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Association of Initial Periodontal Inflammation and Obesity on Oral Wound Healing Outcomes

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By

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Association of Initial Periodontal Inflammation and Obesity on Oral Wound Healing Outcomes

Neil Bealka, DDS

The University of Nebraska Medical Center, 2023

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The purpose of this prospective cohort pilot study was to determine if initial periodontal inflammation limits healing outcomes after tooth extraction, specifically in terms of reduced periodontal attachment on adjacent teeth and changes in radiographic bone quantity and quality. Additionally, the study sought to explore correlations between patients with high body mass index and post-extraction healing outcomes. The study enrolled nine patients at the University of Nebraska Medical Center College Of Dentistry who required the extraction of a posterior tooth due to advanced periodontal bone loss, non-restorable caries, or root fracture. Prior to extraction, a limited field cone beam CT (CBCT), body mass index (BMI), medical history, baseline periodontal indices including periodontal probing depth (PPD), recession (REC), relative clinical attachment level (CAL), and bleeding on probing (BOP) were recorded on the tooth to be extracted as well as adjacent teeth. Patients were then placed into periodontitis and non-periodontitis groups based on initial clinical indices. Routine atraumatic tooth extraction was performed under local anesthesia. Clinical and radiographic assessments were conducted at baseline and three months after extraction to compare healing outcomes between the groups. Horizontal and vertical residual ridge dimensional changes were assessed using CBCT images. Soft tissue dimensional changes were assessed by evaluating changes in the periodontal indices of adjacent teeth. Residual ridge and soft tissue dimensional changes

occurred in both groups of patients, periodontitis and non-periodontitis, following tooth extraction. For residual ridge changes, there were statistically significant differences noted in the horizontal dimension at the apical aspect of the extraction socket nine millimeters from the osseous crest when comparing groups, periodontitis and non-periodontitis, with greater change noted in the non-periodontitis group ($p= 0.03$). In the coronal aspect of the socket, horizontal residual ridge dimensional changes approached statistical significance one millimeter and six millimeters from the osseous crest, with greater change noted in the non-periodontitis group ($p=0.08$ and $p=0.06$, respectively). Radiographic density measurements approached statistical significance in the tangential view ($p=0.06$), with less radiographic density noted in the periodontitis group. For soft tissue dimensional changes, there were no statistically significant differences noted between the two groups. There were no statistically significant correlations that could be drawn between patients with high pre-operative body mass index and post-extraction healing outcomes. Following tooth extraction, soft tissue, and residual ridge dimensional changes occur during the first three months.

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LIST OF ABBREVIATIONS

PPD	Periodontal probing depth
REC	Recession
CAL	Relative clinical attachment level
BOP	Bleeding on probing
CEJ	Cemento-enamel junction
FGM	Free gingival margin
MB	Mesio-buccal
Mid-B	Mid-buccal
DB	Disto-buccal
ML	Mesio-lingual
Mid-L	Mid-lingual
DL	Disto-lingual
BMI	Body Mass Index
DICOM	Digital Imaging and Communication in Medicine
STL	Stereolithography
HU	Hounsfield

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Chapter 1: Introduction

Partial edentulism can have a significant impact on a patient's oral health and overall quality of life. Traditional treatment options for partial edentulism include fixed partial dentures and removable partial dentures. However, dental implant therapy has emerged as an increasingly popular and effective alternative for replacing missing teeth (Duong, 2022). In the last three decades, oral implant placement has progressed into a reliable and standard treatment option for patients to replace missing teeth, with success rates exceeding 95%. (Lambert, 2009) However, not all patients with partial edentulism are good candidates for dental implant therapy. Factors such as a patient's ability to heal, the amount and quality of available bone, periodontal health, and oral hygiene can all affect the success of the procedure.

Periodontitis is a chronic, local inflammatory condition of the mouth that affects the tooth-supporting tissues, including the periodontal ligament, cementum, and alveolar bone. The condition is characterized by progressive destruction of the tooth-supporting apparatus, ultimately leading to tooth loss if left untreated (Papapanou et al., 2018). The local inflammation caused by periodontitis may be associated with suboptimal healing outcomes following tooth extraction, but the mechanisms involved are not fully understood.

Obesity is a prevalent condition that has been shown to impair healing outcomes in extraoral models. Patients with high body mass index (BMI) have been found to have poorer healing outcomes due to their chronic low-grade systemic inflammation and pro-inflammatory reservoirs (Ellulu, 2017.) There is conflicting evidence on the role of BMI in intraoral healing

outcomes, with some studies reporting an increased risk of postoperative complications and others finding no significant effect (Waisath, 2009).

While systemic and local inflammation have been recognized as critical factors in the extraoral wound healing process, their effects on intraoral healing outcomes are not well understood (Politis, 2016). This knowledge gap warrants investigation as inflammation and wound healing play a significant role in several oral diseases, including periodontitis and tooth extractions. Understanding the relationship between inflammation and healing outcomes in the oral cavity can inform clinical decision-making and improve patient outcomes. It remains uncertain whether a patient's baseline periodontal status at the site of tooth extraction or body mass index is linked to an individual's healing outcome both clinically and radiographically. This prospective cohort pilot study aims to investigate the effects of local inflammation and systemic inflammation on oral surgical wound healing outcomes assessed clinically and radiographically by assessing initial local periodontal inflammation at the site of interest as well as a patient's body mass index.

Clinical Significance:

The successful development of adequate bone and soft tissue after tooth extraction relies significantly on the patient's healing ability. Some patients experience suboptimal healing outcomes, necessitating more extensive bone and soft tissue augmentation procedures to facilitate implant placement, for which the patients incur added costs and treatment time. Both periodontitis and body mass index (BMI) are recognized as factors that contribute to local and systemic inflammation and have been implicated in sub-optimal healing outcomes extraorally.

This pilot study seeks to investigate whether clinical periodontal indices and a patient's pre-operative body mass index can serve as predictors for suboptimal healing outcomes following tooth extraction. The threshold periodontal probing depth and body mass index that causes a clinically significant impact on healing is still unknown.

Chapter 2: Wound Healing and Inflammation:

Wound healing is a complex process that involves four integrated phases: hemostasis, inflammation, proliferation, and remodeling. The effectiveness of wound healing is dependent on systemic patient factors as well as local factors present at the site of the wound. Patient factors such as tissue oxygenation, age, stress, obesity, and nutrition, which can interfere with one or more phases of the healing process, can lead to impaired or delayed healing (Guo, 2010). Understanding wound healing and its potential sequelae is crucial for clinicians to avoid unwanted outcomes.

During the normal wound healing response, hemostasis leads to the formation of a thrombus, followed by the inflammatory phase, which involves the influx of neutrophils, monocytes, and M1 macrophages. Macrophages play multiple roles in the wound healing process, releasing cytokines to promote inflammation and apoptotic clearance of cells leading to the resolution of inflammation, then undergoing a phenotypic transition to M2 macrophages to drive tissue regeneration. The proliferative phase overlaps with the inflammatory phase, involving epithelial proliferation, collagen synthesis, and the formation of the extracellular matrix (ECM). Remodeling is the final stage in wound healing, in which the wound undergoes physical contraction (Guo, 2010; Gonzales, 2016).

Local factors affecting wound healing include local inflammation at the site, as noted in skin models. Persistent inflammation can lead to a prolonged pro-inflammatory state which has been shown to impair healing outcomes in skin models. Systemic factors such as obesity have

also been shown to impair wound healing. Obesity is a global epidemic that affects millions of people worldwide. It is associated with a chronic state of low-grade systemic inflammation, which can lead to various health complications, including impaired wound healing (Khanna, 2022; Hruby, 2015). Obese individuals have been found to have poorer healing outcomes after surgery and trauma, likely due to their impaired immune response and increased levels of pro-inflammatory cytokines (Pierpont et al., 2014).

Body mass index, BMI, a measure of body fat based on height and weight, has also been shown to be a predictor of wound healing outcomes in various settings, including oral surgery. Following third molar removal, patients with high body mass index have been shown to have increased postoperative pain and trismus compared to non-obese patients (Coga, 2022). A systematic review and meta-analysis by Monteiro et al. (2019) found that patients with high BMI had an increased risk of peri-implant diseases compared to those with normal BMI. It has been shown that adipose tissue is a highly active and regulated secretory organ that produces bioactive substances collectively called adipokines, including cytokines, chemokines, and hormone-like factors, which impact the immune system and the inflammatory response. The negative impact of these bioactive substances on the systemic immune response may influence the healing process. The systemic effects of these pro-inflammatory reservoirs have been shown to impair wound healing outcomes in skin models (Cristiana, 2005; Gomez, 2022), but their effect on intraoral healing outcomes remains unclear.

Chapter 3: Extraction Socket Healing

Tooth extraction is a common dental procedure that can lead to significant changes in the surrounding bone and soft tissue. The healing process following tooth extraction involves a series of events similar to cutaneous wound healing, including hemostasis, inflammation, proliferation, and remodeling. The outcome of healing can be affected by various factors such as patient age, systemic health, smoking, periodontal status, and surgical techniques.

After tooth extraction, a series of bone and soft tissue changes occur in the socket. The initial phase involves the formation of a blood clot, which serves as a scaffold for the formation of granulation tissue. This is followed by the migration of osteoblasts and fibroblasts into the socket, leading to the formation of new bone and connective tissue. The conclusion of socket healing is generally clinically observed as closure of the socket with epithelium and radiographic bone fill. Generally, radiographic bone fill is observed between three- to six months following extractions, with the majority of those changes occurring within the first three months. During the stages of alveolar socket healing, first, a clot forms to provide initial hemostasis. Following the formation of the clot, granulation tissue replaces the initial clot over a period of 4-5 days with connective tissue infiltration replacing the granulation tissue over the next 13-16 days. At the base and periphery of the socket, calcification of the osteoid takes place. The osteoid generally appears around 7-10 days and by week six, bone trabeculae nearly fills the socket. Substantial bone fill is observed during the course of the following 5-10 weeks (Amler, 1969).

The rate and extent of bone regeneration depend on various factors such as the size of the socket, presence of infection, and surgical technique. (Araujo & Lindhe, 2005) During the first 6 months there can be up to a forty percent reduction in alveolar ridge height and a sixty percent reduction in the alveolar width. (Schropp, 2003) The rate of bone formation is dependent on many factors, including age, sex, health status, and the presence of infection or inflammation. (Kotwal et al., 2017) It can also be affected by the position in the oral cavity, maxilla versus mandible. More changes are noted in the maxilla as compared to the mandible. Soft tissue changes following tooth extraction can also occur and should be monitored. This is evident clinically as changes in clinical attachment level as well as changes in the periodontal probing depths. Clinically, this is important as sub-optimal soft tissue healing can lead to recession of adjacent teeth and the need for soft tissue augmentation.

