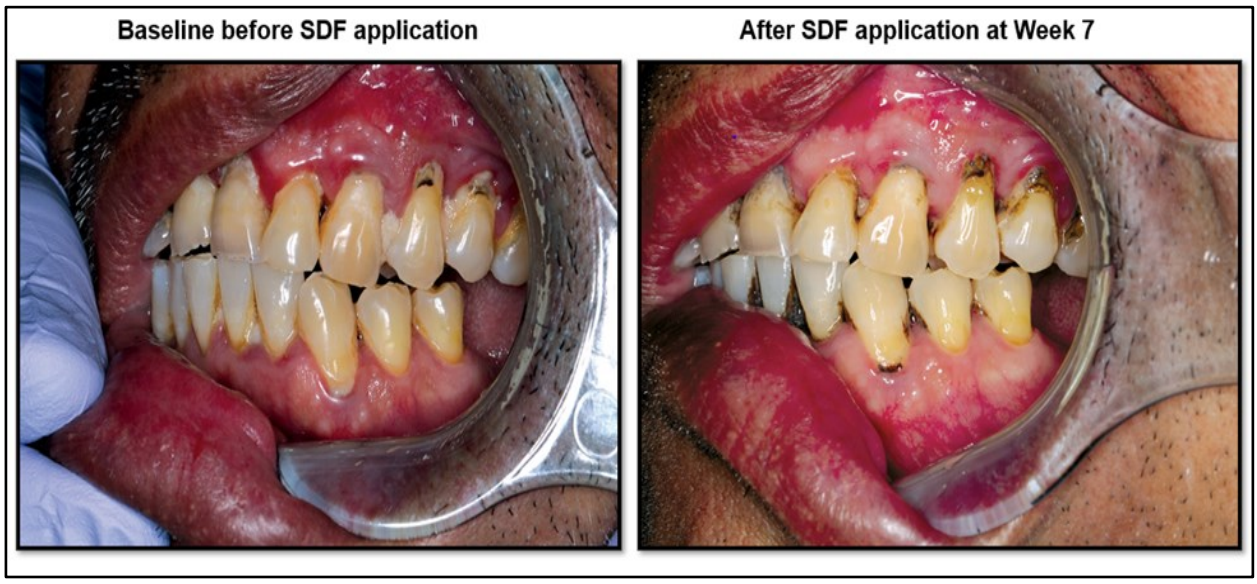

Figure 7 - Chapter 2: Photographic assessment of SDF case before and after SDF application at different time points

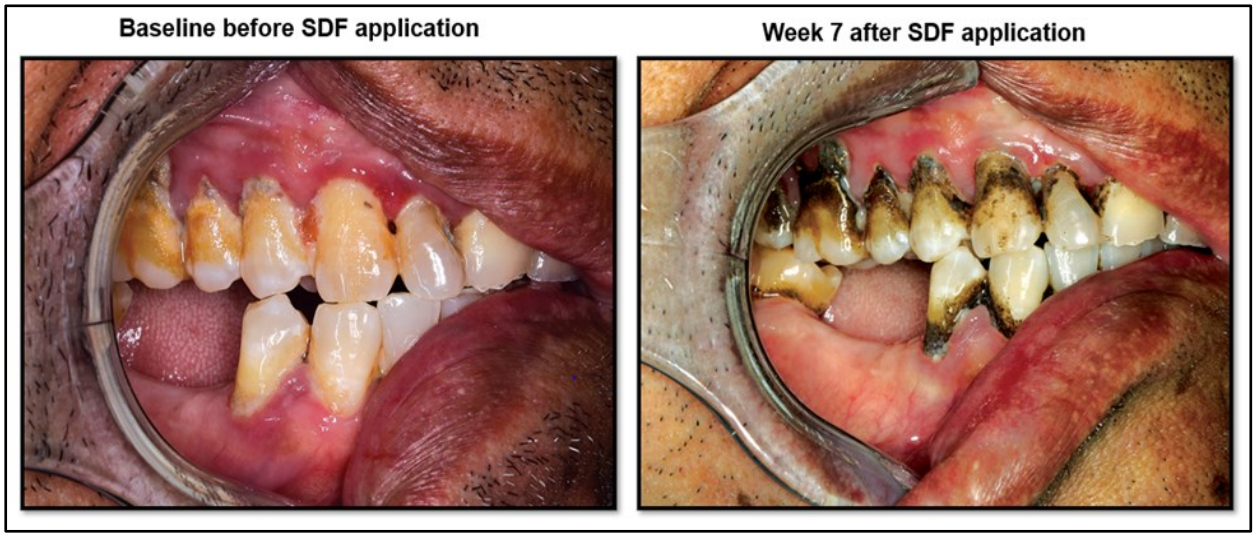

Figure 8 - Chapter 2: Photographic assessment of SDF case before and after SDF application at different time points

