CONNECTIVE TISSUE GRAFTS AND SURGICAL DELAY: CLINICAL AND BIOCHEMICAL CHARACTERIZATION

Thesis

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ABSTRACT

The connective tissue graft (CTG) procedure is among the most predictable and frequently used periodontal surgical procedures, indicated primarily for coverage of exposed root surfaces, i.e., the treatment of gingival recession defects. However, the need to improve CTG periodontal outcomes in challenging clinical situations has not been met. The “surgical delay” or “ischemic preconditioning” or “pre-wounding” technique, which has been used in general plastic surgery, leads to improved clinical outcomes. The aim of this randomized, parallel arm, clinical trial was to examine the feasibility of the “pre-wounding” technique in improving CTG outcomes in deep gingival recession defects.

Adult non-smokers with 3mm or greater recession defects were recruited. Following IRB-approved informed consent, subjects were randomly assigned to receive on a single tooth either a routine CTG (rCTG) or a surgically delayed CTG (pwCTG). In the pwCTG group pre-wounding was performed by means of 2 parallel incisions 1mm apart and the graft was harvested 5 days later. The recipient bed flap design was the same for both CTG types. Each subject contributed one test (rCTG or pwCTG) and one control (contralateral) site. 30-second gingival crevicular (GCF) samples were collected using paper strips immediately pre-operatively, immediately following graft placement, 3 days, and 1, 2, and 3 weeks post-operatively (PO). GCF samples

were analyzed with ELISA for the presence of angiogenin (ANG) and hypoxia-inducible factor 1α (HIF-1α). Recipient and donor sites clinical parameters were assessed immediately pre-operatively, 2, 3, and 6 months PO. Questionnaires completed by subjects were used to evaluate post-operative pain and discomfort, using visual analog scores (VAS) and pain effect scales.

19 subjects (9 rCTG, 10 pwCTG) completed the study. There were no statistically significant intergroup differences regarding pre-operative or PO periodontal clinical parameters.The achieved root coverage (RC) 180 days (D) PO was 89.8±13.1% for the pwCTG group and 85.6±14.9% for the rCTG group with no statistically significant difference between the two groups. Both pwCTG and rCTG showed an intragroup statistically significant difference regarding RC at D180 PO compared to D60 PO. 50% of pwCTG subjects had 100% RC at the D180 PO. 44.4% of the rCTG subjects had 100% RC at D180 PO. GCF analysis did not show an intergroup statistically significant difference in ANG and HIF-1α levels at any of the predetermined GCF sampling. Regarding pain in the donor and recipient sites, there were no intergroup statistically significant differences at any of the predetermined PO visits.

This is the first study to test “pre-wounding” in a CTG procedure. The present study was able to demonstrate that pre-wounding the palate does not have adverse effects on clinical outcomes of CTGs. Additionally, from the patients’ prospective there are no adverse effects by the application of the “pre-wounding” technique. The present

study was not able to prove the clinical or biochemical superiority of the tested technique versus the routine CTG procedure. Further studies on subpopulations with a reduced healing capacity and/or more severe gingival recession are necessary to elucidate the potential of the surgical delay or pre-wounding technique in oral plastic surgery.

Dedicated to my parents

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Finally, I would like to thank my parents for their love and support and for the roots and wings they gave me.

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PUBLICATIONS

1. Tsolaki IN, Tatakis DN. Patient Outcomes Following Harvesting of Routine and Pre-Wounded Connective Tissue Grafts. J Clin Periodontol 39 (S13): Abstract #807, 2012
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FIELDS OF STUDY

Major Field: Dentistry

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CHAPTER 1

INTRODUCTION

Gingival recession is defined as the apical migration of the soft tissue margin leading to the exposure of root surfaces[1]. Due to the resulting root exposure, it can be associated with dentinal hypersensitivity[2-4], root caries[4-6], increased plaque accumulation[7], and esthetic concerns[4, 8]. Left untreated, recession defects will progress, exposing more of the tooth root over time[9-11]. Hypersensitivity, esthetic concerns and persistent localized inflammation due to plaque accumulation are the main indications for root coverage procedures. Gingival recession can affect >50% of certain population segments and its prevalence and severity progressively increase with age[9, 12-17].

Among the various periodontal surgical procedures available for root coverage, the subepithelial connective tissue graft (CTG), introduced by Langer and Calagna[18] as a method of augmentation of edentulous ridges, has been described in detail as a procedure for obtaining root coverage with several variations in surgical technique[19-23]. Several systematic reviews have concluded that CTG is the most predictable of the available periodontal plastic surgery procedures[24-27]. The clinical success of the CTG is dependent on development of blood supply to the graft. The graft tissue's blood supply is disrupted during graft harvesting and new blood supply must be developed in the recipient site. The development of the CTG blood supply is thought to be

assisted by the dual blood supply available from the underlying periosteum and the overlying gingival (mucoperiosteal) flap[28].

Pretreatment or baseline recession depth (RD) is a critical factor in determining root coverage outcomes, particularly complete root coverage (CRC)[24, 29-31]. Studies and systematic reviews that have examined the possible impact of baseline RD on CRC have concluded that with greater baseline RD the probability of CRC decreases significantly[29-31]. When the baseline RD is ≥ 3.5 mm the probability of CRC is significantly less than for defects whose baseline RD is ≤ 2 mm, regardless of the surgical technique used[29]. It should be noted that CRC is an outcome measure considered particularly important by both patients and practitioners[8]. Therefore, further improvements in the outcomes of the various periodontal plastic surgery procedures, such as CTG, indicated for deep (i.e., ≥ 3 mm RD) recession defects would benefit periodontal patients seeking treatment.

Pathophysiology of ischemia-reperfusion

Evidence indicates that periodontal flaps[48], including CTG procedures[49], represent an ischemia-reperfusion flap model[48]. Prolonged ischemia, which routinely occurs in surgical procedures, reduces adenosine triphosphate production and inhibits sodium-potassium adenosine triphosphatase, resulting in the increase of intracellular sodium and calcium. The elevated glycolysis during ischemia causes lactic acid accumulation associated with pH reduction. If the ischemic duration extends beyond a critical point of tolerance, cell necrosis is inevitable. Reperfusion is the only option available to minimize ischemic necrosis. However, reperfusion elicits rapid production of reactive oxygen species in the mitochondria and initiates tissue injury beyond that caused by

the ischemia[52]. Endothelial dysfunction is one of the characteristics of ischemia-reperfusion injury. However, the hallmark of ischemia-reperfusion injury is the mitochondrial dysfunction[53-56]. Under physiologic conditions, the mitochondrial inner membrane is impermeable to maintain the membrane potential and proton gradient that drive adenosine triphosphate synthesis through oxidative phosphorylation. However, under conditions of high calcium and high pH combined with the reactive oxygen species burst during early reperfusion, a nonspecific pore (mitochondrial permeability transition pore) opens in the inner mitochondrial membrane[50]. Mitochondrial permeability transition pore opening leads to immediate depolarization of membrane potential, matrix swelling, outer mitochondrial membrane rupture, and release of proapoptotic molecules such as cytochrome c into the cytosolic compartment, where it activates a program leading to cell apoptosis[50, 51].

Preconditioning procedures

In addition to the fact that ischemia-reperfusion negatively affects the clinical outcomes of surgical procedures, further compromise occurs due to several aggravating factors, such as smoking[32-35], diabetes mellitus, and radiotherapy. Management of these aggravating factors is not always feasible or satisfactory [36]. This has led plastic surgeons to attempt technique modifications that can improve outcomes.

One such powerful modification is the “surgical delay” or “ischemic preconditioning” technique, which amelorates reperfusion injury in various organs/tissues[37-40]. In general, four types of preconditioning have been described:

1) Ischemic preconditioning (*IPC*): brief alternative episode(s) of ischemia (occlusion), and reperfusion (unocclusion) which trigger an adaptive mechanism that protects tissues against injury from a subsequent sustained ischemia and reperfusion[50].
2) Postconditioning (*Post C*): brief alternative episode(s) of reperfusion (unocclusion), and ischemia (reocclusion) which trigger a protective mechanism that attenuates reperfusion injury[50].
3) Remote ischemic preconditioning: brief alternative episode(s) of ischemia and reperfusion in an unrelated organ or tissue that provides protection against injury from subsequent sustained ischemia in other tissues or organs at a distance[50].
4) Remote postconditioning: brief alternative episode(s) of ischemia and reperfusion in an unrelated organ or tissue that provides remote protection against reperfusion injury in other organs or tissues at a distance[50].

Studies are showing that ischemic preconditioning increases functional capillary density[57, 58], prevents leukocyte rolling[57], adhesion[57], and migration[57], reduces leukocyte–endothelial cell interaction[58], reduces cell apoptosis[58], up-regulates endothelial nitric oxide synthase[57], neuronal nitric oxide synthase[57], and inducible nitric oxide synthase isoforms[57].

For skin grafts, a similar approach, termed “pre-wounding” has been used[41]. The concept behind these technique modifications is the same as the “ischemic preconditioning”: the flap (or graft) area gets exposed to ischemic conditions milder than the ones in the actual treatment surgery and the tissue responds by increased

blood supply[37]. Therefore, the odds of successful outcomes at time of surgery are higher. The difference is in the timing of the delay procedure which typically lasts 1-2 weeks prior to the treatment surgery[37, 39, 42].

To the best of our knowledge, the surgical delay approach has not been used or studied in the context of periodontal surgery; thus, the present study is the first one to pursue such an approach in an effort to ultimately improve surgical periodontal therapy outcomes when conditions are challenging, as in deep recession defects.

Biochemical characterization

Changes in GCF concentration of angiogenin correlate with changes in vascularization for gingival grafts during early (3 days – 3 weeks) healing[49,59]. ANG, a 14kDA polypeptide is a growth factor that critically affects the action of other angiogenic growth factors[47]. ANG is present in GCF and there is evidence supporting the angiogenic and antimicrobial properties of ANG[43, 44]. Additionally, levels of detected ANG are higher in chronic inflammatory processes such chronic periodontitis and cigarette smoking[45, 46]. The mechanism of ANG action consists of the stimulation of mRNA transcription. This results in increased ribosome synthesis, protein synthesis and overall cell growth. On the contrary, downregulation of ANG decreases cell growth and proliferation[47].

Hypoxia inducible factor-1 (HIF-1) is a transcription factor composed of the subunits HIF-1α and HIF-1β, which are basic helix-loop-helix DNA-binding proteins. The activity of HIF-1 is predominantly regulated at the post-translational level by regulating HIF-1α protein stability. At normal oxygen tension, HIF- 1α is

hydroxylated in the oxygen-dependent degradation domain (ODDD) by prolyl hydroxylases (PHD). Hydroxylated HIF-1α is recognized by theVon Hippel–Lindau (VHL) protein, ubiquitinated and destined for degradation by proteasome. This process is inhibited during hypoxia[60]. Under hypoxia, stabilized HIF-1α subunits heterodimerize with β-subunits to form the active HIF-1 complex that activates gene transcription by binding to the consensus HIF responsive element (HRE); 5'-RCGTG-3' in promoters and enhancers of target genes[61]. Among these are glucose transporters, glycolytic enzymes, and genes involved in gluconeogenesis, high-energy phosphate metabolism, growth factors, erythropoiesis, haem metabolism, iron transport, vasomotor regulation and nitric oxide synthesis[61-64]. Protein products of the HIF-1 target genes help the cell to survive the hypoxic stress by increasing oxygen delivery (angiogenesis) and by switching to anaerobic glycolysis[61-64]. Increased angiogenesis is an effect of HIF-1α through upregulation of vascular endothelial growth factor (VEGF). VEGF acts through its tyrosine kinase receptors to modulate motility and proliferation of endothelial cells and vascular permeability[65]. Although HIF-1α usually induces prosurvival (CA9, SLC2A1 and VEGF) genes, a role of HIF-1α in regulation of apoptosis has also been described. HIF-1α promotes cell death through an increase in p53 or other proapoptotic proteins like BNIP3[66]. As a result of this dual function of HIF-1α, a "stop-and-go" strategy as a dynamic balance to maintain overall cell growth and survival has been proposed[66].

The present study was designed to test the following **hypotheses**:

i) a surgical delay approach results in improved clinical outcomes for CTG used to treat deep recession defects.

ii) a surgical delay approach improves the patient post-operative experience for CTG donor sites.

iii) a surgical delay approach results in increased angiogenic growth factor levels in GCF of CTG-treated sites during the early healing period.

Specific Aims:

1. To assess and compare the effects of a surgical delay technique versus the routine technique on the clinical outcomes of CTG used to treat deep recession defects.

2. To assess and compare the effects of a surgical delay technique versus the routine technique on patient-based outcomes for the CTG procedure.

3. To assess and compare the effects of a surgical delay technique versus the routine technique on GCF angiogenesis biomarkers in CTG-treated sites.

CHAPTER 2

MATERIALS AND METHODS

Study Population and Experimental Design

The overall design was a randomized, parallel arm, clinical trial. Subjects were recruited through flyers, postings and advertisements in the OSU College of Dentistry, the OSU Health Sciences Colleges bulletin boards, other bulletin board and notice posting sites on the OSU campus. Subjects were also recruited from the patient pool of the OSU College of Dentistry and especially from The Ohio State University College of Dentistry Graduate Periodontics Clinic. Area dental practitioners were contacted with a letter providing information on the study.

Inclusion criteria for the study included:

1) periodontally and systemically healthy adults (aged 18-55 years)
2) non-smokers
3) at least one gingival recession defect with recession depth (RD) $\geq$ 3 mm and classified as type Miller I or Miller II
4) recession defects on the maxillary or mandibular premolars, canines, and incisors

At screening, subjects were assessed for the following additional exclusion criteria: 1) systemic/general: uncontrolled systemic disease; history of systemic disease affecting healing; obesity; medications affecting the gingiva, the immune system, the cardiovascular system, the wound healing process; pregnancy; allergy to iodine, chlorhexidine, impression materials, topical or local dental anesthetic;

unable/unwilling to adhere to study visit schedule; and unable/unwilling to provide informed consent.

2) oral/dental: recession defect(s) with RD < 3 mm; recession defect Miller III or IV; recession defect on molar tooth; history of periodontal surgery resulting in palatal tissue thickness reduction; cleft palate; periodontitis; shallow palatal vault (inadequate palatal height); gingival enlargement; extensive calculus deposits; maxillary removable appliances (orthodontic, restorative); mucosal disease (e.g., candidiasis); lack of maxillary premolars on both sides of palate; and subjects with significant gag reflex (unable to easily tolerate maxillary impression procedure).

All data gathering took place at the Graduate Periodontics Clinic at The Ohio State University. The total duration of the study was 8-9 visits spanning 6 months $\pm$ 10 days from the day of the connective tissue graft treatment (Table 1).

Study Timeline

GROUP rCTG - Routine graft

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8
	D0	D3	D7	D14	D21	D60	D90	D180
Screening visit	Procedure	PO D3	PO D7	PO D14	PO D21 (± 1 d)	PO D60 (± 3 d)	PO D90 (± 5 d)	PO D180 (± 10 d)
If eligible & interested: Consent & HIPAA	Harvest rCTG and use to treat recession	PO visit	PO visit	PO visit	PO visit	PO visit	PO visit	PO visit
ECO Vitals Scr Qstnr	ECO Vitals Pre-op PIX Pre-op GCF Pre-op Qstnr Anesthesia Clin Param *Procedure*	ECO Vitals PIX GCF Qstnr	ECO Vitals PIX GCF Qstnr	ECO Vitals PIX GCF Qstnr	ECO Vitals PIX GCF Qstnr	ECO PIX Clin Param	ECO PIX Clin Param Qstnr	ECO PIX Vitals Anesthesia Clin Param Qstnr

Continued

Table 1. Study Timeline

Table 1. Study Timeline Continued

GROUP pwCTG - Delay of Five (5) Days

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9
	D(-)5	D0	D3	D7	D14	D21	D60	D90	D180
Screening visit	1st Procedure	2nd Procedure	PO D3	PO D7	PO D14	PO D21 (± 1 d)	PO D60 (± 3 d)	PO D90 (± 5 d)	PO D180 (± 10 d)
If eligible & interested: Consent & HIPAA	Pre-wound palate	Harvest rCTG and use to treat recession	PO visit	PO visit	PO visit	PO visit	PO visit	PO visit	PO visit
ECO Vitals Scr Qstnr	ECO Vitals Anesthesia Pre-op Qstnr *Procedure*	ECO Vitals Pre-op PIX Pre-op GCF Pre-op Qstnr Anesthesia Clin Param *Procedure*	ECO Vitals PIX GCF Qstnr	ECO Vitals PIX GCF Qstnr	ECO Vitals PIX GCF Qstnr	ECO Vitals PIX GCF Qstnr	ECO PIX Clin Paramr	ECO PIX Clin Param Qstnr	ECO PIX Vitals Anesthesia Clin Param Qstnr

Screening visit

Subjects were clinically examined (visual oral examination; blood pressure and heart rate), asked to provide an expired air sample for smoking status assessment, and were asked to complete a screening questionnaire (Figures 1, 2). Qualified subjects were given the informed consent form and when they had agreed to participate by signing the form they had their vital signs recorded. Then the subjects were randomly assigned to the test or the control group. The test group included the subjects that would receive a CTG with a pre-wounding technique (pwCTG). The control group included subjects would be treated with a routine CTG (rCTG). In order to achieve the random assignment of the patients in the test or control groups, the following sequence was followed:

- the code “pwCTG” was enclosed in 11 sealed envelopes
- the code “rCTG” was enclosed in another 11 sealed envelopes
- all 22 envelopes were non-transparent and externally identical
- each subject was given the total of the available sealed envelopes and he/she would choose only one
- the chosen envelope would then be opened by the subject
- the subject would be assigned to the respective study group
- the envelope would be destroyed

Each subject contributed one test site (pwCTG or rCTG) and one control site to the the study. The control site was designated as the contralateral tooth in the same arch as the planned CTG; however, outside the surgical field. If the contralateral tooth was in close proximity to the surgical site, a different tooth was used as the control one.

