THE EFFICACY OF VITAMIN C ORAL SUPPLEMENT ON WOUND HEALING AFTER TOOTH EXTRACTION

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Oral and Maxillofacial Surgery
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ประสิทธิภาพของการให้วิตามินซีเสริมชนิดรับประทานต่อการหายของแผลถอนฟัน

น.ส.นันทนัท พิศาลสิทธิ์สกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Thesis Title	THE EFFICACY OF VITAMIN C ORAL SUPPLEMENT ON WOUND HEALING AFTER TOOTH EXTRACTION
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Field of Study	Oral and Maxillofacial Surgery
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นันทนัท พิศาลสิทธิ์สกุล : ประสิทธิภาพของการให้วิตามินซีเสริมชนิดรับประทานต่อการหายของแผลถอนฟัน. (THE EFFICACY OF VITAMIN C ORAL SUPPLEMENT ON WOUND HEALING AFTER TOOTH EXTRACTION) อ.ที่ปรึกษาหลัก : อ. ทญ. ดร.ภัคสินี กมลรัตนกุล

วัตถุประสงค์: เพื่อศึกษาผลของวิตามินซีเสริมชนิดรับประทานต่อการหายของแผลถอนฟันและเปรียบเทียบปริมาณวิตามินซีเสริมที่เหมาะสมต่อการหายของแผลถอนฟันในผู้ป่วย

วิธีการศึกษา: ผู้ป่วยที่ต้องได้รับการถอนฟันกรามน้อยที่ไม่ติดเชื้อที่สมมาตรกันด้านซ้ายและขวาจำนวน 42 คน ถูกแบ่งออกเป็น 3 กลุ่มโดยการสุ่ม (กลุ่มละ 14 คน) ได้แก่ 1.ยาหลอก เทียบกับ วิตามินซี 600 มิลลิกรัมต่อวัน 2.ยาหลอก เทียบกับ วิตามินซี 1,500 มิลลิกรัมต่อวัน และ 3. วิตามินซี 600 มิลลิกรัมต่อวัน เทียบกับ วิตามินซี 1,500 มิลลิกรัมต่อวัน ผู้ป่วยแต่ละคนจะได้รับยาหลอกหรือวิตามินซี 3 ครั้งต่อวันเป็นระยะเวลา 10 วัน มีการวัดแผลในวันที่ถอนฟัน วันที่ 7 และวันที่ 21 หลังถอนฟัน หลังจากนั้นจึงถอนฟันอีกข้างด้วยวิธีการเดียวกับฟันซี่แรก โดยมีผู้วัด 2 คนวัดในแนวด้านแก้ม-ด้านลิ้น แนวใกล้กลาง-ไกลกลาง วัดความลึกของแผล และย้อมสีที่แผลโดยใช้สารละลายโทลูอิดีนบลู 1 เปอร์เซ็นต์ ผู้ป่วยต้องบันทึกคะแนนความเจ็บปวดในช่วง 3 วันแรกหลังถอนฟัน และบันทึกอาหารที่ผู้ป่วยรับประทานโดยเฉพาะอาหารที่มีวิตามินซีสูงในช่วง 7 วันหลังถอนฟัน

ผลการศึกษา: แผลถอนฟันของผู้ป่วยด้านที่ได้รับวิตามินซี 600 มิลลิกรัมต่อวันในด้านใกล้กลาง-ไกลกลางมีขนาดลดลงในสัดส่วนที่มากกว่าด้านที่ได้รับยาหลอกอย่างมีนัยสำคัญทางสถิติระหว่างวันที่ 0 ถึงวันที่ 7 หลังถอนฟัน ($p<0.05$) และคะแนนความเจ็บปวดหลังถอนฟันในช่วง 1-3 วันแรกในด้านที่ได้รับวิตามินซี 600 มิลลิกรัมต่อวันน้อยกว่าด้านที่ได้รับยาหลอกอย่างมีนัยสำคัญทางสถิติ ($p<0.05$)

บทสรุป: การรับประทานวิตามินซีเสริม 600 มิลลิกรัมต่อวันเป็นระยะเวลา 10 วัน มีแนวโน้มช่วยในการหายของแผลถอนฟันและช่วยลดอาการปวดหลังถอนฟัน

สาขาวิชา	ศัลยศาสตร์ช่องปากและแม็กซิลโลเฟเชียล	ลายมือชื่อนิสิต .....
ปีการศึกษา	2562	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

6075818132 : MAJOR ORAL AND MAXILLOFACIAL SURGERY
KEYWORD: ASCORBIC ACID, VITAMIN C, TOOTH EXTRACTION, EXTRACTION WOUND, WOUND HEALING

Nanthanut Pisalsitsakul : THE EFFICACY OF VITAMIN C ORAL SUPPLEMENT ON WOUND HEALING AFTER TOOTH EXTRACTION. Advisor: Paksinee Kamolratanakul, DDS., Ph.D.

Objectives: The purpose of this study was to evaluate the effect and proper dosage of oral vitamin C supplement on post-extraction wound healing.

Methods: This study was a split mouth, double-blind, randomized-controlled clinical trial of 42 patients who underwent symmetric bilateral non-infected premolars extraction. The patients were randomly divided into 3 groups (14 patients for each group); 1.placebo vs vitamin C 600 mg/d, 2.placebo vs vitamin C 1,500 mg/d and 3.vitamin C 600 mg/d vs vitamin C 1,500 mg/d. Each group was prescribed placebo and/or vitamin C three times a day for 10 days after each tooth extraction. The assessment of the wound was performed on day 0, 7 and 21 then then the other side extraction was performed with the same protocol. Size of the extraction wound in bucco-lingual width, mesio-distal width, depth and 1% toluidine blue stained were collected by two examiners. Pain score and high vitamin C-containing diets were recorded by each patient on the first three days and seven days, respectively.

Results: The percentage reduction of the extraction wound size in mesiodistal dimension between day 0 and day 7 of teeth receiving vitamin C 600 mg daily was more than that in placebo ($P < 0.05$). Pain scores on day 1-3 after tooth extraction of teeth receiving vitamin C 600 mg daily was significantly lower than the placebo side ($P < 0.05$).

Conclusion: Taking oral vitamin C 600 mg/d for 10 days after tooth extraction tended to promote extraction wound healing and reduced post-operative pain.

Field of Study:	Oral and Maxillofacial Surgery	Student's Signature .....
Academic Year:	2019	Advisor's Signature .....

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Nanthanut Pisalsitsakul

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CHAPTER I

INTRODUCTION

1.1 Background and Rationale

Tooth extraction is a common dental procedure (1). However, there are regularly minor post-operative complications including dry socket, post-operative bleeding, pain, infection and delayed wound healing which impact on patients' quality of life and increase clinical workloads and cost (2-5).

Normal wound healing process starts with hemostasis and progresses through inflammation, proliferation and remodeling phases, all of which are overlapping (2, 4, 5). Delayed wound healing is failure to progress through the normal phases above and nutrition deficiency such as vitamin C is one of the significant factors (5, 6).

Vitamin C or ascorbic acid, which human have to obtain only from their diet, can promote wound healing by accelerating termination of the inflammatory phase of wound healing in animals (1, 2). Consistently, clinical studies showed that patients who take vitamin C supplement had shorter wound healing time (7, 8).

The role of vitamin C in the healing of wounds has been investigated in a variety of studies in both laboratory investigation and clinical trials (1, 2, 5, 8-15). However, there is limited evidence from human studies, especially in dental field. Few studies reported that dose of vitamin C supplement 500 mg orally 3 or 4 times/day promotes extraction wound healing (8, 15). One study prescribed vitamin C 3 times/day for 3 weeks (8), another prescribed vitamin C 4 times/day for 10 days (15). However, no study reports either an effect of lower dose of vitamin C 3 times/day or effect of vitamin C on extraction wound size. To our knowledge, the effect of vitamin C on cell migration, proliferation or function mostly depends on the dosage reported in in vitro studies (13, 16, 17). Therefore, the purpose of this study

was to investigate the efficacy and to compare dosage of vitamin C oral supplement on extraction wound healing regarding to size of the extraction wound and connective tissue staining in split-mouth clinical study. We hypothesized that proper dosage and administration route might accelerate healing of extraction wound and provide proper tissue for dental substitution and esthetic development.

1.2 Research Question

-Does Vitamin C oral supplement promote tooth extraction wound healing?

-Does the dosage of vitamin C oral supplement relate to tooth extraction wound healing?

1.3 Objective

1. To investigate whether vitamin C oral supplement promotes wound healing after tooth extraction.
2. To investigate whether the dosage of vitamin C oral supplement relates to wound healing after tooth extraction.

1.4 Hypothesis

$H_{0\ (1)}$: Vitamin C oral supplement in patients undergoing tooth extraction does not promote tooth extraction wound healing.

$H_{A\ (1)}$: Vitamin C oral supplement in patients undergoing tooth extraction promotes tooth extraction wound healing.

$H_{0\ (2)}$: Dosage of vitamin C oral supplement does not relate to tooth extraction wound healing.

$H_{A\ (2)}$: Dosage of vitamin C oral supplement relates to tooth extraction wound healing.

