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Treatment of Multiple Gingival Recessions, Class I, with Coronal Advance Flap (CAF) with or without Leucocytes -Platelet rich fibrin (L-PRF). - Randomized Controlled Trial

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TREATMENT OF MULTIPLE GINGIVAL RECESSIONS, CLASS I, WITH CORONAL ADVANCE FLAP (CAF) WITH OR WITHOUT LEUCOCYTES –PLATELET RICH FIBRIN (L-PRF): RANDOMIZED CONTROLLED TRIAL

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TABLE OF CONTENTS

ACKNOWLEDGMENTS ..... **I**
TABLE OF CONTENTS ..... **II**
RESUMO ..... **V**
1.1 INTRODUCÍON V
Antecedentes v
Tratamento das recesións v
L-PRF vi
Tecnoloxía dixital e avaliación tridimensional vi
Hipóteses do estudo vi
1.2 MÉTODOS VII
Deseño do estudo vii
Aleatorización, cegamento e calibración vii
Consideracións éticas vii
Criterios de selección dos participantes vii
Procedementos cirúrxicos viii
Medidas de resultado ix
Análise estatística ix
1.3 RESULTADOS X
Cobertura radicular x
Anchura do tecido queratinizado (ktw) x
Grosor xenxival e volume x
Resultados informados polos pacientes (pros) x
1.4 DISCUSIÓN XI
Relevancia clínica xi
Limitacións e futuros estudos xii
1.5 CONCLUSIÓN XII
ABSTRACT ..... **XIII**
LIST OF FIGURES ..... **XV**
LIST OF TABLES ..... **XVI**
LIST OF PUBLICATIONS ..... **XVIII**
1.1 CONTEXTUALIZATION (INTRODUCTION/BACKGROUND) XVIII
1.2 METHODOLOGY (DIGITAL MEASUREMENTS) XVIII
LIST OF ABBREVIATIONS ..... **XIX**
1.1 ABBREVIATION OF OUTCOMES VARIABLES IN STUDY XIX
1.2 ALL ABBREVIATIONS XIX
1 INTRODUCTION/BACKGROUND **1**
1.1 CLINICAL RELEVANCE AND SCIENTIFIC RATIONAL FOR THE STUDY 1
1.2 GINGIVAL RECESSIONS 1
1.2.1 Definition 1
1.2.2 Etiology 1
1.2.3 Prevalence 2
1.3 CLASSIFICATION OF DENTAL RECESSIONS 2
1.3.1 Classification of Recessions: Simple and Multiple Recessions 2
1.3.2 Miller Classification of Dental Recessions 2
1.3.3 2017 EFP/AAP Classification of Dental Recessions 3
1.5 TREATMENT OF GINGIVAL RECESSIONS 5
1.5.1 Coronally Advanced Flap (CAF) for multiple recessions 6
1.6 PLATELETS CONCENTRATES AND LEUCOCYTE PLATELET RICH FIBRIN (L-PRF) 7
1.6.1 Concept and Definition of Platelet Concentrates and L-PRF 7
1.6.2 Mechanism of action of Platelet Concentrates and L-PRF 8
1.7 USES OF PLATELET CONCENTRATES AND L-PRF IN DENTISTRY 9
1.8 USE OF PRF IN THE TREATMENT OF GINGIVAL RECESSIONS 10
1.8.1 Rationale for combining coronally advanced flap (CAF) with leukocyte- and platelet-rich fibrin (L-PRF) 10

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

1.8.2 Use of L-PRF in the treatment of multiple gingival recessions 12
1.9 LIMITATIONS OF L-PRF 12
2 OBJECTIVES 14
2.1 OVERVIEW 14
2.1.1 Hypotheses 14
3 MATERIALS AND METHODS 16
3.1 STUDY DESIGN AND CENTER 16
3.1.1 Study Design 16
3.1.2 Centers 16
3.1.3 Clinical trial registration 18
3.1.4 Safety, Beneficial and adverse effects 18
3.1.5 Sources of funding 19
3.2 POPULATION 19
3.2.1 Informed consent 19
3.2.2 Selection Criteria 19
3.2.3 Sample size Analysis 21
3.3 GROUPS OF THE STUDY AND RANDOMIZATION 22
3.3.1 Groups 22
3.3.2 Randomization of Participants 22
3.3.3 Randomization implementation and concealment mechanism 23
3.4 BLINDING AND CALIBRATION 23
3.4.1 Blinding 23
3.5 INTERVENTION 25
3.5.1 Description 25
3.5.2 Similarity of interventions 31
3.5.3 Post-operative monitorization 31
3.6 VARIABLES 32
3.6.1 Percentage (%) of root coverage (%RC) at six months (PRIMARY OUTCOME) 32
3.6.2 Complete and mean root coverage at six months 33
3.6.3 Keratinized tissue width gain (ΔKTW) 33
3.6.4 Thickness gain (Δ GT) 34
3.6.5 Analyze of pain and healing 35
3.6.6 Pain, indirect evaluation 36
3.6.7 (PRO and PROMS) Subjective evaluation of results by the patient 37
3.6.8 Data collection 38
3.6.9 Digital assessment Protocols 45
3.7 TEMPORAL MAP 52
3.7.1 Study Time map 52
3.7.2 Data Collection 52
3.8 STATISTICS 54
4 RESULTS AND DISCUSSION 55
4.1 DEMOGRAPHIC AND CLINICAL CHARACTERIZATION OF THE TOTAL SAMPLE 55
4.2 SAMPLE CHARACTERIZATION RELATED TO GROUP DISTRIBUTION 58
4.2.1 Patient- centered sample characterization 59
4.2.2 Site-specific Sample characterization 60
4.2.3 Sample Characterization Conclusions 61
4.3 ROOT COVERAGE RESULTS 63
4.3.1 Primary outcome % root coverage length 63
4.4 SECONDARY OUTCOMES RESULTS 66
4.4.1 Complete root coverage (CRC) 66
4.4.2 Mean root coverage (MRC) 66
4.4.3 Results in terms of keratinized tissue width gain (ΔKTW) 67
4.4.4 Efficacy of results (time/results) 68
4.4.5 Thickness/Volume Results 69
4.4.6 Results in terms of Healing (IPR scale) 73
4.4.7 Results in terms of indirect measurement of pain 74
4.4.8 Results in terms of PROMS 76
4.5 KEY FINDINGS FROM THE STATISTICAL RESULTS 81

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

4.5.2 Summary Table and graphic 83
4.6 DISCUSSION 85
4.6.1 Methods and Overview 85
4.6.2 Root coverage 85
4.6.3 KTW increase 87
4.6.4 Volume increasing 89
4.6.5 Healing and Pain 91
4.6.6 PROs 93
4.6.7 Technical considerations from a surgeon perspective 93
4.6.8 Exploratory data analysis 94
4.6.9 Overall discussion and Study limitations 95
4.6.10 Clinical Implications and Future Research 97
5 CONCLUSIONS 98
5.1 CONCLUSION FOR THE PRIMARY OBJECTIVE (% ROOT COVERAGE LENGTH) 98
5.2 COMPLETE ROOT COVERAGE (CRC) AND MEAN ROOT COVERAGE (MRC) 98
5.3 KERATINIZED TISSUE WIDTH (KTW) GAIN AND THICKNESS GAIN (ΔGT) 98
5.4 HEALING AND INDIRECT MEASUREMENT OF PAIN 98
5.5 PATIENT-REPORTED OUTCOMES S (PROS) 99
BIBLIOGRAPHY 100
APPENDICES 115
APPENDIX I: CONSORT FLOW DIAGRAM 115
APPENDIX II: SELECTION DATA FORM 116
APPENDIX III: PROMS EVALUATION FORM 118
APPENDIX IV: INFORMED CONSENT 121
APPENDIX V: POST-OPERATIVE CARE (PORTUGUESE) 125
APPENDIX VI: DECLARATION OF APPROVAL BY THE RESEARCH ETHICS COMMITTEE 127
APPENDIX VII: CONFLICT OF INTEREST STATEMENT 130
APPENDIX VIII: RIGHTS 131
APPENDIX IX: PERMISSIONS 131

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

RESUMO

Título: Tratamento das Recesións Xenxivais de Clase I de Miller mediante CAF con ou sen Fibrina Rica en Plaquetas: Un Ensaio Clínico Controlado Aleatorizado
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1.1 INTRODUCÍON

ANTECEDENTES

As recesións xenxivais caracterízanse pola migración apical da enxiva, que provoca a exposición da superficie radicular do dente. Este problema non só afecta á estética dos pacientes, senón que tamén pode derivar en hipersensibilidade dentinaria e un maior risco de caries radiculares. As recesións xenxivais poden darse en pacientes de diferentes idades e niveis de hixiene oral, e a súa prevalencia foi aumentando nos últimos anos debido a unha maior preocupación pola saúde e estética dental, así como ao incremento da lonxevidade.

O tratamento das recesións xenxivais ten como obxectivo restaurar a posición da enxiva, cubrindo a superficie radicular exposta, mellorando a estética, reducindo a hipersensibilidade e previndo as caries radiculares. Existen varias técnicas cirúrxicas dispoñibles para o tratamento destas recesións, sendo o retallo avanzado coronal (CAF: Coronal Advanced Flap) unha das técnicas máis utilizadas e consideradas como o estándar de ouro. O CAF consiste na creación dun retallo mucoperióstico que se despraza coronalmente sobre a superficie radicular para cubrir a recesión.

TRATAMENTO DAS RECESIÓNS

O CAF é o tratamento cirúrxico máis común para as recesións xenxivais múltiples, principalmente nas de Clase I de Miller. Nesta técnica, o retallo créase para liberar o tecido xinxival e despois desprazalo coronalmente para cubrir a superficie exposta. Aínda que o CAF é altamente eficaz, nalgúns casos, propúxose a inclusión de enxertos de tecido para mellorar os resultados clínicos. Porén, esta opción ten desvantaxes, como a necesidade de obter tecido dun segundo sitio cirúrxico, o que pode aumentar a morbilidade, ademais de dificultar a integración estética dos tecidos enxertados.

Como alternativa ao uso de enxertos, moitos estudos centraron a súa atención nos biomateriais adxuntos que poderían promover a rexeneración dos tecidos e mellorar os resultados cirúrxicos. Un destes materiais é a fibrina rica en plaquetas (PRF: Platelet Rich Factor), un biomaterial autólogo derivado do sangue do paciente que contén factores de crecemento que promoven a anxioxénese, a proliferación celular e a formación de matriz extracelular. A PRF é unha opción prometedora que podería mellorar a cicatrización e acelerar

a rexeneración do tecido, ofrecendo así un enfoque menos invasivo en comparación cos enxertos tradicionais.

L-PRF

A fibrina rica en leucocitos e plaquetas (L-PRF) é unha forma avanzada de PRF que inclúe leucocitos no composto. Obtida a partir do sangue do propio paciente mediante centrifugación, a L-PRF ten a vantaxe de non requirir anticoagulantes nin axentes xelificantes para a súa produción. Esta matriz de fibrina actúa como un soporte natural que libera de forma progresiva factores de crecemento, os cales poden mellorar a cicatrización e a calidade do tecido rexenerado.

Aínda que o uso de L-PRF en diversas áreas da medicina dental demostrou ser eficaz, a súa eficacia adicional cando se combina coa técnica de CAF para o tratamento de recesións xenxivais aínda está a ser debatida. Algúns estudos suxiren que a combinación de L-PRF co CAF mellora a cobertura radicular e a calidade do tecido xenxival, mentres que outros non atopan diferenzas significativas en comparación co uso do CAF por si só.

TECNOLOXÍA DIXITAL E AVALIACIÓN TRIDIMENSIONAL

Coa evolución das tecnoloxías dixitais, tamén se facilitaron os avances na avaliación dos resultados clínicos. As ferramentas dixitais permiten realizar medicións tridimensionais de alta precisión e avaliar o volume e o grosor dos tecidos rexenerados. Este tipo de avaliación proporciona unha visión máis detallada dos efectos das técnicas cirúrxicas e dos biomateriais utilizados, o que pode axudar a mellorar os protocolos de tratamento e adaptar as intervencións ás necesidades individuais dos pacientes.

HIPÓTESES DO ESTUDO

O obxectivo principal deste estudo é comparar a eficacia da técnica de retallo avanzado coronal (CAF) por si soa e a combinación de CAF con L-PRF no tratamento de recesións xenxivais de Clase I de Miller. Para iso, formúlanse as seguintes hipóteses:

HIPÓTESE NULA:

a) A adición de L-PRF á técnica de CAF non resultará nunha maior cobertura radicular en termos de porcentaxe de cobertura radicular (%CR) en comparación co CAF só, aos seis meses despois da cirurxía.

HIPÓTESES ALTERNATIVAS:

a) A combinación de CAF con L-PRF producirá unha maior cobertura radicular en comparación co CAF só, xa sexa en termos de cobertura radicular media (MRC) ou cobertura radicular completa (CRC).
b) O uso de L-PRF mellorará o grosor xenxival e a calidade do tecido en comparación co CAF só.
c) A cicatrización será máis rápida e haberá menos complicacións no grupo de CAF con L-PRF en comparación co grupo de CAF só.

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d) Os pacientes que se sometan a CAF con L-PRF reportarán un maior nivel de satisfacción cos resultados do tratamento en comparación con aqueles que se sometan a CAF só.

1.2 MÉTODOS

DESEÑO DO ESTUDO

O ensaio clínico controlado e aleatorizado (RCT) foi deseñado cunha estrutura de dous brazos e un deseño paralelo. Desenvolveuse na Clínica Universitaria da Universidade Católica de Viseu, en Portugal, desde xaneiro de 2019 ata xaneiro de 2024. Participaron 19 pacientes (14 mulleres e 5 homes), con idades comprendidas entre os 22 e os 44 anos, cun total de 70 recesións xenxivais de Clase I de Miller. Os pacientes foron asignados aleatoriamente a dous grupos: o grupo de proba (CAF + L-PRF, con 42 sitios) e o grupo de control (CAF só, con 28 sitios).A análise estatística da mostra non indicou diferenzas estatisticamente significativas entre os grupos de control e de proba. Isto suxire que o proceso de aleatorización foi efectivo á hora de crear grupos comparables para o estudo. En consecuencia, calquera diferenza observada nos resultados pode atribuírse ás intervencións probadas, e non a diferenzas nas características iniciais dos participantes

ALEATORIZACIÓN, CEGAMENTO E CALIBRACIÓN

A aleatorización realizouse empregando o sitio web randomization.com mediante o método de bloques permutados aleatoriamente. Para aumentar a validez do estudo, implementouse cegamento para o examinador, quen non tiña coñecemento dos grupos aos que se asignaron os pacientes. Non obstante, o cegamento completo non foi posible para os pacientes e o cirurxián, xa que a extracción de sangue necesaria no grupo de proba facía evidente a asignación do tratamento. Realizouse un exercicio de calibración para garantir que as medicións realizadas polo examinador fosen reproducibles e consistentes ao longo do estudo.

CONSIDERACIÓNS ÉTICAS

O estudo realizouse de acordo cos estándares éticos internacionais, seguindo as recomendacións da Declaración de Helsinki de 1964 e as súas emendas posteriores. Todos os procedementos foron revisados e aprobados pola Comisión de Ética para a Saúde da Universidade Católica Portuguesa (CES-UCP). Todos os participantes proporcionaron o seu consentimento informado por escrito antes de ser incluídos no estudo. A protección dos datos persoais dos pacientes seguiu a normativa vixente en Portugal e España sobre protección de datos persoais, e os pacientes tiveron dereito a acceder, modificar ou eliminar os seus datos segundo o estipulado pola lei.

O ensaio clínico foi rexistrado no portal ClinicalTrials.gov co identificador NCT0659115.

