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นายอะวาลูดิน วิบาว่า

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาชีววิทยาช่องปาก
คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2559
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EFFECT OF   LOW LEVEL LASER STIMULATION ON SALIVARY GLAND FUN
CTION IN DIABETIC PATIENTS WITH HYPOSALIVATION

Mr. Awaludin Wibawa
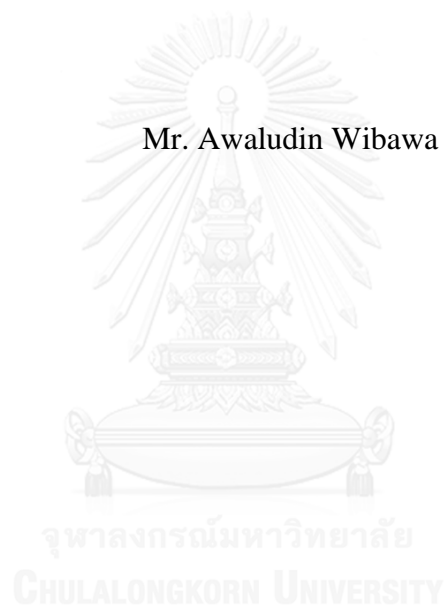
A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Oral Biology
Faculty of Dentistry
Chulalongkorn University
Academic Year 2016

Thesis Title	EFFECT OF LOW LEVEL LASER STIMULATION ON SALIVARY GLAND FUNCTION IN DIABETIC PATIENTS WITH HYPOSALIVATION
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อะ ว า ลู ดิ น   วิ บ า ว่ า   :
ผลของการใช้เลเซอร์พลังงานต่ำกระตุ้นการทำงานของต่อมน้ำลายในผู้ป่วยโรคเบาหวานที่มีภาวะน้ำลายน้อย
(EFFECT OF   LOW LEVEL LASER STIMULATION ON SALIVARY
GLAND   FUNCTION   IN   DIABETIC   PATIENTS   WITH
HYPOSALIVATION) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.   ทพ.   ดร.   จีรัสย์   สุจริตกุล,
อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ประทานพร อารีราชการัณย์, 85 หน้า.

น้ำลายเป็นสารที่ทำหน้าที่หลายอย่างและส่วนที่มีความสำคัญคือการทำหน้าที่ในการหล่อลื่นและในการป้องกันเชื้อโรค ดังนั้นภาวะน้ำลายน้อยกว่าปกติจึงเป็นสาเหตุของอาการผิดปกติที่ตามมาในช่องปาก ภาวะดังกล่าวพบมากในผู้ป่วยโรคเบาหวาน จากรายงานพบว่าความเข้มข้นของมิวซินไฟว์บี   (mucin   5B) ซึ่งมีความสำคัญในการให้คุณสมบัติหล่อลื่นมีค่าน้อยลงในผู้ป่วยเบาหวาน และความเข้มข้นของฮิสแตตินไฟว์ (histatin 5) ซึ่งมีคุณสมบัติในการต้านเชื้อรามีค่าน้อยลงเช่นกัน   ทำให้ผู้ป่วยเบาหวานติดเชื้อในช่องปากได้ง่ายขึ้น มีการศึกษาและรายงานว่าการใช้แสงเลเซอร์ความเข้มต่ำ (low lever laser treatment, LLT) สามารถกระตุ้นการทำงานของต่อมน้ำลาย   และมีผลทำให้อัตราการไหลของน้ำลายเพิ่มขึ้น ดังนั้นการศึกษานี้ต้องการดูผลที่เกิดจากการใช้แสงเลเซอร์ความเข้มต่ำกระตุ้นต่อมน้ำลายในผู้ป่วยเบาหวานที่มีภาวะปากแห้ง การประเมินผลภายหลังการกระตุ้นจะใช้อัตราการไหลของน้ำลาย (ในภาวะที่ไม่ถูกกระตุ้น) ความเข้มข้นของของมิวซินไฟว์บี ฮิสแตตินไฟว์   และการใช้แบบสอบถามความรู้สึกของผู้ป่วยภายหลังการกระตุ้นควบคู่กัน ในการศึกษาจะใช้อาสาสมัครจำนวนสิบสองรายที่เป็นผู้ป่วยที่ได้รับการวินิจฉัยว่าเป็นโรคเบาหวาน และมีภาวะปากแห้งที่เข้ามารับการรักษาที่โรงพยาบาลกรุงเทพ จะได้รับการกระตุ้นต่อมน้ำลายที่สำคัญ (major salivary gland) ด้วยแสงเลเซอร์เป็นเวลา 40 วินาที ต่อพื้นที่ผิวตารางเซนติเมตรของต่อมน้ำลาย โดยฉายแสงจำนวน 6 ครั้งติดต่อกันภายในเวลาสองสัปดาห์การประเมินระดับของมิวซินไฟว์บี มิวซินเซเวน (mucin 7) ฮิสแตตินไฟว์ในน้ำลายจะทำก่อนการกระตุ้นและหลังสิ้นสุดการกระตุ้นแล้ว 6 ครั้ง และในสัปดาห์ที่ 6

ผลการศึกษาพบว่าอัตราการไหลของน้ำลายและระดับมิวซินไฟว์บี เพิ่มขึ้นมีค่านัยสำคัญทางสถิติเมื่อสัปดาห์ที่   6   ภายหลังการกระตุ้น ในขณะที่ความเข้มข้นของฮิสแตตินไฟว์กลับมีค่าลดลงอย่างมีนัยสำคัญ ผลวิเคราะห์แบบทดสอบผู้ป่วยเกี่ยวกับภาวะปากแห้งสอดคล้องกับผลการมิวซินไฟว์บีว่าผู้ป่วยรู้สึกดีขึ้นใน   6   สัปดาห์ ผลการศึกษาชี้ให้เห็นว่าการใช้แสงเลเซอร์พลังงานต่ำมีผลทำให้อัตราการไหลของน้ำลายและระดับมิวซินไฟว์บีเพิ่มขึ้นในผู้ป่วยเบาหวานที่มีภาวะปากแห้ง

สาขาวิชา ชีววิทยาช่องปาก	ลายมือชื่อนิสิต ....
ปีการศึกษา 2559	ลายมือชื่อ อ.ที่ปรึกษาหลัก ....
	ลายมือชื่อ อ.ที่ปรึกษาร่วม ....

5775836832 : MAJOR ORAL BIOLOGY

KEYWORDS: LASER THERAPY, HYPOSALIVATION, SALIVARY PROTEIN, DIABETES, DRY MOUTH

AWALUDIN WIBAWA: EFFECT OF LOW LEVEL LASER STIMULATION ON SALIVARY GLAND FUNCTION IN DIABETIC PATIENTS WITH HYPOSALIVATION. ADVISOR: ASST. PROF. JEERUS SUCHARITAKUL, D.D.S., Ph.D., CO-ADVISOR: ASST. PROF. PRATANPORN ARIRACHAKARAN, D.D.S., Ph.D., 85 pp.

Saliva plays several functions and the most important roles among of those are the lubrication and the protection by defensive proteins. Salivary gland hypofunction showed a high prevalence in patient with diabetes. Previous studies indicated that protein mucin 5B concentration, which is important to lubrication, tended to decrease in hyposalivation patients and a decrease in histatin 5 was found in disease with fungal infection. The several previous studies indicated that low level laser therapy (LLLT) can stimulate salivary gland function with an increase in salivary flow rate in systemic disease patients with dry mouth complication. Therefore, LLLT may be applied as the treatment choice for patients with dry mouth symptom. This study aimed to investigate the effect of low-level laser stimulation on salivary gland function in diabetic patients with hyposalivation. The assessment of salivary flow rates, mucin 5B (MUC5B), mucin 7 (MUC7), histatin 5 concentrations, and questionnaires were performed. A total of twelve diabetic patients under criteria set in this study at Bangkok Hospital were participated on a voluntary basis. A low power laser was used to stimulate major salivary glands with an irradiation time of 40 s on 6 occasions (3 times in 2 consecutive weeks). Questionnaire related to dry mouth symptoms were given. Salivary flow rates and questionnaire were assessed as well as MUC7, MUC5B, and histatin 5 protein concentration in saliva at the 1st visit, 6th visit, and 6th week follow-up visit. The unstimulated salivary flow rate and MUC5B concentration at the 6th week follow-up visit were significantly increased compare with at the 1st visit. By contrast, the concentration of histatin 5 exhibited a significant decrease at the 6th week follow-up visit compare with at the 1st visit . The mean dry mouth score revealed a significant decrease regarding to dry mouth symptoms at the 6th visit and 6th week follow-up visit compared with at the 1st visit. The correlation between overall dry mouth score and flow rate showed the strongest positive correlation at the 6th visit. There were no significant differences found on stimulated salivary flow rate and MUC7 concentration. Our results indicate a beneficial effect of LLLT on diabetic patients in increasing salivary flow rate and maintaining oral lubrication.

Field of Study: Oral Biology	Student's Signature ....
Academic Year: 2016	Advisor's Signature ....
	Co-Advisor's Signature ....

ACKNOWLEDGEMENTS

CONTENTS

ABTRACT

Saliva plays several functions and the most important roles among of those are the lubrication and the protection by defensive proteins. Salivary gland hypofunction is a high prevalence in patient with diabetes mellitus. Previous studies indicated that protein mucin 5B concentration, which is important to lubrication tended to decrease in hyposalivation patients and a decrease in histatin 5 is involved in fungal infection-susceptibility. The several previous studies in xerostomia patients indicated that low level laser therapy (LLLT) can stimulate salivary gland function with an increase in salivary flow rate in systemic disease patients with dry mouth complication. Therefore, LLLT may be applied as the treatment choice for relief the dry mouth symptom. This study aimed to investigate the effect of low-level laser stimulation on salivary gland function in diabetic patients with hyposalivation. The assessment of LLT effect on salivary gland functions was performed using salivary flow rate, mucin 5B and histatin 5 concentrations, and questionnaires. The twelve diabetic patients under criteria set in this study at Bangkok Hospital were participated on a voluntary basis. A low power laser was used to stimulate major salivary glands with an irradiation time of 40 s/cm^2 on 6 occasions (3 times in 2 consecutive weeks). Questionnaire related to dry mouth symptoms were given. Salivary flow rates and questionnaire were assessed as well as MUC7, MUC5B, and histatin 5 protein concentration in saliva at the 1st visit, 6th visit, and 6th week follow-up visit. The unstimulated salivary flow rate and MUC5B concentration at the 6th week follow-up visit were significantly increase. By contrast, the concentration of histatin 5 exhibited a significant decrease at the 6th week follow-up visit. The mean dry mouth score revealed a significant decrease regarding to dry mouth symptoms at the 6th visit and 6th week follow-up visit compared to at the 1st visit. The correlation between overall dry mouth score and flow rate showed the strongest positive correlation at the 6th visit. There were no significant differences found on stimulated salivary flow rate and MUC7 concentration. Our results indicate a

beneficial effect of LLLT to salivary flow rate and MUC5B protein secretion on diabetic patients with hyposalivation.

Chapter 1: Introduction

Saliva is made up of around 99% water, rich of electrolytes such as sodium, potassium, magnesium, phosphate, chloride, bicarbonate, and other antimicrobial factors that plays an important role to maintain the oral health [1]. It has been widely known that saliva plays several important functions, such as for taste, buffer system, digestion, the lubrication and the protection [2].

Several protective proteins have been found in the saliva. Mucins and histatins are the two samples of the protective proteins. In addition, mucins also function as lubricants in the oral cavity [3]. Mucins can be distinguished in two forms based on the molecular weight: high molecular weight, gel forming MUC5B (MW > 1,000 kDa) and lower molecular weight MUC7 (MW 120-150 kDa) [4, 5]. Histatin is another antimicrobial peptides in saliva, especially histatin 5 which is effective against Candida albicans [6].

Hyposalivation is a term for lacking of salivary amount. Several causes have been indicated and the drugs consumption is considered as the most common cause [7, 8]. The existence of systemic diseases, such as diabetes mellitus, Sjögren's syndrome, hypertension, and malnutrition also related to hyposalivation [9, 10]. Numerous studies

have been revealed the relationship between diabetes mellitus and hyposalivation. Salivary dysfunction has been reported in patients with type 1 and 2 diabetes (11, 12). The study showed that patients with both types of diabetes mellitus had lower salivary flow rate compared to the control group.

Low Level Laser Therapy (LLLT) or photobiomodulation utilizes low to mid power lasers with power output in the range of 50-500 mW. The light is in the region of visible (red) or near infrared (NIR) (630-980 nm). The general principle of LLLT is centered on the low dosage that is emitted into tissues where resulting in the primary, secondary, and tertiary effect (13). Studies indicated successful LLLT on decreasing inflammation via TNF-alpha reduction (14), on enhancing regenerative processes of peripheral nerve after trauma (15), and on accelerating collateral circulation and microcirculation on injured area (16). Several studies demonstrated the convincing result of LLLT to the salivary flow rates (17-19).

To our knowledge, the effect of LLLT to the diabetic patients with hyposalivation has not been reported yet. In this recent study, the objective was to investigate the effect of LLLT on salivary gland function in diabetic patients with hyposalivation.

1.1 Research question

Does LLLT stimulate salivary glands function in diabetic patients with hyposalivation?

1.2 Objectives and hypothesis

1.2.1 Objective 1

To investigate the effect of LLLT on salivary flow rate (unstimulated and stimulated) in diabetic patients with hyposalivation

a. Hypothesis

LLLT significantly increases salivary flow rate (unstimulated and stimulated) in diabetic patients with hyposalivation

b. Experimental design

Clinical-experimental study

1.2.2 Objective 2

To investigate the effect of LLLT on mucin 7, mucin 5B, and histatin 5 concentration in diabetic patients with hyposalivation

a. Hypothesis

LLLT significantly increases mucin 7, mucin 5B, and histatin 5 concentration in diabetic patients with hyposalivation

b. Experimental design

Clinical-experimental study

1.2.3 Objective 3

To investigate the effect of LLLT on subjective dry mouth symptoms in diabetic patients with hyposalivation

a. *Hypothesis*

LLLT significantly decreases subjective dry mouth symptoms in diabetic patients with hyposalivation

b. *Experimental design*

Clinical-experimental study

1.3 Expected benefit

The knowledge obtained from this study will potentially assist diabetic patients in increasing salivary flow rate through LLLT approach

1.4 Research design

Clinical-Experimental study

1.5 Conceptual framework
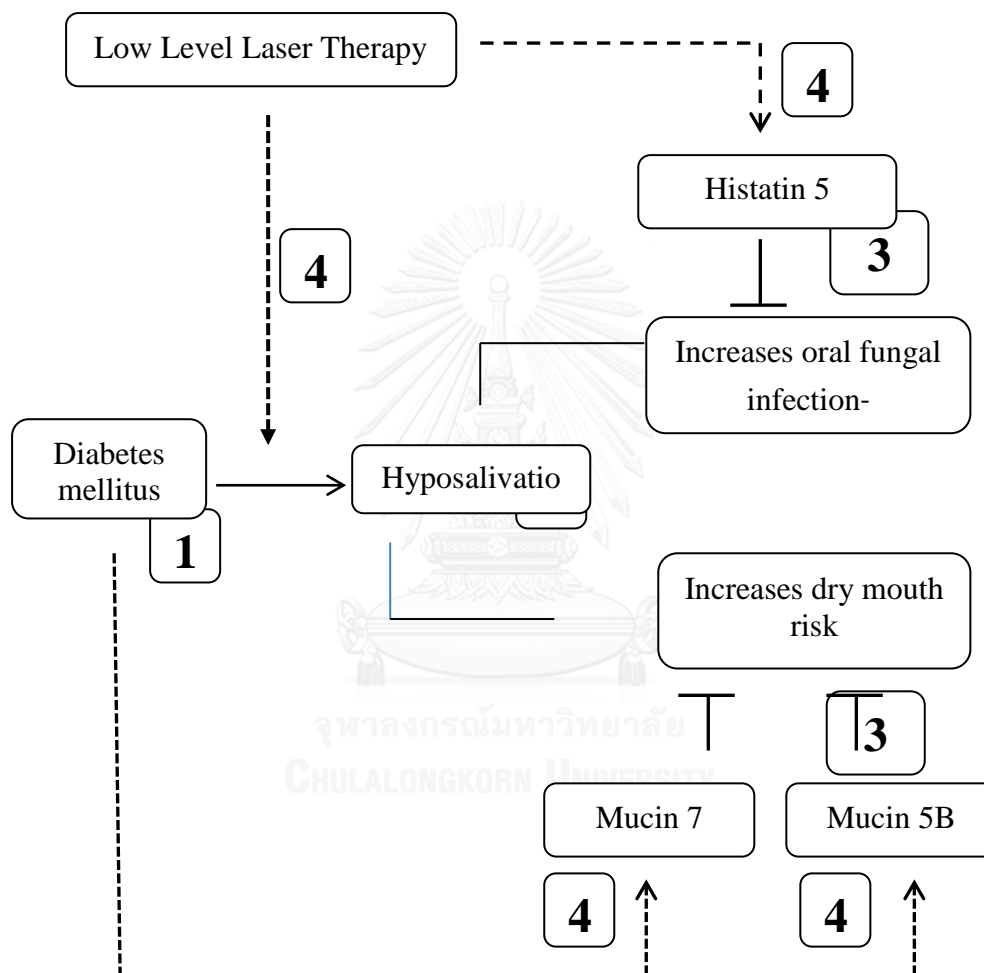
Note :

1. Diabetes mellitus increases the risk of hyposalivation
2. Hyposalivation increases dry mouth risk and oral fungal infection-susceptibility
3. Mucins (MUC7 and MUC5B) and histatin 5 protect the oral environment from dry mouth and oral fungal infection-susceptibility, respectively
4. We want to investigate the effect of LLLT on salivary flow rate, MUC7, MUC5B, and histatin 5 concentration level in diabetic patients with hyposalivation

Chapter 2: Literature review

2.1 Saliva

Humans have two exocrine groups producing salivary glands: major and minor salivary glands. Major salivary glands comprise of two parotid, two submandibular, and two sublingual glands (Fig. 2.1 and Fig.2.2) The major salivary glands are composed of acini which secrete the saliva. Before the saliva is secreted into the oral environment through the excretory ducts, the ducts will bring in the saliva and execute the modification.

