

**EFFECT OF CO₂ LASER AND 38% DIAMMINE SILVER
FLUORIDE TREATMENT ON ENAMEL AND ROOT
DEMINERALIZATION**

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(B.D.S)

A THESIS SUBMITTED FOR THE DEGREE OF

MASTER OF SCIENCE

DEPARTMENT OF PREVENTIVE DENTISTRY

FACULTY OF DENTISTRY

NATIONAL UNIVERSITY OF SINGAPORE

2011

Acknowledgements

With great pleasure in the completion of my project I would like to express my gratitude to all who provided their kind support and motivation.

First and foremost, I would like to express my deepest gratitude to my supervisors, Associate Professor Stephen Hsu and Associate Professor Robert Yee for giving me the opportunity to join as a graduate student and be a part of this prestigious university. I would like to thank my supervisors for their constant encouragement, invaluable guidance and infinite patience throughout the course of this study. Over all, they molded me as a better human being filled with energy and exuberance to go further in the road of academics.

I am deeply thankful to Associate Professor Grace Ong Hui Lian, Dean of the Faculty of Dentistry, for her support towards the completion of my work on time.

The sincere help from all my group members, Dr. Carolina Un Lam, Dr. Chen Huizhen, Liu Yuan Yuan helped me a lot in working and gaining knowledge. I wish to acknowledge their support and friendly working environment. I extend my sincere thanks to Associate Professor Thomas Osipowicz and Ren Minqin for their support and advice on the fluoride uptake measurement by nuclear microscopy. I am also thankful to Mr. Chan Swee Heng and Miss. Lina for their help.

The main backbone of my achievement is contributed to my beloved husband, family and my precious friends. Their faith, encouragement and help push me to become better by day in whatever I do. Without them, my life in Singapore and the pursuit of my graduate degree would not have been the same.

Pradeepa Sivagurunathan

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List of Abbreviations

| | |
|------------------|-------------------------------------------------------|
| ACT | Arresting caries techniques |
| APF | Acidulated phosphate fluoride |
| CO ₂ | Carbon dioxide |
| CHX | Chlorhexidine |
| DSF | Diammine silver fluoride |
| DMFT | Decayed, missing and filled teeth |
| ECC | Early childhood caries |
| ESEM | Environmental scanning electron microscope |
| ICDAS | International caries detection and assessment system |
| KHN | Knoop hardness number |
| NaF | Sodium fluoride |
| Nd: YAG | Neodymium-yttrium aluminium garnet |
| NHANES | National health and examination Survey |
| OHI | Oral hygiene instruction |
| PLM | Polarized light microscope |
| SnF ₂ | Stannous fluoride |
| SEM | Scanning electron microscope |
| SF | Silver fluoride |
| SM | Streptococcus mutans |
| USDHSS | United states department of health and human Services |
| WHO | World health organization |
| YLD | Years lived with disability |

CHAPTER I: Introduction

Effect of CO₂ Laser and 38% Diammine Silver Fluoride Treatment on Enamel and Root Demineralization

ABSTRACT:

Objectives: CO₂ laser and Diammine-Silver-Fluoride have separately been shown to inhibit demineralization. However, the combined effect of the two modalities in inhibiting demineralization of enamel and root has not been investigated yet. The purpose of this study is to evaluate the effect of CO₂ laser combined with 38% DSF treatment on enamel and root demineralization.

Methods: Eight windows (4 in enamel and 4 in root) approximately 3mm x 1mm size were created on fifteen sound extracted human premolars and were randomly assigned to Control (No Treatment), CO₂ laser, DSF, DSF+CO₂ laser treatment groups. 38% DSF was applied for 2 minutes and CO₂ laser with a wavelength of 10.6 μ m, 50HZ repetition rate, 200 μ s pulse was used. A 3-day pH cycling scheme for artificial lesion formation and polarized light microscopy for measuring the lesion depth was performed. Factorial ANOVA was employed to test the main effects and interaction.

Results: The mean lesion depth (in μ m) for each group were 303.75 \pm 12.30 (Control), 224.08 \pm 8.61(DSF), 175.22 \pm 4.10 (CO₂ laser), 152.74 \pm 3.90 (DSF+CO₂ laser) in enamel and 1261.90 \pm 11.68 (Control), 814.85 \pm 8.89(DSF), 935.45 \pm 8.42 (CO₂ laser), 614.37 \pm 4.84 (DSF+CO₂ laser) in root. CO₂ Laser and DSF had a statistically significant effect on lesion depth in enamel and root (all p<0.001). The interaction between CO₂ laser and DSF was significant in enamel (p<0.005) and root (p<0.001). Individual tooth structure had no statistically significant effect on lesion depth formation in both enamel and root.

Conclusion: Combining with CO₂ laser may double the cariostatic effect of DSF.

CHAPTER II: Literature Review

2.1. Dental Caries

2.1.1. *Epidemiology of untreated dental caries*

2.1.1.1. Global burden of oral diseases

Years lived with disability (YLD) is the quantification of the disease burden represented by the severity and the duration of the disability of a population (Baelum et al., 2007). Fig.1 shows that the total disease burden is highest in Sub-Saharan Africa followed by India where communicable diseases play a major part. On the contrary, in Established Market Economy countries, non-communicable diseases play a major role. It also shows that the oral diseases contribute very little to the total YLD/ million populations (Baelum et al., 2007).

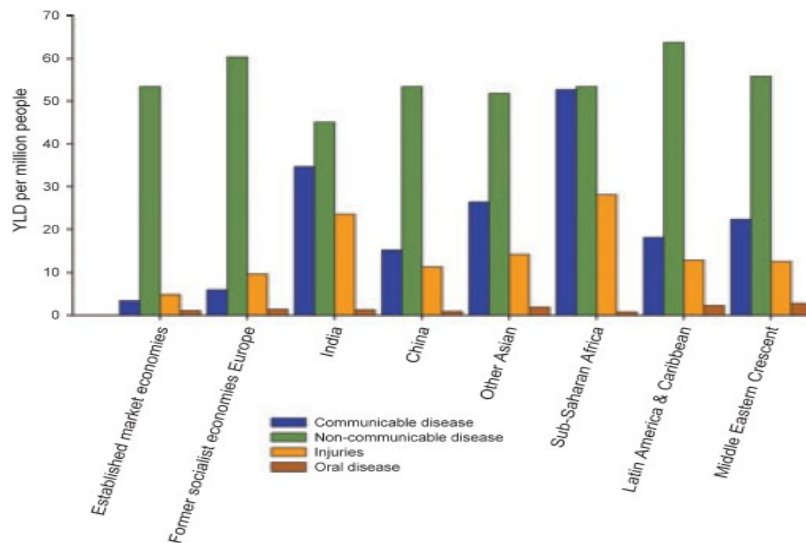


Fig 1. The distribution of the burden of disease measured as years lived with disability (YLD) per million people for different regions of the world (Adopted from Baelum et al., 2007).

The Middle Eastern countries have the highest oral disease contribution followed by Latin America and Caribbean, and other Asian countries. The lowest contributions were in Sub-Saharan Africa & in China (Baelum et al., 2007). Caries, periodontal disease and edentulism have contributed 1.6% towards the total YLD worldwide. Table 1 shows the relative YLD for oral diseases compared to that of other common diseases with comparable YLD values (Baelum et al., 2007).

Table 1. Percentage distribution of years lived with disability (YLDs) for oral diseases compared with some other common diseases in 1990 (Murray and Lopez, 1996).

| World total | Established market economy countries | Sub-Saharan Africa | China and India | Latin American Countries | Middle Eastern Crescent Countries |
|---------------------|--------------------------------------|--------------------|-----------------|--------------------------|-----------------------------------|
| Oral diseases | | | | | |
| 1.6 | 1.8 | 0.6 | 1.1 | 2.4 | 3.0 |
| Diarrhoeal diseases | | | | | |
| 1.0 | 0.4 | 1.1 | 1.3 | 1.3 | 1.0 |
| Malaria | | | | | |
| 0.8 | nil | 4.0 | 0.3 | 0.2 | 0.4 |
| Diabetes mellitus | | | | | |
| 1.1 | 3.1 | 0.3 | 0.7 | 1.3 | 1.5 |
| Tuberculosis | | | | | |
| 0.9 | nil | 1.1 | 1.0 | 0.4 | 0.5 |
| All causes | | | | | |
| 100 | 100 | 100 | 100 | 100 | 100 |

As reported by the WHO Oral Health, an action plan for promotion and integrated disease prevention has to be formulated because oral diseases such as dental caries, periodontal disease, tooth loss, oral mucosal lesions, oropharyngeal cancers, oral manifestations of HIV/AIDS, necrotizing ulcerative stomatitis and orodental trauma can be potentially serious health problems (Petersen, 2008). Its impact on life includes pain and suffering, impairment of regular function and decreased quality of life and is markedly significant at the individual level and in communities (Petersen, 2008). The greatest burden of oral diseases lies with underprivileged and poor populations globally (Petersen, 2008). In the Sixtieth World Health Assembly held in 2007, the WHO, in its provisional agenda, had ranked oral disease as the fourth most expensive disease to treat. Most of the high-income countries deal with oral diseases by establishing advanced oral-health services, which are based mainly on the dental health care provided by private dentists. Public oral health systems are also organised in some high-income countries. In most of the low

and middle income countries oral health care facilities primarily cater to emergency oral care and pain relief (Petersen, 2008).

2.1.1.2. Caries and oral disease burden

As seen in Table 2, dental caries seems to contribute about 10 times more to the YLD measure than the periodontal diseases (Murray and Lopez, 1996). This is mainly due to the fact that the pain is a frequent sequel to caries. Edentulism is an equally important cause for oral disease burden, globally (Murray and Lopez, 1996). It is evident that edentulism is an unfavorable sequelae to caries and they are interrelated. Reports have shown that the prevalence of edentulism decreased as the anti-caries regimens increased (Beltran-Aguilar et al., 2005).

Table 2. The relative contribution of caries, periodontal diseases and edentulism to the oral disease burden for different regions of the world (Murray and Lopez, 1996).

| | Established Market Economies | Former Socialist Economies Europe | India | China | Latin America & Caribbean | Sub-Saharan Africa | Middle Eastern Crescent |
|----------------------|------------------------------|-----------------------------------|-------|-------|---------------------------|--------------------|-------------------------|
| Caries | 46.7 | 50.5 | 64.3 | 58.4 | 89.8 | 73.9 | 50.4 |
| Periodontal diseases | 4.0 | 3.5 | 6.8 | 3.3 | 1.8 | 7.0 | 1.5 |
| Edentulism | 48.8 | 46.0 | 27.7 | 35.8 | 7.7 | 16.8 | 47.7 |

2.1.1.3. Dental caries - the major oral disease burden

Owing to its undesirable sequelae such as oral pain, suffering, disability and tooth loss, dental caries remains as the major oral disease burden (Baelum et al., 2007). In low-income countries dental caries remains untreated in both primary (percentage of untreated caries >94%) and permanent dentitions (percentage of untreated caries >87%) of the children (Yee and Sheiham, 2002). After assessing the WHO Global Oral Data Bank data on the prevalence of caries in many countries, the mean 12-year old DMFT was compiled and calculated for low, medium and high income nations. The mean 12-year old DMFT for the low, medium and high income nations were 1.9, 3.3 and 2.1 respectively (Yee and Sheiham, 2002). For low income African and Asian nations the percentage of untreated dental caries was approximately 95% in the deciduous

dentition and 89% in the permanent dentition (Yee and Sheiham, 2002). The National Institute of Health (NIH) has reported that dental caries is the single most common chronic childhood disease which was 5 times more common than asthma and 7 times more common than hay fever (National Institute of Health, 2000). Recent studies have reported that there is a marked increase in dental caries in both children and adults (Bagramian et al., 2009). This increase appears to be remarkable in children, new immigrants and lower socio economic groups (Bagramian et al., 2009). Table 3 depicts the prevalence of dental caries in various countries across different age groups. Although the sample sizes were variable in the different countries, this table clearly indicates that caries prevalence is a potential threat to good oral health across the globe. Also, from Table 3, it is interesting to note that caries seems to afflict developed countries like USA and UK as much as it affects the developing nations.

Table 3. Prevalence of dental caries in various countries all over the world (Adopted from Bagramian et al., 2009).

| Age | Prevalence (%) | Sample size | Country | Year |
|-------|----------------|-------------|-------------|------|
| 5-9 | 50 | 1,598 | USA | 2004 |
| 17 | 78 | 3,249 | USA | 2004 |
| 6 | 97.1 | 4,050 | Philippines | 2006 |
| 6-12 | 92.3 | 1,200 | Philippines | 2005 |
| 2-6 | 59-92 | 993 | Philippines | 2003 |
| 3-5 | 55 | 2,014 | China | 2007 |
| 5-74 | 100 | 350,000 | China | 2008 |
| 5 | 76 | 140,712 | China | 2002 |
| 5-6 | 84 | 1,587 | China | 2001 |
| 6 | 89.4 | 178 | Taiwan | 2006 |
| 1-6 | 52.9 | 981 | Taiwan | 2006 |
| 0-5 | 40 | 1,487 | Brazil | 2007 |
| 1-2.5 | 20 | 186 | Brazil | 2007 |
| 12 | 53.6 | 1,151 | Brazil | 2004 |
| 7-9 | 78.5 | 121 | Argentina | 2006 |
| 6-12 | 90.2 | 3,048 | Mexico | 2006 |
| 6-9 | 34.7 | 452 | Mexico | 2006 |
| 8 | 50 | 5,580 | UK | 2003 |
| 12 | 59.8 | 48,168 | Norway | 2006 |
| 12 | 86 | 117 | Armenia | 2005 |

Table 4 summarizes the recent studies that have raised concerns with the caries levels that have been identified in deciduous dentitions in various parts of the world.