CRITERIOS DE SELECCIÓN DOS PARTICIPANTES

Os pacientes seleccionáronse da cordo aos seguintes criterios de inclusión:

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CRITERIOS DE INCLUSIÓN

1. Persoas maiores de 18 anos e non fumadoras (segundo autoinforme)
2. Presenza de dúas ou máis recesións xenxivais Clase I de Miller no maxilar superior anterior (incisivos centrais e laterais, caninos, primeiros e segundos premolares).
3. Cantidade mínima de tecido queratinizado apical ás recesións superior a 1,5 mm.
4. Boa hixiene oral, con índices de control de placa de O'Leary e sangrado ao sondar (BOP) inferiores ao 25%.

CRITERIOS DE EXCLUSIÓN

1. Presencia de patoloxías médicas que poidan interferir co proceso de cicatrización, como deficiencias proteicas e vitamínicas, radiación terapéutica, trastornos metabólicos (como a diabetes ou a hipercalcemia), trastornos farmacolóxicos (inmunosupresores) ou alerxias aos compoñentes dos tratamentos.
2. Embarazo o lactancia.
3. Profundidade de sondaxe superior a 3 mm ou nivel de fixación interproximal (CAL) > 0.
4. Presencia de lesións cariosas, trauma oclusal ou procedementos previos de cobertura radicular.
5. Presencia de coroas protéticas nos dentes experimentais.

A inclusión só do maxilar superior anterior débese a que a prevalencia de recesións nesa área é alta e ten un maior impacto na estética dos pacientes, o cal é, se non a máis importante, unha das principais razóns para o tratamento. A opción de incluír unicamente a Clase I de Miller baséase en que, segundo a literatura dos autores da técnica, a técnica cirúrxica de retallo avanzado coronal (sen enxerto) está indicada para a cobertura radicular en pacientes cando a altura do tecido queratinizado apical á exposición radicular é superior a 2 mm. Nestes casos, esta técnica pode proporcionar os mellores resultados estéticos en pacientes con altas expectativas estéticas. Así, estamos comparando o grupo de proba co tratamento estándar de referencia segundo os autores da técnica.

PROCEDEMENTOS CIRÚRXICOS

En ambos grupos, administrouse anestesia local e aplicouse a técnica de retallo avanzado coronal (CAF) modificada para recesións múltiples, segundo a descrición de De Sanctis e Zuchelli. No grupo de proba, despois de elevar o retallo, colocáronse entre 4 e 6 membranas de L-PRF sobre a superficie radicular exposta antes de avanzar o retallo coronariamente. As membranas de L-PRF producíronse mediante centrifugación (Intraspin™, Intra-lock®, Florida, USA) 2700 RPM durante 12 minutos, empregando tubos plásticos de 9 mL (Intra-Lock) para crear unha matriz de fibrina rica en leucocitos e plaquetas (protocolo de Choukroun). No grupo de control, realizouse só o procedemento de CAF sen a adición de L-PRF. Cada paciente recibiu un documento detallado con instrucións postoperatorias despois da cirurxía. Os pacientes foron monitorizados aos 5 días, 14 días, 6 semanas, 3 meses e 6 meses.

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MEDIDAS DE RESULTADO

O **resultado primario** foi a porcentaxe de cobertura radicular (%RC) aos seis meses. Entre os **resultados secundarios**, incluíronse a porcentaxe de cobertura radicular (%RC) en profundidade e área, a frecuencia de cobertura radicular completa (CRC ou %CRC), a cobertura radicular media (MRC) expresada en milímetros, e a área de recesión cuberta ($ROI_{(2)}$ AREA). Ademais, medíronse a ganancia máxima de grosor xenxival (MAXΔGT) e o grosor medio do tecido sobre a raíz exposta (MEANΔGT) na rexión de interese 1 ($ROI_{(1)}$). Estes mesmos parámetros tamén se rexistraron na rexión de interese 2 ($ROI_{(2)}$) como $MAX\Delta GT_{(R)}$ e $MEAN\Delta GT_{(R)}$, respectivamente.

Finalmente, as medidas de volume inclúen a ganancia de volume (ΔVOL) na rexión de interese 2 ($ROI_{(2)}$), expresada en milímetros cúbicos, e a ganancia en anchura do tecido queratinizado (ΔKTW) entre o momento inicial (T_0) e final (T_F), expresada en milímetros.

Para a avaliación da cicatrización utilizouse unha escala de cicatrización de feridas (*IPR scale*), que permite avaliar de forma diferenciada as distintas fases da cicatrización (inflamatoria, proliferativa e de remodelación). Esta escala inclúe un cuestionario sobre a dor mediante unha escala visual analóxica (*VAS*).

A dor postoperatoria avaliouse baseándose no consumo medio de analxésicos (Ibuprofeno), expresado en miligramos. Segundo o protocolo, administráronse 600 mg ao inicio do procedemento cirúrxico. As doses seguintes tomáronse unicamente se fose necesario para controlar a dor. O número de pastillas consumidas foi rexistrado polos pacientes na súa casa e informado durante as visitas de seguimento aos 5 e 14 días.

Os resultados centrados no paciente foron avaliados mediante un cuestionario no que os participantes puntuaron os seus resultados sobre 10 (0 indicando moi malos resultados, 5 medios e 10 excelentes) nunha escala *VAS*, en termos de coincidencia de cor, cantidade de cobertura radicular, satisfacción xeral, satisfacción relacionada coa hipersensibilidade dentinaria cervical e disposición para repetir o tratamento

ANÁLISE ESTATÍSTICA

Unha vez recollidos os datos, estes foron codificados e introducidos nunha base de datos utilizando o programa IBM SPSS versión 26.0 (IBM, Armonk, New York, United States). A análise estatística incluíu unha análise descritiva, con distribucións de frecuencia para datos cualitativos e medidas de tendencia central (media, mediana) e dispersión (desviación estándar) para datos cuantitativos. Para a análise inferencial, empregouse a ANOVA mixta para comparar os resultados entre os grupos de proba e control ao longo do tempo. Ademais, realizáronse probas non paramétricas, incluíndo o test de Mann-Whitney para comparar dous grupos independentes e o test de Kruskal-Wallis para tres ou máis grupos independentes. O nivel de significación estatística establecérase en 5%.

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1.3 RESULTADOS

COBERTURA RADICULAR

A porcentaxe de cobertura radicular (%CR) aos seis meses foi do 89,30% ± 20,33% no grupo de proba e do 81,60% ± 27,93% no grupo de control. Non se atoparon diferenzas estatisticamente significativas entre os dous grupos (p=0,168). A cobertura radicular media (MRC) tamén foi similar entre ambos grupos, cun valor de 1,01 ± 0,59 mm no grupo de proba e 0,92 ± 0,68 mm no grupo de control (p=0,260).

ANCHURA DO TECIDO QUERATINIZADO (KTW)

En ambos grupos observouse un lixeiro aumento da anchura do tecido queratinizado. No grupo de proba, o KTW aumentou de 2,75 ± 0,84 mm a 3,03 ± 0,91 mm, mentres que no grupo de control o aumento foi de 3,00 ± 1,01 mm a 3,06 ± 0,86 mm. A pesar de que o aumento foi significativo ao longo do tempo (p=0,042), non se observaron diferenzas significativas entre os grupos (p=0,474).

GROSOR XENXIVAL E VOLUME

O grupo de proba mostrou un maior incremento no grosor xenxival e no volume en comparación co grupo de control. A ganancia de volume foi de 1,13 ± 1,25 mm^3 no grupo de proba fronte a 0,86 ± 0,84 mm^3 no grupo de control, pero esta diferenza non foi estatisticamente significativa (p=0,415).

RESULTADOS INFORMADOS POLOS PACIENTES (PROS)

Os pacientes en ambos grupos reportaron un nivel semellante de satisfacción cos resultados do tratamento. O consumo de analxésicos foi lixeiramente inferior no grupo de proba (1,70 ± 1,25 pastillas) en comparación co grupo de control (3,11 ± 2,57 pastillas), pero a diferenza non foi significativa (p=0,243).

Táboa I - Táboa de resultados
(Dados recompilados e analizados polo autor, 2024)

PARÁMETRO	GRUPO DE PROVA(CAF+L-PRF)	GRUPO DE CONTROL (CAF)	P-VALOR	UNIDADE
NÚMERO DE SITIOS	42	28		
COBERTURA RADICULAR MEDIA (%RC)	89.30% ± 20.33%	81.60% ± 27.93%	0.168	(%)
COBERTURA RADICULAR MEDIA (MRC, MM)	1.01 ± 0.59	0.92 ± 0.68	0.260	mm
FRECUENCIA DE COBERTURA RADICULAR 100% (CRC)	73.81%	57.14%	0.193	(%)
GAÑANCIA MEDIA DE KTW (ΔKTW, MM)	0.29 ± 0.74	0.06 ± 0.58	0.307	mm
GAÑANCIA MEDIA DE ESPESOR XENXIVAL (ROI 1)	0.16 ± 0.11	0.11 ± 0.10	0.075	mm

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Gañancia Máxima de Espesor Xenxival (ROI 1)	0.58 ± 0.27	0.55 ± 0.20	0.857	mm
Gañancia Media de Espesor Xengival na Area recuberta (ROI 2)	0.33±0.17	0.30±0.16	0.670	mm
Gañancia Máxima de Espesor Xengival na Area recuberta (ROI 2)	0.58 ± 0.27	0.55 ± 0.20	0.857	mm
Gañancia de Volume Medio na Area recuberta -ROI 2 (ΔVOL)	1.13 ± 1.25	0.86 ± 0.84	0.415	mm3
Puntuacións de IPR (Fase Inflamatoria)	6.10 ± 1.37	7.00 ± 0.71	0.182	(%)/max
Puntuacións de IPR (Fase Proliferativa)	4.40 ± 0.52	4.00 ± 0.71	0.278	(%)/max
Puntuacións de IPR (Fase de Remodelación)	3.00 ± 0.00	2.89 ± 0.33	0.720	(%)/max
Puntuación Total de IPR	9.50 ± 1.58	10.11 ± 1.05	0.497	(%)/max
Consumo Medio de Comprimidos (N)	1.70 ± 1.25	3.11 ± 2.57	0.243	n
Satisfacción Estética	9.70 ± 0.67	9.78 ± 0.67	0.780	(%)
Disposición a Repetir o Procedemento	90%	100%	0.16	(%)
Redución de Sensibilidade	100%	77.8%	0.11	(%)

1.4 DISCUSIÓN

Os resultados deste estudo non mostraron diferenzas estatisticamente significativas na cobertura radicular entre os pacientes tratados con CAF combinado con L-PRF e aqueles que se someteron a CAF só. A revisión sistemática e meta-análise de Miron et al. (2020) suxire que o uso de L-PRF pode proporcionar unha lixeira mellora na cobertura radicular cando se combina con CAF, cunha diferenza media que varía entre o 3% e o 14%. Con todo, os nosos resultados non atoparon evidencias suficientemente sólidas para establecer unha diferenza clínica significativa.

Aínda que non se observaron diferenzas significativas nos resultados clínicos, os datos suxiren unha tendencia a mellores resultados no grosor xenxival e na calidade dos tecidos no grupo tratado con L-PRF. Esta mellora non é estatisticamente significativa e o valor absoluto desta diferenza (aproximadamente 0,05 mm) non é clinicamente relevante para a maioría dos casos, pero pode ser considerada en determinadas situacións clínicas.

O tamaño reducido da mostra foi unha das principais limitacións deste estudo. Con só 19 pacientes e 70 recesións tratadas, a capacidade de detectar diferenzas estatisticamente significativas foi limitada. Ademais, a variabilidade na calidade das membranas de L-PRF producidas, debido ás diferenzas individuais na composición do sangue dos pacientes, podería influír nos resultados.

RELEVANCIA CLÍNICA

Aínda que o presente estudo non mostrou diferenzas estatisticamente significativas na maioría dos resultados entre os grupos, ambos tratamentos demostraron ser eficaces para acadar unha alta porcentaxe de cobertura radicular.

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LIMITACIÓNS E FUTUROS ESTUDOS

Unha das limitacións máis importantes deste estudo é o tamaño reducido da mostra, que pode ter afectado a capacidade de detectar diferenzas estatisticamente significativas entre os grupos. Ademais, o período de seguimento foi de só seis meses, o que pode non ser suficiente para avaliar a estabilidade a longo prazo dos resultados.

Futuras investigacións deberían incluír mostras máis grandes e un seguimento a longo prazo para determinar se os beneficios observados coa adición de L-PRF son mantidos ao longo do tempo. Así mesmo, estudos multicéntricos poderían axudar a validar os nosos resultados e proporcionar unha maior xeneralización dos achados.

Ademais, a análise de subgrupos, como pacientes con diferentes biotipos xenxivais ou características específicas, podería proporcionar información máis detallada sobre en que tipos de pacientes o uso de L-PRF podería ser máis beneficioso. O avance das tecnoloxías dixitais tamén xoga un papel importante na avaliación dos resultados clínicos, e futuros estudos deberían continuar explorando o uso de ferramentas dixitais para medir con maior precisión os cambios nos tecidos tras a cirurxía.

1.5 CONCLUSIÓN

Este estudo non atopou diferenzas estatisticamente significativas na maioría dos resultados clínicos entre o tratamento de recesións xenxivais mediante CAF só e a combinación de CAF con L-PRF. Ambos tratamentos resultaron en melloras similares na cobertura radicular, na anchura do tecido queratinizado, no grosor xenxival, no volume rexenerado e na satisfacción dos pacientes. Estudos máis amplos e con seguimento a longo prazo serán necesarios para confirmar esta tendencia e determinar o impacto clínico real da adición de L-PRF no tratamento das recesións xenxivais a longo prazo

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ABSTRACT

Title: Treatment of Miller Class I Gingival Recessions by CAF with or without Platelet-Rich Fibrin: A Randomized Clinical Control Trial
Author: Nuno Bernardo Malta dos Santos
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Background: Gingival recession, characterized by gingival apical migration and subsequent root exposure, poses significant clinical challenges, including aesthetic concerns, hypersensitivity, and potential root caries. The coronally advanced flap (CAF) procedure, with or without the adjunctive use of platelet-rich fibrin (PRF), is used to achieve root coverage and promote periodontal regeneration.

Objective: This study aims to evaluate the clinical outcomes of the treatment of Miller Class I gingival recessions using CAF alone versus CAF combined with leukocyte- and platelet-rich fibrin (L-PRF). The primary outcome measured was the percentage of root coverage (%RC) at six months. Secondary outcomes included mean root coverage (MRC), frequency of achieving 100% root coverage (CRC), keratinized tissue width (KTW), gingival thickness gain, volume gain, recession area reduction, healing quality, pill consumption, and patient-reported outcomes (PROs).

Methods: This randomized clinical trial included 19 patients with a total of 70 Miller Class I gingival recessions. Participants were randomly assigned to two groups: test group (CAF+L-PRF, n=42 sites) and the control group (CAF alone, n=28 sites). Clinical parameters were assessed at baseline and six months post-treatment, including percentage of root coverage (%RC), mean root coverage (MRC), frequency of achieving 100% root coverage (CRC), keratinized tissue width gain (ΔKTW), gingival thickness gain in two different regions of interest (meanΔGT, maxΔGT), volume gain, and healing quality (IPR scores). Pain and patient-reported outcomes were evaluated using the Visual Analog Scale (VAS) and standardized questionnaires, respectively. Pills consumption post-surgery was also recorded. All measurements were made using a digital evaluation protocol. Statistical analyses were performed using Mann-Whitney U test, mixed-effects models, and ANOVA to determine differences between groups and over time.