The healing of extraction sockets can be affected by local and systemic factors such as age, systemic diseases, and medication use. Older individuals have been shown to have a slower rate of bone regeneration following tooth extraction compared to younger individuals. In the elderly, the initial inflammatory stage of healing is extended or delayed because of the overall heightened inflammatory response in comparison to younger adults therefore delaying the wound healing process. (Bertl, 2019) Systemic diseases such as diabetes and osteoporosis can also impair bone healing and increase the risk of complications. Certain medications such as bisphosphonates and corticosteroids can also have adverse effects on bone healing by inhibiting bone turnover and delaying the bone remodeling process. Long-term use of corticosteroids has been shown to decrease bone mineral density. (Beeraka, 2013) Regulation

of local inflammation appears important for the outcome of wound healing.

(Leblebicioglu, 2020)

Overall, inflammation is a critical component of the wound healing response and is necessary for the removal of debris and the recruitment of immune cells to the site of injury. However, excessive inflammation can impair the healing process and lead to delayed or incomplete healing. In the case of tooth extraction, the presence of inflammation can lead to delayed healing, infection, and bone loss. (Kotwal et al., 2017)

Chapter 4: Periodontitis

Periodontitis is a chronic, local inflammatory disease that affects the supporting tissues of teeth, including the gingiva, periodontal ligament, and alveolar bone. It is caused by the accumulation of bacterial biofilm on the teeth, which triggers an immune response in the host and results in local inflammation (Chapple et al., 2018). Its primary features include clinical attachment loss (CAL), radiographically assessed alveolar bone loss, and the presence of periodontal pocketing and gingival bleeding. (Papapanou, 2018) This inflammatory response is characterized by the recruitment of neutrophils and macrophages, which release cytokines and other inflammatory mediators to fight off the invading bacteria. However, if the inflammation persists, it can lead to destruction of the periodontal tissues and ultimately result in tooth loss (Hajishengallis & Chavakis, 2021).

As dental plaque forms, the plaque becomes arranged into highly organized biofilms that consist of microorganisms to form a complex microbial community. Although calculus is not directly involved in the pathogenesis of periodontal disease, it plays a role by serving as a harbor that protects plaque and biofilm. The initiation and progression of periodontal disease requires not only bacterial plaque, but also requires a susceptible host. This susceptibility to disease can be highly variable and depends on a variety of risk factors such as genetics, stress, immunocompromise, and other systemic diseases. (Marsh, 2006)

In addition to its local effects, periodontitis has been implicated in contributing to a systemic inflammatory load in affected individuals. Chronic inflammation in the periodontium

can lead to the release of inflammatory mediators, such as C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), into the bloodstream (Ebersole & Cappelli, 2000). These systemic effects may contribute to the increased risk of various systemic diseases, such as cardiovascular disease, diabetes, and Alzheimer's disease, in individuals with periodontitis (Jepsen et al., 2018).

The local inflammatory response in periodontitis may also have implications for wound healing in other areas of the body. In skin models, it has been shown that local inflammation can impair wound healing by interfering with the normal sequence of events in the wound healing process, including hemostasis, inflammation, proliferation, and remodeling (Guo, 2010). Specifically, pro-inflammatory cytokines such as TNF-α and IL-1β can delay wound healing by inhibiting the migration and proliferation of keratinocytes and fibroblasts, as well as disrupting the extracellular matrix (ECM) deposition and remodeling. The effects of local periodontal inflammation on intraoral wound healing following tooth extraction are still poorly understood.

Chapter 5: Cone Beam CT

Bone remodeling has traditionally been evaluated through two-dimensional radiographs and cast models. However, cone beam computed tomography (CBCT) has become a valuable tool for analyzing bone healing in extraction sockets and for dental implant surgery. CBCT provides a three-dimensional view of the socket and eliminates the need for standardized imaging. Studies have concluded that CBCT is a reliable modality for assessing bone volume measurements and post-operative assessment of bone healing in extraction sockets. (Singh et al., 2019)

In addition to volumetric changes which are important to successful implant placement, bone density and quality within extraction sockets have also been investigated. The Hounsfield unit (HU) is a measure of radiodensity used to interpret CBCT images. Recent studies have used HU values to measure bone density changes in extraction sockets. HU values are obtained through a transformation of the baseline linear attenuation coefficient of the X-ray beam which the software calculates. Higher HU values indicate more dense tissue, while lower HU values indicate less dense tissue. Studies have demonstrated that HU values obtained from CBCT scans reliably correlate with the density of the anatomical object of interest. (Scarfe et al., 2006; Baciut et al., 2017)

Overall, CBCT has become a valuable tool for analyzing bone healing in extraction sockets. It provides a more comprehensive view of the socket and offers a reliable method for assessing bone density and volume changes. The Hounsfield unit can be used to evaluate bone

quality and density, providing information that can help determine optimal implant placement and size for a given patient. Hiasa (2011) demonstrated the usefulness of HU in pre-surgical assessment of bone quality and quantity. Overall, the combination of CBCT imaging and HU analysis can provide valuable information for perioperative implant planning and bone healing assessment following extraction.

CHAPTER 6: RESEARCH HYPOTHESIS AND SPECIFIC AIMS

The central research hypothesis is that extraction sites that exhibit increased clinical signs of periodontitis and higher body mass index will be associated with reduced healing outcomes clinically and radiographically at 3-months postoperatively.

Specific Aims:

1. To determine if periodontitis sites exhibit more clinical attachment loss at the interproximal surfaces of the teeth adjacent to the extraction site at 3-months post-extraction compared to non-periodontitis sites.

2. To determine if periodontitis sites exhibit more horizontal and vertical bone loss visualized on CBCT at the extraction site 3-months post-extraction compared to non-periodontitis sites.

3. To determine if periodontitis sites exhibit less radiodensity at 3-months post-extraction compared to non-periodontitis sites.

4. To determine if patients with higher body mass index exhibit impaired healing outcomes as observed clinically and radiographically.

Chapter 7: Materials and Methods

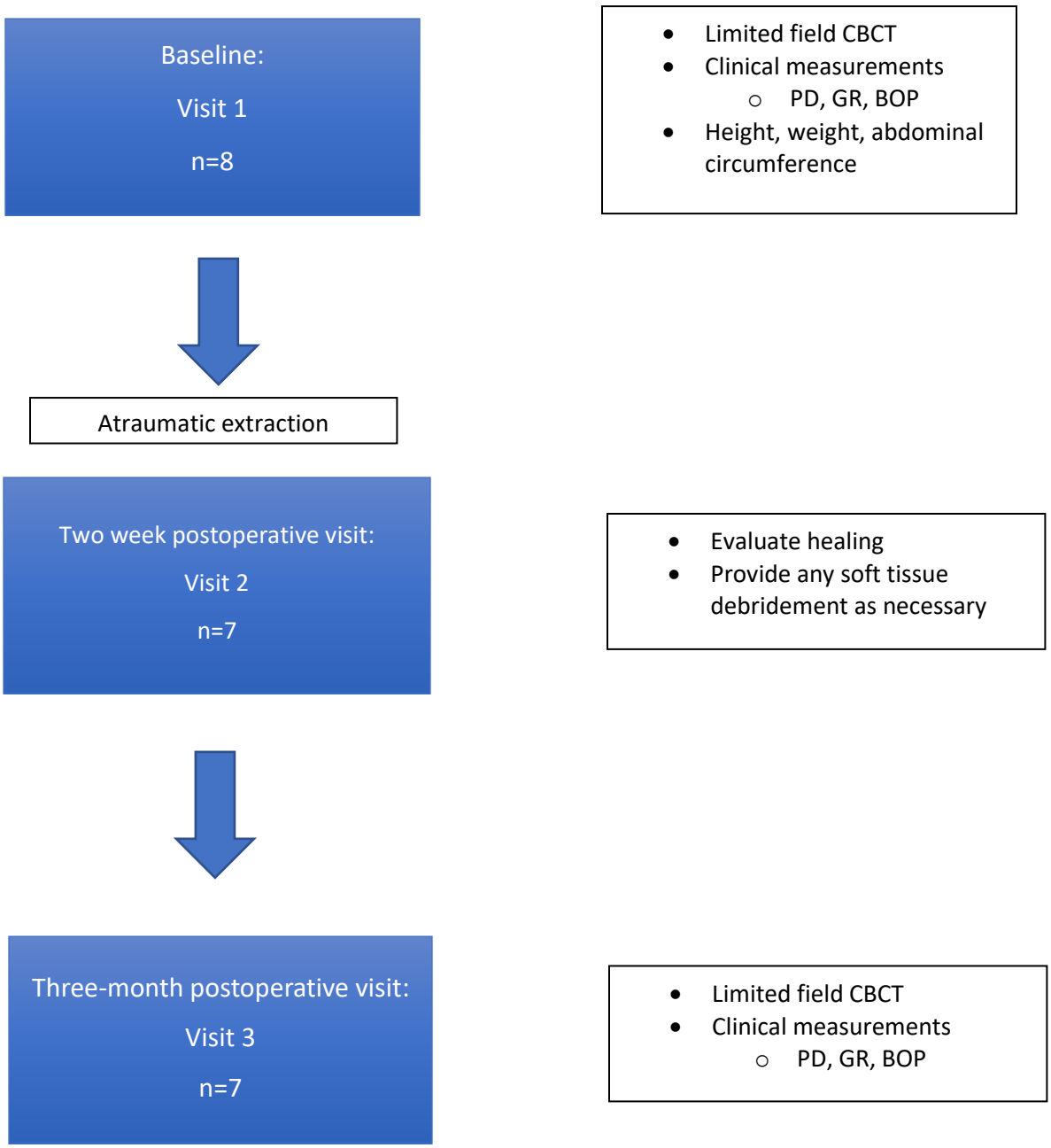
Study Population and Research Design

This three-month prospective cohort, pilot study included patients requiring extraction of a hopeless posterior tooth due to advanced bone loss or non-restorability at the University Of Nebraska Medical Center College of Dentistry in Lincoln, Nebraska. The flow of the study design is included in Figure 1. The following inclusion criteria were used: 1) adult patients seeking care at the UNMC College of Dentistry for extraction of a posterior tooth (premolar/molar) due to a periodontal defect, endodontic defect, non-restorable caries, or fracture; 2) adult patient considering dental implant placement to replace the extracted tooth; 3) no anticipated need for bone grafting following extraction of the tooth; 4) patients between the ages of 21-70 years old. Adult subjects (>21 years old) were chosen to be selected for study inclusion as adults have already completed pubertal-related growth, therefore wound healing will not be affected by pubertal growth hormones. Older patients (>70 years old) typically show decreased wound healing and were therefore excluded from the study. Specific exclusion criteria included the following: subjects with systemic diseases that may affect periodontal inflammation and bone turnover, pregnant patients, and patients <21 years old and > 70 years old. Patients taking chemotherapy agents, those with chronic use of non-steroidal anti-inflammatory medications, bisphosphonates, or any patients with immune-compromised conditions that can delay wound healing were also excluded.