Table 4: Summary of studies that shows the caries status in different parts of the world

| Study | Age group& Sample size | Aim of the study | Findings |
|-----------------------|------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Dye et al., 2007 | 10,000 and 15,000 children, 2-11 years of age | Presented an oral health disease prevalence data by United States National Health and Examination Survey (NHANES) from 1988-1994 and 1999-2004 | A large percentage of untreated tooth decay was found across all age groups and socio-demographic characteristics. No reductions were observed in the prevalence and severity of dental caries in primary teeth during the 10-year period of each survey. |
| Du et al., 2007 | 2,014 children, 3-5 years of age | To determine the current prevalence and severity of caries in primary dentition in a preschool population in two provinces in China | The mean dmft and dmfs values were 2.57 and 4.25 respectively. The caries prevalence and severity increased with age. The data showed that 55% of young children had dental caries and most decayed teeth were untreated. |
| Zeng et al., 2005 | 957 children, 3-5 years of age | To describe the caries status and oral health related behaviors of 3-5 year-old children two ethnic groups in Guangxi Province, China | Overall, 60% of children had caries with a mean dmft value of 3.01. Rampant caries ranged from 9% to 13% for ethnic groups. For both groups decayed teeth dominated the caries index. |
| Wong et al., 2001 | 1587 children aged 5-6 years, 1576 children aged 12 years of age | To describe the oral health status and treatment needs of the 5- to 6-year-old and 12-year-old children in Southern China | The overall weighted prevalence of dental caries (DMFT) was 84% for the younger children while for the older group the DMFT caries score was 42%. Rural children in both groups had higher caries attack rates. Decayed teeth accounted for most of the caries experience. |
| Tsai et al., 2006 | 981 children less than 6 years of age | To investigate the prevalence, patterns, and etiological factors for caries in Taiwan children. | By age 6, 89.38% of children had caries. The prevalence of dental caries for all children combined was 52.9%. |
| Ferreira et al., 2007 | 1487 children from 0-5 years | To investigate the prevalence and severity | Forty percent of the children had caries and the caries increment |

| | | | |
|-------------------------------|------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| | | of dental caries and their association with demographic and socio-economic variables in Brazilian preschoolers | increased with age. Caries was significantly higher in children with mothers of low education and low family income. |
| Scavuzzi et al., 2007 | 186 children from 12-30 months of age. | To investigate caries in a sample of young children in Brazil. | A low prevalence of dental caries (6.4%) was recorded at the initial examination, but caries increased threefold (20%) with new disease being observed during the study period. With children who had caries at baseline the prevalence doubled at the 1-year interval |
| Delgado-Angulo et al., 2006 | sample of 121 children aged 7 to 9 years | To assess the individual and grouped influence of host-related factors on dental caries experience in permanent dentition of 7-9 year-old children in Lima, Peru. | Dental caries in the primary dentition is a risk indicator for caries in the permanent dentition. Clinical examination results showed a prevalence of dental caries in the permanent dentition to be 78.5%. |
| Vallejos-Sanchez et al., 2006 | 452 children, 6-9 year olds | To evaluate the likelihood of caries increment in school children based on their prior caries experience in Campeche, Mexico. | Prevalence of caries in permanent teeth increased from year 1999 to 2000 by over 20%. The percentage of children with new dental caries increased from 14.2% to 34.7%. |
| Gao et al., 2009 | 1,782 children aged 3-6 years | To evaluate caries prevalence and severity and determine the influence of various demographic and socio-economical factors on caries patterns among different ethnic groups of preschoolers in Singapore. | The percentage of affected caries increased with age, being 25.5% in 3-4 yrs, 36.9% in 4-5 yrs and 48.9% in 5-6 yrs old. The mean (SD) deft and defs were 1.54 (2.75) and 3.30 (7.49), respectively. About 90% of the affected teeth were decayed teeth. Rampant caries was found in 16.5% of children and about 61% of affected surfaces were smooth surfaces |

2.1.1.4. Effect of caries on the deciduous dentition

United States Department of Health and Human Services (USDHSS) revealed that poor oral health in children is a major factor that affects their nutrition, growth and development. Untreated childhood oral diseases leads to pain, development of dentofacial anomalies and other serious health problems, such as severe tooth aches, dental abscess, destruction of bone, and

spread of infection via blood stream. The social impact of oral diseases in children is high, as more than 51 million school hours are lost every year due to dental illness. Poor children have almost 12 times more restricted activity days because of the dental illness than children from higher-income families (National Institute of Health, 2000).

Early childhood caries (ECC) is the most common disease of childhood affecting children, their families, the community and the health care system impacting the child's development, school performance and behavior (Casamassimo et al., 2009). Children affected by dental caries have compromised quality of life as their daily routine such as eating and sleeping, weight gain and their normal growth is disrupted. Additionally, they have a lower oral health-related quality of life than children without caries (Elice and Fields, 1990; Filstrup et al., 2003; Low et al., 1999). Children with nursing caries who had received treatment for at least one pulpally involved tooth had lower weight than control children (Acs et al., 1992).

A Turkish study involving children with ECC stated that children with ECC were considerably lighter and shorter than controls without caries. The mean weight of children with caries was between the 25th percentile and 50th percentile compared to controls who were between the 50th percentile and 75th percentiles. Seven percent of cases and 0.7% of controls weighed less than the 20th percentile (Ayhan et al., 1996). After rehabilitation the children with ECC had significantly increased growth velocities (Acs et al., 1999). Moreover, there was a remarkable improvement in children's eating preferences, quality of the food taken, social behavior and sleeping habits reported by their parents (Acs et al., 2001; Filstrup et al., 2003; Thomas and Primosch, 2002). Thus the treatment need for painful carious deciduous teeth cannot be over emphasized. On a different note, painless carious primary teeth that do not have infection can be approached with non-operative, non-invasive alternatives such as topical fluoride applications and oral hygiene maintenance (Baelum et al., 2007). In this way, invasive dental treatment which may cause dental fear and anxiety in young children can be avoided (Milsom et al., 2003).

2.1.2. Role of Fluorides in Caries Prevention

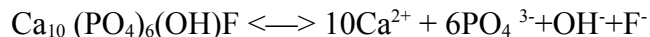
2.1.2.1. Mechanisms of action of fluoride

The mechanism of action of fluoride treatment in caries prevention has been broadly investigated over the years and there is convincing evidence that it acts by inhibiting demineralization and promoting remineralization. Additionally it also inhibits the metabolism and acid production of cariogenic bacteria (Clarkson, 1991; ten Cate, 1999). The results of more recent epidemiological and laboratory studies can be summarized by stating that post eruptive (topical) application of fluoride plays a dominant role in caries prevention (Hellwig and Lennon, 2004). This topical fluoride effect is supported by in vitro and in situ investigations demonstrating that the mode of action of fluoride can be attributed mainly to its influence on de- and remineralization kinetics of dental hard tissues (Hellwig and Lennon, 2004).

2.1.2.1.1. Inhibiting demineralization

Fluoride reduces the solubility of crystals and improves its crystallinity when taken up in the apatite lattice in the form of fluorhydroxyapatite (DePaola, 1991). There have been efforts to increase the fluoride concentration in the outer enamel thereby increasing the lifetime caries resistance of teeth (ten Cate, 1999). This theory forms the basis to the application of fluoride for caries prevention purposes (ten Cate and van Loveren, 1999). Alternatively a small amount of aqueous fluoride in saliva and dental plaque was demonstrated to reduce the rate of mineral loss dramatically (ten Cate, 1999). Since the demineralization was found to be a function of both pH and fluoride concentration the reduction in demineralization could be achieved by influencing either one or both (ten Cate and Duijsters, 1983). Since the dissolved fluoride in the oral environment could be rinsed away, this mechanism implies the requirement of a continued supply of fluoride so that caries prevention can be maintained at anytime with reasonable results (ten Cate and van Loveren, 1999; Wefel, 1990).

The previously mentioned roles of incorporated and aqueous fluoride in inhibiting demineralization can be illustrated by the following reaction:



It is obvious that if the solid material has a low solubility due to incorporated fluoride, less calcium, phosphate, hydroxyl ions and fluoride are required to prevent dissolution. It is however, equally clear that high concentration of any of the ions, including fluoride, in the aqueous phase inhibits dissolution as well (Margolis and Moreno, 1992; Wefel, 1994). Considering these reactions above, it can be concluded that the incorporated and aqueous fluoride work in concert in preventing demineralization (Margolis and Moreno, 1992; Wefel, 1994). Moreover, during the demineralization episodes, the incorporated fluoride could be released into plaque and saliva, while aqueous fluoride could be incorporated into crystalline lattice and replace carbonate, resulting in a mineral with lower solubility (ten Cate, 1999).

2.1.2.1.2. Promoting remineralization

The effect of fluoride on remineralization has received considerable attention during the past decades (ten Cate, 1999). A small amount of fluoride in saliva and plaque has been found to strongly promote remineralization of dentine and enamel, ensuing in a shift from a net negative balance results in caries to a positive balance where the tissue can be further mineralized, remineralized or hypermineralized (Featherstone, 1994). The hyper-mineralization of dentine, evidenced by multiple radio dense bands within the lesion after use of topical fluoride agents was found in vitro, implying that the mineral content and acid resistance exceeded that of sound dentine (Inaba et al., 1996; ten Cate and van Duinen, 1995). In situ studies have shown that fluoride treatments could shift the balance in a demineralizing environment to a condition of remineralization not only for enamel but also for dentine (Stephen et al., 1992; Sullivan et al., 1997; Wefel and Jensen, 1992).

Both the aqueous fluoride and the incorporated fluoride account for the enhanced remineralization. The incorporation of fluoride into crystal lattice with a resultant fluorhydroxyapatite has a lower solubility than hydroxyapatite. Furthermore, it leads to a larger degree of super saturation at a given calcium and phosphate level in saliva and plaque fluid. This thermodynamic driving force for the precipitation determines the rate at which minerals precipitate (Wefel, 1994).

2.1.2.1.3. Interference with bacterial metabolism

Fluoride has been found to be able to disturb the colonization, growth and acid production of bacteria (ten Cate, 1999; Wefel, 1990). The formation of extra cellular polysaccharide, a substance playing a role in bacterial adhesion, was found to be retarded markedly by fluoride in concentrations higher than 40 ppm (Broukal and Zajicek, 1974). The reduction in plaque growth when using topical fluoride treatments has been shown (Birkeland, 1972; Luoma et al., 1973). Several investigators have tried to relate the cariostatic effect of fluoride to changes in microbial composition of plaque on tooth surfaces as found by some studies (Loesche et al., 1975; Woods, 1971). However there was no absolute consensus as to whether fluoride may significantly change the microbial composition (ten Cate, 1999; Wefel, 1990).

The inhibition of carbohydrate metabolism and acid production by fluoride in pure cultures of oral *Streptococci* and *Lactobacilli* was demonstrated as early as 1940 (Van Kesteren et al., 1942). Since then, many studies have been published on direct and indirect effects of fluoride on the metabolism of oral bacteria (Bowden, 1990; Hamilton, 1990; Marquis, 1995). However, there is still debate whether the antimicrobial effects of fluoride do contribute to caries prevention. The most important argument is that, the fluoride concentrations needed for antimicrobial effects significantly surpass the concentrations needed to reduce the solubility of apatite (Tatevossian, 1990).

2.1.2.2. Appropriate use of fluoride interventions in caries control

Various forms of fluoride application has led to the decline of caries in many industrialized countries worldwide even though refined carbohydrate consumption has reached at a comparatively higher level over the past several decades (Brown, 1989; Stephen, 1997; ten Cate, 1999). Most authorities including WHO, have attributed the modern advances in dental caries prevention to the widespread use of fluorides (ten Cate, 1999). The effectiveness of both systemic and topical fluorides in caries prevention is well documented and has been shown effective in many epidemiological, clinical and laboratory studies (Clarkson, 1991; Wefel and Harless, 1984).

2.1.2.2.1. Methods of delivery of fluoride and their indications

The effect of fluoride content in water for the prevention of dental caries was first recognized in the early 1900's (McKay, 1928) and was further investigated by Dean and his colleagues (Dean et al., 1941; Dean et al., 1942). Fluoride levels near 1.0 ppm produced about 60% to 65% reduction in caries incidence in both the primary and permanent dentitions and were considered to be the optimum level as there was only low prevalence of dental fluorosis (Dean et al., 1950). Water fluoridation was thus translated into clinical use and was successfully implemented in communities in the United States (Dean, 1942). Water fluoridation has been regarded as the most cost effective measure available and it should be implemented wherever deemed appropriate and feasible (ten Cate, 1999).

The widespread usage of water fluoridation in many countries has been attributed to the improvement in caries control over the years (Murray, 1993). Water fluoridation serves as the fundamental basis for caries prevention because it has unique advantages in the aspect of delivery, equity, fulfillment and cost-effectiveness compared to other fluoride techniques (Lewis and Banting, 1994). Water fluoridation has been estimated to reduce caries to about 11- 40% and it has been an integral part of oral health programs in many countries (Newbrun, 1989). Singapore started its comprehensive fluoridation programme in 1956, which covered 100 per cent of the population. This fluoridation programme has resulted in a marked decline in dental caries prevalence (Lo and Bagramian, 1997; Loh, 1996; Teo, 1984). Other effective mode of fluoride delivery to the public is through salt and milk fluoridation (Ivanova et al., 1995; Marthaler and Petersen, 2005; Woodward et al., 2001). Dietary fluoride supplements as a targeted preventive procedure should be reserved only those who are at higher caries risk. However, daily administration of fluoride tablet was suggested for children and pregnant women living in communities with suboptimal fluoride concentrations in water supplies (Axelsson, 2000).

Fluoride tooth pastes is the most widespread and significant vehicle used for caries control and their use should augment any caries preventive program (Ogard et al., 1994). Fluoride varnish, mouth rinses and fluoride releasing materials are very effective in inhibiting caries in high risk population (ten Cate and van Loveren, 1999). Professionally applied topical fluoride agents are

recommended for persons with active dental decay, for those who are undergoing head and neck radiation therapy and for older adults experiencing root caries (ten Cate, 1999). Besides efficacy, an appropriate choice of fluoride application for caries prevention depends on practicality and availability, cost, patient acceptance and compliance, caries activity and safety (Newbrun, 2001; Ogard et al., 1994). A variety of fluoride compounds mainly sodium fluoride (NaF), acidulated phosphate fluoride, titanium tetra fluoride, sodium monofluorophosphate, stannous fluoride and Diammine Silver Fluoride have been used for caries prevention (Chu et al., 2002; Gisselsson et al., 1999; Johnson, 1993; Llodra et al., 2005; Wefel, 1994; Yee et al., 2009). Neutral sodium fluoride, the major ingredient of many fluoride mouth rinses, tooth pastes, dentriferics, gels, has been accepted as one of the most important fluoride compounds in caries prevention (Ogard et al., 1994).

Given the improvements in dental manpower and introduction of more specific measures for caries prevention, the non specific systemic use of fluoride in water to prevent caries raised questions because of the risk of dental fluorosis (Clarkson, 1991). As a result, the role of topical fluoride application is becoming more outstanding in modern society (Clarkson, 1991; ten Cate and van Loveren, 1999)

2.1.2.2.2. Professionally applied topical fluoride applications

Over the past several decades, topical fluorides have been shown to be effective in preventing dental caries (Buyukyilmaz et al., 1997; Marinho et al., 2003; Marinho et al., 2004; Ripa et al., 1987). Different types of topical fluoride agents including self applied fluorides (fluoride dentriferics, mouth rinses and gels) and professionally applied fluorides (fluoride varnish and gel), have been widely used and their effectiveness has been well established by clinical trials, laboratory studies and intraoral model systems (Marinho et al., 2003; Marinho et al., 2004). To increase the cariostatic effect, professionally applied topical fluorides, fluoride slow release devices and dental materials, allowing topical fluoride more time to react and taken up by the tooth structure have been developed (Ogard et al., 1994; ten Cate and van Loveren, 1999). Professionally applied topical fluoride agents can result in a caries reduction of about 40% when applied biannually (ten Cate, 1999). Topical application of fluorides to groups or individuals combined with oral health education reduces the progression of caries, which is equal to that of

water fluoridation (Clarkson, 1991; Featherstone, 1994; ten Cate, 1999). The efficacy however depends largely on the concentration of the particular fluoride, the duration and frequency of its application and to some extent the specific fluoride compound used (Newbrun, 2001).