Results:
- **Root Coverage**: The mean percentage of root coverage (%RC) was 89.30% ± 20.33% in the test group and 81.60% ± 27.93% in the control group (p=0.168). The mean root coverage (MRC) was 1.01 ± 0.59 mm in the test group and 0.92 ± 0.68 mm in the control group (p=0.260). The frequency of achieving 100% root coverage (CRC) was 73.81% in the test group and 57.14% in the control group (p=0.193).
- **Keratinized Tissue Width gain (ΔKTW):** The mean KTW, increased from 2.75 ± 0.84 mm to 3.03 ± 0.91 mm in the test group and from 3.00 ± 1.01 mm to 3.06 ± 0.86 mm in the

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control group. The difference over time was statistically significant (p=0.042), but not between groups (p=0.474).

- **Gingival Thickness**: In the first ROI, the mean gingival thickness gain (meanΔGT) was 0.16 ± 0.11 mm in the test group and 0.11 ± 0.10 mm in the control group (p=0.075). In the second ROI, the maximum gingival thickness gain (maxΔGT) was 0.58 ± 0.27 mm in the test group and 0.55 ± 0.20 mm in the control group (p=0.857).

- **Volume Gain**: The mean volume gain was 1.13 ± 1.25 mm^3 in the test group and 0.86 ± 0.84 mm^3 in the control group (p=0.415).

- **Healing Quality (IPR Scores):** No statistically significant differences were found between groups in any phase of healing (p>0.05).

- **Pills Consumption**: The mean number of pills taken post-surgery was 1.70 ± 1.25 in the test group and 3.11 ± 2.57 in the control group (p=0.243).

- **Patient-Reported Outcomes (PROMs):** No statistically significant differences were found between groups in esthetic satisfaction, willingness to undergo the procedure again, or sensitivity reduction (p>0.05).

Conclusion This study found no statistically significant differences in most clinical outcomes between CAF alone and CAF combined with L-PRF in the treatment of Miller Class I gingival recessions. Both treatment modalities resulted in similar improvements in root coverage, keratinized tissue width, gingival thickness, volume gain, healing quality, and patient-reported outcomes. However, the mean gingival thickness gain in the first region of interest showed a trend towards better results in the test group (p=0.075), despite not being statistically significant. Longer studies with larger sample sizes will be needed to confirm this trend.

USC
UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

LIST OF FIGURES

FIGURE 1 - L-PRF BLOOD CLOT 7
FIGURE 2 - L-PRF-BLOOD CLOT. THE LEUKOCYTE-RICH SURFACE OF THE L-PRF BLOOD CLOT, REFERRED TO AS THE 'FACE,' CAN RELEASE GROWTH FACTORS AND SUPPORT CELL PROLIFERATION. 25
FIGURE 3 - CENTRIFUGATION AND PREPARATION OF BLOOD CLOT 26
FIGURE 4 - L-PRF AND INCISION TO CREATE THE "SURGICAL PAPILLA" 27
FIGURE 6 - CORONAL INCISION FOR LIBERATION OF FLAP AND DE-EPITHELIZATION OF PAPILLAE. 28
FIGURE 5 -ENVELOPE FLAP WITH HORIZONTAL INCISIONS AND SPLIT FLAP PREPARATION. 28
FIGURE 8 - POSITION AND SUTURE OF FLAP WITH SINGLE SLING SUTURE AND PTFE. 29
FIGURE 7- L-PRF MEMBRANES SUTURED TOGETHER, AND PLACEMENT OF MEMBRANES. 29
FIGURE 9 - MAGNIFICATION AND MULTI-ANGLE VIEW IN DEFINITION OF REFERENCE POINTS AND MEASUREMENTS. 39
FIGURE 10- PERIODONTAL PROBE SCALE VS DIGITAL MEASUREMENT SCALE 40
FIGURE 11-MEASUREMENTS WITH SUPERIMPOSED DIGITAL MODELS. 41
FIGURE 12 - VOLUME DINAMICS IN TERMS OF 2D (SECCION) AND 3 D EVALUATION 43
FIGURE 13 - MESH STRUCTURE OF A PLY FILE, WITH VERTICES, FACES AND COLORS 44
FIGURE 14 - RECESSION COVERAGE MEASUREMENTS WITH SUPERIMPOSED MODELS 46
FIGURE 15 - RECESSION COVERAGE MEASUREMENTS, WITH SUPERIMPOSED MODELS, IN AN INCOMPLETE COVERAGE 46
FIGURE 16 -GRAPHIC DISPLAY OF THE 3D COMPARE FUNCTION AND WORK AREA IN GEOMAGIC CONTROL X 47
FIGURE 17- STL-ALIGNMENT 48
FIGURE 18-REGION OF INTEREST ($ROI_{(1)}$) FOR A GROUP OF TEETH 49
FIGURE 19- VOLUME INCREASING DECORRENT FROM ARTFACTS 49
FIGURE 20 - REGION OF INTEREST $ROI_{(2)}$ FOR A GROUP OF TEETH 50
FIGURE 21 - VISUAL EVALUATION OF VOLUME DIFFERENCE FOR RO(I) AND ROI(2), RESPECTIVELY 51
FIGURE 22- PATIENT-RELATED -NOMINAL VARIABLES 56
FIGURE 23 FREQUENCY OF NOMINAL VARIABLES FOR THE ENTIRE SAMPLE POPULATION 58
FIGURE 24- HEALING SCORES FOR IPR. 73
FIGURE 25 - PILLS CONSUMPTION AND VAS SCORE AT 5 DAYS (PILLS-N,VAS-SCORE) 75
FIGURE 26- PROM SCORES (%) 77
FIGURE 27 - SUMARY GRAPHIC (SEE TABLE 62 FOR VALUES AND UNITS) 83
FIGURE 28 - ROOT COVERAGE VISUALIZATION OBTAINED BY SUPERIMPOSITON OF STL 85
FIGURE 29 - KERATINIZED TISSUE MEASUREMENTS 88
FIGURE 30- DIGITAL MEASUREMENT OF THICKNES OF MARGINAL GINGIVA 90
FIGURE 31 - BEFORE AND AFTER INTERVENTION, IN A TEST SUBJECT 94

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

LIST OF TABLES

TABLE 1- MILLER CLASSIFICATION FOR GINGIVAL RECESSION 3
TABLE 2. COMPARISON AND OVERLAP BETWEEN MILLER AND CAIRO CLASSIFICATION FOR DENTAL RECESSIONS 3
TABLE 3- SITE-SPECIFIC DIAGNOSTIC CONSIDERATIONS FOR RECESSION COVERAGE 4
TABLE 4-PLATELET CONCENTRATES EVOLUTION 8
TABLE 5- KEY GROWTH FACTOR, RELEASE BY L-PRF, AND THEIR QUANTIFICATION 9
TABLE 6- OTHER GROWTH FACTORS ASSOCIATED WITH PRF AND THEIR FUNCTION 9
TABLE 7-SISTEMATIC OVERVIEW ON SYSTEMATIC STUDIES WITH L-PRF (FERNANDES ET AL., 2021) 11
TABLE 8- TABLE RESUME OF RCT STUDIES COMPARING CAF ALONE AND CAF +L-PRF (FERNANDES ET AL., 2021) 11
TABLE 9- RESEARCH COLABORATORS TASKS 16
TABLE 10- INCLUSION/EXCLUSION CRITERIA 20
TABLE 11- RANDOMIZATION PLAN FROM WWW.RANDOMIZATION.COM 22
TABLE 12- MIRON`S REPORT CRITERIA FOR L-PRF STUDIES 26
TABLE 13- ABBREVIATION OF VARIABLES, FORMULAS AND UNITS USED 34
TABLE 14-INFLAMMATORY, PROLIFERATIVE, AND REMODELING SCALE (IPR). 36
TABLE 15-IPR SCALE. 36
TABLE 16-PATIENT RELATED OUTCOMES (PROMS) IN ESTHETIC AND OVERALL EXPERIENCE 37
TABLE 17-PROMS IN TERMS OF CERVICAL DENTIN HYPERSENSITIVITY (CDH) 38
TABLE 18- RECTANGULAR ROI -$ROI_{(1)}$1LIMITS 48
TABLE 19- POLIGONAL ROI-$ROI_{(2)}$ 50
TABLE 20-ROI/VARIABLES 50
TABLE 21-TIME MAP 52
TABLE 22-DATA COLLECTION AND EXAMINER 53
TABLE 23- CONTINUOUS VARIABLES RELATED TO PATIENTS 55
TABLE 24-PATIENT-RELATED NOMINAL VARIABLES 55
TABLE 25- RECESSION, PATIENT-RELATED 55
TABLE 26- CONTINUOUS VARIABLES SITE-RELATED 57
TABLE 27-CATEGORICAL VARIABLES SITE- RELATED 57
TABLE 28- TOOTH/RECESSION DISTRIBUTION 57
TABLE 29- SENSITIVITY, CEJ B AND STEP(+) BY TYPE OF TOOTH 58
TABLE 30-PATIENT-CENTERED BASELINE CONTINOUOS VARIALBLES COMPARISON 59
TABLE 31-PATIENT-CENTERED BASELINE NOMINAL VARIABLES COMPARISON 59
TABLE 32-SITE ESPECIFIC-SAMPLE CARACTHERIZATION (CONTINUOUS) 60
TABLE 33-SITE-ESPECIFIC SAMPLE CARACTHERIZATION (NOMINALS) 60
TABLE 34-DISTRIBUTION OF RE BY TYPE OF TOOTH 60
TABLE 35-NORMALITY TESTS (SHAPIRO-WILK TEST) 63
TABLE 36VARIANCE HOMOGENEITY TEST (LEVENE'S TEST)- 63
TABLE 37-DESCRIPTIVE STATISTICS AND MANN-WHITNEY U TEST RESULTS FOR % RECESSION COVER LENGTH- 63
TABLE 38-DESCRIPTIVE STATISTICS, MANN-WHITNEY U TEST, AND MIXED-EFFECTS MODEL RESULTS FOR % RECESSION COVER AREA 64
TABLE 39- SAMPLE-DESCRIPTIVE STATISTICS FOR COMPLETE ROOT COVERAGE (CRC) 66
TABLE 40-CRC RESULTS 66
TABLE 41- COMPLEMENTARY STATISTICS TEST FOR COMPLETE ROOT COVERAGE (CRC) 66
TABLE 42- DESCRIPTIVE STATISTICS FOR MEAN ROOT COVERAGE (MRC) 66
TABLE 43- STATISTICAL TESTS FOR MRC 67
TABLE 44-SAMPLE- DESCRIPTIVE ANALYSIS FOR KTW 67
45-SAMPLE-DESCRIPTIVE ANALYSIS FOR KERATINIZED TISSUE GAIN (ΔKTW) 67
TABLE 46-COMPARISON OF ΔKTW DESCRIPTIVE STATISTICS 68
TABLE 47- STATISTICAL TESTS FOR ΔKTW 68
TABLE 48-CHARACTERIZATION OF CLINICAL MEASUREMENTS BY TIME AND GROUP 68

TABLE 49- DESCRIPTIVE STATISICS FOR VARIABLES RELATED TO DIFERENCE IN GINGIVAL THICKNESS(ΔGT) 69
TABLE 50- SHAPIRO-WILK TEST RESULTS FOR NORMALITY OF DATA 70
TABLE 51- STATISTICAL TEST FOR DESCRIPTIVE STATISTICAL O ΔGT 70
TABLE 52COMPARATIVE TABLE BETWEEN GROUPS FOR ΔGT 70
TABLE 53-STATISTICAL MIXED-EFFECTS MODELS TEST FOR ΔGT 71
TABLE 54-HEALING RESULTS 73
TABLE 55-IBUPROFEN CONSUMPTION (NUMBER OF PILLS CONSUMPTION) AND VAS SCORE AT 5 DAYS 74
TABLE 56-PROMS RESULTS IN RECESSION COVERAGE SATISFACTION (SCORE,1-10) 76
TABLE 57- PROMS RESULTS IN SENSITIVITY AND WILLINGNESS TO REPEAT TREATMENT (%) 76
TABLE 58-REGRESSION MODELS RESULT FOR RELATION BETWEEN PREDICTORS AND %RC 78
TABLE 59-INFLUENCE OF TYPE OF TOOT IN %RC 80
TABLE 60-INFLUENCE OF TYPE OF TOOTH IN MRC 80
TABLE 61- SPEARMAN CORRELATION TEST FOR AGE AND OUTCOMES 81
TABLE 62-SUMMARY TABLE: TREATMENT OF MILLER CLASS I GINGIVAL RECESSIONS BY CAF WITH OR WITHOUT PLATELET-RICH FIBRIN 83

USC
UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

LIST OF PUBLICATIONS

1.1 CONTEXTUALIZATION (INTRODUCTION/BACKGROUND)

YEAR	TITLE	AUTHORS	CONTRIBUTION	JOURNAL	IMPACT FACTOR	CITE SCORE
2021	Autologous Platelet Concentrate of 2nd and 3rd generations efficacy in the surgical treatment of gingival recession: an overview of systematic reviews	Fernandes, G.V.O.; **Santos, N.B.M.**; Siqueira, R.A.C.D.; Wang, H.; Blanco Carrion, J.; Fernandes, J.C.H.	Conceptualization, review and editing	Journal of Indian Society of Periodontology	0.88	1.7
2022	Tensile Strength Essay Comparing Three Different Platelet-Rich Fibrin Membranes (L-PRF, A-PRF, and A-PRF+): A Mechanical and Structural In Vitro Evaluation	Simões-Pedro, M.; Tróia, P.M.B.P.S.; **Santos, N.B.M.**; Completo, A.M.G.; Castilho, R.M.; Fernandes, G.V.O.	Conceptualization, review and editing	Polymers	4.7	8.0
2022	Liquid Platelet-Rich Fibrin Coating Implant Surface to Enhance Osseointegration: A Double-Blinded, Randomized Split-Mouth Trial with 1-Year Follow-up	Fernandes GVO, **Santos, N.B.M.**, Correia de Sousa MF, Fernandes JCH	Review	Int J Oral Maxillofacial Implants	1.94	3.3
2024	The Influence of Root Prominence on the Onset of Gingival Recession: A Systematic Review	Raso, G.; **Santos, N.B.M**..; Nassani, L.M.; Mello-Moura, A.C.V.; Fernandes, J.C.H.; Fernandes, G.V.O.	Conceptualization, review and editing	Surgeries	Track	0.8

1.2 METHODOLOGY (DIGITAL MEASUREMENTS)

YEAR	TITLE	AUTHORS	CONTRIBUTION	JOURNAL	IMPACT FACTOR	CITE SCORE
2021	A new digital evaluation protocol applied in a retrospective analysis of periodontal plastic surgery of gingival recessions	Marques, T., **Santos, N. M.**, Fialho, J., Montero, J., & Correia, A.	Conceptualization, Measurements, review and editing	Scientific reports	3.8	7.5
2022	A 3D Digital Analysis of the Hard Palate Wound Healing after Free Gingival Graft Harvest: A Pilot Study in the Short Term	Marques, T., Ramos, S., **Santos, N.B.M.**, Borges, T., Montero, J., Correia, A., Fernandes, G.V.	Conceptualization, Measurements, review and editing	Dent. J.	2.5	3,7
2023	The Effect of the Interproximal Creeping Attachment in Aesthetic Site After Root Coverage Surgery with VISTA Technique - A Case Report	Marques, Tiago M., **Santos, N.B.M.,** Montero, Javier, Correia, André, Fernandes, Gustavo V. O.	Conceptualization, review and editing	Indian Journal of Dental Research	0.88	1.8

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

LIST OF ABBREVIATIONS

1.1 ABBREVIATION OF OUTCOMES VARIABLES IN STUDY

ABBREVIATION	DEFINITION
%RC	% of root coverage (expressed in %)
CRC OR %CRC	Complete root coverage (expressed in frequency %)
MAXΔGT	Maximum gingival thickness gain (at $ROI_{(1)}$)
$MAX\Delta GT_{(R)}$	Maximum thickness of tissue over the denuded root ($ROI_{(2)}$)
MEANΔGT	Mean gingival thickness gain (at $ROI_{(1)}$)
$MEAN\Delta GT_{(R)}$	Mean thickness of tissue over the denuded root ($ROI_{(2)}$)
MRC	Mean Root Coverage (expressed in mm)
$ROI_{(2)}$ AREA	Area of Recession covered (expressed in $mm^{2)}$
ΔKTW	Keratinized tissue width difference between T_F and T_0 (expressed in mm)
ΔVOL	Volume gain (at $ROI_{(2)}$) (expressed in mm^3)