The protocol was approved by the University of Nebraska Medical Center Institutional Review Board, Omaha, Nebraska (IRB protocol #: 0257-21-FB) and was in accordance with the

declaration of Helsinki of 1975. The clinical trial was conducted from January of 2022 to March of 2023. There were no conflicts of interest for this study.

Figure 1: Research Design

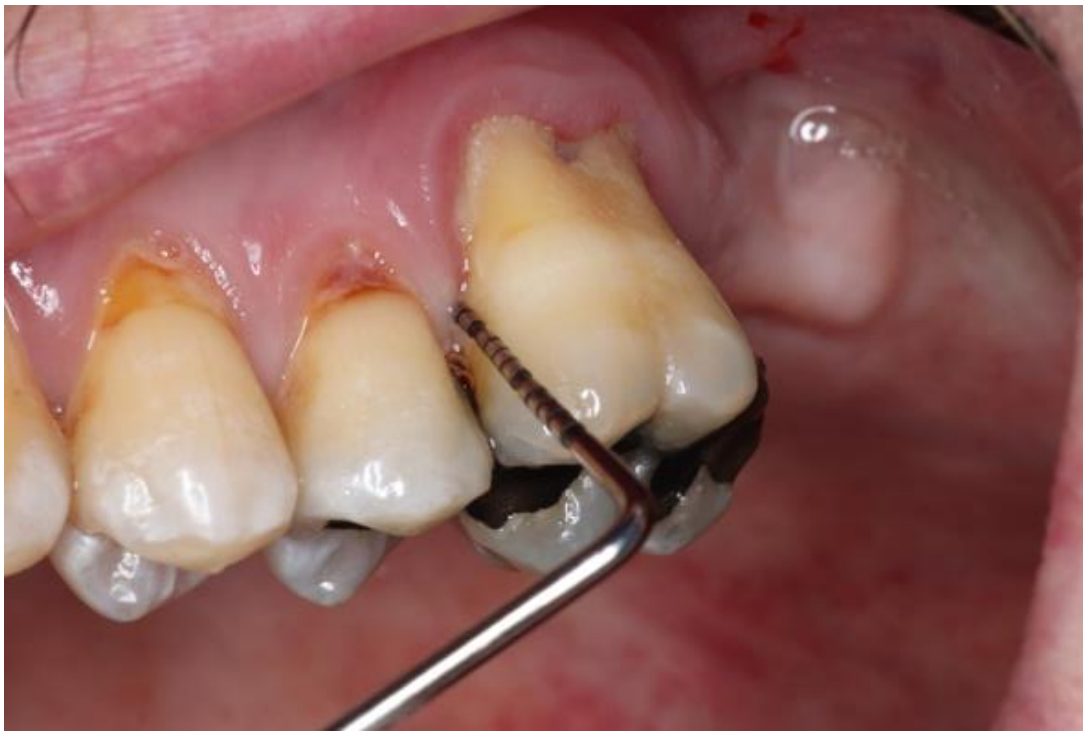


Sample Collection and Clinical/ Radiographic Measurements

Three examiners (NB, SM, PS) were calibrated for reproducibility for periodontal probing depth measurements (PPD), gingival recession measurements (REC), and detection of bleeding on probing (BOP) after 30 seconds. Probing depths were measured from the free gingival margin (FGM) to the base of the pocket for test teeth and adjacent teeth, as indicated in Figure 2. Gingival recession was recorded from the cemento-enamel junction (CEJ) to the free gingival margin, and bleeding on probing was recorded 30 seconds after probe removal. These measurements were taken at six sites on test teeth as well as adjacent teeth: mesio-facial, mid-facial, disto-facial, mesio-lingual, mid-lingual, and disto-lingual.

Before tooth extraction, baseline data was collected which included the following: a limited field cone beam computed tomography (CBCT) of the tooth to be extracted, subject height, weight, gender, smoking status, and medical history. Clinical periodontal data using a UNC 15 periodontal probe was recorded. The clinical attachment level (CAL) was calculated by adding the probing depth and recession measurements. Patients were grouped into the periodontitis group and non-periodontitis groups based on their probing depth measurements. Criteria for inclusion into the periodontitis group was a patient that had a probing depth of ≥ 4 mm with bleeding on probing. Patients were categorized into the non-periodontitis group if they had probing depths of < 4 mm. Body mass index was calculated using the patient's height and weight measurements. Abdominal circumference was taken and recorded. Radiographic measurements were taken at baseline and 3 months to assess initial bone topography, final osseous healing and volumetric changes, as shown in Figures 3 and 4.

Figure 2: Probing Depth Measurement



Treatment Protocol

After data collection, local anesthesia was obtained around the test tooth. A sulcular incision extending to the neighboring papillae was made. Papillae were reflected and a routine atraumatic extraction was performed by assigned residents (SM, NB). Surgical sectioning was performed as necessary to avoid any damage to the underlying bone topography. The extraction sites were curetted of any granulation tissue and irrigated with normal saline.

Postoperative Care

Following the procedure, hemostasis was obtained and 2x2 gauze was placed. Patients were instructed to avoid brushing experimental site for 2 weeks during the initial healing phase. Patients were instructed to take over-the-counter Tylenol for management of any post-operative discomfort.

2-week follow up

Subjects were scheduled for a routine postoperative visit two weeks following the extraction. At this visit, an assessment of healing as well as suture removal as necessary was performed. At this appointment, soft tissue debridement was performed as if impaired healing was noted.

3-month follow up

Subjects were seen for a final post-operative visit at three months to evaluate bone healing and for implant planning. Bone height, width, and density were assessed at the three-month post-operative visit. Also, all baseline clinical measurements were repeated to assess final soft tissue healing and periodontal status of the adjacent teeth.

Assessment of Bone Dimensional Changes on CBCT

Cone beam computed tomography (CBCT) images were obtained on the Planmeca Romexis prior to extraction as well as three months postoperatively. This was used to assess the dimensional changes pre- and post-extraction of the alveolar ridge. A series of cross-

sectional and sagittal views were used for measurements. The CBCT was set to have a slice thickness of 0.5 mm to ensure measurement accuracy. Alveolar bone measurements were performed using the integral tool of the software. Figure 3 demonstrates the pre-operative status prior to tooth extraction. Figure 4 demonstrates the post-operative status following three months healing. The CBCT images were then imported into BlueSky Plan (Libertyville, IL). The CBCT images had common reference points identified on the images and the common reference points were used to align the pre-and post-operative images. The software then calculated the differences between the images and displayed them as color-coded map that highlights areas of change or discrepancy. The pre-extraction and post-extraction results were then evaluated and measurements made using the integral tool of the software. The long axes of the study sites were used to measure the five different levels of alveolar bone width and height before and after surgery. Given these sites would be future implant sites, horizontal measurements were taken at 1, 2, 3, 6, and 9 mm below the osseous crest to represent the coronal, middle, and deep layers of the extraction socket. The vertical height change was measured by evaluating the pre-operative and post-operative images. Figure 5 demonstrates the map generated from the overlays of the CBCT images with blue representing the preoperative status of the alveolar ridge and tooth, and green representing the postoperative status of the alveolar ridge.

Figure 3: Preoperative CBCT- Baseline

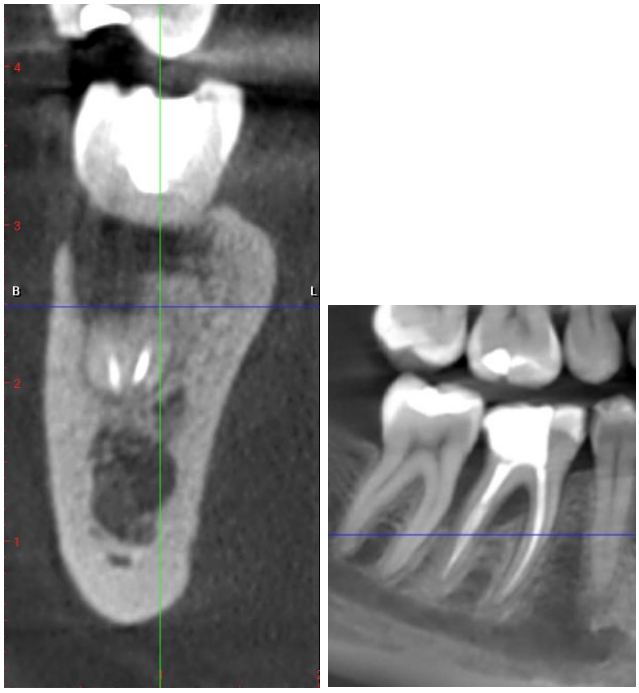


Figure 4: Postoperative CBCT- 3 months

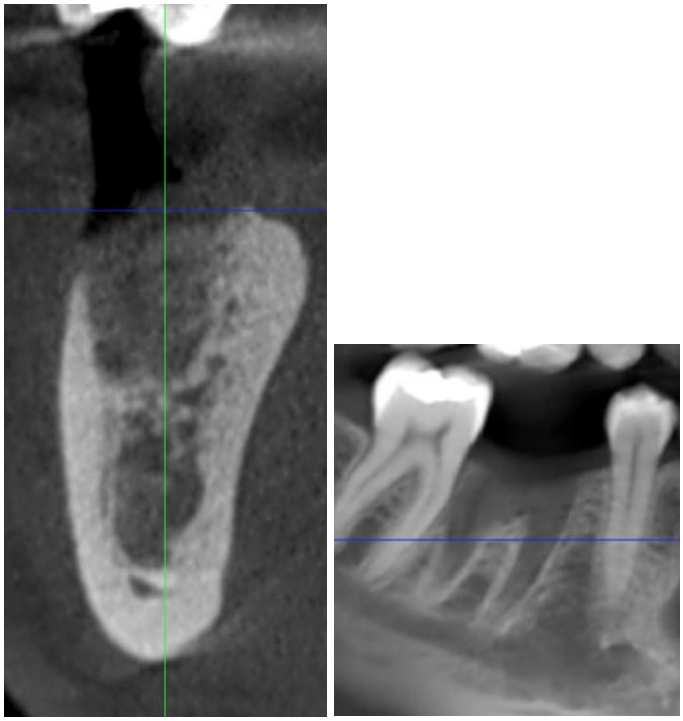
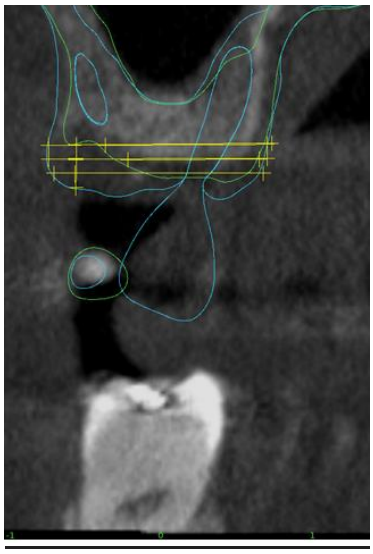