2.1.2.3. Fluoride in teeth

2.1.2.3.1. Fluoride uptake and its role in caries inhibition

Fluoride uptake, in both loosely bound form (calcium fluoride which is readily to be dissolved in the oral environment) and the firmly bound form (apatitic fluoride which is incorporated into the crystal structure therefore not easily released) has been recognized as a marker of caries resistance in teeth (Caslavaska et al., 1975; DePaola, 1991). Firmly bound fluoride in the form of apatitic fluoride resulting in a less soluble mineral than the original enamel apatite through the compositional and crystallographic alterations has drawn significant interest among dental researchers for many years (Caslavaska et al., 1975; DePaola, 1991; ten Cate, 1997). The study done by Larsen and Fejerskov showed that the formation of fluorapatite could increase the caries resistance because subsurface lesions were developed when the enamel was exposed to a liquid unsaturated with respect to hydroxyapatite and supersaturated with respect to fluorapatite (Larsen and Fejerskov, 1977). Another study done by Takagi et al. 2000 removed the loosely bound fluoride in the teeth after topical fluoride application and they proved that the mineral loss after 5-day pH cycling process was reduced by 55% due to the formation of firmly bound fluoride. When dicalcium phosphate dihydrate (DPCD) was adopted to increase the firmly bound fluoride formation, the mineral loss was reduced by 77% (Takagi et al., 2000). This study concluded that the enamel resistance to lesion formation was closely related to firmly bound fluoride while the loosely bound fluoride was absent (Takagi et al., 2000). In addition to reducing solubility of tooth during acid attack, the dissolved apatitic fluoride can also prevent demineralization as observed in in-vitro and in-vivo studies (LeGeros et al., 1983; Ogaard et al., 1988). Apatitic fluoride which could be released during initial dissolution of mineral may also serve as a reservoir of fluoride for the inhibition of acid production especially under low pH conditions (Birkeland and Charlton, 1976; Harper and Loesche, 1986).

The cariostatic effect of loosely bound fluoride has been well acknowledged by several studies (Arends et al., 1983; Margolis et al., 1986). When the hard tissues of teeth exposed to high concentration of fluoride application CaF_2 -like globules were formed on the surface and in the intercrystal region (Arends et al., 1983; Tsuda and Arends, 1993). In the oral environment, loosely-bound fluoride is readily dissolved and released fluoride into the plaque and saliva, resulting in a beneficial fluoride levels. This may account for a shift in mineral uptake and loss pattern thereby facilitating overall remineralization (ten Cate, 1997; Wefel, 1990). During the demineralization episodes the loosely bound fluoride release may also be incorporated into tooth crystal to form apatitic fluoride (Wefel, 1990).

The loosely bound fluoride could be readily washed away, resulting in an exponential decrease of fluoride levels in saliva and plaque after a topical fluoride application. However, the important finding is that the loosely bound fluoride tend to be released at the time it is most needed, namely during a cariogenic challenge (ten Cate, 1997; ten Cate and van Loveren, 1999). In addition CaF_2 was found to be less soluble and stay within the tooth surface for a long time in in-vivo conditions (Caslavaska et al., 1975; ten Cate, 1997). The presence of CaF_2 like deposits in tooth may therefore act as a reservoir for fluoride to be mobilized into the underlying tooth surface (Westerman et al., 1999).

2.1.2.3.2. Efforts to increase fluoride uptake

Conventional topical fluoride treatments do not lead to a significant increase in fluoride uptake (Takagi et al., 1992; Takagi et al., 2000). Considerable efforts have been directed towards increasing the concentration and application time, prolonging the reaction time of fluoride with hydroxyapatite by fluoride varnish, pre-treating the tooth with the complexing agent dicalcium phosphate dihydrate (DPCD) and combining fluoride with cationic surfactants (Chow and Brown, 1975; Sieck et al., 1990). Although related studies have shed light into enhancing fluoride uptake in tooth the practical and efficient methods needs further exploration (Takagi et al., 1992; Takagi et al., 2000).

2.1.3. Role of DSF in Dental caries

2.1.3.1. The Technique

2.1.3.1.1. Arresting Caries Techniques

Arrest of caries techniques (ACT) are known as minimal intervention techniques because it helps in arresting caries without the mechanical preparation of the tooth for a restoration. Arresting caries techniques include those using silver fluoride, stannous fluoride, diammine silver fluoride, low viscosity glass ionomer cement, and supervised tooth brushing programmes using fluorides. The main goal of this technique is to arrest caries rather than restoring the damaged tooth structure. This is non-invasive, painless and quick compared to traditional curative approach of cavity preparation and restoration. This procedure minimizes the pain and suffering of mechanical preparation of the teeth especially in children who were young and difficult to manage. Moreover, the WHO dental databases of caries in children showed that 90% of caries were left untreated in developing countries due to the cost of treating them with traditional restorative treatment (Yee and Sheiham, 2002). Therefore, these techniques are particularly useful in community dental health procedures in disadvantaged communities as many of the young children are burdened with dental caries from an early age. Ultimately the aim of remineralization therapies in caries management for enamel lesions is to slow lesion progression, promote lesion arrest, and ideally achieve lesion regression (Pitts and Wefel, 2009).

2.1.3.1.2. Arresting non-cavitated lesions

All the non cavitated smooth surface lesions should receive preventive therapy to arrest the carious lesion, to remineralize and to avoid cavitation and eliminate the need of surgical treatment (Stahl and Zandona, 2007). The International Caries Detection and Assessment System (ICDAS), based on visual inspection can detect cavitated and non-cavitated lesions with adequate reliability for use in clinical research as well as in epidemiological surveys (Pitts, 2004; Ismail et al., 2007). Inclusion of non-cavitated lesions is necessary because these lesions can be arrested through preventive management thereby lowering the costs of restorative treatment (Pitts and Fyffe, 1988; Pitts, 2004). The non-cavitated lesions in primary and permanent teeth can be managed professionally by non-invasive means such as fluoride varnish and sealants and

supervised daily home tooth brushing using fluoride toothpaste to arrest the progression of the lesion so that restorations will not be necessary (Evans and Dennison, 2009).

2.1.3.1.3. Arresting cavitated lesions

Various topical agents such as silver nitrate, stannous fluoride, sodium fluoride, silver fluoride and Diammine Silver Fluoride have been applied clinically at high concentrations to arrest the active cavitated carious lesions and to prevent further caries progression. High fluoride concentration compounds such as silver fluoride [AgF] and Diammine Silver Fluoride $\text{Ag}(\text{NH}_3)_2\text{F}$ were used to arrest more advanced carious lesions in several countries (Zero, 2006). AgF and DSF are particularly appropriate in children with moderate and severe caries involving more than one surface of the tooth. Such minimal intervention techniques mainly targeted children who were phobic to dental treatment.

2.1.3.1.4. Silver Fluoride

Silver fluoride (AgF) was developed by Craig in 1978. The technique used AgF with stannous fluoride to limit caries progression in the primary molars of children living in a low socioeconomic background in New South Wales, Australia (Craig et al., 1981). Subsequently, the atraumatic approach started in Western Australia using 40% Silver fluoride solution in arresting residual caries followed by the insertion of glass ionomer restoration in carious primary teeth (Gotjamanos, 1996). This approach used a modified preparation of the cavity without local anaesthesia followed by the application of AgF and restorations with GI cement (Gotjamanos, 1996). The clinical follow-up of approximately 400,000 cases of these deep carious lesions indicated a success rate of 100% based on the absence of symptoms (Gotjamanos, 1996).

2.1.3.1.5. Diammine Silver Fluoride

The usage of Diammine Silver Fluoride [$\text{Ag}(\text{NH}_3)_2\text{F}$] to arrest dental caries dates back to 1969 (Nishino *et al.*, 1969). This simple and non-invasive application method of Diammine Silver Fluoride has been used in many countries for years to arrest dental caries and it is commercially

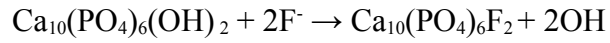
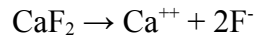
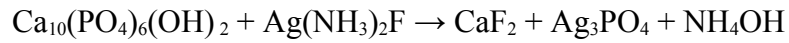
available in markets for many years. DSF is a colorless solution containing fluoride ions. The ammonia ions in DSF combines with silver ions to form diammine-silver ion $[\text{Ag}(\text{NH}_3)_2]^+$ and this formation is a reversible reaction. This complex is very stable and the equilibrium lies within diammine-silver ions. DSF is more stable than AgF and it can be kept constant concentration for a longer time (Chu and Lo, 2008). Moreover, DSF is not alkaline as AgF solution. The main advantages of DSF pointed out by Bedi and Infirri (1999) were:

1. DSF is effective in preventing and arresting caries progression which if left untreated will cause pain and infection.
2. The cost of DSF treatment is low and affordable.
3. The application procedures are simple so that the non-dental professionals can be easily trained to apply DSF.
4. It does not require expensive equipment to perform the treatment.
5. The risk of spreading infection is low.

DSF is available in two concentrations of 38% (44,800 ppm F) and 12%. The 38% Diammine Silver Fluoride is commonly used to arrest caries in children, who would not accept normal dental treatment (Chu and Lo, 2008) and used as an alternative where restorative treatment for primary teeth is not an option (Yee et al., 2009). Its low cost and simplicity in application makes DSF an appropriate therapeutic agent for use in community dental health projects. DSF's ability to halt the caries process and simultaneously prevent the formation of new caries makes it superior to the other fluoride based caries preventive agents (Rosenblatt et al., 2009).

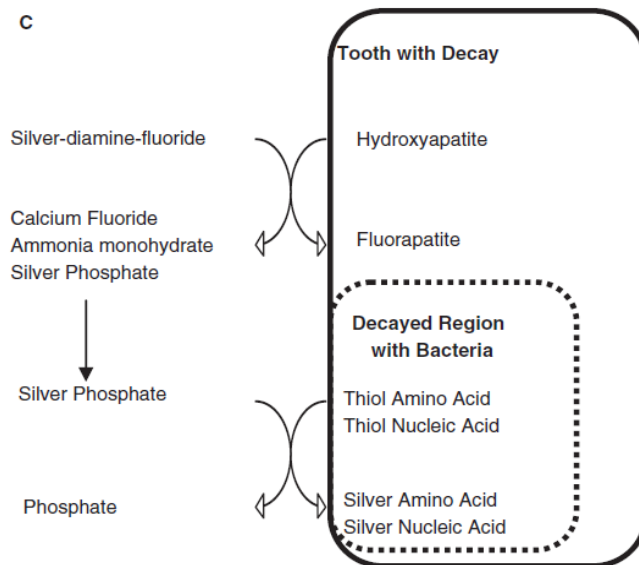
2.1.3.2. The mode of action

Diammine Silver Fluoride reacts with hydroxyapatite and forms calcium fluoride and silver phosphate. Further dissociation of calcium fluoride into calcium and fluoride leads to the formation of fluorapatite (Rosenblatt et al., 2009).



In the decayed teeth, silver phosphate reacts with bacterial amino and nucleic acid thiol groups to form silver amino and nucleic acids (Figure 2) (Rosenblatt et al., 2009).

Fig 2. Mode of action of Diammine Silver Fluoride (Adopted from Rosenblatt et al., 2009).



2.1.3.3. Efficacy

2.1.3.3.1. In vitro studies involving SF and DSF

Over the last 40 years silver fluoride has been used in dentistry as an anticaries agent. In vitro studies demonstrated that silver fluoride is effective in inhibiting *S. mutans* growth (Thibodeau et al., 1978) and caries progression (Klein et al., 1999). Histological assessment of dental pulps following the application of 40% AgF and GI cement showed a good response in the pulp which includes the formation of reparative dentin and increased odontoblastic activity (Gotjamanos, 1996).

In order for fluoride to be effective as a remineralizing agent, it has to be present near the tooth surface in a stable form. When DSF reacts with enamel apatite, it forms calcium fluoride and silver phosphate which are insoluble in oral environment and therefore are more stable. Thus the decalcification of teeth due to constant acid attack is minimized. The penetration assessed by electron probe microanalyser showed that the fluoride penetrated through the enamel for about 25 μ depth. Moreover, the fluoride retained after the immersion in synthetic saliva is higher compared to other fluorides NaF, NaF-PO₄ and SnF₂ (Suzuki et al., 1974). An in-vitro study on demineralized dentin disks in a diffusion apparatus compared the efficacy of AgF and AgF followed by KI on the penetration and viability of *S. mutans*. The results showed significant inhibitory effects on *S. mutans* penetration and growth during a 14 day exposure (Knight et al., 2005). The same group has demonstrated that the AgF/KI treatment of demineralized and non-demineralized dentine prevented biofilm formation and reduced further demineralization by *S. mutans* (Knight et al., 2007, Knight et al., 2009). The micro hardness of measurements of arrested dentinal caries on primary teeth receiving DSF and NaF after 30 months was harder than that of active carious lesions (Chu and Lo, 2008). Within the outer 25– 200 μ m, the median micro hardness of dentin in arrested carious lesions (range, 20–46 or 196–451 MPa) were greater than those of soft carious lesions (range, 5–20, or 49–196 MPa) (Chu and Lo, 2008).

The recent study evaluated the effect of 3.8% DSF as an antibacterial agent against *E. faecalis* biofilms (Hirashi et al., 2010). They demonstrated that DSF can be used as an antimicrobial root canal irrigant or interappointment dressing, especially in locations in which potential blackening

of dentin by metallic silver is not a major concern. Table 5 summarizes the in-vitro studies involving Diammine Silver Fluoride and Silver Fluoride.