TELEPHONE SCREENING CHECKLIST

	YES	NO
Have you completed your 18th birthday?		
Have you completed your 56st birthday?		
Are you a smoker?		
If no, have you ever been a smoker?		
Do you have any systemic disease, such as diabetes, high blood pressure, etc.?		
Are you taking any medications affecting the gums, the immune system, the cardiovascular system, wound healing?		
Are you obese?		
Are you taking any medications?		
(Females only) Is there any chance you might be pregnant?		
Are you allergic to Iodine, dental materials or dental anesthetic?		
Would you be available for 9 study visits over 6 months?		
Do you have receeding gums?		
Did you ever have a soft tissue graft (skin graft) from the roof of your mouth?		
Do you have or did you have cleft palate?		
Has any dentist/hygienist ever told you that you have gum disease?		
Do you have your upper teeth?		
Do you have any removable appliances on the upper jaw (e.g., denture)?		
Do you easily gag? Is it difficult for you to have a mold made of your upper jaw?		

Figure 1. Telephone Screening Checklist

SCREENING CHECKLIST

	YES	NO
18th birthday not completed		
56th birthday completed		
Smoker		
Last smoked less than 48months (4 years) ago		
Current usage of smokeless tobacco		
Last usage of smokeless tobaco less than 48 months (4 years) ago		
Nicotine therapy in the last 48 months (4 years)		
Systemic disease – not controlled		
Hx disease affecting wound healing		
Obesity		
Medications affecting gingiva, cardiovascular or immune systems, wound healing		
Quantitative and/or qualitative PMN defects		
Organ transplant(s)		
Diabetes (type I or II)		
Pregnant		
Can not comply/be available for study visit schedule (specific days - duration)		
Cannot provide informed consent		
Allergy to Iodine, Chlorexidine, impression materials, topical/local dental anesthetic		
Recession RD < 3mm		
Miller III or IV recession		
Recession on molar tooth		
HX of soft tissue graft harvest from palate		
Hx of other soft tissue Sx on palate		
Hx of Cleft Palate		
Periodontitis		
Shallow palatal vault		
Gingival enlargement		
Extensive calculus present		
Maxillary removable appliances		
Mucosal disease		
Lack of maxillary premolars on both sides of the palate		
Significant gag reflex		

Figure 2. Screening Checklist

Pre-wounding visit (pwCTG group only)

Following routine preparation (vital signs and routine local anesthesia) and exhaled air sample, subjects completed a questionnaire (pre-op questionnaire) (Figure 3). Then pre-wounding was performed on left or right palate for subsequent sdCTG harvest.

Treatment visit

Preoperatively, exhaled air sample was collected, clinical photographs of the recipient area were taken, GCF samples were collected, routine preparation (vital signs and routine local anesthesia) was provided, a pre-operative questionnaire (Figure 4) was completed by the subject, and clinical periodontal measurements were recorded.

After the CTG procedure was performed, postoperative clinical photographs of the recipient and donor areas were taken.

Post-operative visits

Exhaled air sample was collected, clinical photographs of the donor and recipient sites were taken, vital signs were taken, GCF samples were collected on D3 PO, D7 PO, D14 PO and D21 PO, clinical measurements were taken on D60 PO, D90 PO, D180 PO, and questionnaires were completed on D3 PO, D7 PO, D14 PO, D21 PO, D90 PO, D180 PO. (Figures 5 – 7).

QUESTIONNAIRE (PRE-OPERATIVE)

Date Completed _____
Please answer as best you can. If you would like an explanation, please feel free to ask. If you have difficulty answering a question, please leave it blank. Your answers are strictly confidential and will remain anonymous.

1. How old are you? _____years _____months

2. _____Male _____Female

3. Are you currently taking any medications (including non-prescription/over the counter medications, such as herbals and vitamins)?
   _____Yes _____No

   If yes, please list the medication, reason for taking it, and dosage (if known)

Medication	Reason	Dosage

4. Do you have any allergies? _____Yes _____No
   If yes, what are you allergic to?
   _____

5. Did you ever have reaction/complication from dental anesthesia? _____Yes _____No
   If yes, please describe
   _____
   _____
   _____

6. Do you currently smoke cigarettes or use other tobacco products?
   ____Yes _____No
   If yes: a) what do you use? (circle all that apply)

   cigarettes pipe cigar chewing tobacco

   b) If you smoke cigarettes, how many do you smoke each day? _____

c) If you smoke cigarettes, how long have you been smoking? ____years ____months

7. If you currently do not smoke, have you ever smoked cigarettes or used other tobacco products? ____Yes _____No

   If yes:

   a) what did you use? (circle all that apply)

   cigarettes pipe cigar chewing tobacco

   b) If you smoked cigarettes, how many did you smoke each day? _____

   c) If you ever smoked cigarettes or used other tobacco products, <u>when was the last time</u> you smoked or used tobacco? __year ___month

Thank you for completing this questionnaire

Figure 3. Pre-operative questionnaire

QUESTIONNAIRE - Study Day 5 (PRE-sdCTG HARVEST)

Date Completed _____
Please answer as best you can. If you would like an explanation, please feel free to ask. If you have difficulty answering a question, please leave it blank. Your answers are strictly confidential and will remain anonymous.

1. Did you have any **pain** since the end of the procedure (wounding of the roof of your mouth) five days ago? ____Yes ____No

   If yes, please describe the pain as best you can (for example: throbbing, stabbing, sharp, dull, duration, etc…)

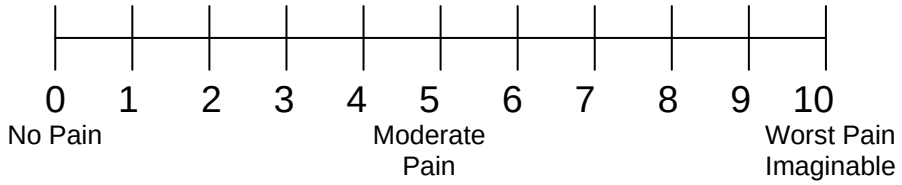
   _____

   _____

   _____

   _____

2. How much pain did you have? Please **circle** number, with '0' being no pain and '10' being the most severe pain imaginable



3. Please **circle** the number that best describes the pain that you experienced and how it affected your activities

   0 = No pain
   1 = Tolerable and pain does not prevent any activities
   2 = Tolerable and pain prevents some activities
   3 = Intolerable and pain does not prevent use of telephone, TV viewing, or reading
   4 = Intolerable and pain prevents use of telephone, TV viewing, or reading
   5 = Intolerable and pain prevents verbal communication.

   If you experienced pain that you rated 2 or higher, please list or describe all the activities that were prevented by the pain:

   _____

   _____

_____
_____

4. Did you take any pain medication since the end of the procedure (wounding of the roof of your mouth) five days ago? ____Yes ____No

   If yes, please indicate when you took it, how much you took, and how often you took it:

When taken (Day/Date)	Amount taken (Number of pills)	How often taken (example: twice a day)

5. Did you use the prescribed mouth rinse since the end of the procedure (wounding of the roof of your mouth) five days ago? _____Yes _____No

   If yes, how much and how often did you use it?

   _____
   _____
   _____
   _____
   _____
   _____

6. Did you feel any discomfort (example: itching, pulling) **from the stitches**?

   _____Yes _____No

   If yes, please describe the discomfort, when and how often you felt it:

   _____
   _____
   _____
   _____

_____

_____

7. Did you experience any bleeding from the wound? _____Yes _____No
   If yes, please describe when and how often you experienced it:
   _____
   _____
   _____

8. Did you have any swelling in the wound area? _____Yes _____No
   If yes, please describe when did it start (when you first felt it) and whether it prevented you from any activities:
   _____
   _____
   _____

9. Have you smoked rinse since the end of the procedure (wounding of the roof of your mouth) five days ago? _____Yes _____No
   If Yes: a) how long after the procedure did you begin smoking? _____Days _____Hours
   b) how many cigarettes did you smoke per day, on average, since you began
   smoking after the end of procedure? _____

Thank you for completing this questionnaire

Figure 4. Pre-pwCTG harvest questionnaire

QUESTIONNAIRE – 3, 7, 14, 21 Day Post-Op

Date Completed _____

Please answer as best you can. If you would like an explanation, please feel free to ask. If you have difficulty answering a question, please leave it blank. Your answers are strictly confidential and will remain anonymous.

1. Did you have any **pain** since the end of last visit's procedure (when the graft was placed over the tooth)? _____Yes _____No

   If yes, please describe the pain as best you can (for example: throbbing, stabbing, sharp, dull, duration, etc…)

   _____

   _____

   _____

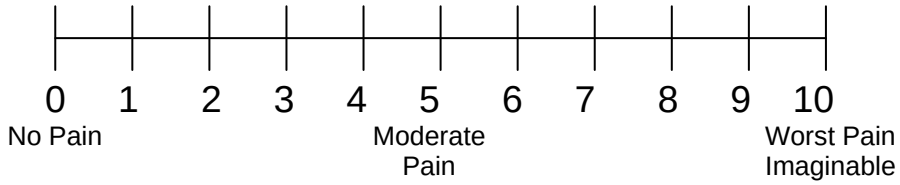
2. If you answered YES above, where did you experience the pain ? (Please check only one)

   _____ ONLY at the roof of mouth (palate)

   _____ONLY at the site where the graft was placed (treated tooth area)

   _____BOTH at roof of mouth and where graft was placed

3. If you experienced pain at the roof of the mouth (palate), how much pain did you have? Please **circle** number, with '0' being no pain and '10' being the most severe pain imaginable



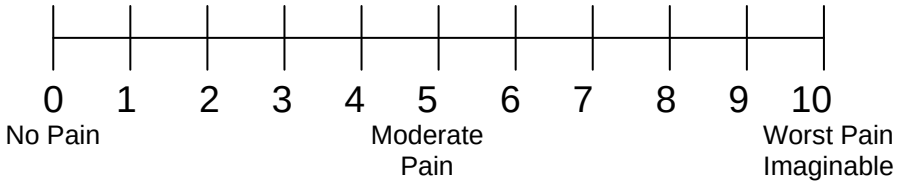
4. Please **circle** the number that best describes the pain that you experienced at the roof of your mouth and how it affected your activities

   0 = No pain
   1 = Tolerable and pain does not prevent any activities
   2 = Tolerable and pain prevents some activities
   3 = Intolerable and pain does not prevent use of telephone, TV viewing, or reading
   4 = Intolerable and pain prevents use of telephone, TV viewing, or reading
   5 = Intolerable and pain prevents verbal communication.

If you experienced pain that you rated 2 or higher, please list or describe all the activities that were prevented by the pain:

_____
_____
_____

5. If you experienced pain at the site where the graft was placed (treated tooth area), how much pain did you have? Please **circle** number, with '0' being no pain and '10' being the most severe pain imaginable



6. Please **circle** the number that best describes the pain that you experienced at the site where the graft was placed and how it affected your activities

0 = No pain
1 = Tolerable and pain does not prevent any activities
2 = Tolerable and pain prevents some activities
3 = Intolerable and pain does not prevent use of telephone, TV viewing, or reading
4 = Intolerable and pain prevents use of telephone, TV viewing, or reading
5 = Intolerable and pain prevents verbal communication.

If you experienced pain that you rated 2 or higher, please list or describe all the activities that were prevented by the pain:

_____
_____
_____

7. Did you take any of the pain medication since your last visit? _____Yes

_____No

If yes, please indicate when you took it, how much you took, and how often you took it:

When taken (Day/Date)	Amount taken (Number of pills)	How often taken (example: twice a day)

8. Did you use the prescribed mouth rinse since your last visit? _____Yes _____No

If yes, how much and how often did you use it?

_____

_____

_____

_____

9. Did you feel any discomfort (example: itching, pulling) **from the stitches**? _____Yes _____No

If yes, please describe the discomfort, when and how often you felt it:

_____

_____

_____

_____

If yes, the discomfort you felt from the stitches was (Please **circle one**):

Only on the **ROOF** of mouth Only on **GRAFT** placement area On **BOTH** sites

10. Did you experience any bleeding from the wounds? _____Yes _____No

If yes, please describe when and how often you experienced it:

_____

_____

_____

_____

If yes, the bleeding you experienced was from (Please **circle one**):

Only on the **ROOF** of mouth Only on **GRAFT** placement area On **BOTH** sites

11. Did you have any swelling in the wound area? _____Yes _____No

If yes, please describe when did it start and whether it prevented you from any activities:

_____

_____

_____

_____

If yes, the swelling you experienced was from (Please **circle one**):

Only on the **ROOF** of mouth Only on **GRAFT** placement area

On **BOTH** sites

12. Have you smoked since your last visit? _____Yes _____No

If Yes: a) how long after the procedure did you begin smoking? _____Days _____Hours

b) how many cigarettes did you smoke per day, on average, since you began

smoking after the end of procedure? _____

Thank you for completing this questionnaire

Figure 5. D3, 7, 14 and 21 PO Questionnaire

QUESTIONNAIRE - 90 Day Post-Op

Date Completed _____

Please answer as best you can. If you would like an explanation, please feel free to ask. If you have difficulty answering a question, please leave it blank. Your answers are strictly confidential and will remain anonymous.

1. Did you have any **pain** related to your gum grafting procedure since your last visit (1 month ago)? _____Yes _____No

   If yes, please describe the pain as best you can (for example: throbbing, stabbing, sharp, dull, duration, etc…)

   _____

   _____

   _____

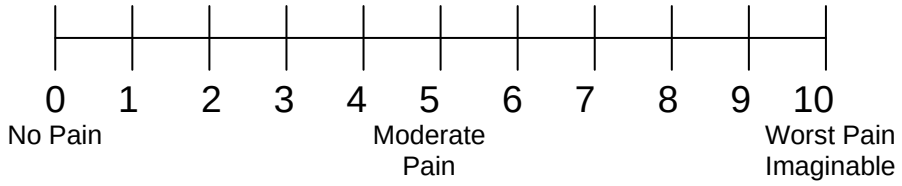
2. If you answered YES above, where did you experience the pain ? (Please check only one)

   _____ ONLY at the roof of mouth (palate)

   _____ONLY at the site where the graft was placed (treated tooth area)

   _____BOTH at roof of mouth and where graft was placed

3. If you experienced pain, how much pain did you have? Please **circle** number, with '0' being no pain and '10' being the most severe pain imaginable



4. Please **circle** the number that best describes the pain that you experienced and how it affected your activities

   0 = No pain
   1 = Tolerable and pain does not prevent any activities
   2 = Tolerable and pain prevents some activities
   3 = Intolerable and pain does not prevent use of telephone, TV viewing, or reading
   4 = Intolerable and pain prevents use of telephone, TV viewing, or reading
   5 = Intolerable and pain prevents verbal communication.

5. If you experienced pain that you rated 2 or higher, please list or describe all the activities that were prevented by the pain:

_____
_____
_____
_____

6. Did you experience any discomfort (examples: itching; altered sensation; numbness) or anything else unusual (examples: redness or change in tissue color; trauma in the grafted area) related to your gum grafting procedure during the past 30 days? _____Yes _____No

If yes, please describe what you experienced and where you experienced it, as best you can:

_____
_____
_____
_____
_____

7. Have you smoked since your last visit (1 month ago)? _____Yes _____No

If Yes: a) how long after your last visit did you begin smoking? _____Days

b) how many cigarettes did you smoke per day, on average, since you began

smoking after your last visit? _____

Thank you for completing this questionnaire

Figure 6. D90 PO Questionnaire

QUESTIONNAIRE - 180 Day Post-Op

Date Completed _____

Please answer as best you can. If you would like an explanation, please feel free to ask. If you have difficulty answering a question, please leave it blank. Your answers are strictly confidential and will remain anonymous.

1. Did you have any **pain** related to your gum grafting procedure since your last visit (3 months ago)? _____Yes _____No

   If yes, please describe the pain as best you can (for example: throbbing, stabbing, sharp, dull, duration, etc…)

   _____

   _____

   _____

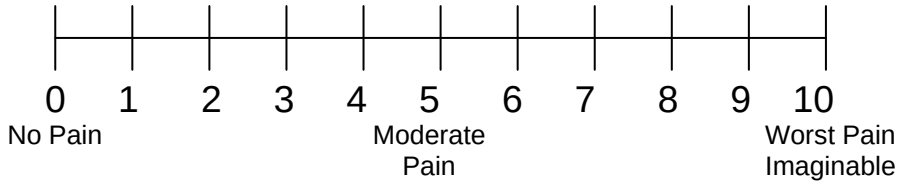
2. If you answered YES above, where did you experience the pain ? (Please check only one)

   _____ ONLY at the roof of mouth (palate)

   _____ONLY at the site where the graft was placed (treated tooth area)

   _____BOTH at roof of mouth and where graft was placed

3. If you experienced pain, how much pain did you have? Please **circle** number, with '0' being no pain and '10' being the most severe pain imaginable



4. Please **circle** the number that best describes the pain that you experienced and how it affected your activities

   0 = No pain
   1 = Tolerable and pain does not prevent any activities
   2 = Tolerable and pain prevents some activities
   3 = Intolerable and pain does not prevent use of telephone, TV viewing, or reading
   4 = Intolerable and pain prevents use of telephone, TV viewing, or reading
   5 = Intolerable and pain prevents verbal communication.