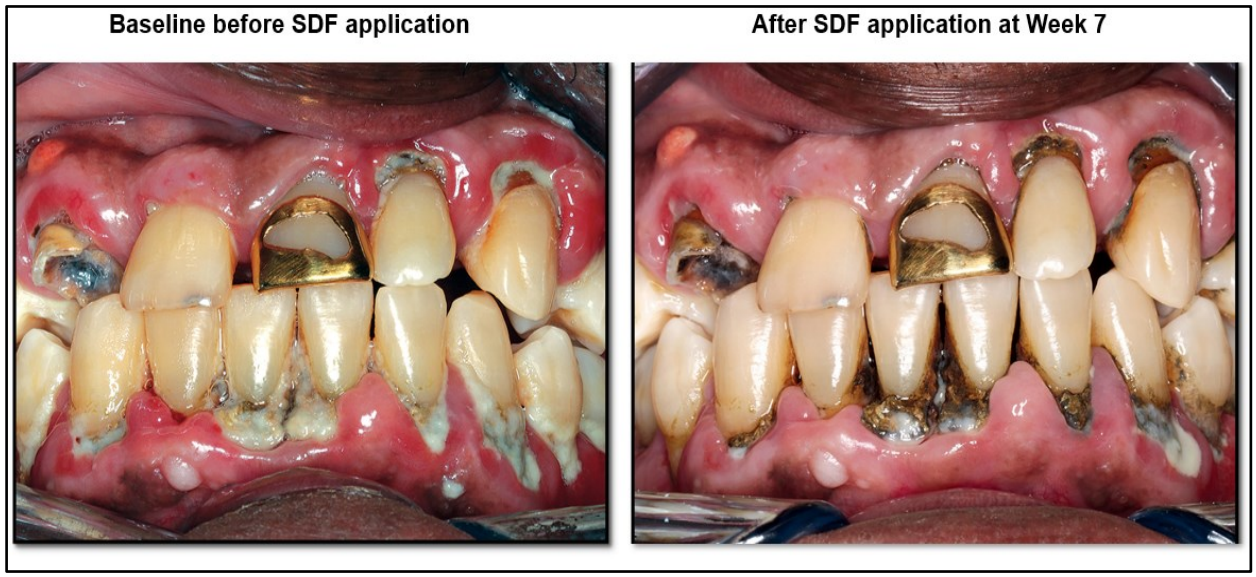

Figure 9 - Chapter 2: Photographic assessment of SDF case before and after SDF application at different time points

