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ผลของน้ำเกลือที่ระดับความเข้มข้นต่างกันต่อเนื้อเยื่ออ่อนของแผลถอนฟัน: การศึกษาทางคลินิก

น.ส.วลัยลักษณ์ กุนทีกาญจน์

จุฬาลงกรณ์มหาวิทยาลัย
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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาศัลยศาสตร์ช่องปากและแม็กซิลโลเฟเชียล ภาควิชาศัลยศาสตร์
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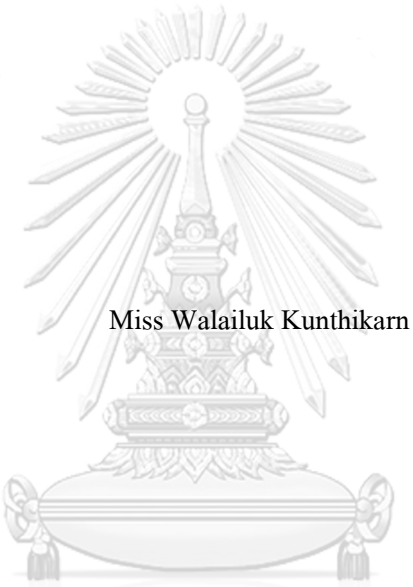
ปีการศึกษา 2565
ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

EFFECT OF NORMAL SALINE IN DIFFERENT CONCENTRATION ON SOFT TISSUE HEALING OF EXTRACTION WOUND: A CLINICAL STUDY

Miss Walailuk Kunthikarn



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A Thesis Submitted in Partial Fulfillment of the Requirements

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Department of Oral and Maxillofacial Surgery

FACULTY OF DENTISTRY

Chulalongkorn University

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หัวข้อวิทยานิพนธ์	ผลของน้ำเกลือที่ระดับความเข้มข้นต่างกันต่อเนื้อเยื่ออ่อนของแผลถอนฟัน: การศึกษาทางคลินิก
โดย	น.ส.วลัยลักษณ์ กุนทีกาญจน์
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คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย อนุมัติให้นับวิทยานิพนธ์ฉบับนี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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วลัยลักษณ์ กุนทีกาญจน์ : ผลของน้ำเกลือที่ระดับความเข้มข้นต่างกันต่อเนื้อเยื่ออ่อนของแผลถอนฟัน: การศึกษาทางคลินิก. (EFFECT OF NORMAL SALINE IN DIFFERENT CONCENTRATION ON SOFT TISSUE HEALING OF EXTRACTION WOUND: A CLINICAL STUDY) อ.ที่ปรึกษาหลัก : ผศ. ทพญ. ดร. ภัคสินี กมลรัตนกุล, อ.ที่ปรึกษาร่วม : รศ. ทพญ. ดร.รัชนี อัมพรอร่ามเวทย์

สารละลายโซเดียมคลอไรด์ (NaCl) ถือเป็นสารน้ำทางการแพทย์ที่เหมาะสมสำหรับการล้างแผล เนื่องจากมีคุณสมบัติไม่เป็นพิษและเป็นไอโซโทนิก การศึกษาในห้องทดลองก่อนหน้านี้แสดงให้เห็นว่าสารละลายโซเดียมคลอไรด์ ทำให้เกิดการเคลื่อนที่ของเซลล์ไฟโบรบลาสต์จากเซลล์เหงือกของมนุษย์ที่มากขึ้น และมีการผลิตสารภายนอกเซลล์เพิ่มขึ้น อย่างไรก็ตาม ยังไม่มีรายงานเกี่ยวกับผลของการล้างแผลถอนฟันด้วยสารละลายโซเดียมคลอไรด์จากการศึกษาทางคลินิก การศึกษาวิจัยนี้เป็นการทดลองทางคลินิกแบบสุ่มที่มีกลุ่มควบคุม และแบ่งเป็นสองฝั่งซ้ายขวา เพื่อตรวจสอบว่าการล้างแผลถอนฟันด้วยสารละลายโซเดียมคลอไรด์ 0.9% และ 1.8% จะสามารถส่งเสริมการหายของเนื้อเยื่ออ่อนของแผลถอนฟันได้หรือไม่ โดยศึกษาในแผลถอนฟัน 60 ตำแหน่ง ในผู้ป่วยที่ได้รับการวางแผนสำหรับการถอนฟันกรามน้อยสองข้าง โดยแบ่งออกเป็น 3 กลุ่มที่ใช้สารละลายที่แตกต่างกัน ได้แก่ น้ำสเตอไรด์ (ตัวควบคุม) สารละลายโซเดียมคลอไรด์ 0.9% และสารละลายโซเดียมคลอไรด์ 1.8% ตามลำดับ โดยวัดความกว้างของด้านใกล้กลาง-ไกลกลาง ความกว้างด้านแก้ม-ลิ้น และความลึกของแผลถอนฟัน ณ วันที่ 0 (หลังการถอนฟันทันที) วันที่ 7 และ 21 ตามลำดับ ผลการวิจัยพบว่าในด้านความกว้างของด้านใกล้กลาง-ไกลกลางมีการลดลงอย่างมีนัยสำคัญทางสถิติ ในกลุ่มน้ำสเตอไรด์และกลุ่มสารละลายโซเดียมคลอไรด์ 1.8% ในสัปดาห์แรก แต่ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติในสัปดาห์ที่สาม ไม่พบความแตกต่างที่มีนัยสำคัญทางสถิติในผลลัพธ์ของความกว้างด้านแก้ม-ลิ้น แต่ความลึกของแผลถอนฟันลดลงอย่างมีนัยสำคัญในกลุ่มสารละลายโซเดียมคลอไรด์ 0.9% ในสัปดาห์ที่สาม ผลการศึกษานี้อาจเป็นประโยชน์ในเรื่องของการกระตุ้นการหายของแผลถอนฟันในผู้ป่วยที่มีภาวะภูมิคุ้มกันบกพร่อง

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สาขาวิชา	ศัลยศาสตร์ช่องปากและแม็กซิลโลเฟเชียล	ลายมือชื่อนิสิต .....
ปีการศึกษา	2565	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

6370025632 : MAJOR ORAL AND MAXILLOFACIAL SURGERY

KEYWORD: Extraction wound, Normal saline solution, wound healing

Walailuk Kunthikarn : EFFECT OF NORMAL SALINE IN DIFFERENT CONCENTRATION ON SOFT TISSUE HEALING OF EXTRACTION WOUND: A CLINICAL STUDY. Advisor: Asst. Prof. PAKSINEE KAMOLRATANAKUL, D.D.S., Ph.D. Co-advisor: Assoc. Prof. RUCHANEE AMPORNARAMVETH, D.D.S., Ph.D.

Sodium chloride solution (NaCl) is regarded as the most suitable and recommended medical irrigation, because of the nontoxic and isotonic properties. The previous in vitro study showed that NaCl solution induced migration and extracellular matrix excretion from human gingival fibroblast. However, there is no report concerning the effect of rinsing extraction wound with NaCl solution in clinical trial study. This research study is split mouth single-blind randomized controlled clinical trial to investigate if rinsing with 0.9% and 1.8% NaCl solution on extraction wound can promote soft tissue healing. The operation was performed in 60 socket sites. The patients, who had been planned for bilateral premolar extraction, were randomly divided into 3 groups with different solutions used including sterile water solution (control), 0.9% NaCl solution, and 1.8% NaCl solution, respectively. Mesio-distal dimension, bucco-lingual dimension and the depth of sockets were evaluated to determine the socket wound healing property on day 0 (immediate post-operation), 7 and 21, respectively. The results showed that there was a significant reduction in Mesio-Distal dimension of the sterile water and 1.8% NaCl group in the first week, but not in the third week. The Bucco-Lingual dimension did not show a significant difference in the results. And the socket depth, there was a significant difference in the percentage reduction in socket depth in the 0.9% NaCl group in the third week. The result of this study may be helpful in term of the acceleration of the healing period in the complicated case or in immunocompromised patients.