If you experienced pain that you rated 2 or higher, please list or describe all the activities that were prevented by the pain:

_____
_____
_____

5. Did you experience any discomfort (examples: itching; altered sensation; numbness) or anything else unusual (examples: redness or change in tissue color; trauma in the grafted area) related to your gum grafting procedure during the past 3 months? _____Yes_____No

If yes, please describe what you experienced and where you experienced it, as best you can:

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

6. Have you smoked since your last visit (3 months ago)? _____Yes _____No

If Yes: a) how long after your last visit did you begin smoking? _____Days

b) how many cigarettes did you smoke per day, on average, since you began

smoking after your last visit? _____

7. How would you rank the experience of being in this study?

_____Fantastic _____Great _____Average _____Poor _____Never Again

8. Please tell us what, if anything, was worst about taking part in this research study:

_____

_____

_____

_____

_

9. Please tell us what, if anything, was best about taking part in this study:

_____

_____

_____

_____

_

Thank you for completing this questionnaire and for being a participant in this research study

Figure 7. D180 PO Questionnaire

Study Procedures

Smoking status assessment. Expired air carbon monoxide (ECO) analysis.

ECO analysis was be performed at D(-5), at D0, and at D3, D7, D14, D21(±1 day), D60 (± 3 days), D90 (± 5 days), and D180 (± 10 days) PO. The cut-off point for distinguishing smokers from non-smokers was 8 parts per million (ppm)[67]. Subjects were instructed to exhale completely, draw a deep breath, hold for 15 seconds, and slowly exhale into the instrument (Bedfont Smokerlyzer). A digital readout immediately displayed the CO level in ppm.

Vital Signs:

Blood pressure was measured with a standard blood pressure cuff and stethoscope. Heart rate was measured from the radial artery pulse. Temperature was measured using a disposable thermometer.

Photographs:

Standard intraoral photographs of the treated sites were taken with a digital camera prior to each procedure, at the end of each procedure and at each postoperative visit. All photographs were taken at 1:1 magnification using appropriate intraoral photographic mirrors and cheek retractors.

Gingival Crevicular Fluid (GCF) Samples:

Teeth of interest were isolated with cotton rolls without touching the gingiva. Supra-gingival dental plaque was then gently removed using a curette, without touching the gingiva, and the surface was gently air-dried using the air syringe. Four GCF samples (strips) were then collected per site, one at a time, with a 1-minute interval between samples. Two samples were collected from the mesiobuccal aspect and two from the

distobuccal aspect of the respective tooth. GCF was obtained using standardized filter paper strips (PerioPaper strips, OraFlow Inc., Plainview, New York) placed non-traumatically in the entrance of the gingival sulcus until mild resistance was felt. Each strip was placed at the specific site and left in place for 30 sec. Samples contaminated with blood were discarded. GCF volume was determined immediately thereafter using a Periotron 8000 (Electronic micro-moisture meter, OraFlow Inc., Plainview, New York) and the strips were placed in a sterile 500 µl polypropylene centrifuge tube. The strips from the same site were pooled in the same tube which was kept on ice until the end of the clinical session. All GCF samples were then stored at –80°C until assay time. GCF samples were obtained from both recipient and contralateral sites as described above, at the following times: pre-operatively (treatment day), and D3, D7, D14, D21 PO.

Periodontal clinical parameters

Clinical measurements were obtained by a trained and calibrated examiner on the tooth of interest. All vertical, apico-coronal linear measurements were obtained on the midbuccal surface of the tooth (one site per tooth, except Plaque and Gingival Index). Horizontal, mesio-distal measurements were obtained at the level of the CEJ. All measurements were obtained using a UNC-15 probe and recorded to the nearest 0.5 mm.

• Plaque Index (PlI). Measured on three buccal sites (disto-, mid-, and mesio-buccal). Recorded at baseline and D60, D90 and D180 PO.

• Gingival Index (GI). Measured on three buccal sites (disto-, mid-, and mesio-buccal). Recorded at baseline and D60, D90 and D180 PO.

• Recession depth (RD). Distance from the CEJ to the gingival margin (GM). When GM was coronal to the CEJ the RD value was recorded as a negative number. Recorded at baseline and PO days 60, 90 and 180.

• Probing depth (PD). Distance from GM to sulcus depth. Recorded at baseline and D60, D90 and D180 PO.

• Attachment level (CAL). Calculated by the formula: CAL=PD+RD. Recorded at baseline and D60, D90 and D180 PO.

• Keratinized tissue width (KT). Distance from GM to mucogingival junction (MGJ). MGJ position was determined following Lugol solution application on the oral mucosa. Recorded at baseline and D60, D90 and D180 PO.

• Attached gingiva width (AG). Calculated by the formula: AG=KT-PD. When PD>KT, then AG was recorded as zero. Recorded at baseline and D60, D90 and D180 PO.

• Recession width (RW). Distance from mesial to distal line angle of the recession defect. Defining points will be the mesial and distal points where GM intersects CEJ. Recorded at baseline and D60, D90 and D180 PO.

• Alveolar bone crest (ABC=distance from CEJ to alveolar bone crest). Measured by transgingival probing (sounding) immediately prior to recipient bed preparation and confirmed after envelope flap elevation at baseline. Measured again (after anesthesia) by transgingival probing (sounding) at D180 PO.

Surgical procedures

With the exception of the sdGTC preparation and harvesting, all surgical procedures were performed in the routine fashion CTG is used for patients seeking root coverage

in the Graduate Periodontics Clinic at the College of Dentistry, The Ohio State University. Medical history and vital signs were reviewed prior to all surgical procedures. Immediately prior to graft harvesting, subjects received topical and local anesthesia in the palate, followed by 1-minute rinse using antimicrobial rinse (chlorhexidine 0.12%). For all CTG graft harvesting graft thickness (buccolingual dimension) was standardized at 1.0 mm. Graft length (mesio-distal dimension) and width (apico-coronal dimension) were determined by the formula: graft area (=length x width) = visible recession area (VRA) x 10 (see below for VRA determination). Donor sites were routinely sutured with non-absorbable sutures. Following topical anesthesia, the exposed root surfaces were scaled and root planed, and the recipient bed were prepared with an envelope flap design. Non-absorbable sutures were used to secure the graft and flap in place at the end of graft placement. For subjects randomized to receive sdCTG, the sdCTG procedure (pre-wounding), which was performed 5 days prior to graft harvesting and placement, consisted of all the steps included in the routine harvesting of a CTG described above, except for the fact that the routine distal and apical incisions were not performed, thus preventing complete removal of the graft. The pwCTG was secured in situ with a single absorbable suture until the next appointment (graft harvesting and placement).

VRA determination.

A piece of sterile aluminum foil was cut to fit the recession surface area. The piece was then photographed under standardized conditions (magnification 1:1) and the surface area of the calibrated digital image was determined using image analysis software (Adobe Photoshop 2007).

Postoperative protocol

Routine postoperative instructions included modified oral hygiene, analgesics (acetaminophen and, if needed as rescue medication, ibuprofen) and chlorhexidine rinse. Plaque control was performed by the subjects using only twice daily rinse with 0.12% chlorhexidine gluconate solution for two weeks. The subjects were instructed to change the self performed oral hygiene by abstaining from brushing and flossing the treated arch until suture removal (D14 PO). The patients were also instructed to avoid mechanical trauma in the treated sites by consuming only soft foods during the first week, and by avoiding hard foods until suture removal. The patients were maintained with professional care for plaque control, weekly for the first 3 PO weeks and then at each subsequent appointment (D60, D90, and D180).

Patient outcomes

Questionnaires that included a visual analog scale (VAS) were used to assess pain experience and analgesic use by the subjects, as previously described[68]. Questionnaires were given to the subjects pre-operatively and at D3, D7, D14, and D21(±1), D90(±5), and D180(±10).

Gingival Crevicular Fluid Sample Processing

Gingival crevicular fluid samples were analyzed for the presence of ANG and HIF-1α. Periopaper strips were thawed on ice and GCF was eluted from each Periopaper strip using a previously decribed method[69]. An extraction buffer containing 50mM Tris/HCL with 5mM $CaCl_2$, 0.2 NaCl. pH 7.6 containing 1mg/L antipan, 1mg/L aprotinin, 1mg/L leupeptin, 125 mg N-ethylaleimide and 50mg Zwittergent 3-12 (inhibitor coctail) was used. The 4 Periopaper strips collected from each site (CTG or control) were placed into 1.5ml centrifuge tubes along with 220µl of extraction buffer. The Periopaper strips, in combination with the GCF extraction buffer, were vortexed vigorously three times every fifteen minutes over a period of one hour. A hole was created at the bottom of a 400µl Eppendorf tube using a 25-Gauge needle. Periopaper strips were then placed into the 400µl tube, and that tube was then fitted on top of the 1.5ml centrifuge tube. The tubes were centrifuged (10,000g, 3min, 4^oC) forcing excess elution fluid from the Periopaper strips housed in the Eppendorf tube down into the 1.5ml centrifuge tube. A 200µl sample was stored at -80^oC until analysis.

Molecular Analysis

Angiogenin

GCF fluid samples were analyzed for the presence of ANG using a commercially available double antibody sandwich enzyme-linked immunosorbent assay (ELISA) following manufacturer's instructions*. Previously prepared GCF samples were diluted 10 fold. Phosphate buffered saline was used to dilute the GCF samples. A standard curve was created by serial dilution of a provided standard and plotting the absorbance at 450nm versus the log of recombinant human ANG concentration. Based on this curve, ANG concentrations in GCF and serum were calculated. GCF ANG concentrations were calculated taking into account the volume of GCF on each Periopaper strip.

Hypoxia-inducible Factor-1α

GCF fluid samples were analyzed for the presence of human HIF-1α using a commercially available immunoassay kit (ELISA) following manufacturer's instructions**. Previously prepared GCF samples were diluted 1.5 fold. The product provided by Abnova was used to dilute the GCF samples. A standard curve was created by serial dilution of a provided standard and plotting the absorbance at 450nm versus the log of recombinant human HIF-1α concentration. Based on this curve, HIF-1α concentrations in GCF were calculated. GCF HIF-1α concentrations were calculated taking into account the volume of GCF on each Periopaper strip.

*R&D Systems, Mineapolis, MN, USA

**ABNOVA, Taiwan

CHAPTER 3

DATA ANALYSIS

The subject was the unit of analysis. Descriptive statistics were expressed as mean ± SD. For the clinical parameters, intragroup comparisons between baseline and 6-months were performed by paired T-test. Unpaired T-test was used for intergroup comparisons.

Repeated measures analysis of variance (ANOVA) was used for analysis of mean differences in GCF values between baseline and follow-up visits within groups, and factorial ANOVA was used for examination of mean differences in GCF between groups at each time point. Post hoc testing was performed for differences between groups when significant differences were found. The significance level for rejection of the null hypothesis was set at $a = 0.05$

CHAPTER 4

RESULTS

General Observations

122 subjects went through the screening process. 21 subjects who fulfilled the inclusion criteria were recruited in the study. 10 of these subjects were randomly assigned to the pwCTG group and the other 11 subjects were randomly assigned to the rCTG group.

All CTGs were considered successful. 1 subject from the rCTG group discontinued after the 1 week post-operative visit. 1 subject from the rCTG group discontinued after the 60 day post-operative visit. Therefore, there were 10 subjects in the pwCTG group and 9 subjects in the rCTG group who completed the 6 month post-operative visit. (Table2)

According to post operative questionnaires, subjects followed postoperative instructions and used the prescribed analgesics. The only deviation was 1 subject that used 2 tablets of Aspirin 325mg postoperatively on the day of the pre-wounding procedure.

Smoking or use of any tobaco products was denied by all the study participants. The non-smoking status was confirmed by ECO analysis. Each subject never exceded 4ppm CO. (Data not shown here)

Two subjects developed post-operative complications. One pwCTG subject, who used 2 tablets of Aspirin 325mg after the pre-wounding procedure, developed an organized clot on the donor and the recipient site. He presented to the clinic with this condition on the 3rd postoperative day after the pwCTG procedure. No adverse outcome came to our attention regarding the 5 days interval between the pre-wounding procedure and the pwCTG procedure in this individual. The organized clot on the palate was removed and the organized clot on the recipient site was trimmed. No bleeding followed. No further complications occurred.

The 2nd post-operative complication occurred in one rCTG subject. The subject presented with a soft tissue abscess in the recipient site at the 7 days post-operative visit. Amoxicillin 500mg t.i.d. for 7 days was administered. The abscess resolved within 2-3 days after the initiation of the antibiotic therapy. No further complications ocurred.

Demographics		
	pwCTG	rCTG
Gender (females:males)	6:4	8:3
Age (years)	34.9±9.2	39.2±10.7

Table 2. Demographics

Subject Reported Outcomes

Donor site

According to D0 pre-operative questionnaires, 7 out of the 10 pwCTG subjects reported experiencing pain after the palate pre-wounding procedure. The reported VAS score was 3±2.45. The pain effect score was 1.6±1.07. However, based on analgesics consumption, the discomfort ocurred mainly immediately after the pre-wounding procedure and lasted for approximately 24 hours. (Table 3)

At D3 PO visit, 8 out of 10 pwCTG subjects reported they had experienced some pain during the first 3 days after the CTG procedure was completed. The corresponding report from the rCTG subjects was 9 out of 11 subjects. Pain prevalence decreased in subsequent PO visits. Specifically, regarding the pwCTG group, reported pain prevalence was 4 out of 10 subjects at D7 PO, 2 out of 10 subjects at D14 PO and 1 out of 10 subjects at D21 PO. The respective prevalence for the rCTG group was 7 out of 11 subjects, 2 out of 10 subjects and 1 out of 10 subjects. (Figure 8) The comparison of reported pain prevalence between the two study groups, i.e. pwCTG and rCTG, at any of the predetermined PO visits, i.e. D3, D7, D14, D21, did not yield any statistically significant differences. No subjects reported any pain at the 60D, 90D and 180D PO visits. The intragroup comparisons showed a statistically significant decrease in reported pain prevalence between D3 PO and D21 PO for pwCTG, D3 PO and D14 PO for rCTG, and D3 PO and D21 PO for rCTG ($p<0.005$).

The mean ± SD VAS values for the pwCTG group were 4.3±2.0, 1.3±1.89, 0.5±1.27 and 0.1±0.32 on D3, D7, D14 and D21 respectively. The mean ± VAS values for the rCTG group were 4.14±2.12, 1.27±1.35, 0.2±0.42 and 0±0 on day 3, 7, 14 and 21

respectively. The intergroup comparisons by means of an independent two-tailed t-test did not reveal any statististically significant difference at any of the predetermined PO visits. Both pwCTG and rCTG showed a statistically significant intragroup VAS difference between D3 and D7, D3 and D14, and D3 and D21 ($p<0.005$) (Figure 9)

The mean$\pm$ SD pain effect values for the pwCTG group at day 3, 7, 14 and 21 were 1.7 ± 0.64, 0.65 ± 0.78, 0.3 ± 0.67, and 0.1 ± 0.32 respectively. The corresponding values for the rCTG group were 2.09 ± 1.16, 0.55 ± 0.50, 0.2 ± 0.42, and 0 ± 0. There were no intergroup statistically significant differences at any of the predetermined PO visits. There were statistically significant differences between D3 PO and D7 PO for both the pwCTG and the rCTG groups. (Figure 10)

D0 Pre-op Report	VAS value	Pain Effect Value	Analgesics	Time/Quantity of Analgesics
N	1	1	N	
Y	2	2	Y	D(-5)/2 tablets Aspirin 325mg
N	2	0	N	
Y	2	2	Y	D(-5)/ 1 tablet Tylenol 325mg D(-4)/ 1 tablet Tylenol 325mg b.i.d.
Y	2	2	Y	D(-5)/ 1 tablet Tylenol 325mg D(-4)/ 1 tablet Tylenol 325mg
N	0	0	N	
Y	7	3	Y	D(-5)/5 tablets Tylenol 325mg
Y	5	3	Y	D(-5)/ 1 tablet Tylenol 325mg
Y	7	2	N	
Y	2	1	Y	D(-5)/ 1 tablet Tylenol 325mg