1.5 Conceptual framework

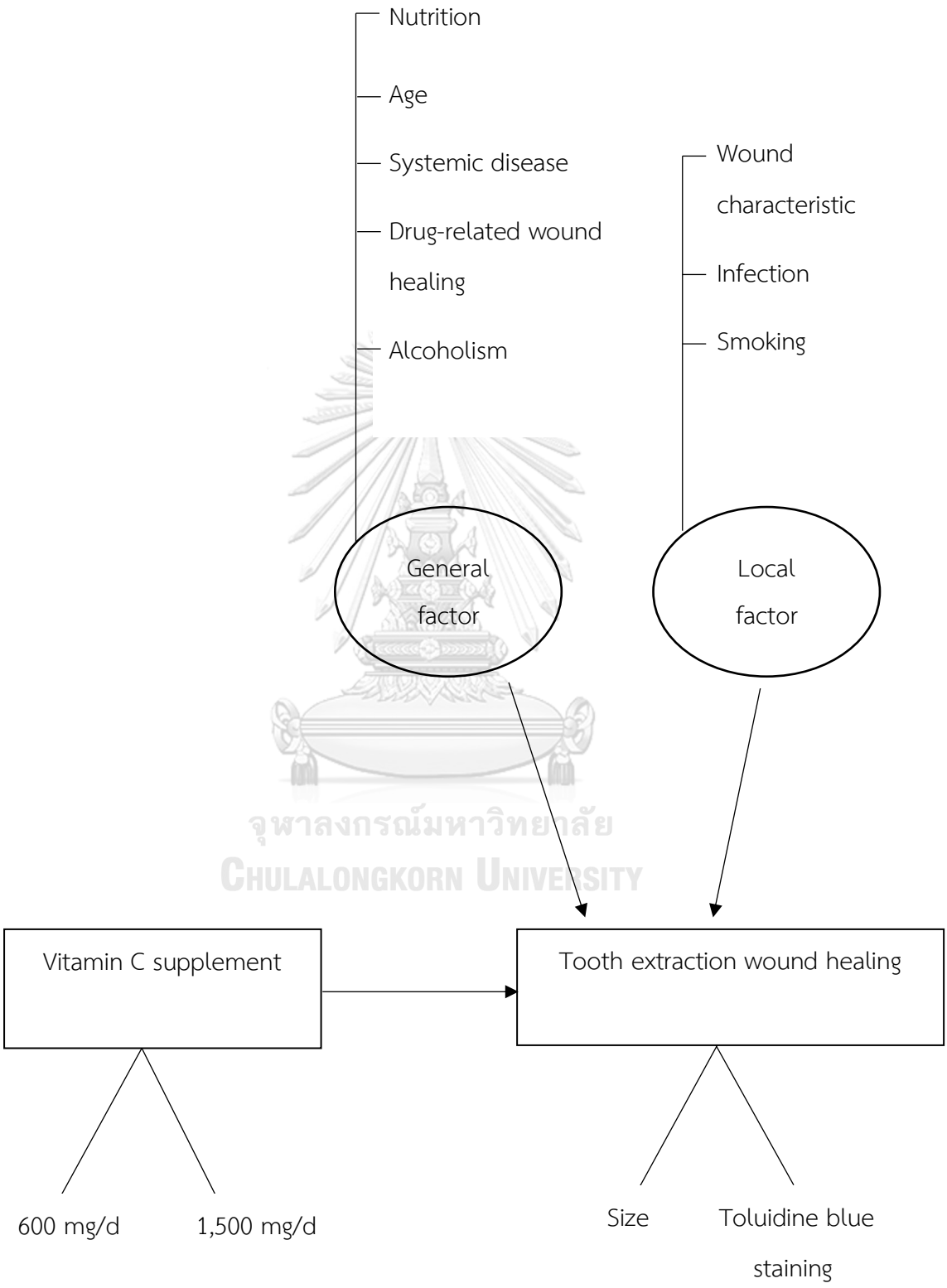


Figure 1 Conceptual framework

1.6 Keywords

Vitamin C, Ascorbic acid, Tooth extraction, Wound healing

1.7 Defined terms

Delayed wound healing: fails to progress through the normal stages of healing (5)

1.8 Limitation of the study

1. We could not control vitamin C in the circulation and we did not know how much vitamin C released from the organs or deposited at injured tissue.
2. Patients might also receive vitamin C from their regular diet, so we did not know the exactly amounts of vitamin C they received. As we assumed that each patient had a similar diet during the study, adding on vitamin C supplement might increase the level of vitamin C serum and achieved the treatment dose which needs more dose than daily recommended dose.

1.9 Expected benefit

This study ensured the efficacy of vitamin C oral supplement on tooth extraction wound and provide the suitable concentration of vitamin C that should be prescribed for promoting healing of tooth extraction wound. Moreover, we established further knowledge about vitamin C and dental healing and vitamin C may be routinely prescribed in proper dosage and administration to patients after minor oral surgery in the future.

CHAPTER II

REVIEWS OF LITERATURES

2.1 Wound healing

Wound healing is a normal physiological process in the human body which responses to restore the structure and function of injured tissue (2, 5, 9). The process requires both macronutrients and micronutrients (18, 19). Normal wound healing consists of four phases: hemostasis, inflammation, proliferation, and remodeling (2, 4, 5, 20). These dynamic processes are all overlapping phases which have to occur in the appropriate sequence at a specific time in order to heal successfully (2, 5, 9, 21).

Chemokines and cytokines play important roles on cellular events of the wound healing process. When the wounds or injuries occur, hemostasis begins immediately by vasoconstriction and fibrin clot formation. Pro-inflammatory cytokines and growth factors such as transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF) are released from the clot and surrounding wound tissue. When bleeding is stopped, the inflammatory phase continues and proceeds with the migration of polymorphonuclear neutrophils (PMN), macrophages and lymphocytes.

Neutrophil infiltration is to clear invading microbes and tissue debris at the wound site. Macrophages clear apoptotic cells and stimulate fibroblast migration which promotes the change to proliferation phase. T-lymphocyte population is highest during the late-proliferation to the early-remodeling phase but the role of them are still controversial.

Overlapping with the inflammation phase, the proliferative phase associated with epithelialization. Fibroblasts and endothelial cells promote angiogenesis and collagen formation. Apart from collagen, fibroblasts produce glycosaminoglycans and

proteoglycans which are the composition of the extracellular matrix (ECM). ECM synthesis shows that the process comes to the final phase of healing (2, 5).

2.2 Extraction socket healing

Dental socket healing is classified into five stages. In first stage, red and white blood cells form the coagulation. The second stage which occurs in 4- to 5- day period involves in the replacement of the clot by granulation tissue. The third stage is in 14- to 16-day period. This stage involves the replacement of the granulation tissue by connective tissue which contains spindle-shaped fibroblasts, collagen, and ground substance. Osteoid calcification takes place at the base of the socket in the fourth stage. Osteoid formation is initially found around the base of the socket at 7 to 10 days post-extraction, following by bone trabeculae formation at 6 weeks post extraction. In the fifth stage, the socket is completely close by epithelial cell in 24 to 35 days post-extraction (22, 23).

2.3 Vitamin C

Vitamin C or L-ascorbic acid (2,3-didehydro-L-threo-hexano-1,4-lactone) is an essential water-soluble vitamin for humans (1, 6, 10, 19). It plays an important role of various biochemical and physiological processes in the body and also acts as an antioxidant (1, 2, 5, 6). In order to survival, humans need ascorbic acid from their diet such as fresh fruits and vegetables or other supplement because normally, humans cannot synthesize vitamin C (2, 6, 10, 24). The example amount of vitamin C in foods is presented in Table 1 (25). The high concentration of vitamin C is found in many organs in humans and shown in Table 2 (26).

Table 1 The example amounts of vitamin C in foods.

Food	Serving size	Vitamin C (mg)
Vegetables		
Asparagus, frozen, cooked	6 spears	22
Broccoli, raw	125 ml (½ cup)	42
Broccoli, cooked	125 ml (½ cup)	54
Cabbage, red, raw	250 ml (1 cup)	42
Cabbage, cooked	125 ml (½ cup)	30
Cauliflower, raw or cooked	125 ml (½ cup)	27-29
Peppers (red, green), raw	125 ml (½ cup)	101-144
Peppers (red, green), cooked	125 ml (½ cup)	121-132
Potato, with skin, cooked	1 medium	14-31
Snow peas, raw	125 ml (½ cup)	20
Snow peas, cooked	125 ml (½ cup)	41
Sweet potato, with skin, cooked	1 medium	22
Tomato, raw	1 medium	17
Tomato sauce, canned	125 ml (½ cup)	8-9
Fruit		
Avocado	½ fruit	26
Berries (raspberries, blueberries, blackberries)	125 ml (½ cup)	14-17
Guava	1 fruit	206
Grapefruit, pink or red	½ fruit	38-47
Kiwi	1 large	84
Lychee	10 fruits	69
Mango	½ fruit	38
Orange	1 medium	59-83
Papaya	½ fruit	94
Pineapple	125 ml (½ cup)	42-49
Strawberries	125 ml (½ cup)	52

Table 2 Vitamin C concentration in organs and body fluids.

Organ/ Body fluid	***Vitamin C (mg/100g)***
Pituitary gland	40-50
Adrenals	30-40
Eye lens	25-31
Liver	10-16
Pancreas	10-15
Spleen	10-15
Kidney	5-15
Heart muscle	5-15
Brain	3-15
Lungs	7
Skeletal muscle	3-4
Plasma	0.4-1

Vitamin C deficiency results in scurvy, the major symptoms of which include easy bruising, pinpoint hemorrhages, bleeding gums and poor wound healing (2, 10, 11, 18, 19, 24, 27). Recently, high doses of vitamin C have been widely prescribed for the treatment of many diseases and disorders including common cold, cancer, cataracts, glaucoma, atherosclerosis, diabetes, heart disease, wounds and other physical injuries (6, 8, 10, 28).

Base on the previous studies regarding the mechanism of vitamin C, the process involves two types of proteins: sodium-ascorbate co-transporters (SVCTs) and hexose transporters (GLUTs). SVCTs specifically import ascorbate, the reduced form of vitamin C, across plasma membrane (29, 30). SVCT1 is involved in the homeostasis of vitamin C, while SVCT2 protects cells from oxidative stress. Oral vitamin C is

transported through the intestinal epithelium via SVCT1, diffuses into the surrounding capillaries and then to the circulatory system (6, 29). To maintain the homeostasis of vitamin C, the kidney takes part in reabsorption and excretion (29). The reabsorption of vitamin C occurs at the proximal renal tubule and it depends on glomerular filtration rate (31). Vitamin C is transported in the bloodstream and has a short half-life in plasma, approximately 2 hours (32). Plasma level relates to dietary intake and tissue levels show the bioavailability of vitamin C (33). Normal level of vitamin C in plasma range from 23 to 84 μmol/l (34). Excess amounts of vitamin C are excreted in the urine (35, 36).

The increasing dose of oral vitamin C makes the absorption less efficient due to saturation of the transporters. The study in healthy volunteers indicated that receiving 100-200 mg of vitamin C provided plasma saturation (35). Bioavailability of vitamin C was complete at 200 mg as a single dose and declined at doses of 500 mg and higher. Urinary excretion did not occur until the dosage reached 100 mg and nearly all of the absorbed vitamin C was excreted at the dosage of 500 mg (35, 36). Complete plasma saturation occurred at 1,000 mg daily at the expense of decreased bioavailability and increased urinary excretion. Although the absorption of oral vitamin C was completed at dose of 200 mg, the absorption at doses of 500 mg and 1,250 mg were <75% and <50%, respectively (35). Melethil et al. (1984) reported that both gastrointestinal absorption and renal tubular reabsorption of vitamin C were dose-dependent processes and renal excretion occurs only when plasma vitamin concentration exceeds a threshold value (37). Similarly, Friedman et al. (1940) supported that the mentioned processes were saturable (38).