Figure 5: CBCT Overlay Map



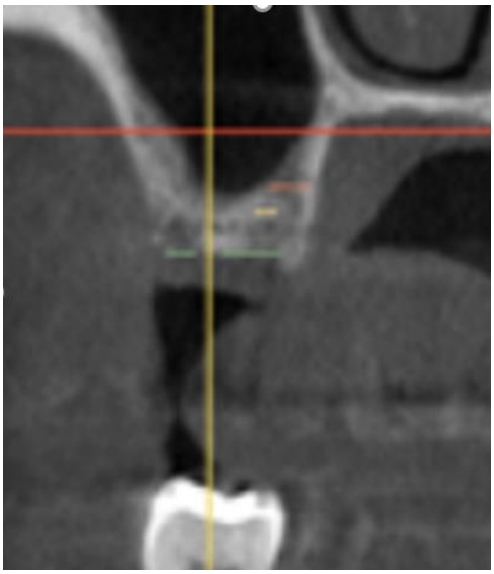
Radiographic Density:

In order to evaluate the bone density, DICOM data of the final post-operative CBCT images were imported into the CBCT software in order to obtain the density measurement for each extraction site. Figure 6 demonstrates a tangential cross section through the post-operative site. The green highlighted area illustrates the extraction socket. The software within 3D Slicer software calculates the mean Hounsfield (HU) unit for the extraction site indicating radiographic density. The Hounsfield unit for each site was assessed in the tangential view and axial view as seen in Figures 6 and 7, respectively. The axial view was used to assess the Hounsfield measurements at three different points. These points were twenty five percent of the extraction socket depth, fifty percent of the extraction socket depth, and seventy-five

percent of the extraction socket depth representing the coronal, middle and deep layer of each extraction socket.

Figure 6: Hounsfield Unit Tangential View

Figure 7: Hounsfield Unit Axial View



Statistical Analysis

Two of the eight teeth in this sample came from the same patient but were treated independently in the analysis. Differences in measurements over time were calculated by subtracting the pre value from the post value. Descriptive statistics for continuous data are given as medians and interquartile ranges (IQRs, representing the range of the middle 50% of the data). Associations between categorical variables were assessed using Chi-Square tests, or Fisher's exact tests when expected cell counts were low. Wilcoxon Rank Sum tests were used to examine differences in distributions of variables of interest between dichotomous variables. Spearman Rho correlations were used to assess correlations between numeric variables. All analyses were performed using SAS software version 9.4 (SAS Institute Inc., Cary, NC).

When interpreting the results of differences which are significant, it would be good to see if the pre-values also significantly differed between groups. If one group had particularly large values at the start, it would make sense that their differences from pre to post would also be large, and thus it might not be the grouping variable that is relevant, but rather that patients in that group happened to have high starting values.

Chapter 8: Results

Patient Characteristics

Following screening, nine patients were enrolled in the study and consented to participate. The median age of the patients for the periodontitis group was 46.5 and 57.0 for the non-periodontitis group with no significant difference in age between the two groups (p=0.77) (Table 1). There was no significant difference in gender enrollment between groups. There were no smokers in either the periodontitis group or the non-periodontitis group. One patient failed to return after the initial extraction appointment and was therefore not included in the analysis. Patient number 5 elected to participate in the study providing two different teeth in two different quadrants that met inclusion. Overall, there were no significant differences between groups in regard to age, gender, or body mass index as seen in Table 1.

TABLE 1: Demographics between Groups

	Periodontitis	Non-Periodontitis	
	n=4	**n=4**	**p-Value**
Gender			
Male	2	2	1 †
Female	2	2	1 †
Smoking Status			
Non-smoker	4	4	
Body Mass Index	34.9	32.1	0.77 ††
Waist Circumference (cm)	112	107	
Median Age	56.0	46.5	0.77 ††

† P-value from Fisher's exact test
†† P-values from Wilcoxan Rank Sum

Clinical Outcomes

The mean baseline and three-month final post-operative results for respective changes in clinical outcomes between groups is reported in Tables 2-6. There were no significant differences between groups for baseline PD or baseline CAL of the test tooth.

Mean difference in radiographic bone width measurements, shown in Table 2, illustrates that there were no statistically significant differences between the periodontitis group and the non-periodontitis group at 1 mm, 2 mm, 3 mm, and 6 mm from the osseous crest. The difference in horizontal measurements between baseline and three months was statistically significant only for H9, nine millimeters from the original osseous crest (p=0.03). The buccal crest height change showed a difference of 1.7 mm for the non-periodontitis group and 1.9 mm for the periodontitis group which was not statistically significant (p=0.89).

Table 2: Radiographic Horizontal and Vertical Residual Ridge Change

P-values from Wilcoxan Rank Sum

	H1 Δ	**H2 Δ**	**H3 Δ**	**H6 Δ**	**H9 Δ**	**Buccal Crest Height Δ**
Periodontitis	-3.0	-1.7	-1.0	-0.0	-0.3	1.9
Non-Periodontitis	-8.0	-5.5	-2.5	-1.9	-0.9	1.7
p-Value	0.08	0.11	0.31	0.06	0.03	0.89

The radiographic density measurements are illustrated in Table 3. There were no statistically significant differences between the periodontitis group and the non-periodontitis

groups in regard to radiographic density measurements in the tangential view or the three axial views representing the coronal, middle, and deep layers of the socket. However, radiographic density measurements approach statistical significance for the tangential view in which periodontitis sites had a mean radiographic density of 325.7 HU and non-periodontitis sites had a mean radiographic density of 568.3 HU (p=0.06).

Table 3: Radiographic Density- Tangential and Axial Views

	Hounsfield Tangential	**Hounsfield Axial 25%**	**Hounsfield Axial 50%**	**Hounsfield Axial 75%**
Periodontitis	325.7	296.6	304.8	512.3
Non-Periodontitis	568.3	625.1	618.4	573.4
p-Value	0.06	0.19	0.11	0.89

For soft tissue dimensional changes on the teeth adjacent to the study site, there were no statistically significant differences between baseline and three months post-operatively for periodontal probing depth and relative clinical attachment level between the periodontitis group and the non-periodontitis groups as seen in Tables 4 and Table 5. Both groups demonstrated a reduction in probing depths from baseline to three-months postoperatively.

Table 4: Change in PPD between Baseline and Three-month Postoperatively

	Ant_DB_ PPD_Diff	Ant_DL_ PPD_Diff	Post_MB_ PPD_Diff	Post_ML_ PPD_Diff
Periodontitis	-0.5	-1.5	-2.0	-1.0
Non-Periodontitis	-1.5	-1.5	-2.0	-1.0
p-Value	0.54	1.00	1.00	1.00

Table 5: Change in CAL between Baseline and Three-month Postoperatively

	Ant_DB_ CAL_Diff	Ant_DL_ CAL_Diff	Post_MB_ CAL_Diff	Post_ML_ CAL_Diff
Periodontitis	0.5	-1.0	-1.5	-1.0
Non-Periodontitis	0.0	-0.5	-3.0	-0.5
p-Value	0.88	0.88	0.41	0.62

In regard to body mass index, Spearman's rho correlation found that there were no statistically significant correlations for patients with higher body mass index and changes in the clinical and radiographic outcomes for patients as seen in Table 6. There was a strong positive correlation between BMI and horizontal dimensional changes at H3, however, the results were not statistically significant (Spearman's rho= 0.61, p= 0.01).

In regard to abdominal circumference, Spearman's rho correlation found that there was positive, statistically significant correlation between patients with larger abdominal circumference and horizontal residual ridge dimensional changes 6 and 9 mm from the osseous crest. Table 7 illustrates the Spearman's Rho Correlation Coefficients and p-values for abdominal circumference.