Table 5. In-vitro studies involving Diammine Silver Fluoride and Silver Fluoride

| Study | Objective | Methods | Results |
|----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Suzuki et al.,1974 | To find out the subsequent changes of CaF ₂ and AG ₃ PO ₄ produced by the application of DSF in oral environment, penetration of fluoride and fluoride uptake | 1. X-Ray diffraction analysis. 2. Electron probe microanalysis 3. Chemical Analysis | Ag ₃ PO ₄ reacted with SCN to form AgSCN which retained longer period of time. The fluoride penetrated through the enamel for about 25μ depth. DSF Retained after the immersion in synthetic saliva for a week was highest. |
| Afonso et al., 1996 | An in vitro test system involving application of 40% AgF solution to prepared cavities of extracted teeth to demonstrate the passage of significant amounts of fluoride into the dental pulp | Fluoride analysis Histology is to determine the distribution of reduced silver. | Failed to demonstrate the passage of significant amounts of fluoride into the dental pulp, despite a very high concentration of fluoride (100,000 ppm) in the applied solution. |
| Gotjanos et al.,1996 | This study investigated pulp response in primary teeth with deep residual caries treated with silver fluoride and glass ionomer cement | Histological assessment of the dental pulps of 55 carious primary teeth at 3 to 58 months after treatment by atraumatic technique of 40% AgF application followed by restoration GI cement | Fifty of the 55 teeth examined showed a favourable pulpal response, inducing presence of abundant reparative dentine and a wide odontoblast layer. |
| Klein et al.,1999 | This study compares four chemotherapeutic regimens AgNO ₃ , AgF/SnF ₂ , DSF, CHX used for inhibiting carious lesion progression | A bacterial model system containing MS and LC was used to generate carious lesions on 85 extracted sound permanent 3 rd molars which were randomly assigned to 4 test groups and control group. | Six weeks later lesions treated with a single AgNO ₃ , AgF/SnF ₂ application demonstrated 29% and 19% less lesion progression respectively than the control group (p<0.05). |
| Knight et al.2005 | The aim of this study was to develop an in vitro model that would provide an indication of the permeability of demineralized dentine to | Forty dentine discs were bonded to the base of forty 5mL polycarbonate screw top vials filled with nutrient medium, sterilized and placed into a continuous culture of | Samples treated with AgF and AgF/KI had significantly lower optical densities than the corresponding controls. The range of optical densities was least amongst |

| | | | |
|-----------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| | SM after treatment of the dentine with AgF followed by KI. | SM. | demineralized samples treated with AgF/KI |
| Knight et al.2007 | To observe the difference between demineralized and non-demineralized dentine treated with AgF/KI after exposing to <i>S. mutans</i> . | 10 samples of demineralized dentine 10 samples of demineralized dentine treated with AgF/KI 5 samples of non-demineralized dentine. 10 samples of non-demineralized dentine treated with AgF/KI. | AgF/KI treatment prevented biofilm formation. AgF/KI treatment of demineralized dentine was more effective in reducing dentine breakdown and the growth of SM. Significantly higher levels of silver and fluoride were deposited within demineralized dentine |
| Chu and Lo, 2008 | This study measured the micro hardness of arrested dentinal caries on primary teeth receiving regular fluoride applications after 30 months. | 38% DSF every 12 months or 5% NaF varnish every 3 months. At 30 months very mobile Knoop Hardness Number measurements at sites below the surface at the center of the carious lesion every 25 mm toward the pulp | Within the outer 25–200 mm, the median KHN of arrested carious lesions (range, 20–46 or 196–451 MPa) were greater than those of soft carious lesions (range, 5–20, or 49–196 MPa). |
| Knight et al.2009 | The purpose of this study was to measure whether a topical application of DSF followed by KI on partially demineralized dentine affected the formation of a SM biofilm. | 10 Disks as control 10 Disks treated with AgF followed by KI 10 Disks treated with KI 10 Disks treated with AgF. The outer surfaces of the disks were subjected SEM. EPMA Analysis. | An SM biofilm covered with entire exposed surfaces of all control and KI treated disks. No discernible bacterial biofilm was detected on disks treated with AgF or AgF/KI. Detectable amounts of silver and fluoride were found up to 450 μm in the AgF or AgF/KI sections |
| Hiraishi et al., 2010 | This study investigated the use of 3.8% DSF as an antibacterial agent against <i>Enterococcus faecalis</i> biofilms and its ability to penetrate dentinal tubules by the formation of silver salts. | Biofilms were generated on membrane filter discs and subjected to 15-minute and 60-minute exposure times with 3.8% DSF, saturated Ca (OH) ₂ , 5.25% NaOCl (negative control), 0.9% NaCl (positive control). Ag (NH ₃) ₂ F application is for 24, 48, and 72 hours. SEM analysis to find the deposition of silver salts. | Both NaOCl and Ag (NH ₃) ₂ F were effective against <i>E. faecalis</i> biofilms. Silver deposits were present on 66.5% of the radicular dentin surfaces after 72-hour application of Ag (NH ₃) ₂ F. Penetration of the silver deposits was observed at most 40 mm into dentinal tubules. |

2.1.3.3.2. Clinical trials involving SF and DSF

The clinical studies have shown that AgF inhibits caries progression in both primary and permanent teeth (McDonald and Sheiham, 1994) and is effective in preventing caries in newly erupted first permanent molars when applied with SnF₂ (Green, 1989).

The clinical trial study involving 375 Chinese preschool children showed that yearly application of DSF was effective in arresting dentin caries and preventing new carious lesions in primary anterior teeth compared to the other group which had 3 monthly application of NaF (Chu et al., 2002). Children who received DSF annually had more arrested caries lesions than the other groups. The mean number of arrested carious tooth surfaces in DSF group with or without excavation were 2.5 and 2.8 compared to the NaF group which is 1.5 and 1.5 (Chu et al., 2002). There was no evidence to show that the removal of carious dentin prior to the application of fluoride agents had an effect on their ability to arrest dentin caries (Chu et al., 2002).

Llodra et al. (2005) showed that six monthly application of a 38% DSF was effective in reducing caries in both primary teeth and first permanent molars in 6 to 15 year old school children. This clinical study was about 36 months and the total number of samples was 452 Cuban school children. The mean baseline dmfs (decayed, missing and filled surface index) scores of the children were 3.68 ± 0.30 in the DSF group and 3.35 ± 0.26 in the control group. The mean number of surfaces with active caries was 3.29 ± 0.28 in the DSF group and 2.91 ± 0.22 in the control group (Llodra et al., 2005). After 36-month follow-up, the mean number of new decayed surfaces in primary teeth was 0.29 in DSF compared to 1.43 in controls. The mean number of new decayed surface in first permanent molars was 0.37 in DSF group and 1.06 in controls after follow-up (Llodra et al., 2005). Compared with the control, DSF group children had more surfaces with inactive caries and a higher proportion of black stains in inactive lesions (Llodra et al., 2005).

Another clinical study performed arresting caries treatment to manage untreated dental caries in primary teeth of 976 Nepalese school children with ages ranging from 3-9 years for the period of 2 years (Yee et al., 2009). This is the first clinical study to evaluate the effectiveness of one time application of DSF in both 38% and 12% concentrations and tested the effect of DSF with or without a reducing agent (Yee et al., 2009). The mean number of arrested caries surfaces with

38% DSF alone and with reducing agent was 4.5 and 4.2 after 6 months, 4.1 and 3.4 after one year and 2.2 and 2.1 after 2 years. A single application of 38% DSF, with or without tea as a reducing agent, was significantly more effective in both the anterior and posterior primary dentitions of young children than 12% DSF and control (Yee et al., 2009).

The randomized control clinical trial study by Tan et al. (2010) assessed effectiveness of four methods including DSF in preventing root caries. A total number of 306 generally healthy elders having at least 5 teeth with exposed sound root surfaces were selected for the study. They received either oral hygiene instruction (OHI), OHI and applications of 1% chlorhexidine varnish every 3 months, OHI and applications of 5% sodium fluoride varnish every 3 months and OHI and annual applications of 38% Diammine Silver Fluoride (DSF) as treatment (Tan et al., 2010). Two thirds (203/306) of the elders were followed for 3 years. Mean numbers of new root caries surfaces in the four groups were 2.5, 1.1, 0.9, and 0.7 respectively (ANOVA, $p < 0.001$). Results showed that DSF solution, sodium fluoride varnish, and chlorhexidine varnish were more effective in preventing new root caries than OHI alone (Tan et al., 2010).

The recent multi-center randomized clinical trial study assessed the effectiveness and safety of topical Diammine Silver Fluoride in reducing root sensitivity (Castillo et al., 2011). From Lima and Cusco, Peru, 126 adults with at least one tooth sensitive to compressed air were randomly assigned to either the experimental treatment group or sterile water (Control). The pain was assessed by means of a 100-mm visual analogue scale at 24 hours and 7 days post-treatment (Castillo et al., 2011). The Diammine Silver Fluoride reduced pain at 7 days at both sites. In Lima, the average change in pain scores assessed using a Visual Analog Scale between baseline and day 7 for the silver fluoride group was -35.8 (SD = 27.7) mm vs. 0.4 (SD = 16.2) mm for the control group ($P < 0.001$). In Cusco, the average change in pain scores for the silver fluoride group was -23.4 (SD = 21.0) mm and -5.5 (18.1) mm for the control group ($P = 0.002$) (Castillo et al., 2011). No tissue ulceration, white changes, or argyria were observed. No changes were observed in the Gingival Index and the study concluded that Diammine Silver Fluoride is a clinically effective and safe tooth desensitizer (Castillo et al., 2011)

2.1.3.4. Safety

Application of silver fluoride solution to prepared cavities of extracted teeth did not demonstrate the passage of significant amounts of fluoride into the dental pulp, despite using a very high concentration of fluoride (100,000 ppm) in the solution (Afonso and Gotjamanos, 1996). Therefore, this in vitro study confirmed that the application of 40% silver fluoride as a cavity varnish or liner and its use in the 'atraumatic' technique for treating deep carious lesions can be considered as a safe clinical procedures (Afonso and Gotjamanos, 1996).

The possible acute toxicity to the pulp or the induction of fluorosis through the use of DSF has been widely debated (Gotjamanos, 1997; Neesham, 1997). The black discoloration of carious teeth is the only drawback of using DSF for clinical purposes. Chu and his associates stated that there was no significant change in parental satisfaction due to the staining (Chu et al., 2002). Appearance of mildly painful white lesions on the mucosa which disappeared in 48 hrs without treatment has been mentioned in one study (Llodra et al., 2005). Considering the dose related safety, DSF's dosage is approximately one drop for each quadrant, applied with a brush and rinsed off afterward. This minimal amount of application keeps the adverse events low (Rosenblatt et al., 2009).

2.1.3.5. Summary

Studies have shown that DSF and SF can inhibit progression of caries (Klien et al., 1999; McDonald and Sheiham, 1994) and investigations have shown that enamel and dentin are harder and less soluble after application of DSF (Chu and Lo, 2008). The silver component in AgF may cause destruction of plaque bacteria including *S. mutans* and result in the mechanical sealing of sound and carious dentinal tubules (Gotjamanos, 1996; Thibodeau et al., 1978). Studies have demonstrated that DSF can prevent biofilm formation and reduce further demineralization by *S. mutans* (Knight et al., 2005; Knight et al., 2007) and have shown effective against *E. faecalis* biofilm (Hirashi et al., 2010). Clinical trials have found DSF to be an effective agent in preventing new caries and arresting caries especially in young children who are less cooperative (Chu et al., 2002; Llodra et al., 2005; Yee et al., 2009). In addition to this, recent clinical trials have shown that DSF is effective in preventing root caries (Tan et al., 2010) as well as root

sensitivity (Castillo et al., 2011). However, there has not been any study reporting the effect of CO₂ laser combined with Diammine Silver Fluoride treatment in inhibiting caries.

2.2. Lasers in Dentistry

2.2.1. *Laser principles*

2.2.1.1. Laser light production

The basic system of laser contains an active element, a resonator and a pump source. The pump source illuminates a working substance which is the active element of the laser and leads to exponential light amplification (Akhmanov, 1997). The light going sideways abandons the active element without gaining substantial energy. On the other hand, the light wave travelling along the axis of the optical resonator and passing repeatedly through the active element, gains energy constantly (Akhmanov, 1997). Because of the partial transmission of light through one of the resonant mirrors, the radiation comes out as a laser beam from the resonator (Akhmanov, 1997).

2.2.1.2. Characteristics of laser light

There are mainly three important characteristics with the laser beam (Akhmanov, 1997).

2.2.1.2.1 Coherence

The light produced by a laser differs from ordinary light in that it is made up of waves of the same wavelength and all waves are in one phase both spatially and temporally.

2.2.1.2.2 Monochromaticity

Laser has one specific color because laser light travels in a specific wavelength.

2.2.1.2.3. Collimation

Laser light travels in a collimated or parallel beam and it does not diverge significantly even over long distances. This leads to ultimate precision and minimal energy loss.

2.2.1.3. Laser Parameters

Laser radiation is characterized primarily by its wavelength, energy, pulse duration, continuous wave power, spectral bandwidth, tuning range and beam divergence (Akhmanov, 1997). These parameters vary extensively for different type of lasers. For example, excimer lasers produce ultra violet radiation in the 200-300 nm wavelength range and CO₂ lasers operate in the infrared (10.6 μm) (Akhmanov, 1997). According to wavelength, lasers can be categorized ultraviolet (1-400 nm), visible (400-750 nm), and infrared (750 + nm) levels. Energy density, defined as the amount of energy that is distributed over a surface area or volume, expressed in j/cm², is an important parameter representing the intensity of laser radiation (Birngruber, 1989).

2.2.2. *Laser-tissue interactions*

2.2.2.1. Factors modulating biological effects of laser

The optical properties of tissue elements determine the nature and degree of the tissue response through the processes of absorption, transmission, reflection and scattering of the laser beam (Dederich, 1991). The extent of interaction of laser light as a form of radiant energy with tissue is determined by two dependant variables, the specific wavelength of the laser emission and the optical characteristics of the particular target tissues (Dederich, 1991).

Biologic systems have different optical absorption characteristics and so predicting the effect of a particular laser emission may appear problematic. Water is the predominant element which controls the tissue effects of laser emission within the infrared spectrum such as CO₂. So, the CO₂ laser at a wavelength of 10.6 μm is well absorbed by oral tissue fluids and has a minimal penetration beyond the surface. Conversely, water is transparent to the emission of Nd: YAG laser which may lead to deeper penetration into the tissue.

The characteristic of preferential absorption of specific wavelengths of radiant energy by chromophores such as hemoglobin and melanin exert a significant influence over the interaction with tissues. Hemoglobin readily interacts with 488 and 514 nm wavelengths. As a result the ability of the argon lasers for coagulation and hemostasis is proved effective.

2.2.2.2. Tissue effects of laser irradiation

Photo-thermal effect is the major biological effect of laser. Under varied irradiation conditions, diverse thermal effects can be achieved resulting in warming, coagulation, tissue shrinkage, vaporization, ablation and carbonization of biological tissues respectively. The photo acoustic effects can also be created by lasers. The pulse of laser energy on hard dental tissues can produce a shockwave which ultimately pulverizes the tissue creating an abraded crater. Lasers can create photo-chemical effects by stimulating chemical reactions and rupturing inter molecular and atomic bonds. The applications of lasers in the curing of composite resin and photodynamic therapy for treating cancer are based on the photochemical effects of laser. Moreover laser induced fluorescence has been used effectively as a diagnostic tool in clinical practice (Fisher, 1992).

2.2.2.3. Laser- tissue interactions on dental hard tissues

The absorption and transmission of laser light in human teeth is mainly dependent on the wavelength of laser light (Frentzen and Koort, 1991). Ultraviolet laser light is well absorbed but in the mid-infrared spectrum the absorption in water and in hydroxyapatite differs enormously depending on the wavelength of the laser light. In water and in HA, there is a very low absorption at a wavelength of 2 μm in comparison to the high absorption of laser energy at 3 μm and 10 μm . At 1 μm the absorption in water and in HA becomes about 10,000 times less than that at 3 μm (Nagasawa, 1983; Legros, 1999).