The recommended daily allowance (RDA) is the amount of vitamin that yields the least risk of inadequacy and the least risk of toxicity. Factors which determine the RDA for vitamin C include the relationship between vitamin C dose and plasma

concentration, bioavailability, urinary excretion, cell concentration, and potential adverse effects. The RDA for vitamin C is 60 mg daily. (39). Based on the data from the study of Levine at el. (1996) and Institute of Medicine criteria, the RDA of 60 mg daily for vitamin C was recommended to increase to 200 mg daily (35). In 2000, the Food and Nutrition Board of the Institute of Medicine raised the RDA for vitamin C for men and women to 90 and 75 mg, respectively, with an Upper Limit (UL) for safety established at 2,000 mg per day (40).

2.4 Vitamin C and wound healing

Vitamin C plays an important role in all phases wound healing (19). It is required for neutrophil apoptosis and clearance in the inflammatory phase (41). In the proliferative phase, vitamin C involves in the process of synthesis, maturation, secretion and degradation of collagen by acting as a cofactor for hydroxylation of proline and lysine during collagen synthesis and associates with fibroblast proliferation which has effect on angiogenesis and capillary strength (2, 9, 12, 19, 42) Vitamin C deficiency impedes the collagen production in the maturation phase and affects scar formation in consequence (43). Patients with vitamin C deficiency not only often have poor wound healing, but also suspect to wound infection (5, 6, 11, 24).

Rapidly utilization of vitamin C during post-operative period indicates that adequate vitamin C is necessary for normal healing process (2, 6). There were studies which indicated that both surgical and infectious disease patients had significantly lower vitamin C level than normal and required 500 to 3,000 mg of vitamin C for restoration to normal status (44, 45). There is no specific guideline of vitamin C for wound healing (46). Because the evidence of toxicity is low, vitamin C is often supplemented in high doses (6). Some studies had shown the benefits of vitamin C supplement even though the baseline intake was as high as 500 mg per day (24). In

order to maximize uptake and plasma concentrations of vitamin C, Padayatty et al. (2004) recommended to prescribe vitamin C in several smaller doses over the day (47). Demling (2009) recommended the dosage between 500 mg and 2,000 mg, which was more than 10 times the recommended daily intake as suggested by the Food Standards Agency (FSA) (48), for supporting of the production of energy in the hypermetabolic state (49). For promoting the healing process, the recommended dose of vitamin C supplement was 500 to 1,000 mg per day (6).

2.5 Related research

2.5.1 In vitro studies

There was an in vitro study of L-ascorbic acid 2-phosphate magnesium salt (APM) which was an L-ascorbic acid (AsA) derivative developed to improve AsA stability on the properties of human gingival fibroblasts. Two cell lines of human gingival fibroblasts were obtained from biopsies of healthy volunteers and were incubated in the presence or absence of 50 μM APM or L-ascorbic acid sodium salt (AsANa). HPLC was used for intracellular AsA analysis. Collagen synthesis and expression of TNF-α-induced interleukin-8 which increased during inflammatory reactions were measured by ELISA and real-time RT-PCR. This laboratory observation indicated that type I collagen synthesis, cell damage reduction and the decreased expression of interleukin-8 in human gingival fibroblast were related to the amount of intracellular AsA and the effects of APM were superior to AsANa (13).

2.5.2 In vivo studies

There were many animal studies conducted to study the effect of vitamin C on wound healing. The study of gingival wound healing in twelve guinea pigs using 1% toluidine blue for connective tissue detection in 1967 revealed that the healing time in group given vitamin C intraperitoneally was shorted than control group (50). In 2008, Omeroğlu et al. made an experimental study in healthy rat model. Forty-

two rats of which the right Achilles tendons were ruptured into vitamin C group and control group. The first group were intraperitoneally injected 150 mg of vitamin C in 1.5 ml, while the latter received 0.9% of NaCl in 1.5 ml once for every 2 days. Seven rats from each group were euthanized on the 3rd, 10th and 21th days and histological examination of the healing tissues were compared under microscope and analyzer software. Their experiment showed that this high-dose of vitamin C supplement enhanced the Achilles tendon healing of healthy rats due to early neovascularization and collagen synthesis (12). In 2010, Kamer et al. carried out a study in streptozotocin-induced diabetic rats to investigate whether vitamin C improved incisional wound healing. They randomly divided 20 rats into two groups of 10 each. Study group was given 200 mg/kg of vitamin C once a day orally with orogastric tube for 10 days. At post-operative day 14, the rats were sacrificed then the tensile strength, histological examination and tissue hydroxyproline level were analyzed. According to what they found, they concluded that vitamin C significantly promoted wound healing of laparotomy incisions in diabetic rats (9). In 2016, Mohammed et al. carried out a study in Gulo-/- mice which cannot synthesize vitamin C and found that the results were consistent with the previous study. They conducted the study by divided mice into three groups. The first group was provided vitamin C 330 mg/l in water twice a week, the second group was reduced vitamin supplement to 33 mg/l for a week followed by removal of vitamin C supplement for an additional week and the third group was daily injected 200 mg/kg of vitamin C intraperitoneal and received 330 mg/l vitamin C drinking water for 14 days after wounds were made. At the 7th and 14th day post-wounding, mice were sacrificed and their tissues were collected for histological and cytological assessments. The results showed that mice in the first and third group had more rapid wound healing due to higher expression of healing mediators, induction of self-renewal genes and fibroblast proliferation. They concluded that the vitamin C promote tissue repair and

shortened healing duration (2). Chen et al. (2017) also summarized that vitamin C promoted epithelial wound healing in the cornea of mouse in vivo and also enhanced the cultured corneal epithelial stem/progenitor cells of mouse in vitro (10).

Moreover, Chen et al. (2015) carried out a study of healing in rabbits. All rabbits were randomly numbered from 1 to 75. After atraumatically extraction of bilateral first premolars, the right side socket of rabbits number 1 to 50 were placed with absorbable gelatin sponges soaked with 75 mg of osteogenic inducer whereas the left side sockets were placed with gelatin sponges. The rest rabbits, numbered 51 to 75, had nothing placed into the extraction sockets. The osteogenic inducer was composed of 10-8 mol/l dexamethasone, 50 mg/l vitamin C, and 10 mmol/l β-sodium glycerophosphate. Ten of the rabbits numbered 1-50 and five of those numbered 51-75 were sacrificed at 1, 2, 4, 8, 12 weeks after tooth extraction and their specimens were investigated in radiographic examination using CBCT, histological and histomorphometric examination. Significant differences were found in the results that osteogenic inducer containing vitamin C accelerated alveolar wound healing of extraction socket in rabbits (1).

Recently, in 2018, there was a study of the effect of high dose oral vitamin C and mesenchymal stem cells on wound healing in a diabetic mouse model. The mice were divided into 5 groups including control group, diabetes group (from a streptozotocin injection), diabetes treated with mesenchymal stem cells group, diabetes treated with vitamin C group and diabetes treated with mesenchymal stem cells and vitamin C group. The skin wounds measured on day 7 and day 14 showed that the diabetes group had lowest wound closure percentage whereas the diabetes treated with mesenchymal stem cells and vitamin C group had highest wound

closure percentage. Moreover, the highest capillary formation was found in diabetes treated with mesenchymal stem cells and vitamin C group (51).

2.5.3 Clinical studies

Vitamin C deficiency also impaired wound healing in humans. Ringsdorf et al. (1982) revealed that there was interrelationship between vitamin C and human's healing potential. After administration 500 to 3,000 mg per day of vitamin C, approximately 8 to 50 times the RDA of 60 mg, to patients recovering from surgery and injuries, decubital ulcers and leg ulcers due to hemolytic anemia, they concluded that vitamin C significantly accelerated the healing. They also observed case of an 8-year-old boy with Type VI Ehlers-Danlos syndrome who had inability to synthesize collagen due to hydroxylysine deficiency, the formation of hydroxylysine was significantly increased after daily administration 4,000 mg of vitamin C for 2 years. These resulted in clinical improvement of wound healing (14).

Moreover, there were case reports about vitamin C and wound healing in surgical patients. Bikker et al. (2016) found that patients with low or subclinical levels of vitamin C were often seen in Meander Medical Center in The Netherlands. Despite not showing clinical symptoms of scurvy, collagen synthesis might be impaired. After measurement of vitamin C plasma levels of 4 surgical patients who had poor wound healing, it was found that their vitamin C levels were below the reference limit of 25 μmol/l. When patients were given intravenous or oral supplement of 1000 mg vitamin C for 3-4 weeks, the healing of wounds were improved and showed full recovery (27).

Another of Ringsdorf et al. (1982)'s experiment was conducted in two healthy volunteers whose circular-shaped gingival tissue size 3 mm were removed and the wounds were observed by using 1% toluidine blue solution which adhered only to connective tissue. The volunteers had a regular diet after the first wound. Each day

the wounds were painted with the solution and rinsed until there was no toluidine blue stain which was defined as complete healing. After a 2-week rest period, the second wound were made and they were given 1,000 mg of vitamin C daily: 250 mg after each meal and at bedtime. When comparing the results, they found that the healing duration decreased 40 percent after having vitamin C supplement. The study was repeated in another pair of students by giving 500 mg of vitamin C instead and it revealed 50 percent decrease in healing time after taking a daily dose of vitamin C 2,000 mg. Complete healing took 9 days in this group whereas it took 18 days with a regular diet (14).

Another study in Thai population performed supplementary of vitamin C after tooth extraction. After tooth extraction, the study group received 500 mg of vitamin C 4 times daily for 10 days. The healing duration of the control group was about a month whereas in the study group was about 7 day earlier. The conclusion was that the healing of extraction wound was accelerated significantly by taking 2,000 mg of vitamin C daily (15).

The effect of vitamin C on dental healing was also reported in clinical study. In 1993, Abrahmsohn et al. performed a double-blind experimental trial by administered vitamin C to patients after tooth extraction. After the operation, the study group were given 500 mg of vitamin C orally with meal three times a day for 3 weeks, while the controlled group were given placebo with the same regimen. Patients were appointed a week after tooth extraction for suture removal and post-operative symptoms were interviewed (8). Dental healing rates were defined as two categories which were ‘slow healing’ and ‘rapid healing’. The criteria was recommended by Dubois et al. (1982). Pain and request for additional analgesic, edema and purulent material, weak to moderate granulation-bed formation and administration of antibiotic prophylactic were defined as ‘slow healing’, whereas no

pain or mild pain, no need for additional analgesic, no edema or other swelling, no sign of infection, complete granulation-bed formation and no need for antibiotic or other medications were defined as ‘rapid healing’ (52). The study evidenced that vitamin C supplement benefited in accelerating wound healing after tooth extraction and reducing the incidence of alveolar osteitis (8).