The three main excretory ducts in the major salivary glands are the Stensen's, the Wharton's, and the Bartholin. In the parotid gland, the main excretory duct is the Stensen's duct, it enters the oral cavity in the buccal mucosa area adjacent to second maxillary molar, and penetrate through the buccinator muscle. Parotid gland is located in the preauricular area and along the posterior part of the mandible (Figure 2.1) [20]. In the submandibular gland, the main secretory duct is the Wharton's duct. The Wharton's duct comes into oral cavity under the tongue by the lingual frenum at a structure called the sublingual carancula, while the sublingual gland has small ducts called ducts of Rivinus and the Barthollin duct. The connection between the Barthollin duct and Wharton's duct is at the sublingual carancula [21, 22]. Submandibular gland is located in the posterior part of the submandibular triangle, while sublingual gland lies in a submucosal plane within the anterior floor of the mouth (Figure 2.2) [20, 23]

The types of saliva are different between one gland to other glands. The parotid glands are comprised of serous acini and therefore they secrete more watery saliva. The submandibular and sublingual glands are comprised of both mucous and serous acini, so they secrete mixed watery and mucous type of saliva secretion. The submandibular glands are dominated with serous acinar cells, whereas the sublingual glands are comprised of mucous acinar cells as the majority. The major salivary glands normally produce over 90% of the total saliva secretion amount. The salivary amount could be more with the addition from minor salivary glands that are located around the palate and are distributed all over in the oral mucosa (24). Table 2.1 describes the differences between major salivary glands.
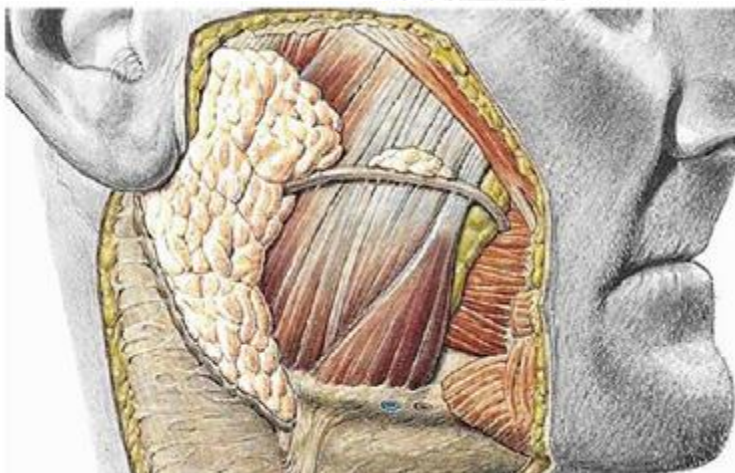
Figure 2.1 Anatomy of parotid gland (20)
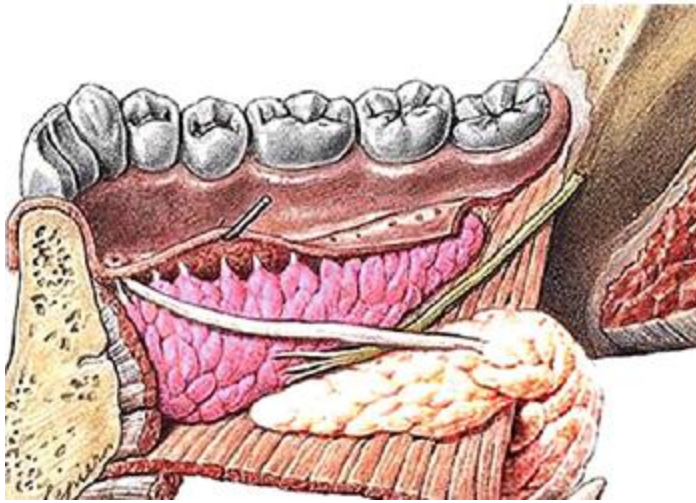
Figure 2.2 Anatomy of sublingual and submandibular gland (20)

Table 2.1 The difference between major salivary glands (25)

	Parotid	Submandibular	Sublingual
Shape	Largest salivary gland. Irregular, wedged shape and unilobular	Submaxillary gland. Irregular and walnut-like shaped	Smallest of the major salivary glands
Weight	14-28 grams in weight	10-15 grams in weight	3-4 grams
Ducts	Stenson duct or Parotid duct	The Wharton's duct	Ducts of Rivinus and a common duct, the

			Bartholin duct.
Acinar type	Mainly serous	Mixed serous and mucous	Mainly mucous

Salivary secretion process is occurred in parts of the salivary gland system (Fig.2.3). The system comprises of acinus, intercalated duct, striated duct, excretory duct, and myoepithelial cells. The acini first produce primary saliva which is isotonic. In intercalated duct, bicarbonate ion is secreted and chloride is absorbed. Reabsorption of sodium and secretion of potassium are took place in striated ducts later on. As for secondary saliva, which is hypotonic, is occurred in excretory duct subsequently [20]
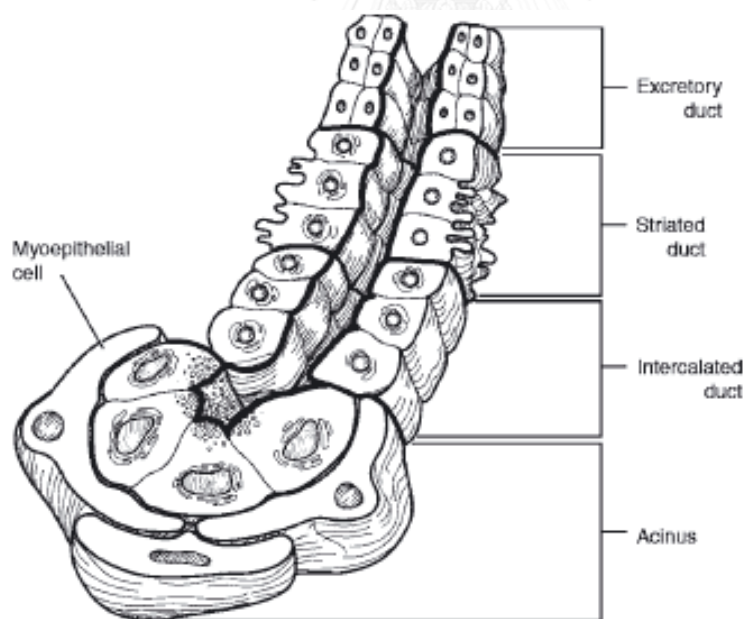
Figure 2.3 Secretory units of saliva [20]

The secretion of salivary glands are predominantly regulated by autonomic nervous system. Parasympathetic nervous system takes more control for salivary secretion rate. Parasympathetic stimulates acinar and ductal transport process and subsequently leads to myoepithelial cell contraction. Acetylcholine functions as parasympathetic neurotransmitter that acts on muscarinic receptors of the salivary glands. Calcium ions serve as second messenger involved in salivary secretion (20, 26).

Sympathetic effect results in biphasic vasoconstriction and vasodilatation of blood flow. Norepinephrine functions as sympathetic neurotransmitter that acts on alpha-adrenergic receptors. Cyclic AMP serves as a second messenger involved in secretion of various proteins (20, 26)

Cyclic AMP activation, as a secondary messenger, by beta-adrenergic receptor stimulation is involved in small volume fluid secretion and salivary protein secretion by exocytosis, while Ca^{2+} activation, as another secondary messenger, by muscarinic and alpha adrenergic receptors is involved with large volumes fluid secretion and protein secretion via vesicular secretion and exocytosis (27). In addition, both autonomic innervations also take part in protein synthesis. Non-adrenergic and noncholinergic effects are involved in protein synthesis which is induced by parasympathetic nerves, while alpha and beta adrenergic receptors are involved in protein synthesis induced by sympathetic nerves (28, 29)

Saliva is made up of approximately 99% water, rich of electrolytes such as sodium, potassium, magnesium, phosphate, chloride, bicarbonate, and other antimicrobial factors that plays an important role to maintain our oral health system

(1). It has been widely known that saliva plays several important functions, such as for taste, buffer system, digestion, the lubrication and the protection as well (2). Water in saliva functions as cleanser and to prepare the food for swallowing.

Saliva secretion type is divided into unstimulated salivary flow rate (USFR) and stimulated salivary flow rate (SSFR). USFR is the basal secretion of saliva which is the mixture of secretions flowing into oral cavity in the absence of exogenous stimuli. SSFR is a saliva secretion as a result of masticatory, gustatory, or other form of stimulation. In stimulated salivary flow rate measurement; wax, chewing gum, and citric acid are agents that are commonly applied to stimulate the saliva secretion. Table 2.2 is provided to describe the main differences between USFR and SSFR

Table 2.2 The difference between USFR and SSFR (30, 31)

	USFR	SSFR
Typical value (mL/min)	0.25-0.40	1-2
Main source (gland)	Submandibular	Parotid
Protein produced	Mucin-rich	Almost mucin-free
Main function	Basal production	Assist in deglutition

2.2 Mucins and histatins

Several defense proteins have been found in the saliva. Mucins and histatins are examples of innate defense system in the saliva. Mucins (Fig.2.4) play an important function in the salivary defense system. The mucins are produced mostly by submandibular glands and other minor glands such as palatal and labial glands (32, 33). The submandibular glands secrete the largest amount of the mucin (34). The salivary mucins have the thickness around 10-22 μm covering all over the oral surfaces (35, 36). Salivary mucins trap and agglutinate the oral microorganisms due to their high affinity (35).
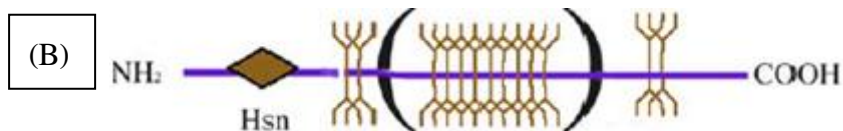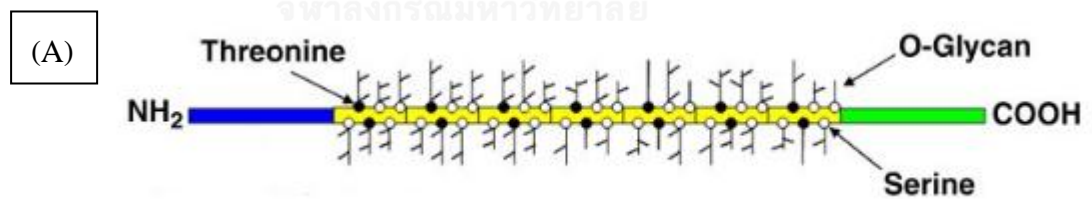
Figure 2.4 Illustrative salivary mucins forms, (A) high molecular weight MUC5B with long oligosaccharide chain. *O*-glycans are attached to threonine and serine site (37) and (B) low molecular weight MUC7 with shorter oligosaccahride chain, with histatin-like domain (Hsn) near its *N*-terminus. (38)

Salivary mucins protect the tissues from the outer environment and play critical role in the oral lubrication (39). The mucins have two forms based on the molecular weight : high molecular weight, gel forming MUC5B (MW > 1,000 kDa) and low molecular weight MUC7 (MW 120-150 kDa) (40-42).

Several studies proposed that MUC5B has the characteristic to protect the tissues against dryness better than MUC7 (43, 44). Moreover, it has been noted that MUC5B can be found in many variations of glylosylated forms (32, 45, 46).

MUC5B is mostly expressed in submandibular, sublingual, and labial glands and other sites, such as submucosal tracheobronchial glands, endocervix, respiratory tracts, and urogenital tracts. It has stretched thread-like structure containing heavily glycosylated domains and huge amount of carbohydrate content which is able to form hydrophilic viscoelastic gels functioning as mechanical protector and prevention to the microorganism infiltration. Moreover, MUC5B also has the ability to protect enamel from acidic exposure.

MUC5B has larger dimension and longer structure than MUC7 and in combination with a hydrophilic carbohydrate properties, MUC5B "traps" water and give "wet sensation" in oral mucosa. However, MUC5B binds to relatively few oral

microorganisms, for example *Hemophilus parainfluenzae* and *Helicobacter pylori* (47, 48). This can be explained due to extreme heterogeneity of its carbohydrate chain that leads to low surface density of binding site. This makes multivalent binding of bacteria sometimes physically difficult (49). Oral microorganisms, such as *H.pylori*, has been identified to attach to sulfated glycans present in MUC5B (50)

MUC7 is decorated with short oligosaccharide side chain. Different from MUC5B, MUC7 binds and aggregates a wide variety of oral microorganisms including *Streptococcus sanguinis, S.mitis, and Escherichia coli* (51-53). MUC7 is rich of sialic acid containing oligosaccharide side chain, the site that oral microorganisms bind to (54).

Histatins are antimicrobial peptides that also important in oral health system. The histatins are secreted from parotid and submandibular salivary glands (55). A study revealed that histatin 5 is responsible for both fungicidal and fungistatic activities against *Candida albicans* (6). It has demonstrated that they produce positive effects in patients with AIDS and denture stomatitis (56). In addition to candida species, histatins are also highly potent against *Cryptococcus neoformans* and *Saccharomyces cerevisiae* (57, 58).
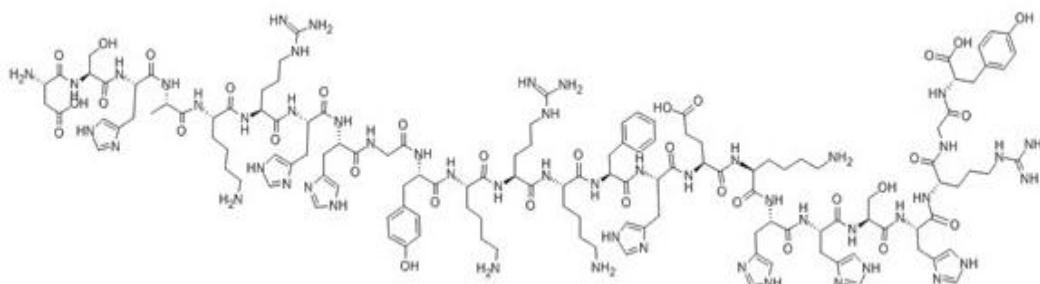
Figure 2.5 Histatin 5 chemical structure. The *C*-terminal sequence, a peptide chain length of 14 residues, and helical conformation are important for anticandida activity (59)

Histatin 5 is the most widely known type of histatin (60, 61). Histatin 5 (Fig.2.5) has "functional domain" sited around region of 11-24 residues at the C terminal that postulated to be the center of fungicidal activity (62). It needs at least 12 residues from C terminus to be effective for antifungal activity, with longer length means produce better activity (63).
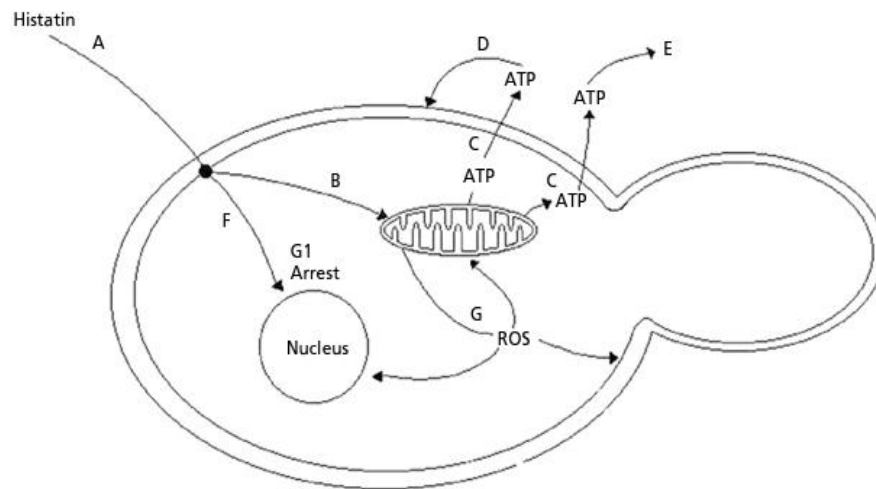
Figure 2.6 Histatin mechanisms against *C.albicans* (64)

Histatins give impact on mitochondria after it pass through the plasma membrane of fungi (65). They trigger cell death via ATP release (66), produce reactive oxygen species (ROS) that cause damage on organelles (67), and interfere the cell cycle (68). First theory, histatin 5 triggers the ATP release into the cytoplasm once it attaches to mitochondria. When the ATP is released, it induces cell death to the fungi. The second theory, histatin 5 produces ROS resulting in fatal DNA damage. ROS disrupt the cell organelle structures leading to cell death. The third theory, histatin 5 disturbs cell cycle in G1 phase. This is related to the perturbation of regulation of the cell and therefore led to cell death (Fig.2.6).