Table 6: Spearman's Rho Correlation Coefficients and p-values for BMI

	Spearman Correlation	p-value
Buccal Crest Height Δ	0.07	0.87
Hounsfield Tangential	0.05	0.91
Hounsfield Axial 25 %	0.20	0.62
Hounsfield Axial 50%	0.07	0.87
Hounsfield Axial 75%	0.16	0.69
Ant_DB_PPD_Diff	0.06	0.88
Ant_DL_PPD_Diff	-0.22	0.59
Post_MB_PPD_Diff	0	1
Post_ML_PPD_Diff	0	1
Post_MB_AL_Diff	-0.32	0.68
Post_ML_AL_Diff	0.26	0.74
Ant_DB_AL_Diff	-0.54	0.16
Ant_DL_AL_Diff	-0.13	-0.75
H1 Δ	0.40	0.42
H2 Δ	0.08	0.84
H3 Δ	0.61	0.10
H6 Δ	0.50	0.20
H9 Δ	0.20	0.62

Table 7: Spearman's Rho Correlation Coefficients and p-values for Abdominal Circumference

	Spearman Correlation	p-value
Buccal Crest Height Δ	0.34	0.42
Hounsfield Tangential	-0.34	0.42
Hounsfield Axial 25 %	-0.56	0.15
Hounsfield Axial 50%	-0.26	0.53
Hounsfield Axial 75%	0.05	0.91
Ant_DB_PPD_Diff	0.44	0.27
Ant_DL_PPD_Diff	-0.53	0.18
Post_MB_PPD_Diff	0	1
Post_ML_PPD_Diff	0	1
Post_MB_AL_Diff	0.95	0.06
Post_ML_AL_Diff	-0.77	0.23
Ant_DB_AL_Diff	0.12	0.77
Ant_DL_AL_Diff	-0.28	0.50
H1 Δ	0.58	0.23
H2 Δ	0.35	0.40
H3 Δ	0.63	0.09
H6 Δ	0.72	0.04*
H9 Δ	0.78	0.02 *

Indicates statistical significance: *

Chapter 9: Discussion

This prospective cohort pilot study compared healing outcomes following tooth extraction in two groups of patients, periodontitis and non-periodontitis, after three months of healing. The aim was to assess differences in radiographic and clinical outcomes between sites with initial periodontal inflammation and those without. Healing outcomes were assessed radiographically by evaluating changes in the horizontal and vertical residual ridge dimensions using CBCT analysis. The differences in radiographic density between the two groups following healing were evaluated using the Hounsfield unit for assessment of postoperative osseous healing. Clinically, soft tissue dimensional changes were assessed by evaluating changes in the relative clinical attachment level and probing depth measurements on the adjacent teeth. The study also evaluated any potential correlations between patients with high preoperative body mass index and impaired healing outcomes. There were no statistically significant differences in baseline probing depth measurements between the two groups.

The clinical and radiographic healing outcomes between the periodontitis and non-periodontitis group were the primary outcomes evaluated. Body mass index (BMI) was evaluated secondarily to determine if any correlations exist between preoperative BMI and post-extraction healing outcomes. The current study demonstrates that horizontal and vertical changes in the residual ridge occurred following tooth extraction in both groups of patients, which agrees with previous studies evaluating extraction socket healing (Schropp, 2003; Hansson, 2012; Couso-Queiruga, 2021). Six months following extraction, up to a forty percent reduction in alveolar ridge height and a sixty percent reduction in alveolar width has been

reported, with two-thirds of this reduction occurring within the first three months. These changes are generally more pronounced in the coronal one-third of the socket.

This study reveals that both patient groups experienced more significant changes in the horizontal dimension at the crest of the residual ridge, while the changes were less pronounced at the apical extent of the ridge. However, no statistically significant differences were observed between the two groups, except at H9, which is situated nine millimeters from the osseous crest ($p=0.03$). Both the periodontitis group and the non-periodontitis group exhibited the most substantial changes at H1, with volumetric changes decreasing as we moved apically. The values at H1 and H6 approached statistical significance ($p=0.08$ and 0.06, respectively). However, this study revealed an unexpected finding where the non-periodontitis group exhibited more significant changes compared to the periodontitis group. This finding contradicts the central research hypothesis, as one would anticipate a greater horizontal loss in patients with a higher level of local inflammation. One possible explanation for this difference could be attributed to the criteria used to classify patients into the periodontitis and non-periodontitis groups. It is possible that recruiting patients with a more substantial local inflammatory load, characterized by 6-9 mm periodontal pockets, would have resulted in findings consistent with the central research hypothesis. In this study, patients with only mild periodontal inflammation were included, which may have influenced the observed outcomes. In regard to soft tissue dimensions, changes were noted in the periodontal indices of the adjacent teeth following tooth extraction, which is in agreement with previous studies (Lin,

2019). However, there were no statistically significant differences noted between the groups so results need to be interpreted with caution in the context of this pilot study.

A clinically relevant finding related to the contribution of local inflammation on the postoperative healing following tooth extraction was the radiodensity of the residual ridge three months postoperatively. At each level within the extraction socket, the Hounsfield unit measurements exhibited less radiodensity for the periodontitis group compared to the non-periodontitis group, which is consistent with the central research hypothesis. Sites that had local inflammation present at baseline were less radiodense three months postoperatively compared to sites without local inflammation present. This is also in agreement with previous studies pointing to the impact that local inflammation can play on osseous healing (Leblebicioglu, 2020). However, these results were not statistically significant, so would need to be interpreted with caution.

Body mass index was not found to have any statistically significant correlations with postoperative healing outcomes both clinically and radiographically. This is likely attributable to the low sample size of the study. Perhaps in future studies, the recruitment of more patients with larger variations in body mass index would yield statistically significant results. Abdominal circumference did show a strong positive correlation with osseous healing at H6 and H9 (Spearman's rho= 0.72 and 0.78, respectively). This could implicate a possible correlation to the impact of systemic inflammation on the postoperative healing process; however, more studies need to be completed to establish both statistical and clinical significance.

Direct comparisons of the results of this study to other extraction and wound healing studies are challenging as no previous studies have evaluated clinical and radiographic changes between these two groups of patients, periodontitis and non-periodontitis. Also, there are few studies that have evaluated post-extraction healing with CBCT measurements. Although previous studies have evaluated hard and soft tissue dimensional changes following tooth extraction, these studies have primarily evaluated healing outcomes through the use of two-dimensional radiographs and study casts. Subtraction radiography was used in these studies to evaluate horizontal and vertical residual ridge dimensional changes (Schropp, 2003). This differs from the current study in that a three-dimensional CBCT image was used to make residual ridge measurements. This study agrees with previous extraction studies in the sense that major dimensional changes occur in the alveolar ridge following extraction within the first three months; however, some studies report that additional residual ridge dimensional changes occur up to 6 months post-extraction.

These dimensional changes include both changes in the hard tissue architecture as well as the soft tissue architecture. Perhaps in future studies, a longer follow-up period would be beneficial to evaluate hard and soft tissue healing.

There were some limitations to this study. One major limitation of this study is the small sample size. Inherent limitations with a small sample size include reduced statistical power as well as the inability to draw conclusions that can be generalized to a larger group. The lack of heterogeneity within the sample limits the ability of the results to be applied to a larger population, potentially reducing the validity of the results. Perhaps in future studies, the

recruitment of a greater number of patients would yield more statistically significant results. Another limitation of the study was the criteria used to group patients into periodontitis and non-periodontitis groups. The patients selected for inclusion into the periodontitis group had only mild inflammation present at the sites that were being evaluated. Potentially, more statistically significant changes would have been observed if patients with more severe forms of periodontal disease were selected for inclusion. This may have led to larger dimensional changes as patients with more severe forms of disease have more local inflammation present at the site of interest. Also, a full mouth periodontal charting was not completed; therefore, the overall inflammatory load within the oral cavity was not assessed. A novel index that has been introduced in the periodontal literature is called the periodontal inflamed surface area (PISA). This index represents the sum of the periodontal pocket depth of bleeding on probing-positive sites. This is advantageous for research studies as it is treated as a continuous variable that can help quantify the amount of local inflammation present within the oral cavity (Park, 2017). Perhaps in future studies, the consideration of full mouth periodontal charting and calculation of the periodontal inflamed surface area (PISA) to give an accurate representation of the overall oral inflammatory load would be beneficial (Park, 2017). Finally, the study had a significant limitation regarding the field of view utilized for obtaining baseline and postoperative CBCT measurements. Both baseline and final healing measurements were acquired using a limited field CBCT at three months. Although this allowed for overlaying the STL and DICOM data, a more precise overlay could have been achieved by utilizing a full arch CBCT for the patients. However, opting for a full arch CBCT was not feasible due to the potential

increase in radiation exposure for patients. Consequently, the limited field CBCT might have led to less accurate measurements of volumetric bone changes.

It is important to note that the effects of both local and systemic inflammation on post-extraction healing warrant further investigation in future studies. In particular, evaluating patients with higher levels of periodontal inflammation and those individuals with greater variability in body mass index (BMI) may provide valuable insight into the potential influence inflammatory factors have on extraction site healing as well as an evaluation of differences in healing between maxillary and mandibular sites. Such investigations would help determine the interplay between local and systemic inflammatory responses and their impact on postoperative outcomes. Future studies with more patients would allow us to gain a more comprehensive understanding of the complex interactions between inflammation and the wound healing processes. These insights would contribute to providing treatment strategies and improving outcomes in patients undergoing extractions, particularly those with heightened inflammatory conditions.

Chapter 10: Conclusion

Following tooth extraction, patients experience both residual ridge dimensional changes and soft tissue changes which can preclude them from future implant placement. Knowledge of the wound healing process is essential in order to provide optimal patient care. The present study demonstrated residual ridge changes in both the horizontal and vertical dimensions in both groups which is in agreement with previous studies. Although this pilot study did not demonstrate statistically significant differences in dimensional changes between patients with either local or systemic inflammation, it provides a framework for future studies which may help further elucidate the differences between these two groups of patients and postoperative healing outcomes following tooth extraction. We recognize the importance of considering both statistical and clinical significance, and we acknowledge the need for further research to evaluate the impact of these inflammatory factors by recruiting more patients. By integrating these findings into practice, clinicians can make informed decisions and manage patient expectations regarding their expected healing outcomes.

References

Acar B, Kamburoğlu K. Use of cone beam computed tomography in periodontology. World J Radiol. 2014 May 28;6(5):139-47. doi: 10.4329/wjr.v6.i5.139. PMID: 24876918; PMCID: PMC4037540.

Al-Fakeh H, Sharhan HM, Ziyad TA, Abdulghani EA, Al-Moraissi E, Al-Sosowa AA, Liu B, Zhang K. Three-dimensional radiographic assessment of bone changes around posterior dental implants at native bone site in Gansu Province, Northwest of China: A retrospective cohort study. J Stomatol Oral Maxillofac Surg. 2022 Sep;123(4):e186-e191.

Amler MH. The time sequence of tissue regeneration in human extraction wounds. Oral Surg Oral Med Oral Pathol. 1969; 273:309-318.

Anaya DA, Dellinger EP. The obese surgical patient: a susceptible host for infection. Surg Infect (Larchmt). 2006 Oct;7(5):473-80. doi: 10.1089/sur.2006.7.473. PMID: 17083313.

Araújo MG, Silva CO, Misawa M, Sukekava F. Alveolar socket healing: what can we learn? Periodontol 2000. 2015 Jun;68(1):122-34. doi: 10.1111/prd.12082. PMID: 25867983.

Araujo, M., & Lindhe, J. (2005). Dimensional ridge alterations following tooth extraction. An experimental study in the dog. Journal of Clinical Periodontology, 32(S6), 212-218.