1.2 ALL ABBREVIATIONS

ABBREVIATION	DEFINITION
%CRC	Percentage of complete root coverage
%RC	% of root coverage
%RC	% of root coverage
APC	Autologous Platelet Concentrate
APC	Autologous Platelet Concentrate
ASA	American society of anesthesiologists
BOP	Bleeding on probe
CAD	Computer-Aided Design
CAF	Coronally advanced flap
CAL	Clinical attachment level
CBCT	Cone-beam computed tomography
CDH	Cervical dentin hypersensitivity
CEJ	Cementoenamel junction
CRC OR %CRC	Complete root coverage (expressed in %)
CTG	Connective tissue graft
DHS	Dentine hypersensitivity
DICOM	Digital Imaging and Communications in Medicine
ICC	Interclass correlation coefficient
IOS	Intraoral scanner
IPR	Inflammatory, Proliferative, and Remodeling (Wound Healing Scale)
KTW	Keratinized tissue width

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L-PRF	Leukocyte and Platelet Rich Fibrin
MAGRs	Multiple adjacent gingival recession
MAXΔGT	Maximum gingival thickness gain (at $ROI_{(1)}$)
$MAX\Delta GT_{(R)}$	Maximum thickness of tissue over the denuded root ($ROI_{(2)}$)
MCAF	Modified coronally advanced flap (Zucchelli and Sanctis)
MEANΔGT	Mean gingival thickness gain (at $ROI_{(1)}$)
$MEAN\Delta GT_{(R)}$	Mean thickness of tissue over the denuded root ($ROI_{(2)}$)
MGL	Mucogingival line
MRC	Mean Root Coverage
PC	Platelet Concentrate
PCR	O'Leary Plaque Control Record (PCR)
PD	Pocket depth
PLY	Polygon File Format
PRC	% of root coverage
PRC	% of root coverage
PRF	Platelet-rich fibrin
PROMs	Patient-reported outcome measures
RC	Root/Recession coverage
RCT	Randomized controlled trial
RE	Gingival recession
REAREA	Area of gingival recession
ROI	Region of interest
$ROI_{(1)}$	Region of interest 1 (see table 26)
$ROI_{(2)}$	Region of interest 2 (see table 27)
RT1	Cairo recession type 1
RT2	Cairo recession type 2
RT3	Cairo recession type 3
SCTG	Subepithelial connective tissue graft
STL	Standard tessellation language
T_0	The baseline time point
T_F	Final time point (6 months in this study)
ΔGT	Gingival thickness gain
ΔKTW	Keratinized tissue width difference between T_F and T_0
ΔVOL	Volume gain

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1 INTRODUCTION/BACKGROUND

1.1 CLINICAL RELEVANCE AND SCIENTIFIC RATIONAL FOR THE STUDY

The demand for cosmetic medical treatments has increased markedly in the past few years. In periodontology, the treatment of gingival recessions (Re), especially in anterior teeth, is increasingly requested, and patient expectations are high (Chambrone & Prato, 2019). Platelet-rich fibrin (PRF) has been used in various procedures in both the medical and dental fields, such as in root coverage procedures. The results of randomized clinical controlled trials (RCTs) have suggested that PRF has the ability to promote soft tissue wound healing and boost regeneration in mucogingival recessions. However, the evidence can be considered non-sufficient and/or sometimes contradictory (Fernandes et al., 2021; Mancini et al., 2021; Miron et al., 2020).

Despite the increased use of PRF in clinical treatments in recent years, the predictability remains controversial, and more scientific evidence regarding the effects of L-PRF is necessary (Amine et al., 2018; Kuka et al., 2018). Moreover, association between technological evolution and new techniques has permitted the use of digital apparatus for the evaluation of clinical results, with the capacity of 3D and volume assessment which can bring new insights into this field.

Decision making in clinical scenarios should preferably be supported by scientific evidence, and randomized clinical trials (RCTs) on the use of L-PRF are crucial to support the clinical decision and choice of protocols and materials (Ehrenfest et al., 2009; Fernandes et al., 2021; Mancini et al., 2021; Miron, Zucchelli, & Choukroun, 2017; Miron et al., 2020).

1.2 GINGIVAL RECESSIONS

1.2.1 DEFINITION

According to the American Academy of Periodontology (AAP) Glossary, *gingival recession (Re)* is "the migration of the gingiva to an apical point to the cement-enamel junction." It is commonly used as synonymous with "*periodontal recession",* a marginal soft tissue recession in which the periodontal attachment apparatus (gingiva, periodontal ligament, and alveolar bone) migrates to an apical point to what occurs in a state of health" (AAP, 2018).

Exposure of the root surface, due to attachment loss, has been associated with several conditions, such as compromised esthetics, hypersensitivity, root caries, cervical abrasion, and complex oral hygiene maintenance (Cortellini & Bissada, 2018).

1.2.2 ETIOLOGY

The etiology of dental (gingival) recessions is multifactorial and involves a combination of anatomical, physiological, and behavioral factors. In terms of anatomical factors, a thin periodontal phenotype, the absence of attached gingiva, and reduced alveolar bone thickness due to the abnormal tooth position in the arch are considered risk factors for the development of gingival recession (Cortellini & Bissada, 2018).

Clinically, chronic inflammation and an improper tooth brushing method have also been reported as risk factors. Improper tooth brushing technique as been considered as the most important mechanical factor contributing to the development of gingival recessions; however, the existing evidence may be inconclusive (Cortellini & Bissada, 2018; Rajapakse et al., 2007). Furthermore, the influence of orthodontic treatment has been reported. Certain orthodontic treatments can also contribute to gingival recession, particularly if they result in the movement of teeth outside the alveolar bone envelope (Cortellini & Bissada, 2018; Joss-Vassalli et al., 2010). Recently, our group reviewed the influence of root protuberance on the onset and treatment outcomes of recession coverage. We observed negative correlations when comparing root prominence with variables such as linear root cover, covered root surface area, and linear tissue thickness gain. However, we were only able to include three studies in our systematic review, which hindered us in drawing definite conclusions (Raso et al., 2024).

1.2.3 PREVALENCE

A gingival recession can occur in populations with high and low oral hygiene standards (Löe et al., 1992; Matas et al., 2011; Serino et al., 1994). Even in populations with a high level of oral hygiene and despite improvements in tooth-brushing technique, the number and severity of gingival recession tend to increase over time (Daprile et al., 2007).

Recessions tend to increase with age, and studies show a substantial increase for each decade of life. By the age of 60, it is expected that almost 90% of people have at least one site with >1 mm of recession, while about 40% have at least one site with more than 3 mm. Recession sites are likely to progress over time, so this prevalence is expected to increase as patients retain their teeth longer (Heasman et al., 2017).

However, treatment can alter this situation. The sites treated with gingival augmentation surgery showed a tendency for coronal displacement of the gingival margin with a reduction in recession. Untreated contralateral sites tend to cause apical displacement of the gingival margin with increasing existing recessions (G. Agudio et al., 2009; Giancarlo Agudio et al., 2016, 2017).

1.3 CLASSIFICATION OF DENTAL RECESSIONS

1.3.1 CLASSIFICATION OF RECESSIONS: SIMPLE AND MULTIPLE RECESSIONS

Gingival recessions can affect one or more surfaces of the teeth and can be localized or multiple if they affect more than one tooth. In this work, we considered multiple recessions, recessions affecting two or more teeth, which may or may not be adjacent.

1.3.2 MILLER CLASSIFICATION OF DENTAL RECESSIONS

Several classification systems of gingival recession have been proposed in recent decades. The 1985 Miller classification is a widely accepted and commonly used classification scheme for gingival recession defects worldwide (Kasaj, 2018). In Miller's classification, gingival recessions are divided into four categories. In Classes I and II, the interdental periodontal support is intact, and the difference is that in Class II, the gingival margin reaches, or surpasses, the mucogingival line. According to Miller, complete root coverage can be anticipated up to the cemento-enamel junction in classes I and II. In Class III, marginal tissue recession extends

to or beyond the mucogingival line with loss of periodontal support in the interdental area or tooth malposition. In these cases, only a partial root coverage can be anticipated. Finally, in Miller Class IV, gingival recession extends to or beyond the mucogingival line, and loss of interproximal periodontal support and tooth malposition is so severe that no root coverage can be anticipated. (Miller, 1985).

Table 1- Miller classification for gingival recession
(Adapted from Miller. (Miller, 1985)

CLASS	INTERDENTAL PERIODONTAL SUPPORT	POSITION OF THE GINGIVAL MARGIN	ANTICIPATED COVERAGE
I	Intact	Above mucogingival line	Complete root coverage
II	Intact	Reaches / passes mucogingival line	Complete root coverage
III	Loss in the interdental area or malposition of the tooth	Extends to/beyond the mucogingival line	Partial root coverage
IV	Severe loss of interproximal support and malposition of the tooth	Extends to/beyond the mucogingival line	No root coverage

1.3.3 2017 EFP/AAP CLASSIFICATION OF DENTAL RECESSIONS

The more recent Cairo classification (Cairo et al., 2011) addresses some limitations of Miller systems and offers a more comprehensive approach. His implementation was recommended by the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions, cosponsored by the American Academy of Periodontology (AAP) and the European Federation of Periodontology (EFP). The Cairo classification has some overlap over Miller classification with classes I and II corresponding to RT1 and classes III and IV corresponding to RT2 and RT3, respectively (Table 2).

Table 2. Comparison and overlap between Miller and Cairo classification for dental recessions
(Analysis by author from Miller and Cairo, 2024 (Cairo et al., 2011; Miller, 1985)

FEATURE	MILLER CLASSIFICATION	CAIRO CLASSIFICATION
YEAR INTRODUCED	1985	2011
BASIS OF CLASSIFICATION	Extent of recession relative to the mucogingival junction and loss of interproximal bone/soft tissue	Interproximal clinical attachment level (CAL) in relation to buccal attachment loss
CLASSES/TYPES	4 (Class I-IV)	3 (RT1, RT2, RT3)
Class Type Description		
CLASS I / RT1	The recession does not extend to the mucogingival junction, and there is no loss of interproximal bone / soft tissue.	Gingival recession with no loss of interproximal attachment; interproximal CEJ not clinically detectable
CLASS II / RT1	The recession extends to or beyond the mucogingival junction, with no loss of interproximal bone / soft tissue.	Same as RT1
CLASS III / RT2	The recession extends to or beyond the mucogingival junction and there is loss of interproximal bone / soft tissue.	Gingival recession with interproximal attachment loss less than or equal to buccal attachment loss
CLASS IV / RT3	Recession extends to or beyond the mucogingival junction and there is severe loss of interproximal bone / soft tissue.	Gingival recession with interproximal attachment loss greater than buccal attachment loss
PREDICTABILITY OF ROOT COVERAGE	Full root coverage possible for Classes I and II; partial for Class III; unpredictable for Class IV	The predictability of root coverage is aligned with the severity of loss of interproximal attachment.

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The same workshop suggests the evaluation of each site regarding recession depth, gingival thickness, interdental attachment level, and root conditions (Table 3) (Cairo et al., 2011; Cortellini & Bissada, 2018; G. Prato et al., 2010).

Table 3 - Site-specific diagnostic considerations for recession coverage (Adapted from Cortellini (Cortellini & Bissada, 2018))

ABBREVIATIONS	DESCRIPTION	CLASS	DIAGNOSTIC
RT (CAIRO CLASSIFICATION)	Recession Type	RT1 RT2 RT3	
RE DEPTH	Depth of the Gingival Recession	Distance from gingival margin to CEJ	
GT	Gingival Thickness	Observing a periodontal probe detectable through soft tissues after being inserted into the sulcus (transparency).	
KTW	Keratinized Tissue Width	Distance from the gingival margin to the mucogingival line	
CEJ (PINI-PRATO CLASSIFICATION)	Cement Enamel Junction Status	A B	Detectable CEJ Undetectable CEJ
STEP (PINI-PRATO CLASSIFICATION)	Root surface concavity	Class + Class -	Presence of a Cervical Step >0.5 mm Absence of the Cervical Step

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1.5 TREATMENT OF GINGIVAL RECESSIONS

Root-covering surgeries are indicated by aesthetic demand, to reduce radicular hypersensitivity, or to create or increase the width of keratinized tissue. In terms of treatment objectives, it has been considered that complete success was obtained when the following criteria were met: gingival margin (GM) located at or coronal to the cement-enamel junction (i.e., complete root coverage [100% root coverage]), a sulcus depth up to 2 mm, the presence of clinically attached gingiva and no bleeding at examination (BOP) of the treated sites and the aesthetic integration of the regenerated tissues and patient satisfaction (Chambrone et al., 2019; Mounssif et al., 2018).

Several surgical techniques have been developed to treat exposed roots. Coronal advance flap techniques (CAF) and his modifications (D. M. Sanctis & Zucchelli, 2007; G Zucchelli et al., 2009; Giovanni Zucchelli, Mounssif, Mazzotti, Stefanini, et al., 2014) are techniques that can be applied to the treatment of multiple or unitary recessions. In these techniques, the residual gingival tissue, apical to the recession, is surgically displaced coronal to obtain the root cover. This technique can allow the use of a subepithelial connective tissue graft (SCTG) to increase the thickness of subepithelial tissue (G Zucchelli et al., 2003).

CAF and SCTG have been considered the gold standard for treating gingival recession (Chambrone et al., 2018; Chambrone & Tatakis, 2015). From a clinical point of view, SCTG-based procedures might be selected to treat any kind of recession. However, their current/recent indications also suggest that selective approaches could be preferable to prevent some kind of 'overtreatment' (i.e., thickening of the gingiva at sites that already presenting GT >0.8 to 1.00 mm (Chambrone & Prato, 2019). However, this technique has disadvantages. This technique (CAF+SCTG) requires a donor site, which is usually the palatal area, thus increasing the morbidity of surgery (Blanco-Carrion et al., 2004; Cairo et al., 2016; Giovanni Zucchelli et al., 2010; Giovanni Zucchelli & Mounssif, 2015). In addition, if the patient has a 'low' palate or very thin tissue, it can be difficult to get enough tissue from one donor tissue. Furthermore, an additional donor site may be necessary when multiple defects are present, leading to multiple surgeries (Zuhr et al., 2014).

Some recent evidence suggests the selective use of graft procedures to treat gingival recession. Cairo and collaborators (Cairo et al., 2016) pointed out that the use of SCTG for the treatment of multiple recession-type defects in esthetic sites (i.e., the anterior area of the maxilla) could be avoided at sites with a keratinized thickness > 0.8 mm, where the use of CAF alone is associated with similar clinical results and better esthetics.

Excessive augmentation beyond a critical threshold may not provide additional benefits and may negatively impact patient satisfaction due to an unnatural appearance. Patients may perceive overly thickened gingival tissues as less aesthetically pleasing, which can detract from their overall satisfaction with the procedure (Rebele et al., 2014; Zuhr et al., 2021). It appears crucial to prevent too much thickening that could result in an artificial look and negatively affect patient satisfaction.

Zucchelli and collaborators (Giovanni Zucchelli, Mounssif, Mazzotti, Stefanini, et al., 2014) found better postoperative course and better color match using CAF alone in short- and long-term results, but also found that CAF+SCT provided better complete root coverage (CRC) at five years.