The major differences between MUC7, MUC5B, and histatin 5 salivary protein were described in Table 2.3

Table 2.3 The differences between MUC7, MUC5B, and histatin 5 salivary

protein

	MUC7	MUC5B	Histatin 5
Structure	Short oligosaccharides glycoprotein	Long oligosaccharides glycoprotein	Positively charged peptides
Typical value in saliva (ng/mL)	0.06-0.32	0.05-0.78	2,000-14,000
Molecular weight (kDa)	120-150	> 1,000	> 3
Main source	Mucous and serous cells of SMG, SLG, and minor glands	Exclusively from mucous cells of SMG, SLG, and minor glands	Serous cells of parotid and submandibular glands
Function	Lubrication, more interaction with microorganisms	Lubrication, less interaction with microorganisms	Broad antimicrobial activity against bacteria and yeasts

2.3 Salivary glands dysfunction

Hyposalivation is a term for lacking of salivary amount, while xerostomia is more like subjective feelings. The normal unstimulated salivary flow rate in human is 0.25- 0.40 ml/min (31). Several causes have been indicated and the drugs consumption is considered as the most common cause (7). It has been found the groups of drugs induce hyposalivation such as : 1) the drugs that directly damage the salivary gland, 2) drugs with anticholinergic activity, 3) drugs that affect central nervous system, 4) drugs acting on sympathetic system, 5) drugs that deplete body fluid, 6) and anti-hyperglycemic drugs (7, 31, 69). The existence of some systemic diseases, such as diabetes mellitus, Sjögren's syndrome, hypertension, and malnutrition, are also related to hyposalivation (9, 10). The exposure of x-ray in radiotherapy is one cause of hyposalivation, including other physiological alterations, such as age and hormones (10). Some psychogenic causes also leads to hyposalivation, such as anxiety, depression, and stress (70).

The hyposalivation management depends on different causes. For example, drugs-induced xerostomia may be changed by other alternative medications, and systemic condition-induced xerostomia, such as diabetes mellitus, has to be treated first (7). The management depends on the severity of the salivary gland damage that includes etiologic, stimulative, symptomatic, or palliative approach. Nowadays, the well-known therapies are saliva stimulants (sialogogues) and saliva substitutes (71)

Some of the treatments are able to generate stimulation on the salivary glands, such as laser treatment, chewing and acidic stimulation, acupuncture, and electrical stimulation. Low level laser theraphy (LLLT) is believed as a stimulant for the cell regeneration effect to the gland (18). Other systemic stimulants such as cevimeline HCl, pilocarpine, and bromhexine are provided in the large-scale in the clinic, despite their different final results (72). In some particular conditions, such as the severe diabetic condition and irreversible damage of salivary glands because of radiotherapy, the only choice left is palliative treatment (71).

The technology has provided alternatives that imitate the saliva texture, such as dry mouth gel (GC, Japan) and oral balance gel (Biotene,USA). However, these products have important flaw which cannot mimic the genuine rheologic properties of the saliva; therefore, saliva production from salivary glands stimulation is still the best choice to overcome the problem. The patients are also being informed about how to maintain good oral health. Education for the patients is the major key to change the patient paradigm. The proper hydration and stay away from high-sugar diet and low-acidic liquids/food are also being advised to the patients. They also should be kept away from caffeine-containing drinks and tea. Other bad habits such as alcohol consumption and smoking should be stopped to prevent further oral cavity drought. Mouth rinses with bactericidal effect and alcohol-free contain, such as chlorhexidine, can be instructed as home care treatment as well (73). Several etiology of xerostomia and its management are shown in table 2.4.

Table 2.4 Etiology of hyposalivation/xerostomia

A. ETIOLOGY

1. Physiological alterations of salivary gland function (age, sex, hormonal change in women, time of the day) (10)

2. Xerogenic drugs (7):

   - drugs that directly damage salivary glands (e.g cytotoxic drugs),

   - drugs with anticholinergic activity (e.g atropine and omeprazole),

   - central acting psychoactive agents (e.g phenothiazine, benzodiazepine, opioids),

   - drugs acting on the sympathetic system (e.g ephredine, terazosin, propanolol),

   - drugs that deplete body fluid (diuretics)

3. Oral hypoglycemic drugs

4. Systemic disorder (Sjögren's syndrome, depression, malnutrition, diabetes mellitus, hypertension) (9, 10)

5. Head and neck radiotherapy (10)

6. Psychogenic causes (depression, anxiety, stress, fear) (70)

B. MANAGEMENT (74)

1. Patient education (75)

2. Identification and management of underlying causes (70)

3. Palliative management (water, sugarless chewing gum, lozenges, saliva substitute)

4. Salivary secretion stimulating treatment,

- systemic stimulation: saliva-stimulating medication (pilocarpine, cevimeline)

- local stimulation : non-medication salivary stimulation (chewing and acidic taste, LLLT, acupuncture, electrical stimulation)

5. Non-saliva stimulating medication

- Tooth bleaching agent: 10% carbamide peroxide, palifermine

6. Early diagnosis and treating of oral complications (e.g dental caries and candidiasis due to hyposalivation (70)

2.4 Diabetes mellitus and oral candidiasis

Diabetes mellitus (DM) is suffered by 9% of adults population worldwide (76, 77) or around 382 million people. It is a very common metabolic disease that if left untreated, it leads to many complication such as amputation, blindness, kidney failure and damage to the heart, nerves and blood vessels (77). DM also cause death of around 4.9 million people in 2014 and is predicted as the 7th leading cause of death in 2030 (78).

Besides many systemic complications, DM can also lead to various oral health problems, including periodontal disease, dental caries, burning mouth syndrome, impaired healing, various potentially malignant disorders, dysfunction of salivary flow and opportunistic fungal infections (79, 80).

DM can be classified into two types. DM type 1 is caused by the destruction of pancreatic beta cells. This is characterized by dependence on insulin due to lack of insulin production. DM type 2 is dominated the diabetic population by 90%. It is caused by insulin resistance and also known as non-insulin dependent (81). DM has effect on saliva despite the DM type (82, 83) with type 2 DM has the highest risk to cause xerostomia (84). According to WHO, DM is now stated as a pandemic with an increased prevalence over the past few decades and is predicted to triple in the next ten years (85). DM is associated with various inflammatory diseases and soft tissue pathologies in oral cavity (86, 87). Periodontal diseases, salivary and taste dysfunction, oral infection, poor oral wound healing, non-candidal oral soft tissue lesion, oral mucosal disease, neuro-sensory oral disorder, and dental caries and tooth loss have been reported as most common complications in diabetes mellitus patients (88)

Salivary dysfunction has been reported in patients with DM (11). A study showed that patients with type 1 DM had lower salivary flow rate when compared to the control subjects without DM. Diabetic patients who had neuropathy symptoms also linked to hyposalivation (12). Another study with type 2 DM patients also confirmed that salivary reduction is more prevalent in this group of patients (89).

Parotid gland in poorly controlled type 2 DM patients produced less stimulated salivary flow rate compared to well-controlled patients and patients without diabetes (83).

Neuropathy and microvascular abnormalities from diabetic condition is believed to be the cause of hyposalivation. These chronic complications lead to microcirculation disturbance that contribute to the reduction salivary flow (90). In addition, the use of metformin as antidiabetic drugs also related to the reduction of salivary flow rate (91).

Patients with DM are frequently reported with the existence of opportunistic infections caused by *Candida albicans*. Smoking, xerostomia, endocrine and metabolic diseases are some of the predisposing factors of this infection (92). Fungal infections are very common in diabetic patients for many years (93). Dentures, poor glycemic control, smoking, use of steroid and broad spectrum antibiotics increase the risk of this infection (94). Several types of oral candidiasis that commonly found are pseudomembranous candidiasis, chronic hyperplastic candidiasis, and angular cheilitis (Fig. 2.7, 2.8, and 2.9)

Figure 2.7 Pseudomembranous candidiasis

(courtesy of Dr. Steve Debbink, AIDS Resource Center of Wisconsin)
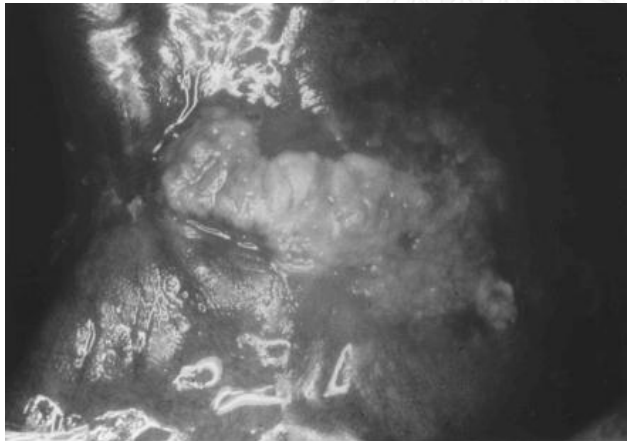
Figure 2.8 Chronic hyperplastic candidiasis

(96)

Figure 2.9 Angular cheilitis

(95)

2.5 Low level laser therapy

Laser light is defined as monochromatic, coherent, and polarized (97). This process results in an electron or molecule undergoing a stimulated quantum jump from a higher to a lower energy state, giving a laser beam more potency than other unmodified optical radiations.

Low Level Laser Therapy (LLLT) or photobiomodulation utilizes low to mid power with power output in the range of 50-500 mW. The light is in the region of red visible to near infrared (630-980 nm) (98). Moreover, LLLT produces lower or even no heating of tissue. A common reference is the "Guidelines for Skin Exposure to Laser Light" in the International Standard Manual (IEC-825) which considers an exposure below 200 mW as a standard exposure (99).

LLLT emission results in a photo biomodulation effect to its object of therapy. The historical starting point was occurred in 1967, a few years after the first laser was invented, when Endre Mester, a Hungarian scholar, was running an experiment. The experiment question was whether laser application can stimulate cancer in mice. The dorsal hair of the mice was shaved and then treated with a low-power ruby laser (694 nm in wave length). The treated group then was compared with an untreated group. The result was surprising, as the laser-treated group did not get cancer and their hair grew back more quickly than the untreated group (100). Starting from this positive result, a lot of studies were then conducted to reveal more about the unique usage of LLLT to many medical problems.

The general principle of LLLT is centered on the low dosage that is emitted into tissues where resulting in the primary, secondary, and tertiary effect (13). Primary cellular effects is generally related to the interaction of photons and the intracellular molecules that absorb them, the cytochromes. Cells contain porphyrins, polarization-sensitive molecules. They are located in mitochondria where they form part of the respiratory chain. When the cytochromes in mitochondria absorb light, they interact with it subsequently. The primary photoreceptor is cytchrome c oxidase (CcO) (13). In the secondary effect, after CcO absorbs the light, it increases mitochondrial membrane potential and leads to the enhancement of ATP production. The increase of Ca^{2+}, cAMP and the alteration of intracellular pH are also noticed (101). The mechanisms then continue to the tertiary effect. The changes of mitochondrial

membrane potential, ATP, Ca^{2+}, cAMP, and intracellular pH induce the activation of signaling molecules and changes in downstream cascades involved in gene transcription and consequently control cellular response such as proliferation and migration (101).

Numerous treatments have been performed using LLLT. Studies indicated successful LLLT on decreasing inflammation via Tumor Necrosis Factor-alpha reduction (14), on enhancing regenerative processes of peripheral nerve after trauma (15), and on accelerating collateral circulation and microcirculation on injured area (16).

Laser therapy devices utilize various resources or active medium. Old type of laser used helium neon (HeNe), ruby, argon, and krypton. Nowadays, gallium arsenide (GaAs) and gallium aluminium arsenide (GaAlAs) semiconductor laser diodes have become available with producing light in range wavelength of 820 - 940 nm (102) (Table 2.5).

Table 2.5 Low Energy Lasers in Clinical Use (102)

Laser	Wavelength
Helium Neon (HeNe)[a]	632.8 nm
Gallium aluminium arsenide (GaAlAs)[a]	820, 830 nm
Gallium arsenide (GaAs)[a]	940 nm

Neodymium-ytrim-aluminium garnet (Nd:YAG)[b]	1064 nm
Argon (Ar)[c]	488, 514 nm
Krypton (Kr)[c]	521, 530, 568, 647 nm
Ruby[c]	694 nm

[a] Commonly used

[b] Less commonly used, but still reported

[c] Used in the past

Different papers have discussed about different parameters. However, Basford summarized the most common LLL characteristics (Table 2.6). The incident dose of LLLT can be measured as either power density = output power in watts/area of irradiation, or energy density = output power x irradiation time/area of irradiation. Energy density that usually used in therapeutic reason is ranged from 0.1 J/cm^2 to 4 J/cm^2 (103).

Table 2.6 Low Energy Laser Treatment Parameters (102)

Wavelength	Typically 632.8, 820, 830, or 940 nm
Powers	Average power 10-90 mW (rarely a few hundred)
Dosage/site	0.1 - 4 J/cm^2

Treatment Administration	On daily or every other day basis, usually 3-4 times a week

~~Several~~ A few studies have demonstrated the positive effects of the LLLT related to salivary flow rate. Juras, et al.2010 investigated the effects of low power semiconductor diode laser (Table 2.7) with frequency 5.2 Hz on salivation of patients suffering from mouth dryness (MD). The study involved 17 patients with mouth dryness. All participant were non-smokers and without clinical signs and symptoms of oral diseases other than MD. None of them were taking any medications that related to salivary secretion. MD was diagnosed when subjects produced less than 0.1 ml/min period. The laser diode approximately 5 mm far from surface over glands anatomic site. The whole saliva quantities were measured just before the 1st, after the 10th and thirty days following the last (10th) treatment. The result revealed the significant improvement of quantity of saliva and sIgA.

Table 2.7 LLLT on different studies

	Juras et al	Loncar et al	Pazelj-Ribaric et al
Energy Density (J/cm^2)	1.8	29.5	3
Output Power	30	6	30

(mW)			
Occasions	10 sessions in consecutive 2 weeks	10 consecutive days	5 days a week for 4 consecutive weeks
Duration	5 min for parotid gland, 2 min for submandibular gland, and 1 min for sublingual gland per treatment	2 min	10 min
Wavelength (nm)	-	904	685

Loncar, et al.2010 also investigated the effect of semiconductor Ga-As laser (Table 2.7) related to xerostomia. Thirty four (34) patients with xerostomia (mean age 56 yo) were included in this study with additional 16 people (mean age 54 yo) as control group. Nominal operating distance was 5 mm and delivered energy per exposure was 0.72 J with treatment area 2.44 mm^2. The result showed the significant increase of total amount of the saliva in LLLT group when compared to control group.

Pazelj-Ribaric, et al.2010 also investigated the effect low level GaAlAs diode laser (Table 2.7) on salivary flow rate. Twenty (20) subjects were involved in this study. The saliva collection procedure was repeated after the final treatment, after four

weeks. The treatment areas, each one being a 1 cm^2. The result showed that salivary flow rate after LLLT were significantly greater than before LLLT.

However, the parameters vary in different papers. The "upper limit" of the LLL dosage is still debatable. There is no strong evidence yet to decide what the toxic dosage of LLL is which can harm the cells. However, few studies suggested the safety margin of the energy density yet still far from conclusive. Plavnik et al. revealed that the energy density above 11 J/cm^2 is toxic to submandibular glands of guinea pigs (104), while another study performed by Sharma et al. showed that the energy density at 30 J/cm^2 is toxic to human neurons (105)

It seems promising that LLLT improves the quality and the quantity of the saliva based on these studies as long as we utilize the proper dosage. To our knowledge, the effect of LLLT to the diabetic patients with hyposalivation has not been reported yet. In this recent study, the objective was to investigate the effect of LLLT on salivary gland function in diabetic patients with hyposalivation. LLLT treatment is potential for a safe way to enhance the quality and quality of saliva.