In 2018, Li et al. conducted a clinical study of vitamin C in wound healing after dental implant surgery. Patients who required dental implant surgery were divided into 4 groups, group A dental implants supported by guided bone regeneration (GBR), group B dental implants and Bio-Oss collagen grafts, group C dental implants in patients with chronic periodontitis and group D dental implants without any bone grafts or periodontal disease. Each group was divided into 2 subgroups, one was control subgroup and another was experimental subgroup which received oral vitamin C 300 mg/d for 7 days. Landry index was used for evaluating the soft tissue healing on day 3, 7 and 14 post-surgery. The results showed that, on day 7 and 14, the experimental subgroup of group B significantly improved wound healing comparing with the control group. In group A and group C, the experimental subgroup also presented better healing than control group on day 14 post-surgery (53).

According to the pain aspect, although the analgesic mechanism is unclear, there were a number of recent clinical studies showed that vitamin C administration decreased symptoms of pain in patients with chronic regional pain syndrome (54). From a double-blind placebo-controlled RCT of Terezhalmy et al. (1978), the pain duration of herpes labialis was shortened by 51% when patients received 1,000 mg daily of vitamin C together with bioflavonoids (55). Furthermore, high dose vitamin C administration decreased herpetic neuralgia and cancer-related pain (54). For example, patients with cancer who received regular doses of opioid became less

pain after 5 or 7 days of vitamin C supplement and there was the observation that vitamin C administration significantly decreased the requirement for opioid analgesics (54, 56, 57). Therefore, Carr et al. (2017) suggested vitamin C as an effective adjunctive therapy for reducing acute and chronic pain in patients (54).

CHAPTER III

MATERIALS AND METHODS

3.1 Research design

A split mouth double-blind randomized-controlled clinical trial

3.2 Population

Patients who required tooth extraction at the Faculty of Dentistry, Chulalongkorn University during August 2018 to August 2019.

3.3 Samples

3.3.1 Inclusion criteria

- Patients who had bilaterally symmetrical non-infected premolars needed for extraction, which were usually extracted to make space for orthodontic treatment. Dental caries and retained roots with no periapical lesions or abscess were included.
- No odontogenic related local or space infection
- Aged 14-40 years old
- Absence of uncontrolled medical condition including diabetes mellitus, hematological disorders, immunocompromised or American Society of Anaesthesiologist (ASA) Physical Status I or II

3.3.2 Exclusion criteria

- Smokers or alcoholics
- On drug therapy which may interfere with wound healing such as corticosteroids
- Pregnant or lactating women and taking estrogen-containing contraceptive drugs

- Psychiatric patients
- Patients who could not cooperate with the study and post-operative follow-up
- patients who missed more than 1 dose of vitamin C
- Intra-operative and post-operative complication including space infection, bleeding, bone or root fracture

3.3.3 Sample size calculation

The software, G*Power version 3.1 was used for sample size calculation. According to our pilot study with considering an α error of 5%, the study power (1-β) of 0.80, the calculated sample size was 8 per group. There might be sample losses due to leave, quit, drop out or in case of excluded from the study, so after compensating, the sample size was 14 per group. Therefore, the total sample size of 3 groups was 42, with 84 extraction sites.

3.3.4 Sample grouping

The enrolled patients were randomly divided into by running number into 3 groups (14 patients for each group); 1.placebo vs vitamin C 600 mg/d (P/600), 2.placebo vs vitamin C 1,500 mg/d (P/1,500) and 3.vitamin C 600 mg/d vs vitamin C 1,500 mg/d (600/1,500).

3.4 Methods

3.4.1 Ethic consideration

The study protocol was approved by the Human Research Ethic committee of the Faculty of Dentistry, Chulalongkorn University, code HREC-DCU 2018-031 and approved by Thai Clinical Trial Registry committee with identification number TCTR20180830002. All subjects were informed and explained about the experimental

study and their co-operation required. Patients who fulfilled all the criteria had to sign the informed consent.

3.4.2 Surgical protocol

In the first visit, each patient was randomly assigned the experimental site by alternating. The vital sign was recorded before the extraction. All operations were performed by the same surgeon with standardized technique, using elevators and adapted forceps under local anesthesia consisting of 2% mepivacaine with 1:100,000 epinephrine. After tooth extraction, bleeding was stopped completely using gauze pressure. Every patient was given the same post-operative instructions. The analgesic drug, acetaminophen 500 mg was prescribed as needed. The other side of the tooth was extracted with the same methods on day 21 after the first site extraction.

There were two different interventions given to each patient after tooth extraction in each side. Patients were blinded which one they were prescribed in each visit. The interventions of each group depending on which group they were assigned (Table 3).

Table 3 The interventions assignment.

Group	Extraction site	Intervention (3 times/day)		Vitamin C dosage per day (mg)
		Placebo (tab)	Vitamin C 100 mg (tab)	
P/600	1st site	5	0	0
	2nd site	3	2	600
P/1,500	1st site	5	0	0
	2nd site	0	5	1,500
600/1,500	1st site	3	2	600
	2nd site	0	5	1,500

Patients were prescribed to take each intervention three times daily after meal for 10 days post-extraction. Vitamin C used in the study was the product from Patar Lab Co. (Pathumthani, Thailand). Whereas placebo which consists of calcium carbonate, sucrose and coated with carnauba wax was manufactured under the license of the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

3.4.3 Data collection

Their demographic data including age, gender, medical condition, allergies and medication were collected in a form. Panoramic radiograph was taken to confirm that the teeth were bilateral symmetry.

Each extraction wound site was measured 3 times (immediately after tooth extraction as a baseline, on day 7 and day 21 post-extraction) by two blinded examiners who were not the operator to avoid bias. Inter-rater reliability was calibrated by two examiners who measured the extraction wound sizes. The data

was compared and the reliability test showed that intraclass correlation coefficient was 0.771 which indicated good reliability (58).

The measurement procedures were described as following:

- The size of the extraction socket (rounded up to the nearest 0.5 mm) in both bucco-lingual (BL) width and mesio-distal (MD) width were measured by a caliper (Figure 2) using cementoenamel junction (CEJ) level of the adjacent teeth as reference level.
- The depth of the extraction (rounded up to the nearest mm) was measured at the middle of buccal plate between the adjacent teeth by a periodontal probe (PCPUNC15, Hu-Friedy) (Figure 3).

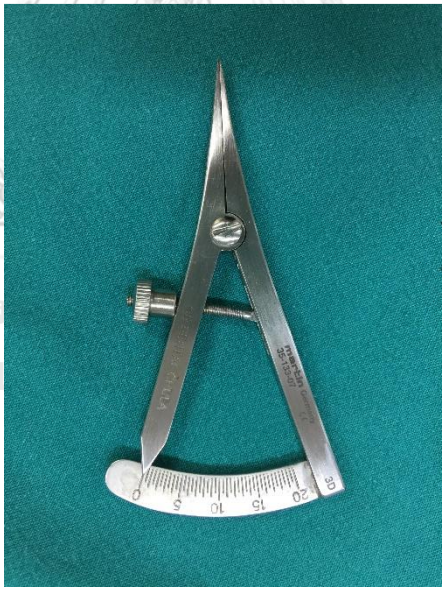


Figure 2 Caliper

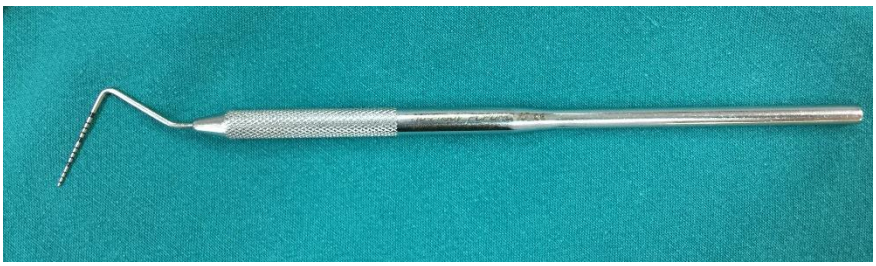


Figure 3 Periodontal probe

On day 21 post-extraction, the extraction wound was stained with 1% toluidine blue solution (Sigma-Aldrich) (Figure 4) and rinsed. Normally, toluidine blue does not adhere to intact gingival tissue but adheres only to the connective tissue. Therefore, the less staining site referred to 'less connective tissue exposed' or 'better healing'. After staining, the photos of extraction wounds were taken and two observers compared which extraction site had less staining area. The reliability test showed that Cohen's kappa coefficient was 0.60 which indicated moderate agreement (59).

Figure 4 1% Toluidine blue solution

All patients were asked to list their meals in a form during the wound healing period for 7 days to determine the amount of vitamin C in their diet. Moreover, pain

score using the 10-point visual analog scale from 0 (no pain) to 10 (extreme pain) was recorded by each patient on the first three days post-extraction at 8 am, 12 pm and 6 pm. Every patient was instructed to record pain score before taking analgesic drug.

3.4.4 Data analysis

To analyze the data, the statistical program SPSS version 22.0 (Chicago, USA) was used. Patients' demographic data was presented as descriptive statistic. Normal distribution of the data were tested by Kolmogorov-Smirnov test. The reduced size of the extraction wound at each time point were calculated in proportion as follows:

$$\text{Size BL}_{\text{(Between day 7 and immediate)}} = \frac{\text{Size BL}_{\text{(Immediate post-extraction)}} - \text{Size BL}_{\text{(Day 7 post-extraction)}}}{\text{Size BL}_{\text{(Immediate post-extraction)}}}$$

$$\text{Size MD}_{\text{(Between day 7 and immediate)}} = \frac{\text{Size MD}_{\text{(Immediate post-extraction)}} - \text{Size MD}_{\text{(Day 7 post-extraction)}}}{\text{Size MD}_{\text{(Immediate post-extraction)}}}$$

$$\text{Depth}_{\text{(Between day 7 and immediate)}} = \frac{\text{Depth}_{\text{(Immediate post-extraction)}} - \text{Depth}_{\text{(Day 7 post-extraction)}}}{\text{Depth}_{\text{(Immediate post-extraction)}}}$$

$$\text{Size BL}_{\text{(Between day 21 and immediate)}} = \frac{\text{Size BL}_{\text{(Immediate post-extraction)}} - \text{Size BL}_{\text{(Day 21 post-extraction)}}}{\text{Size BL}_{\text{(Immediate post-extraction)}}}$$

$$\text{Size MD}_{\text{(Between day 21 and immediate)}} = \frac{\text{Size MD}_{\text{(Immediate post-extraction)}} - \text{Size MD}_{\text{(Day 21 post-extraction)}}}{\text{Size MD}_{\text{(Immediate post-extraction)}}}$$

$$\text{Depth}_{\text{(Between day 21 and immediate)}} = \frac{\text{Depth}_{\text{(Immediate post-extraction)}} - \text{Depth}_{\text{(Day 21 post-extraction)}}}{\text{Depth}_{\text{(Immediate post-extraction)}}}$$

$$\text{Size BL}_{\text{(Between day 7 and day 21)}} = \frac{\text{Size BL}_{\text{(Day 7 post-extraction)}} - \text{Size BL}_{\text{(Day 21 post-extraction)}}}{\text{Size BL}_{\text{(Day 7 post-extraction)}}}$$

$$\text{Size MD}_{\text{(Between day 7 and day 21)}} = \frac{\text{Size MD}_{\text{(Day 7 post-extraction)}} - \text{Size MD}_{\text{(Day 21 post-extraction)}}}{\text{Size MD}_{\text{(Day 7 post-extraction)}}}$$

$$\text{Depth}_{\text{(Between day 7 and day 21)}} = \frac{\text{Depth}_{\text{(Day 7 post-extraction)}} - \text{Depth}_{\text{(Day 21 post-extraction)}}}{\text{Depth}_{\text{(Day 7 post-extraction)}}}$$