Stefanini and collaborators (Stefanini et al., 2018) recently advocated that the coronally advanced flap is the most suitable surgical technique in patients with high aesthetic expectations, as it provides the best aesthetic results. This procedure is recommended when the height of the keratinized tissue above the exposed root(s) is more than 2mm. However, some

anatomic factors could limit its clinical applicability and efficacy, such as the absence or only a minimal amount (≤ 1 mm) of thickness of tissue apical to the recession defect, the presence of loss of interdental clinical attachment, the presence of a gingival clef extending into the alveolar mucosa, high pull of the frenulum at the soft tissue margin, loss of deep root structure, buccal dislocated root and very shallow depth of vestibulum (Stefanini et al., 2018). Rasperini (Rasperini et al., 2018), assessing the long-term (9 years) clinical outcomes of CAF and CAF+CTG, concluded that isolated gingival recessions can be successfully treated with CAF+CTG or CAF alone.

1.5.1 CORONALLY ADVANCED FLAP (CAF) FOR MULTIPLE RECESSIONS

Multiple gingival recessions are usually more challenging defects than single recession defects because the surgical field is more extensive with greater anatomical variability that can include prominent roots, shallow vestibules, enamel-root abrasions and unevenness in residual keratinized tissue. Furthermore, the treatment of multiple recessions must consider the total number of surgical procedures, the amount of donor tissue that can be obtained from the palate and the aesthetic requests of the patients (Cairo, 2017).

Bernimoulin and collaborators (Bernimoulin et al., 1975) described in the 1970s a technique to treat multiple gingival recessions, which included a free gingival graft for gingival augmentation that was followed three months later by a coronal position of the gingival margin. The authors performed a surgical papilla incision based on the depth of the gingival recessions, elevated a combined split (at the surgical papillae)–full (from the gingival margin to the mucogingival junction)–split (beyond the mucogingival junction) thickness flap, and included two vertical release incisions to mobilize the flap that was sutured coronally. Complete root coverage was obtained in 43% of the treated sites at one year of follow-up (Bernimoulin et al., 1975).

In 2000, Zuccheli and De Santis (G. Zucchelli & Sanctis, 2000) proposed a modification for the CAF in multiple recession-type defects. This technique avoids vertical incisions and uses a flap-type envelope that preserves the blood flow of the flap and reduces the chance of scars after surgery. Other advantages are derived from the split-full-split flap elevation. More thickness (and thus better opportunity to achieve root coverage) is provided for that portion of the flap residing over the previously exposed root exposures; it facilitates the coronal displacement of the flap and guarantees anchorage and blood supply to the surgical papilla in the interproximal areas between the root exposures (G. Zucchelli & Sanctis, 2000). This technique, for the treatment of multiple gingival recessions Miller class I and II, was shown to be effective in terms of root coverage and increased height of keratinized tissue, and these successful results were well maintained during the long-term observation period. On a 5-year examination, 94% of the root surfaces initially exposed due to gingival recession were still covered with soft tissue, and 85% of the treated recession defects showed complete coverage. Complete root coverage was maintained in all recessions in 15 of 22 patients (68%) (G Zucchelli & Sanctis, 2005).

Since its publication in 2000, this technique has been widely used, in the treatment of multiple recessions, with success, for more than 20 years. It has been tested in more than 100 studies and cited in 403 papers to date, proving its efficacy and repeatability (Stefanini et al., 2020).

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1.6 PLATELETS CONCENTRATES AND LEUCOCYTE PLATELET RICH FIBRIN (L-PRF)

1.6.1 CONCEPT AND DEFINITION OF PLATELET CONCENTRATES AND L-PRF



Figure 1 - L-PRF Blood Clot
(Created by author, 2024)

A relatively recently popular innovation in dentistry is the use of second-generation platelet concentrate, which is a platelet-rich autologous fibrin membrane (L-PRF) with growth factors and cicatricial properties that can be used for more diverse procedures (Choukroun et al., 2006a; Dohan et al., 2006c)

Autologous platelet concentrates (APC) collected from whole blood were introduced more than 20 years ago. The first platelet concentrate protocols were described by the so-called autologous platelet-derived wound healing factors (PDWHF) and were published in 1986 by Knighton (Knighton et al., 1986), in 1997 by Whitman (Whitman et al., 1997), and in 1998, also by Marx (Marx et al., 1998), with the name platelet-rich plasma (PRP)(to emphasize platelets and growth factor content). This platelet concentrate system requires the addition of anticoagulants during its processing procedure and aims to concentrate platelets and eliminate blood-derived leukocytes. One of the advancements of PRP was the plasma rich in growth factors (PRGF) developed by Anitua. Expanding on the PRP concept, Eduardo Anitua introduced PRGF in 1999 (Anitua, 1999, 2001), aiming to augment the regenerative properties of PRP. The initial notable clinical trials evaluating PRGF's efficacy were conducted in the dental and orthopedic fields, indicating its potential to enhance healing outcomes in various surgical procedures. Over time, PRGF attained recognition and approval for clinical use in both Europe and the U.S., further substantiating its application in regenerative therapies. Subsequent research has led to the development of improved PRGF protocols, such as PRGF-A and PRGF-B, which optimize platelet recovery and growth factor concentration, thus enhancing its therapeutic efficacy. Nonetheless, some research associated with the Anitua group has been

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subject of controversy, particularly regarding potential conflicts of interest (Ehrenfest et al., 2014).

In 2001, as an alternative to PRP and PGRF, with numerous advantages, Choukroun developed platelet-rich fibrin (L-PRF) (Choukroun et al., 2001), a second generation of PCs that does not require the addition of anticoagulants. These second-generation platelet concentrates, greatly facilitated their application and reproducibility in daily practice, making the procedure less technic sensitive, with less preparation time and affordable.

Table 4-Platelet Concentrates evolution
(Data compiled by author, 2024)

YEAR	DEVELOPMENT
1954	Kingsley first used the term "platelet-rich plasma" (PRP).
1970s	Fibrin glue introduced by Matras for improved wound healing
1986	Knighton et al. demonstrated that platelet concentrates promote healing
1997	Whitman introduced PRP in oral and maxillofacial surgery.
1998	Marx et al. popularized the use of PRP in bone grafts
1999	Anitua developed the plasma rich in growth factors (PRGF) technique
2000	Choukroun et al. introduced platelet-rich fibrin (PRF) in France
2001	Choukroun et al. introduced leukocyte- and platelet-rich fibrin (L-PRF).
2006	Sacco introduced concentrated growth factors (CGF)
2009	Dohan Ehrenfest et al. proposed the first classification of platelet concentrates
2010	Sohn introduced the concept of "sticky bone" (autologous fibrin glue with bone graft)
2014	Choukroun introduced advanced PRF (A-PRF).
2014	Tunal et al. introduced titanium-prepared PRF (T-PRF).
2015	Mouro et al. described the injectable PRF (i-PRF) technique.
2016	Kobayashi et al. introduced advanced PRF+ (A-PRF+)
2020s	Extended PRF (e-PRF) was developed to extend the resorption time by up to 4-6 months by Choukroun et al

1.6.2 MECHANISM OF ACTION OF PLATELET CONCENTRATES AND L-PRF

The autologous platelet concentrates (APCs) are a source of autologous growth factors (Bielecki & Ehrenfest, 2012; Ehrenfest et al., 2012). Platelets are vital components that affect the early stages of tissue regeneration, essential during hemostasis and fibrin clot formation. Platelets have also been shown to secrete several important growth factors, including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), coagulation factors, adhesion molecules, cytokines/chemokines, and a variety of other angiogenic factors capable of stimulating the proliferation and activation of cells involved in the wound healing process, including fibroblasts, neutrophils, macrophages, and mesenchymal stem cells (Choukroun & Miron, 2017).

The addition of leukocytes is believed to enhance regenerative properties and immune response, making L-PRF more effective for healing and tissue regeneration. L-PRF with its synergistic combination of host cells, a three-dimensional fibrin matrix, and accumulation of growth factors has frequently been recognized for its favorable effects on wound healing and reduction of postoperative discomfort (J. Blanco et al., 2024; Castro et al., 2017).

In various clinical applications, L-PRF's can enhance wound healing, tissue regeneration, and bone formation. The combination of a fibrin scaffold, sustained growth factor release, and presence of leukocytes and platelets makes L-PRF a potent autologous biomaterial for regenerative medicine (C. Blanco et al., 2022; Choukroun et al., 2006b; Dohan et al., 2006a, 2006b).

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PRF releases several growth factors, such as PDGF (Platelet-Derived Growth Factor), TGF-β (Transforming Growth Factor-Beta), VEGF (Vascular Endothelial Growth Factor), and EGF (Epidermal Growth Factor). These are essential for cell proliferation, angiogenesis, and tissue regeneration.

1.6.2.1 Key Growth Factors Released by PRF and Their Function

L-PRF releases various growth factors sustainably over up to 14 days, with the exact quantities influenced by preparation protocols and patient-specific factors. This controlled release is beneficial for enhancing tissue regeneration and healing in various clinical applications (Table 5).

Table 5- Key growth factor, release by L-PRF, and their quantification
(Data compiled by author from cited articles, 2024)

Growth factor		Function:	Concentration	reference
Platelet-Derived Growth Factor	(PDGF)	Function: PDGF is crucial for cell proliferation, angiogenesis, and new connective tissue formation.	10 to 100 ng/mL	(Ehrenfest et al., 2009)
Transforming Growth Factor-Beta	(TGF-β)	TGF-β regulates cell growth, differentiation, and matrix production	20 to 100 ng/mL	(Simonpieri et al., 2012)
Vascular Endothelial Growth Factor	(VEGF)	VEGF is essential for angiogenesis, the formation of new blood vessels	0.13 ± 0.02 ng/mL	(Schär et al., 2015)
Epidermal Growth Factor	(EGF)	EGF promotes cell growth, proliferation, and differentiation	0.1 to 1 ng/mL.	(Choukroun et al., 2001)
Insulin-like Growth Factor	(IGF)	GF supports cell growth and survival, particularly in bone formation and repair.	1 to 5 ng/mL.	(He et al., 2009)

Table 6- Other Growth factors associated with PRF and their function
(Data compiled by author from cited articles, 2024)

Growth factor	Function:
Insulin growth factor-1 (IGF-1)	Stimulates chemotaxis and activation of osteoblasts and bone formation; induces differentiation and mitogenesis of mesenchymal cells
Interleukin-1b (IL-1b)	increases expression of adhesive molecules on endothelial cells; stimulates helper T cell, chemotaxis of lymphocytes; activates osteoblasts
Interleukin-6 (IL-6)	Stimulates B-cell differentiation and antibody secretion; induces differentiation of naive T cells in cytotoxic T lymphocytes
Tumor necrosis factor-a (TNF-a)	Induces neutrophil cytotoxicity; stimulates cell survival and proliferation; enhances the remodeling capacities of fibroblasts
Interleukin-4 (IL-4)	Induces B-cell differentiation into plasmocytes, B-cell class switching to IgE, differentiation of naive helper T cells in Th2 cells

1.7 Uses of Platelet Concentrates and L-PRF in Dentistry

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Since L-PRF was first introduced in 2001 as a second-generation platelet concentrate, it has been used in various types of surgeries, including dentistry surgeries. In the last decade,

there has been a constant increase in published articles on the clinical applications of PRF, with a great diversity of experimental methods and results (Ghanaati et al., 2018). L-PRF has now been widely used in dentistry for various procedures to help facilitate the repair and regeneration of oral tissues. In the early years, it was used as a scaffold matrix alone or in combination with other biomaterials. Early attempts were made both successfully and unsuccessfully to investigate the benefits and limitations of this treatment modality in dentistry. Since then, a great deal of information has been learned about the regenerative potential of PRF and its effect on various tissues in the oral cavity.

Platelet concentrates (and specifically L-PRF) use, and research, have been described in the regeneration of intrabony and furcation defects, extraction socket management, sinus lifting procedures, gingival recession treatment, and guided bone regeneration (GBR), including horizontal/vertical bone augmentation procedures (Castro et al., 2017; Choukroun & Miron, 2017; Ghanaati et al., 2018; Meza-Mauricio et al., 2014; Miron, Zucchelli, & Choukroun, 2017; Miron, Zucchelli, Pikos, et al., 2017).

1.8 USE OF PRF IN THE TREATMENT OF GINGIVAL RECESSIONS

1.8.1 RATIONALE FOR COMBINING CORONALLY ADVANCED FLAP (CAF) WITH LEUKOCYTE- AND PLATELET-RICH FIBRIN (L-PRF)

As discussed early, Leukocyte-Platelet Rich Fibrin (L-PRF) is an autologous biomaterial created from the patient's own blood, rich in platelets and leukocytes. These components release essential growth factors and cytokines that significantly promote wound healing and tissue regeneration (Miron, Fujioka-Kobayashi, Bishara, et al., 2017). L-PRF's benefits include enhanced healing, improved soft tissue and bone regeneration, and reduced inflammation, making it a valuable adjunct in periodontal therapy.

Combining CAF with L-PRF could leverages the results of CAF, potentially achieve superior clinical outcomes. The synergy of CAF and L-PRF is hypothesized to improve the degree and predictability of root coverage, enhance the quality and thickness of gingival tissue, and accelerate the healing process. This combination aims to provide a more stable and durable result, reduce recovery time, and improve patient satisfaction by minimizing postoperative complications through L-PRF's anti-inflammatory and anti-microbial (Moraschini et al., 2024) properties. Previous studies and pilot results have suggested that using L-PRF as an adjunct to various periodontal procedures, including CAF, can lead to better root coverage and, fast and better, tissue regeneration than CAF alone (Kuka et al., 2018). Thus, the rationale for combining these techniques lies in the potential for improved clinical outcomes and patient benefits.

1.8.1.1 CLINICAL TRIALS ON THE TREATMENT OF GINGIVAL RECESSIONS AND THE USE OF L-PRF

Our group, in support for this work, recently conducted an umbrella review; we studied the use of autologous platelet concentrates (APC) and platelet-rich fibrin (PRF) (2nd and 3rd generations membranes), in periodontal surgeries for root coverage procedures. We accessed nine systematic reviews, finding mixed results. Although some studies show that APC/PRF effectively reduces postoperative discomfort, they do not consistently outperform traditional connective tissue graft (CTG) in clinical outcomes such as recession coverage (%Re) and clinical attachment levels. The review concludes that while APC/PRF is a feasible alternative, CTG remains superior (Fernandes et al., 2021).

Table 7-Sistematic overview on systematic studies with L-PR.
(Adapted from Fernandes (Fernandes et al., 2021)

ARTICLE	CONCLUSIONS
AMINE ET AL., 2017	APC/PRF reduces postoperative discomfort and is a viable alternative to CTG.
CASTRO ET AL., 2017	No significant benefit of APC/PRF over CTG in terms of clinical outcomes.
CHAMBRONE AND TATAKIS, 2015	APC/PRF does not provide additional benefits compared to CTG.
CHAMBRONE ET AL., 2019	APC/PRF shows favorable results in reducing gingival recession.
DEL FABBRO ET AL., 2011	APC/PRF is effective in reducing postoperative discomfort and improving clinical outcomes.
HOFMÄNNER ET AL., 2012	APC/PRF does not significantly improve clinical outcomes compared to CTG.
LI ET AL., 2019	APC/PRF shows promising results, but more research is needed.
LUO ET AL., 2015	APC/PRF is effective in reducing postoperative discomfort and shows favorable clinical outcomes.
MORASCHINI AND BARBOZA, 2016	APC/PRF does not provide significant benefits over CTG.