Chapter 3: Materials and methods

3.1 Participant recruitment

The study protocol was approved by the Ethics Committee of Bangkok Hospital, Bangkok, Thailand. Twelve diabetic patients attended the diabetic clinic in Bangkok Hospital between November 2015 to April 2016 were recruited on a voluntary basis. All volunteers were informed of the effects of diabetes mellitus on oral health. Those who fulfilled the criteria of hyposalivation (unstimulated salivary flow rate (USFR) less than 0.25 mL/min), were recruited into this study. Patients aged <18 years, pregnant, diagnosed with oral or maxillofacial neoplasms, patients with alcohol use of more than 1 drink per day for women or 2 drinks per day for men (106), and illicit drug users, which is long-term regular injecting use of opioids, amphetamines or cocaine (107), were excluded. Study objectives and procedures were then informed to each participant. A brief medical history data were taken as supportive information. The number of patientsubjects was based on the following formula:

$$\left[\left(\frac{Za}{2} + \frac{Zb}{2}\right) \times SD \div \Delta\right]^2$$

Za/2 = corresponding to the level of confidence (1.96)

Zb/2 = corresponding to the chosen level of power (1.28)

SD = referred to standard deviation form our pilot study (0.14)

Δ = expected difference after LLLT. As our inclusion criteria is USFR less

than 0.25 ml/min and mean of adult USFR is 0.40 ml/min (31). Expected

difference is 0.15 ml/min.

$$[(1.96 + 1.28)x\ 0.14 \div 0.15\ ]^2$$

Estimated sample size = 10 participants

3.2 Low level laser stimulation

An oral examination of each participant was performed prior to laser

stimulation. The laser stimulation procedures were performed by a dentist at

Bangkok Hospital. The parotid and submandibular glands were exposed to laser

stimulation extraorally, and the sublingual glands were exposed intraorally. Slow

circulating laser movements were performed during the stimulation to ensure

that the gland area was treated comprehensively. The salivary glands were

stimulated with a 940 nm Indium-gallium-arsenide-phosphide (InGaAsP) low

power semiconductor diode laser (Epic^{TM}10, Biolase Inc, Irvine, CA, USA).

Stimulation was performed 3 times a week for 2 consecutive weeks. Eye-glasses

protectors were worn by the patients and dentist. Each parotid, submandibular,

and sublingual gland was stimulated using 0.1 W output power for 40 sec/cm^2

area. Total energy density (ED) of 4 J/ cm^2 was used based on previous studies

(108, 109) and the equation as follows : ED (J/ cm^2) = Power (W) x Time per cm^2

(sec/ cm^2).

3.3 Salivary flow rate measurement

Saliva was collected 3 times at the 1st visit before laser stimulation, at the 6th visit after laser stimulation, and at the 6-week follow-up visit. The collection of unstimulated and stimulated whole saliva was performed from 9:00 a.m.–noon using standard techniques as described by Navazesh and Christensen (110). The participants were instructed to stop eating, drinking, and smoking 1 h before saliva collection. For unstimulated saliva collection, the participants were directed to lean forward and spit their saliva for 5 min into a sterilized plastic cup that was pre-weighed using a digital scale (Denver Instrument balance, Bohemia, NY, USA). The collection procedure was repeated two more times. The unstimulated salivary flow rate was calculated using the mean weight of the three saliva samples divided by 5 min.

To stimulate saliva flow, the patients were instructed to chew 1 g of tasteless paraffin (Parafilm, Neenah, WI, USA). The patients were told to not swallow their saliva during chewing. The patients with dentures were directed to chew the paraffin without removing their dentures. The patients were instructed to spit their saliva into a pre-weighed plastic cup every 30 seconds for 2 min. The collection procedure was repeated two more times. The stimulated salivary flow rate was calculated using the mean weight of the three saliva samples divided by 2 min.

3.4 Salivary protein measurement

Enzyme linked immunosorbent assay (ELISA) (MyBioSource, San Diego, CA, USA) was performed to determine the MUC7, MUC5B, and Histatin 5 salivary protein levels. Unstimulated saliva was used for mucins analysis. The ELISA procedures followed the manufacturer's instructions and were performed in triplicate.

In brief, in the first incubation step, the target biomarker in the samples was bound to polyclonal antibodies. A washing step was then carried out. In a second incubation step, a Peroxidase-labeled conjugate was added, which recognized the bound wanted protein specifically. After another washing step, the solid phase was incubated with Tetramethylbenzidine. An acidic solution was then added to stop the reaction. A dose response curve of the optical density at 450 nm vs concentration was generated, using the results obtained from the plate readers. The target protein in the participants samples was then determined directly from the curve.

3.5 Dry mouth symptom

A questionnaire related to xerostomia was given to each participant 3 times at the 1st visit before laser stimulation, the 6th visit after laser stimulation, and at the 6-week follow-up visit. Because dry mouth symptoms are subjective, a self-administered 11-item questionnaire modified from the Xerostomia Inventory-

Dutch version (112) was used to assess the xerostomia symptoms as shown in

Table 3.1.

Table 3.1 Questionnaire items

Number	Question
1	I sip liquids to aid in swallowing food (SIP-LIQ)
2	My mouth feels dry when eating a meal (DRY-MEL)
3	I get up at night to drink (NGT-DRK)
4	My mouth feels dry (MTH-DRY)
5	I have difficulty in eating dry foods (DIF-DRY)
6	I suck sweets or cough lollies to relieve dry mouth (SWT-DRY)
7	My lips feel dry (LIP-DRY)
8	I have a lot of dental caries (DEN-CAR)
9	I have a bad breath (BAD-BRH)
10	My tongue stick to my palate (TNG-PLT)
11	Bleeding when brushing (BLD-BRS)

A visual analogue scale (VAS) was used to quantify the response of each item

(not agree (0) to totally agree (10). The mean dry mouth score and the correlation

between dry mouth score and salivary flow rate were analyzed for each visit.

3.6 Data analysis

Statistical analysis was performed using the SPSS program version 22 for Windows. Because the data were not normally distributed, statistical analysis was performed using non-parametric tests. Salivary flow rate, salivary proteins, and dry mouth score of each item were assessed using the Friedman test followed by the post hoc. Wilcoxon signed-rank test to determine significant differences. The dry mouth scores were reversed prior to the correlation analysis using Spearman rank test (i.e. 8 became 2). A p value < 0.05 was considered significant.

Chapter 4: Results

4.1   Demographic data

Twelve diabetic patients, (6 males and 6 females ranging from 37–86-years old), were recruited into this study. All patients participated until the 6th visit and 10 returned for the 6-week follow up visit

Characteristics	
Number of Participants	
No. of Participants recruited	12
No. of Participants completed all visits	10
Sex*	
Male (%)	6 (50)
Female (%)	6 (50)

Age*	
Mean age (yr±SD)	60.83±14.36
Age range (yr)	37-86
Mean HbA1c* (%±SD)	8.4±2.51
Mean diabetes duration* (yr±SD)	6.45±5.59
Systemic disease*	
Hypertension (%)	5 (41.7)
Thyroid (%)	1 (8.3)
Autoimmune (%)	1 (8.3)
Heart disease (%)	1 (8.3)
Medication list*	
Antidiabetic (%)	9 (75)
Anticholesteremic (%)	6 (50)
Antihypertensive (%)	4 (33.3)
Analgesic (%)	5 (41.6)
Antidepressant (%)	3 (25)
Antispasmodic (%)	2 (16.7)
Thyroid agent (%)	1 (8.3)
Antihistamine (%)	1 (8.3)
Antitussive (%)	1 (8.3)
Antibiotic (%)	1 (8.3)
Antioxidant (%)	1 (8.3)
Antibacterial agent (%)	1 (8.3)
Anticoagulant (%)	1 (8.3)
Renal drug (%)	1 (8.3)
Anticonvulsant (%)	1 (8.3)

PDE inhibitors (%)	1 (8.3)
Antacid (%)	1 (8.3)
Antidiarrheals (%)	1 (8.3)
Vitamin (%)	3 (25)

4.2 Unstimulated salivary flow rate (USFR) and stimulated salivary flow rate (SSFR)

The results demonstrated a trend of increased USFR over the duration of our study (Table 4.2). There were significant increases in USFR between the 1st visit and the 6th visit (p=0.005) and between the 1st visit and the 6-week follow up (p=0.005). No significant difference was found between the 6th visit and the 6-week follow up (p=0.241). The results exhibited a trend of increased SSFR during the duration of the study (Table 4.2). However, there were no significant differences among the 3 different visits (p>0.05)

	1st visit (baseline) N= 12	6th visit (the end of stimulation) N = 12	6 week follow up N = 10	p-value
USFR (mL/min±SD)	0.14±0.08[a,b]	0.29±0.16[a]	0.32±0.16[b]	$p < 0.01$

SSFR (mL/min±SD)	0.79±0.47	0.92±0.43	0.94±0.42	0.232
MUC7 (ng/mL±SD)	3.29±5.36	2.49±4.05	2.43±5.04	0.519
MUC5B (ng/mL±SD)	9.15±5.15^a	8.08±2.92^b	13.78±8.65^{a,b}	$p < 0.05$
Histatin 5 (ng/mL±SD)	192.10±141.52^a	234.86±245.98^b	100.89±8.65^{a,b}	$p < 0.05$
Dry mouth score ($\bar{x}$±SD)	4.05±3.25^{a,b}	1.26±1.18^a	1.03±1.19^b	$p < 0.001$

Table 4.2 Mean score of salivary flow rate, salivary protein concentration, and dry mouth score

* Friedman test

[a,b] Groups with the same superscript letters are significantly different per the Wilcoxon signed-rank test.

4.3 MUC5B, MUC7, and histatin 5 concentration

We found a trend of decreased MUC7 concentration during the duration of the study (Table 4.2). Although slight decreases were noticed at the 6th visit and the 6-week follow-up, the differences were not significant among the 3 different visits ($p>0.05$). The results showed a trend of increased MUC5B salivary concentration, however a slight decrease was found at the 6th visit (Table 4.2). There was no significant difference between the 1st visit and the 6th visit ($p = 0.875$). In contrast, significant increases were found between the 1st visit and the 6-week follow up ($p = 0.037$) and between the 6th visit and the 6-week follow up ($p=0.028$). The overall results indicate a decreasing trend of histatin 5, although a slight increase was found at the 6th visit (Table 4.2). There was no significant difference between the 1st visit and the 6th visit ($p = 0.530$). Nonetheless, significant decreases were found between the 1st visit and at 6 weeks follow up ($p = 0.047$) as well as between the 6th visit and the 6- week follow up ($p = 0.022$)

4.4 Dry mouth score

The mean dry mouth scores indicated a trend of decreasing scores during the study duration (Table 4.2). Significant decreases in mean dry mouth score were found between 1st visit and 6th visit (p=0.000) and between the 1st visit and the 6-week follow up (p=0.000). Although a slight decrease was observed between the 6th visit and 6-week follow up the difference was not significant (p = 0.268). The mean dry mouth scores of each questionnaire item are seen in Table 4.3. Item 7, "my lips feel dry" received the highest mean score (5.67±3.14) on the 1st visit followed by item 4 (4.75±2.89), and item 9 (4.5±3.87), "my mouth feels dry" and "I have a bad breath", respectively. Items 3, 4, 7, 8, and 9 showed significant decreases from the 1st visit to the 6-week follow up (p<0.05 for items 3, 8, 9 and p < 0.01 for items 4 and 7) (Table 4.3). However, only item 4, "my mouth feels dry", exhibited a significant decrease in a time-dependent manner

Table 4.3 The mean dry mouth scores of each questionnaire item referring to visits

Questionnaire Items	V1 (mean±SD) N = 12	V2 (mean±SD) N = 12	V3 (mean±SD) N = 10	*p*-value*
1. SIP-LIQ	2.42±2.87	0.92±0.99	0.9±0.99	0.291
2. DRY-MEL	3.08±3.53	1.5±0.79	1.1±0.99	0.483
3. NGT-DRK	3.83±3.27[a]	0.92±0.79[a]	1.3±1.06	0.042
4. MTH-DRY	4.75±2.89[a,b]	1.5±1[a,c]	0.8±0.63[b,c]	0.002
5. DIF-DRY	2.42±2.87	1.33±0.98	1.2±1.13	0.965
6. SWT-DRY	2.67±2.96	1.08±1.38	0.6±0.84	0.070

7. LIP-DRY	$5.67\pm3.14^{a,b}$	1.17 ± 0.83^{a}	1.6 ± 1.50^{b}	0.001
8. DEN-CAR	$3.92\pm3.50^{a,b}$	1.75 ± 1.91^{a}	1.4 ± 1.78^{b}	0.042
9. BAD-BRH	4.5 ± 3.87^{a}	1.58 ± 1.62^{a}	1.7 ± 1.83	0.016
10. TNG-PLT	4.0 ± 3.69	1 ± 0.74	0.7 ± 0.67	0.072
11. BLD-BRS	2.17 ± 2.17	0.83 ± 0.94	1.2 ± 1.39	0.28

V1 : 1st visit; V2 : 6th visit; V3 : 6 weeks follow-up

* Friedman test

[a,b,c] Groups with the same superscript letters are significantly different per the Wilcoxon signed-rank test

Table 4.4 Correlation between mean dry mouth score and salivary flow rate on different visits

	USFR		SSFR	
	6th visit N = 12	6 week follow up N = 10	6th visit N = 12	6 week follow up N = 10
Dry mouth score item 4	0.467 (P = 0.125)	0.437 (P = 0.207)	0.437 (P = 0.156)	0.668 (P = 0.035)
Dry mouth score item 7	0.419 (P = 0.176)	0.013 (P = 0.971)	0.200 (P = 0.534)	0.052 (P = 0.886)

*Used questionnaire item no.4 and no.7 only

Only questionnaire items 4 and 7 at the 6th visit and 6-week follow up were used in the correlation analysis because these items best represent dry mouth. The results revealed no significant differences between mean dry mouth score and salivary flow rate on the 6th visit and 6-week follow up, except between item 4 and SSFR at the 6-week FU, which was the strongest correlation (Table 4.4).

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Chapter 5: Discussion

Total number of diabetics is predicted to rise from 366 million in 2011 to 552 million in 2030 (113). Those whose age > 65 years old are more prone to be affected with DM (114). A huge investigation project was done by Rawdaree et al in Thailand finding that almost 95% of diabetics are type 2 diabetes. The mean age affected by DM was 59.4 years with the age group of 61-70 was the majority. The mean DM duration was 10 years with DM duration of 6-10 years was the highest percentage. Only 30.7% of the patients had HbA1C below 7% (115). The cut-off point of HbA1C for diabetes was 6.1%. HbA1C levels showed 3 months average of blood glucose concentrations. HbA1C can be measured anytime regardless during fasting or the content of previous meal. In addition, HbA1C test can be done with only small blood sample using a small portable device (116). In 2009 the prevalence of diabetes in Thailand was 10.6%. The diabetics proportions were higher in men than in women (47.3% vs 23.3%) (117).

Xerostomia or dry mouth, a subjective symptoms that commonly occurred in diabetics, affects around 30% of the population with the age above 65 years old. Dehydration and reduced biting force in elderly also contribute to the reduction in salivary secretion (118).

The present study evaluated the effect of LLLT on salivary gland function in diabetic patients with hyposalivation. The findings indicated that using LLLT on the major salivary glands significantly increased the unstimulated salivary flow

rate and MUC5B salivary concentration, and alleviated the dry mouth symptoms in these patients.

Our results demonstrated that LLLT increased the USFR of diabetic patients; however, the elevation of the stimulated salivary flow rate was not significant. The normal USFR value is at least 0.25 mL/min (30). It has been suggested that salivary collection procedure was performed at a fixed time interval in the morning to minimize the outcome variation (119). In our study, salivary collection procedure were performed mostly in the morning between 8.00 and 11.00. The prevalence of hyposalivation was higher in women with the percentage of 22%, more than in men (only 15%) (120). This is in agreement with our results that showed a higher salivary flow rates in men than in women (appendix 1).

The mean USFR of 0.14 mL/min found at the 1st visit was below the typical value. After LLLT was applied, the mean USFR, but not SSFR, increased to within the normal range. Our results are consistent with previous studies in hyposalivation subjects (18, 121). These findings may result from LLLT generating ATP production by activating the electron transport chain in mitochondria (122); stimulating cell function. However, LLLT did not improve either USFR or xerostomia in patients undergoing radiotherapy (123). This may be due to acinar atrophy and chronic inflammation of the salivary glands and may lead to necrosis (124), implying that LLLT is not effective on atrophic glands. This suggests the major salivary gland response to LLLT is different under physiological and pathological conditions.

The salivary flow rate varies in one person in different period of time. A study found that a difference of 45% in salivary flow rate in different period of time was still considered as a normal deviation, meaning that a difference of below or equal to 45% can be deemed as a "not true difference" (125). Three participants (25%) and 8 participants (66.7%) showed differences in USFR and SSFR, respectively, below or equal to 45% between 6th visit and 1st visit, while 8 participants (80%) and 9 participants (90%) showed differences in USFR and SSFR, respectively, below or equal to 45% between 6-week follow up and 6th visit (appendix 2 and 3).

These results imply that LLLT's biostimulation was somehow more effective at 6th visit compare with 6-week follow up. Apart from multifactorial that were involved in salivary flow rate variation, the LLLT's biostimulation effect might reach "plateau" state since there was no laser exposure between 6th visit and 6-week follow up. The high percentage of SSFR that were below or equal to 45% validates another result showing insignificant increase in SSFR at 3 different periods.