All proportions were calculated into percentage. Because of the abnormal distribution, the reduced size proportion in percentage of the extraction wound between 2 extraction sites were compared using Wilcoxon Signed-Rank test. Pain scores after 2 extractions were also compared using Wilcoxon Signed-Rank test. McNemar Chi-square was used to analyze the toludine blue stain. Differences were considered significant if the *p*-value was less than 0.05 (the confidence level of 95%). Moreover, patients' vitamin C-containing diet after each tooth extraction was also compared and presented in percentage of difference.

3.4.5 Conflict of interest

The authors confirmed that this study had no conflict of interest to declare.

CHAPTER IV

RESULTS

4.1 Patient demographic data

Total twenty-eight patients were enrolled completly in this study. There were 10 patients in group P/600 and 9 patients in group P/1,500 and 600/1,500. The patients were 9 males and 19 females. The mean age of these patients was 18.68±3.95 years, with a range from 14 to 28 years. The extraction site was mostly in the maxilla (57.1%). The demographic data and *p*-value were shown in Table 4. Chi-square was used for comparing the difference in gender and the location of the extraction site among 3 groups, whereas ANOVA was used for comparing the age of patients in 3 groups. There were no significant differences in age, gender and extraction site between 3 groups.

Table 4 Patients' demographic data.

Variables	All (n=28)	Group P/600 (n=10)	Group P/1,500 (n=9)	Group 600/1,500 (n=9)	*p*-value
Age (years) mean±SD	18.68±3.95	19.60±4.97	18.89±3.72	17.44±2.88	0.444
Gender (n,%)					
Male	9 (32.1%)	5 (50%)	1 (11.1%)	3 (33.3%)	0.348
Female	19 (67.9%)	5 (50%)	8 (88.9%)	6 (66.7%)	
Extraction site (n,%)					
Maxilla	16 (57.1%)	6 (60%)	5 (55.6%)	5 (55.6%)	0.543
Mandible	12 (42.9%)	4 (40%)	4 (44.4%)	4 (44.4%)	

4.2 Wound size reduction

When compared the percentage reduction of the extraction wound sizes between 2 extraction sites, in group P/600, wound size in MD dimension of teeth receiving vitamin C 600 mg/d (200 mg three times daily) was reduced more compared with placebo (57.3% vs. 48.3%, 0.090±0.12 mm difference) between day 0 and day 7 significantly (*p*-value = 0.036). However, wound size reduction between 2 extraction sites of group P/1,500 and 600/1,500 showed no significant difference ($p > 0.05$) (Table 5, 6, 7).

Table 5 %Reduction of mean wound size between 2 extraction sites of group P/600.

Group P/600 (Placebo vs Vitamin C 600 mg/d)

	%reduction of extraction wound of placebo side	%reduction of extraction wound of vitamin C 600 mg/d side	Size difference between 2 extraction sites (site 2 - site 1) (mean±SD)	*p*-value [a]
Immediate and day 7				
Bucco-lingual	45.9	47.5	0.016±0.16	0.575
Mesio-distal	48.3	57.3	0.090±0.12	0.036*
Depth	61.1	68.2	0.071±0.13	0.122
Immediate and day 21				
Bucco-lingual	54.8	58.9	0.041±0.12	0.445
Mesio-distal	54.6	60.2	0.056±0.15	0.262
Depth	83.3	86.2	0.029±0.08	0.398

Day 7 and day 21				
Bucco-lingual	13.8	18.7	0.049±0.18	0.575
Mesio-distal	11.3	5.9	-0.054±0.19	0.416
Depth	56.8	49.6	-0.072±0.28	0.540

SD, standard deviation.

[a] *p*-value, Wilcoxon Signed-Rank test.

* $p < 0.05$.

A negative value indicates that the site 1 value was greater than that of site 2.

Table 6 %Reduction of mean wound size between 2 extraction sites of group P/1,500.

Group P/1,500 (Placebo vs Vitamin C 1,500 mg/d)

	%reduction of extraction wound of placebo side	%reduction of extraction wound of vitamin C 1,500 mg/d side	Size difference between 2 extraction sites (site 2 - site 1) (mean±SD)	*p*-value [a]
Immediate and day 7				
Bucco-lingual	52.2	47.5	0.047±0.11	0.233
Mesio-distal	37.0	36.5	0.005±0.22	0.953
Depth	47.1	60.2	0.131±0.19	0.066
Immediate and day 21				
Bucco-lingual	59.2	58.4	0.008±0.08	0.575
Mesio-distal	49.4	48.9	-0.005±0.10	0.674
Depth	82.3	88.1	0.058±0.10	0.069

Day 7 and day 21				
Bucco-lingual	12.6	20.0	0.074±0.16	0.225
Mesio-distal	16.6	16.8	0.002±0.19	0.917
Depth	66.7	67.2	0.005±0.17	0.953

SD, standard deviation.

[a] *p*-value, Wilcoxon Signed-Rank test.

* $p < 0.05$.

A negative value indicates that the site 1 value was greater than that of site 2.

Table 7 %Reduction of mean wound size between 2 extraction sites of group 600/1,500.

Group 600/1,500 (Vitamin C 600 mg/d vs Vitamin C 1,500 mg/d)

Mean difference	%reduction of extraction wound of vitamin C 600 mg/d side	%reduction of extraction wound of vitamin C 1,500 mg/d side	Size difference between 2 extraction sites (site 2 - site 1) (mean±SD)	*p*-value [a]
Immediate and day 7				
Bucco-lingual	48.2	42.3	-0.059±0.23	0.767
Mesio-distal	45.0	44.6	-0.004±0.21	0.953
Depth	69.4	70.0	0.006±0.05	0.753
Immediate and day 21				
Bucco-lingual	58.1	56.4	-0.017±0.13	0.953
Mesio-distal	52.7	53.0	0.003±0.21	0.674
Depth	86.6	83.0	-0.036±0.08	0.237

Day 7 and day 21				
Bucco-lingual	20.2	22.0	0.018±0.15	0.674
Mesio-distal	14.1	13.8	-0.003±0.15	1.000
Depth	53.0	41.1	-0.119±0.24	0.160

SD, standard deviation.

[a] *p*-value, Wilcoxon Signed-Rank test.

* $p < 0.05$.

A negative value indicates that the site 1 value was greater than that of site 2.

4.3 Toluidine blue stain

The less staining site of toluidine blue represented better healing, however, no significant differences were found in all groups ($p > 0.05$) (Table 8). The vitamin C 600 mg/d side tended to have the least staining. There was more less staining site of vitamin C 600 mg/d side in group P/600 and group 600/1,500 when comparing to another side, but in group P/1,500, the less staining site of placebo was more. The representative photos of the toluidine blue staining in both more stain and less stain compared in each group were presented in Figure 5-10.

Table 8 Number of less toluidine blue stain sites of 3 groups.

Group	Number of less staining sites		*p*-value [a]
	Extraction site 1	Extraction site 2	
Group P/600	4	6	0.754
Group P/1,500	6	3	0.508
Group 600/1,500	7	2	0.180

[a] *p*-value, McNemar Chi-square.

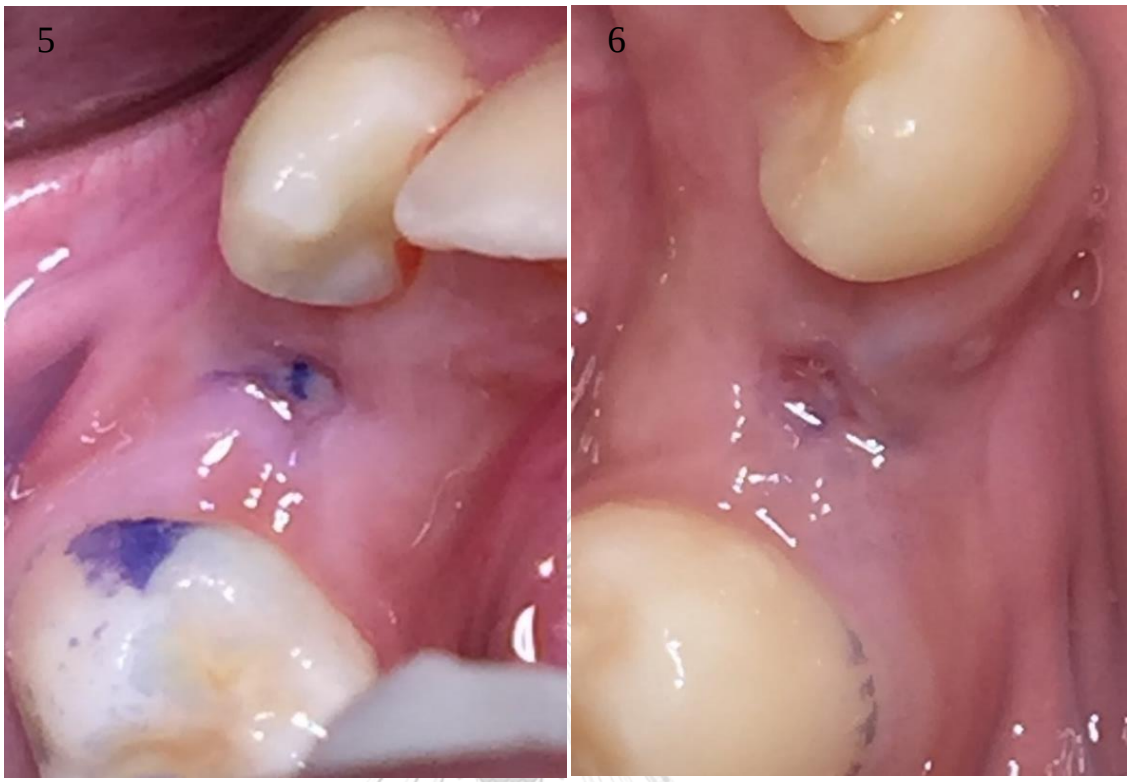
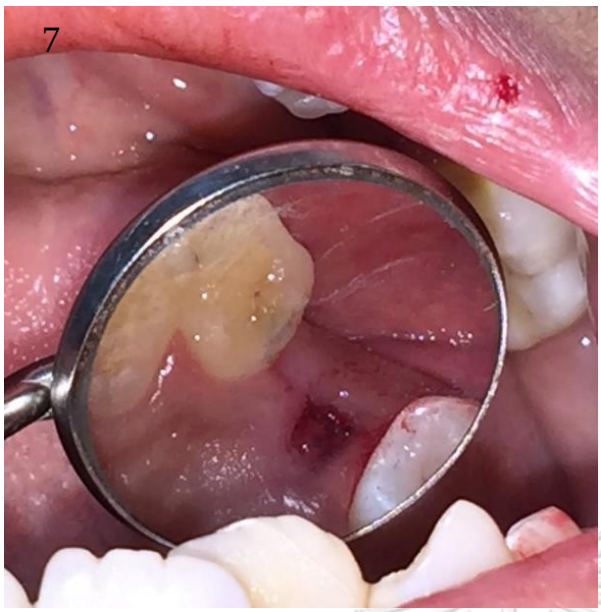