Table 3. Analgesics Consumption After Pre-wounding

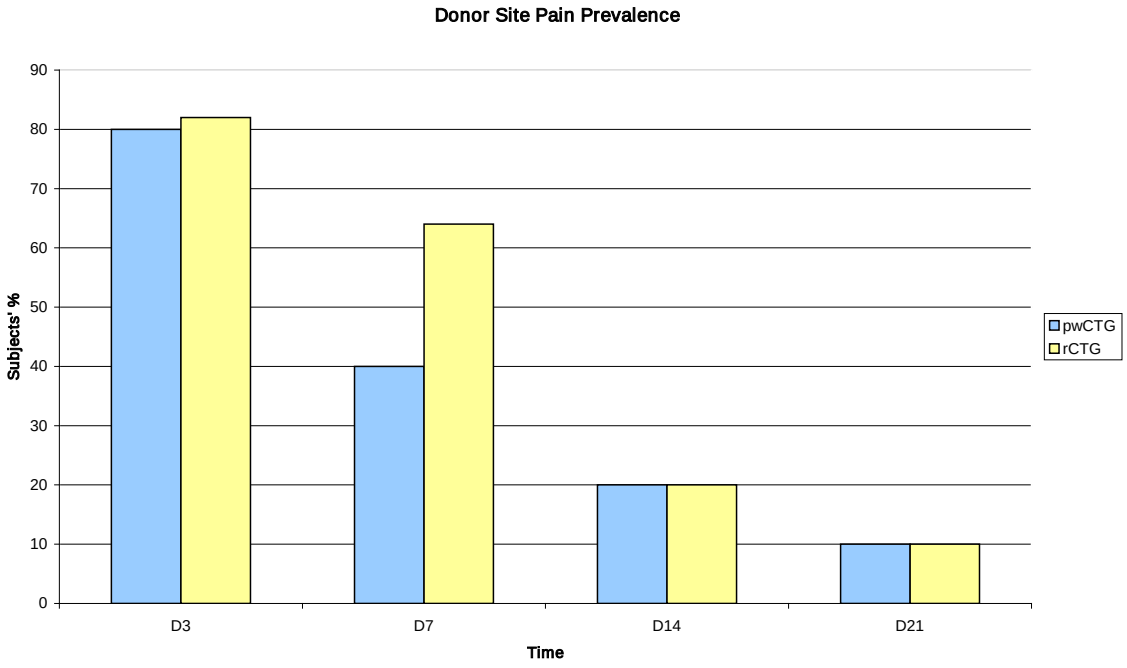


Figure 8. Donor Site Pain Prevalence

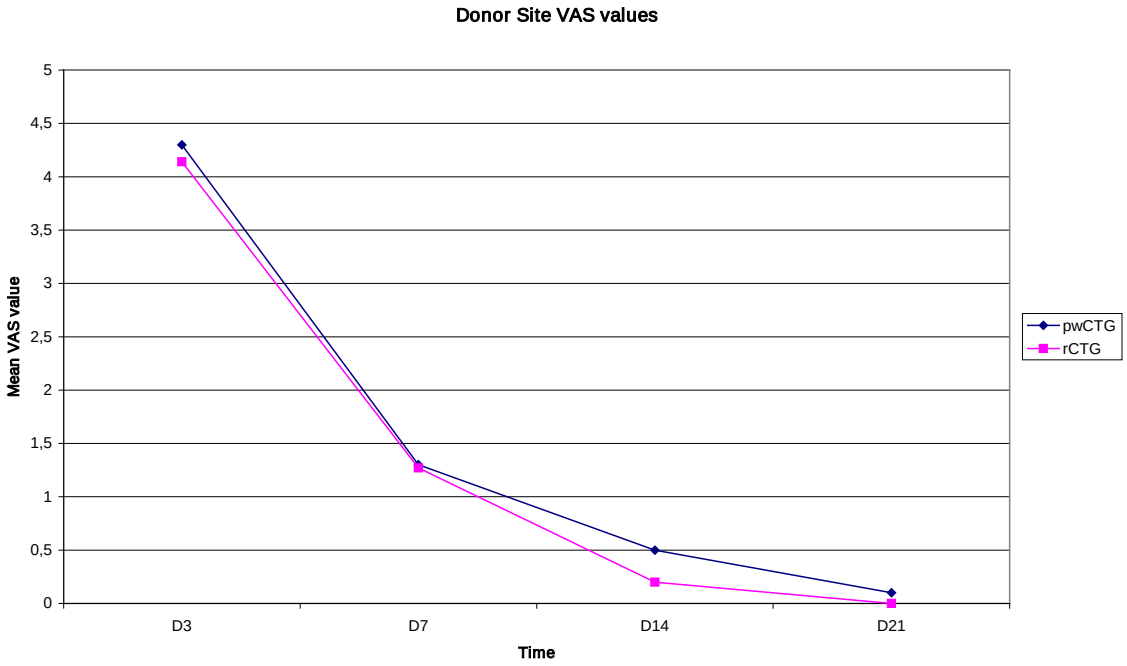


Figure 9. Donor Site Average VAS Scores

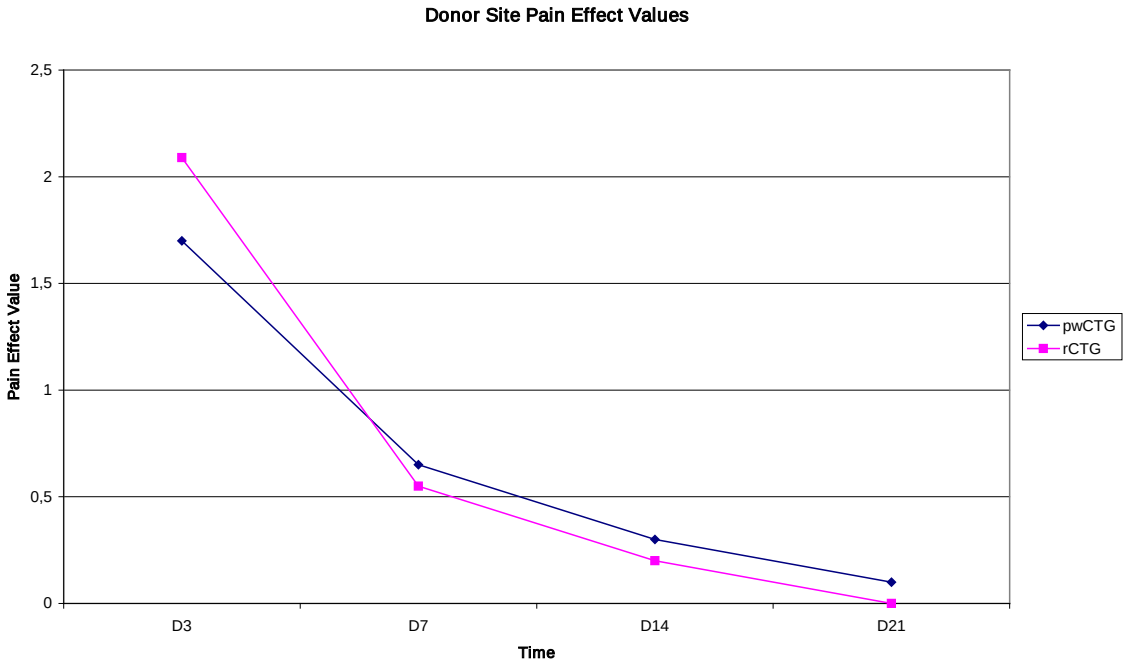


Figure 10. Donor Site Average Pain Effect Scores

Recipient site

At D3 PO visit 9 out of 10 pwCTG subjects reported they had experienced some pain between the completion of the CTG procedure and D3 PO visit. The corresponding report regarding the rCTG group was 9 out of 11 subjects. Pain prevalence decreased with time. Regarding pwCTG group, 4 out of 10 subjects, 1 out of 10 subjects, and 0 out of 10 subjects reported pain at D7, D14 and D21 PO. Regarding the rCTG group, 5 out of 10 subjects, 1 out of 10 subjects, and 1 out of 10 subjects reported pain during the same sequence of PO visits. The comparison of reported pain prevalence between the two study groups, i.e. pwCTG and rCTG, at any of the predetermined PO visits, i.e. D3, D7, D14, D21, did not yield any statistically significant differences. No subjects reported any pain at the 60D, 90D and 180D PO visits. The intragroup comparisons showed a statistically significant decrease in reported pain prevalence between D3 PO and D14 PO, and also between D3 PO and D21 PO for both pwCTG and rCTG groups ($p<0.005$). (Figure 11)

The mean $\pm$ SD VAS values for the pwCTG group were 4.35 ± 2.91, 1.4 ± 1.71, 0.4 ± 1.26 and 0 ± 0 on D3, D7, D14 and D21 respectively. The mean $\pm$ VAS values for the rCTG group were 3.91 ± 2.34, 1.32 ± 1.06, 0.2 ± 0.42 and 0.1 ± 0.32 on day 3, 7, 14 and 21 respectively. The intergroup comparisons by means of an independent two-tailed t-test did not reveal any statististically significant difference at any of the predetermined PO visits. Both pwCTG and rCTG showed a statistically significant intragroup VAS difference between D3 and D14, and D3 and D21 ($p<0.005$). Additionally, rCTG showed a statistically significant difference between D3 and D7 PO. (Figure 12)

The mean+ SD pain effect values for the pwCTG group at day 3, 7, 14 and 21 were 1.7±0.64, 0.65±0.78, 0.3±0.67, and 0.1±0.32 respectively. The corresponding values for the rCTG group were 2.09±1.16, 0.55±0.50, 0.2±0.42, and 0±0. There were no intergroup statistically significant differences at any of the predetermined PO visits. There were statistically significant differences between D3 PO and D14 PO for the rCTG group and also between D3 PO and D21 PO for both pwCTG and rCTG groups. (Figure 13)

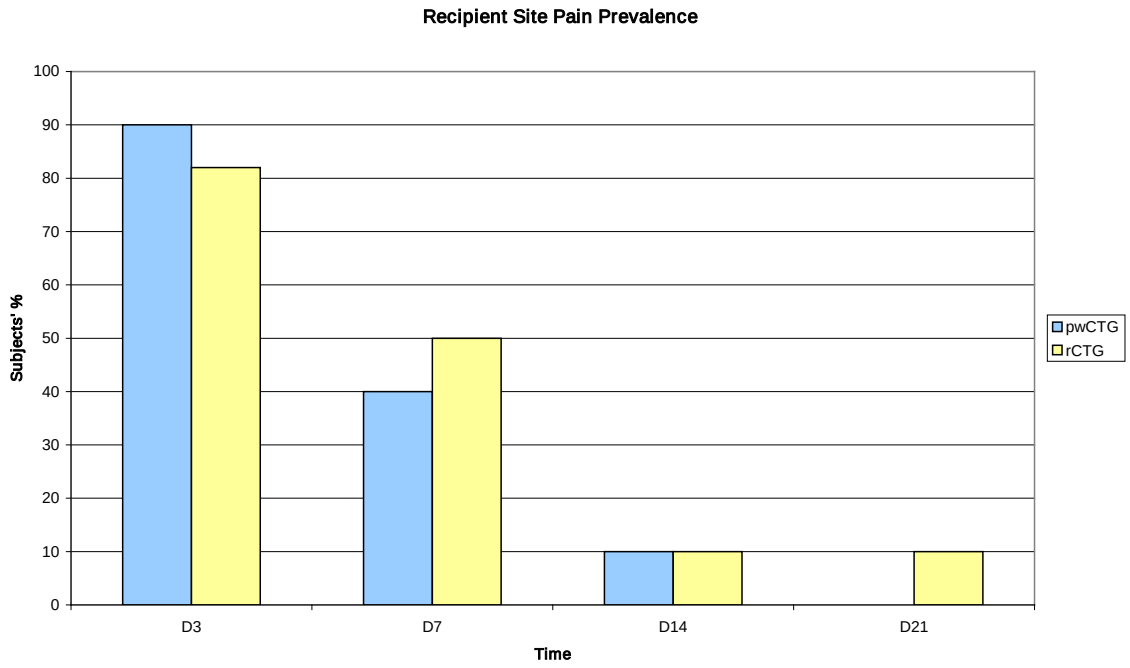


Figure 11. Recipient Site Pain Prevalence

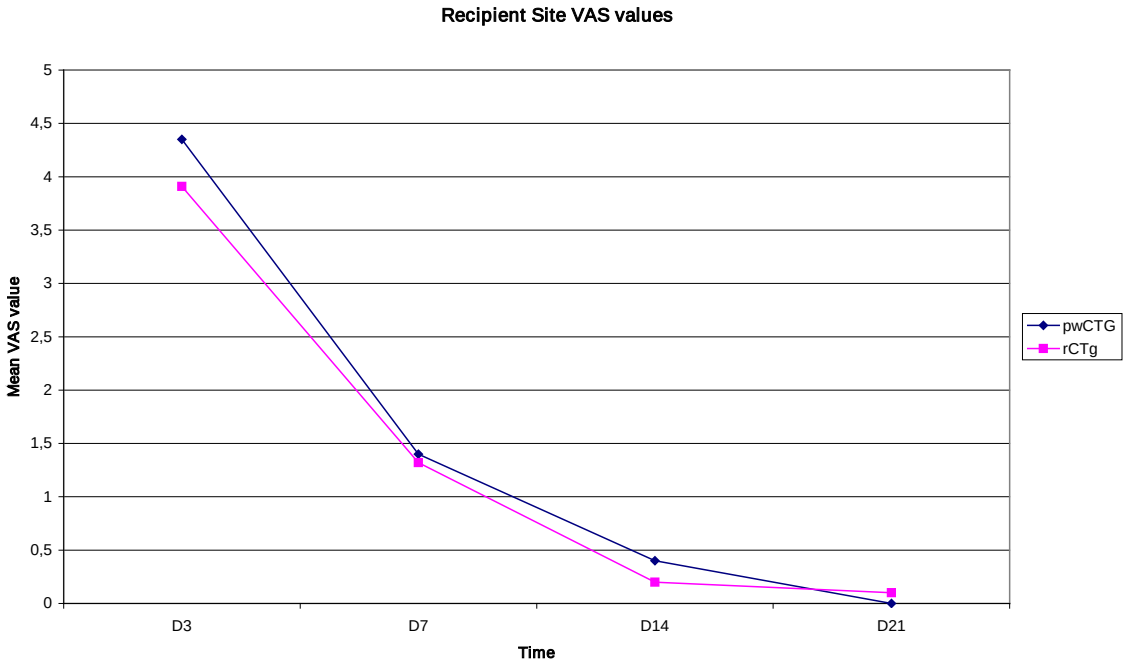


Figure 12. Recipient Site Average VAS Scores

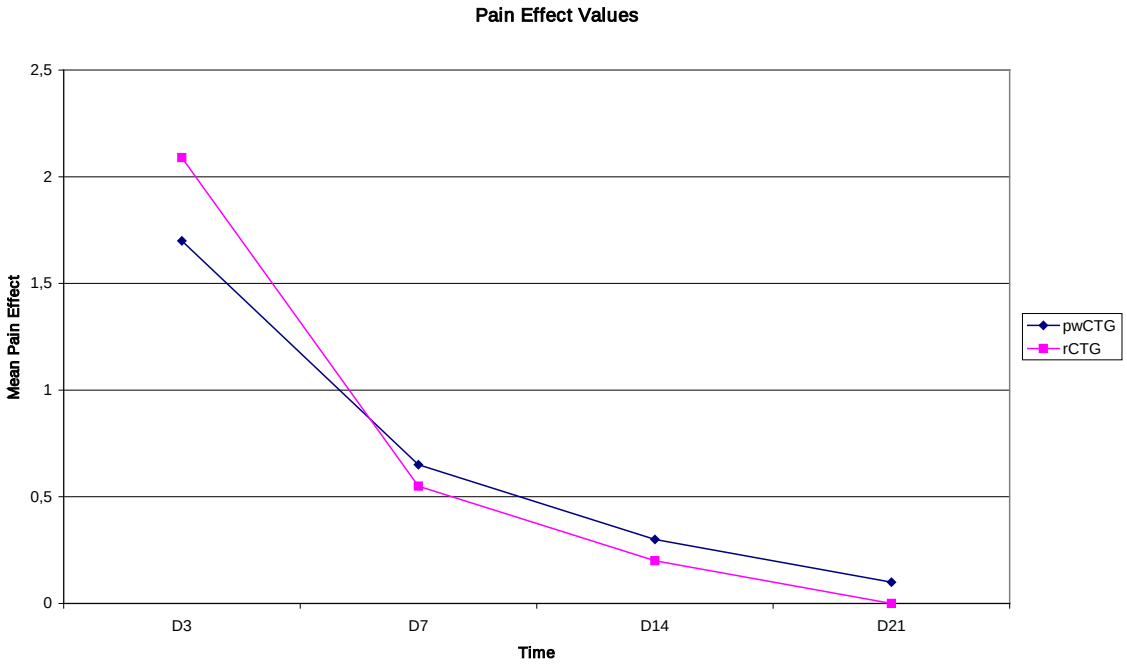


Figure 13. Recipient Site Average Pain Effect Scores

Clinical Outcomes

Figures 14-22 present a clinical case treated with the pwCTG technique. Figures 23-31 present a clinical case treated with the rCTG technique.