To explore more about factors that may play a role in salivary flow rate variation, some additional analysis were performed. USFR was chosen as a main parameter since USFR is a basal physiological state of the saliva (126). It represents how much salivary amount that can be produced in resting state. The multiple regression analysis was performed between HbA1C, age, DM duration and USFR at 3 different visits. A factor changed dependent variable value when all other factors remained constant, which was demonstrated by "Beta" value. For instance, at the 1st visit, 1 unit increased in HbA1C, there was an increase in

USFR in 0.005 mL/min, while 1 unit increased in age, there was a decrease in USFR of 0.002 mL/min (appendix 4)

These results imply that in our study, LLL's biostimulation effect was not affected by HbA1C value, while DM duration might be the most influential factors affecting LLL's biostimulation effect. The variation of "Beta" at each visit may be due to other habit and psychological factors such as poor hydration and stress. However, at all of these 3 visits, there were no potentially significant association between these 3 factors to USFR, implying that there was no a dominant risk factor associated to the result, yet it was rather multifactorial. These results also conclude that the regression model was not a good fit for the data(appendix 4). Hyposalivation is mostly related to drug consumption. Almost 80% of the commonly prescribed drugs are causing hyposalivation (127), so the amount of the drugs consumed should be linear with the degree of hyposalivation. In this study we performed additional analysis to explore whether amount of drugs consumed was related to hyposalivation.

Cut-off value of USFR is 0.25 mL/min (30). At the 1st visit, 12 participants (100%) showed USFR below the cut off value. The lowest USFR was 0.013 mL/min with only 5 drugs consumed. A participant with the highest amount of drugs consumed (14 drugs) showed USFR of 0.094 mL/min, meaning that it was not the lowest USFR although the drug consumption was high. At the 6th visit, 5 participants (41.6%) were still below the cut-off value of USFR. One of these 5 participants consumed 14 drugs which was the highest. The lowest USFR shown was 0.104 mL/min, with 6 drugs consumed. At the 6 week FU, 2 participants (20%) were still below the cut-off value of USFR. These 2

participants consumed the highest amount of drugs (16 and 20 drugs) (appendix 2 and 5)

Cut-off value for SSFR is 1 mL/min (30). At the 1st visit, 8 participants (66.7%) showed SSFR below cut-off value. A participant with the lowest SSFR, 0.251 mL/min, consumed the highest amount of drugs (14 drugs). At the 6th visit, 7 participants (58.3%) were still below the cut off value. Among of them was a participant with the highest amount of drugs consumed (14 drugs). However, a participant with the lowest SSFR consumed 6 drugs only. At the 6 week follow up, 6 participant (60%) were still below the cut-off value, among of them was a participant with the highest amount of drugs consumed (20 drugs). However, a participant with the lowest SSFR (0.506 mL/min) consumed only 7 drugs (appendix 3 and 5)

Considering the analysis of the result, it showed that the amount of drugs consumed was related to the degree of hyposalivation, yet not the only factor which was involved. For instance, the lowest USFR at the 1st visit only consumed 5 drugs, which was categorized as "low" compared with other 5 participants who consumed more than 5 drugs (appendix 2 and 5) Other factors may play role in this issue, such as duration of DM disease and age. This participant, who only consumed 5 drugs, has experienced DM for 20 years with current age of 86. The high amount of drugs consumed in the past periods, which was before the study begun, may not be well-documented. The severity of salivary glands damage due to long duration of DM and drugs consumption may result in non-optimal effect of LLL's biostimulation (appendix 2, 5 and 6).

Some participants showed decrease in USFR and SSFR even after LLL stimulation. Another example, a participant has experienced DM for only 2 years and consumed only 2 drugs at the 1st visit, yet the cut-off value of USFR could not be reached, implying these demographic data obtained were not the only factors that were involved in hyposalivation (appendix 2, 5, and 6). Other factors such as poor hydration and psychologic factors, such as anxiety and stress, also could be related (128)

The amount of drugs analysis demonstrated that at the 6th visit, "Beta" value showed that 1 unit increased in total amount of drugs consumed, there was an increase in USFR by 0.003 mL/min. At the 6-week FU, the "beta" value showed that 1 unit increased in total amount of drugs consumed, there was a decrease in USFR by 0.005 mL/min. There were no potentially significant association between total amount of drugs consumed and USFR(P - value 0.717 and 0.651 at the 6th visit and 6 week FU, respectively). These results also concluded that the regression model was not a good fit for the data (appendix 7).

Moreover, scatterplot analysis between each factor and USFR demonstrated that there were no strong association between each factor and USFR. Thus, the flow rate cannot be associated with one factor alone, but rather multifactorial (appendix 8, 9, 10, and 11)

The typical MUC5B concentration in unstimulated whole saliva ranges from 0.05–0.78 ng/ml (129). Surprisingly, the patients in our study showed much higher concentrations of both mucins compared with normal values. A possible explanation could be the difference in salivary protein content in diabetics compared with non-diabetics. Increased MUC1 in saliva is associated with pro-

inflammatory cytokines (130). Diabetes mellitus is an inflammatory disease, thus, the higher mucin concentration found in our study is likely related to changes in pro-inflammatory cytokine levels in the salivary glands of diabetic patients (131). The LLLT induced significant increase in MUC5B found at the 6-week follow up visit may have resulted from LLLT's biomodulatory effect on the salivary glands (Table 4.2).

We observed that MUC7 was present at a lower concentration compared with that of MUC5B. This finding is consistent with a previous report showing that the mucin in saliva is predominantly MUC5B (45). Our results showed that MUC7 was higher than normal value (3.29±5.36 ng/mL versus 0.06-0.32 ng/ml) (129) and was not significantly increased by the LLLT (Table 4.2). MUC7, but not MUC5B, has been found to be localized in serous acini in sublingual, submandibular, lingual, and palatine glands (41). A slight decreased of MUC7 concentration in our study may be due to damaged serous acini cells in diabetic patients. Moreover, MUC7 are more susceptible to proteolysis compare with MUC5B (132). The proteolysis might occur due to sialidases, an enzyme that was secreted by oral microorganisms (132)

A previous study revealed salivary histatin concentrations were lower in diabetic children compared with controls (133). This indicates that their anti-fungal and bacterial enzyme inhibition activity cannot be optimally achieved in diabetic patients. However, further investigation to resolve these issues is needed. Interestingly, we found that the concentration of salivary histatin 5 was significantly decreased at the 6-week follow up visit. This result does not agree with that of a previous investigation demonstrating that LLLT had a mild

disinfecting effect against *C. albicans* and reduced inflammation in denture stomatitis patients (134). A previous study concluded that the parotid gland serous cells in diabetics patients were prone to intracellular lipid accumulation (135). This may explain the reduced amount of histatin 5 found in our study (Table 4.2), because the serous cells in the parotid glands are involved in secreting this protein (111)**.** Moreover, dissimilarity in the diabetes severity levels of the patients in our study may have resulted in different acinar cell function between patients, because most diabetic patients are taking multiple drugs which is related to salivary gland hypofunction (8). In addition, histatin 5 may form complexes with other protein such as amylase, statherin, and PRP due to hydrophobic interactions and hydrogen bonds. This could lead to a decrease in histatin 5 concentration level (136). The decreased histatin 5 concentration at the follow up visit supports the insignificant increase in SSFR found in our study, suggesting that the parotid glands of diabetic patients may be more sensitive to salivary gland impairment, given that the parotid glands contribute to both stimulated salivary secretion and histatin 5 production.

In our study, however, the protein activity was not the main concern. The protein analysis was performed to investigate concentration levels only in physiological condition. It is difficult to avoid proteolysis completely. A certain amount of the salivary protein might undergo degradation when was being collected from oral cavity.

The standard procedures was performed to minimize protein degradation activity. After saliva collection, the saliva was immediately moved into a tube

and was then put into an ice box. The sample was then centrifuged to remove debris and stored in -20 degrees fridge.

The dry mouth score results indicated that LLLT decreased the subjective dry mouth symptoms throughout the duration of the study (Table 4.2). These results are in line with a previous report that found LLLT effectively reduced dry mouth symptoms (137). Item 4 on the questionnaire, "my mouth feels dry", demonstrated the highest mean score among the items prior to laser stimulation (Table 4.3). This response indicates that the major subjective sign of dry mouth is the feeling of dryness inside the mouth, as found in a previous study (112).

The questionnaire item analysis revealed that item 4 (my mouth feels dry) and 7 (My lips feel dry), significantly decreased after LLLT, indicating that laser stimulation reduces the dry mouth symptoms induced by diabetes mellitus. A previous study found an association between diabetes mellitus and dry mouth symptoms (138). It is important to note that item 4 (my mouth feels dry) significantly decreased at each visit, indicating that LLLT alleviated the most dominant dry mouth symptom in this study.

Correlation analysis between mean dry mouth score and salivary flow rate showed the strongest positive correlation at the 6 week FU between item 4 and SSFR with a significant difference. However, other results demonstrated insignificant differences with weak correlation. These results are similar to those of a previous study (123) which concluded that decreased dry mouth symptoms are not always directly proportional to increased salivary flow rates, and vice versa. Multifactorial etiologies such as stress and anxiety may vary perception of the dry mouth.

One aspect concerning our statistical analysis is that because 2 patients did not attend the 6-week follow up visit, the SPSS Friedman test was not able to assess uneven participant numbers between the 3 visits. To resolve this issue, the Friedman test was performed using 10 patients, excluding the data from the missing patients. However, the mean scores presented in this study represent the values from the 12 patients, except for the 6-week follow up data that were based on the 10 patients who attended this visit.

In our study, we used a 4 J/cm^2 ED, which has been suggested as the most effective ED to stimulate cells (108, 109), however, we found that the LLLT induced increase in SSFR was not significant and histatin 5 concentration decreased. Because the parotid glands are responsible for the SSFR and secretion of histatin 5, we hypothesize that a higher laser ED may be required on these glands to achieve the optimum result in diabetic patients. Damage to sublingual, submandibular, lingual, and palatine gland serous cells in diabetic patients may contribute to the slight decrease of MUC7 observed in our study. It may be necessary to also apply laser stimulation over the minor glands area stimulate their cell function. Dental Health Education (DHE) given by the dentist is strongly required to maintain oral health in hyposalivation patients. Lack of salivary flow decreases oral clearance leading to caries, candidal infection, and other oral health problems. Proper hydration, routine tooth brushing, and avoiding stress and anxiety are amongst the important advices that must be given to the patients.

A previous study reported that intracellular lipid accumulation may exist in diabetic's salivary glands (139), thus a high LLL's wavelength is required in

order to perform deeper penetration to the tissue. A 940 nm low level laser was used in our study, which is still in typical wavelength range used in treatments (630-980 nm) (13). Energy density is a critical factor that determine whether the treatment would be beneficial or detrimental (140). To increase the energy density of LLL, a higher power output is needed. A higher power output means the greater the photons number will be present at any given depth. Moreover, a higher power output resulting in a shorter irradiation times, thus makes the treatment duration more efficient (141). Even though a dosage of 4 J/cm2 has showed optimum results, several studies applied higher dosage than 4 J/cm2 to severe cases, such as rheumatoid arthritis (142). However, it is important to note that increasing energy density should be applied only if the final outcome is unsatisfying. The gradual manner in increasing energy density is highly recommended (143). A 40 s treatment time per targeted point was applied in our study, which is a typical time given in LLL treatment (about 30 s) (102). There is no consensus reported about the recommended frequency of treatment, however giving treatment too often is not recommended (143). In our study, the participants were exposed to LLL 3 times a week for 2 consecutive weeks. A small dosage applied between periods of time are more effective than giving treatments that are administered close together (144). This is because the dosage given are cumulative. Giving the treatment too close will accumulate the dosage above the biostimulating range or even in bioinhibitory range (145). Another study concluded that treatment in every other day is safer than exposure for 7 consecutive days in muscle injury in toad (146). This treatment interval is also a typical required treatment that commonly performed (102) A previous study

assessed the effect of LLL stimulation to salivary glands and investigated the LLL's effect until 30 days FU (17). It is then interesting to investigate whether the LLL's biostimulation effect still persists in more than 30 days FU. In our study, a 6-week FU analysis was performed.

When the final outcomes are not satisfying, the higher energy density can be applied in order to achieve a better outcome. LLLT showed insignificant effect on healthy tissue and on experimental wounds in healthy participants.. This may explain the variation in final results between clinical and laboratory research. In addition, it means that the healthy tissue is not affected by LLLT (147)

Our report is the first using LLLT on salivary glands in diabetic patients. However, due to the limitation of the study duration and the difficulties of recruiting patients, we only evaluated a small sample size of diabetic patients. In conclusion, LLLT is a beneficial approach to elevate unstimulated salivary flow rate and MUC5B concentration, as well as to decrease dry mouth symptoms in diabetic patients.

Chapter 6 : Conclusion and Future Direction

6.1 Conclusion

This thesis has investigated the effect of LLL stimulation to salivary gland function on diabetic patients with hyposalivation. In our study, the salivary gland function was represented by unstimulated and stimulated salivary flow rate, mucin 7, mucin 5B, and histatin 5 concentration levels. Subjective dry mouth symptoms were also assessed using a questionnaire.

As diabetics are prone to hyposalivation as well as to oral fungal infections, mucins and histatin concentration levels were assessed in our study. Mucin 7 (MUC7) and mucin 5B (MUC5B) were chosen because these type of mucins showed abundant levels in the saliva, while histatin 5 (Hist5) has been found as the strongest type of histatin to kill C.albicans, a common fungal species causing oral candidiasis.

Although different type of approaches have been performed to overcome hyposalivation, none of them has been proven one hundred percent free of unpleasant side effects. LLLT offers a safe and effective approach to stimulate salivary gland function without side effects. The light from LLL enhances mitochondria activity leading to an increase in ATP production. Thus, cell functions are expected to be more optimum after being exposed by LLL.

The results demonstrated that LLLT significantly increases unstimulated salivary flow rate (USFR) but not stimulated salivary flow rate (SSFR). The explanation could be the sensitivity of parotid glands to salivary gland impairment, as parotid glands more contribute to SSFR secretion.

As for salivary proteins assessment, only MUC5B concentration level showed a significant increase after LLLT. MUC7 concentration level showed an insignificant decrease while Hist5 concentration level demonstrated a significant decrease after LLLT. Given that MUC5B concentration level in saliva is higher than MUC7 in healthy individuals, it is clear that MUC7 concentration level was lower than that of MUC5B in our study . MUC7, but not MUC5B, is secreted by serous cells of the submandibular, sublingual, and minor glands. The severe damage of the serous cells may explain the decrease in MUC7 concentration level. Moreover, MUC7 are more susceptible to proteolysis compare with MUC5B. The proteolysis might occur due to sialidases, an enzyme that was secreted by oral microorganisms.

A significant decrease in Hist5 concentration level validated another result in our study, which is an insignificant increase in SSFR. Parotid glands contibute to both hist5 and stimulated saliva secretion. Severe damage on parotid glands may explain a significant decrease in hist5 concentration level. In addition, hist5 may form complexes with other protein such as amylase, statherin, and PRP due to hydrophobic interactions and hydrogen bonds. This could lead to a decrease in hist5 concentration level.

Dry mouth symptom assessment using questionnaire showed a significant decrease of dry mouth symptom after LLLT. Item no.4 (my mouth feels dry) and item no.7 (my lips feel dry) were used for correlation analysis as these 2 items best represent dry mouth. Correlation analysis between USFR-SSFR and item no.4 and 7 at the 6th visit and 6-week FU showed insignificant differences except between item no.4 and SSFR at the 6-week FU. These results imply that an increase in salivary flow rate is not always followed by a decrease in dry mouth symptom as it is a subjective perception.

Many factors may play a role in salivary flow rate amount. In our study, factors like sex, age, DM duration, HbA1C, and amount of drug consumption may affect the result. It has been concluded that men secrete more saliva than women and this is in agreement with our result even though only with slight differences. However, other factors showed insignificant association with USFR, meaning that salivary flow rate cannot be associated with only one factor, but it is connected with other factors as well, thus it is rather multifactorial.

To sum up, these are the main points of our study conclusion :

1.   LLL stimulation significantly increases unstimulated salivary flow rate and MUC5B concentration level in diabetic patients with hyposalivation

2.   LLL stimulation decreases subjective dry mouth symptoms in diabetic patients with hyposalivation

3.   Salivary flow rate was affected by multifactorial, such as age, HbA1c, DM duration, and amount of drugs consumption. Thus the flow rate was not associated with only one factor.

6.2 Future direction

Considering the limitation and results of our study, constructive suggestions came up and can be used as future direction for further studies. These are the main points of the future direction :

1.   The difficulties to persuade participant at Bangkok Hospital caused only a small number of participants involved in our study. More intense communication and teamwork with the physicians at Diabetes Clinic may be needed in order to increase

the chance to recruit more participants, since all diabetics at this hospital were going through examination by the physicians.

2.	To strengthen the output data, more information may be required to explore more about the effect of LLL to the quality of saliva. Analysis of salivary pH, buffer capacity, and viscosity before and after LLL stimulation are highly encouraged for further studies.

3.	The exposure of LLL to all minor glands are recommended to achieve optimum salivary flow rate and mucin secretion result, especially MUC7, since MUC7 is also secreted by serous cells in minor glands.

4.	A higher energy density of LLL may be required to achieve optimum result, especially when applying exposure to parotid glands. This is based on unsatisfactory results of SSFR and Hist5 concentration level, since they both are associated with parotid glands. It is important to note that a gradual increase in energy density is highly recommended

References

1.	Edgar W. Saliva: its secretion, composition and functions. Brit Dent J. 1992;172(8):305-12.
2.	de Almeida PDV, Gregio A, Machado M, De Lima A, Azevedo LR. Saliva composition and functions: a comprehensive review. J Contemp Dent Pract. 2008;9(3):72-80.
3.	Chang W-I, Chang J-Y, Kim Y-Y, Lee G, Kho H-S. MUC1 expression in the oral mucosal epithelial cells of the elderly. Archives of oral biology. 2011;56(9):885-90.