Baciut, M., Hedesiu, M., Bran, S., Baciut, G., & Silosi, I. (2017). Hounsfield units in cone beam computed tomography: a literature review. Clinical, Cosmetic and Investigational Dentistry, 9, 43-48.

Barone, A., Aldini, N. N., Fini, M., Giardino, R., & Guirado, J. L. (2014). Xenograft versus extraction alone for ridge preservation after tooth removal: a clinical and histomorphometric study. Journal of periodontology, 85(10), 1333-1340.

Beeraka SS, Natarajan K, Patil R, Manne RK, Prathi VS, Kolaparthi VS. Clinical and radiological assessment of effects of long-term corticosteroid therapy on oral health. Dent Res J (Isfahan). 2013 Sep;10(5):666-73. PMID: 24348627; PMCID: PMC3858744.

Bertl K, Ebner M, Knibbe M, Pandis N, Kuchler U, Ulm C, Stavropoulos A. How old is old for implant therapy in terms of early implant losses? J Clin Periodontol. 2019 Dec;46(12):1282-1293. doi: 10.1111/jcpe.13199. Epub 2019 Nov 8. PMID: 31529723; PMCID: PMC6899847.

Chapple, I. L. C., Mealey, B. L., Van Dyke, T. E., & Bartold, P. M. (2018). Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and

Peri-Implant Diseases and Conditions. Journal of Clinical Periodontology, 45(Suppl 20), S68-S77.

Chen H, Liu N, Xu X, Qu X, Lu E. Smoking, radiotherapy, diabetes and osteoporosis as risk factors for dental implant failure: a meta-analysis. PLoS One 2013; 8: e71955. doi: 10.1371/journal. pone.0071955

Cho YD, Kim KH, Lee YM, Ku Y, Seol YJ. Periodontal Wound Healing and Tissue Regeneration: A Narrative Review. Pharmaceuticals (Basel). 2021 May 12;14(5):456. doi: 10.3390/ph14050456. PMID: 34065862; PMCID: PMC8151433

Coga M, Jerkovic D, Gavic L, Tadin A, Jerkovic K, Macan D. Correlation between Body Mass Index and the Occurrence of Postoperative Complications after Surgical Removal of the Lower Third Molar. Acta Stomatol Croat. 2022 Mar;56(1):12-21. doi: 10.15644/asc56/1/2. PMID: 35382480; PMCID: PMC8972475.

Cristiana E. Juge-Aubry, Elvire Henrichot, Christoph A. Meier, Adipose tissue: a regulator of inflammation,Best Practice & Research Clinical Endocrinology & Metabolism,Volume 19, Issue 4, 2005,Pages 547-566,ISSN 1521-690X, https://doi.org/10.1016/j.beem.2005.07.009.

Couso-Queiruga, E., Stuhr, S., Tattan, M., Chambrone, L. and Avila-Ortiz, G. (2021), Post-extraction dimensional changes: A systematic review and meta-analysis. J Clin Periodontol, 48: 127-145. https://doi.org/10.1111/jcpe.13390

Duong HY, Roccuzzo A, Stähli A, Salvi GE, Lang NP, Sculean A. Oral health-related quality of life of patients rehabilitated with fixed and removable implant-supported dental prostheses. Periodontol 2000. 2022 Feb;88(1):201-237. doi: 10.1111/prd.12419. PMID: 35103325; PMCID: PMC9304161.

Ebersole, J. L., & Cappelli, D. (2000). Acute-phase reactants in infections and inflammatory diseases. Periodontology 2000, 23, 19-49.

Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity and inflammation: the linking mechanism and the complications. Arch Med Sci. 2017 Jun;13(4):851-863. doi: 10.5114/aoms.2016.58928. Epub 2016 Mar 31. PMID: 28721154; PMCID: PMC5507106.

Fokas G, Vaughn VM, Scarfe WC, Bornstein MM. Accuracy of linear measurements on CBCT images related to presurgical implant treatment planning: A systematic review. Clin Oral Implants Res. 2018 Oct;29 Suppl 16:393-415. doi: 10.1111/clr.13142. PMID: 30328204.

Gonzalez AC, Costa TF, Andrade ZA, Medrado AR. Wound healing - A literature review. An Bras Dermatol. 2016 Sep-Oct;91(5):614-620. doi: 10.1590/abd1806-4841.20164741. PMID: 27828635; PMCID: PMC5087220.

Gomez I, Robert V, Alayrac P, Arlat A, Duval V, Renoud ML, Vilar J, Lemitre M, Silvestre JS, Cousin B. Identification of Adipose Tissue as a Reservoir of Macrophages after Acute Myocardial Infarction. Int J Mol Sci. 2022 Sep 10;23(18):10498. doi: 10.3390/ijms231810498. PMID: 36142416; PMCID: PMC9499676

Guo S, Dipietro LA. Factors affecting wound healing. J Dent Res. 2010 Mar;89(3):219-29. doi: 10.1177/0022034509359125. Epub 2010 Feb 5. PMID: 20139336; PMCID: PMC2903966.

Hajishengallis, G., & Chavakis, T. (2021). Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. Nature Reviews Immunology, 21(7), 426-440.

Hämmerle, C. H., Jung, R. E., & Feloutzis, A. (2012). A systematic review of the survival of implants in bone sites augmented with barrier membranes (guided bone regeneration) in partially edentulous patients. Journal of clinical periodontology, 39(suppl 12), 52-66

Hansson S, Halldin A. Alveolar ridge resorption after tooth extraction: A consequence of a fundamental principle of bone physiology. J Dent Biomech. 2012;3:1758736012456543. doi: 10.1177/1758736012456543. Epub 2012 Aug 16. PMID: 22924065; PMCID: PMC3425398.

Hiasa K, Abe Y, Okazaki Y, Nogami K, Mizumachi W, Akagawa Y. Preoperative computed tomography-derived bone densities in hounsfield units at implant sites acquired primary stability. ISRN Dent. 2011;2011:678729. doi: 10.5402/2011/678729. Epub 2011 Nov 30. PMID: 22203910; PMCID: PMC3235676.

Hruby A, Hu FB. The Epidemiology of Obesity: A Big Picture. Pharmacoeconomics. 2015 Jul;33(7):673-89. doi: 10.1007/s40273-014-0243-x. PMID: 25471927; PMCID: PMC4859313.

Hu K-F, Lin S-W, Lin Y-C, Jeng J-H, Huang Y-T, Liu P-F, Tseng C-J, Chou Y-H. Using Cone-Beam Computed Tomography to Assess Changes in Alveolar Bone Width around Dental Implants at Native and Reconstructed Bone Sites: A Retrospective Cohort Study. *Journal of Personalized Medicine*. 2021; 11(10):1011. https://doi.org/10.3390/jpm11101011

Jepsen, S., Caton, J. G., Albandar, J. M., Bissada, N. F., Bouchard, P., Cortellini, P., ... & Zijnge, V. (2018). Periodontal manifestations of systemic diseases and developmental and acquired conditions: Consensus report of workgroup 3 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. Journal of clinical periodontology, 45(Suppl 20), S219-S229. doi: 10.1111/jcpe.12946

Khanna D, Khanna S, Khanna P, Kahar P, Patel BM. Obesity: A Chronic Low-Grade Inflammation and Its Markers. Cureus. 2022 Feb 28;14(2):e22711. doi: 10.7759/cureus.22711. PMID: 35386146; PMCID: PMC8967417.

Knapik DM, Perera P, Nam J, Blazek AD, Rath B, Leblebicioglu B, Das H, Wu LC, Hewett TE, Agarwal SK Jr, Robling AG, Flanigan DC, Lee BS, Agarwal S. Mechanosignaling in bone health, trauma and inflammation. Antioxid Redox Signal. 2014 Feb 20;20(6):970-85. doi: 10.1089/ars.2013.5467. Epub 2013 Aug 12. PMID: 23815527; PMCID: PMC3924811.

Kotwal GJ, Chien S. Macrophage Differentiation in Normal and Accelerated Wound Healing. Results Probl Cell Differ. 2017;62:353-364. doi: 10.1007/978-3-319-54090-0_14. PMID: 28455716; PMCID: PMC5841920.

Lambert FE, Weber HP, Susarla SM, Belser UC, Gallucci GO. Descriptive analysis of implant and prosthodontic survival rates with fixed implant-supported rehabilitations in the edentulous maxilla. J Periodontol 2009; 80: 1220–30. doi: 10.1902/jop. 2009.090109

Lin JH, Tu CC, Chen YW, Wang CY, Liu CM, Kuo MY, Chang PC. Influence of Adjacent Teeth Absence or Extraction on the Outcome of Non-Surgical Periodontal Therapy. Int J Environ Res Public Health. 2019 Nov 7;16(22):4344. doi: 10.3390/ijerph16224344. PMID: 31703336; PMCID: PMC6888517.

Marsh PD. Dental plaque as a biofilm and a microbial community - implications for health and disease. BMC Oral Health. 2006 Jun 15;6 Suppl 1(Suppl 1):S14. doi: 10.1186/1472-6831-6-S1-S14. PMID: 16934115; PMCID: PMC2147593

Monteiro JLGC, Pellizzer EP, Araújo Lemos CA, de Moraes SLD, do Egito Vasconcelos BC. Is there an association between overweight/obesity and dental implant complications? A systematic review and meta-analysis. Int J Oral Maxillofac Surg. 2019 Sep;48(9):1241-1249. doi: 10.1016/j.ijom.2019.01.015. Epub 2019 Feb 19. PMID: 30792086.

Nemtoi, A., et al. "Cone beam CT: a current overview of devices." *Dentomaxillofacial Radiology* 42.8 (2013): 20120443.

Papapanou, Panos N., et al. "Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions." Journal of Clinical Periodontology, vol. 45, supplement 20, 2018, pp. S162-S170.

Park SY, Ahn S, Lee JT, Yun PY, Lee YJ, Lee JY, Song YW, Chang YS, Lee HJ. Periodontal inflamed surface area as a novel numerical variable describing periodontal conditions. J Periodontal Implant Sci. 2017 Oct;47(5):328-338. doi: 10.5051/jpis.2017.47.5.328. Epub 2017 Oct 30. PMID: 29093989; PMCID: PMC5663669.

Pauwels R, Jacobs R, Singer SR, Mupparapu M. CBCT-based bone quality assessment: are Hounsfield units applicable? Dentomaxillofac Radiol. 2015;44(1):20140238. doi: 10.1259/dmfr.20140238. PMID: 25315442; PMCID: PMC4277442.