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Field of Study:	Oral and Maxillofacial Surgery	Student's Signature .....
Academic Year:	2022	Advisor's Signature .....
		Co-advisor's Signature .....

กิตติกรรมประกาศ

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วลัยลักษณ์ กุนทีกาญจน์

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CHAPTER 1

Introduction

Background and rationale

The healing process comprises of a consecutive overlapping event, divided into 4 phases, coagulative, proliferative, osteogenic-remodeling and epithelium formation. These processes were regulated by many cytokines, chemokines and growth factors that determine cellular recruitment.(1, 2)

The healing of extraction sockets is similar to normal wound healing, which combines soft tissue and alveolar socket healing and associated with individual factors, such as age, area of extraction, pathology of the teeth, physical health and the difficulty of extraction. The cells that come to take this critical place in soft tissue healing process are initially fibroblasts.(3, 4)

Fibroblasts play an essential role in healing, one of the most critical cells in the stroma. They have various functions and composes the basic framework for tissues and organs. Fibroblasts at different body sites have different characteristics depending on locations and activities. The primary function of fibroblasts is to maintain the integrity of tissues by secreting the components of the extracellular matrix, producing collagen fibers, and synthesizing ground substances. They play an essential role in the immune response to tissue injury. They also initiate and stimulate inflammation response to invading microorganisms through cytokine production to continue stimulating a cascade of events to eliminate the microorganism and repair tissues.(5, 6)

The gingival fibroblasts have a high regeneration potential, which makes intraoral wound heals faster than skin or many other structures. In some studies,

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gingival fibroblasts have shown faster collagen contraction than skin fibroblasts.(5, 6) Gingival fibroblasts contract and degrade a fibrin lattice-typically present in early wounds significantly faster than dermal fibroblasts because of an increased fibrinolytic tissue plasminogen activator expression.(7) This may be important in the early stages of healing, allowing cells to remodel the provisional wound matrix quickly and, at least in part, contribute to the fast gingival wound healing.(8) Gingival fibroblasts secrete pro-angiogenic vascular endothelial growth factors to promote endothelial cell growth and angiogenesis and produce keratinocyte growth factors to promote re-epithelialization. As mentioned, wound closure re-epithelialization and angiogenesis are faster than in skin wounds.

The most common complication of wound care is the infection of a wound. Therefore cleansing or irrigation of wounds is an essential part of wound care. Proper cleansing of wounds may create the optimal environment for healing. Sterile normal saline is considered the most appropriate and preferred cleansing solution because it is a non-toxic, isotonic solution that does not damage healing tissues. Moreover, Huynh et al. (2016) stated that rinsing human gingival fibroblast cells with 0.9%-1.8% NaCl for 2 minutes three times daily can promote fibroblast migration.(9)

Many studies,(9, 10) support the efficacy of rinsing wounds with NaCl to promote wound ulcer healing. And the different concentrations of NaCl can give different results on cell activities. To our knowledge, no study reported rinsing extraction wounds with 0.9% and 1.8% NaCl solution affect the healing of soft tissue on extraction wound in the clinical trial study. Thus, this study aims to investigate the efficacy of rinsing extraction wounds with 0.9% and 1.8% NaCl in the extraction wound soft tissue healing.

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Objective

To investigate the efficacy of rinsing the mouth with saline solution in two different concentrations (0.9% and 1.8%) on extraction wounds in the aspect of socket size reduction.

Research question

Does rinsing orally with two different concentrations of a saline solution affect clinical extraction wound healing?

Research hypothesis

H0: Rinsing extraction wound with saline solution 0.9% and 1.8% concentrations does not promote the soft tissue healing

H1: Rinsing extraction wound with saline solution 0.9% and 1.8% concentrations promotes the soft tissue healing

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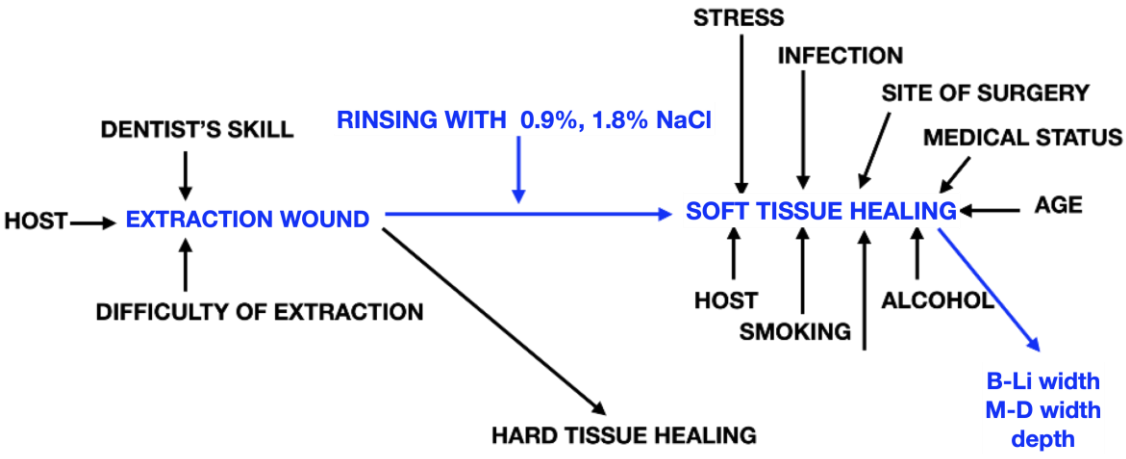
Research design

A split-mouth single-blind randomized-controlled clinical trial

Research Framework



CHAPTER 2

Literature review

The extraction socket wound healing

There are several risks and complications of a tooth extraction that may affect some patients who are indicated a tooth removed, common post-operative effects such as pain, inflammation, bruising and infection. Pain swelling or bruise in the area associated with the operation can be expected for most patients, which will subside shortly.(11) Analgesic and anti-inflammatory medications can help to alleviate the symptoms in the meantime. Continued bleeding and infection of the area are less common, but can occur in some patients or situations and require further management. Dry socket, nerve injury and maxillary sinus exposure can also occur in some cases, which need complicated treatment until the symptoms are cured or subside.(12, 13)

According to the extraction socket healing, soft and hard tissue healing must be concerned. The healing of extraction sockets is similar to normal wound healing. Still, it takes a more extended period, according to factors associated with an individual, such as age, area of extraction, pathology of the teeth, physical health, and the difficulty of extraction.(4)

The healing process comprises a consecutive overlapping event, including extracellular matrix deposition, cell proliferation, cell migration, and remodeling, regulated by many cytokines, chemokines, and growth factors that determine cellular recruitment.(11)

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The basic healing process has been divided into 4 phases

1. coagulative phase
2. proliferative phase
3. osteogenic-remodeling phase
4. epithelium formation

Coagulative phase

When the tooth is extracted, the endothelium is damaged, and the collagen is exposed to the circulating platelet. Collagen will bind with these platelets by collagen-specific glycoprotein and be strengthened by the von Willebrand factor.(14, 15) The localization of the platelets to the extracellular matrix promotes the interaction of collagen and platelets; the other process is the adhesion of the platelet to the injury site. After that, platelets will secrete cytokines to activate a further process of other cells to complete primary hemostasis.(16)

The cascade of secondary hemostasis has two initial pathways that lead to fibrin formation, comprising intrinsic and extrinsic pathways. The final product of these pathways is cross-linked fibrin. Activation of coagulation from the extrinsic pathway happens only when the injury occurs. The first step of this pathway is the interaction between tissue factor and factor VII underneath the endothelial layer. The further process is the activation of factor X to become factor Xa. This latest factor Xa will adhere with factor Va, phospholipid, on the surface of platelets and calcium. This complex is called the prothrombinase complex, which converts prothrombin into thrombin. After that, thrombin will convert fibrinogen to fibrin.(17, 18)