The baseline RD was 4.2±1.1mm for the pwCTG group and 3.8±1.0mm for the rCTG group. The D60 PO RD was 1.3±0.8mm for the pw CTG group and 1.1±1.1mm for the rCTG group. The D90 PO RD was 1.0±0.9mm for the pw CTG group and 0.8±0.7mm for the rCTG group. The D180 PO RD was 0.45±0.55mm for the pwCTG group and 0.6±0.7mm for the rCTG group. There was no statistically significant difference between the pwCTG group and rCTG group at any of the PO visits, i.e. D0, D60, D90, and D180. There was a statistically significant difference when comparing baseline RD to D90 RD and to D180 RD ($p<0.0001$) (Table 4)

The achieved root coverage (RC) at D60 PO was 69±20.4% for the pwCTG group and 72.2±24.7% for the rCTG group with no statistically significant difference between the two groups. The achieved RC at D90 PO was 79±19.4% for the pwCTG group and 80.6±15% for the rCTG group with no statistically significant difference between the two groups. 40% of the pwCTG subjects (n=4) had 100% RC at D90 PO. 33.3% of the rCTG subjects (n=3) had 100% RC at D90 PO. There was no statistically significant difference when comparing RC at D60 and D90 for any of the two study groups. The achieved root coverage 180 days PO was 89.8±13.1% for the pwCTG group and 85.6±14.9% for the rCTG group with no statistically significant difference between the two groups. Both pwCTG and rCTG showed an intragroup statistically significant difference regarding RC at D180 compared to D60. 50% of

pwCTG subjects (n=5) had 100% RC at the D180 PO. 44.4% of the rCTG subjects had 100% RC at D180 PO. (Table 4)

The baseline RW was 3.9±0.9mm for the pwCTG group and 4.2±0.8mm for the rCTG group. The D60 PO RW was 1.7±1.6mm for the pwCTG group and 2.2±1.8mm for the rCTG group. The D90 PO RW was 1.7±1.6mm for the pwCTG group and 1.9±1.5mm for the rCTG group. The 180 days RW was 1.4±1.42mm for the pwCTG group and 1.4±1.5mm for the rCTG group. There was no difference between the pwCTG group and the rCTG group at any of the PO visits. There was a statistically significant difference between baseline and D90 PO ($p<0.005$) for each of the two groups. There was also a statistically significant difference between baseline and D180 PO ($p<0.0005$) for each of the two groups. (Table 4)

The baseline KT was 1.2±0.8mm for the pwCTG group and 1.8±1.1mm for the rCTG group. The D60 PO KT was 4.0±1.2mm for the pw CTG group and 3.7±1.1mm for the rCTG group. The D90 PO KT was 4.3±1.2mm for the pw CTG group and 4.1±1.1mm for the rCTG group. The D180 PO KT was 4.6±0.8mm for the pwCTG group and 3.7±1.5mm for the rCTG group. There was no statistically significant differnce between the two groups at any of the PO visits. There was a statistically significant difference when comparing the D90 PO and D180 PO data of each of the two study groups to the respective baseline data ($p<0.0001$). (Table 4)

The baseline AG was 0.2±0.4mm for the pwCTG group and 0.6±1.1mm for the rCTG group. The D60 PO AG was 2.8±1.4mm for the pw CTG group and 2.6±1.2mm for the rCTG group. The D90 PO AG was 2.7±1.3mm for the pw CTG group and 2.9±1.3mm for the rCTG group. The D180 PO AG was 3.2±0.7mm for the pwCTG

group and 2.1±1.9mm for the rCTG group. There was no statistically significant differnce between the two groups at any of the PO visits. There was a statistically significant difference when comparing the D90 PO data ($p<0.0005$ for pwCTG and $p<0.0001$ for rCTG) and the D180 PO data ($p<0.0000005$ for pwCTG and $p<0.05$ for rCTG) of each of the two study groups to the respective baseline data. (Table 4)

PD remained constant throughout the study. The baseline recordings were 1.6±0.5mm for both study groups. At D60 PO the PD was 1.2±0.42mm for the pwCTG group and 1.1±0.33mm for the rCTG group. At D90 the PD was 1.5±0.5mm for both study groups. At D180 PO the PD was 1.3±0.48mm for the pwCTG group and 1.6±0.49mm for the rCTG group. There were no statistically significant changes between the two groups at any time. There were no statistically significant changes with time in any of the two groups. (Table 4)

The baseline CAL was 5.8±1.3mm for the pwCTG group and 5.3±1.1mm for the rCTG group with no statistically significant difference between the two groups. The D60 PO CAL was 2.3±1.3mm for the pw CTG group and 2±1.5mm for the rCTG group. The D90 PO CAL was 2.0±1.6mm for the pw CTG group and 1.7±1.1mm for the rCTG group. The D180 PO CAL was 1.1±1.19mm for the pwCTG group and 1.7±1.1mm for the rCTG group. There were no statistically significant differences between the the two groups. Within each study group, i.e. pwCTG and rCTG, there was a statistically significant difference between baseline and each of D90 PO and D180 PO data ($p<0.0001$). (Table 4)

PI was low throughout the study. Baseline PI was 0 for the pwCTG group and 0.09±0.30 for the rCTG group. D60 PO PI was 0.5±0.97 for the pwCTG group and

0.44±0.53 for the rCTG group. D90 PO PI was 0.2±0.42 for the pwCTG group and 0.67±0.71 for the rCTG group. D180 PO PI was 0.1±0.32 for the pwCTG group and 0.44±0.53 for the rCTG group. (Table 4)

GI was also low throughout the study. It was 0 for both groups at baseline, D90 PO and D180 PO. It was 0.2±0.42 for the pwCTG group and 0.11±0.33 for the rCTG group at D60 PO. (Table 4)

The baseline pre-operative ABC was 7.8±1.7mm for the pwCTG group and 7.3±1.0mm for the rCTG group. The baseline ABC measured with the flap elevated was 8.0±1.6mm for the pw CTG group and 7.3±1.2mm for the rCTG group. The D180 PO ABC was 4.7±1.2mm for the pwCTG group and 5.5±0.8mm for the rCTG group.

Both pwCTG and rCTG presented a statistically significant intragroup difference regarding ABC measured by transmucosal probing pre-operatively and direct measurement with the full flap elevated. Additionally, both pwCTG and rCTG showed an intragroup statistically significant difference regarding ABC measurement at day 0 and day 180 ($p<0.0001$). (Table 4)