2.2.3. Laser Application in general dentistry

Following the discovery of laser (Maiman, 1960), the effects of laser on the teeth were explored (Goldman et al., 1964; Stern et al., 1966). With subsequent growth in laser technology, several types of laser such as CO₂ laser, Nd: YAG laser, Argon laser, Er: YAG laser and He: Ne lasers

were developed and used in dental research and treatment (Coluzzi, 2000; Wigdor et al., 1995). The applications of these different kinds of lasers in dentistry includes oral surgery, analgesia, treatment of hypersensitive dentin and aphthous ulcers, tooth whitening, sulcular debridement, caries removal, cavity preparation, polymerization of light cured composite restorative materials, pulpotomy, various endodontic treatments, welding dental prostheses, bone ablation and cartilage reshaping (Coluzzi, 2000; Wigdor et al., 1995).

Until now, lasers were mainly used in soft tissue surgeries and produced desirable effects. Due to the unpredictability of laser induced effects in dental hard tissue treatment, its clinical application is very limited in dental hard tissues (Wigdor et al., 1995)

2.2.4. Laser Applications in caries prevention

Dental laser research was actually started in the year 1963 at the University Of Los Angeles School Of Dentistry with the investigations of the researchers named Ralph S. Stern and Reider F. Sognnaes (Miserendino and Pick, 1995). Various types of lasers in the prevention of dental caries have been documented over the past several years. These studies have used different types of lasers, namely ruby lasers (Mannerberg et al., 1969; Stern et al., 1966; Stern, 1969; Vahl, 1968), Nd: YAG laser (Stern, 1969; Yamamoto and Ooya, 1974; Yamamoto and Sato, 1980), argon lasers (Hattab, 1987; Nammour et al., 1987) and CO₂ lasers (Brune, 1980; Kuroda and Fowler, 1984; Lobene et al., 1968; Nelson et al., 1987; Scheinin and Kantola, 1969; Stern, 1969; Stern et al., 1972).

2.2.4.1. The cariostatic effects of laser

The CO₂ laser is more effective and safer in caries preventive application, because the emission wavelengths of a CO₂ laser coincide with the strong infrared absorption regions of apatite. Stern and his associates have investigated the macroscopic enamel surface changes induced by a pulsed CO₂ laser at an energy density ranging from 13 to 50 J/cm² (Stern et al., 1972). They showed that CO₂ laser was more efficient than ruby laser, which required an energy density > 200 J/cm² to generate similar inhibitory effects on enamel demineralization (Stern et al., 1972). About 50-60% reduction in subsurface demineralization was achieved by CO₂ laser (Fox et al., 1992; Stern et al., 1972). In another study there was an 87% reduction in caries like lesions,

observed by using pulsed CO₂ laser on enamel (Featherstone and Nelson, 1987). The CO₂ laser irradiation was capable of converting the enamel surface apatite to lower soluble one (Fox et al., 1992). The critical pH of enamel was lowered from 5.5 to 4.78 after the laser treatment (Fox et al., 1992).

Borggreven et al. (1980) used 200 µm thick slices of bovine enamel mounted as membranes in diffusion cells and tested the permeability of enamel before and after laser irradiation. They found that repeated pulsed CO₂ laser irradiation caused a significant permeability increase, rather than a decrease. They concluded that irradiation of enamel at an energy density that increases the acid resistance of enamel did not decrease the rate of transport of various compounds, even when the irradiation was repeated up to 40 times. It was suggested that the cariostatic mechanisms of laser irradiation on enamel might be due to laser-induced chemical modifications rather than due to permeability changes (Borggreven et al., 1980).

One study used Nd: YAG laser at an energy density of 10 J/cm² and 20 J/cm² and they assessed the enamel surface changes by using micro-radiography. At energy density of 20 J/cm² the Nd: YAG lased enamel samples showed no subsurface demineralization in microradiographs. It was concluded that the apparent difference in acid resistance between Nd: YAG lased and unlased enamel may be due to a physical change in the size of enamel crystallites as a result of the loss of water and carbonate from minerals at the surface of enamel (Yamamoto and Ooya, 1974). Another study concluded that reduced acid solubility of the Nd: YAG lased enamel resulted from a decrease in permeability caused by the changes in organic matter that accounted for the smooth appearance of the enamel surface (Yamamoto and Sato, 1980).

Nelson et al. (1987) evaluated the effect of low energy, pulsed CO₂ laser radiation on human enamel at wavelengths ranging from 9.3 to 10.6 µm. Fractured cross-sections of these zones revealed that the effect of the laser extended approximately 5 µm below the enamel surface, where melting, fusion and recrystallization of enamel crystallites were observed. The apparent inhibition of demineralization was more effective at the higher energy treatment of 50 J/cm² than lower energy treatment of 10 J/cm² (Nelson et al., 1987).

The study by Ferreria et al. (1989) revealed that laser irradiated enamel surface was altered. They exhibited either crazing alone or crater in combination with crazing. In the crazed enamel, prism boundaries were present and most crystals generally resembled those of normal enamel, even though some crystals have unusual shapes and sizes. They concluded that lased enamel exhibiting a greater number of voids did not specify an ultra structural improvement compared with normal enamel. However, the larger apatite crystal size and loss of prism boundaries in the crazed and cratered enamel may decrease acid dissolution, since the dissolution rates are proportional to the crystal surface area. Therefore, larger particles in crazed and cratered enamel would probably result in reduced acid reactivity (Ferreira et al., 1989).

The study by Hsu et al (2000) demonstrated the role of the organic matrix in CO₂ laser induced inhibition of enamel demineralization. The effects of low-energy CO₂ laser irradiation on demineralization of both normal human enamel and human enamel without its organic matrix were investigated. The laser irradiation resulted in a 98% reduction in mineral loss, but the laser effect dropped to about 70% when the organic matrix in the enamel was removed. The study also showed that melting of the crystals was not necessary for achieving laser-induced inhibition of demineralization (Hsu et al., 2000).

2.2.4.2. Mechanisms involved in laser induced caries prevention

Several mechanisms have been proposed for years to explain the caries preventive effects of lasers (Hicks et al., 1997; Oho and Morioka, 1990).

2.2.4.2.1. Morphological, physical and chemical changes

Surface melting and fusion on lased enamel were suggested as the main reasons for the increased acid resistance in the earlier studies (Stern and Sognaes, 1965; Stern, 1969). However, the favorable changes in acid resistance of the mineral have occurred at temperatures below the melting point of the tooth mineral (Kantorowitz et al., 1998). On the other hand they showed that melting and fusion were not necessary to achieve caries inhibition (Kantorowitz et al., 1998). The laser irradiance melted the enamel apatite and this melt composed of traces of alpha tri calcium phosphate and tetra calcium phosphate which has the higher solubility than the original apatite (Kuroda and Fowler, 1984). These remarkable findings have raised questions about the

melting and fusion in laser induced caries prevention and recommended that there may be other mechanisms involved in the cariostatic effects of lasers (Kantorowitz et al., 1998). An increase in the enamel micro hardness after the application of lasers has been suggested to play a role in caries inhibition (Marquez et al., 1993).

The laser induced changes in the composition and crystal structure of enamel have caught the interest of the researchers in recent years (Hsu et al., 1998; Oho and Morioka, 1990; Zuerlein et al., 1999). There was an alteration in the composition of enamel with the reduction in organic, carbonate and water substances after the CO₂ laser irradiation at different fluence levels (Fox et al., 1992; Hsu et al., 1998; Oho and Morioka, 1990). Thirty percent of the carbonate was removed from the enamel at temperatures between 400-600° C (Zuerlein et al., 1999). This carbonate reduction in hydroxyapatite crystals produced a tooth surface and its underlying structures less acid soluble than the unlased hydroxyapatite (Hsu et al., 1998; Oho and Morioka, 1990).

The studies in the past have demonstrated that the CO₂ laser was capable of producing recrystallization and increasing the size of hydroxyapatite crystals and resulting in more caries resistant enamel (Goodman and Kaufman, 1977; Kantola et al., 1973). A high positive birefringence, signifying the formation of microspaces in enamel and a decrease of lattice strain added with slight a-axis contraction contributed to the laser induced acid resistance of enamel. The removal of carbonate and recrystallization of enamel crystals occurred at a lower temperature compared to the melting and formation of unwanted additional calcium phosphate phases (Zuerlein et al., 1999).

The mechanism for the increased acid-resistance was explained by laser induced reduction in solubility and the alterations of the inorganic component of the tooth hard tissues (Stern et al., 1966). Moreover, the thermal changes in the organic content such as protein denaturation and micropores expansion which leads to the decrease in permeability of enamel was attributed to play a significant role in caries inhibition (Stern et al., 1972; Yamamoto and Sato, 1980). The increased acid resistance of lased enamel was mainly due to the reductions in both permeability and solubility of tooth enamel (Nelson et al., 1987).

2.2.4.3. Possible side effects

The main hindrance of using lasers in prevention of dental caries clinically are the possible side effects on the hard and soft tissue of tooth including the formation of unwanted calcium phases that are more soluble than the original apatite; creation of enamel cracks that could permit the penetration of bacterial products into deeper layers of teeth and lead to the reduction in micro hardness; and possible laser-induced thermal damages to pulp (Cox et al., 1994; Hashiguchi and Hashimoto, 2000; Hicks et al., 1995a; Tagomori and Morioka, 1989; Zuerlein et al., 1999). On the other hand the laser tissue interactions differ enormously depending upon the type of laser beam, operational mode and energy output. Therefore, it is necessary to explore appropriate laser therapy for efficient protection against caries without causing adverse effects on tooth structures (Tagomori and Morioka, 1989)

2.3. Combined treatment with fluoride and laser

2.3.1. Synergistic cariostatic effect of fluoride and laser treatment

Several studies have reported that laser irradiation combined with fluoride treatment have a greater effect on inhibiting caries in both enamel caries (Table 6) and root caries (Table 7) compared to laser or fluoride treatment alone.

Combined argon laser and APF treatment resulted in 50-60% reduction in lesion depth compared with control in enamel caries initiation and progression. The fluoride treatment before laser irradiation resulted in greater reduction in lesion depth compared to fluoride treatment after laser irradiation (Flaitz et al., 1995; Hicks et al., 1995a). CO₂ laser in combination with fluorides has shown greater efficacy in inhibiting enamel caries (Hsu et al., 2001; Hsu et al., 2004; Tepper et al., 2004). There was enamel caries inhibition of about 91% as shown by the combined CO₂ laser treatment in a pH-cycling system (Featherstone et al., 1991). Treatment with low-intensity CO₂ laser irradiation in combination with 2% NaF gel has resulted in a 98.3% reduction in mineral loss (Hsu et al., 2001). The study by Tepper and his associates reported that the treatment with continuous wave CO₂ laser immediately after applying amine fluoride led to 50% increase

fluoride uptake in the enamel compared to the control group (Tepper et al., 2004). The recent study which compared the effectiveness of CO₂ laser and diode laser on the fluoride uptake revealed that the uptake is increased on the diode laser (57%) and CO₂ laser (69%) irradiated enamel surface and better pulp safety was obtained with CO₂ laser than with diode laser (Gonzalez-Rodriguez et al., 2009).

For roots, the reduction in lesion depth ranged from 54% to 66% compared with control (Hicks et al., 1995b; Hicks et al., 1997). Another study by Gao et al. (2006) reported a significant synergistic effect of combined CO₂ laser and fluoride treatment on the inhibition of root demineralization which may be caused by the laser-enhanced fluoride uptake of about 84.5% in the root (Gao et al., 2006).

Table 6. Studies on the effect of combined fluoride and laser treatment in inhibiting enamel caries

| Study | Sample | Characterization Technique | Lesion formation | Laser Parameters | Fluoride treatment | L& F timings* | Results (% Reduction in comparison with control) |
|----------------------------|----------------------------|-------------------------------------------------|---------------------------------------------|----------------------------------------------------|--------------------------|---------------|----------------------------------------------------------------------------------------------------------------------------|
| Goodman and Kaufman, 1977 | 18 incisor enamel sections | Atomic absorption spectroscopy | Dissolved in 150 µl of 2M perchloric acid | Argon laser (5145 Å) 4.0 Watts | NaF, 4% | L/F | 50% reduction |
| Tagomori and Morioka, 1989 | Enamel sections | Atomic absorption spectroscopy | Etched with 1 ml of 0.5 M HClO ₄ | Nd:YAG, 40 J/cm ² | 2% NaF, APF, 5 minutes | L → F & F → L | laser +APF- 43% APF+ laser- 60% laser+ NaF- 38% NaF+ laser- 47% |
| Featherstone et al., 1991 | 90 human Pre molars | Micro hardness measurement | pH cycling for 5 days | CO ₂ , 15/25 mJ per pulse | 1.23% APF gel, 5 minutes | L → F | 91% |
| Fox et al., 1992 | Human enamel | Initial dissolution rate | Bisphosphonic acid | CO ₂ , 65 J/cm ² 2 sec. | NaF | L → F | 84% |
| Flaitz et al., 1995 | Human enamel | PLM microscopy | Acidified gel | Argon 2 w for 10s 100 J/cm ² | 1.23% APF gel, 4 minutes | L → F & F → L | laser alone: 26-32% L+F- 50% |
| Hicks et al., 1995 | 20 sound human molars | PLM microscopy | Acidified gel | Argon 0.25 watts 10,12.0 +/- 0.5 J/cm ² | 1.23% APF gel, 4 minutes | L → F & F → L | 52 % (L → F) 56% (F → L) |
| Hsu. J et al., 1998 | Human enamel blocks | PLM microscopy & micro radiography | Demineralizing solution | CO ₂ , 42.5-170 J/cm ² | 0.2 ppm fluoride | L → F | 42.5 J/cm ² - 37.3%, 85 J/cm ² - 85.7%, 127.5 J/cm ² - 94.5%, 170 J/cm ² - 55% |
| Haider et al., 1999 | 10 human premolars | 16 X Bisecting microscope, caries detection dye | Acidified gelatin gel with HCl | Argon, 100J/cm ² | 2% NaF , 4 minutes | L → F | 60% |

| | | | | | | | |
|---------------------------------|--------------------------|-----------------------------------------------------------------------|--------------------------|----------------------------------------------------------------------------------------|----------------------------------|---------------|--------------------------------------------------------------------------------------------|
| Hsu et al., 2001 | 24 human pre molars | Micro radiography | pH cycling | CO ₂ , 0.3 J/cm ² , 2 W, 5 ms | 2% neutral NaF, 4 minutes | F → L | F+L treatment led to 98.3% and 95.1% with and without organic matrix |
| Santos et al., 2001 | 110 human enamel blocks | PLM microscopy | pH cycling | CO ₂ , 9.6 um, 1.0, 1.5 J/cm ² , 10 Hz, 5 μs | APF gel, 5 minutes | L → F & F → L | 35% to 76% achieved. Best inhibition seen when treated with APF before laser. |
| Santos et al., 2002 | 120 human enamel blocks | PLM microscopy | pH cycling | CO ₂ , 9.6 um, 2.0 & 3.0 J/cm ² , 5-8 μs | APF gel, 5 minutes | L → F & F → L | 87% to 170% (together with reversal of lesions) |
| Hsu et al., 2004 | 5 human molars | Secondary ion mass spectroscopy, ESEM | KOH extraction technique | CO ₂ laser at 2 and 4 W 5, and 4 s | 2.0% NaF, 4 minutes | F → L | KOH and Non KOH groups have higher fluoride uptake of 60% |
| Tepper et al., 2004 | 40 human enamel sections | Selective electrode, SEM | Etching with 10μl 2M HCl | CO ₂ laser wavelength- 10.6 um, 2 W, irradiation time 15 s. | 1% Amine fluoride 15 seconds | F → L | laser treatment with amine fluoride solution caused almost 50% increase in fluoride uptake |
| Gonzalez Rodriguez et al., 2009 | 45 sound human molars | Selective ion electrode Thermocouple probe to measure thermal changes | Etching with 2M HCl | 1. Diode - 2 settings 5w, 7w for 30 ms 2. CO ₂ laser- 10.6 um, 1w, 15 ms | 0.1 mg Amine fluoride 15 seconds | F → L | AmF only- 38% AmF+Diode 5w - 44% AmF+Diode 7w -57% AmF+ CO ₂ 1w- 69% |