The intrinsic pathway is inactive in the bloodstream, which will be activated by the substance in the subendothelium, mostly collagen fibers. These substances will activate factor XII into factor XIIa, which can activate factor XI to factor XIa, then further activation of factor IX by factor XIa into factor IXa happens, and after the

adhesion of factor IXa, factor VIIIa phospholipid, and calcium becomes a Tenase complex, which can activate factor X and converse factor X into Xa. Then factor Xa will adhere to factor Va, phospholipid, and calcium, becoming a prothrombinase complex that can convert prothrombin into thrombin. Thrombin will convert fibrinogen into fibrin. Then the process comes to a common pathway to complete the coagulative phase.(12, 15)

Proliferative phase

Many processes occur in the proliferative phase, which are blood clot degradation, connective tissue formation, angiogenesis, traveling of osteoclastic cells into the extraction wound area, and the converting of osteoprogenitor cells into osteoblasts. All of these processes occur at the same time. Endothelial cells and Fibroblasts take the role of replacing blood clots with granulation tissues about day seven. (8) The formation of new tissue, made up of collagen, extracellular matrix and composed of red blood cells, white blood cells, fibrin, and plenty of microvascular. In the second week, the osteoprogenitor cells in granulation tissues will be induced to transform into osteoblasts. The connective tissue will replace granulation tissue around day 20. In the proliferative phase, the wound contracts as new tissues are built. A new network of blood vessels must be constructed so that the granulation tissue can receive sufficient oxygen and nutrients. And the myofibroblasts cause wound contraction by pulling the wound edges together. The epithelial cells will resurface the wound, called epithelialization. Most importantly, the wound should be moist, hydrated, and clean to optimize epithelialization.(19, 20)

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Osteogenic-remodeling phase

The osteogenesis comprises osteoid production, mineralization of osteoid, and bone remodeling. Once the tooth extraction occurs, the alveolar bone is resorbed by osteoclast in the inflammatory phase. After that, the osteoblast will secrete a collagen-rich ground substance. The production of extracellular matrix

vesicles, composed of hydroxyapatite, enzyme alkaline pyrophosphatases, and ATPase initiated follow the process of secretion of ground substance. These two enzymes have been studied and believed to be a promotor of calcification by inhibiting the pyrophosphate and ATP to increase phosphate concentration to enhance calcification. At the time of mineralization, the vesicles of hydroxyapatite ruptured, then deposited into the calcified tissue.(20, 21)

The period of bone formation takes two weeks to six months. The bone remodeling phase takes the role, following the bone formation phase.(22) Bone formation starts at the socket's rim, at the endosteum area ingrowth into the central area.

Epithelium formation

The mucosal tissue gradually closes the socket, and collagen fibers can lie closer together and cross-link. Small extraction sockets will be able to close in two weeks, but in large extraction sockets, it may take about three to four weeks to close. Fibroblasts are The cells that take this critical place in the healing process.(23)

Fibroblasts

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Fibroblast is one of the most important cells in the stroma. It has various function and composes the basic framework for tissues and organs.(24) Fibroblasts are flat elongated (spindle-shaped) cells with processes extending out of the cell body with multiple cytoplasmic projections. Their nucleus is flat and ovoid. (14) The cytoplasm of fibroblast has an abundance of rough endoplasmic reticulum (rER) and the large Golgi apparatus. Fibroblasts exist in an alternative state, which are fibrocytes and fibroblasts. Fibroblast is in the activated state, but fibrocytes are in a less active state.(25)

The origin of fibroblasts is primitive mesenchymal stem cells. In some cases, epithelial cells are also shown to produce fibroblasts. That process is called

epithelial-mesenchymal transition (EMT). Conversely, fibroblasts sometimes undergo a mesenchymal-epithelial transition (MET) to produce epithelium.(26)

Fibroblasts at different body sites have different characteristics depending on location and activity. The primary function of fibroblasts is to maintain the integrity of tissues by secreting the components of the extracellular matrix, producing collagen fibers, and synthesizing ground substances. They play an important role in the immune response to tissue injury. They also initiate and stimulate inflammation response to invading microorganisms through cytokine production to continue stimulating a cascade of events to eliminate the microorganism and repair tissues.(27)

The fibroblasts can differentiate into more specialized tissue cells, chondrocytes, osteocytes, adipocytes, and smooth muscle cells by responding to the environmental factors and mediators released from other cell types. Fibroblasts can also modify their extracellular matrix components secreted in response to mediators released from different cell types.(27)

Fibroblasts adapt to their environment during stress and can respond and send local signals. In times of injury, the fibroblast can transform phenotypes and synthesize the building blocks necessary to replace wounded tissue.(28)

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Fibroblasts produce a variety of tissue products, including collagen type I, III, and IV, proteoglycans, fibronectin, laminins, glycosaminoglycans, metalloproteinases, prostaglandins and many more extracellular matrices found in many organs such as skin, bone, lung, heart, liver, kidney, eyes, and the others. The extracellular matrix constantly communicates with the surrounding cells as they can secrete and respond to cells' cytokines.(13)

One well-described fibroblast transformation is to transform into myofibroblast, with the same feature as fibroblast and smooth muscle cells.

Myofibroblast line at the endothelial cells and work with endothelial cells to form granulation tissue during wound healing.(27)

Gingival fibroblasts

The gingival fibroblast has a high regeneration potential, which makes intraoral wound heals faster than skin or many other structures. The reasons that may relate to the specific properties of saliva, which contains molecules to promote wound healing, the arrangement of oral connective tissues that are denser than the skin, in some areas, they attach to the underlying bone so that they can induce the surrounding cells and tissue to migrate to the wound area. In some studies, gingival fibroblasts have shown faster collagen contraction than skin fibroblasts.(5)

Compared with the skin fibroblasts, the gingival fibroblasts express a specific phenotype in which molecules involved in regulating inflammation and extracellular matrix remodeling are elevated.(29, 30) These properties of gingival fibroblasts may then underlie the ability of intraoral or gingival wounds to heal faster with no or less scar formation when compared with skin fibroblasts.(28)

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Gingival fibroblasts contract and degrade fibrin lattice-typically present in early wounds significantly faster than dermal fibroblasts because of an increased fibrinolytic tissue plasminogen activator expression.(7) This may be important in the early stages of healing, allowing cells to remodel the provisional wound matrix quickly and, at least in part, contribute to the fast gingival wound healing.(8) Gingival fibroblasts secrete pro-angiogenic vascular endothelial growth factors to promote endothelial cell growth and angiogenesis and produce keratinocyte growth factors to promote re-epithelialization. As mentioned, wound closure re-epithelialization and angiogenesis are faster than in skin wounds.(28)

Factors influencing the extraction socket wound healing

There are five stages of the healing process that occur in the extraction socket. After tooth extraction treatment, the coagulation from red, white blood cells and the coagulation factors create blood clots in the socket, which leads to the eventual closure of the extraction wound. The second stage represents transforming blood clots into granulation tissue over 4 to 5 days. Afterward, the connective tissues, composed of spindle-shaped fibroblasts, collagen, and the ground substance, replace the granulation tissue in the third stage. The process occurs over a 14 to 16 days period. In the fourth stage, noncalcified osteoid presents at the base of the socket at about 7 to 10 days, and it takes about six weeks to complete the trabecular bone in the socket. For the fifth stage, epithelial closure of the socket wound will meet for 24 to 35 days. After 5 to 10 weeks of healing, the substantial bone fill has taken place in the healing process. The osteogenic activity begins to slow down at week 8. The bone formation will be complete at week 16, but only minimal osteogenic activity remains.(31, 32)

When tooth loss occurs, bone remodeling is a subsequent process in certain patterns. Maxilla and mandible have different ways of ridge resorption. In the maxilla, after the tooth has been extracted, the labial wall of the socket tends to resorb more rapidly, which is different from the mandible. The mandibular alveolar ridge tends to resorb from the lingual aspect first. The alveolar ridges resorb in a horizontal direction before the supero-inferior direction.(32)

Tooth extraction results in remodeling the edentulous alveolar process, including the bone surrounding the tooth socket. The extraction leads to bone loss that affects both the vertical and horizontal dimensions of the socket, causing a three-dimensional volume shrinkage. This shrinkage is accompanied by a lingual shift of the central, meaning that the center of the bone loss tends to be more towards the lingual side.