4. Prakobphol A, Levine MJ, Tabak LA, Reddy MS. Purification of a low-molecular-weight, mucin-type glycoprotein from human submandibular-sublingual saliva. Carbohydrate research. 1982;108(1):111-22.

5. Levine M, Reddy M, Tabak L, Loomis R, Bergey E, Jones P, et al. Structural aspects of salivary glycoproteins. J Dent Res. 1987;66(2):436-41.

6. Pollock JJ, Denepitiya L, MacKay B, Iacono V. Fungistatic and fungicidal activity of human parotid salivary histidine-rich polypeptides on Candida albicans. Infection and immunity. 1984;44(3):702-7.

7. Scully C, Felix D. 3: Oral Medicine—Update for the dental practitioner: Dry mouth and disorders of salivation. Brit Dent J. 2005;199(7):423-7.

8. Sreebny LM, Schwartz SS. A reference guide to drugs and dry mouth–2nd edition. Gerodontology. 1997;14(1):33-47.

9. Huang Y-f, Cheng Q, Jiang C-m, An S, Xiao L, Gou Y-c, et al. The immune factors involved in the pathogenesis, diagnosis, and treatment of Sjogren's syndrome. Clinical and Developmental Immunology. 2013;2013.

10. Puy CL. The role of saliva in maintaining oral health and as an aid to diagnosis. Med Oral Patol Oral Cir Bucal. 2006;11(5):449-55.

11. Moore PA, Guggenheimer J, Etzel KR, Weyant RJ, Orchard T. Type 1 diabetes mellitus, xerostomia, and salivary flow rates. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001;92(3):281-91.

12. Sandberg GE, Wikblad KF. Oral dryness and peripheral neuropathy in subjects with type 2 diabetes. Journal of Diabetes and its Complications. 2003;17(4):192-8.

13. Dyson M, editor Primary, secondary, and tertiary effects of phototherapy: a review. Biomedical Optics 2006; 2006: International Society for Optics and Photonics.

14. Aimbire F, Albertini R, Pacheco M, Castro-Faria-Neto H, Leonardo P, Iversen V, et al. Low-level laser therapy induces dose-dependent reduction of TNFα levels in acute inflammation. Photomed Laser Surg. 2006;24(1):33-7.

15. Mohammed IF, Kaka LN. Promotion of regenerative processes in injured peripheral nerve induced by low-level laser therapy. Photomed Laser Surg. 2007;25(2):107-11.

16. Ihsan FM. Low-level laser therapy accelerates collateral circulation and enhances microcirculation. Photomedicine and Laser Therapy. 2005;23(3):289-94.

17. Vidović Juras D, Lukač J, Cekić-Arambašin A, Vidović A, Canjuga I, Sikora M, et al. Effects of low-level laser treatment on mouth dryness. Coll Antropol. 2010;34(3):1039-43.

18. Lončar B, Mravak Stipetić M, Baričević M, Risović D. The effect of low-level laser therapy on salivary glands in patients with xerostomia. Photomed Laser Surg. 2011;29(3):171-5.

19. Pezelj-Ribarić AS, Gržetić N, Urek MM, Glažar I, Kuiš D. Salivary flow rate before and after low level laser therapy. International magazine of laser dentistry. 2010;2(4):6-8.

20. Holsinger FC, Bui DT. Anatomy, function, and evaluation of the salivary glands. Salivary gland disorders: Springer; 2007. p. 1-16.

21. Carlson GW. The salivary glands. Surgical Clinics. 2000;80(1):261-73.

22. Johns M. The salivary glands: anatomy and embryology. Otolaryngologic clinics of North America. 1977;10(2):261.

23. Bialek EJ, Jakubowski W, Zajkowski P, Szopinski KT, Osmolski A. US of the Major Salivary Glands: Anatomy and Spatial Relationships, Pathologic Conditions, and Pitfalls 1. Radiographics. 2006;26(3):745-63.

24. Ten Cate A. Salivary gland. Oral Histology: Development, Structure, and Function 5th edition St Louis, Missouri: Mosby. 1998.

25. Holmberg KV, Hoffman MP. Anatomy, biogenesis and regeneration of salivary glands. Saliva: Secretion and Functions. 24: Karger Publishers; 2014. p. 1-13.

26. Ekström J, Khosravani N, Castagnola M, Messana I. Saliva and the control of its secretion. Dysphagia: Springer; 2011. p. 19-47.

27. Ekström J. Muscarinic agonist-induced non-granular and granular secretion of amylase in the parotid gland of the anaesthetized rat. Experimental physiology. 2002;87(02):147-52.

28. Ekström J, Havel GE, Reinhold AC. Parasympathetic Non-Adrenergic, Non-Cholinergic-Induced Protein Synthesis and Mitogenic Activity in Rat Parotid Glands. Experimental physiology. 2000;85(2):171-6.

29. Sayardoust S, Ekström J. Nitric oxide-dependent protein synthesis in parotid and submandibular glands of anaesthetized rats upon sympathetic stimulation or isoprenaline administration. Experimental physiology. 2004;89(2):219-27.

30. Ericsson Y, Hardwick L. Individual diagnosis, prognosis and counselling for caries prevention. Progress in Caries Prevention: Karger Publishers; 1978. p. 94-102.

31. Sreebny LM. Dry mouth: A multifaceted diagnostic dilemma. In: Sreebny LM, Vissink A, editors. Dr mouth. The malevolent symptom: a clinical guide. London: Wiley-Blackwell Publishing; 2010. p. 33-51.

32. Veerman EC, van den Keybus PA, Valentijn-Benz M, Amerongen AN. Isolation of different high-Mr mucin species from human whole saliva. Biochem J. 1992;283(3):807-11.

33. Denny P, Denny P, Klauser D, Hong S, Navazesh M, Tabak L. Age-related changes in mucins from human whole saliva. J Dent Res. 1991;70(10):1320-7.

34. Veerman E, Keybus Pvd, Vissink A, Amerongen A. Human glandular salivas: their separate collection and analysis. European journal of oral sciences. 1996;104(4):346-52.

35. Fábián TK, Fejérdy P, Csermely P. Saliva in health and disease, chemical biology of. Wiley encyclopedia of chemical biology. 2007.

36. Pramanik R, Osailan SM, Challacombe SJ, Urquhart D, Proctor GB. Protein and mucin retention on oral mucosal surfaces in dry mouth patients. European journal of oral sciences. 2010;118(3):245-53.

37. Rose MC, Voynow JA. Respiratory tract mucin genes and mucin glycoproteins in health and disease. Physiol Rev. 2006;86(1):245-78.

38. Gipson IK. Distribution of mucins at the ocular surface. Exp Eye Res. 2004;78(3):379-88.

39. Tabak LA. In defense of the oral cavity: structure, biosynthesis, and function of salivary mucins. Annual review of physiology. 1995;57(1):547-64.

40. Baughan L, Robertello F, Sarrett D, Denny P, Denny P. Salivary mucin as related to oral Streptococcus mutans in elderly people. Oral Microbiol Immunol. 2000;15(1):10-4.

41. Nielsen P, Mandel U, Therkildsen M, Clausen H. Differential expression of human high-molecular-weight salivary mucin (MG1) and low-molecular-weight salivary mucin (MG2). J Dent Res. 1996;75(11):1820-6.

42. Cohen RE, Aguirre A, Neiders ME, Levine MJ, Jones PC, Reddy MS, et al. Immunochemistry and immunogenicity of low molecular weight human salivary mucin. Archives of oral biology. 1991;36(5):347-56.

43. Tabak LA, Levine MJ, Mandel ID, Ellison SA. Role of salivary mucins in the protection of the oral cavity. Journal of Oral Pathology & Medicine. 1982;11(1):1-17.

44. Levine M, Herzberg M, Levine M, Ellison S, Stinson M, Li H, et al. Specificity of salivary-bacterial interactions: role of terminal sialic acid residues in the interaction of salivary glycoproteins with Streptococcus sanguis and Streptococcus mutans. Infection and immunity. 1978;19(1):107-15.

45. Thornton DJ, Khan N, Mehrotra R, Howard M, Sheehan JK, Veerman E, et al. Salivary mucin MG1 is comprised almost entirely of different glycosylated forms of the MUC5B gene product. Glycobiology. 1999;9(3):293-302.

46. Bolscher J, Veerman E, Amerongen AVN, Tulp A, Verwoerd D. Distinct populations of high-Mr mucins secreted by different human salivary glands discriminated by density-gradient electrophoresis. Biochem J. 1995;309(3):801-6.

47. Veerman E, Ligtenberg A, Schenkels L, Walgreen-Weterings E, Amerongen AN. Binding of human high-molecular-weight salivary mucins (MG1) to Hemophilus parainfluenzae. J Dent Res. 1995;74(1):351-7.

48. Veerman E, Bolscher J, Appelmelk B, Bloemena E, Van den Berg T, Amerongen AN. A monoclonal antibody directed against high Mr salivary mucins recognizes the SO3-3Galβ1-3GlcNAc moiety of sulfo-Lewisa: a histochemical survey of human and rat tissue. Glycobiology. 1997;7(1):37-43.

49. van't Hof W, Veerman EC, Nieuw Amerongen A, Ligtenberg AJ. Antimicrobial defense systems in saliva. Saliva: Secretion and Functions. 24: Karger Publishers; 2014. p. 40-51.

50. Silva DG, Stevens RH, Macedo JM, Hirata R, Pinto AC, Alves LM, et al. Higher levels of salivary MUC5B and MUC7 in individuals with gastric diseases who harbor Helicobacter pylori. Archives of oral biology. 2009;54(1):86-90.

51. Tabak LA. Structure and function of human salivary mucins. Crit Rev Oral Biol Med. 1990;1(4):229-34.

52. Murray P, Prakobphol A, Lee T, Hoover C, Fisher S. Adherence of oral streptococci to salivary glycoproteins. Infection and immunity. 1992;60(1):31-8.

53. Moshier A, Reddy MS, Scannapieco FA. Role of type 1 fimbriae in the adhesion of Escherichia coli to salivary mucin and secretory immunoglobulin A. Current microbiology. 1996;33(3):200-8.

54. Groenink J, Ligtenberg A, Veerman E, Bolscher J, Amerongen AN. Interaction of the salivary low-molecular-weight mucin (MG2) with Actinobacillus actinomycetemcomitans. Antonie Van Leeuwenhoek. 1996;70(1):79-87.

55. Puri S, Edgerton M. How does it kill?: understanding the candidacidal mechanism of salivary histatin 5. Eukaryot Cell. 2014;13(8):958-64.

56. Rayhan R, Xu L, Santarpia R, Tylenda C, Pollock J. Antifungal activities of salivary histidine-rich polypeptides against Candida albicans and other oral yeast isolates. Oral Microbiol Immunol. 1992;7(1):51-2.

57. Driscoll J, Zuo Y, Xu T, Troxler R, Oppenheim F, editors. Investigation of the anticandidal mechanism of histatins. JOURNAL OF DENTAL RESEARCH; 1996: AMER ASSOC DENTAL RESEARCH 1619 DUKE ST, ALEXANDRIA, VA 22314.

58. Tsai H, Bobek LA. Human salivary histatin-5 exerts potent fungicidal activity against Cryptococcus neoformans. Biochimica et Biophysica Acta (BBA)-General Subjects. 1997;1336(3):367-9.

59. Almståhl A, Wikström M, Groenink J. Lactoferrin, amylase and mucin MUC5B and their relation to the oral microflora in hyposalivation of different origins. Oral Microbiol Immunol. 2001;16(6):345-52.

60. Oppenheim F, Xu T, McMillian F, Levitz S, Diamond R, Offner G, et al. Histatins, a novel family of histidine-rich proteins in human parotid secretion. Isolation, characterization, primary structure, and fungistatic effects on Candida albicans. Journal of Biological Chemistry. 1988;263(16):7472-7.

61. Xu T, Levitz S, Diamond R, Oppenheim F. Anticandidal activity of major human salivary histatins. Infection and immunity. 1991;59(8):2549-54.

62. Driscoll J, Zuo Y, Xu T, Choi J, Troxler R, Oppenheim E. Functional comparison of native and recombinant human salivary histatin 1. J Dent Res. 1995;74(12):1837-44.

63. Raj PA, Edgerton M, Levine M. Salivary histatin 5: dependence of sequence, chain length, and helical conformation for candidacidal activity. Journal of Biological Chemistry. 1990;265(7):3898-905.

64. Kavanagh K, Dowd S. Histatins: antimicrobial peptides with therapeutic potential. Journal of pharmacy and pharmacology. 2004;56(3):285-9.

65. Helmerhorst EJ, Wim VTH, VEERMAN EC, Simoons-Smit I, Arie V. Synthetic histatin analogues with broad-spectrum antimicrobial activity. Biochem J. 1997;326(1):39-45.

66. Koshlukova SE, Lloyd TL, Araujo MW, Edgerton M. Salivary histatin 5 induces Non-lytic release of ATP fromCandida albicans leading to cell death. Journal of Biological Chemistry. 1999;274(27):18872-9.

67. Helmerhorst EJ, Troxler RF, Oppenheim FG. The human salivary peptide histatin 5 exerts its antifungal activity through the formation of reactive oxygen species. Proceedings of the National Academy of Sciences. 2001;98(25):14637-42.

68. Baev D, Li XS, Dong J, Keng P, Edgerton M. Human salivary histatin 5 causes disordered volume regulation and cell cycle arrest in Candida albicans. Infection and immunity. 2002;70(9):4777-84.

69. Shetty SR, Bhowmick S, Castelino R, Babu S. Drug induced xerostomia in elderly individuals: An institutional study. Contemporary clinical dentistry. 2012;3(2):173.

70. Anurag Gupta B, Epstein JB, Sroussi H. Hyposalivation in elderly patients. J Can Dent Assoc. 2006;72(9):841-6.

71. Mravak-Stipetić M. Xerostomia–diagnostics and treatment. Rad Hrvatske akademije znanosti i umjetnosti Medicinske znanosti. 2012(514= 38):69-90.

72. Grisius MM, Fox PC. Salivary gland diseases. Oral medicine—diagnosis and treatment 10th edn Spain: BC Decker Inc. 2003:248.

73. Twetman S. Treatment protocols: nonfluoride management of the caries disease process and available diagnostics. Dental Clinics of North America. 2010;54(3):527-40.

74. Borgnakke W, Taylor G, Anderson P, Shannon M. Oral and General Health-Exploring the Connection. Dry Mouth (Xerostomia): Diagnosis, Causes, Complications and Treatment Research Review DDPA. 2011:1-35.

75. Fox PC. Xerostomia: recognition and management. Dent Assist. 2008;77(5):18-48.

76. Organization WH. Global data on visual impairments 2010. Geneva. 2013.

77. Organization WH. Global status report on noncommunicable diseases 2010. Geneva, 2011. 2014.

78. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. Plos med. 2006;3(11):e442.

79. Negrato CA, Tarzia O. Buccal alterations in diabetes mellitus. Diabetology & metabolic syndrome. 2010;2(1):1.

80. Lamster IB, Lalla E, Borgnakke WS, Taylor GW. The relationship between oral health and diabetes mellitus. The Journal of the American Dental Association. 2008;139:19S-24S.

81. Association AD. Standards of Medical Care in Diabetes—2014. Diabetes Care 2014; 37 (Suppl. 1): S14–S80Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2014; 37 (Suppl. 1): S81–S90. Diabetes Care. 2014;37(3):887-.

82. Busato IMS, de Lima AAS, Deantoni CC, Brancher JA, Azevedo-Alanis LR, Machado MÂN, et al. Impact of hyperglycemia on xerostomia and salivary composition and flow rate of adolescents with type 1 diabetes mellitus: INTECH Open Access Publisher; 2011.

83. Chavez EM, Taylor GW, Borrell LN, Ship JA. Salivary function and glycemic control in older persons with diabetes. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000;89(3):305-11.

84. Carda C, Mosquera-Lloreda N, Salom L, Gomez de Ferraris M, Peydró A. Structural and functional salivary disorders in type 2 diabetic patients. Medicina Oral Patologia Oral y Cirugia Bucal. 2006;11(4):209.

85. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes estimates for the year 2000 and projections for 2030. Diabetes care. 2004;27(5):1047-53.

86. Bell G, Large D, Barclay S. Oral health care in diabetes mellitus. Dental update. 1999;26(8):322-8, 30.

87. Baldwin E. Oral health. The Lancet. 2009;373(9664):628-9.

88.   Al-Maskari AY, Al-Maskari MY, Al-Sudairy S. Oral manifestations and complications of diabetes mellitus: a review. Sultan Qaboos University Medical Journal. 2011;11(2):179.

89.   Chávez EM, Borrell LN, Taylor GW, Ship JA. A longitudinal analysis of salivary flow in control subjects and older adults with type 2 diabetes. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001;91(2):166-73.

90.   Chomkhakhai U, Thanakun S, Khovidhunkit S-oP, Khovidhunkit W, Thaweboon S. Oral health in Thai patients with metabolic syndrome. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2009;3(4):192-7.