Table 8- Table Resume of RCT studies comparing CAF alone and CAF +L-PRF
(Adapted from Fernandes (Fernandes et al., 2021))

AUTHOR & YEAR	TITLE	RECESSION TYPE	NO. OF PATIENTS	DEFECTS (TEST/CONTROL)	FOLLOW-UP (MONTHS)	%RC CAF (%)	%RC CAF+PRF (%)	SIGNIFICANCE
Aroca et al. 2009	Clinical evaluation of a modified coronally advanced flap alone or in combination with a platelet-rich fibrin membrane for the treatment of adjacent multiple gingival recessions: A 6-month study	Multiple	20	67/67	6	91.5 ± 11.4	80.7 ± 14.7	$p < 0.05$
Padma et al. 2013	A split mouth randomized controlled study to evaluate the adjunctive effect of platelet-rich fibrin to coronally advanced flap in Miller's class-I and II recession defects	Single	15	15/15	6	68.44 ± 17.42	100 ± 0.00	NR
Gupta et al. 2015	Clinical evaluation and comparison of the efficacy of coronally advanced flap alone and in combination with platelet rich fibrin membrane in the treatment of Miller Class I and II gingival recessions	Single	26	15/15	6	86.6 ± 23.83	91 ± 19.98	NR
Thamarai selvan et al. 2015	Comparative clinical evaluation of coronally advanced flap with or without platelet rich fibrin membrane in the treatment of isolated gingival recession	Single	20	10/10	6	65 ± 44.47	74.16 ± 28.98	NR
Kumar A et al. 2017	Comparative evaluation of platelet-rich fibrin membrane and connective tissue graft in the treatment of gingival recession: A randomized controlled study	Single	36	15/15	6	NR	NR	NR
Dixit N et al. 2018	Root coverage by modified coronally advanced flap with and without platelet-rich fibrin: A clinical study	Multiple	12	12/12	6	79.5	82.87	NR
Kuka S et al. 2018	Clinical evaluation of coronally advanced flap with or without platelet-rich fibrin for the treatment of multiple gingival recessions	Multiple	24	28/24	12	74.63 ± 8.05	88.36 ± 15.45	NR

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The results of the reported RCTs that compared CAF alone and CAF with PRF for root coverage are not conclusive (Fernandes et al., 2021). Most studies indicated similar or slightly

improved results for the CAF+PRF group compared to CAF alone, with mean root coverage from 74.16% to 100% for CAF+PRF and 56.50% to 91.5% for CAF alone. However, two studies reported better results for CAF alone (Aroca et al. 2009 and Oncü et al. 2017). Most studies found no statistically significant differences between the two techniques. Some benefits reported for CAF+PRF include increased gingival thickness, faster healing, and less patient discomfort. Most of the trials were followed for 6 months, some for 12 months. Overall, while some studies suggested CAF+PRF had potential, the evidence does not clearly show its advantage over CAF alone for root coverage procedures.

1.8.2 USE OF L-PRF IN THE TREATMENT OF MULTIPLE GINGIVAL RECESSIONS

The benefits of using platelet-rich fibrin (PRF) in the treatment of multiple gingival recessions are less consistent compared to its use in single recessions. Although some studies report significant improvements in clinical parameters such as root cover (%RC), clinical attachment level (CAL) and gingival thickness (GT) when PRF is used in conjunction with coronally advanced flap (CAF) in multiple recessions (Kuka et al., 2018), others do not find substantial differences between CAF alone and CAF+PRF (Aroca et al., 2009). Furthermore, while PRF has been associated with improved soft tissue healing and increased gingival thickness in single recessions, its benefits in multiple recessions are not as clear-cut. This inconsistency may be due to factors such as the heterogeneity of study designs, differences in patient populations, and variations in PRF preparation and application techniques (Fernandes et al., 2021). Therefore, more research is needed to fully understand the benefits of PRF in treating multiple gingival recessions and to establish standardized protocols for its use.

1.9 LIMITATIONS OF L-PRF

1.9.1.1 RAPID DEGRADATION AND LACK OF STRUCTURAL INTEGRITY ONCE IMPLANTED

L-PRF is known to undergo rapid degradation when exposed to enzymes in the body. This property results in a gradual release of growth factors that can be beneficial for tissue healing and regeneration. However, rapid degradation can also be a limitation in certain applications where prolonged mechanical structural support is needed.

1.9.1.2 VARIABILITY IN QUALITY AND CONSISTENCY OF MEMBRANES PRODUCED

The preparation of L-PRF is highly sensitive to the centrifugation protocol used, including the speed (RPM), time, and type of centrifuge (fixed-angle vs. horizontal). Variations in these parameters can significantly affect the quality and consistency of the L-PRF(Marc Quirynen et al., 2024).

The type of blood collection tube (glass vs. silica-coated plastic or titanium (Tunalı et al., 2013; Yamaguchi et al., 2020)) can, also, influence the activation of the coagulation cascade and the final quality of the L-PRF membrane. Issues such as silica particle contamination from silica-coated tubes have been reported, which could potentially affect the biocompatibility and safety of the L-PRF membrane (Tsujino et al., 2019).

Hematocrit levels affect the size (Miron, Dham, et al., 2019) and quality of L-PRF membranes since they determine how blood components separate during centrifugation. For example, older women and patients usually produce larger but thinner L-PRF membranes than

older men and young patients due to lower hematocrit levels. The optimal hematocrit range for the preparation of L-PRF is unclear. Patients with coagulation problems or anticoagulant medications may have trouble forming L-PRF membranes, but this is not well studied. Even the time of day/ time since the last meal or the type of last meal can potentially affect the membrane preparation. The volume of L-PRF membranes is also limited by the amount of blood that can be drawn safely from the patient.

1.9.1.3 TECHNICAL ISSUES/LIMITATIONS

One technical issue is timing: blood must be centrifuged soon after drawing. If not, the clots will be smaller and weaker and the L-PRF membrane will be of lower quality. Another thing is that L-PRF membranes must be used as soon as possible. Waiting for or handling them wrong can damage their structure and function (Marc Quirynen et al., 2024). Final, temperature plays a crucial role in the production and quality of leukocyte- and platelet-rich fibrin (L-PRF).

The process involves several temperature-sensitive steps that can significantly impact the structural integrity, cellular content, and overall effectiveness of the final L-PRF product.

The importance of temperature has dictated some alternative protocols for PRF membranes. Pompac® uses cold storage of blood tubes and butterflies during blood collection before centrifugation to improve mechanical properties. On the other hand, the preparation of e-PRF involves controlled heating to change the properties of produced PRF (Miron et al., 2023).

1.9.1.4 LIMITED TIME EFFECT

L-PRF matrices may not last long after implantation, which could affect their ability to regenerate tissues over time. Growth factors in L-PRF do not persist long, which can reduce its long-term advantages. L-PRF membranes dissolve relatively quickly in 2-4 weeks.

1.9.1.5 LACK OF EFFECTIVENESS AND LONG-TERM EVIDENCE

There is a lack of long-term clinical data on the outcomes of L-PRF treatments. Most studies have follow-up periods of less than 18 months, making it difficult to assess the long-term stability and effectiveness of L-PRF in various clinical applications. Clinical outcomes can be inconsistent due to the variability in preparation methods and patient-specific factors. There is no explicit 'cutoff' for the healing factor that makes PRF work, and the relationship between dose and response is unknown.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

2 OBJECTIVES

2.1 OVERVIEW

PRIMARY OBJECTIVE

The primary objective of this study was to compare the efficacy of Coronal Advanced Flap (CAF) alone versus CAF combined with Leukocyte-Platelet-Rich Fibrin (L-PRF) to achieve root coverage in patients with gingival recession. This was assessed by measuring the percentage of root coverage at six months post-surgery. By focusing on the degree of root coverage (%RC), the study aimed to determine whether the addition of L-PRF to CAF provided a significant improvement over the use of CAF alone.

SECONDARY OBJECTIVES

The secondary objectives of this study aimed to provide a comprehensive assessment of the outcomes related to root coverage and overall patient satisfaction. Moreover, we assessed other outcomes related to recession coverage, including the percentage of complete root coverage (CRC) and mean root coverage (MRC). These evaluations were intended to offer a more detailed understanding of the possible success of each surgical technique. Additionally, we attempted to measure the difference in gingival tissue thickness, and keratinized tissue width (KTW) between the two groups at six months post-surgery to determine whether the addition of L-PRF to CAF improved tissue quality and stability. The study also aimed to determine the overall volume gain in gingival tissue in the specific area of recession covered by both techniques, providing information on their regenerative capabilities and the three-dimensional changes in tissue structure.

Furthermore, we sought to analyze the healing and recovery processes by comparing postoperative complications, patient discomfort, and overall recovery time between the two groups. Finally, we tried to investigate patient satisfaction with the aesthetic and functional outcomes of the procedures, evaluating their perceptions of the success of treatment and the quality of life after surgery. This comprehensive approach was designed to ensure that all relevant outcomes were considered, trying to offer a robust evaluation of the efficacy and benefits of combining CAF with L-PRF and providing comparability with previous and futures studies.

2.1.1 HYPOTHESES

In order to achieve this goal, we aimed to test the following hypotheses to address this subject. Specifically, we hypothesize that:

- Null hypothesis: The addition of L-PRF to CAF will not result in significantly greater root coverage in terms of percentage of root coverage compared to CAF alone at six months post-surgery.
- Alternative hypothesis: CAF combined with L-PRF will:

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(a) result in significantly greater root coverage compared to CAF alone at six months post-surgery in terms of mean root coverage or complete root coverage.
(b) will lead to thicker gingival tissue and better tissue quality (KTW) compared to CAF alone.
(c) will have a faster healing and recovery, with fewer complications in the CAF with L-PRF group compared to the CAF alone group.
(d) will report higher satisfaction levels with the treatment outcomes compared to those undergoing CAF alone.

The validity of these hypotheses was examined by evaluating the relevant outcome variables.

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3 MATERIALS AND METHODS

3.1 STUDY DESIGN AND CENTER

3.1.1 STUDY DESIGN

Randomized clinical trial (RCT) with two arms and parallel design.

3.1.2 CENTERS

CENTERS

Study conducted at the University Clinic of 'Universidade Católica Portuguesa' in Viseu, Portugal, from January 2019 to January 2024

RESEARCH TEAM

Principal investigator (PI),Lead Researcher
Nuno Bernardo Malta dos Santos (NS)

Monitoring (Spain):
Prof. Dr. D. Juan Blanco Carrión (Universidade de Santiago Compostela)
Prof. Maria Pilar Batalla Vazquez (Universidade de Santiago Compostela)

Monitoring (Portugal):
Prof. Dr. André Correia (Universidade Católica Portuguesa)
Prof. Dr. Gustavo Fernandes (Universidade Católica Portuguesa)

Research Collaboration:
Prof. Dr. Tiago Marques (TM)
Dr Manuel Correia Sousa (MS)
Prof. Dr. Gustavo Fernandes (GF)
Dr Filipe Araujo (FA)

Table 9- Research colaborators tasks
(Data from the author,2024)

	TIME	FORM (APPENDIX)	COLLABORATOR
PRINCIPAL INVESTIGATOR (PI) AND STUDY COORDINATOR	T_0-T_F		NS
PRE-EVALUATION/RECRUITMENT	Not defined	Appendix II	NS, TM,MS, GF
ASSESSMENT OF ELIGIBILITY AND SELECTION DATA FORM	Not defined	Appendix III	TM,
PROFESSIONAL CLEANING AND HYGIENE INSTRUCTION	4 weeks before T_0		-
RANDOMIZED GROUP ALLOCATION	Time zero - T_0	Table 11	FA

BASELINE ASSESSMENT T_0	Time zero - T_0	Appendix IV	MS,
INTERVENTION	Time zero - T_0		NS
FOLLOW UP FORM	5 days	Appendix V	GF
FOLLOW-UP FORM AND SUTURE REMOVAL	2 weeks after	Appendix VI	GF
FOLLOW-UP FORM AND PROPHYLAXIS	6 weeks	Appendix VII	GF
DATA COLLECTION (T_F)	final time (T_f) -6 months	Appendix VIII	TM
STATISTICS AND DATA ANALYSIS	>6 months		NS,GF
PROTOCOL MANAGER	T_0-T_F		NS

In this research, the surgeon also serves as the principal investigator (NS). Although involving the surgeon as the principal investigator (PI) in a randomized controlled trial (RCT) can introduce potential biases, it is not inherently unethical or methodologically flawed (Friedman et al., 2015). The primary concern stems from the possibility that the surgeon, due to their dual role as both the PI and the clinician performing the intervention, may inadvertently influence multiple facets of the trial . To mitigate the potential biases introduced under these circumstances, several strategies were employed. To prevent selection bias, where the surgeon might unconsciously select patients more likely to succeed with the intervention, patient eligibility assessment was conducted by an independent clinician strictly adhering to the predefined inclusion criteria. Performance bias, wherein the surgeon's awareness of treatment allocation might affect the quality of surgical procedures, was minimized by implementing standardized surgical protocols to ensure consistency across interventions. Detection bias, which occurs when the surgeon assesses outcomes, particularly subjective ones, was addressed by utilizing independent, blinded outcome assessors to ensure that evaluations were made objectively and without knowledge of the treatment group (Friedman et al., 2015). Reporting bias, wherein the Principal Investigator (PI) may selectively present outcomes that align with their expectations and surgical preferences, posed a more formidable challenge to mitigate, given that the requirements of this PhD research necessitate the PI's (and in this case surgeon) direct involvement in data analysis and reporting. However, oversight by the PhD tutorial team ensured impartial review of the trial's progress, and decisions were made objectively while managing any conflicts of interest appropriately.

3.1.2.1 ETHICAL APPROVAL.

The Study protocol, guidelines, consent forms, and all other related items were submitted for approval of the “Comissão de Ética para a Saúde” - Universidade Católica Portuguesa (CES-UCP) with positive approval (Appendix VI)

3.1.2.2 ETHICAL DECLARATION

The researchers declare compliance with good clinical practice according to the interventions performed and the regulations for managing blood products.

The investigators oversee all medical and administrative aspects of this study and ensure that the activities related to it comply with the guidelines set out in the Protocol and its

Modifications, with those set by the Centre's Clinical Research Ethics Committee, and with the regulations and Standards of Good Clinical Practice and other regulations relevant to the conduct of Clinical Trials in Portugal and Spain.
The investigators are also accountable for the accuracy and validity of the data entered in the Data collection booklet.

3.1.3 CLINICAL TRIAL REGISTRATION

This clinical trial is registered with ClinicalTrials.gov, under the identifier NCT06591156

3.1.4 SAFETY, BENEFICIAL AND ADVERSE EFFECTS

Possible complications may occur with any surgery. The patients were informed regarding risks/ benefits before, through informed consent and oral explanation (Appendix IV).

The Application of L-PRF can carry the following effects:

3.1.4.1 BENEFICIAL EFFECTS:

Research in recent years has provided experimental and clinical evidence showing that growth factors favor tissue regeneration. Platelets have growth factors within them, proteins that can accelerate tissue healing processes. When centrifuged, the growth factors found in the alpha granules of the platelets are released to the tissue where they have been applied.

Although no adverse effect can be ruled out, previous experiences have shown that it is a safe method. With this technique, the patient's plasma is applied, and there is no risk of immunologic reactions to its application.

The obtaining and application of autologous Plasma is carried out under strict aseptic conditions, minimizing the risk of contamination and the possibility of infection in application.

3.1.4.2 RISKS:

Injury of structures adjacent to the venipuncture (Risks related to blood extraction)

Postoperative infection is a risk, as with any surgical procedure. Proper oral hygiene and following postoperative care and instructions are crucial to minimize this risk.

Some bleeding can occur after the surgery, but excessive bleeding can be a concern. Patients are advised to avoid activities that could exacerbate bleeding, such as vigorous brushing or flossing in the treated area.

Swelling and discomfort can occur after surgery. These symptoms typically resolve within a few days but can be managed with prescribed medications.

Pain is usually mild and can be controlled with pain relievers according to protocol.

There is a possibility that the gum recession may recur over time, mainly if the underlying causes (e.g., aggressive brushing) are not addressed.