Pischon N, Heng N, Bernimoulin JP, Kleber BM, Willich SN, Pischon T. Obesity, inflammation, and periodontal disease. J Dent Res. 2007 May;86(5):400-9. doi: 10.1177/154405910708600503. PMID: 17452558.

Pierpont YN, Dinh TP, Salas RE, Johnson EL, Wright TG, Robson MC, Payne WG. Obesity and surgical wound healing: a current review. ISRN Obes. 2014 Feb 20;2014:638936. doi: 10.1155/2014/638936. PMID: 24701367; PMCID: PMC3950544.

Politis C, Schoenaers J, Jacobs R, Agbaje JO. Wound Healing Problems in the Mouth. Front Physiol. 2016 Nov 2;7:507. doi: 10.3389/fphys.2016.00507. PMID: 27853435; PMCID: PMC5089986.

Scarfe, W. C., Farman, A. G., & Sukovic, P. (2006). Clinical applications of cone-beam computed tomography in dental practice. Journal of the Canadian Dental Association, 72(1), 75-80.

Schropp L, Wenzel A, Kostopoulos L, Karring T. Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. Int J Periodontics Restorative Dent. 2003 Aug;23(4):313-23. PMID: 12956475.

Singh, A., Sun, Y., & Wadhwani, C. P. (2019). Cone beam computed tomography: a useful tool for assessing bone regeneration in ridge preservation and augmentation. Journal of Oral Implantology, 45(5), 402-408.

Stadelmann, W.K., et al. "Endogenous interferon gamma impairs healing and induces local immunosuppression in skin wound healing." Journal of Immunology, vol. 162, no. 12, 1999, pp. 7462-7470.

Vignoletti, F., Matesanz, P., Rodrigo, D., Figuero, E., Martin, C., Sanz, M., & Salvi, G. E. (2012). Surgical protocols for ridge preservation after tooth extraction. A systematic review. Clinical oral implants research, 23, 22-38.

Waisath TC, Marciani RD, Waisath FD, James L. Body mass index and the risk of postoperative complications with dentoalveolar surgery: a prospective study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009 Aug;108(2):169-73. doi: 10.1016/j.tripleo.2009.04.028. PMID: 19615654.

Appendix A:

Raw Clinical Data- Patient Characteristics

Patient	Periodontitis	Age	BMI	Abd Circ. (cm)	Gender	Smoking	Tooth Extracted
1	1	39	38.1	112	F	0	Mand. 1st molar
2	1	70	31	113	M	0	Max. 2nd premolar
3	1	54	39.1	121	F	0	Mand 1st molar
4	0	58	38.8	111	F	0	Max 2nd molar
5.1	0	37	31.6	108	M	0	Max 1st molar
5.2	1	37	31.6	108	M	0	Max 1st molar
6	0	56	26	106	M	0	Max 1st molar
8	0	62	32.6		F	0	Max 2nd premolar

Appendix B:

Raw Clinical Data- Extracted Tooth

PATIENT	MB PPD	MB REC	MB CAL	MID-B PPD	MID-B REC	MID-B CAL	ML PPD	ML REC	ML CAL	DB PPD	DB REC	DB CAL	MID-L PPD	MID-L REC	MID-L CAL	DL PPD	DL REC	DL CAL
1	2	0	2	3	0	3	4	0	4	3*	0	3	1	0	1	3	0	3
2	5	0	5	2	1	3	4	0	4	3*	0	3	3	0	3	4	0	4
3	5*	0	5	4*	6	10	4*	0	4	3*	0	3	2	0	2	4*	0	4
4	2	0	2	1	0	1	2	0	2	2	0	2	2	0	2	3	0	3
5.1	3*	1	4	2*	4	6	2	0	2	2*	4	6	1	7	8	3*	4	7
5.2	3	0	3	2	0	2	2	0	2	4*	0	4	2	0	2	3*	0	3
6	3*	0	3	1*	2	3	3	0	3	3	0	3	2	0	2	3	0	3
8	3	0	3	1	0	1	2	0	2	1	0	1	2	0	2	1	0	1

Appendix C:

Raw Clinical Data- Adjacent Teeth Baseline

Patient	Ant DB PPD	Ant DB REC	Ant DB CAL	Ant DL PPD	Ant DL REC	Ant DL CAL	Post MB PPD	Post MB REC	Post MB CAL	Post ML PPD	Post ML REC	Post ML CAL
1	2	0	2	3	0	3	4 *	0	4	3	0	3
2	3	0	3	4	0	4	3	0	3	3	0	3
3	3	0	3	4	0	4	3	0	3	3	0	3
4	3	0	3	3	0	3	n/a	n/a	n/a	n/a	n/a	n/a
5.1	2	0	2	2	0	2	n/a	n/a	n/a	n/a	n/a	n/a
5.2	2	0	2	1	0	1	n/a	n/a	n/a	n/a	n/a	n/a
6	3	0	3	3	0	3	3	0	3	3	0	3
8	3	0	3	2	0	2	3	3	6	2	0	2

Appendix D:

Raw Clinical Data- Adjacent Teeth 3 months Postoperative

Patient	Ant DB PPD	Ant DB REC	Ant DB CAL	Ant DL PPD	Ant DL REC	Ant DL CAL	Post MB PPD	Post MB REC	Post MB CAL	Post ML PPD	Post ML REC	Post ML CAL
1	2 *	0	2	2	0	2	2	0	2	2	0	2
2	3	2	5	2 *	1	3	1 *	1	2	2 *	0	2
3	1	1	2	1	0	1	2 *	0	2	3 *	0	3
4	3	0	3	1 *	2	3	n/a	n/a	n/a	n/a	n/a	n/a
5.1	1	4	5	1	3	4	n/a	n/a	n/a	n/a	n/a	n/a
5.2	1	2	3	2	3	5	n/a	n/a	n/a	n/a	n/a	n/a
6	1	2	3	1	0	1	1	0	1	2	0	2
8	1	0	1	1	0	1	1	1	2	1 *	1	2

Appendix E

Raw Clinical Data: Horizontal and Vertical Residual Ridge Measurements

Patient	Periodontitis	Pre-H1	Post-H1	H1 Δ	Pre-H2	Post-H2	H2 Δ	Pre-H3	Post-H3	H3 Δ	Pre-H6	Post-H6	H6 Δ	Pre-H9	Post-H9	H9 Δ	Buccal Crest Height Δ
1	1	13.53	10.63	2.90	14.12	14.00	0.12	14.18	14.37	-0.19	13.26	13.52	-0.26	12.04	12.28	-0.24	2.22
2	1	10.97	0	10.97	11.35	10.52	0.83	11.32	10.76	0.56	10.56	8.91	1.65	11.17	10.65	0.52	1.50
3	1	10.74	7.75	2.99	11.59	8.95	2.64	11.02	9.51	1.51	13.29	13.29	0	13.77	13.73	0.04	3.69
4	0	9.42	1.44	7.98	14.07	4.46	9.61	15.30	15.11	0.19	15.73	15.47	0.26	14.12	13.29	0.83	1.09
5.1	0	13.77	3.17	10.60	14.16	8.91	5.25	14.47	11.30	3.17	14.90	12.91	1.99	14.99	13.95	1.04	2.13
5.2	1	6.47	2.39	4.08	6.95	3.91	3.04	6.56	4.26	2.30	6.34	6.30	0.04	8.25	7.76	0.60	1.01
6	0	13.78	0	13.78	14.36	8.63	5.73	14.67	10.64	4.03	14.21	12.34	1.87	14.51	13.73	0.78	2.87
8	0	10.30	3.63	6.67	10.64	8.47	2.17	10.60	8.86	1.74	9.60	6.81	2.79	8.69	7.45	1.24	1.24

Appendix F: Hounsfield Units 3 months Post-operatively

Patient	Hounsfield_Ax25	Hounsfield_Ax50	Hounsfield_Ax75	Hounsfield_Tang
1	411.29	321.05	817.57	366.91
2	158.10	140.78	223.15	189.14
3	358.92	597.28	536.72	455
4	573.50	584.97	527.07	484.60
5.1	198.93	759.51	671.45	737.07
5.2	234.34	288.53	487.82	284.43
6	676.69	651.73	619.79	652.09
8	776.62	418.29	388.11	453.53

Appendix G: Consent Form

University of Nebraska Medical Center

IRB PROTOCOL # 0257-21-FB **Page 1 of 7**

CONSENT FORM

Title of this Research Study
Association of Periodontal Inflammation on Immune Response in Wound Healing

Invitation and Summary

You are invited to be in this research study. Taking part in this research is voluntary. You do not have to take part. For the purposes of this document: "You" can refer to:

- Yourself
- The person for whom you are the Legally Authorized Representative (LAR)
- Your child under the age of 19.

"Organization" can refer to: University of Nebraska Medical Center (UNMC), Nebraska Medicine (NM), University of Nebraska at Omaha (UNO) or Children's Hospital & Medical Center (CH&MC).

Here is a summary of the purpose, methods, risks, benefits, and alternatives, to help you decide whether or not to take part in the research.

The study will help to understand healing after a tooth extraction.

You have a tooth that needs to be removed. A 3D xray will be taken prior to removal of the tooth. Numbing medicine will be given and the tooth will be taken out. Stitches will be put in the area and you will be taught how to keep the area clean. Two weeks later, you will return to the clinic to have the stitches taken out.

If you decide to take part in this study, the following things will also be done. Before the tooth removal, the study investigator will take measurements from the teeth next to the one to be removed. Your height, weight and waist measurements will be taken. A small blood sample will be taken from a finger prick. You will be asked to spit in to a cup. A small amount of blood will be drawn from your arm. After the tooth is taken out, a small piece of gum from the extraction area will be removed and saved for analysis. Two weeks later, when you return to the clinic to have the stitches taken out, a small piece of gum from the area will be removed again and saved for analysis. Three months later, you will come back to the clinic and have a 3D xray taken.

Risks of being in this study could include temporary soreness, bleeding and swelling of the area where the gum sample was taken.

There are no benefits to being in this study.

IRB
Approved 08/19/2022
Valid until 03/15/2023

You can proceed with tooth removal and implant planning without being a part of this study.

Why are you being asked to be in this research study?
You are being asked to be in this research study because you are a healthy adult, aged 21-70 years old, need to have a back tooth removed and are planning to get an implant. Up to 25 people will be in this study.

What is the reason for doing this research study?
The study will help us learn more about healing after a tooth extraction.