* L → F- Laser treatment before fluoride treatment; F → L- Laser treatment after fluoride treatment; L → F & F → L- Laser treatment before and after fluoride treatment; L/F- Laser treatment concurrent with the fluoride treatment

Table 7. Studies on the effect of combined fluoride and laser treatment in inhibiting root caries

| Study | Sample | Charecterization technique | Lesion formation | Laser Parameters | Fluoride tx | L & F timings* | Results (% reduction in comparision with control) |
|---------------------|--------------------------|----------------------------|------------------|--------------------------------|----------------------------|------------------|----------------------------------------------------------|
| Hicks et al., 1995b | Sound human root | PLM microscopy | pH cycling | Argon, 12 J/cm ² | 1.23% APF gel, 4 minutes | L → F & F → L | 54 %(L → F) 57% (F → L) |
| Hicks et al., 1997 | Sound human root | PLM microscopy | pH cycling | Argon, 12 J/cm ² | 1.23% APF gel, 4 minutes | L → F & F → L | 64 %(L → F) 66% (F → L) |
| Gao et al., 2006 | 15 sound human premolars | PLM microscopy | pH cycling | NaF 1.14 J/cm ² | 2% neutral NaF , 4 minutes | F → L | laser only: 29.8% fluoride only:30.8% F → L: 84.5% |

* L → F- Laser treatment before fluoride treatment; F → L- Laser treatment after fluoride treatment; L → F & F → L- Laser treatment before and after fluoride treatment

2.3.2. Possible mechanisms of the combined cariostatic effect of laser and fluoride

So far, the mechanism of the combined fluoride and laser treatment in inhibiting dental caries remains unclear. Several studies have reported that CO₂ (Gao et al., 2006; Hsu et al., 2004; Tepper et al., 2004), Argon (Goodman and Kaufman, 1977), Diode (Gonzalez-Rodriguez et al., 2009) and Nd:YAG (Zhang et al., 1996) laser irradiation may enhance fluoride uptake on tooth surfaces. Two theories have dominated the studies on the mechanism behind the cariostatic effect of combined laser and fluoride treatment. Scanning electron microscopic studies have demonstrated numerous spherical and globular precipitates that resembled calcium fluoride on root surfaces after fluoride and laser treatment (Westerman et al., 1999; Zhang et al., 1996). These precipitates acted as a fluoride reservoir to replenish the fluoride released during periodic episodes of demineralization thereby interfering with the dynamic process of the caries formation (Haider et al., 1999; Westerman et al., 1999). The other theory emphasized the role of lasers on enhancing fluoride uptake into the tooth structure and thus optimizing its crystallinity instead of producing surface deposits (Goodman and Kaufman, 1977; Hsu et al., 2004; Meurman et al., 1997; Zhang et al., 1996).

Using the enamel powder, the study showed a 14-fold increase in fluoride uptake and an increase in crystallite size after laser irradiation (Goodman and Kaufman, 1977). The penetration of fluoride into a depth of 20 microns in root surface was successfully achieved by Nd: YAG laser was reported by another study (Zhang et al., 1996). It was believed that the application of fluoride before laser irradiation could lead to the mobilization of surface coating of fluoride allowing the incorporation of these surface precipitates into the underlying cementum and dentine, thus increasing their caries resistance (Hicks et al., 1997). With the use of synthetic hydroxyapatite one study proved that the CO₂ laser treatment in the presence of fluoride could even transform hydroxyapatite into fluorapatite, resulting in a reduced surface area and less solubility (Meurman et al., 1997).

2.4. Aims and Objectives

The aim of this study is to evaluate the effect CO₂ laser combined with Diammine Silver Fluoride treatment on demineralization and fluoride uptake in both enamel and root.

2.5. Hypothesis

Diammine Silver Fluoride (DSF) when combined with CO₂ laser will have a synergistic effect in preventing enamel and root demineralization and increasing the fluoride uptake in comparison with DSF or laser treatment alone

CHAPTER III: Materials and Methods

3.1. Part I: Effect of CO₂ Laser and Diammine Silver Fluoride treatment on demineralization of Enamel and Root

3.1.1. Tooth collection and cleaning

The teeth collected for this study were collected from Dr. R. Baskaran, Manikandan Memorial Dental Clinic, Tamilnadu, India. They were premolars extracted for orthodontic purposes. Through clinical examination, all the teeth were ensured to be free from caries and other defects of tooth hard tissues. Only forceps were used during the extraction. Those collected teeth were stored at 4° C in 0.1% thymol solution. The procedure for tooth collection was approved under the exemption category of the Institutional Review Board of the National University of Singapore (NUS-IRB reference code: 11-106E).

All the extracted teeth were cleaned in the Cariology Lab, Level-3, DSO Building, NUS through careful scaling to remove the debris, attached soft tissues and calculus and examined under a

stereo microscope (Olympus SZ40, Olympus Optical Co. LTD, Japan) at 10X magnification to select the teeth with no caries lesion.

3.1.2. *Sample preparation and grouping*

Fifteen human sound premolars were selected and cleaned. A hole was drilled with a dental turbine (Super- Torque LUX 3 turbine 650, Ka Vo Dental GmbH, Germany) at the apical portion of each tooth, to allow a dental floss (Oral-B Essential floss, Gillette Company, South Boston, MA, U.S.A.) to pass through. This could facilitate suspension of the teeth in the solutions during pH cycling. Each tooth was varnished two times using an acid resistant varnish (Express Finish, Maybelline Inc., NY, U.S.A), leaving eight windows: two on the buccal surface and two on the lingual surface in enamel and two on the mesial and two on the distal surface in root (Fig 3). Windows were approximately of 1 mm (height) by 3mm (length). The windows were created on the buccal and lingual surfaces of enamel and mesial and distal surfaces of root. The distance between the two windows on the same enamel and root surface was 2mm and the windows were located at 1 mm below the cemento enamel junction (CEJ) in the root.

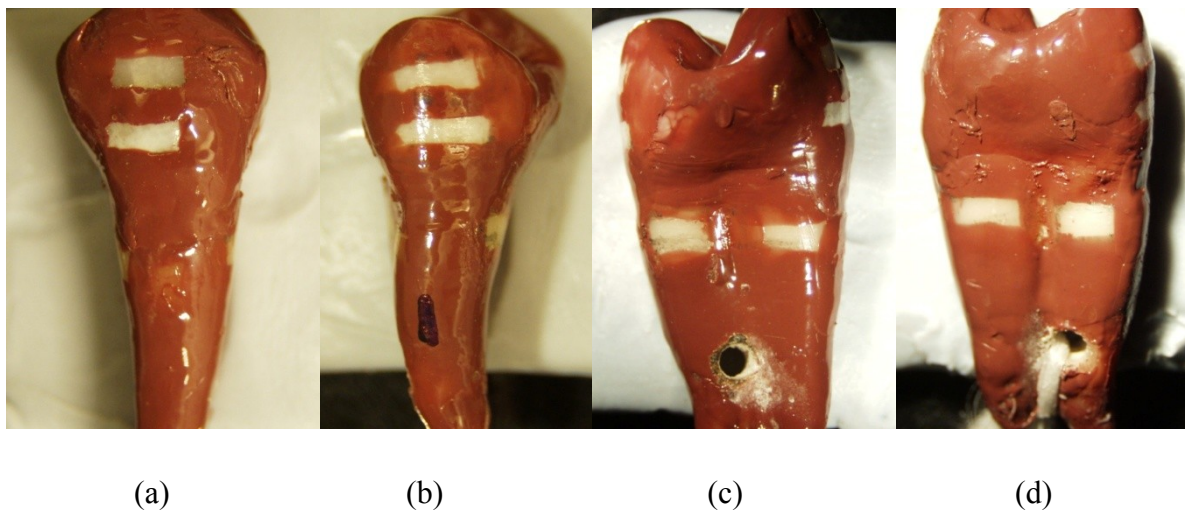


Fig.3: (a) Buccal view. (b) Lingual view. (c) Mesial view. (d) Distal view

Randomly, each of the four windows on the same tooth was assigned into four groups listed below by using a research randomizer program (www.randomizer.org). The random assignment was performed by an independent laboratory assistant in order to minimize selection bias.

Group 1: Control

Group 2: Laser treatment alone

Group 3: Fluoride treatment alone

Group 4: Fluoride followed by laser treatment

3.1.3. Fluoride treatment

38% Diammine Silver Fluoride (Bee Brand Medical Dental Company Ltd, Osaka, Japan) was applied in the windows of 3 and 4 for 2 minutes using an applicator brush (Fig 4). After the treatment, the excess DSF solution was wiped off with tissue paper before further treatment.



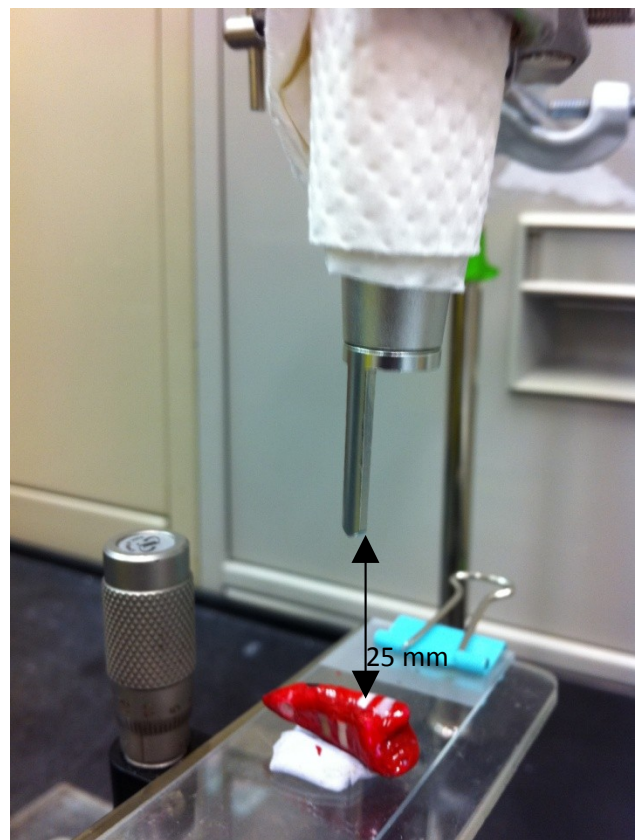
(a)

(b)

Fig 4: (a) Diammine Silver Fluoride. (b) Application of DSF on windows

3.1.4. Laser treatment

The windows of group 2 and 4 received CO₂ laser irradiation treatment. A single pulse mode of a carbon dioxide laser system (SMARTPS laser system, Shin Han Systek Co Ltd, Korea) with a wavelength of 10.6 μm , 50 HZ repetition rate, 200 μs pulse was applied (Fig 5a and 5c). The laser tip was clamped on a chemical stand to prevent movement (Fig 5b). The spot size was about 1.01 m in diameter (Fig 5d) ensured by a stylus-object distance of about 25 mm adjusted through a X-Y-Z positioner. After laser treatment, all the teeth were rinsed in a beaker of de-ionized and distilled water for 5 minutes at a stirring speed of 130 rpm at 37° C.



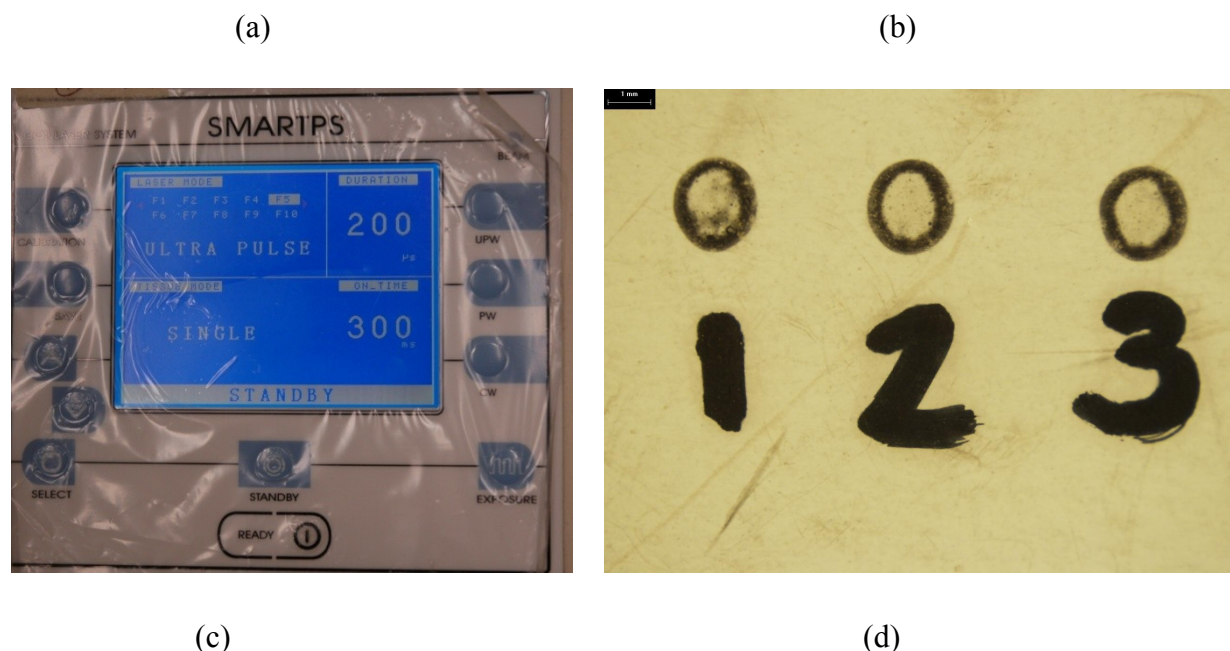


Fig 5: (a) SMARTPS CO₂ laser System. (b) Placement of tooth 25mm from laser tip. (c) Laser Settings. (d) Laser dimensions with average spot size of 1.01mm.