Figure 5 The representative photo of the toluidine blue staining of group P/600, placebo side, more stain.

Figure 6 The representative photo of the toluidine blue staining of group P/600, vitamin C 600 mg/d side, less stain.



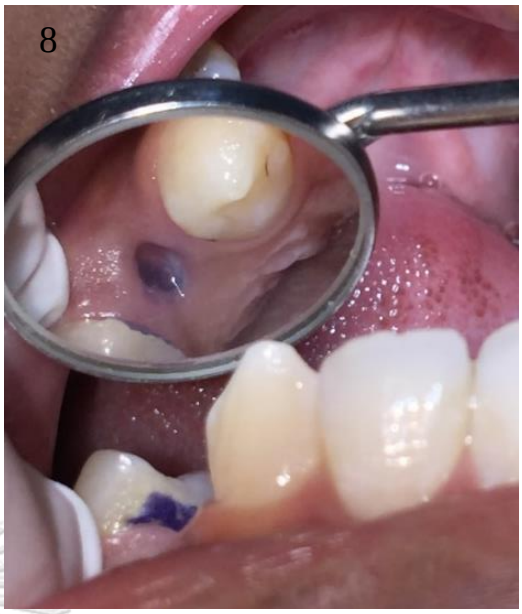
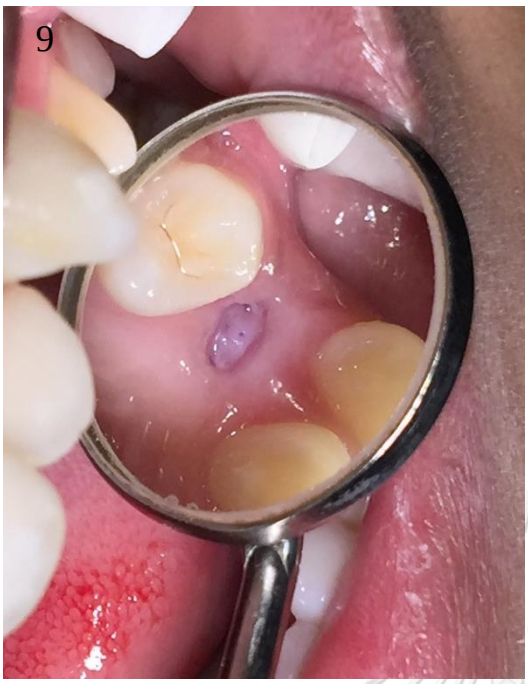


Figure 7 The representative photo of the toluidine blue staining of group P/1,500, placebo side, less stain.

Figure 8 The representative photo of the toluidine blue staining of group P/1,500, vitamin C 1,500 mg/d side, more stain.



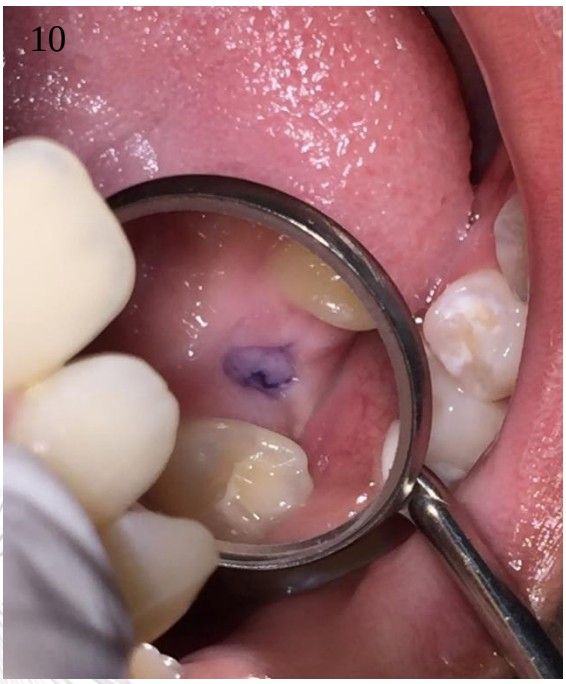


Figure 9 The representative photo of the toluidine blue staining of group 600/1,500, vitamin C 600 mg/d side, less stain.

Figure 10 The representative photo of the toluidine blue staining of group 600/1,500, vitamin C 1,500 mg/d side, more stain.

4.4 Post-operative pain

Moreover, mean VAS scores on day 1-3 of patients receiving vitamin C was significant lower than placebo in group P/600 ($p < 0.05$) (Table 9).

Table 9 Pain scores assessed by VAS (in mm).

Group	Time	Placebo	Vitamin C 600 mg/d	*p*-value [a]
Group P/600 (n=10)	Post-operative day 1 (mean±SD)	4.012±2.71	1.664±1.34	0.021*
	Post-operative day 2 (mean±SD)	2.725±2.40	1.126±1.25	0.045*
	Post-operative day 3 (mean±SD)	1.116±1.33	0.582±0.76	0.017*
Group	Time	Placebo	Vitamin C 1,500 mg/d	*p*-value [a]
Group P/1,500 (n=9)	Post-operative day 1 (mean±SD)	2.001±3.26	1.033±2.05	0.063
	Post-operative day 2 (mean±SD)	1.550±3.07	0.327±0.65	0.157
	Post-operative day 3 (mean±SD)	1.326±2.63	0.017±0.34	0.157
Group	Time	Vitamin C 600 mg/d	Vitamin C 1,500 mg/d	*p*-value [a]
Group 600/1,500 (n=9)	Post-operative day 1 (mean±SD)	1.622±1.78	1.799±1.15	0.608
	Post-operative day 2 (mean±SD)	1.019±1.58	1.088±1.54	0.458
	Post-operative day 3 (mean±SD)	0.776±2.21	0.395±0.69	0.892

VAS, visual analog scale.

[a] *p*-value, Wilcoxon Signed-Rank test. * $p < 0.05$.

4.5 Patients' high vitamin C diet

In group P/600, 60% of the patients had the same high vitamin C-containing diet after 2 extractions and 40% had higher vitamin C-containing diet after the first extraction than the second one. In group P/1,500, the percentage of patients who had high vitamin C-containing diets after the first extraction than second extraction was 66.7%, whereas 22.2% had higher after the second extraction. There was 66.7% of the patients who had the same high vitamin C-containing diet in group 600/1,500 and 22.2% had higher vitamin C-containing diet after the second extraction. The percentage of difference between 2 extractions of patients' high vitamin C-containing diet in each group was presented in Figure 11-13.

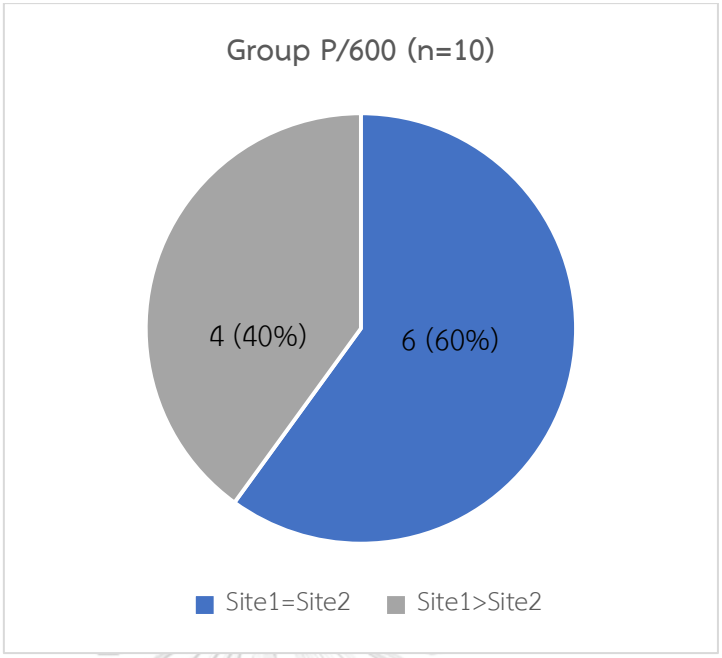


Figure 11 The difference of patients' high vitamin C-containing diet after each tooth extraction of group P/600.