The bone resorption primarily affects the buccal wall of the socket. This is mainly due to the structural space created by the absence of the tooth and the loss of functional stimulation at the alveolar level. The buccal bone wall tends to undergo partial or complete resorption following tooth extraction.

Studies have reported average horizontal resorption of 3.8 mm and vertical resorption of 1.2 mm within six months after tooth extraction. These measurements serve as general guidelines, but the amount of resorption can vary depending on factors such as the specific tooth extracted, patient age, systemic conditions, and local factors like infection or use of bone grafting materials.

The phenomenon of bone resorption following tooth extraction has been historically associated with the atrophy of the bundle bone. The bundle bone refers to the part of the alveolar bone that receives the functional fibers of the periodontal ligament, which attach the tooth to the surrounding bone. The bundle bone undergoes resorption With tooth extraction and subsequent loss of periodontal ligament function.

It is important to note that these remodeling patterns are generalizations, and individual cases may exhibit variations. The resorption and remodeling process can also continue beyond the initial six-month period (figure 1).(33)

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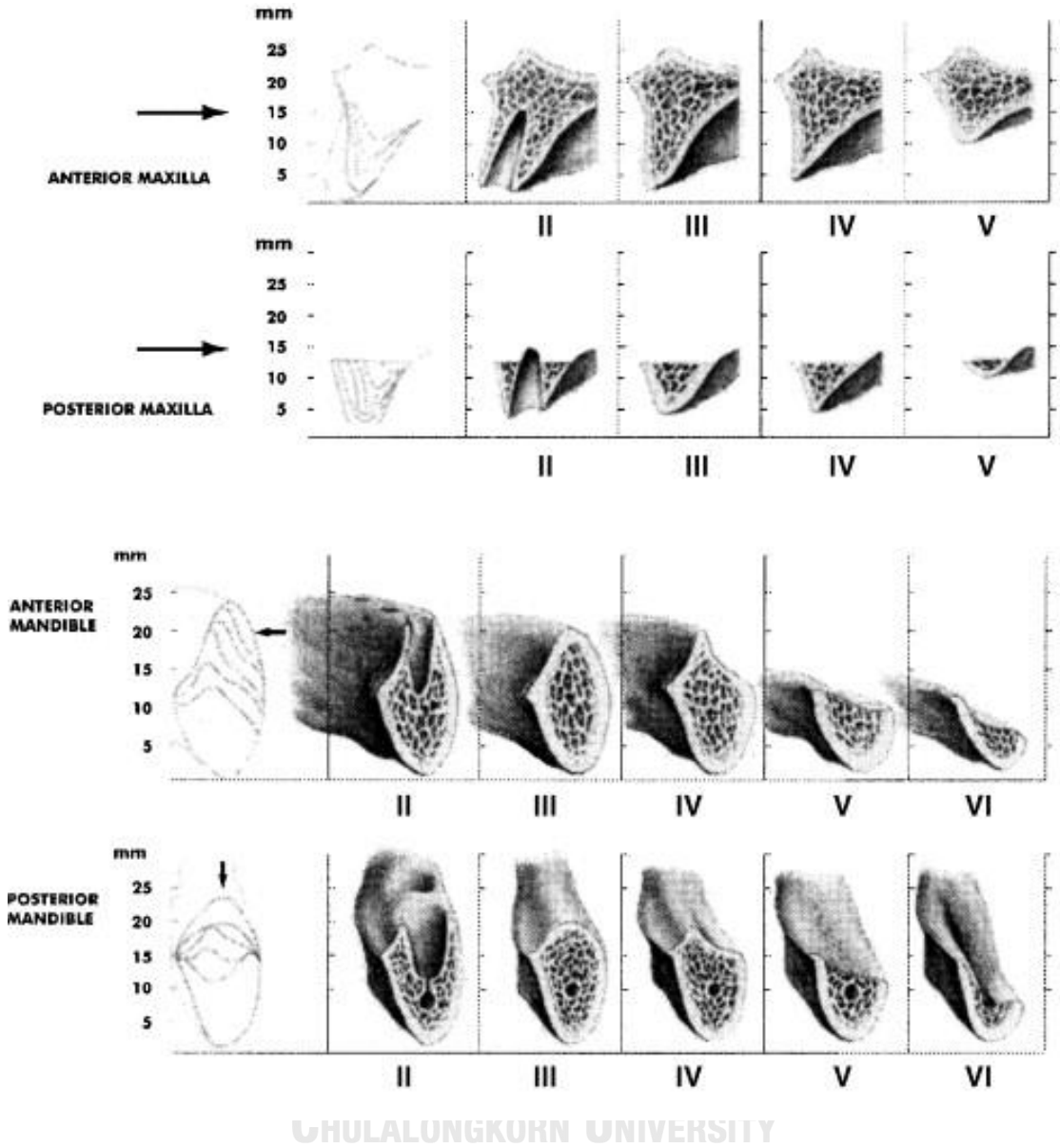

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Figure.1 Pattern of alveolar ridge resorption

Generally, several local and systemic factors were found to influence the healing process. They are causing improper or impaired wound healing. Wounds that exhibit poor healing, including delayed acute and chronic wounds, generally have failed to progress through the normal stages of healing. First, the patient's age is associated with decreasing bone elasticity. Some may associate with a medically compromised host, the medical history which impaired wound healing, such as Diabetes mellitus (DM), where healing impairment is diminished by reducing

angiogenesis, decreasing collagen synthesis, and reducing the capacity and number of immune cells. Patients with long-term steroid use, HIV-infected patients, radio-chemotherapy patients, hepatic disease, and kidney failure. Some drug consumption may affect coagulation and cause prolonged or delayed bleeding after the operations.(29)

Smoking: Smoking also affects clot formation. Post-operatively, the patient who smokes shows delayed wound healing and increased risk of infection, wound rupture, and flap necrosis. Nicotine negatively affects osteoblasts and reduces blood supply and oxygen supply.(23) It stimulates the release of epinephrine which casein peripheral vasoconstriction and decreases tissue perfusion, inhibit fibroblast growth, affects the production of collagen and fibronectin, and promotes collagen breakdown.(34)

The sites of surgery and the number of tooth extraction also relate to the healing process. Multiple extractions are associated with the size of the wound. The larger, the longer time it takes for the healing process. And the extraction site, such as maxillary arch, may cause oro-antral communications or fistula, or in the mandible, associated with the inferior alveolar nerve. The type of tooth, number of roots, and shape of tooth or root are also related to the difficulty of extraction. The following factors are factors affecting the healing process.(34)

Age: Increasing age is one of the strong factors impairing the healing process. But not an actual impairment interns of the quality of healing. Which is associated with delayed immune response, the immune cells' activity, reduced chemokine, and city kine production. Delayed re-epithelialization, collagen synthesis, and agiogenesis have also been observed in aged mice compared to young mice.(35)

Stress: Stress is one of the most impact factors on human health. Many studies show stress-induced disruption of neuroendocrine equilibrium. Stress can

cause delayed wound healing. Stress can up-regulate glucocorticoid hormone and reduce the level of pro-inflammatory cytokines, affecting the healing phase.(35)

Medications: Many medications can interfere with coagulation, inflammatory response, or cell proliferation, such as glucocorticoid steroids, which inhibit cytokine production, and reduce cell production and proliferation. And it also may increase the risk of wound infection.(36)

NSAIDs (Non-steroidal Anti-inflammatory Drugs) are widely used as analgesics. It affects platelet aggregation. It can also decrease the number of fibroblasts, reduced wound contraction, and delayed epithelialization.(36, 37)

Alcohol consumption: Alcohol intoxication can increase susceptibility to wound infection. Acute ethanol exposure can lead to impaired wound healing by impairing the early inflammatory response, inhibiting wound closure, angiogenesis, and collagen production. Chronic alcohol consumption can impair immune cell activity and destroy liver tissue, which affects coagulating factors.