3.1.5. Artificial lesion formation

A three-day pH-cycling scheme was performed, with 18-hours of demineralization followed by 6-hours of remineralization, at a stirring speed of 150 rpm at 37° C. The demineralization solution used in this study was Yakult. A pH meter (Model 370, ORION Basic Selective Benchtop Meter; Orion Research, Inc, Boston, MA, USA) was used to measure the pH value of the solutions. A calibration range of pH 7 to pH 4 was selected. Measurement of pH was repeated for 5 separate bottles of Yakult® and the mean pH was 3.65. The remineralizing solution, pH of 7.0, containing 0.15M potassium chloride, 1.5mM calcium and 0.9mM phosphate ions, was prepared. A 10-minute wash in the de-ionized and distilled water were performed between the demineralization and remineralization phases and at the end of the pH-cycling. Both

demineralization and remineralization solutions were changed daily. All teeth were stored in plastic containers with 100% humidity after pH cycling.

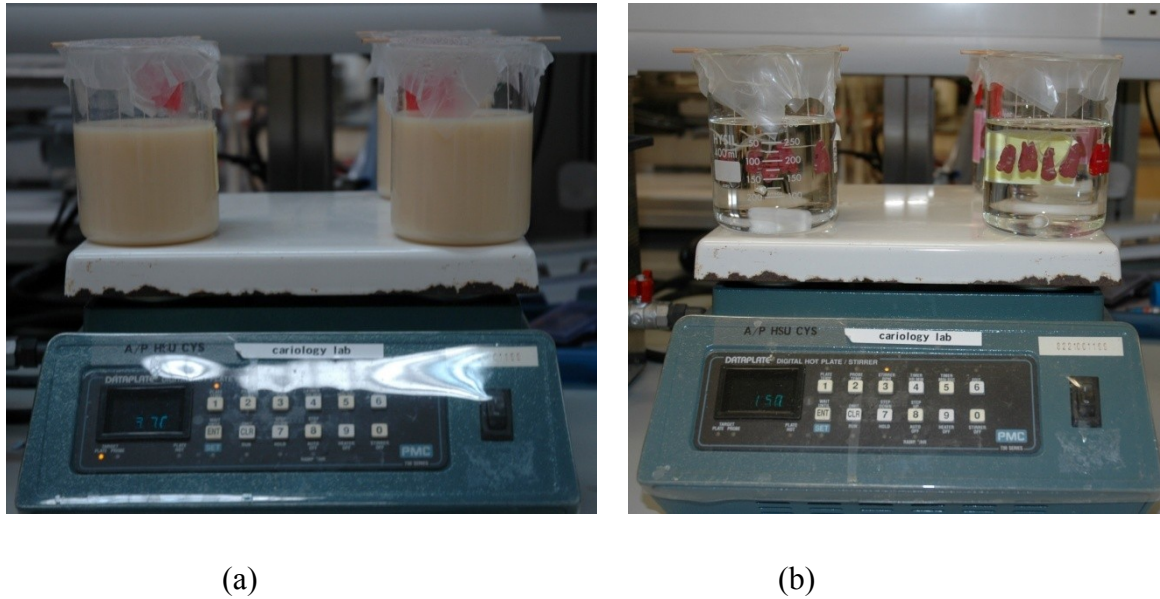


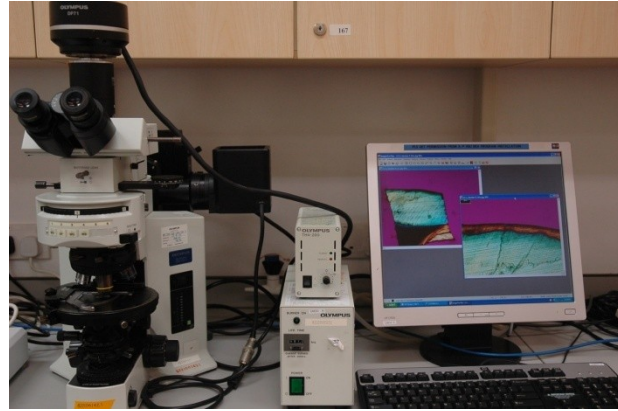
Fig 6: (a) Teeth suspended in Yakult® solution. (b) Teeth suspended in remineralizing solution.

3.1.6. Sectioning

All teeth were sectioned longitudinally, perpendicular to the crown surfaces through the central part of the each window with a Silverstone- Taylor hard-tissue microtome (Series 1000 deluxe. Sci Fab, Littleton, CO, USA) equipped with a Buehler Diamond Wafering Blade (Series 15LC, Buehler Ltd. Lake Bluff, IL, USA) (Fig 7). An opportune air mist for cooling was maintained during the whole sectioning process. The sections were detached from the crown using a disposable microtome blade (LEICA model 818. LEICA Instruments GmbH, Nussloch, Germany). From central area of each window four sections, with the thickness of about 150 μm , were obtained.



(a)



(b)

Fig 7: (a) Silverstone-Taylor hard-tissue microtome. (b) Polarized light microscope

3.1.7. Polarized Light Microscopy (PLM) Characterization

Amplly imbibed in water all the sections were characterized at 10x magnification under a polarized light microscope (Model BX51, Olympus, Japan), to produce PLM digital images with a color video digital camera (Model ssc-DC58AP, Exwave HAD, Sony, Japan) as in Figure 7b.

3.1.8. Lesion Depth Measurement

On each of the PLM image, by using the image analysis software, (Micro Image Olympus, Japan), the lesion area was traced and measured within the central 400 μm of the artificial

enamel caries lesion (Fig. 8). The area value was divided by 400, to produce the average lesion depth. Area measurements were performed by a blinded evaluator in order to minimize observer bias in the study.

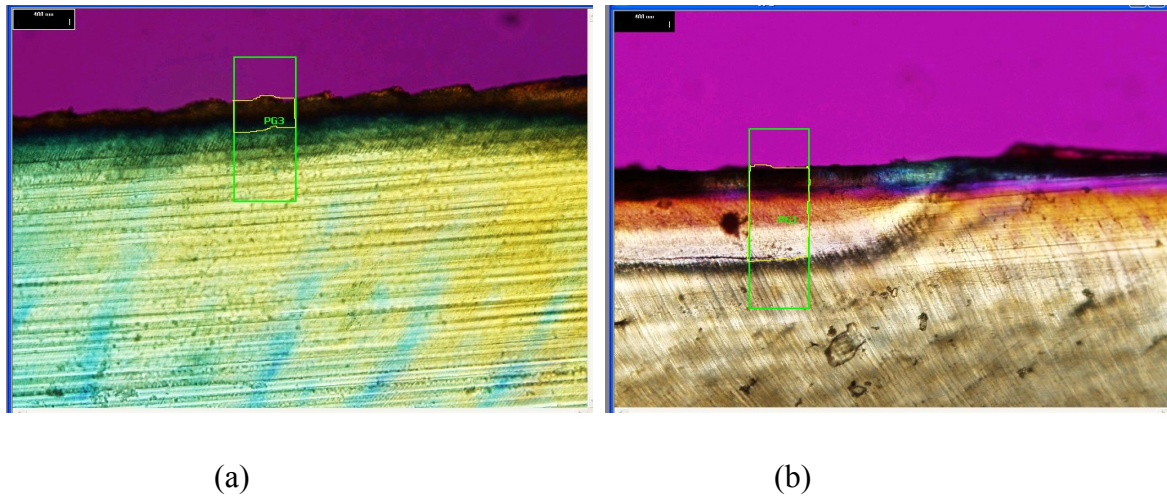


Fig 8: (a) Lesion depth measurement- enamel (b) Lesion depth measurement- root

3.1.9. *Statistical Analysis*

The dependant variable was lesion depth (in micrometers). The independent variables included, laser treatment, fluoride treatment and tooth structure.

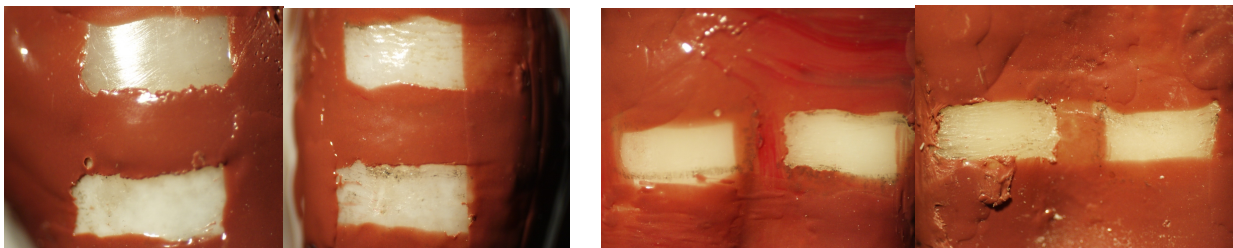
After testing the homogeneity of variance by the Levene test, a general linear model was constructed to evaluate the main effects of independent variables and the laser-fluoride interaction. A post hoc test, the Tukey-Kramer test for multiple comparisons, was adopted to evaluate the significance of the between-group differences with level of statistical significance set at $p < 0.05$. The univariate general linear model (SPSS Statistics 16.0) was used to analyze the

main effects and the potential interactions between the independent variables on enamel and root respectively. The dependant variable was lesion depth (in micrometers). The independent variables were laser and fluoride. Tooth number was kept as a random variable.

3.2. Part II: Effect of CO₂ Laser and Diammine Silver Fluoride treatment on fluoride uptake in Enamel and Root

3.2.1. Sample preparation and grouping

Five human sound premolars were selected and cleaned. Each tooth was varnished for two times using an acid resistant varnish (Express Finish, Maybelline Inc., NY, U.S.A), leaving eight 1 mm (height) x 3mm (length) windows with two on the buccal surface and two on the lingual surface) in enamel and root. The distance between the two windows on the same enamel and root surfaces was 2mm and the windows were located at 1 mm below the cemento enamel junction (CEJ) in the root.



(a)

(b)

Fig 9: (a) Buccal and Lingual windows -enamel (b) Left and Right windows- root

Each of the four windows on the same tooth was randomly assigned to one of the four groups listed below by using a research randomizer program (www.randomizer.org). The randomization was performed by an independent laboratory assistant in order to minimize selection bias.

Group 1: Control

Group 2: Laser treatment alone

Group 3: Fluoride treatment alone

Group 4: Fluoride followed by laser treatment

3.2.2. Fluoride treatment

38% Diammine Silver Fluoride (Bee Brand Medical Dental Company Ltd, Osaka, Japan) was applied in the windows of group 3 & 4 for 2 minutes using an applicator brush. After the treatment, the excess of DSF solution was wiped off with tissue papers before further treatment.

3.2.3. Laser treatment

The windows of group 2 and 4 received CO₂ laser irradiation treatment. The single pulse mode of a SMARTPS carbon dioxide laser system (SMARTPS laser system, Shin Han Systek Co Ltd, Korea) with a wavelength of 10.6 μm, 50 Hz repetition rate, 200 μs pulse was applied. The laser tip was clamped on a chemical stand to prevent movement and to ensure the spot size of about 0.5 mm in diameter. After laser treatment, all the teeth were rinsed in the de-ionized and distilled water for 5 minutes at a stirring speed of 130 rpm at 37° C.

3.2.4. Sectioning

The first two cuts were done with a Buehler Isomet Low Speed Saw (Model no: 11-1280-250, Lake Bluff, IL, USA) equipped with a Buehler Diamond Wafering Blade (Series 15 LC, Buehler Ltd, Lake Bluff, IL, USA). Then the samples were sectioned using a Silverstone-Taylor hard-tissue microtome (Series 1000 Deluxe, Scifab, Littleton, CO, USA) equipped with a Buehler Diamond Wafering Blade (Series 15 LC, Buehler Ltd, Lake Bluff, IL, USA) for the rest of the cuts. An opportune water air mist for cooling was maintained during the whole sectioning process. From each window 0.6mm thick sections were obtained.

3.2.5. Fluoride uptake measurement by nuclear microscopy

The measurement of fluoride concentration using nuclear microscopy was assisted by the Department of Physics, NUS, Singapore with the 2 MeV proton beam focused onto a spot approximately 5 micrometers. The tooth samples were irradiated with a 2 MeV proton beam at currents of 2-3nA from Singletron accelerator at the Center for Ion Beam Applications. Proton induced Gamma Emission (PIGE), Proton Induced X-ray Emission PIXE and (non)-Rutherford Backscattering (RBS) were applied. The characteristic X-rays produced by the various elements in the tooth samples were detected by a Si(Li) detector and the total proton fluence was measured by RBS. From this data, elemental distributions and concentrations were calculated. The calcium distribution maps were used for the straightforward identification of the tooth surfaces during the measurements. The fluorine content in the teeth was simultaneously determined by observing the 6-7 MeV gammas from the nuclear reaction $^{19}\text{F}(p, \text{ag})^{16}\text{O}$ with a NaI (Tl) detector. During the

measurement, the beam spot on the tooth was clearly visible due to the fluorescence. This allowed to select and identify the various regions of the teeth for analysis purposes.

3.2.6. Statistical Analysis

The dependant variable was “fluoride uptake”. The independent variables included “laser treatment”, “fluoride treatment” and “tooth structure”.

A factorial ANOVA model with level of statistical significance set at $p < 0.05$ was constructed for the assessment of the main effects and potential interactions between factors. After testing the homogeneity of variance by the Levene test, a general linear model was constructed to evaluate the main effects of independent variables and the laser-fluoride interaction on enamel and root respectively. One-way ANOVA was used to evaluate the difference between the four groups. A post hoc test, the Tukey-Kramer test for multiple comparisons, was adopted to evaluate the significance of the between-group differences with level of statistical significance set at $p < 0.05$.

CHAPTER IV: RESULTS

4.1. Results for demineralization study

4.1.1. Enamel

The mean lesion depths (in μm) were 303.75 ± 12.30 for the control group, 224.08 ± 8.61 for the DSF group, 175.22 ± 4.10 for the laser group, and 152.74 ± 3.90 for DSF + laser group in enamel. Figure 10 shows the mean lesion depths in enamel for the different treatments.

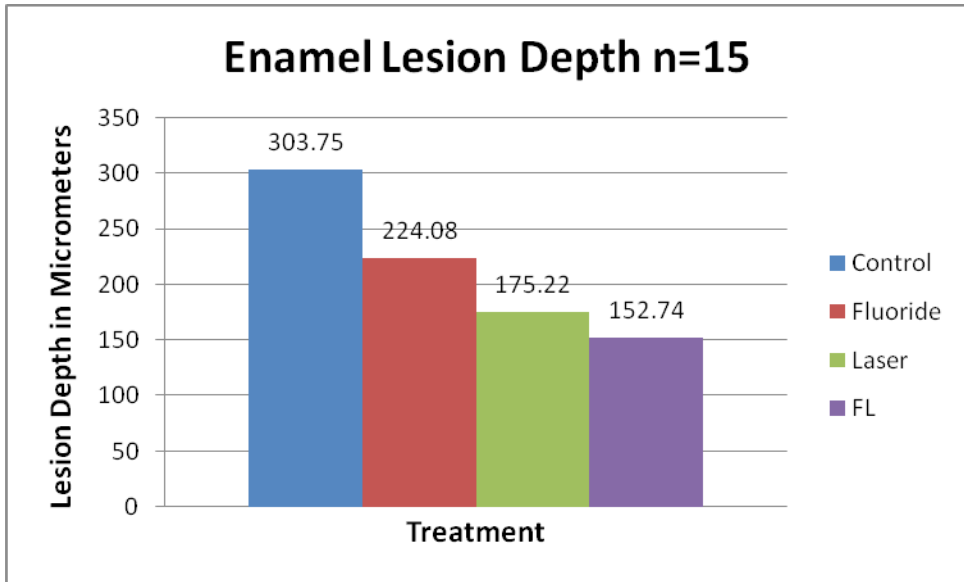


Figure 10: Mean lesion depth for different treatments in enamel.