The aesthetic outcome may not always meet the patient’s expectations. Variability in tissue color and texture can affect the overall appearance.

Some patients may experience altered sensation or numbness in the treated area. This symptom is usually temporary but can be permanent in rare cases.

Allergic reactions to anesthesia or ibuprofen used during the procedure are possible but rare.

USC DE SANTIAGO DE COMPOSTELA

MEASURES TO MINIMIZE RISKS

To minimize risks, participants will undergo a complete clinical history to identify potential increased risks, and procedures will be conducted using surgical techniques and materials in accordance with the 'gold standard' of surgical infection control.

An experienced nurse will carry out blood collection, and the surgical intervention will be performed by a specialist in Periodontology, certified by the "Ordem dos Médicos Dentistas," with more than ten years of experience in the field.

Written information on postoperative care and necessary medication will be provided to the patient to minimize the risks. Participants will receive postoperative follow-up according to the research protocol.

3.1.5 SOURCES OF FUNDING

Self-financed by the investigators and their institutions. All investigators declare that they have no conflict of interest.

3.2 POPULATION

Patients attending the 'Clínica Dentária Universitária' da 'Universidade Católica Portuguesa'

3.2.1 INFORMED CONSENT

Informed consent was obtained from all individual participants included in the study (see Appendix IV for the form).

3.2.2 SELECTION CRITERIA

The sample was chosen based on the following criteria:

3.2.2.1 INCLUSION CRITERIA

The sample was chosen based on the following criteria:

1. People over 18 years of age. Nonsmokers (self-reported).
2. At least, the presence of two or more neighboring, or not, gingival recessions Miller class I in the anterior upper jaw (central and lateral incisors, canine, first and second premolars
3. A minimal amount (>1,5 mm) of keratinized tissue width (KTW) apical to the recession area.
4. Oral hygiene and oral health, with the O'Leary plaque control record (PCR) (O'Leary et al., 1972) and bleeding on probing (BOP) (Lang et al., 1990) less than 25%.

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3.2.2.2 EXCLUSION CRITERIA

1. Medical pathology that prevents the application of treatments affects the response of these and the healing process: protein and vitamin deficiency, therapeutic radiation, metabolic disorders (diabetes, hypercalcemia, and others.), drug disorders (antimetabolic, immunosuppressive) and hormonal, allergy to some component of the treatments applied in the study.
2. Pocket depth > 3mm or interproximal clinical attachment loss (CAL) >0
3. Pregnancy.
4. Presence of Carious lesions, occlusal trauma, or previous root cover procedures.
5. Presence of Prosthetic crown on experimental teeth

Table 10- Inclusion/Exclusion criteria
(Data from the author)

CRITERIA	DESCRIPTION
AGE	Over 18 years old
SMOKING STATUS	Non-smokers or smokers with less than ten cigarettes (self-reported)
MEDICAL PATHOLOGY	No medical pathology that prevents the application of treatments
PREGNANCY STATUS	No pregnant
ORAL HEALTH – PCR	O'Leary Plaque Control Record (PCR) < 25%
ORAL HEALTH – BOP	Bleeding on probing (BOP) < 25%
GINGIVAL RECESSIONS	At least two or more neighboring gingival recessions Miller class 1 in the anterior upper jaw
KERATINIZED TISSUE (KT)	Minimal amount (> 1mm) of KT apical to recession area
POCKET DEPTH (PD)	≤ 3 mm
CLINICAL ATTACHMENT LOSS (CAL)	Interproximal = 0
CARIOUS LESIONS	Absence of carious lesions
OCCLUSAL TRAUMA	Absence of occlusal trauma
PREVIOUS ROOT COVERAGE	Absence of previous root coverage procedures
PROSTHETIC CROWN	Absence of Prosthetic crown at experimental teeth

CONSIDERATIONS OF INCLUSION AND EXCLUSION CRITERIA

The inclusion of only the anterior upper jaw is because the prevalence of recession is high in that area and has a greater impact factor on the esthetics of patients, which is one if not the most important reason for treatment (Wennström, 1996). The option to include only Miller Class I is based on the fact that, according to the literature from the authors of the technique (Stefanini et al., 2018), the coronal advanced flap surgical technique (without graft) is indicated for root coverage in patients when the height of the keratinized tissue apical to the root exposure is greater than 2 mm (Stefanini et al., 2018; Giovanni Zucchelli & Mounssif, 2015). In these cases, this technique can provide the best aesthetic results in patients with high aesthetic

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expectations (Stefanini et al., 2018). Thus, we are comparing the test group with the standard reference treatment according to the authors of the technique.

3.2.2.3 PREOPERATIVE INTERVENTION AND ELIGIBILITY ASSESSMENT

Patients will have a thorough diagnostic evaluation, followed by professional cleaning and instruction on how to brush appropriately without causing trauma (roll technique). Surgical treatment of recession defects will be postponed until the patient can maintain a satisfactory level of plaque control. After four weeks, the eligibility for the intervention was verified by a single operator (TM).

3.2.2.4 MEASURE OF COMPLIANCE

WITHDRAWAL CRITERIA:

Non-compliance with study protocols, such as missing scheduled visits or failing to adhere to treatment regimens, can lead to withdrawal.

The participant does not wish to continue the study.

If a participant loses the capacity to provide informed consent, they must be withdrawn from the study.

Participants may lose eligibility during the trial due to changes in their health status or the onset of conditions that interfere with the study objectives.

3.2.3 SAMPLE SIZE ANALYSIS

3.2.3.1 ASSUMPTIONS AND ESTIMATES

To determine the appropriate sample size for our randomized controlled trial (RCT) comparing Coronally Advanced Flap (CAF) alone with CAF combined with Platelet Rich Fibrin (PRF), we used data from a previous study by Kuka et al. (2018). This study reported a mean root cover of 88.36% (SD = 15.45%) for CAF + PRF and 74.63% (SD = 8.05%) for CAF alone.

We calculated an effect size (Cohen's d) of approximately 1.12. Aiming for a statistical power of 0.80 and a significance level (α) of 0.05, we determined the required sample size per group. Given that each patient could contribute multiple recession sites, we adjusted the sample size calculation to account for the clustering effect using an intraclass correlation coefficient (ICC) of 0.1 and an average of 2 recession sites per patient.

The calculation indicated that we need approximately 10 patients per group, resulting in a total sample size of 20 patients. This corresponds to approximately 20 recession sites per group, or a total of 40 recession sites for the study.

By selecting an effect size of 1.12 and adjusting for the clustering of recession sites within patients, our objective is to ensure that our study is adequately powered to detect a clinically meaningful difference between the treatment groups, providing robust and significant results.

To ensure the reliability of our results, we decided to include 10 patients per group, considering that the average of recessions per patient is greater than two. This adjustment ensures the ability to detect clinically significant differences while adjusting for potential dropouts and variability.

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3.3 GROUPS OF THE STUDY AND RANDOMIZATION

3.3.1 GROUPS

The total sample where19 patients with a total of 70 recessions (se appendix 1 for CONSORT flow diagram) categorized as Class I Miller.

Participants were randomly assigned to one of two groups using a computer-generated randomization sequence: the control group, which received the CAF procedure alone have 9 patients, and the test group, which received the CAF procedure in combination with L-PRF, have 10 patients.

The recession sites were distributed with 28 in the control group and 42 in the test group.

The Results and Discussion chapter presents a comprehensive analysis of the entire sample and its group distribution.

3.3.2 RANDOMIZATION OF PARTICIPANTS

3.3.2.1 OVERVIEW

Randomization ensured that each participant had an equal chance of being assigned to either group, thereby reducing selection bias and ensuring that the groups were comparable at the start of the study

3.3.2.2 RANDOMIZATION METHOD, SEQUENCE GENERATION

We applied Dallal randomization, which uses the techniques created by Gerard E. Dallal to make randomization plans for clinical trials and other studies. This tool can be accessed on Randomization.com (Dalllal, n.d.). This website allows the use of different randomization generators.

Using the method of randomly permuted blocks (Friedman et al., 2015; J. Kim & Shin, 2014), one treatment was assigned to each of 20 subjects split into blocks of "6,4,4,6". This plan was made on 26/06/2020, 15:47:59, using sed 2948 with the same number of subjects per block/number of blocks and treatment labels as originally entered.

Table 11- Randomization plan from www.randomization.com
(Data produced from the site randomization.com)

NUMBER	TYPE
1.	PRF
2.	Control
3.	Control
4.	PRF
5.	Control PRF
7.	Control
8.	PRF
9.	Control
10.	PRF
11.	Control
12.	PRF
13.	Control
14.	PRF

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15.	PRF
16.	PRFI
17.	Control
18.	PRF
19.	Control
20.	Control

3.3.3 RANDOMIZATION IMPLEMENTATION AND CONCEALMENT MECHANISM

Random generation was performed by one study collaborator (FA) using the method described. Concealment was performed by the same clinician not involved in participant care or data collection. This study collaborator (FA) conceals the table that assigns participants to the appropriate group. Before the intervention, the investigator (NS) called the study collaborator, who told them the assignment of the patient in the test or control group (Friedman et al., 2015).

3.4 BLINDING AND CALIBRATION

3.4.1 BLINDING

Blinding was implemented to enhance the validity of the trial where possible. However, complete blinding was not feasible for all parties involved:

3.4.1.1 PARTICIPANTS

Blinding participants was not possible because the collection of blood for L-PRF preparation was required for the test group, which would have made them aware of their group allocation. Additionally, informed consent required that participants be aware of the two different settings, with or without blood collection, making blinding impractical.

3.4.1.2 SURGEONS

Blinding the surgeons was not feasible as they needed to perform specific procedures depending on the group allocation, particularly the preparation and application of L-PRF.

3.4.1.3 OUTCOME ASSESSORS

Only the outcome assessors were successfully blinded to the group assignments. This was crucial to ensure that the assessment of clinical outcomes remained unbiased.

The inability to blind participants and surgeons was justified by the practical and ethical necessity of blood collection for the L-PRF procedure and the requirement for precise execution of different surgical techniques. Ensuring that outcome assessors were blinded helped to mitigate potential bias in the evaluation of the study's results.

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3.4.1.4 CALIBRATION OF THE CLINICAL AND DIGITAL EXAMINERS

The examiner has no knowledge of the patient-allocated group (blinded).

A calibration exercise was performed to determine the acceptable intra-examiner reproducibility. Five patients not enrolled in the study, who exhibited at least two teeth with a recession height (RH) of >1 mm, were evaluated by the examiner twice at 1-week intervals. Recession depth (Re), recession Area (ReArea), and keratinized tissue width (KTW) were measured in an STL file. The examiner was accepted as measurements at baseline and at one week were similar to the 0,5 mm at ≥90% (Ozenci et al., 2015). The intra-examiner reproducibility was considered excellent for ReArea and Re (>90), and good for KTW (>75).

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3.5 INTERVENTION

3.5.1 DESCRIPTION

An operator, preferentially a clinician not involved with the care of participants, assessed eligibility and filled out the data form. This operator proceeded to the baseline examination if the participants confirmed their eligibility. After receiving the allocation information from the randomization unit (FA), the surgeon, a clinician not involved in data collection (NS), started the protocolized procedure.

3.5.1.1 TEST GROUP (CAF PLUS L-PRF)

BLOOD COLLECTION

If a patient was assigned to the test group (L-PRF), a designed nurse proceeded to the blood collection to obtain L-PRF (Choukroun et al., 2001). A vein (basilica, cephalic, or median), usually on the inside of the elbow or the back of the hand, was the source of blood. The area where the puncture occurred was cleaned with an antiseptic with 0.2% chlorhexidine. An elastic band or pressure cuff was wrapped around the forearm to squeeze the vein and make it more noticeable under the band. This helped the needle find the vein more easily.

As soon as that was done, a needle was placed in the vein and blood was collected in a clean and sealed plastic tube (Intra Spin™ – Intra-Lock), between 4 and 6 tubes, without anticoagulant. During the process, the band was removed to allow blood flow normally and, after the blood was collected, the needle was removed, and the puncture site was covered to prevent bleeding. The FRP-L membranes were prepared according to the technique described



Figure 2 - L-PRF-Blood clot. The leukocyte-rich surface of the L-PRF blood clot, referred to as the 'face,' can release growth factors and support cell proliferation.
(Created by author, 2024)

by Choukroun et al. 2001(Choukroun et al., 2001; M. Quirynen et al., 2024). Tubes were centrifuged immediately at 2700 rpm for 12 minutes on the centrifugation machine (Intraspin™, Intra-lock®, Florida, USA). .

After centrifugation, the gelatinous portion obtained from each tube was removed and separated from the red part with the help of toothed tweezers. The membranes obtained were placed on a sterile metal surface.

To gently compress the L-PRF clots by gravity, a light metal plate and the compression box (e.g., Xpression™ Intra-Lock, Boca Raton, FL, USA) were used. The L-PRF membranes could be used after five minutes. The L-PRF membranes remained viable for at least 3 h, as long as they were kept covered in the compression box, preventing them from drying out (NR et al., 2017; M. Quirynen et al., 2024). Efforts were made to minimize the time between production and use.

RELATIVE CENTRIFUGAL FORCES TO OBTAIN L-PRF

A protocol of 2700RPM for 12 minutes (RCF-clot = 408g) was used to produce L-PRF membranes with leukocytes and platelets. L-PRF membranes were produced utilizing an



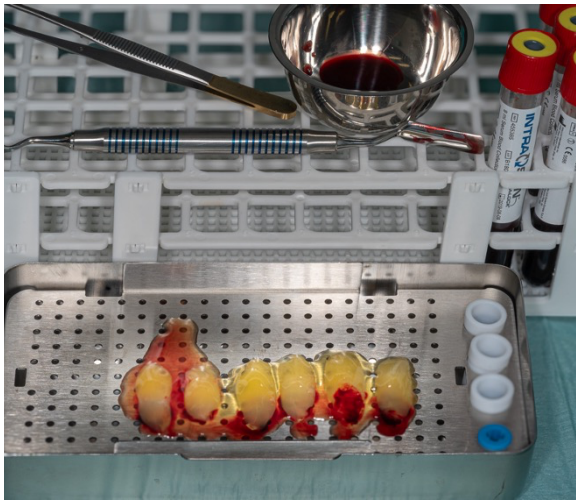


Figure 3 - Centrifugation and preparation of blood clot (Created by author, 2024)

Intraspin ™ . Miron (Miron, Pinto, et al., 2019) highlighted a minimum of 6 criteria that must be reported in all studies related to PRF to increase the transparency and comparability, of research data in publications. In Table 12 we report that.

Table 12- Miron`s Report Criteria for L-PRF studies (Template from Miron, (Miron, Pinto, et al., 2019) data from the author, 2024)

TECHNICAL CONSIDERATIONS FOR PRODUCTION OF L-PRF IN OUR STUDY		
1	DIMENSIONS OF THE ROTOR (RADIUS AT THE CLOT AND END OF THE TUBE)	50 mm-80 mm
2	ROTOR ANGULATION FOR THE TUBE HOLDER	33°
3	REVOLUTIONS PER MINUTE (RPM) AND TIME	2700 RPMx12 mints
4	RCF VALUE CALCULATED AT EITHER THE RCF-MIN, RCF-CLOT OR RCF-MAX	RCF-clot = 408g
5	COMPOSITION AND SIZE OF TUBES UTILIZED TO PRODUCE PRF	9 mL glass-coated plastic tubes (Intra-Lock)
6	CENTRIFUGATION MODEL UTILIZED	Intra-Lock®, Boca Raton, Florida, USA

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SURGICAL PROCEDURE

In case of CEJ undetectable or with 'step', CEJ restoration (G. Prato et al., 2010) was performed prior to surgical procedure and digital scan (T_0).