Some local factors influence healing, such as oxygenation and infection.(36)

Oxygenation: Oxygen is important for cell metabolism and critical for the healing process. Oxygen prevents infection, induces angiogenesis, re-epithelialization, and keratinocyte differentiation, enhancing fibroblast proliferation and collagen synthesis. Therefore, the proper oxygen level is important for optimum wound healing.(37)

Infection: the infected wound has a complex community of bacteria in the biofilms. Bacteria in the biofilm can secrete extracellular polysaccharide matrix, which plays the role of delaying wound healing and causing infection of both acute and chronic wounds. Mature biofilms are resistant to conventional antibiotic treatment.(35)

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Normal saline solution

Normal saline is a prescription medicine for fluid and electrolyte replenishment for intravenous administration. Normal Saline may be used alone or with other medications. Normal Saline belongs to a class of drugs called Crystalloid Fluid. It can come in various concentrations, mostly 0.9% and 0.45%.

0.9% Normal Saline is an isotonic concentration of sodium chloride, best suited for parenteral replacement of chloride losses that exceed or equal the sodium loss. Within each 100 mL of 0.9% sodium chloride Injection USP, there is 154 mEq of sodium ions and 154 mEq of chloride ions. Additionally, the osmolarity is 308 mOsmol/liter and a pH range of 4.5 to 7.(38)

Normal saline is a crystalloid fluid. By definition, it is an aqueous solution of electrolytes and other hydrophilic molecules. The main indication for using crystalloid fluids in humans is their isotonic nature compared to serum plasma. There is less of an osmotic effect than different types of fluids (hypertonic, hypotonic).(39)

Sodium ions are the main ion in saline solution. They are the main electrolytes of extracellular fluid, which regulate many actions in many body processes. However, too much sodium intake can have negative health consequences. The kidneys play a crucial role in regulating the amount of sodium in the body, but if there is an excess of sodium intake, the kidneys may struggle to keep up and excrete the excess sodium. This can lead to sodium buildup in the bloodstream, which can contribute to high blood pressure (hypertension). Hypertension is a significant risk factor for heart disease, stroke, and other health problems. The chloride ions are responsible as buffering agents and help to facilitate binding between carbon dioxide and oxygen to hemoglobin and many more actions. Chloride ions play a critical role in many cellular functions, including pH regulation, cell volume regulation, transepithelial salt and water transport, and membrane potential stabilization. In the nervous system, chloride ions are particularly important

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in synaptic signaling mediated by the neurotransmitter GABA (gamma-aminobutyric acid). Normal saline functions to expand intravascular volume without disturbing ion concentration or causing large fluid shifts between intracellular, intravascular, and interstitial spaces.(39)

Hyperosmolarity is when bloody osmolarity is higher than 290 mOsmol/kg. Too intense hyperosmolarity can cause cell damage. But several studies stated that the exposure of cells to a suitable hyperosmotic medium could increase cell diameter and cell adhesion area, enhancing fibroblasts' cell adhesion, which is a critical biological response to wound healing.(40-42)

The study of Huynh et al. 2016, states that rinsing human gingival fibroblast cells with 0.9%-1.8% Nacl for 2 minutes three times a day to mimic the practical habit of performing oral rinse can promote fibroblast proliferation. Appropriate doses of NaCl (0.9%-1.8%) promote hGFs cell migration and extracellular matrix production. Using too high a concentration of NaCl (7.2%) was shown to have adverse effects. Too intense dose of NaCl, especially with long-term exposure, can cause DNA damage and unable to repair. And there is no evidence to support the effect of NaCl on the wound-healing process.(43)

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Wound cleansing

The most common complication of wound care is the infection of the wound. Cleansing or irrigation of wounds is an important part of wound care. Wound irrigation aims to remove the debris, exudate, and foreign material to reduce the risk of bacterial infection and contamination.(44)

Proper cleansing of wounds may create the optimal environment for healing. A variety of cleansing solutions have been launched in the market, and will be selected according to the appropriateness, as long as it does not cause cytotoxicity. Many cleansing solutions have demonstrated safe and effective results. In contrast,

others may damage and destroy cells essential to healing.(10) Sterile normal saline is considered the most appropriate and preferred cleansing solution because it is a non-toxic, isotonic solution that does not damage healing tissues.(45)

Pual et al. (2015) compared the outcomes of normal saline and antiseptic solution for Negative-Pressure Wound Therapy with installation. Antiseptics are the most chosen solutions for irrigating the wound and were reported as the most effective solution for wound cleansing. But for this study, they wanted to compare with normal saline solution for negative-pressure wound therapy. 0.1% polyhexanide plus 0.1% betaine was the antiseptic chosen in this experiment compared to normal saline, and they reported that the use of normal saline gave the effective result of 98% wound closure, which is quite impressive.(10)

A study by Joel et al. (2006) compared cleansing wounds with water versus normal saline solution. This study's literature review also has some studies that support the use of tap water for routine cleansing of acute and chronic wounds. They claimed that using tap water on surgical and sutured wounds did not increase infection rates. Conversely, there is a standard practice of avoiding showering and irrigation for the first operative period. But as this study reviewed, the quality of tap water has a strong impact on microbial growth or wound healing. They suggest that the water should be produced from a potable drinking water supply. Athletic trainers should base decisions on the use of tap water on the quality of the tap water. The type of wound and the availability and cost of other solutions and equipment needed for cleansing.(13)

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According to the study of Yingcharoenthana et al. (2020), they studied the results administration of local and systemic vitamin C to investigate whether it can promote intraoral socket wound healing. They clarified the results supporting the combination of the local and systemic use of vitamin C, which resulted in better overall socket healing. Local administration of vitamin C can be absorbed directly across the wound, and the systemic Vitamin C diffuses into the circulatory system to

the cells. Only a small amount of Vitamin C is biologically available and active in the oral mucosa.(37)

Clinical manifestations of disturbed extraction wound healing can manifest in many pathologic lesions at every phase. The manifestations include excessive bleeding, absence of blood clot formation, granulomatous formation, sinus polyps, wound dehiscence, wound necrosis, pus formation, infection, fibrosis, and trismus. (44, 45) Hom et al. (2009) stated that the following clinical signs indicate poor wound healing: persistent inflammation for longer than seven days, malodor wound, increased exudate, delayed epithelialization, wound dehiscence, and necrotic tissue.(46)

According to the study of Almer et al. (1999), they reported that alveolar osteitis is the result of a disturbance in the progression of healing from blood clot to granulation tissue. Interference or failure in the transformation mechanism from blood clot into granulation tissue gives rise to the well-known symptoms of dry socket, which is one of the most common complications from disturbance of extraction socket wound healing.(47)

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The clinical study from Osundae (2014) revealed that rinsing the extraction wound with warm saline 2 times and 6 times daily after 24 hours of extraction for 2 days can statistically significantly reduce post-operative alveolar osteitis than the non-rinsing group. However, there were no significant differences between patients who gargled six times daily with warm saline and those who gargled twice daily regarding either alveolar osteitis or acutely inflamed socket.(48)

The study of Collins (2021) on the effectiveness of rinsing periodontal surgery wounds with sodium chloride and 0.12% chlorhexidine mouth rinse. Interestingly, the results show a statistically significant reduction in the gingival index at 1 week and 3 months after surgery, comparable to the anti-inflammatory effects of 0.12%

chlorhexidine. Additionally, their pilot study suggests that sodium chloride may be more effective in reducing plaque regrowth.(49)

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CHAPTER 3

Materials and Methods

This study was a split-mouth single-blind, randomized, controlled clinical trial to investigate the rinsing extraction socket with 0.9% and 1.8% saline concentrations affecting soft tissue healing of the extraction socket. The size of sockets in bunco-lingual width, mesiodistal width, and depth of sockets was analyzed to evaluate socket healing.