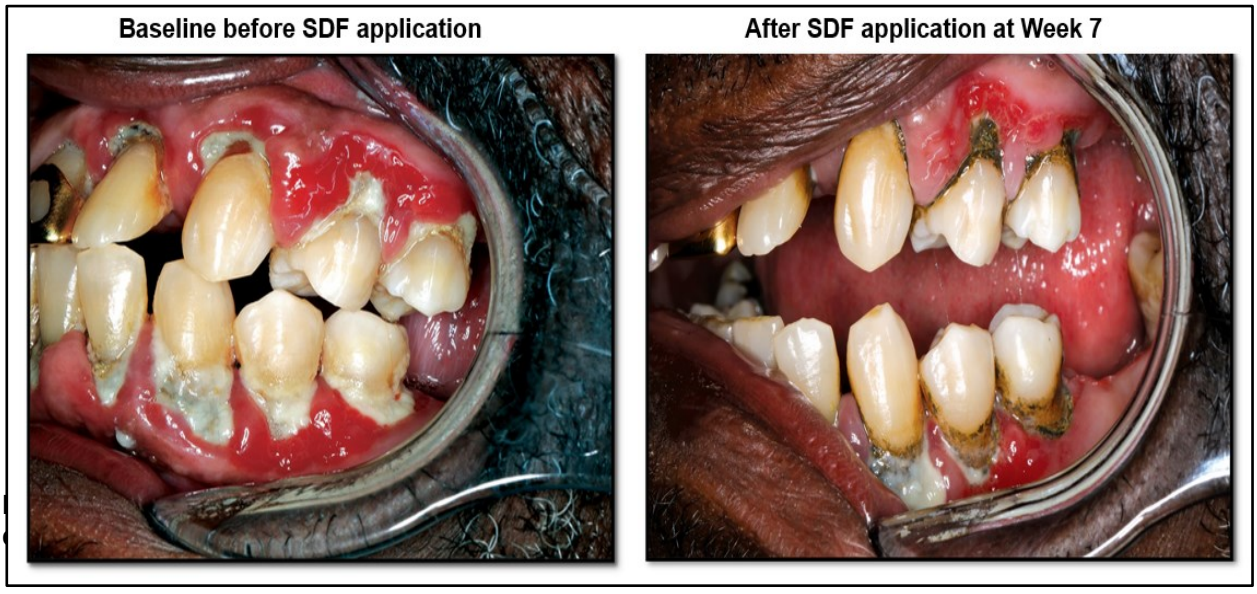

Figure 10 - Chapter 2: Photographic assessment of SDF case before and after SDF application at different time points

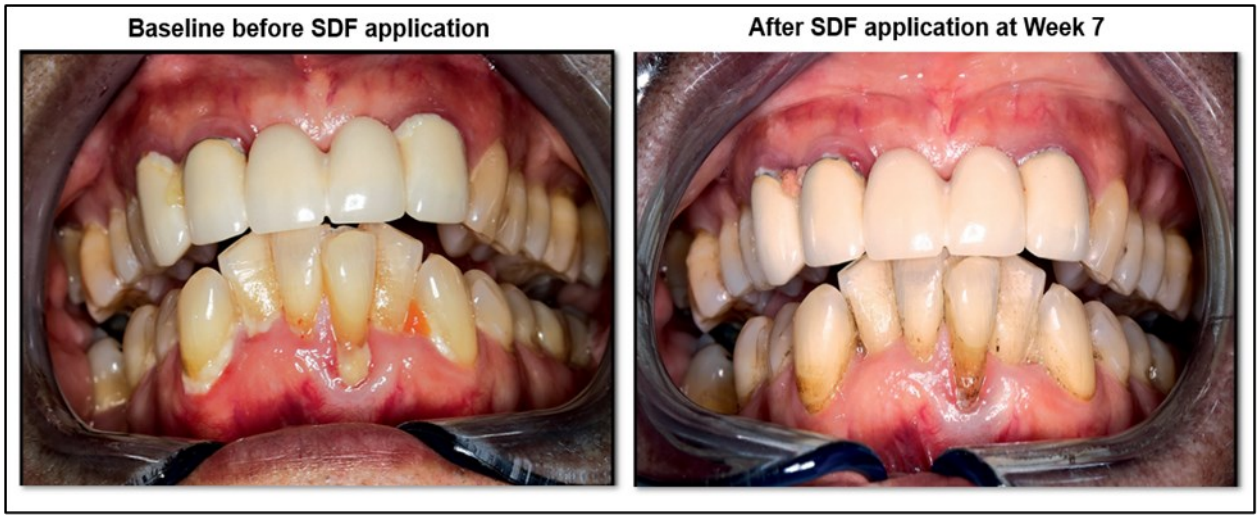

Figure 11 - Chapter 2: Photographic assessment of SDF case before and after SDF application at different time points