What will be done during this research study?
This study will start by taking measurements of the gums around the tooth to be removed and the two teeth on either side of the tooth to be removed, as well as your height, weight and waist measurements. A small piece of paper will be used to soak up a small amount of liquid around your tooth. These paper pieces will be frozen and the liquid analyzed at a later time. You will also have a 3D xray taken.

A small drop of blood will be collected from a finger prick. A collection of your saliva will be gathered. A small amount of blood will be collected from your arm.

Numbing medicine will be given to the tooth and it will be removed.

A small piece of gum from the area will be taken and saved for analysis. This is different from usual care following having a tooth removed. The gum samples will be send to a laboratory in Denver, CO at National Jewish Hospital for analysis. The gum samples will be labeled with a number and no identifying information. Stitches will be placed at the area and you will be sent home. A simple blood test (finger prick) will be done to determine the level of glucose in your blood at the time of the tooth removal. Glucose levels in the blood can help to understand wound healing following tooth removal. You will be asked to spit in to a vial so that your saliva can be evaluated to see if any markers of inflammation are present. These markers can also help to understand wound healing. A small vial of blood will be drawn from your arm and the blood will be sent to Denver with the gum sample. The blood sample will help to understand if there are differences in inflammation between your body and the tooth extraction site.

Two weeks after the tooth removal, you will return to the College of Dentistry. At that appointment, stitches will be removed and another small piece of gum will be

Institutional Review Board (IRB)

What Do I Need To Know Before Being In A Research Study?

You have been invited to be in a **research study**. Research studies are also called "clinical trials" or "protocols." **Research** is an organized plan designed to get new knowledge about a disease or the normal function of the body. The people who are in the research are called **research subjects**. The **investigator** is the person who is running the research study. You will get information from the investigator and the research team, and then you will be asked to give your **consent** to be in the research.

This sheet will help you think of questions to ask the investigator or his/her staff. You should know all these answers before you decide about being in the research.

What is the **purpose** of the research? Why is the investigator doing the research?

What are the **risks** of the research? What bad things could happen?

What are the possible **benefits** of the research? How might this help me?

How is this research different than the care or treatment I would get if I wasnt in the research? Are there other treatments I could get?

Does **everyone** in this research study get the same treatment?

Will being in the research **cost** me anything extra?

Do I have to be in this research study? Will the doctor still take care of me if I say **no**?

Can I **stop** being in the research once Ive started? How?

Who will look at my **records**?

How do I reach the investigator if I have more **questions**?

Who do I call if I have questions about being a **research subject**?

Make sure all your questions are answered before you decide whether or not to be in this research.

Academic Research & Services Building 3000 / 987830 Nebraska Medical Center / Omaha NE 68198-7830
402-559-6463 / FAX 402-559-3300 / Email: irbora@unmc.edu / http://www.unmc.edu/irb

removed. This gum sample will again be sent to Denver, Colorado for analysis. The removal of this gum sample is different from the usual care of a removed tooth.

Three months later, you will return to the College of Dentistry for a final appointment. At that time, measurements will be taken of the two teeth on either side of the area as well as a final 3D xray.

The sample(s) we collect will not be used for other research studies by us, or by any other investigator after this research is over.

What are the possible risks of being in this research study?
The risks of being in this study include minor soreness, bleeding and slight swelling at the site where the gum sample was taken.

The gum and liquid samples will not be labeled with your name or any identifying personal information.

What are the possible benefits to you?
You will not receive benefits from being in this research study.

What are the possible benefits to other people?
Possible benefits to society include a better understanding of wound healing in the mouth.

What are the alternatives to being in this research study?
Instead of being in this research study, you can choose not to take part. The usual care of having your tooth extracted and implant planning will be available to you whether or not you choose to participate in the study.

What will being in this research study cost you?
There will be no additional costs to you for being in this study.

You will have to pay any insurance deductibles and co-payments for usual care. If you want to speak with someone about your insurance, just tell us.

Will you be paid for being in this research study?
You will receive a gift card for each study visit you participate in, an $80.00 gift card for Visit 1; a $60.00 gift card for Visit 2; and a $60.00 gift card for Visit 3.

The gift card will either be to Target or a Visa gift card.

Who is paying for this research?
The Windsweep Farm Fund (departmental funding) gives us money to do this study.

What should you do if you are injured or have a medical problem during this research study?
Your health and safety is our main concern. If you are injured or have a medical problem because of this study call someone listed at the end of this consent form. You can get emergency medical treatment at Nebraska Medicine. You can also go to your doctor, the nearest emergency room or call 9-1-1.

We have no plans to pay for your treatment or give you any other money or compensation. Your insurance may pay. If they do not you will have to pay.

Signing this does not mean you have given up any of your legal rights.

How will information about you be protected?
In the course of this research we will collect information about you. These can be things that could be used to find out who you are (like your name, age). We call this "identifiable private information". We will keep this information as confidential as possible.

The information will not be used for other research by us, or by any other researcher.

Who can see information about you?
We also will get medical information about you (like medical record number, medical history, or the results of physical exams, blood tests, x-rays or other medical or research procedures). We call this "protected health information" or PHI. PHI is protected by a law called the HIPAA Privacy Rule. We will collect the smallest amount of PHI that we can. We will keep your PHI as confidential as possible.

By signing this consent form, you are letting us (the researchers listed on this consent form and other people involved in this research at the UNMC COD) have access to your PHI. Your PHI will be used only for the purposes described in the section "What is the reason for doing this research study?"

You can change your mind and tell us to stop collecting your PHI for use in this research at any time by writing to the principal investigator. We can still use the PHI we have already collected. If you tell us to stop collecting your PHI, you will have to stop being in this research.

We may share your PHI with other groups listed below:

- The UNMC Institutional Review Board (IRB)
- Institutional officials designated by the UNMC IRB
- The HHS Office for Human Research Protections (OHRP)

We may share your PHI with other groups listed below. The HIPAA Privacy Rule requires these groups to protect your PHI.

- Your health insurance company

How will results of the research be made available to you during and after the study is finished?

In most cases, the results of the research can be made available to you when the study is completed, and all the results are analyzed by the investigator or the sponsor of the research. The information from this study may be published in scientific journals or presented at scientific meetings, but your identity will be kept strictly confidential.

If you want the results of the study, contact the Principal Investigator at the phone number given at the end of this form or by writing to the Principal Investigator at the following address: [UNMC College of Dentistry, 4000 Easter Campus Loop South, Lincoln, NE 68583-0740]:

What will happen if you decide not to be in this research study?

You can decide not to be in this research study. Deciding not to be in this research will not affect your medical care or your relationship with the investigator or the organization. Your doctor will still take care of you and you will not lose any benefits to which you are entitled.

What will happen if you decide to stop participating once you start?

You can stop being in this research (withdraw) at any time. Just call the researcher or any research staff

If you stop being in the research study it will not affect your care or your relationship with the investigator or the organization. You will not lose any benefits to which you are entitled.

Will you be given any important information during the study?

We will tell you right away if we get any new information that might make you change your mind about being in the study.

What should you do if you have any questions about the study?
We gave you a copy of *"What Do I Need to Know Before Being in a Research Study?"*

If you ever have any questions about this study, call the Principal Investigator or anyone else listed on this consent form.

What are your rights as a research participant?
You have rights as a research subject. These rights have been explained in this consent form and in The Rights of Research Subjects that you have been given. If you have any questions concerning your rights, or want to discuss problems, concerns, obtain information or offer input, or make a complaint about the research, you can contact any of the following:

- The investigator or other study personnel
- Institutional Review Board (IRB)
  - Telephone: (402) 559-6463.
  - Email: IRBORA@unmc.edu
  - Mail: UNMC Institutional Review Board, 987830 Nebraska Medical Center, Omaha, NE 68198-7830
- Research Subject Advocate
  - Telephone: (402) 559-6941
  - Email: unmcrsa@unmc.edu

Documentation of informed consent
You are deciding whether to be in this research study. Signing means that:

- You have read and understood this consent form.
- You have had the consent form explained to you.
- You have been given a copy of The Rights of Research Subjects You have had your questions answered.
- You have decided to be in the research study.
- You have been told you can talk to one of the researchers listed below on this consent form if you have any questions during the study.
- You will be given a signed and dated copy of this consent form to keep.

Signature of Subject_____Date_____

My signature certifies that all the elements of informed consent described on this consent form have been explained fully to the subject. In my judgment, the subject

University of Nebraska Medical Center

possesses the legal capacity to give informed consent to participate in this research and is voluntarily and knowingly giving informed consent to participate

Signature of Person Obtaining Consent_____Date_____

Authorized Study Personnel
Principal
* Killeen, Amy
phone: 402-472-7848
alt #: 402-472-7848
degree: DDS

Secondary
* Reinhardt, Rick (Rick)
phone: 402-472-1287
alt #: 402-472-1287
degree: DDS

Participating Personnel
* Bealka, Neil
phone: 402-472-1311
alt #: 402-472-3249
degree: DDS

Davis, Richard
phone: 402-472-3249
alt #: 402-472-3249
degree: DDS

* Mohammadi, Sasha
phone: 402-472-1311
alt #: 402-472-3249
degree: DDS

* Schlemmer, Paula
phone: 402-472-3249
alt #: 402-472-3249
degree: DDS

Institutional Review Board (IRB)

THE RIGHTS OF RESEARCH SUBJECTS
AS A RESEARCH SUBJECT YOU HAVE THE RIGHT

to be told everything you need to know about the research before you are asked to decide whether or not to take part in the research study. The research will be explained to you in a way that assures you understand enough to decide whether or not to take part.

to freely decide whether or not to take part in the research.

to decide not to be in the research, or to stop participating in the research at any time. This will not affect your medical care or your relationship with the investigator or the Nebraska Medical Center. Your doctor will still take care of you.

to ask questions about the research at any time. The investigator will answer your questions honestly and completely.

to know that your safety and welfare will always come first. The investigator will display the highest possible degree of skill and care throughout this research. Any risks or discomforts will be minimized as much as possible.

to privacy and confidentiality. The investigator will treat information about you carefully, and will respect your privacy.

... to keep all the legal rights you have now. You are not giving up any of your legal rights by taking part in this research study.

to be treated with dignity and respect at all times

The Institutional Review Board is responsible for assuring that your rights and welfare are protected. If you have any questions about your rights, contact the Institutional Review Board at (402) 559-6463.

Academic Research & Services Building 3000 / 987830 Nebraska Medical Center / Omaha NE 68198-7830
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