Population

All patients plan to undergo tooth extraction at the Faculty of Dentistry, Chulalongkorn University.

Samples

Inclusion criteria

1. Patients who have bilaterally symmetrical non-infected premolars needed for extraction such as extraction for orthodontic treatment
2. Age between 15 to 35 years old
3. Patients who have American Society of Anesthesiologist (ASA) class I or II
4. No antibiotic usage
5. Simple/atraumatic extraction

Exclusion criteria

1. Infection is found at the site of extraction
2. Smokers or alcoholics
3. On drug therapy which may affect wound healing such as glucocorticoid steroids and non-steroidal anti-inflammatory drugs and oral contraceptive
4. Pregnant women, lactating women and patients who take estrogen-containg contraceptive drugs

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5. Patients who have blood disorders like thalassemia, G6PD deficiency, sickle cell disease and hemochromatosis
6. Psychiatric patients
7. Patients who cannot participate with the study and post-operative follow-up
8. Patients who cannot rinse their mouth three times a day

Discontinuing criteria

1. Dry socket
2. Buccal bone fracture
3. Patients discontinue the study
4. Late infection
5. Extraction with soft tissue flap operation with or without bone removal

Sample size calculation

The sample size is calculated by using the software G*Power version 3.1. Mean and SD from the pilot study of Pisalsitsakul N, et al. are used. The calculated effect size is 1.16. According to an α error of 5%, study power of 80%, and effect size of 1.16, the sample size of this study will be 8 in each group, allowing for a 25% dropout rate if they are in exclusion criteria. Thus, the study's sample size will be 30, then the sample was calculated as a split-mouth design of 60.

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Surgical procedure

All patients were informed about the objectives, study procedures and the consent forms. Their demographic data including age, gender, medical condition, allergies and medication were also recorded. Panoramic radiographs were used to confirm that teeth are bilateral symmetry.

Teeth extraction were performed by a single experienced operator. Vital sign of patients was recorded before the extraction. Teeth extraction followed

standardized technique. 20% Benzocaine Topical anesthesia gel was applied, local anesthesia with 2% Mepivacaine with 1:100,000 epinephrine was given. The teeth were extracted by elevator and adapted forceps. A gauze was pressed against the extraction site for 1 hour. Each patient was given the same post-operative instructions and medication, which are 15 tablets of 500 mg of paracetamol.

The intervention of this study was a rinsing mouth with saline in two different concentrations, which are 0.9% normal saline which is sold over the counter of drug store and 1.8% saline solution, which composed of 1.8 g. of Sodium Chloride in 100 ml of distilled water, and the sterile water as a control. The solution was prepared by researcher for the accuracy of the outcomes of the study. The solution 15 ml. was instructed to keep in the mouth for 2 minutes and split out, 2 times a day. After brushing the teeth as a typical mouth rinse for 14 days. The patients were instructed to brush their teeth as usual and not using any other mouth rinse while they were in the research project.

Intros split mouth study, the patients were divided into 3 groups randomly as following;

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Group	One side	Another side
1	Control	0.9% saline
2	Control	1.8% saline
3	0.9% saline	1.8% saline

In the first visit, each patient was randomly selected, which side of the tooth was extracted and which intervention was prescribed under allocation concealment for the accurate and non-bias results. After tooth extraction by one

surgeon, the first intervention of each group was prescribed followed our research protocol for 14 days. The intervention was started 24 hours after tooth extraction to preserve blood clot in the socket.

The post-operative evaluations is the size of the sockets measurement (mesio-distal width, bucco-lingual width and socket depth), which was performed by one blinded examiner.

Outcome measurement

One examiner evaluated the size of the sockets. The size of the socket in both buccolingual width and mesiodistal width was measured by a caliper, and the depth of the socket was measured by the periodontal probe using the gingival margin of the adjacent teeth as a reference point. The socket was examined and measured immediately as a baseline and again at day 7 and day 21 after the operation date.

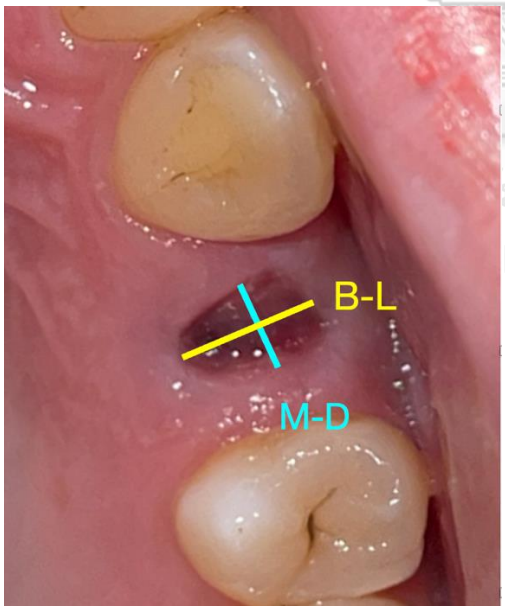

Figure 2. Socket size measurement.
Measurement bucco-lingual width (yellow line) and mesio-distal width (blue line) by caliper

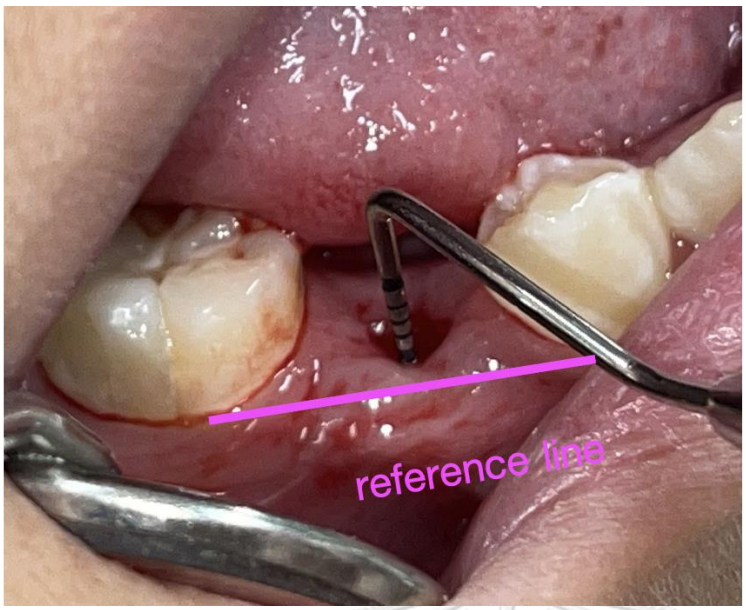

Figure 3. Depth of the socket measurement.

Measurement depth of the socket by periodontal probe using the gingival margin of the adjacent tooth as a reference point.