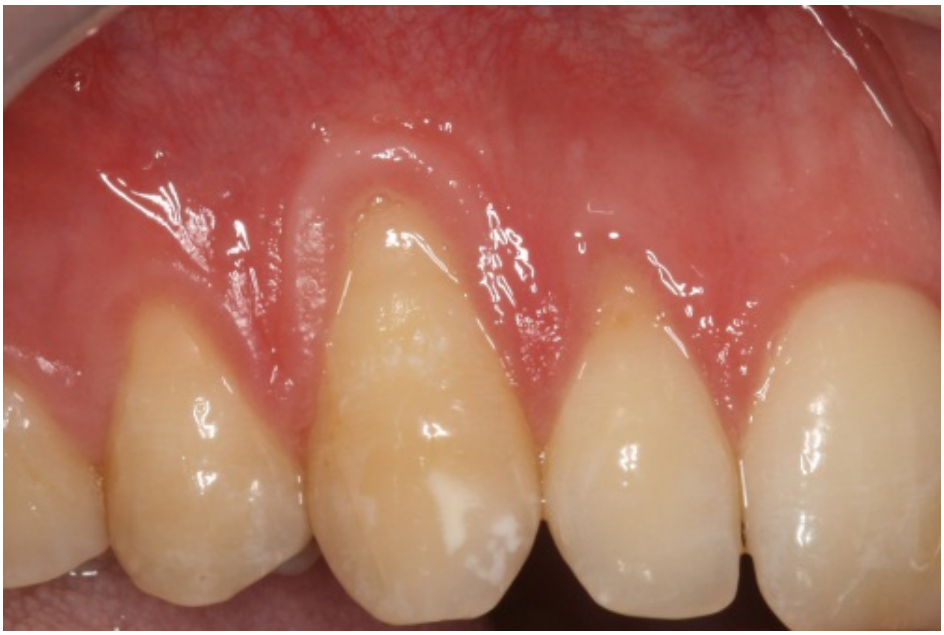


Figure 14. pwCTG Case Pre-operatively

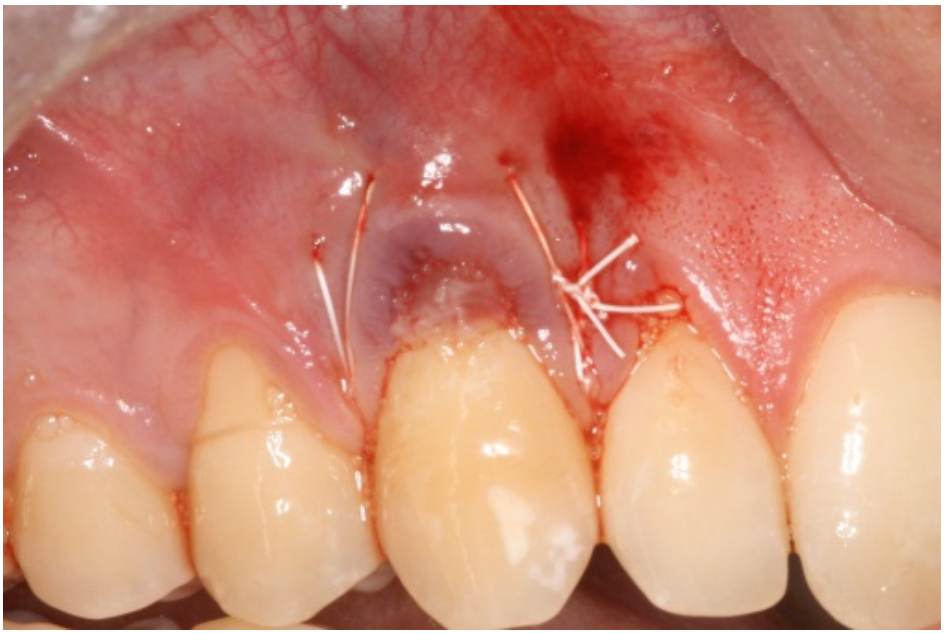


Figure 15. pwCTG Case D0 PO

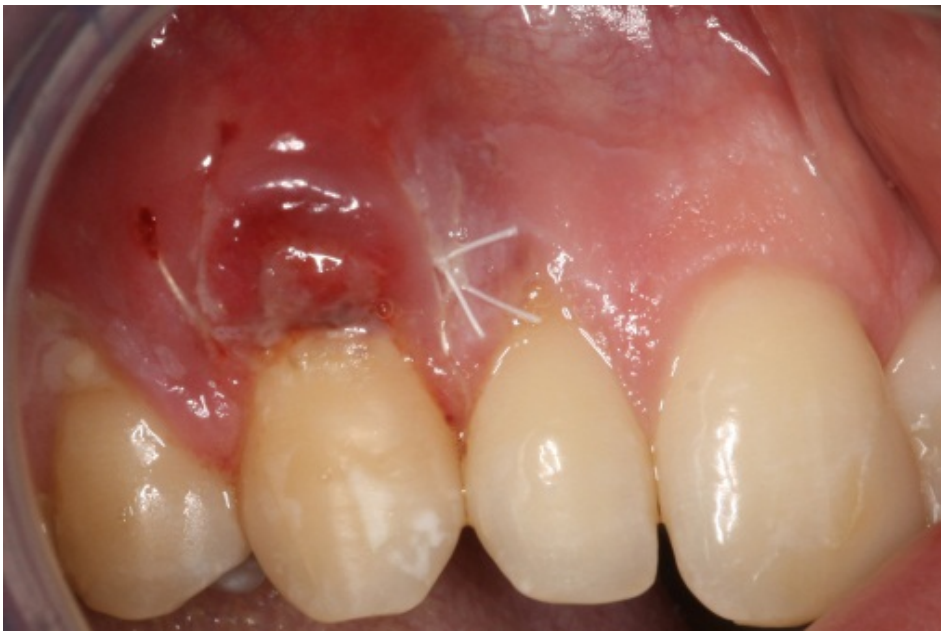


Figure 16. pwCTG Case D3 PO

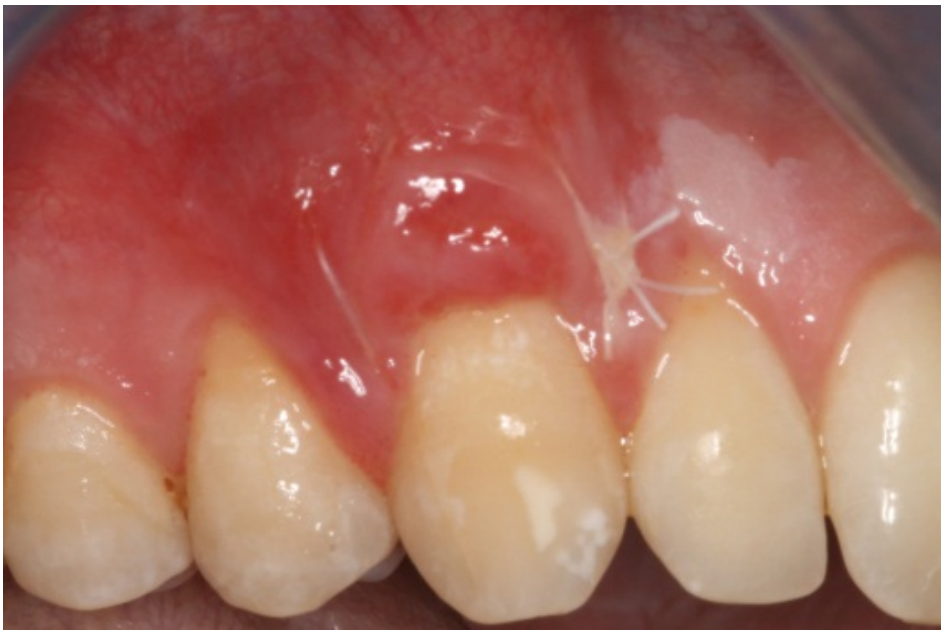


Figure 17. pwCTG Case D7 PO

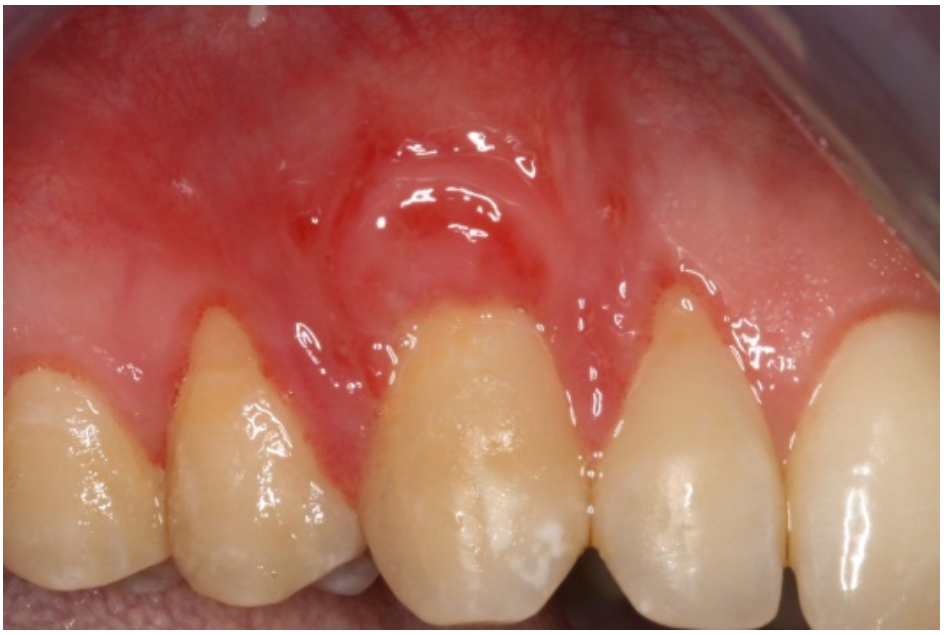


Figure 18. pwCTG Case D14 PO

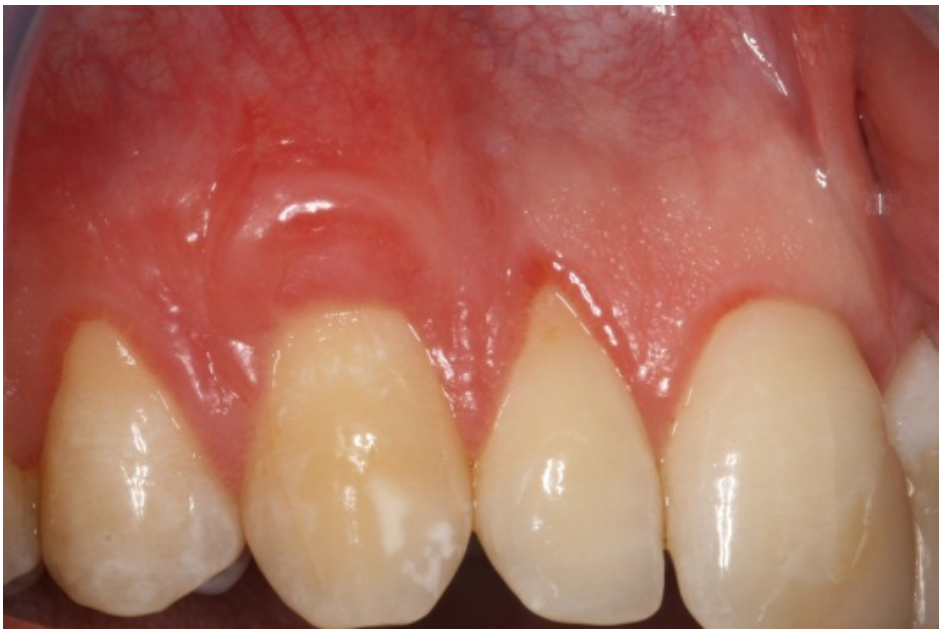


Figure 19. pwCTG Case D21 PO

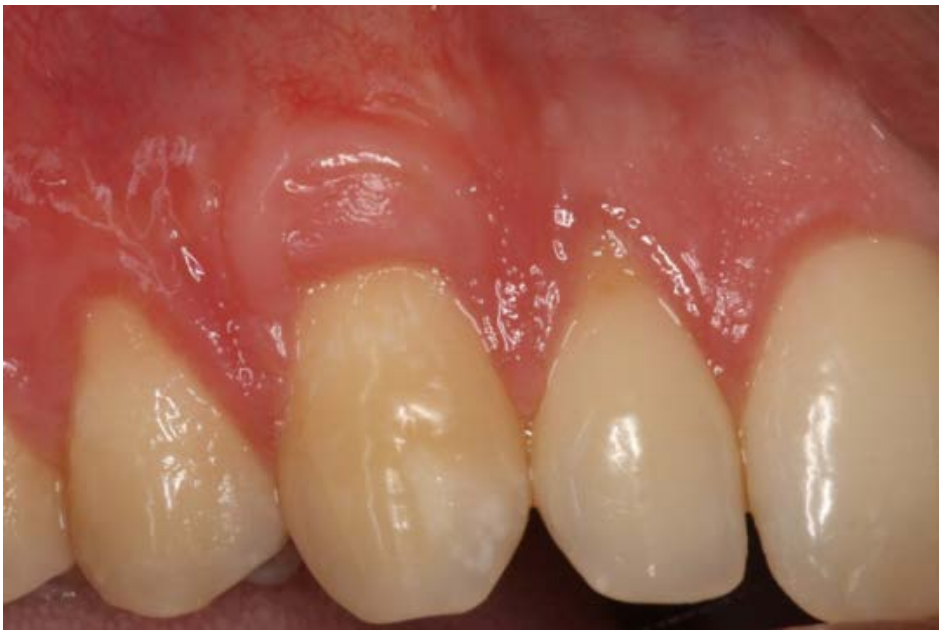


Figure 20. pwCTG Case D60 PO

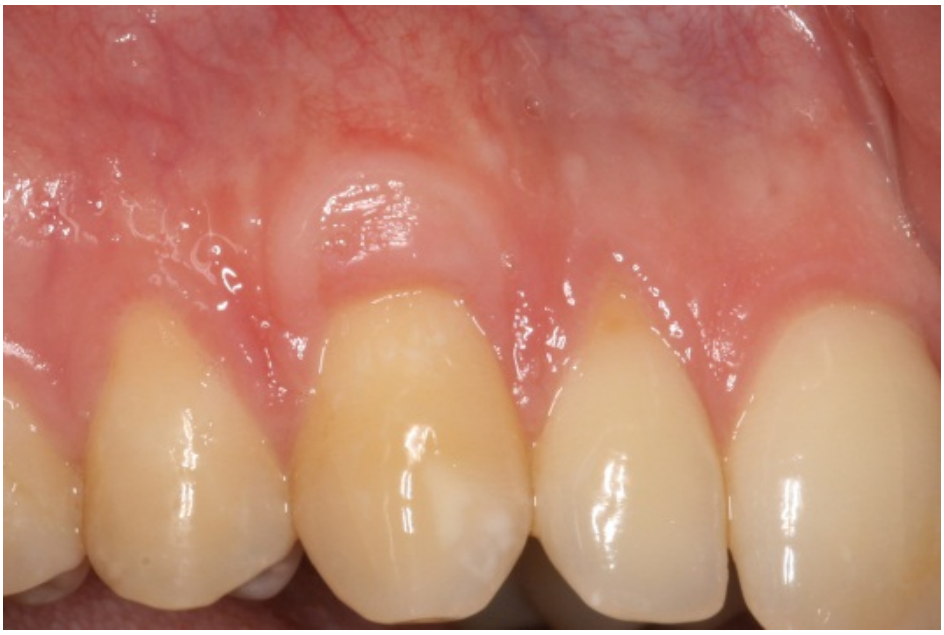


Figure 21. pwCTG Case D90 PO

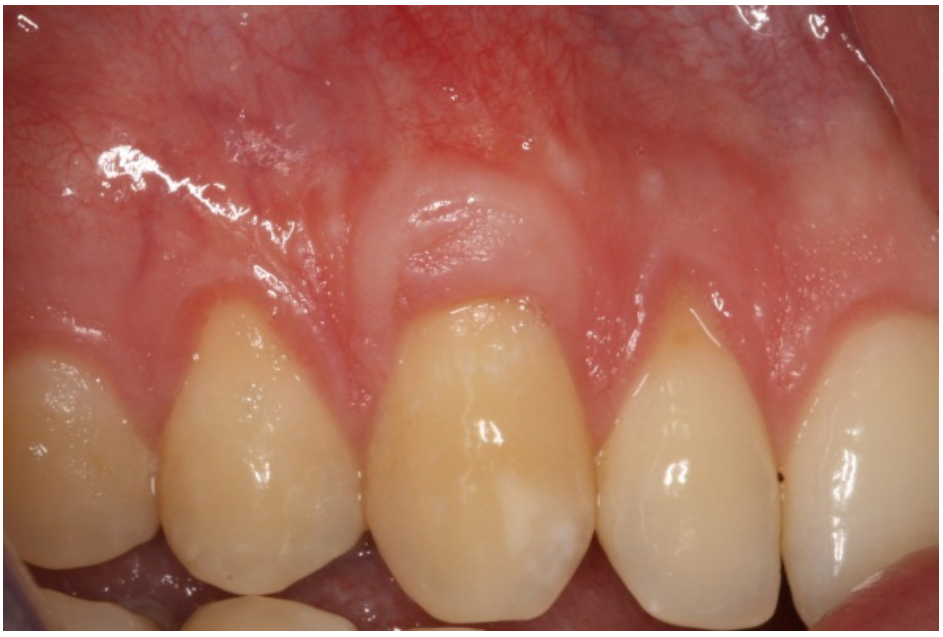


Figure 22. pwCTG Case D180 PO

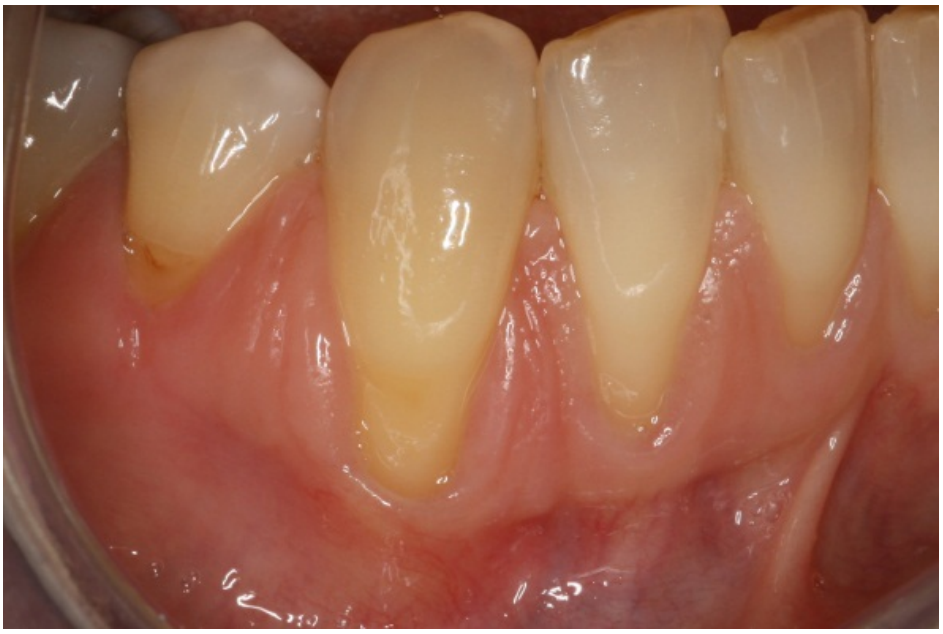


Figure 23. rCTG Case Pre-operatively

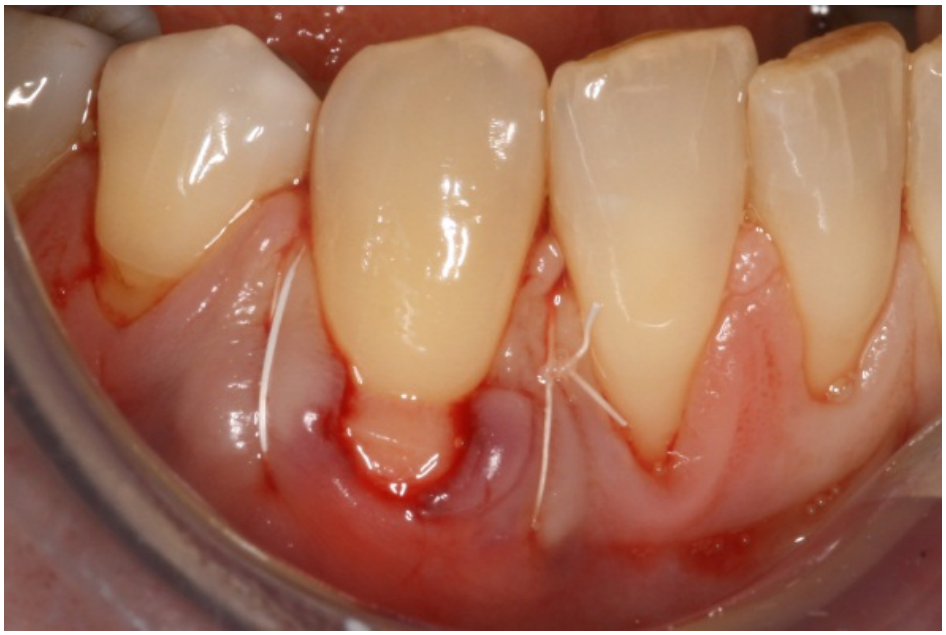


Figure 24. rCTG Case D0 PO

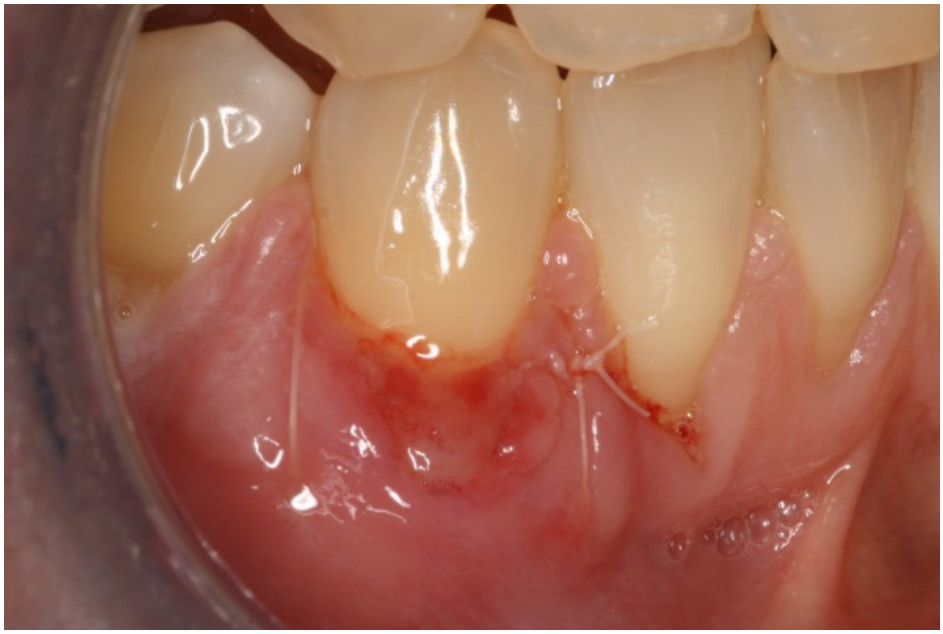


Figure 25. rCTG Case D3 PO

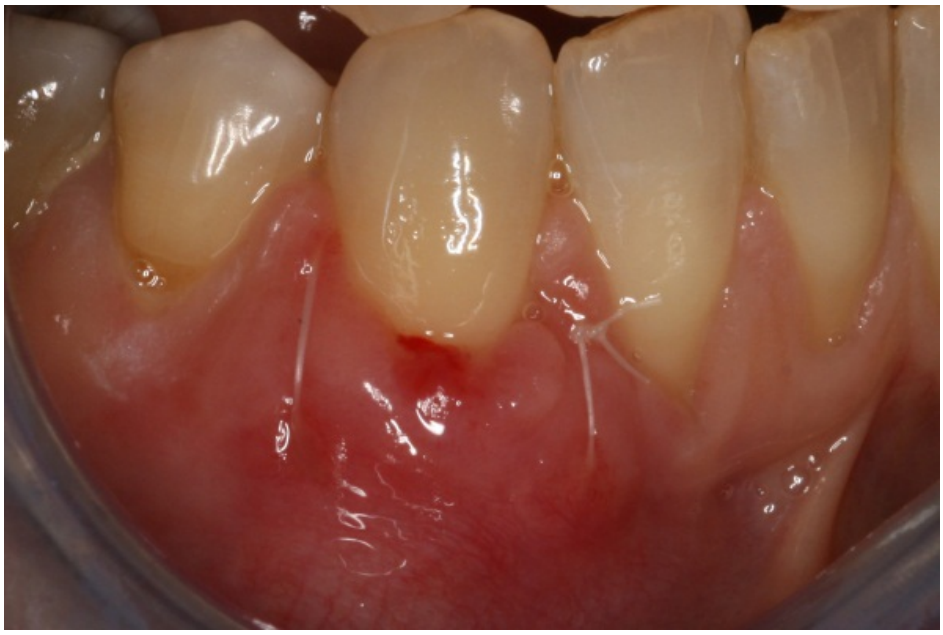


Figure 26. rCTG Case D7 PO

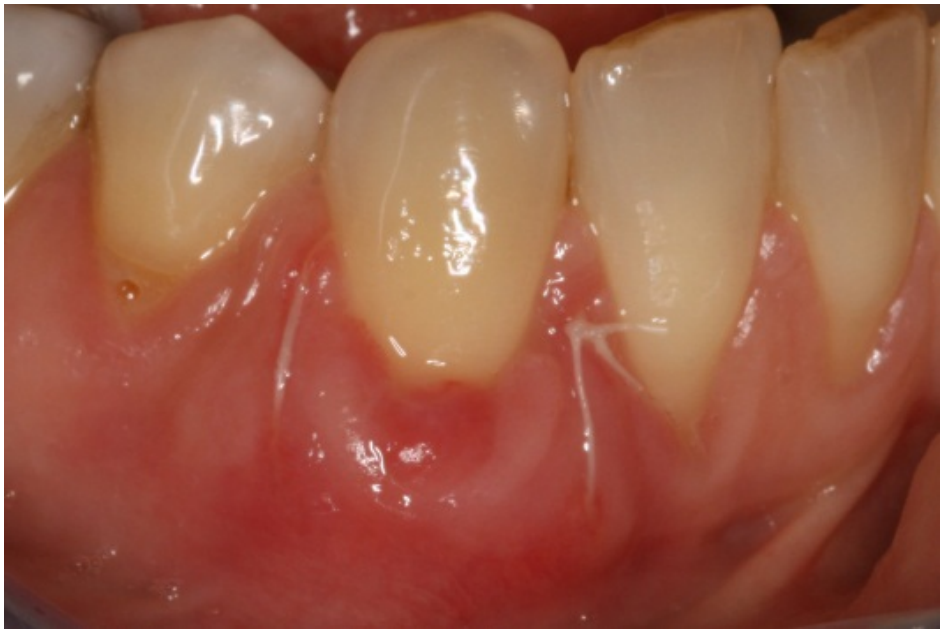


Figure 27. rCTG D14 PO

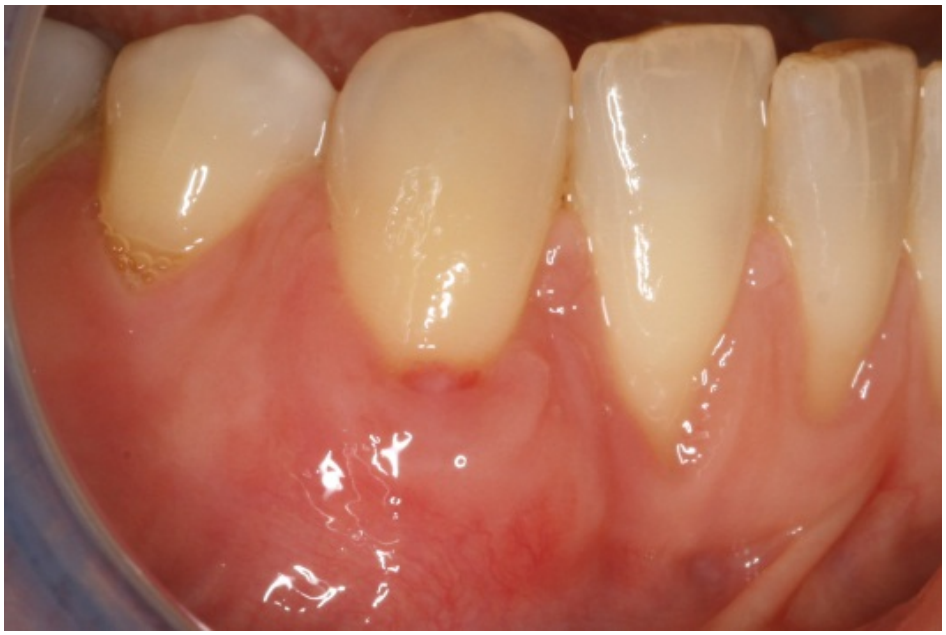


Figure 28. rCTG Case D21 PO

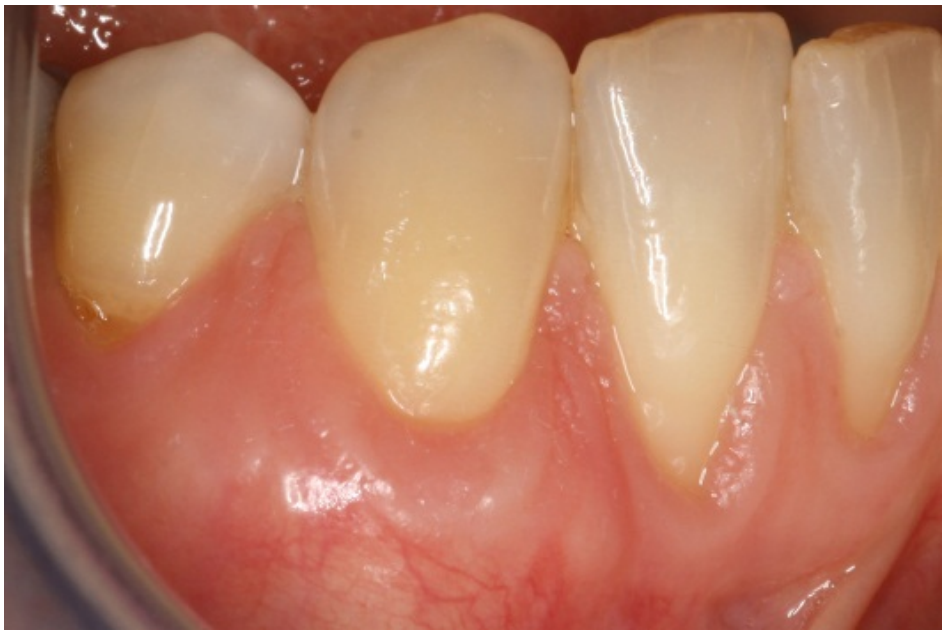


Figure 29. rCTG Case D60 PO

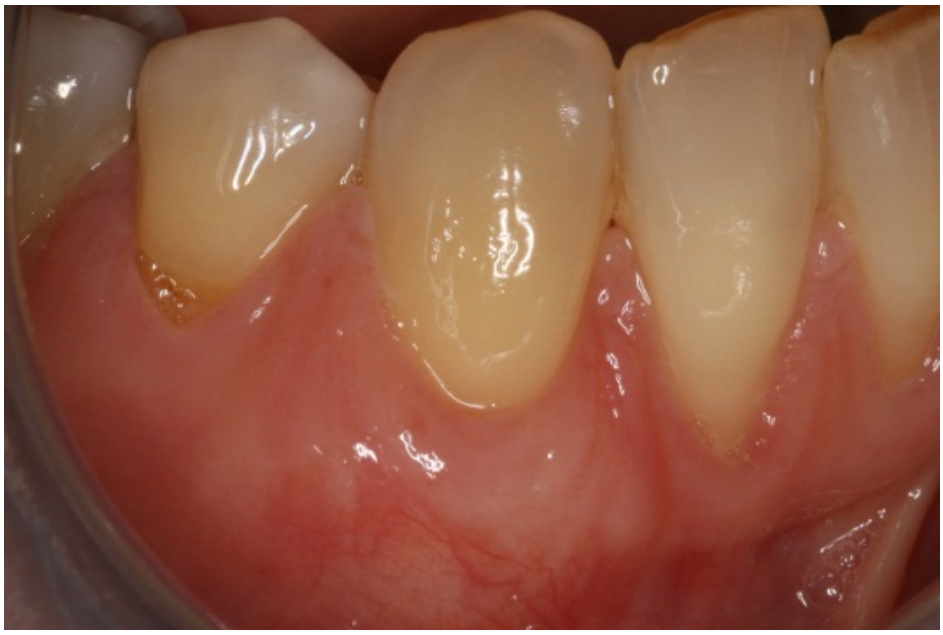


Figure 30. rCTG Case D90 PO

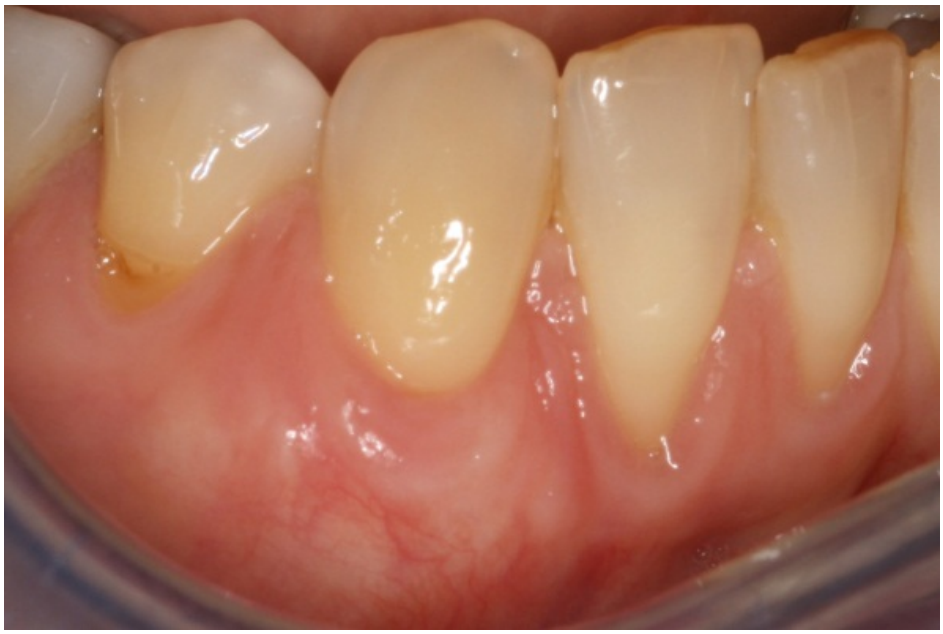


Figure 31. rCTG Case D180 PO

Clinical Parameters (mean ± SD)									
	pwCTG				rCTG				
	Baseline		90 Days	180 Days	Baseline		90 Days	180 Days
RD (mm)	4.2±1.1		1.0±0.9	0.45±0.55	3.8±1.0		0.8±0.49	0.6±0.7
RW (mm)	3.9±0.9		1.7±1.6	1.4±1.42	4.1±0.8		1.9±1.5	1.4±1.42
PD (mm)	1.6±0.5		1.5±0.5	1.3±0.48	1.6±0.5		1.5±0.5	1.6±0.49
CAL (mm)	5.8±1.3		2.0±1.6	1.1±1.19	5.3±1.1		1.7±1.1	1.7±1.15
KT (mm)	1.2±0.8		4.3±1.2	4.6±0.84	1.8±1.1		4.1±1.1	3.7±1.5
RC (%)			79.0±19.4	89.8±13.1			80.6±15	85.6 ± 14.9
ABC (mm)	*	**		*	*	**		*
	7.8± 1.72	8.0 ±1.63		4.7±1.25	7.3±1	7.3±1.22		5.5±0.76

* - ABC measurement by transmucosal probing (no flap elevated)

** - direct ABC measurement (flap elevated)

Table 4. Clinical Outcomes

Gingival Crevicular Fluid Angiogenin

GCF ANG concentration was measured at control and test sites at D0 pre-operatively (baseline) and D3, D7, D14, and D21 PO.

The baseline GCF ANG was 331.72±113.25pg/µl for the test site in the pwCTG group and 295.37±77.87pg/µl for the test site in the rCTG group. The baseline GCF ANG was 276.58±175.15pg/µl for the control site in the pwCTG group and 329.33±154.26pg/µl for the control site in the rCTG group. The D3 PO GCF ANG was 492.47±324.83pg/µl for the test sites in the pw CTG group and 363.37±161.67pg/µl for the test sites in the rCTG group. The D3 PO GCF ANG was 286.46±169.55pg/µl for the control sites in the pw CTG group and 241.95±67.11pg/µl for the control sites in the rCTG group. The D7 PO GCF ANG was 475.17±223.03pg/µl for the test sites in the pw CTG group and 390.13± 66.86pg/µl for the test sites in the rCTG group. The D7 PO GCF ANG was 334.62±132.16pg/µl for the control sites in the pw CTG group and 295.25±82.90pg/µl for the control sites in the rCTG group. The D14 PO GCF ANG was 413.42±168.42pg/µl for the test sites in the pw CTG group and 428.69±70.25pg/µl for the test sites in the rCTG group. The D14 PO GCF ANG was 330.93±57.29pg/µl for the control sites in the pw CTG group and 331.79±130.31pg/µl for the control sites in the rCTG group. The D21 PO GCF ANG was 302.42±123.17pg/µl for the test sites in the pw CTG group and 303.73±57.43pg/µl for the test sites in the rCTG group. The D21 PO GCF ANG was

269.00±124.91pg/µl for the control sites in the pw CTG group and 298.20±31.00pg/µl for the control sites in the rCTG group. (Table 5) (Figures 32-35) Repeated measures ANOVA revealed no statistically significant GCF ANG differences over time for each of the groups (pwCTG, rCTG), for either test or control sites ($p>0.4$). There were no statistically significant differences in GCF ANG between groups at any time point ($p>0.1$).