91.   Khovidhunkit S-oP, Suwantuntula T, Thaweboon S, Mitrirattanakul S, Chomkhakhai U, Khovidhunkit W. Xerostomia, hyposalivation, and oral microbiota in type 2 diabetic patients: a preliminary study. 2009.

92.   McIntyre G. Oral candidosis. DENTAL UPDATE-LONDON-. 2001;28(3):132-9.

93.   Lamey PJ, Darwaza A, Fisher B, Samaranayake L, MacFarlane T, Frier B. Secretor status, candidal carriage and candidal infection in patients with diabetes mellitus. Journal of Oral Pathology & Medicine. 1988;17(7):354-7.

94.   Willis A, Coulter W, Fulton C, Hayes J, Bell P, Lamey PJ. Oral candidal carriage and infection in insulin-treated diabetic patients. Diabetic medicine. 1999;16(8):675-9.

95.   Akpan A, Morgan R. Oral candidiasis. Postgraduate medical journal. 2002;78(922):455-9.

96.   Sitheeque M, Samaranayake L. Chronic hyperplastic candidosis/candidiasis (candidal leukoplakia). Critical Reviews in Oral Biology & Medicine. 2003;14(4):253-67.

97.   Kleinkort J, Foley R. Laser: a preliminary report on its use in physical therapy. Clin Manag Phys Ther. 1982;2:30-2.

98.   Tunér J, Beck-Kristensen PH, Ross G, Ross A. Photobiomodulation in Dentistry. Principles and Practice of Laser Dentistry. 2nd ed: Mosby; 2015. p. 251.

99.   Commission IE. IEC 60825-1 Safety of laser products-part 1: equipment classification and, requirements. Ed; 2007.

100.   Hamblin MR, Demidova TN, editors. Mechanisms of low level light therapy. Biomedical Optics 2006; 2006: International Society for Optics and Photonics.

101.   Gao X, Xing D. Molecular mechanisms of cell proliferation induced by low power laser irradiation. Journal of biomedical science. 2009;16(1):1.

102.   Basford JR. Low intensity laser therapy: still not an established clinical tool. Lasers in surgery and medicine. 1995;16(4):331-42.

103.   Ohshiro T, Calderhead RG. Low level laser therapy: a practical introduction: John Wiley & Sons; 1988.

104.   Plavnik LM, De Crosa ME, Malberti AI. Effect of low-power radiation (helium/neon) upon submandibulary glands. Journal of clinical laser medicine & surgery. 2003;21(4):219-25.

105.   Sharma SK, Kharkwal GB, Sajo M, Huang YY, De Taboada L, McCarthy T, et al. Dose response effects of 810 nm laser light on mouse primary cortical neurons. Lasers in surgery and medicine. 2011;43(8):851-9.

106. Dietary Guidelines for Americans, 2010. In: Services UDoAaUDoHaH, editor. 7th ed. Washington DC: U.S Government Printing Office; December 2010. p. 31.
107. Degenhardt L, Hall W, Warner-Smith M, Lynskey M. Illicit drug use. Comparative quantification of health risks: global and regional burden of disease attributable to selected major risk factors. 2004;1:1109-76.
108. Simões A, Nicolau J, De Souza DN, Ferreira LS, de Paula Eduardo C, Apel C, et al. Effect of defocused infrared diode laser on salivary flow rate and some salivary parameters of rats. Clin Oral Investig. 2008;12(1):25-30.
109. Kana JS, Hutschenreiter G. Effect of low—power density laser radiation on healing of open skin wounds in rats. Arch Surg. 1981;116(3):293-6.
110. Navazesh M, Christensen C. A comparison of whole mouth resting and stimulated salivary measurement procedures. J Dent Res. 1982;61(10):1158-62.
111. Ahmad M, Piludu M, Oppenheim FG, Helmerhorst EJ, Hand AR. Immunocytochemical localization of histatins in human salivary glands. J Histochem Cytochem. 2004;52(3):361-70.
112. van der Putten G-J, Brand HS, Schols JM, de Baat C. The diagnostic suitability of a xerostomia questionnaire and the association between xerostomia, hyposalivation and medication use in a group of nursing home residents. Clin Oral Investig. 2011;15(2):185-92.
113. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract. 2011;94(3):311-21.
114. Rathmann W, Giani G. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes care. 2004;27(10):2568-9.
115. Rawdaree P, Ngarmukos C, Deerochanawong C, Suwanwalaikorn S, Chetthakul T, Krittiyawong S, et al. Thailand diabetes registry (TDR) project: clinical status and long term vascular complications in diabetic patients. J Med Assoc Thai. 2006;89(Suppl 1):S1-9.
116. Bennett C, Guo M, Dharmage S. HbA1c as a screening tool for detection of type 2 diabetes: a systematic review. Diabetic Medicine. 2007;24(4):333-43.
117. Aekplakorn W, Chariyalertsak S, Kessomboon P, Sangthong R, Inthawong R, Putwatana P, et al. Prevalence and management of diabetes and metabolic risk factors in Thai adults. Diabetes care. 2011;34(9):1980-5.
118. Nagler RM, Hershkovich O. Relationships between age, drugs, oral sensorial complaints and salivary profile. Archives of Oral Biology. 2005;50(1):7-16.
119. Flink H, Tegelberg Å, Lagerlöf F. Influence of the time of measurement of unstimulated human whole saliva on the diagnosis of hyposalivation. Archives of oral biology. 2005;50(6):553-9.
120. Bergdahl M. Salivary flow and oral complaints in adult dental patients. Community Dent Oral Epidemiol. 2000;28(1):59-66.
121. Terlević Dabić D, Jurišić S, Vučićević Boras V, Gabrić D, Bago I, Vrdoljak DV. The Effectiveness of Low-Level Laser Therapy in Patients with Drug-Induced Hyposalivation: A Pilot Study. Photomed Laser Surg. 2016;34(9):389-93.

122. Yu W, Naim JO, McGowan M, Ippolito K, Lanzafame RJ. Photomodulation of oxidative metabolism and electron chain enzymes in rat liver mitochondria. J Photochem Photobiol. 1997;66(6):866-71.

123. Saleh J, Figueiredo MAZ, Cherubini K, Braga-Filho A, Salum FG. Effect of low-level laser therapy on radiotherapy-induced hyposalivation and xerostomia: a pilot study. Photomed Laser Surg. 2014;32(10):546-52.

124. Guchelaar H-J, Vermes A, Meerwaldt J. Radiation-induced xerostomia: pathophysiology, clinical course and supportive treatment. Support Care Cancer. 1997;5(4):281-8.

125. Ghezzi E, Lange L, Ship J. Determination of variation of stimulated salivary flow rates. J Dent Res. 2000;79(11):1874-8.

126. Petrone D, Condemi JJ, Fife R, Gluck O, Cohen S, Dalgin P. A double-blind, randomized, placebo-controlled study of cevimeline in Sjögren's syndrome patients with xerostomia and keratoconjunctivitis sicca. Arthritis & Rheumatism. 2002;46(3):748-54.

127. Smith RG, Burtner AP. Oral side-effects of the most frequently prescribed drugs. Spec Care Dentist. 1994;14(3):96-102.

128. Bergdahl M, Bergdahl J, Johansson I. Depressive symptoms in individuals with idiopathic subjective dry mouth. Journal of oral pathology & medicine. 1997;26(10):448-50.

129. Gabryel-Porowska H, Gornowicz A, Bielawska A, Wójcicka A, Maciorkowska E, Grabowska SZ, et al. Mucin levels in saliva of adolescents with dental caries. Med Sci Monit. 2014;20:72-7.

130. Li X, Wang L, Nunes D, Troxler R, Offner G. Pro-inflammatory cytokines up-regulate MUC1 gene expression in oral epithelial cells. J Dent Res. 2003;82(11):883-7.

131. Pradhan A. Obesity, metabolic syndrome, and type 2 diabetes: inflammatory basis of glucose metabolic disorders. Nutr Rev. 2007;65(suppl 3):S152-S6.

132. Takehara S, Yanagishita M, Podyma-Inoue KA, Kawaguchi Y. Degradation of MUC7 and MUC5B in human saliva. PLoS One. 2013;8(7):e69059.

133. Cabras T, Pisano E, Mastinu A, Denotti G, Pusceddu PP, Inzitari R, et al. Alterations of the salivary secretory peptidome profile in children affected by type 1 diabetes. Mol Cell Proteomics. 2010;9(10):2099-108.

134. Maver-Biscanin M, Mravak-Stipetic M, Jerolimov V. Effect of low-level laser therapy on Candida albicans growth in patients with denture stomatitis. Photomedicine and Laser Surgery 2005;23(3):328-32.

135. Pışırıcıler R, Çalişka-Ak E, Emeklı-Alturfan E, Yarat A, Canberk Y. Impact of Experimental Hyperlipidemia on Histology of Major Salivary Glands. Medical Journal of Trakya University/Trakya Universitesi Tip Fakultesi Dergisi. 2009;26(4).

136. Iontcheva I, Oppenheim F, Troxler R. Human salivary mucin MG1 selectively forms heterotypic complexes with amylase, proline-rich proteins, statherin, and histatins. J Dent Res. 1997;76(3):734-43.

137. Simoes A, de Campos L, de Souza DN, de Matos JA, Freitas PM, Nicolau J. Laser phototherapy as topical prophylaxis against radiation-induced xerostomia. Photomed Laser Surg. 2010;28(3):357-63.

138. Busato IMS, Ignácio SA, Brancher JA, Moysés ST, Azevedo-Alanis LR. Impact of clinical status and salivary conditions on xerostomia and oral health-related quality of life of adolescents with type 1 diabetes mellitus. Community Dent Oral Epidemiol. 2012;40(1):62-9.

139. Anderson L, Garrett J. Lipid accumulation in the major salivary glands of streptozotocin-diabetic rats. Archives of oral biology. 1986;31(7):469-75.

140. Enwemeka CS, Parker JC, Dowdy DS, Harkness EE, Harkness LE, Woodruff LD. The efficacy of low-power lasers in tissue repair and pain control: a meta-analysis study. Photomedicine and Laser Therapy. 2004;22(4):323-9.

141. Kert J, Rose L. Clinical Laser Therapy Low Level Laser Therapy: Scandinavian Medical Laser Technology; 1989.

142. Bliddal H, Hellesen C, Ditlevsen P, Asselberghs J, Lyager L. Soft-laser therapy of rheumatoid arthritis. Scand J Rheumatol. 1987;16(4):225-8.

143. Laakso L, Richardson C, Cramond T. Factors affecting low level laser therapy. Aust J Physiother. 1993;39(2):95-9.

144. Mester E, Mester AF, Mester A. The biomedical effects of laser application. Lasers in surgery and medicine. 1985;5(1):31-9.

145. Hawkins D, Abrahamse H. Effect of multiple exposures of low-level laser therapy on the cellular responses of wounded human skin fibroblasts. Photomedicine and Laser Therapy. 2006;24(6):705-14.

146. Bibikova A, Oron U. Attenuation of the process of muscle regeneration in the toad gastrocnemius muscle by low energy laser irradiation. Lasers in surgery and medicine. 1994;14(4):355-61.

147. Steinlechner CW, Dyson M. The effects of low level laser therapy on the proliferation of keratinocytes. Laser Therapy. 1993;5(2):65-73.

APPENDIX

Appendix 1

Salivary flow rate comparison between male and female

	1st visit		6th visit		6 week FU	
	Male (n=6)	Female (n=6)	Male (n=6)	Female (n=6)	Male (n=6)	Female (n=4)
USFR	0.141	0.140	0.363	0.228	0.365	0.264
SSFR	0.822	0.763	0.922	0.911	0.967	0.901

Appendix 2

Raw data of USFR

Patient	USFR (mL/min)			Difference between 6th visit and 1st visit (mL/min)	Difference between 6-week FU and 6th visit (mL/min)
	1st visit	6th visit	6-week FU		
001	0.013	0.137	0.202	0.124	0.065
002	0.228	0.387	■	0.159	■
003	0.176	0.32	0.334	0.144	0.014
004	0.072	0.104	■	0.032	■
005	0.202	0.41	0.746	0.208	0.336
006	0.094	0.112	0.139	0.018	0.027
007	0.214	0.387	0.376	0.173	-0.011
008	0.217	0.673	0.37	0.456	-0.303
009	0.073	0.278	0.302	0.205	0.024
010	0.236	0.195	0.26	-0.041	0.065
011	0.05	0.32	0.26	0.27	-0.06
012	0.12	0.23	0.26	0.11	0.03

Appendix 3
Raw data of SSFR

Patient	SSFR (mL/min)			Difference between 6th visit and 1st visit (mL/min)	Difference between 6-week FU and 6th visit (mL/min)
	1st visit	6th visit	6-week FU		
001	0.496	0.841	0.521	0.345	-0.32
002	0.37	0.528	■	0.158	■
003	1.326	1.508	1.461	0.182	-0.047
004	0.503	0.341	■	-0.162	■
005	0.648	1.533	1.73	0.885	0.197
006	0.251	0.375	0.78	0.124	0.405
007	1.795	1.01	1.26	-0.785	0.25
008	1.156	1.385	0.86	0.229	-0.525
009	0.416	0.63	0.506	0.214	-0.124
010	1.233	1.31	1.01	0.077	-0.3
011	0.67	0.6	0.67	-0.07	0.07
012	0.65	0.94	0.615	0.29	-0.325

Appendix 4
Multiple regression analysis between HbA1C, age, DM duration and USFR

	Beta (Unstandardized coefficient)*			P – value*		
	USFR at 1st visit[#]	USFR at 6th visit[##]	USFR at 6-week FU[###]	USFR at 1st visit[#]	USFR at 6th visit[##]	USFR at 6-week FU[###]
HbA1C	0.005	0.014	0.039	0.688	0.517	0.054
Age	-0.002	-0.005	0.002	0.409	0.146	0.480
DM duration	-0.002	-0.002	-0.008	0.613	0.825	0.215

*Multiple regression analysis. $P < 0.05$ was considered significant

$^{\#}R^2 = 0.230$ $^{\#\#}R^2 = 0.393$ $^{\#\#\#}R^2 = 0.683$. R^2 represents coefficient of determination for the regression model

Appendix 5
Amount of drugs consumed per visit

Patient	Drugs consumed			Total drugs consumed		
	1st visit	Between 1st visit and 6th visit	Between 6th visit and 6-week FU	1st visit	Until 6th visit	Until 6-week FU
001	Rocatrol	Elmetacin Spray	Bioflor	5	7	20
	Myonal	Celebrex	Paramed			
	Lyrica		Reparil			
	Lorazepam		MOM suspension			
	Pyridium		Forlax			
			Madopar			
			Plendil Dimenhydrinate			
			Betalol			
			Zimmex			
			Prevacid FDT			
			Allopurinol			
			Metformin			
			Betamed			
002	thyroxine	Celebrex	■	2	10	■
	amlodipine	Elmetacin spray				
		Baclofen				
		Galvus Met TAB				

		diamicron				
		Lipitor				
		Euthyrox				
		diprospan inject				
003		Sodium carbonate mouthwash		2	4	4
	metformin		NC			
	bistatin	Floucinolone orabase				
004	glipizide	NC	■	6	6	■
	amlodipine					
	losartan potassium					
	simvastatin					
	aspirin					
	hydrochlorothiazide					
005	diamicron	Niflec	NC	6	9	9
	actos-MET	Hyoscine				
	Niflec	Midazolam inj				
	ultravist-300					
	aspirin baby					
	viagra					
006	ezetrol	NC	Paramed	14	14	16
	trajenta		Cardura			
	hemax					
	alprazolam					
	soda mint					
	folic acid					
	hydralazine					
	concor					
	crestor					

	plavix					
	Novonorm					
	apresoline					
	ketosteril					
	ferli-6					
007	Telfast	NC	NC	11	11	11
	dextromethor phan					
	mucosolvan-PL					
	zithromax					
	hidresac					
	colofac					
	medicnor					
	medicplex					
	samarin					
	lorazene					
	paramed					
008	Kombiglyze	NC	NC	8	8	8
	novolin penfill					
	levemir					
	actos MET					
	vytorin					
	diamicron					
	aspirin baby					
	forxiga					
009	triliplix	Prednisolon	NC	4	7	7
	enalapril	Arcoxia				
	glucophage	Norgesic				
	diamicron					
010	metformin	NC	NC	3	3	3

	paramed					
	celebrex					
011	forxiga	NC	NC	4	4	4
	januvia					
	crestor					
	glucophage					
012	glucophage	NC	NC			
	glipizide			2	2	2

Appendix 6

Demographic data

Patient	Age	Sex	HbA1C	DM duration
001	86	F	7	20
002	58	F	8.8	7
003	73	F	7.1	2
004	76	F	6	5
005	65	M	14.4	0.25
006	65	M	7.2	20
007	44	M	6.5	6
008	44	M	9.7	10
009	37	M	11.8	0.17
010	57	F	8.8	10
011	57	M	6.5	10
012	68	F	7	2

Appendix 7

Multiple regression analysis between amount of drugs consumed and USFR

	USFR at 6th visit*		USFR at 6-week FU*	
	Beta (Unstandardized coefficient)	P - value	Beta (Unstandardized coefficient)	P - value

	0.005	0.717		
Amount of drugs until 6th visit[#]	0.005	0.717		
Amount of drugs until 6-week FU[##]			-0.005	0.651

*Multiple regression analysis. $P < 0.05$ was considered significant.