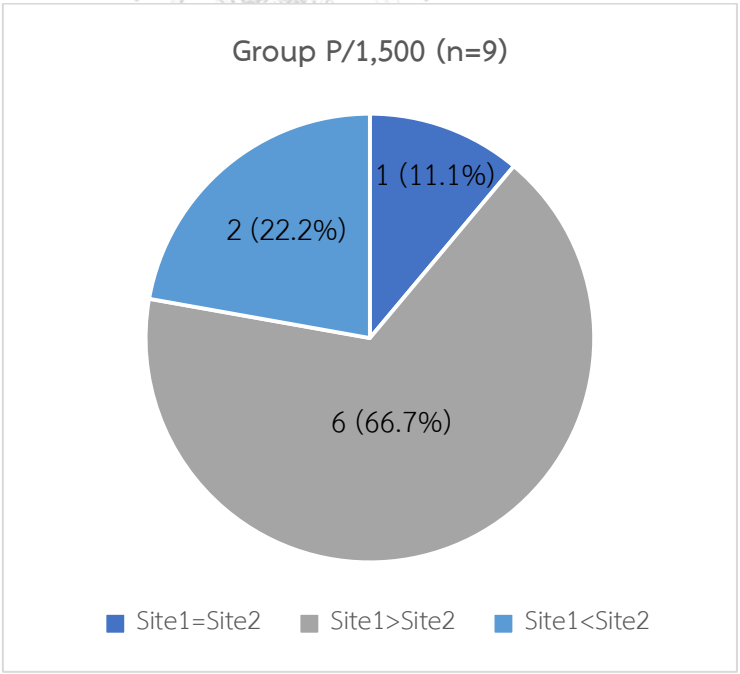


Figure 12 The difference of patients' high vitamin C-containing diet after each tooth extraction of group P/1,500.

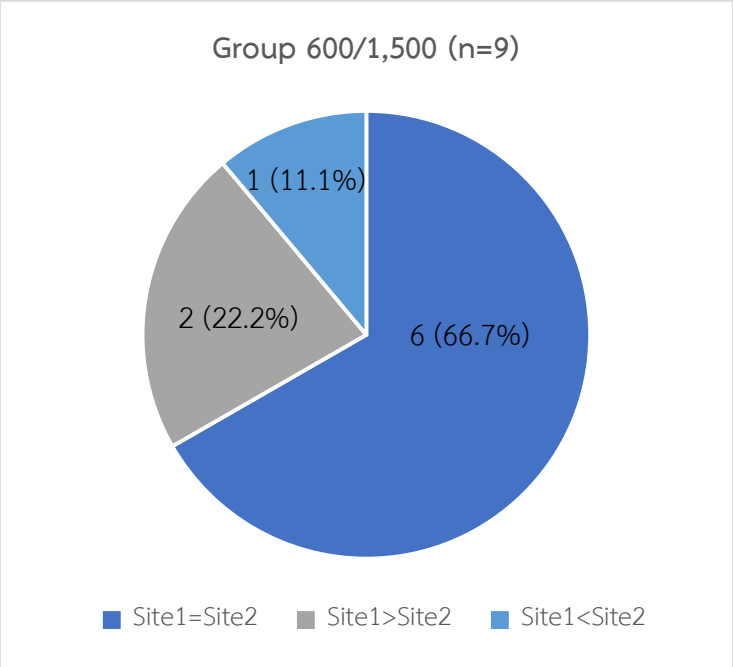


Figure 13 The difference of patients' high vitamin C-containing diet after each tooth extraction of group 600/1,500.

CHAPTER V
DISCUSSION

In this study, we carried out as a double-blind, randomized, controlled clinical trial: split mouth design; this type of study has the distinct advantage of removing confounding factors from inter-subject variability (60). We created this study in order to evaluate the pattern of extraction wound healing related to vitamin C supplement.

Total 14 patients out of 42 patients were excluded from the study due to intraoperative root fracture or missing more than 1 dose of vitamin C. These drop-out patients were 4 patients from group P/600, 5 patients from group P/1,500 and 5 patients from group 600/1,500. A patient in group P/600 and 2 patients in group 600/1,500 were excluded due to intraoperative root fracture. Other patients were excluded because they missed more than 1 dose of vitamin C.

The previous studies in the aspect of extraction wound healing investigated the effect of vitamin C 1,500 mg/d and 2,000 mg/d (8, 15). Our hypothesis that divided total dose of 600 mg per day into 200 mg three times daily might show more effective for promoting wound healing due to the bioavailability. We compared two regimens of vitamin C, i.e. 600 mg/d vs 1,500 mg/d in this study. Recently, Vitamin C regimen is still controversy and there is no specific guideline of vitamin C for wound healing (46). High doses of vitamin C is widely used without the evidence of toxicity (6). However, the previous study in healthy volunteers indicated that receiving 100-200 mg of vitamin C was able to achieve saturation of vitamin C concentration in plasma. Bioavailability of vitamin C was complete at 200 mg as a single dose and declined at doses of 500 mg and higher (35, 36). Normally, oral vitamin C is transported through the intestinal epithelium via sodium-ascorbate co-transporters (SVCTs) and SVCT1 is involved in the homeostasis of vitamin C (29). The increasing

dose of oral vitamin C makes the absorption less efficient due to saturation of the transporters. Although the absorption of oral vitamin C was completed at dose of 200 mg, the absorption at doses of 500 mg and 1,250 mg were <75% and <50%, respectively (35). Melethil et al. and Friedman et al. reported that both gastrointestinal absorption and renal tubular reabsorption of vitamin C were dose-dependent processes and renal excretion occurs only when plasma vitamin concentration exceeds a threshold value (37, 38). In order to maximize uptake and plasma concentrations of vitamin C, it was recommended to prescribe vitamin C in divided doses over the day (47). In the hypermetabolic state, the dosage recommended was between 500 mg and 2,000 mg per day, which was more than 10 times the recommended daily intake as suggested by Food Standards Agency (FSA) (49). For promoting the healing process, the recommended dose of vitamin C supplement was 500 to 1,000 mg per day (6).

Although vitamin C has a short half-life in plasma, it is also stored in organs which could be the source of vitamin C and release to plasma during healing period (26, 32). The retention of vitamin C in several organs has concentration-dependent mechanism (61). Therefore, in each group, we designed to prescribed placebo for the first intervention in both group P/600 and group P/1,500 then followed by prescribing vitamin C 600 mg/d and vitamin C 1,500 mg/d in each group respectively for the second intervention. Consistently, patients in group 600/1,500 were prescribed the lower dose of vitamin C (600 mg/d) for the first intervention then followed by higher dose of vitamin C (1,500 mg/d).

We found that in group P/600, the significant of percentage reduction of the extraction wound size in mesiodistal dimension of vitamin C 600 mg/d side on day 7 ($P < 0.05$) was possibly due to differentiation of fibroblasts and wound contraction which represented faster wound closure. The blood clot in the extraction wound is replaced by granulation tissue in 1 week and granulation tissue formation and

collagen deposition take place from day 4 to day 14 after injury (62). Wound contraction begins after the granulation tissue is well-established (63).

Bone loss and collapse of the surrounding gingiva is found as the normal healing response to tooth extraction (64). The bone resorption occurs both in buccolingual and apicocoronal dimensions (65). Whereas, in mesiodistal dimension, there were interdental bone supported by the adjacent teeth. Therefore, we assumed that change of the extraction wound in mesiodistal dimension was possibly due to gingival tissue healing without the effect of interdental bone resorption. On the other hand, bone resorption might affect in buccolingual dimension. Consistently, soft tissue healing in this mesiodistal dimension showed the significant difference. However, we suggested to observe bone healing in further study.

Higher dose of vitamin C (1,500 mg/d) showed no difference in extraction wound healing compared to lower dose of vitamin C (600 mg/d) ($P > 0.05$). Vitamin C possibly enhanced wound healing in both 600 mg/d and 1,500 mg/d but there might not be obvious to show the significant difference in group 600/1,500.

The less toluidine blue stain of vitamin C 600 mg/d side in both group P/600 and group 600/1,500 represented better healing than another side. Re-epithelialization starts 24 hours post-extraction and epithelialization is one of the processes in the proliferative phase (62). The data implied that vitamin C 600 mg/d tended to promote the re-epithelialization. Moreover, this result was found to be concordant with the lower pain score of patients who received vitamin C 600 mg/d in group P/600 and explained that the re-epithelialization associated with less pain. According to the free nerve endings in the connective tissue, the more exposed connective tissue, the more pain feeling of the patients (66).

Although wound size reduction showed the significant result in group P/600, toluidine blue stain had no significant difference in all groups. Toluidine blue adheres only to the connective tissue so it can detect the re-epithelialization, whereas,

wound size reduction represented the wound contraction. When the shrinkage of wound edges occurred, the wound size reduction was found (62). Despite the smaller size on the top of the wound, there was still the exposed connective tissue in the socket.

There was an in vitro study of local effect of vitamin C and human gingival fibroblast reported that the different dosages of vitamin C affect wound healing in different ways. Low dose of vitamin C induced fibroblast migration which tended to increase gingival wound closure, but in high dose, vitamin C delayed gingival wound closure due to the inhibition of fibroblast proliferation. However, fibroblast function and gene expression which related to wound healing were increased in high dose of vitamin C. These implied that extraction wound healing in patients were various due to different dosages of vitamin C patients received (67).

Although the analgesic mechanism of vitamin C is unclear, the previous study reported a correlation between vitamin C and pain control (54). Li et al. showed that vitamin C dose of 300 mg/d did not decrease post-operative pain in dental implant surgery (53), but this study found that vitamin C 600 mg/d reduced post-operative pain after tooth extraction. Mohammed et al. also reported that vitamin C accelerated termination of the inflammatory phase (2). As pain is one of the inflammation symptoms, the shorter inflammation may cause less pain. Since vitamin C acts as an antioxidant, it can reduce cell damage and also reduce inflammation by decreasing in markers of inflammation such as C-reactive protein and pro-inflammatory cytokines (54). We also found that the pain score of placebo side of group P/600 and P/1,500 were rather different. This might be due to the different of pain perception of each patient. Pain is multi-dimensional perception which involves in both physiological and psychological factors (68). Although the physical factor in this study was the same due to the same clinical intervention, the psychological

factor depended on each patient including negative individual experience of pain, anxiety, emotional distress and anticipatory pain (69).

Since vitamin C can be obtained from patients' normal diets such as fruits and vegetables, we tried to reduce the individual variations between patients by designing the study as split mouth. We believed that vitamin C oral supplement which patients received was a top-up vitamin C from their baseline, however, there were some differences in patients' vitamin C-containing diet after 2 extractions. These considered as the limitation of the study because we did not really determine the exact amount of vitamin C taken by each patient. To control patients' vitamin C-containing diet, more defined diet was recommended in the future study.

CHAPTER VI

CONCLUSION

In conclusion, the study ensured the efficacy of vitamin C oral supplement on extraction wound healing with the dosage of 600 mg/d (200 mg three times daily) which had a trend to promote extraction wound healing and reduced post-operative pain. The results of the study provide for further knowledge about vitamin C and dental healing and could be generalized to a larger population with other dental procedures including patients prior to pre-prosthetic treatment and dental implantation, immunocompromised patients and patients with risk of delayed wound healing. Owing to the faster healing of soft tissue compared with hard tissue, further studies are suggested to assess by a long-term follow-up

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APPENDIX

Appendix A Data recorded form of size of the extraction wound

Name.....HN.....Group.....