Date	size of the socket		
	MD	BL	Depth
On the day of the extraction			
Day 7			
Day 21			

Then, the study was continued on the other side. The tooth on the other side was extracted on Day 21 after the records of the previous extraction socket were completely done by the same method and measured by the same procedures. Before starting the extraction on the other side, there was a 7-day washout period to remove all the effects of the previous intervention and make the results more precise.

Data analysis

Statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL). All p- values less than 0.05 was considered significant. The reliability of the intra-interclass correlation coefficient was performed before measuring the study results. And the measurements from one blinded examiner must be accurate. The distribution test was analyzed by the Kolmogorov-Smirnov test, and the data were analyzed by the Kruskal-Wallis test.

Budget

Tooth extraction operation	18,000 baht
Saline solution	10,000 baht
Compensation payment for volunteers	18,000 baht
Documentation fees	2,500 baht
Others	2,500 baht

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Timeline

Activities	JAN 21-MAR 22	APR 22	MAY 22-JULY 22	AUG 22	SEP 22-DEC 22
Literature review and research proposal	■				
Research ethics approval		■			
experiment and data collection			■		
Data and statistical analysis				■	
Report and publication					■

Ethical issues

The characteristics and objectives of the study were informed to all patients. Informed consent forms that the Committees have approved for the Protection of Human Subjects and Scientific Review was signed by all patients. The Ethics Review Committee for Research Involving Human Research Subjects, Health Science Group, Thai Clinical Trials Registry (TCTR20230222004), Chulalongkorn University (REC-DCU 2022-027) approved the study and the patient's informed consent forms.

Results

Thirty patients (60 extraction sites) were included in this pilot study. There were 21 extraction sites for the sterile water group, 19 for the 0.9% NaCl solution group, and 20 for the 1.8% NaCl solution group. The age, gender, and location of the teeth are seen in Table 1. The distribution of the demographic data in Table 1 was in the normal distribution.

Table 1. Demographic data of the patients.

Group	Gender	Age	Location
Sterile water	Male 57.14%	22.63_3.79	Maxillary 66.67%
	Female 42.86%		Mandible 33.33%
0.9% NaCl	Male 63.16%	19.11_4.16	Maxillary 63.16%
	Female 36.84%		Mandible 36.84%
1.8% NaCl	Male 35%	20.15_4.74	Maxillary 30%
	Female 65%		Mandible 70%
P-value	0.311	0.879	0.828

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Comparing the percentage reduction in the mesiodistal width of the socket between the extraction sites, the sterile water group and 1.8% NaCl group demonstrated more reduction in mesiodistal width compared to the 0.9% NaCl group on day 7. However, there was no significant difference in the percentage reduction of mesiodistal width on day 21 in all groups. And the result showed a significant difference in percentage reduction between the sterile water group and 0.9% NaCl group and between 0.9% NaCl and 1.8% NaCl group, as in Table 2.

Table 2 The percentage reduction of mesio-distal width

Group	% MD reduction 1 week			% MD reduction 3 week		
	Mean	SD	95% CI	Mean	SD	95% CI
Sterile water	47.05	1.68	Lower 43.53 Upper 50.56	54.77	1.60	Lower 51.42 Upper 58.12
0.9% NaCl	40.89	1.73	Lower 37.25 Upper 44.53	55.08	1.33	Lower 52.30 Upper 57.88
1.8% NaCl	46.11	1.77	Lower 42.40 Upper 49.81	56.14	1.74	Lower 52.50 Upper 59.79

Kruskal-wallis test revealed significant difference (P = 0.04) in percentage reduction in mesio-distal width at first week

Pairwise comparisons revealed significant difference between group sterile water - 0.9% (P = 0.02) and group 0.9% - 1.8% (P = 0.03)

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No statistically significant difference in percentage reduction in Bucco-Lingial dimension in all groups at both first and third week, as seen in Table 3.

Table 3 The percentage reduction of bucco-lingual width

Group	% BL reduction 1 week			% BL reduction 3 week		
	Mean	SD	95% CI	Mean	SD	95% CI
Sterile water	50.58	1.39	Lower 47.69 Upper 53.48	58.46	1.05	Lower 56.26 Upper 60.66
0.9% NaCl	48.91	2.28	Lower 44.12 Upper 53.69	60.26	1.94	Lower 56.19 Upper 64.34
1.8% NaCl	47.36	1.59	Lower 44.03 Upper 50.70	58.12	1.26	Lower 55.47 Upper 60.77

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The percentage reduction in the socket depth of the 0.9% NaCl group demonstrated a significant difference in socket depth reduction in the third week but not in the first week, and the result also showed a significant difference between the sterile water group and 0.9% NaCl group, as seen in table 4.

Table 4 The percentage reduction of socket depth

Group	% Depth reduction 1 week			% Depth reduction 3 week		
	Mean	SD	95% CI	Mean	SD	95% CI
Sterile water	65.43	1.63	Lower 62.03 Upper 68.83	87.47	2.04	Lower 83.20 Upper 91.74
0.9% NaCl	70.47	2.00	Lower 66.28 Upper 74.67	92.85	1.51	Lower 89.67 Upper 96.03
1.8% NaCl	64.92	2.61	Lower 59.46 Upper 70.38	89.99	1.12	Lower 87.64 Upper 92.33

Kruskal-wallis test revealed significant difference (P = 0.03) in percentage reduction in depth at third week

Pairwise comparisons revealed significant difference between group sterile water - 0.9% (P = 0.009).