[#]$R^2 = 0.014$ [##]$R^2 = 0.027$

Appendix 8
HbA1C and USFR scatterplot at the 1st visit, 6th visit, and 6-week FU
1st visit
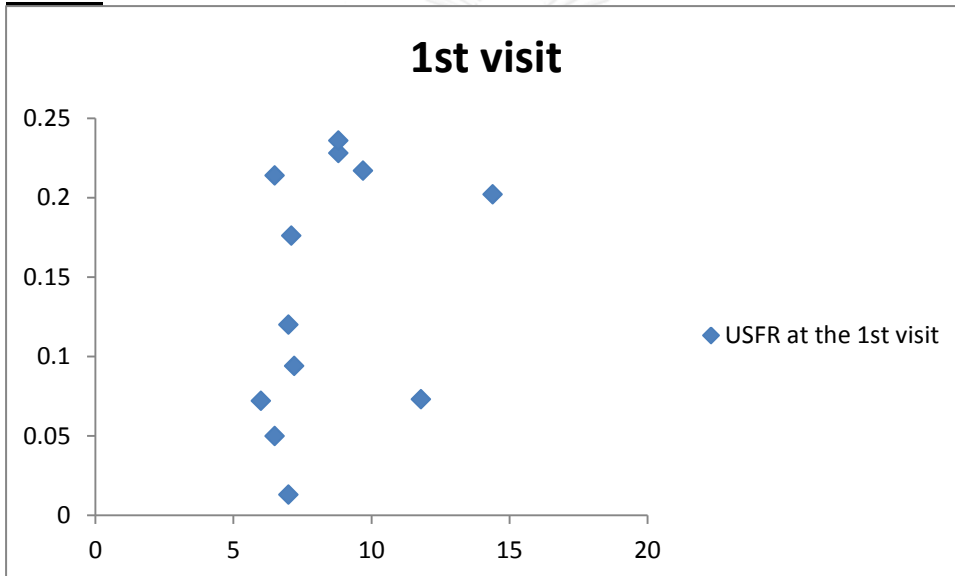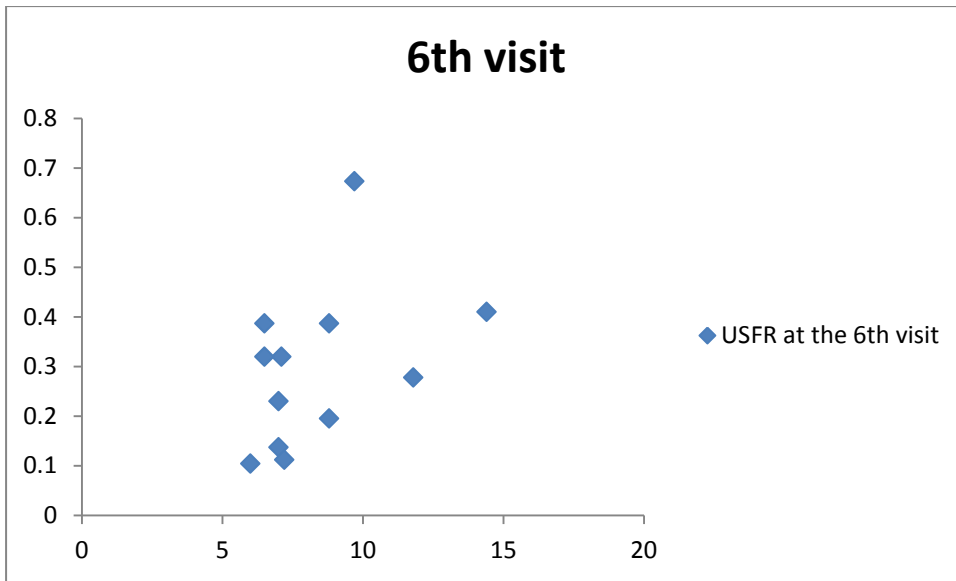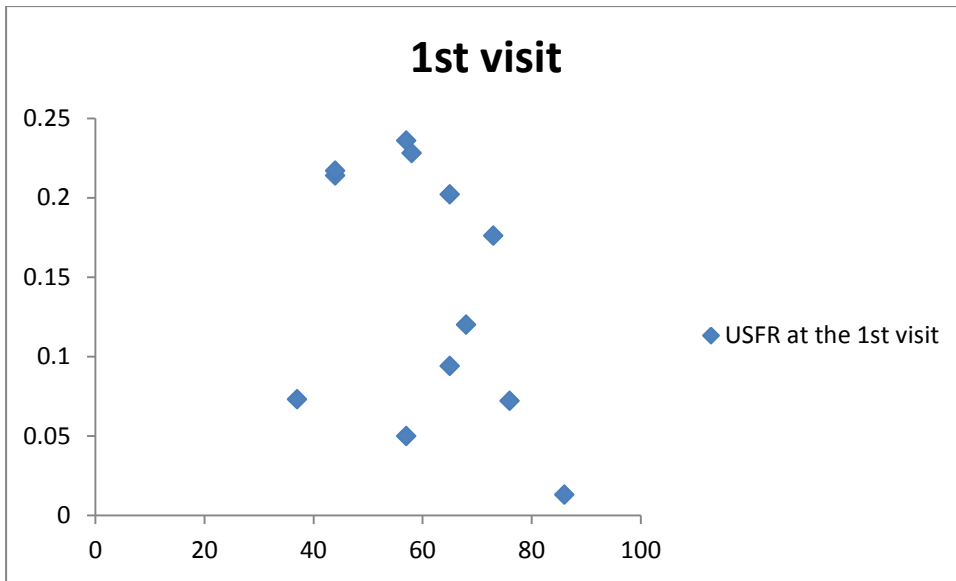

6th visit
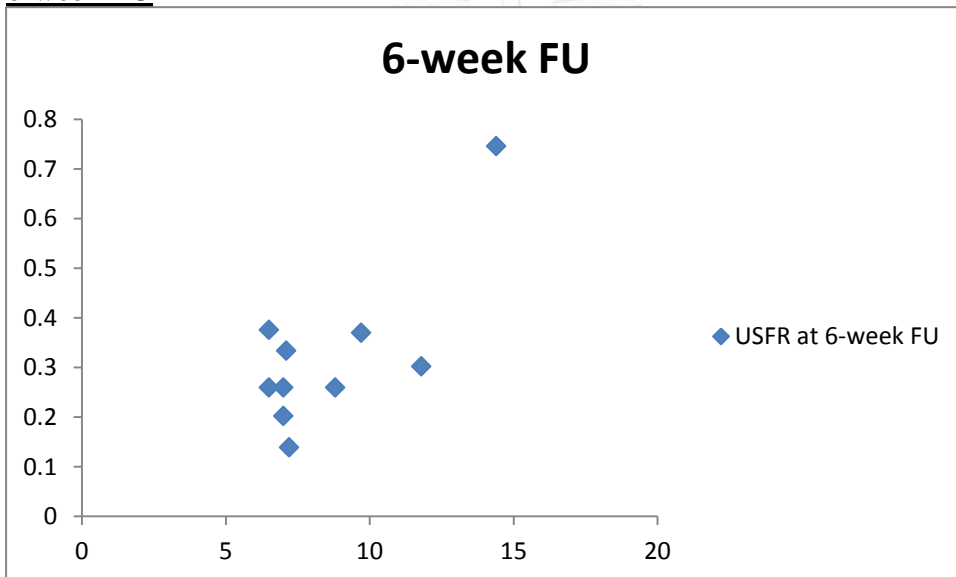
6th visit

6-week FU

Appendix 9
Age and USFR scatterplot at the 1st visit, 6th visit, and 6-week FU

1st visit
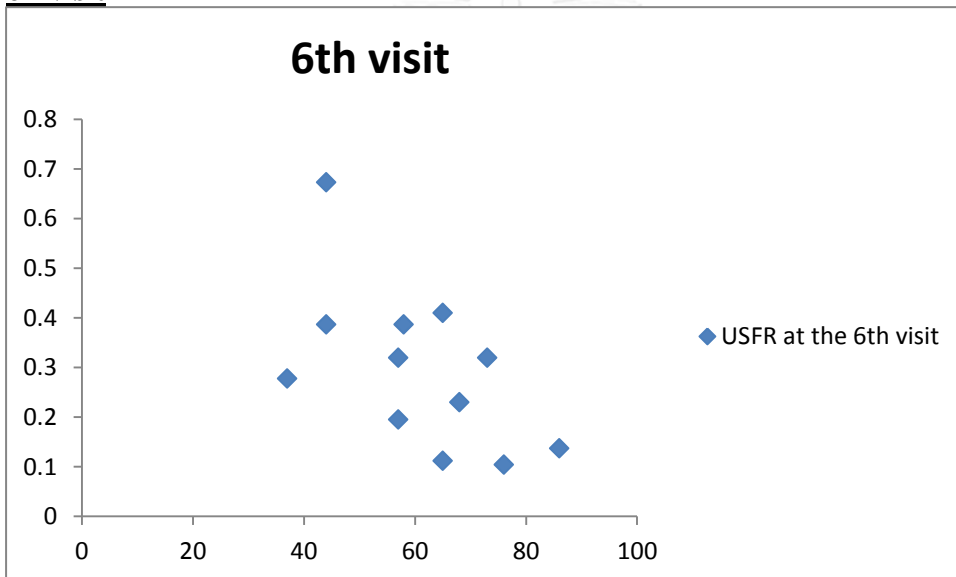
6th visit
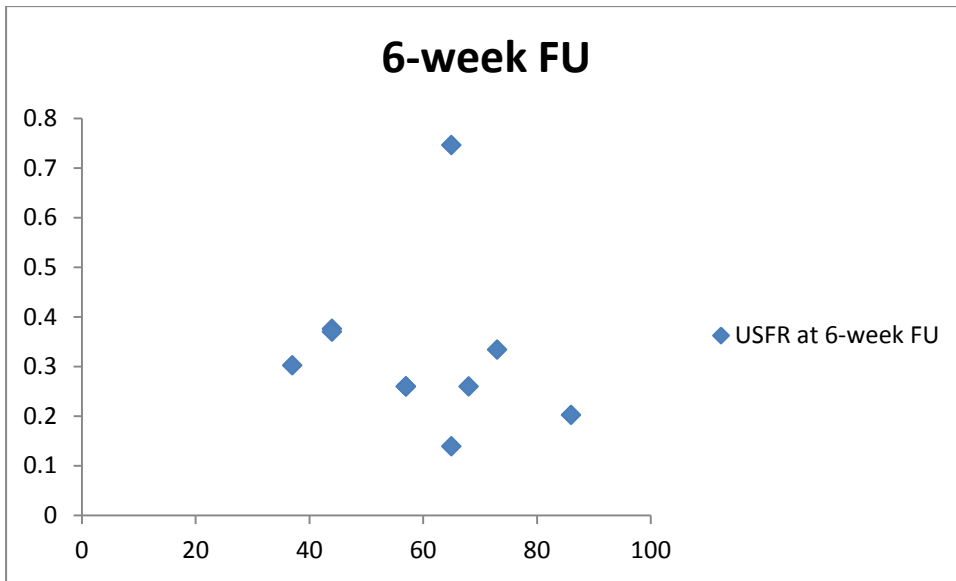
6-week FU

6-week FU

Appendix 10

DM duration and USFR scatterplot at the 1st visit, 6th visit, and 6-week FU

1st visit

1st visit

6th visit
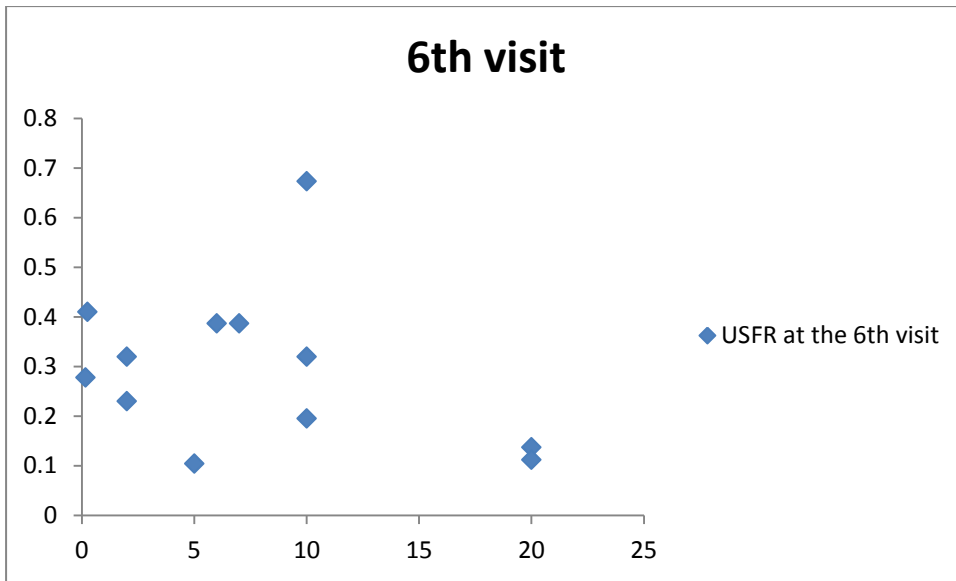
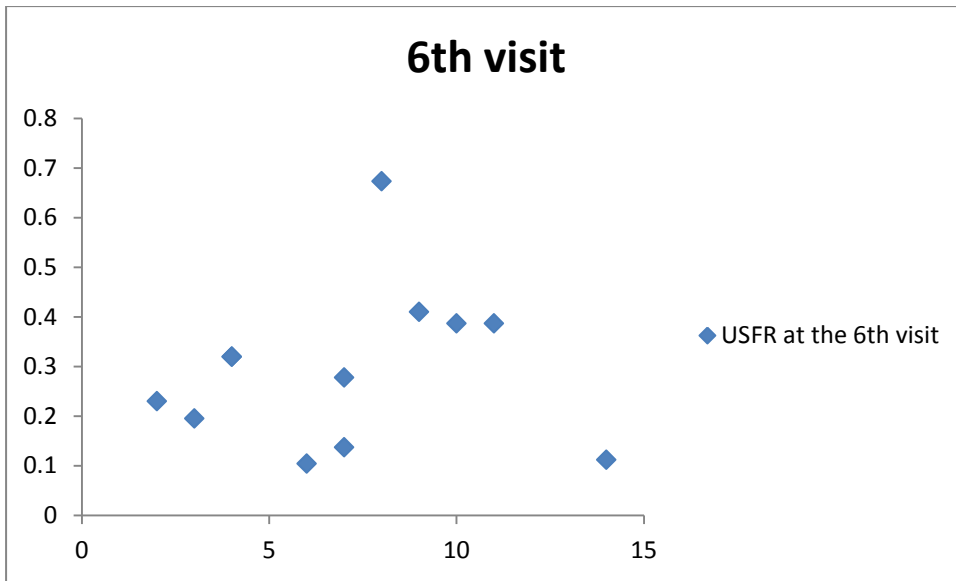**6th visit**
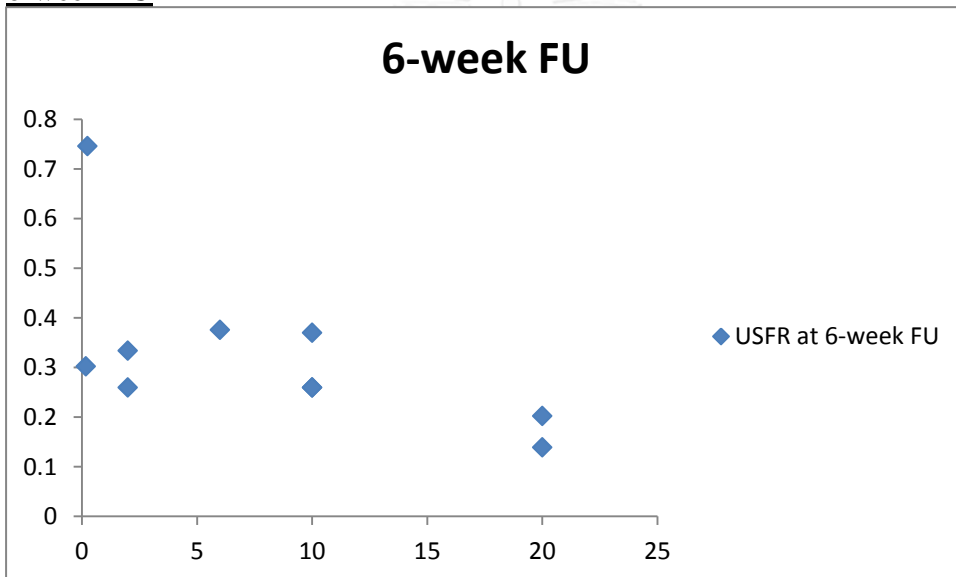
6-week FU

6-week FU

Appendix 11
Amount of drugs consumed and USFR scatterplot at the 6th visit and 6-week
FU
6th visit

6-week FU

REFERENCES

(1)

APPENDIX

VITA