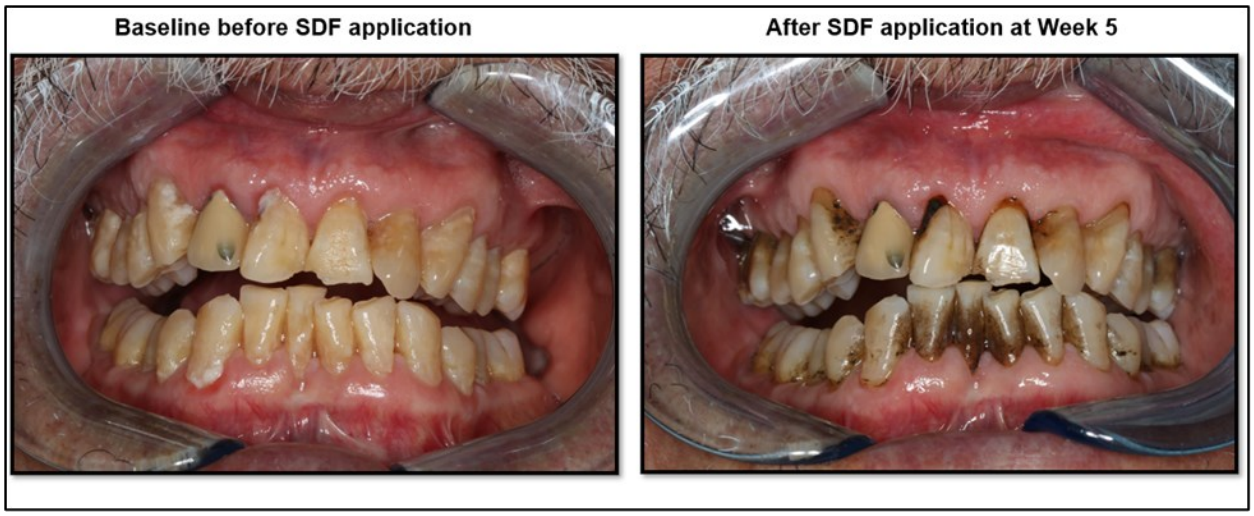

Figure 12 - Chapter 2: Photographic assessment of SDF case before and after SDF application at different time points

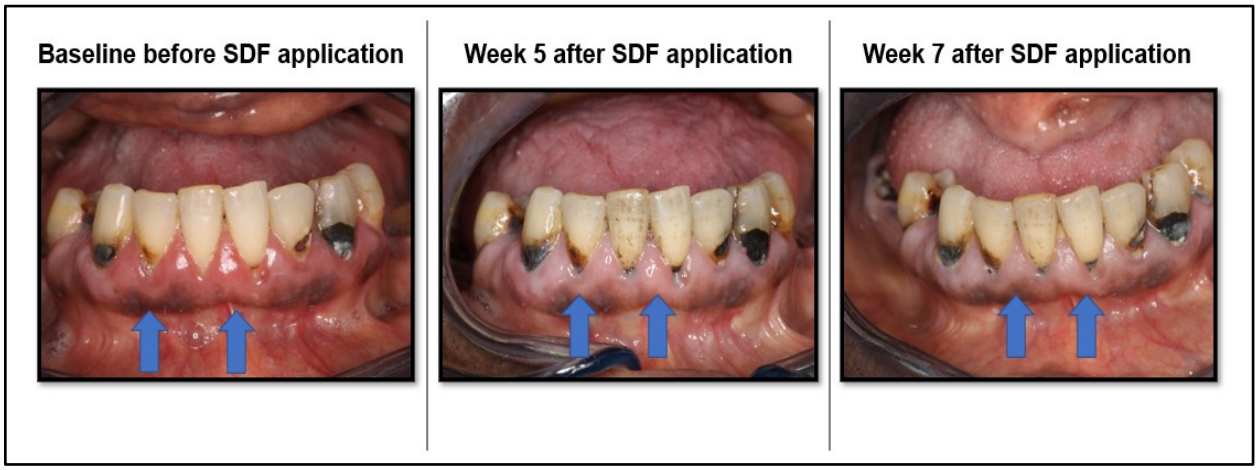

Figure 13 - Chapter 2: Photographic assessment of SDF case before and after SDF application at different time points