When the percentage reduction of different treatments was compared to the control it showed that the combined DSF- laser treatment nearly doubled the cariostatic effect of DSF (Table 8).

Table 8: Percentage reduction of lesion depth compared to control

| Enamel | Control | Fluoride | Laser | FL |
|--------------------|----------|----------|---------|----------|
| Mean | 303.7507 | 224.08 | 175.22 | 152.74 |
| Standard Deviation | 47.63173 | 33.35123 | 15.8747 | 15.11813 |
| % reduction | | 25% | 42% | 49% |
| Ranking | IV | III | II | I |

CO₂ Laser and DSF had a statistically significant effect on lesion depth in enamel (all $p < 0.001$). The interaction between CO₂ laser and DSF was significant in enamel ($p = 0.001$). Individual tooth structure had no significant effect on lesion depth formation in enamel ($p = 0.310$).

4.1.2. Root

The mean lesion depths (in μm) were 1261.90 ± 11.68 for the control group, 814.85 ± 8.89 for the DSF group, 935.45 ± 8.42 for the laser group, 614.37 ± 4.84 for the DSF + laser group in root. Figure 11 depicts the mean lesion depths in root.

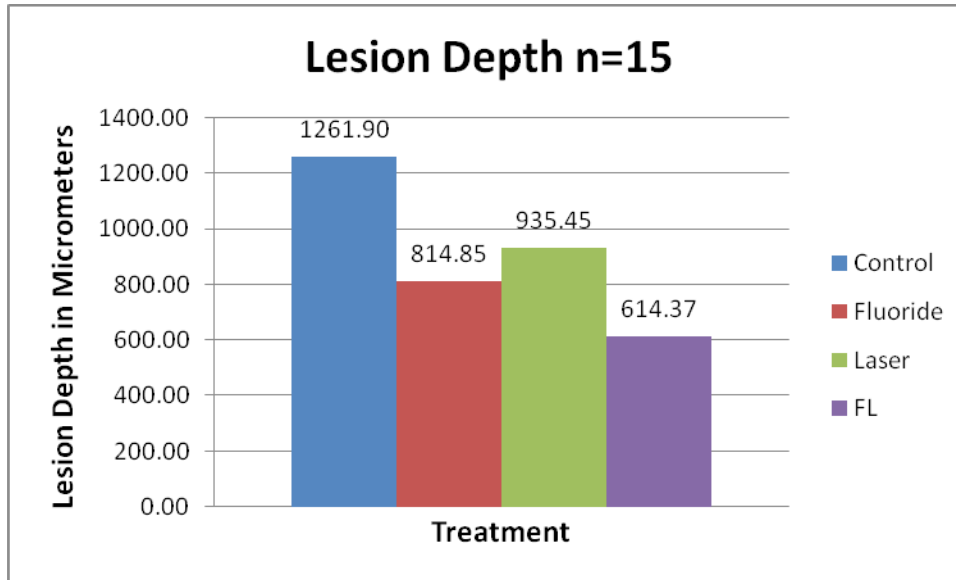


Fig 11: Mean lesion depth for different treatments in root

When the percentage reduction of different treatments was compared to the control the combined DSF-laser treatment was double the cariostatic effect of the laser group.

Table 9: Percentage reduction of lesion depth compared to control

| Root | Control | Fluoride | Laser | FL |
|--------------------|---------|----------|----------|----------|
| Mean | 1261.90 | 814.85 | 935.45 | 614.37 |
| Standard Deviation | 45.2098 | 34.43197 | 32.61967 | 18.75995 |
| % reduction | | 35% | 25% | 51% |
| Ranking | IV | II | III | I |

CO₂ Laser and DSF had a statistically significant effect on lesion depth in root (all $p < 0.001$). The interaction between CO₂ laser and DSF was significant in root ($p < 0.001$). Individual tooth structure had no significant effect on lesion depth formation in root ($p = 0.401$).

4.2. Results for Fluoride Uptake study

The effect of laser and DSF on fluoride uptake in enamel or root was not statistically significant (all $p > 0.05$). The results were different from those of the PLM study and summarized in Table 10 for enamel samples and Table 11 for root samples.

4.2.1. Enamel

Table 10: Percentage increase in fluoride uptake compared to Enamel

| Enamel | Control | Fluoride | Laser | FL |
|--------------------|----------|----------|----------|----------|
| Mean | 3514 | 3573 | 3964 | 4290 |
| Standard Deviation | 1175.671 | 1867.359 | 997.6629 | 1988.796 |
| % of uptake | | 1.67 | 12 | 22 |
| Ranking | I | I | I | I |

4.2.2. Root

Table 11: Percentage increase in fluoride uptake

| Enamel | Control | Fluoride | Laser | FL |
|--------------------|----------|----------|----------|----------|
| Mean | 820 | 591.2 | 867 | 1165 |
| Standard Deviation | 800.0761 | 86.31164 | 734.4733 | 849.8286 |
| % uptake | | -27 | 5.73 | 42 |
| Ranking | I | I | I | I |

CHAPTER V: Discussion

5.1. Main Findings

5.1.1. Significant effect of combined CO₂ laser and DSF in inhibiting enamel and root demineralization

Compared to the control group, the reduction in lesion depth for laser treatment alone and DSF treatment alone was about 42% and 25% in enamel and 25% and 35% in root respectively. Moreover, the combination of DSF treatment with the CO₂ laser irradiation demonstrated almost 50% reduction in lesion depth compared to control (no treatment) in enamel and root. This was similar to the results of previous studies which showed that the combined argon laser and APF treatment had resulted in a 50-60% reduction in lesion depth compared with the controls in enamel and root caries initiation and progression (Flaitz et al., 1995; Hicks et al., 1995a; Hicks et al., 1995b; Hicks et al., 1997). Fluoride treatment before laser irradiation resulted in greater reduction in lesion depth compared to fluoride treatment after laser irradiation (Flaitz et al., 1995; Hicks et al., 1995a; Hicks et al., 1995b). In contrast, another study reported a significantly higher (60%) inhibition of Ca release from enamel treated with acidulated phosphate fluoride after laser irradiation (Tagomori and Morioka, 1989). As for the CO₂ laser treatment, its combination with fluorides has shown greater efficacy than laser and fluoride treatment alone in inhibiting enamel caries (Hsu et al., 2001; Hsu et al., 2004; Tepper et al., 2004). There was 91% inhibition of enamel demineralization after the combined CO₂ laser and fluoride treatment evaluated in a pH-cycling system (Featherstone et al., 1991). Treatment with low-intensity CO₂ laser irradiation in combination with 2% NaF gel resulted in a 98.3% reduction in enamel mineral loss (Hsu et al., 2001).

5.1.2. Combination of CO₂ laser and DSF – A promising method in caries prevention

In the studies with the combined fluoride and laser treatment for caries prevention, Argon and Nd: YAG were used. An important concern regarding laser irradiation of teeth which limits laser application in dental practice and which leads to many laser strategies being abandoned is the possibility of overheating the tooth pulp that could cause pulp-necrosis (Zuerlein et al., 1999). This is more important when laser irradiation is conducted on the root surface where thinner tooth structure may result in a higher temperature change in pulp (Powell et al., 1990).

Regarding CO₂ laser, it is highly absorbed by tooth surface, resulting in a temperature rise limited to a thin layer near the surface (Stern, 1968; Nelson et al., 1987; Ferreira et al., 1989, Zuerlein et al., 1999). Previous studies have shown that CO₂ laser irradiation will not cause thermal injury to the pulp if the total energy delivered in a few seconds is less than 4-8 J (Fisher and Frame, 1984; Powell et al., 1990). In the current study, the CO₂ laser irradiation with an

energy density of 1.14 J/cm² was adopted. Although the effects of the treatment of pulp were not investigated in this experiment, a few in vitro and in vivo studies have suggested the safety of the irradiation conditions (Fowler and Kuroda, 1986; Anic et al., 1992; Yu et al., 1993). The unfavorable high temperature alterations, such as melting and formation of new calcium phosphate phases, reported as a consequence of CO₂ laser at a high energy density (Kantola et al., 1973; Kuroda and Fowler, 1984), were probably not formed under the irradiation conditions used in this study (Hsu et al., 2000; Hsu et al., 2001; Zuerlein et al., 1999). Therefore, the combination of DSF and CO₂ laser treatment may offer a clinically promising safe therapy with a higher efficacy in caries protection than DSF and CO₂ laser treatment alone.

5.1.3. Will low cost laser therapy enhance DSF's cariostatic effect in the rural communities?

The clinical trials have demonstrated that DSF's lowest prevented fractions for caries arrest and prevention were 96.1% and 70.3%, respectively (Rosenblatt et al., 2009). In contrast, fluoride varnish's highest prevented fractions for caries arrest and prevention were 21.3% and 55.7%, respectively. Similarly, DSF's highest numbers needed to treat for caries arrest and caries prevention were 0.8 (95% CI = 0.5–1.0) and 0.9 (95% CI = 0.4–1.1), respectively (Rosenblatt et al., 2009). For fluoride varnish, the lowest numbers needed to treat for caries arrest and prevention were 3.7 (95% CI = 3.4–3.9) and 1.1 (95% CI = 0.7–1.4), respectively (Rosenblatt et al., 2009). These promising results suggest that DSF is more effective than fluoride varnish, and may be a valuable caries-preventive intervention. Moreover, DSF has been shown to be a safe, affordable, effective, efficient, and equitable caries-preventive agent to meet the criteria of both the WHO Millennium Goals and the US Institute of Medicine's criteria for 21st century medical care (Rosenblatt et al., 2009).

Due to the high cost of high power lasers, they are still not widely employed in private practice, particularly in developing countries. Low power red and near-infrared lasers may be an alternative approach, since reports in the literature have suggested that their use with or without topical fluoride, can lead to enhanced tooth resistance against dental caries (Slujaiev et al., 1990). This study used a 5-minute rinse after DSF application to simulate the intra-oral swallowing or rinsing effect and found that the cariostatic effect of DSF nearly doubled in enamel when combined with CO₂ laser. Even though low power lasers are less expensive than high power lasers, its use in low-income rural communities would still be prohibitive.

5.1.4. Current relevance of the combined treatment strategy in caries prevention

Although fluoride has been validated to be an effective agent in caries prevention and has been widely applied for several decades, it has not resulted in the elimination of dental caries (Winn et al., 1996). Conventional fluoride treatments appeared to be not as effective for root as for enamel, due to the greater critical pH of dentine/cementum and the quicker progression rate of root caries (Herksroter et al., 1991; ten Cate et al; 1995; ten Cate, 1997). The preventive measures for root caries may have to be more aggressive due to the highly porous structure of root. As a result, the combination of fluoride with other preventive methods, instead of fluoride alone was proposed to enhance its efficacy (Ogard et al., 1994, ten Cate, 1997; ten Cate. 1999). The data of this present study substantiates this approach.

5.1.5. Possible mechanisms of laser effect in enhancing fluoride uptake

The fluoride uptake after topical fluoride application has been validated as a process of diffusion with simultaneous chemical reaction (Duckworth and Braden, 1967). However, several potential mechanisms have been speculated in laser-enhanced fluoride uptake. The significant carbonate loss induced by laser irradiation has been revealed by previous studies (Oho and Morioka, 1990; Zuerlein et al., 1999). The high concentration of fluoride ions may replace the carbonate and be incorporated into the crystal structure, resulting in the formation of fluoridated hydroxyapatite or fluorapatite (Goodman and Kaufman, 1977). An experiment on synthetic hydroxyapatite has demonstrated the phase transformation to fluorapatite by laser above 38 J/cm² (Meurman et al., 1997). Using low-energy laser, fluoridated hydroxyapatite has been formed as well (Hsu et al., 2004).

5.1.6. Potential problems in evaluating F-uptake in this study

5.1.6.1. Sample Preparation

The potential factor which may affect the results in fluoride uptake is the size of the samples. Some of the samples were shaped after the fluoride and laser treatment to fit into the loading post

during the fluoride measurement. Since the four windows on the same tooth were assigned into four groups by an independent laboratory assistant, selection bias was minimized in this study.

5.1.6.2. Fluoride measurement using nuclear microscopy technique

In most of the fluoride uptake studies, two traditional methods, namely the acid-etch and microdrill techniques, were used for the assessment of fluoride uptake in teeth (Sakkab et al., 1984; Grobler and Kotze, 1990; Duckworth and Gilbert, 1992; Dunipace et al., 1997; Soyman et al., 1997). With these two methods, accuracy cannot be guaranteed during the processes of etching and fluoride measurement (Duckworth and Gilbert, 1992), and may result in considerable over- or under-estimation of the amount of fluoride (Sieck et al., 1990). The PIGE technique used in this study gives positive and specific identification of fluorine (Lenglet et al., 1988). But the major drawbacks of this technique were poor intrinsic resolution and long analysis times (12-16 hour per scan). The samples were cut after the fluoride and laser treatment. There may be contamination of the treated surface while cutting and rinsing of samples after the fluoride/laser treatment. Other potential problems may include the inherent heterogeneous distribution of fluoride in the control group and the potentially erroneous sampling of treatment sites for F-counts. These issues might possibly contribute to the inconclusive fluoride uptake data.

5.2. Limitations, Future directions and Conclusion

5.2.1. Limitations

We would like to highlight some limitations in this study.

The duration and frequency of exposure of tooth samples to the demineralizing and remineralizing solution for 3 days is not exactly the case in the oral environment.

The mechanism of action by which the CO₂ laser and DSF acted synergistically to reduce the lesion depth has not been fully investigated in this study.

The results of this study provide in-vitro data only. Other variables, such as the role of biofilm, bacteria, and saliva, have not been taken into account.

5.2.2. Future directions

In view of the above-mentioned limitations of this study, our future directions will be

1. To develop a realistic exposure time and frequency for the combined CO₂ laser and DSF treatment so that it can be implemented at the chair-side. This will be accomplished by performing time-series in-vitro experiments similar to this study, followed by choosing the shortest and most effective time-point at which the combined DSF + CO₂ laser treatment may maximize its cariostatic effect.
2. To characterize the crystallographic alterations in enamel and root after the combined fluoride and laser treatment. This can be accomplished using electron and micro X-ray diffraction, coupled with transmission electron microscopy studies.
3. To examine the possibility of using a dynamic model which could simulate the environment of oral cavity including oral biofilm in order to study the cariostatic effect of DSF+CO₂ lasers.

5.2.3. Conclusion

The CO₂ laser when combined with DSF will potentiate the cariostatic effect of DSF. Further in-vitro, in-vivo and epidemiological studies are necessary to fully understand the combined effects of this CO₂ laser and DSF application in the clinical setting.

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