Tooth.....

Collected data / Day	Size of the extraction wound			Other complication
	BL	MD	Depth	
Immediately after tooth extraction				
Day 7 post-extraction				
Day 21 post-extraction				

Appendix B Data recorded form of toluidine blue stain

Name.....HN.....Group.....

Tooth.....

Collected data / Day	Stain	No stain
Day 21 post-extraction		

Appendix C VAS and dietary recorded form

Day \ Time	8 am	12 pm	6 pm
The extraction day			VAS 0 ——— 10 Dinner
Day 1 post-extraction	VAS 0 ——— 10 Breakfast	VAS 0 ——— 10 Lunch	VAS 0 ——— 10 Dinner
Day 2 post-extraction	VAS 0 ——— 10 Breakfast	VAS 0 ——— 10 Lunch	VAS 0 ——— 10 Dinner
Day 3 post-extraction	VAS 0 ——— 10 Breakfast	VAS 0 ——— 10 Lunch	VAS 0 ——— 10 Dinner

Day 4 post-extraction	Breakfast	Lunch	Dinner
Day 5 post-extraction	Breakfast	Lunch	Dinner
Day 6 post-extraction	Breakfast	Lunch	Dinner
Day 7 post-extraction	Breakfast	Lunch	Dinner

Appendix D Top 5 of high vitamin C vegetables and fruit recorded form

Amounts	Top 5 high vitamin C vegetables	Amounts	Top 5 high vitamin C fruit
	☐ Pepper (red, yellow), raw		☐ Guava
	☐ Pepper (red, green), cooked		☐ Papaya
	☐ Broccoli, cooked		☐ Kiwi
	☐ Cabbage, red, raw		☐ Orange
	☐ Snow peas, cooked		☐ Strawberries

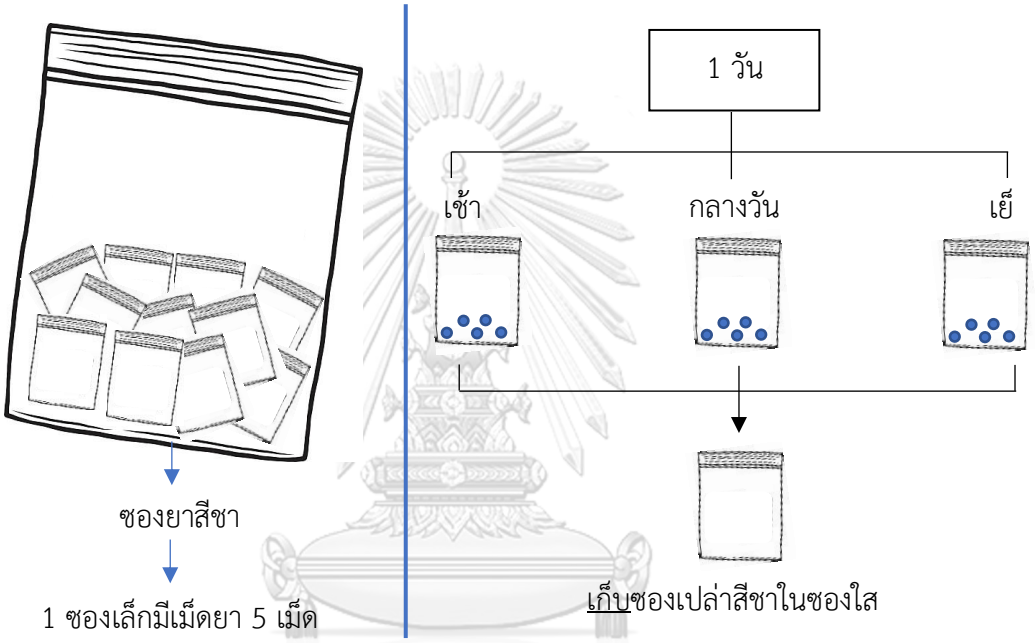
Appendix E Thai recorded form for patients

คำอธิบายเกี่ยวกับการเข้าร่วมวิจัยเรื่อง

ประสิทธิภาพของการให้วิตามินซีเสริมชนิดรับประทานต่อการหายของแผลถอนฟัน

สิ่งที่จะขอความร่วมมือจากอาสาสมัคร คือ

1. ให้อาสาสมัครรับประทานเม็ดยาที่จ่ายไปให้ จำนวน **5 เม็ดต่อมื้อ** เป็นเวลานาน **10 วัน**



2. บันทึกความเจ็บปวด 3 วันแรกหลังถอนฟัน โดยให้กากบาทลงบนเส้นบันทึกความเจ็บปวดจาก 0-10
3. บันทึกอาหารที่อาสาสมัครรับประทาน **7 วัน**หลังถอนฟัน
4. มาตามนัดตรวจแผลหลังถอนฟัน โดยจะนัดเมื่อ ครบ **7 วัน** และ ครบ **21 วัน**หลังถอนฟัน **แต่ละซี่**

หมายเหตุ: รบกวนนำเอกสารฉบับนี้และซองใส่ยามาคืนในวันนัดตอนครบ 21 วันด้วยค่ะ

ขอขอบคุณอาสาสมัครทุกท่านที่ให้ความร่วมมือค่ะ

ทญ.นันทนัท พิศาลสิทธิ์สกุล (นิสิตปริญญาโทผู้ทำวิจัย)

อ.ทญ.ดร.ภัคสินี กมลรัตนกุล (อาจารย์ที่ปรึกษาวิจัย)

ตารางบันทึก<u>ความเจ็บปวด</u>และ<u>อาหาร</u>ที่รับประทาน

วัน \ เวลา	8:00 น.	12:00 น.	18:00 น.
วันที่ถอนฟัน ...../...../.....			ความปวด 0 ——— 10 มื้อเย็น
1 วันหลังถอนฟัน ...../...../.....	ความปวด 0 ——— 10 มื้อเช้า	ความปวด 0 ——— 10 มื้อกลางวัน	ความปวด 0 ——— 10 มื้อเย็น
2 วันหลังถอนฟัน ...../...../.....	ความปวด 0 ——— 10 มื้อเช้า	ความปวด 0 ——— 10 มื้อกลางวัน	ความปวด 0 ——— 10 มื้อเย็น
3 วันหลังถอนฟัน ...../...../.....	ความปวด 0 ——— 10 มื้อเช้า	ความปวด 0 ——— 10 มื้อกลางวัน	ความปวด 0 ——— 10 มื้อเย็น

4 วันหลังถอนฟัน ...../...../.....	มื้อเช้า	มื้อกลางวัน	มื้อเย็น
5 วันหลังถอนฟัน ...../...../.....	มื้อเช้า	มื้อกลางวัน	มื้อเย็น
6 วันหลังถอนฟัน ...../...../.....	มื้อเช้า	มื้อกลางวัน	มื้อเย็น
7 วันหลังถอนฟัน ...../...../.....	มื้อเช้า	มื้อกลางวัน	มื้อเย็น

จำนวน	ผักที่มีวิตามินซีสูง	จำนวน	ผลไม้ที่มีวิตามินซีสูง
	☐ พริกหวานสดหรือพริกหยวกสด สีแดง สีเหลือง		☐ ฝรั่ง
	☐ พริกหวานหรือพริกหยวก สีแดง สีเขียว		☐ มะละกอ
	☐ บร็อคโคลี่		☐ กีวี่
	☐ กะหล่ำสีม่วง		☐ ส้ม
	☐ ถั่วลันเตา		☐ สตรอเบอรี่

Appendix F Thai consent form

เอกสารยินยอมเข้าร่วมการวิจัย

(Consent Form)

การวิจัยเรื่อง ประสิทธิภาพของการให้วิตามินซีเสริมชนิดรับประทานต่อการหายของแผลถอนฟัน

ข้าพเจ้า (นาย/ นาง/ นางสาว/ เด็กชาย/ เด็กหญิง).....

อยู่บ้านเลขที่.....ถนน.....ตำบล/แขวง.....

อำเภอ/เขต.....จังหวัด.....รหัสไปรษณีย์.....

ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้

1. ข้าพเจ้าได้รับทราบรายละเอียดข้อมูลคำอธิบายสำหรับอาสาสมัครที่เข้าร่วมในการวิจัย รวมทั้งได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย วิธีการทำวิจัย อันตรายหรืออาการที่อาจเกิดขึ้นจากการทำวิจัยหรือจากยาที่ใช้รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียดและมีความเข้าใจดีแล้ว
2. ผู้วิจัยได้ตอบคำถามต่างๆ ที่ข้าพเจ้าสงสัยด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าพอใจ
3. ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าเป็นความลับและจะเปิดเผยได้เฉพาะในรูปที่เป็นสรุป ผลการวิจัย การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่างๆ ที่เกี่ยวข้องกระทำได้เฉพาะกรณีจำเป็นด้วยเหตุผลทางวิชาการเท่านั้น และผู้วิจัยรับรองว่าหากเกิดอันตรายใดๆ จากการวิจัยดังกล่าว ข้าพเจ้าจะได้รับการรักษาพยาบาลโดยไม่คิดมูลค่า
4. ข้าพเจ้ามีสิทธิที่จะบอกเลิกการเข้าร่วมในโครงการวิจัยนี้เมื่อใดก็ได้และการบอกเลิกการเข้าร่วมการวิจัยนี้จะไม่มีผลต่อการรักษาโรคที่ข้าพเจ้าจะพึงได้รับต่อไป

ข้าพเจ้าจึงสมัครใจเข้าร่วมโครงการวิจัยนี้ตามที่ระบุในเอกสารข้อมูลคำอธิบายสำหรับอาสาสมัครและได้ลง นามในใบยินยอมนี้ด้วยความเต็มใจ และได้รับสำเนาเอกสารใบยินยอมที่ข้าพเจ้าลงนามและลงวันที่ และเอกสารยกเลิกการเข้าร่วมวิจัย อย่างละ 1 ฉบับ เป็นที่เรียบร้อยแล้ว ในกรณีที่อาสาสมัครยังไม่บรรลุนิติภาวะจะต้องได้รับการยินยอมจากผู้ปกครองด้วย

ลงนาม..... (อาสาสมัคร) (.....) วันที่...../...../.....	ลงนาม..... (ผู้ปกครอง) (.....) วันที่...../...../.....

ลงนาม..... (ผู้วิจัยหลัก) (.....) วันที่...../...../.....	ลงนาม..... (พยาน) (.....) วันที่...../...../.....

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