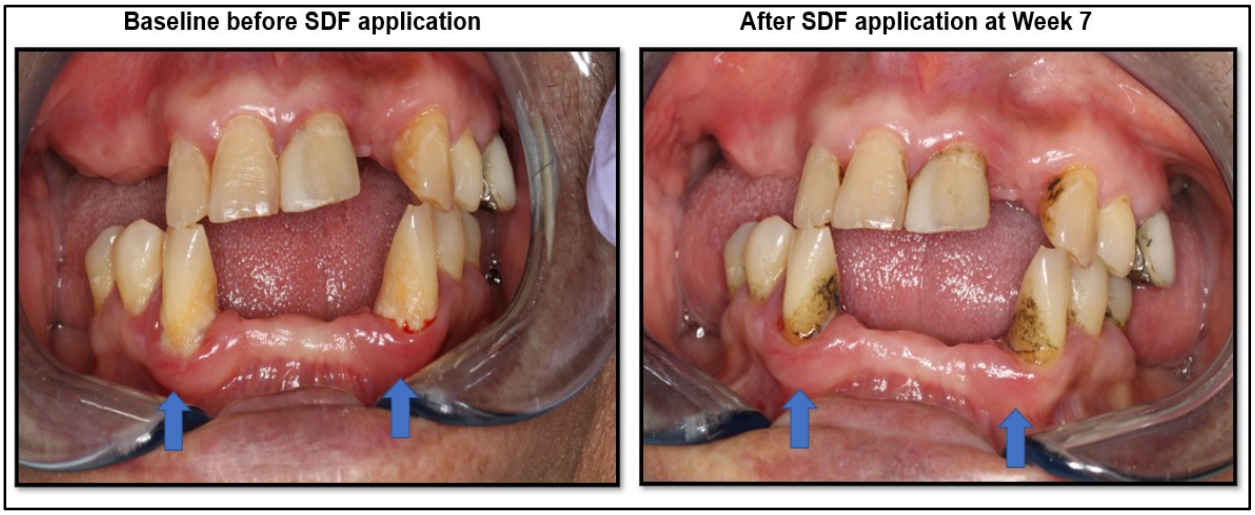

Figure 14 - Chapter 2: Photographic assessment of placebo case before and after saline application at different time points

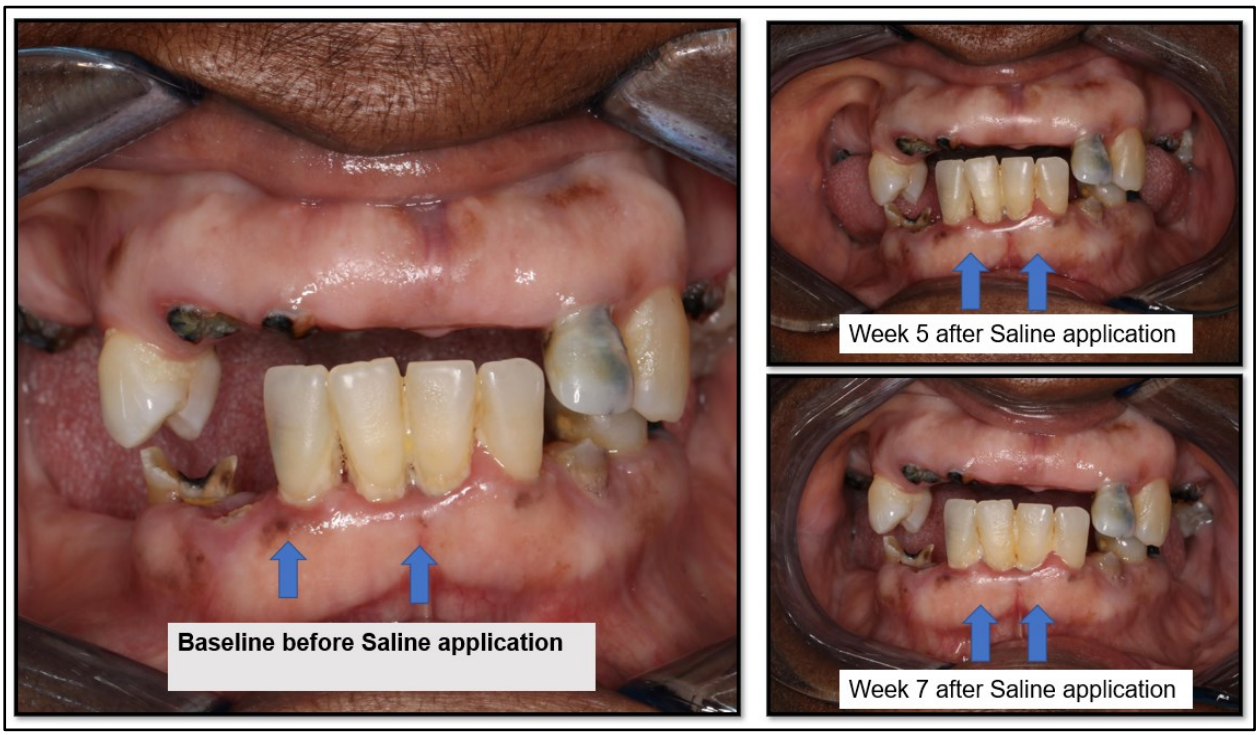

Figure 15 - Chapter 2: Photographic assessment of placebo case before and after saline application at different time points