Gingival Crevicular Fluid HIF-1α

GCF HIF-1α concentration was measured at test sites at D0 pre-operatively (baseline) and D3, D7. Additionally, GCF HIF-1α concentration was measured at the control sites of two subjects (one subject belonging to the pwCTG group and one subject belonging to the rCTG group).

The baseline GCF HIF-1α was 96.81±57.05ng/ml for the test site in the pwCTG group and 162.73±122.51ng/ml for the test site in the rCTG group. The baseline GCF HIF-1α was 292.78 for the control site in the pwCTG subject and 64,06ng/ml for the control site in the rCTG subject. The D3 PO GCF HIF-1α was 53.19±35.00ng/ml for the test sites in the pw CTG group and 228.62±30.43ng/ml for the test sites in the rCTG group. The D3 PO GCF HIF-1α was 77.70ng/ml for the control site in the pw CTG subject and 107.32ng/ml for the control site in the rCTG subject. The D7 PO GCF HIF-1α was 88.11±67.95ng/ml for the test sites in the pw CTG group and 167.98±97.64ng/ml for the test sites in the rCTG group. The D7 PO GCF HIF-1α was 49.98ng/ml for the control site in the pw CTG subject and 66.24ng/ml for the control site in the rCTG subject. (Table 6) (Figure 36)

Repeated measures ANOVA revealed no statistically significant GCF HIF1a differences over time for each of the groups (pwCTG, rCTG), for the test sites ($p>0.07$). There were no statistically significant differences in test site GCF HIF1a levels between groups at any of the tested time points ($p>0.1$).

ANG concentration (ng/µl)					
	pwCTG			rCTG	
		Test Site	Control Site	Test Site	Control Site
	Day 0 Pre-Op	331.72±113.25	276.58±175.15	295.37±77.87	329.33±154.26
	Day 3 PO	492.47±324.83	286.46±169.55	363.38±161.67	241.95±67.11
	Day 7 PO	475.17±223.03	334.62±132.16	309.13±66.86	295.25±82.90
	Day 14 PO	413.42±168.42	330.93±57.29	428.69±70.25	331.79±130.31
	Day 21 PO	302.42±123.17	269.00±124.91	303.73±57.43	298.2±31.00

Table 5. Angiogenin Concentration (mean±SD)

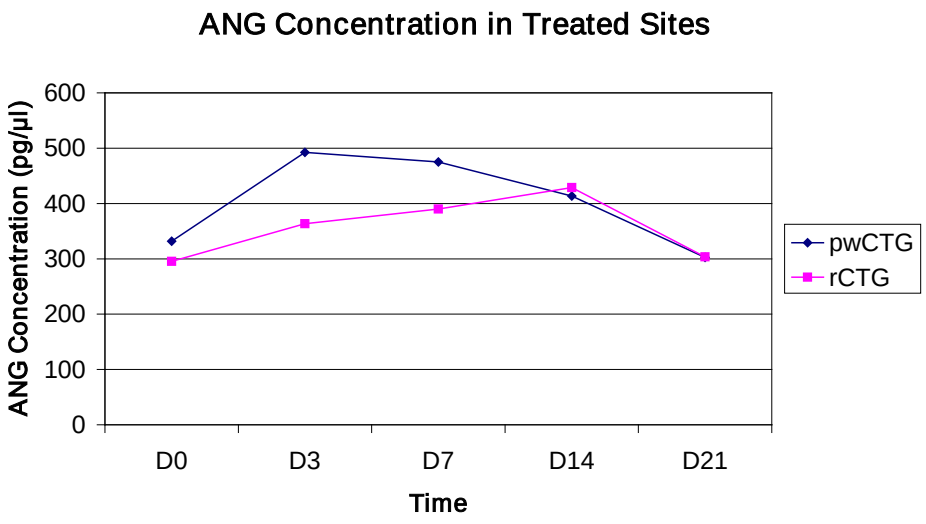


Figure 32. Average ANG Concentration in Treated Sites

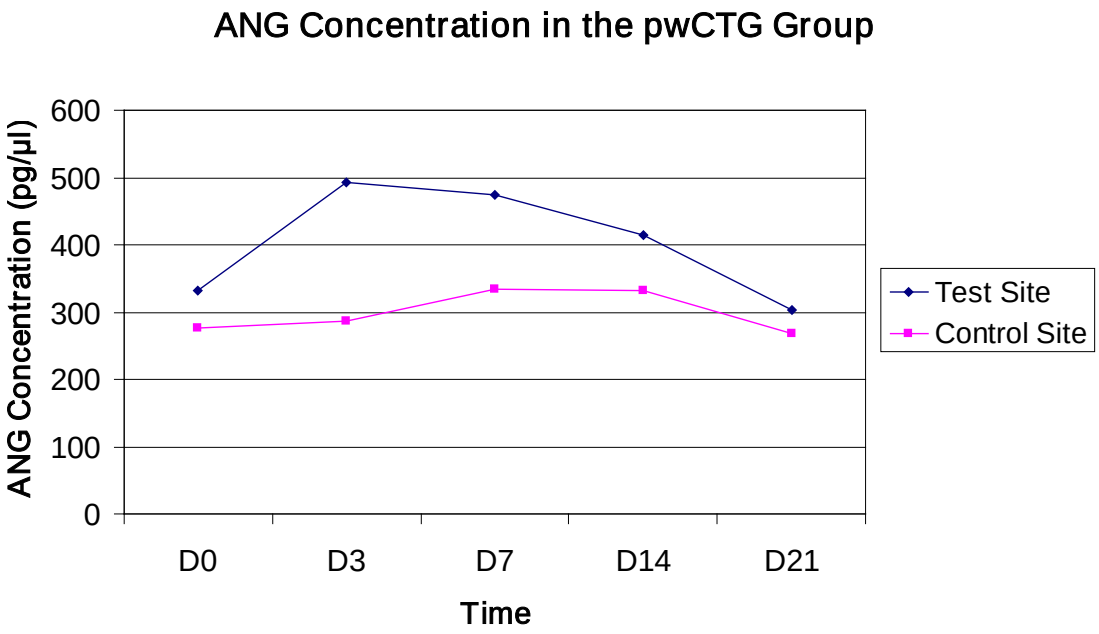


Figure 33. Average ANG Concentration in the pwCTG Group

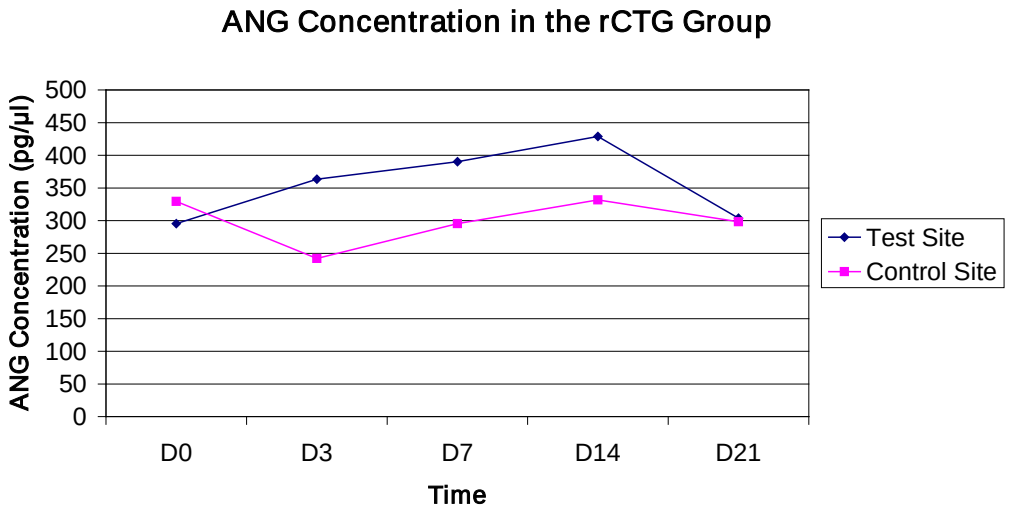


Figure 34. Average ANG Concentration in the rCTG Group

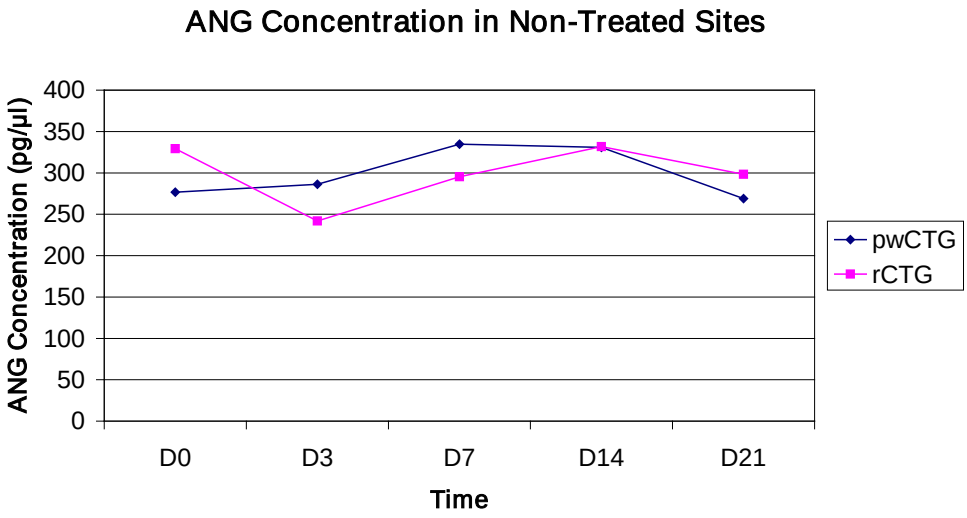


Figure 35. Average ANG Concentration in Non-Treated Sites

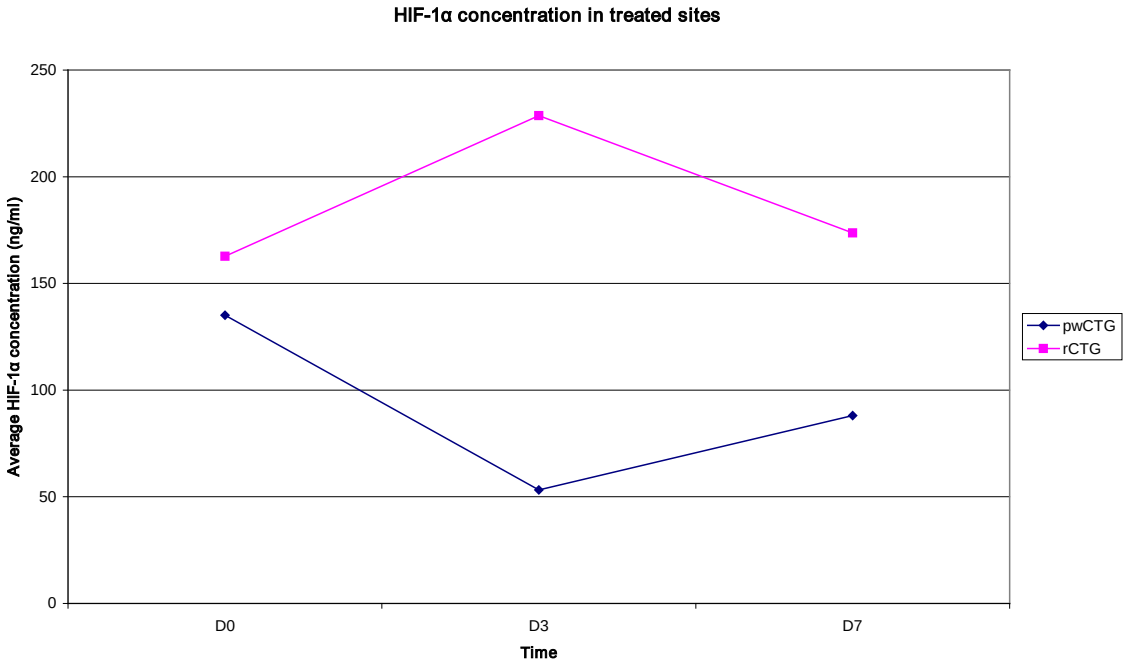


Figure 36. Average HIF-1α Concentration in Treated Sites

HIF-1α concentration (pg/ml)					
	rCTG		Test Site	162.73±122.51	228.62±30.43	173.63±90.46
			Control Site	167.98	107.32	66.24
	pwCTG		Test Site	135.10±99.90	53.19±35.00	88.11±67.95
			Control Site	292.77	77.70	66.20
		Day 0 PO	Day 3 PO	Day 7 PO		

Table 6. HIF-1α Concentration (mean±SD)

CHAPTER 5

DISCUSSION

The present study attempted to incorporate the pre-wounding technique in the CTG procedure. The results were evaluated in three different levels:

a) Subjects reports

b) Clinical outcomes

c) Biochemical characterization by means of GCF analysis

a) Subjects reports

Pain and discomfort are the main parameters traditionally investigated as subject based outcomes. Seven studies[49, 59, 70-74], two[49, 59] of which were completed in the Department of Periodontology, The Ohio State University, have evaluated patients' outcomes in terms of pain and discomfort. There are several reasons that perplex comparisons though. Differences in pain assessment methods, study design and surgical techniques could account for the discrepancies between studies.