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Fig. 4A Fig. 4B Fig. 4C

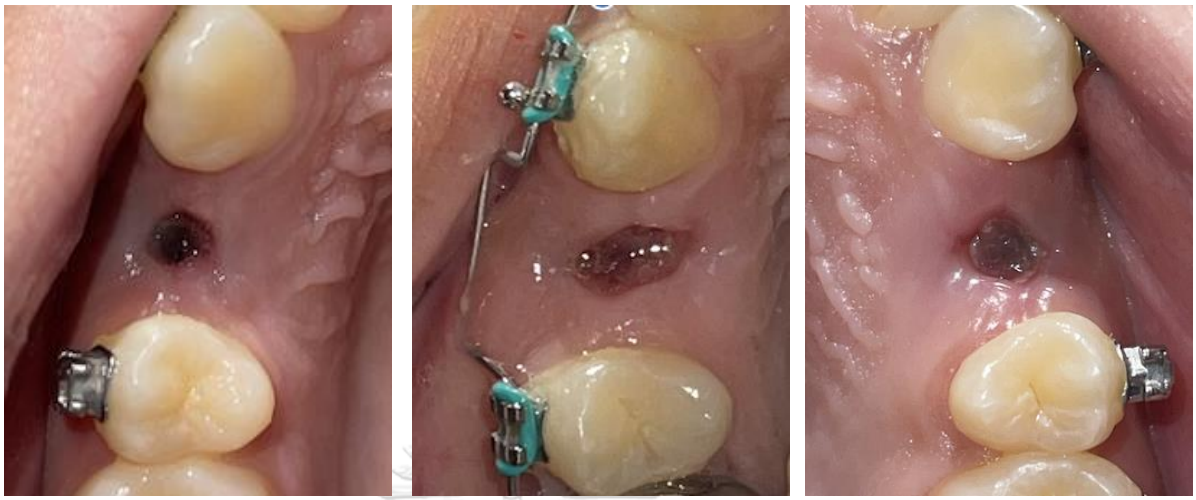

Fig 4A. Showed the socket in sterile water group at first week

Fig 4B. Showed the socket in 0.9% NaCl group at first week

Fig 4C. Showed the socket in 1.8% NaCl group at first week

Fig. 5A Fig. 5B Fig. 5C

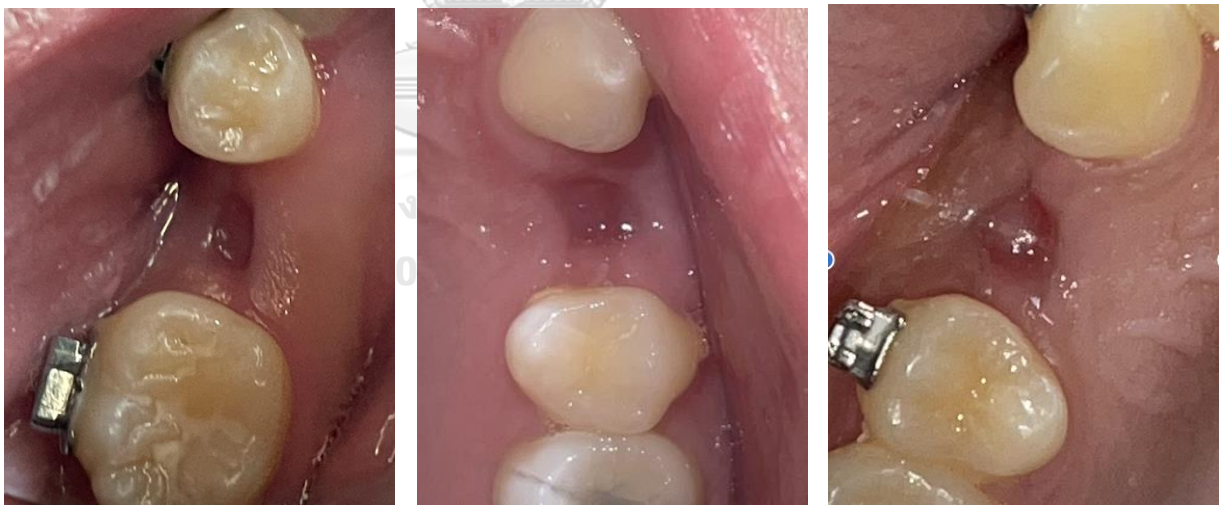

Fig 5A. Showed the socket in sterile water group at third week

Fig 5B. Showed the socket in 0.9% NaCl group at third week

Fig 5C. Showed the socket in 1.8% NaCl group at third week

No complication occurred during the study in all groups of the samples.

Discussions

Wound healing is how the body repairs damaged tissue or wounds. It is a complex process that involves various biological and physiological mechanisms. Delayed healing and infection can occur due to factors such as poor oral hygiene, smoking, systemic diseases, age, and gender. These factors can interfere with the normal wound-healing process and increase the risk of complications. Delayed healing can lead to more complications, particularly in immunocompromised patients more prone to infections. The spread of infection in immunocompromised patients is harder to manipulate. Accelerating wounds to heal faster is the only way to prevent complications.(8, 27, 50)

In normal socket healing, starting with the socket fills with blood and coagulates to form a clot. Within 24 hours of the extraction, the clot stabilizes by fibrin cross-linking. Then, the clot is broken down by fibrinolytic activity of the enzyme plasmin during the next 48 hours. After approximately five days, fibroblasts migrate from the socket wall, and angiogenesis occurs to the formation of capillaries. The clot is replaced by granulation tissue, and gingival epithelium proliferates. In the next two weeks, the granulation tissue is replaced by connective tissue with fibroblasts, collagen, and ground substance. Osteoid is produced by induced mesenchymal cells and osteoprogenitor cells in the remaining periodontal ligament. Then, bone formation occurs and fills in the socket. Bone trabeculae form in 6 weeks post extraction. The epithelial cells completely heal the socket in 24 to 35 days post-extraction, and complete normal healing is approximately three months after the extraction.(51-53)

Interestingly, the blood clot in an extraction wound is replaced by granulation tissue in about a week, and granulation tissue formation and collagen deposition occur from days 4 to 14 after the injury. The depth of the extraction socket decreases once the granulation tissue is well-established, which can take several weeks to a month or longer in larger defects.

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The potential implications for the use of saline in medical and dental applications. In cases of dehydration, saline solution is commonly used to restore bodily fluids and electrolytes. It can also be injected into the venous system to maintain blood volume and pressure, particularly in shock or blood loss cases.

Saline solution is also frequently used for wound irrigation to clean and remove debris from wounds in medical and dental fields, as well as to moisten dressings and promote healing. Additionally, it can be applied topically to soothe and cleanse the skin or mucous membranes.

The clinical study by Osundae (2014) demonstrated that rinsing the extraction wound with warm saline solution has a statistically significant impact on reducing the occurrence of post-operative alveolar osteitis. The study compared two groups: one group rinsed their extraction wounds with warm saline solution either 2 times or 6 times daily, and another did not rinse their extraction wounds . The study results showed that the group that rinsed their extraction wounds with warm saline solution had a significantly lower incidence of post-operative alveolar osteitis than the non-rinsing group. The statistical analysis indicated that the difference between the two groups was highly significant, with a p-value of less than 0.001. These findings supported rinsing extraction wounds with sodium chloride solution, specifically warm saline. Rinsing with a warm saline solution can help cleanse the extraction site, promote healing, and reduce the risk of postoperative complications such as alveolar osteitis.(48)

Moreover, a study by Collins(2021) on the effectiveness of rinsing periodontal surgery wounds with sodium chloride and 0.12% chlorhexidine mouth rinse. Interestingly, the results show a statistically significant reduction in the gingival index at 1 week and 3 months after surgery, comparable to the anti-inflammatory effects of 0.12% chlorhexidine. Additionally, their pilot study suggests that sodium chloride may be more effective in reducing plaque regrowth. (Collins et al., 2021). This study

indicates that sodium chloride could be a promising alternative to chlorhexidine as a post-surgery mouth rinse, with similar anti-inflammatory benefits and potentially better plaque control. Consequently, sodium chloride solution reduced the risk of alveolar osteitis, and its anti-inflammation effect is comparable to chlorhexidine.(49)

The findings from the study of Huynh et al. (2016) reported that the use of NaCl solution within 0.9% to 1.8% NaCl solution for 2 minutes three times a day can be beneficial in promoting the migration of human gingival fibroblasts and the production of extracellular matrix. However, excessive use of NaCl solution at a high concentration (3.6% and 7.2%) can be harmful and may cause cell death and damage to DNA. The interesting results of Huynh et al. (2016) led to this clinical trial.(43)

Based on the results presented, the demographic data analysis showed no statistically significant difference. This indicated that there was no discernible variation among the groups. These findings suggested that the factors, which were age, gender, and location, had no statistically significant difference, which could be implied to the overall population without any bias. In the mesiodistal dimension, there was a significant reduction in the mesiodistal dimension of the sterile water and 1.8% NaCl group in the first week but not in the third week. The Bucco-Lingual dimension did not show a significant difference in the results. Additionally, for the socket depth, there was a significant difference in the percentage reduction in socket depth in the 0.9% NaCl group in the third week, which was consistent with our previous pilot study and also the result from the study of Huynh et al. (2016), which demonstrated the better result of fibroblast migration and extracellular matrix production in 0.9% NaCl group than 0% NaCl group. The results of the reduction in mesiodistal and Bucco-lingual dimension might be inconclusive according to the gingival phenotype, the keratinized band, or even the buccal frenum, which might affect the contraction or the reduction in the size of the socket. The result might be because of the small sample size and study in healthy patients, which can make the results indistinctly different than in immunocompromised patients, especially in

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patients who had a tendency of healing impairment. Therefore, more data collection may need to be done in the future.

Conclusion

In conclusion, this study demonstrated that rinsing the mouth with 0.9% NaCl solution showed a statistically significant difference in socket depth reduction at third week, consistent with our previous pilot study. Nevertheless, there are no complications from rinsing with 0.9% and 1.8% NaCl solution. The result of the study may help reduce the healing time and prevent post-op complications from delayed wound healing in complicated cases or immunocompromised patients.

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