Pain assessment methods include self-assessment scales and records of consumed analgesics. Both of these methods were used in the present study. Regarding self-assessment scales, the results of the present study are in agreement with the results of previous studies[49, 59, 70, 72, 74] that used the same pain assessment scales (i.e. a VAS scale with 10 divisions and a pain descriptive pain effect scale with 5 categories). Opposite to the present study, Wessel & Tatakis[49] did not find a statistically significant difference in the reported pain between post-operative week 1 and post-

operative week 3. There is no available data providing a correlation between different pain self-assessment scales. Therefore, the comparison of the findings of the present study with the findings of previous studies[71, 73] that used different pain assessment scales is not possible.

Regarding analgesics use, Tylenol 325mg was the primary analgesic prescribed in the present study. Addiotionally, Ibuprofen was used in case Tylenol was insufficient for pain control. Previous studies administered a variety of post-operative analgesics and analgesic regimens[49, 59, 70-74]. Therefore, a comparison of analgesics consumption in the present study with the analgesics consumtion in previous studies is usually not possible. The analgesics consumption was lower in the present study compared to Wessel & Tatakis[49].

There are several additional factors that do not allow a precise comparison of the results of the present study with the results of previous studies. Source of pain was not always clarified, i.e. palate or recipient site[71], or the recipient site was not monitored at all[70, 73]. Some studies recorded pain separately from discomfort[49] while others considered that pain and discomfort are the same[73]. Some studies included smokers[71] although there are indications that pain perception is altered in smokers[71]. With the exception of one study[71], all the other existing studies had a small study population which does not allow for the detection of existing small differences.

Regarding study design, the present study included two parallel groups and may have created another limitation. Differences in patient perceptions can influence the levels of reported postoperative pain[75]. Although the patients were provided with literal descriptions and a numerical scale to minimize the differences in cognitive and

comprehensive understanding, they were asked to grade complications according to their individual perceptions. This may have induced a bias to the study because the patients could have had different thresholds and standards.

In the present study the surgical procedures were performed by one highly experienced periodontist (D.N.T.) and the results showed a decrease in reported pain with time regardless of the surgical technique. In some of the previous studies, the procedures are performed by multiple surgeons[49, 70] possibly with a variety of skills and experience and this may have contributed to the lack of statistically significant differences regarding reported pain between post-operative week 1 and week 3[49]. Operator experience could affect the duration of surgery, and the duration of soft tissue grafting surgical procedures has been identified as the most important risk indicator for the development of moderate or severe postoperative pain[71] and analgesics consumption[71]. The speculation behind this is that lengthy surgical procedures may create extensive tissue injury, prolong vasodilation that permit more fluid to accumulate in the interstitial spaces, and result in higher levels of biologic mediators released by inflammatory and resident cells.

Regarding surgical design, there are several details that can affect the experienced pain. In the present study, the donor site was prepared with a two incision technique and the recipient site was prepared with a pouch technique. In previous studies, some used two incisions technique with no vertical incision for donor site[71,72], single incision technique for donor site[73], trap door technique for donor site[70, 73], free gingival graft harvest[70], Bruno technique for recipient site[49,71], Bruno technique with

the addition of a vertical incision[72], trapezoid split thickness flap for recipient site[70], Raetzke technique for recipient site[49].

Harvested from the palate grafts varied in thickness and surface. In the present study, the graft thickness was standardized at 1.0mm. In previous studies it was 1-2mm[71], 1.5mm[73] and 1.3mm[70] or non-specified[ref]. Regarding surface, there is only one study[73] that standardized this parameter. The number of sites that were treated with a single graft varies also. In one study[73] one site/patient was treated, in another one 2.6±1.4 teeth/patient[71] and in other cases isolated and multiple defects were included in the same study[72]. There is evidence that shallower and thinner grafts result in less PO analgesics consumption[70]. Similarly, the height of redrawal has been related to PO analgesics consumption[70]. Periosteum was taken with the graft in some studies[49, 71, 72] and not removed in others[70, 73]. A surgical dressing was used at the donor[71, 72] and recipient sites[71, 72] in some studies and this may have masked the actual discomfort. Subjects responses regarding pain were not collected in the same time intervals. In the present study questionnaires were completed at D3, D7, D14, D21 PO. In previous studies questionnaires were completed daily for the 1st PO week[72], at 1 week PO only[70, 71], at 1 and 3 weeks PO[49], at D3 PO and at 1, 2 & 3 weeks PO[59], and at 1, 2, 3, 4, 6 and 8 weeks PO[73]. The importance of the above numerous details is that only a rough comparison between studies is at best possible and any existing smaller differences cannot be detected.

Study	RC (%)	CRC (% cases)
Tsolaki & Tatakis	89.8±13.1 85.6±14.9	50
Raetzke[76]	80	42
Nelson[77]	91	
Allen[78]	84	61
Harris[79]	97.9±7.6 97.9±6.8	
Bouchard[80]	69.2	
Borghetti[81]	70.5	
Paolantonio[82]	85.23±17.86	48.57
Cordioli[83]	89.6±15	
Da Silva[84]	75	

Table 7. CTG Clinical Studies Outcomes

b) Clinical outcomes

RC is the main clinical outcome traditionally studied following a CTG. As already mentioned, 6 months after the surgical procedures, the present study found 89.8±13.1% RC for the pwCTG group and 85.6±14.9% RC for the rCTG group. 50% of pwCTG subjects had 100% RC. 44.4% of the rCTG subjects had 100% RC. At a first glance, these clinical outcomes are in very good agreement with most of the previous clinical studies on CTG. (Table 7)

Raetzke et al.[76] reported 80% average RC and CRC in 42% of the cases. The baseline RD in this study was 3.29mm (2.0-5.0mm range). The baseline RW was 3.63mm (1.5-6.0mm). The baseline RD in our study was 4.2±1.1mm for the pwCTG and 3.8±1.0mm for the rCTG. The baseline RW in our study was 3.9±0.9mm for the pwCTG group and 4.2±0.8mm for the rCTG group. Raetzke et al.[76] is the only other study besides the present one that measured ABC, although only pre-operatively. The

average ABC was 2.5 and never more than 3.5mm (from the gingival margin)[76]. Given the fact that PD was usually 1mm, and if the measurements of ABC were accurate, the cases treated in this study did not have any bone dehiscence. In the present study, there was no statistically significant difference between the ABC mesurements before and after flap elevation at D0. There was a statistically significant difference, though, between D0 and D180 PO measurements. There is no evidence that periodontal regeneration predictably occurs after a CTG procedure. Therefore, the only explanation is that the presence of the connective tissue graft caused an error in PO ABC measurement.

Nelson et al.[77] reported regarding the use of subpedicle connective tissue grafts 100% RC in cases with baseline RD 1-3mm, 92% RC in cases with baseline RD 4-6mm, 88% RC in cases with baseline RD 7-10mm. The average RC for all cases was 91%. The follow up period was 6-42 months and few teeth per category were treated.

Allen et al.[78] reported CRC in 61% of the cases and the average RC was 84%. CRC was achieved in 83% of the 1-3mm baseline RD; 50% of the 3mm baseline RD cases (n=4); average RC was 85.5% in the 3mm baseline RC with a CRC in 40% of the cases with 4-5mm baseline RD; average RC when baseline RD was 4-5mm was 73%. Based on pre-op RW, the RC was 95% in the 2mm baseline RW, 87% in the 3mm baseline RW, 76% in the 4mm baseline RW cases.

Harris[79] after treating 100 consecutive cases reported 89% of the cases achieved CRC. The mean baseline RD was 3.3$\pm$0.9mm (range 2-7mm), the mean baseline RW was 3.5$\pm$1.1mm (range 2-9mm). More specifically in the group with baseline RW 3-4.5mm (mean 3.5mm SD 0.6) the mean RC was 97.6$\pm$7.6 % and in the group >5mm

baseline RW the mean RC was 95±10%. In the group with baseline RD 3-4.5mm (mean 3.4mm SD 1) the mean RC was 97.9±7.6 % and in the group >5mm mean RC was 97.9±6.8%. The follow up ranged from 8 to 72 weeks.

Bouchard et al.[80] reported 69.2% RC. The baseline RD was 4.2/4.53mm (2 groups). The follow up period was 6 months.

Borghetti et al.[81] reported 70.5% RC when baseline RD was 3.66±0.16 mm, RW 3.26±0.26mm with a 1 yr follow up. One of the factors that may have contributed to the relatively low RC is that Miller III cases were also included. Paoloantonio et al.[82], in a study on subpedicle CTG and a 5 year follow up, demonstrated 85.23±17.86% RC with 48.57% CRC after treating cases with baseline RD 3.43±0.39mm. Da Silva et al.[84] in a 6 month study demonstrated 75% RC after treating cases with baseline RD 4.20±0.78mm and the use of a trapezoidal flap.

When Cordioli et al.[83] compared the clinical results of CTG with the envelope versus the coronally positioned flap combined with CTG, they found mean RC 89.6±15% for the envelope technique and 94.7±11.4% for the CTG - coronally positioned flap combination. Baseline RD was 3.5±1.1mm and 3.6±1.2mm in the two groups. Follow up was 1 to 1.5 years.

Root coverage in all the previously mentioned studies, including the present one, was assessed in relation to the identified CEJ. How acurate, though, is the identification of the CEJ? How realistic is the anticipation of complete root coverage? How does this aspect of our methodology affect clinical outcomes evaluation?

According to Zucchelli et al.[85], who examined 900 teeth with gingival recession (360 patients), the CEJ was completely detectable in 30% and partially recognizable in

25% of the selected cases. In the great majority (>90%) of these teeth, cervical abrasions were associated with the recession of the soft tissue margin. In many cases of gingival recessions associated with cervical abrasion, a line separating the enamel from the coronal dentin (exposed due to the abrasion defect) does appear, and this is frequently confused with the anatomic CEJ. This error in the localization of the CEJ leads to other measurement mistakes, obviously making the desired root coverage unobtainable. The CEJ line has a curved, convex outline, more or less scalloped, according to the patient's biotype. In the great majority of cases, the abrasion lines are flat. However, the differential diagnosis between abrasion line and anatomic CEJ is sometimes difficult in posterior teeth, which are characterized by a flatter outline of the CEJ even in a thin and scalloped patient's biotype. In the present study, teeth that did not have detectable CEJ were excluded but we cannot exclude the posibility that the ones included were actually affected by some degree of cervical abrasion. Additionally, there are some local conditions at the tooth with the recession defect that may limit root coverage even in the absence of interdental attachment and bone loss:

- loss of the interdental papilla(e) height
- tooth rotation
- tooth extrusion
- occlusal abrasion

These situations impair complete root coverage in cases that are not surgical failures. More specifically, during mucogingival surgery, a loss of papilla height decreases the potential advancement of the coronal flap and reduces the vascular exchanges

between the root covering soft tissues and the interdental connective tissue. As a result, coverage up to the CEJ cannot be achieved in a tooth with gingival recession and with no loss of interdental attachment and bone but with some papilla(e) loss. Given the protagonistic role of papilla and underlying interproximal bone in CTG clinical outcomes, any other condition that affects the dimensions of papilla results in affecting the clinical outcomes of CTG procedures. For example, in a rotated tooth or a tooth with occlusal abrasion the topographic relationship between the CEJ and the interdental papillae changes. In cases of tooth rotation at one tooth side the CEJ gets closer to the tip of the papilla, whereas at the other side, it gets further.

Studies[85] have shown that the consideration of clinical CEJ, as described by Zucchelli, instead of the anatomical CEJ would help us to determine in a more objective manner the maximum root coverage that can be achieved in each specific site irrespective of the surgical technique.

c) Biochemical characterization by means of GCF analysis

The expresion of severeal wound healing proteins can be investigated as an outcome measurement of surgical trauma and/or healing phase. ANG and HIF-1 α were chosen for the current study. The present study reported a maximum of ANG concentration at D3 PO for the pwCTG group. This is in agreement with previous studies[49, 59]. The present study showed a maximum of ANG levels at D14 for the rCTG group, which is not in agreement with previous studies[49, 59]. There was a trend for higher ANG levels in the pwCTG group in comparison to the rCTG group. However, the results were not statistically significant. Bleeding was more frequent during GCF samples collection at D3 PO for the test sites of pwCTG subjects in comparison with test sites

of rCTG subjects. Therefore, there were seven D3 PO GCF samples available for analysis from the pwCTG group and only four D3 PO GCF samples available from analysis from the rCTG group. More GCF samples from the rCTG group are required to allow for a better statistical evaluation of the ANG results.

Regarding HIF-1α, the present study is the first one to evaluate HIF-1α concentration during CTG healing. According to the results of the present study, HIF-1α levels reach their mimium at D3 PO for the treated sites of pwCTG subjects and their minimum at D3 PO for the treated sites of rCTG subjects. However, statistical significance was not reached. Therefore, despite the existing trends, the biochemical findings of the present study cannot support a faster healing response in the pre-wounded subjects.

Surgical delay technique

There is significant evidence that the surgical delay technique contributes to reduced flap necrosis or fat necrosis in transverse rectus abdominis musculocutaneous (TRAM) flap breast reconstruction, especially in high risk populations (obesity, history of cigarette smoking, radiation therapy, or abdominal scar) [86, 87, 88]. However, the dimensions of the surgical sites and the blood vessels in these cases are many times greater than the CTG sites in the oral cavity. This may have an effect on what level of pre-wounding in the oral cavity will lead to an improved clinical outcome.

The optimum time course from the pre-wounding to flap elevation, as it relates to survival, has not been well studied--specifically whether the potential benefit of a surgical delay lessens at any particular time after the pre-wounding procedure. In general plastic surgery, there are clinical studies where the surgical delay lasted for 1

week[89-91], 2 weeks[89], 13.9 days on average[88], 3.6 months on average[92]. The benefit of delay in an animal model was maintained even up to 7 months[93]. Once again, the present study is the first one to test pre-wounding for a CTG treatment in the oral cavity. Therefore, there is no data on the ideal delay interval. There is data however, showing that oral mucosa heals faster than skin[94]. Based on this fact, it may be speculated that the surgical delay intervals in the oral cavity should probably be kept shorter than in the skin.

The optimum surgical delay is not precisely determined either. The surgical delay procedure consisted of a combination of contralateral rectus perforator ligation and ipsilateral dominant pedicle ligation achieved with two minimal skin incisions and no significant flap undermining[86], bilateral deep inferior epigastric and superficial inferior epigastric artery and vein ligation[89], ligature of both deep inferior epigastric arteries and veins[88], selective embolization of the deep inferior epigastric arteries[95] by an angiographic procedure[92], skin delay only[90], an extended skin island delay that essentially divides the unipedicle TRAM flap into two stages[91], and acute ischemic preconditioning[96]. Data from human trials and animal studies demonstrated that arterial division is critical for TRAM flap delay and that arbitrary venous interruption is unnecessary[94, 97]. There is evidence from animal studies that the combination of surgical delay with an intramuscular injection of human vascular endothelial growth factor (hVEGF) can furthermore enhance angiogenesis and flap survival[98].

In conclusion, according to existing research, the level of induced ischemia that is necessary to cause an improved treatment outcome in general plastic surgery is

determined empirically. Of course, this experience does not exist yet regarding a surgical procedure in the oral cavity. Further research is necessary to determine the most efficient surgical delay approach for the oral mucosa.

CHAPTER 6

CONCLUSION

The present study did not prove that a CTG with the pre-wounding of the palate results in better clinical outcomes than a routine CTG for single deep gingival recession defects. There was a tendency for better results with the tested technique; however, statistical significance was not reached. Similarly, GCF biochemical markers, ANG and HIF-1α did not show a statistically signifficant difference in the two treatment groups. Further research is necessary to expose the full capacity of ischemic preconditioning in oral plastic surgery, especially in subpopulations with gingival recession of greater severity and/or with a compromised healing potential. According to the patients' reported experience, there are no adverse effects from the application of the pre-wounding technique. Therefore, should future research show better clinical outcomes, the pre-wounding technique seems to be acceptable by the patients.

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