The coronally advanced flap (CAF) technique for multiple gingival recessions, as described by de Sanctis and Zucchelli (G. Zucchelli & Sanctis, 2000), is a minimally invasive surgical procedure with intrasulcular incisions around the affected teeth and horizontal incisions in the interdental areas, preserving the papillae. A split-full-split thickness envelope flap is then elevated, with split-thickness dissection in the papillae and beyond the mucogingival junction, and full-thickness at the recession level This technique avoids vertical releasing incisions, which contributes to better blood supply, improved esthetics, and a more comfortable postoperative experience for the patient (Stefanini et al., 2020). All surgeries (both groups) were performed using microsurgery principles, with the use of magnification, (loupes), illumination and micro instruments (Gianfilippo et al., 2021).

SURGICAL STEP BY STEP

The anesthetic was applied to the intervened area, and a coronal advance flap was created, according to the technique described by Zucchelli (G. Zucchelli & Sanctis, 2000).

After local anesthesia, a horizontal incision was made delimitating the coronal part of flap. This horizontal incision of the envelope flap consists of an oblique submarginal incision in the interdental areas that create a surgical papilla (Fig. 4)

The incisions continue with the intrasulcular incision in the recession defects (Fig.5) and

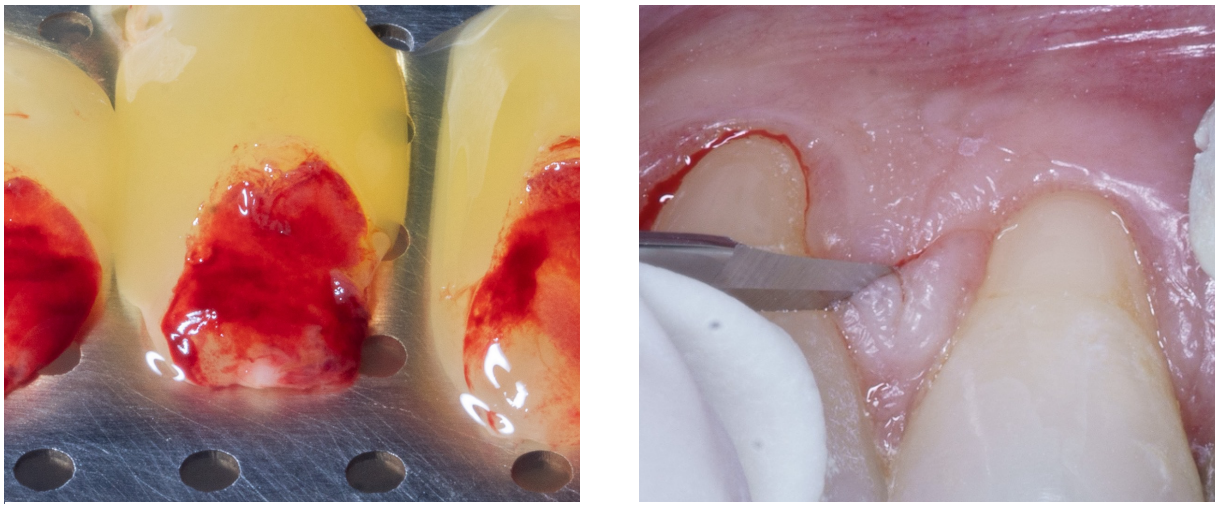


Figure 4 - L-PRF and incision to create the "surgical papilla" (Created by author,2024)

are extended to form an envelope flap between central and molar. This design facilitates the planned coronal repositioning of the flap tissue on the exposed root surfaces.

The envelope flap is raised with a split-full-split approach in the coronal-apical direction, and the oblique interdental incisions are performed, keeping the blade parallel to the long axis of the teeth to dissect the surgical papilla in a split thickness manner (Fig.5).

The apical gingival tissue at root exposures is raised to a full thickness to provide the critical part of the flap for root coverage with more thickness. Finally, the most apical portion of the flap is elevated in a split thickness to facilitate coronal displacement of the flap (Fig.6).

USC DE COMPOSTELA

At limit of flap (mesial and distal) a hemi-tunnelization was performed to assure release of tension and better mobilization of the flap at extremities.



Figure 6 -Envelope flap with horizontal incisions and split flap preparation.
(Created by author, 2024)

Then a sharp dissection was performed in the mucosa of the vestibular lining to eliminate muscle tension (Fig. 8). It must be considered that the "adequate" coronal displacement of the flap results from the elimination of muscle and lip tensions in the apical part of the flap. (G. Zucchelli & Sanctis, 2000). This way, the flap design incorporates a wide base to ensure adequate blood supply, with the base of the flap being wider than the coronal portion to promote better vascularization and healing. The flap's length should be sufficient to cover the recession defects fully and extend coronally beyond the CEJ of the involved teeth, ensuring complete coverage of the exposed roots and promoting optimal healing.

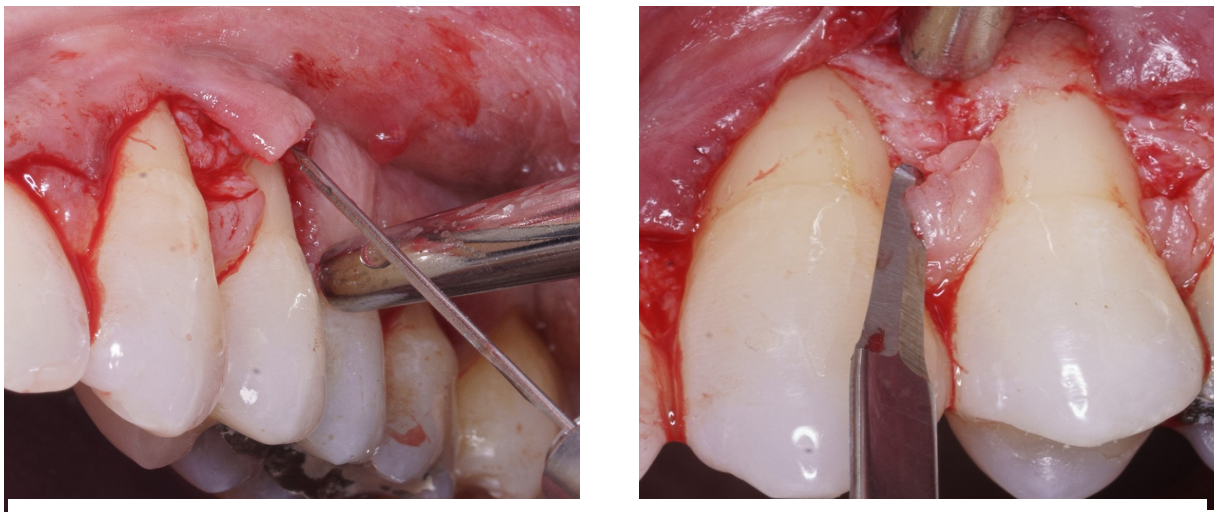


Figure 5 - Coronal incision for liberation of flap and de-epithelization of papillae.
(Created by author, 2024

The epithelium is removed from the anatomical papillae to allow them to fit the surgical papillae at the time of suture (Fig.6). Then, root cleaning is performed with manual Mini-Gracey curettes (LM®, Finland)

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In the CAF+L-PRF group, ideal 6 (minimum 4) L-PRF membranes (with dimensions of receptor bed) are fixed together, with resorbable suture seven zeros (Novosyn®, BBraun, Melsungen, Germany) and adapted to the root surface at CEJ level with face of the inner membrane towards the recession.

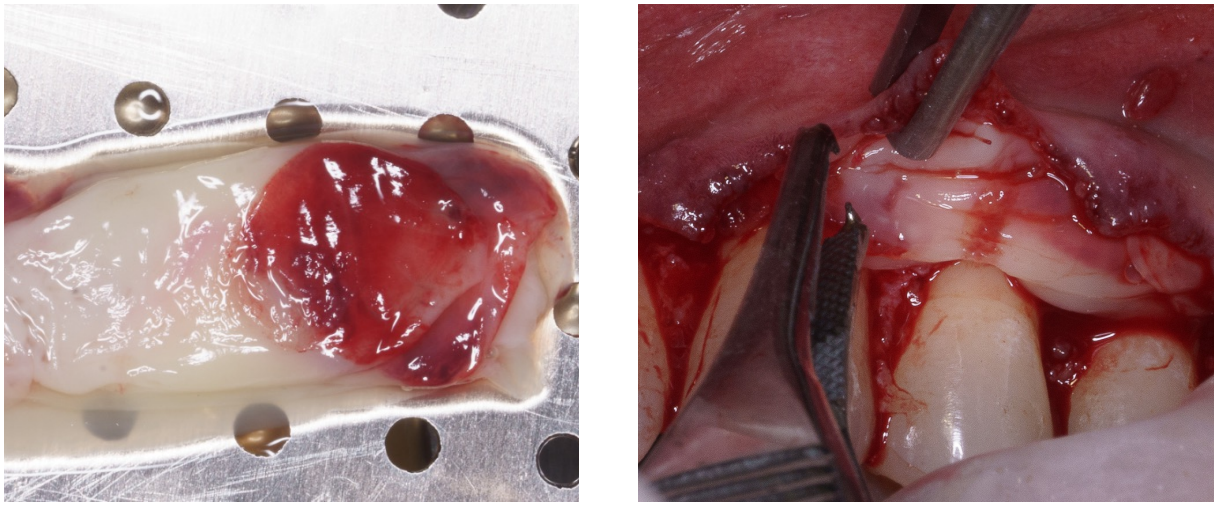


Figure 8- L-PRF membranes sutured together, and placement of membranes.
(Created by author, 2024)

The flap is then repositioned. Each surgical papilla, that was dislocated surgically (with respect to the anatomic papilla by the oblique submarginal interdental incisions), are now placed over the de-epithelialized papilla, promoting a rotation of the flap toward the canine and in coronal direction (Fig.8). One of key factors for recession coverage techniques is ensuring that the flap can be advanced coronally without any tension (Burkhardt & Lang, 2010; Mörmann & Ciancio, 1977; G. P. Prato et al., 2000). So, if considered necessary, when

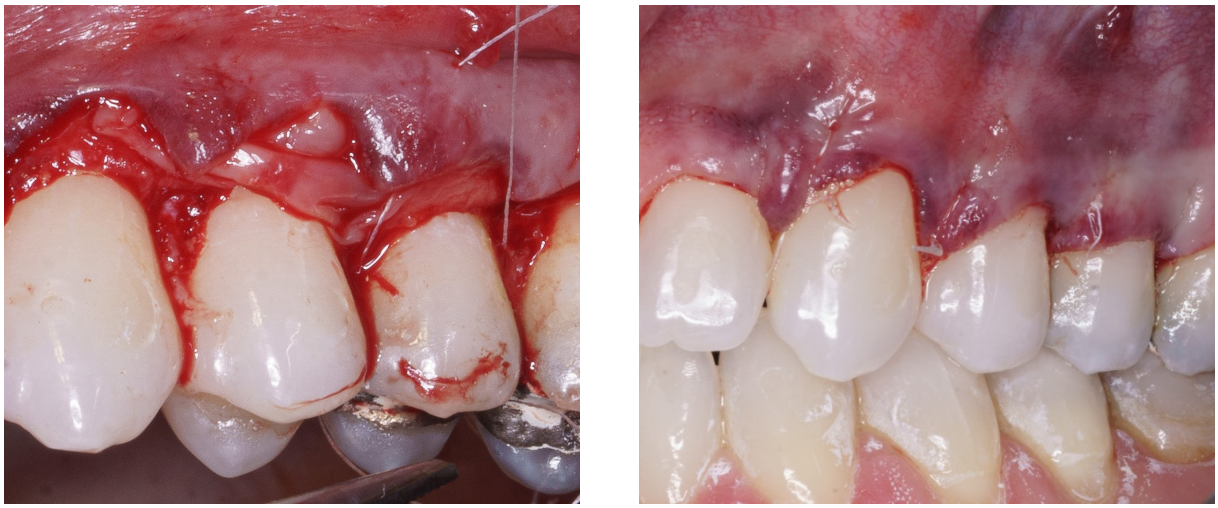


Figure 7 - Position and suture of flap with single sling suture and PTFe.
(Created by author, 2024)

verifying the reposition of the flap, additional partial-thickness dissection apically was performed to relieve tension and allow for proper positioning of the flap before suture.

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After repositioning the flap, it was stabilized with a suspended suture (M. Quirynen et al., 2024). Sling sutures, with PTFE six zeros (Elasyn®, BBraun, Melsungen, Germany) were used to precisely adapt the buccal flap on the exposed root surfaces and stabilize every surgical papilla (Fig.10)

To assure standardization of the technique, and less variability, we always performed the flap from central to first molar (no vertical incisions), even if there no recession at level of lateral or central.

NUMBER OF MEMBRANES USED

Instead of using one PRF membrane/graft over the teeth like in standard grafting, using more L-PRF membranes in recession coverage is suggested (M. Quirynen et al., 2024).

PRF and conventional graft materials used in recession coverage work through different mechanisms; SCTGs work in a way that the connective tissue of the graft influences the tissue phenotype of the surrounding tissue (Karring et al., 1975). So, when using SCTG, one ”membrane” is enough. On the other hand, PRF is a biological fibrin matrix. It helps cover exposed roots by promoting and inducing angiogenesis and new tissue at the site of recession. PRF seems to capitalize on the increase in growth factors and leukocytes (Wong, 2019). In theory, increasing the number of membranes used, increases the number of growth factors (GF) released in wound healing.

There is a lack of evidence regarding the ideal number of membranes to use in recession coverage. Pinto, in 'Guidelines for the use of L-PRF flow charts: Step by Step Approach Leucocytes and Platelet Rich Fibrin in Different Intraoral Applications Applying the IntraSpin ™ Concept” (NR et al., 2017) advise the use of 'minimal' two to three L-PRF membranes (with correct dimension) sutured together associated with CAF. Aalam recommends using three to four membranes for every two teeth using a VISTA approach (Aalam et al., 2017). In the most recent instructions, Quiryen suggests “at least two and preferably three or four” (M. Quirynen et al., 2024) .Using more PRF membranes per tooth seems to be a tendency.

In a recent review, Tavelli (Lorenzo Tavelli, McGuire, et al., 2019) summarized that platelet concentrates have negligible enrichment of growth factors (GF) levels and do not show significant improvements in wound healing and root coverage. Speculating that can be a problem of a “dose-response relationship.”

The only known study by the authors that studies this variable is from Culhaoglu and colleagues. They conducted an RCT to assess the impact of PRF combined with CAF in the treatment of gingival recession. They compared two groups that used different numbers of membranes (2 and 4) with the CTG control group. They reported better outcomes within the group with more membranes for the procedure. That result implies that the effect of using PRF membranes may depend on the dose (Culhaoglu et al., 2018)

Therefore, we join 4 to six membranes with resorbable 7-0 sutures to obtain a thickness of 2 to 3 mm at most (Fig. 9). We believe that this is the optimal number of membranes we can apply without raising the flap tension while maintaining a satisfactory 3d shape in terms of thickness.

TECHNICAL VARIATIONS AND METHODOLOGICAL CONSIDERATIONS

Regarding the technique, in cases involving multiple recessions, it was necessary to adapt the technique to facilitate surgical feasibility. According to the literature, the placement of the L-PRF membrane is suggested to be placed on the receptor bed and over the recession (with the face portion of the deepest membrane oriented toward the exposed root) (M. Quirynen et al., 2024). As previously described, the membranes were sutured together in groups of 4 to 6

with 7-0 absorbable sutures (Fig 9), but to ensure that the membranes covered the maximum number of recessions, they were sutured together with half of the membrane 'faces' oriented distally and the other half oriented mesial. After that, the membranes were positioned horizontally, parallel to the flap over the recession, in a similar manner to what is done with a connective tissue graft. If the membranes did not cover all the recessions, they were preferentially placed over the deepest recessions. Efforts were made to ensure that the innermost membrane had its face in contact with the largest recession.

From the description, we can infer that not all recessions included in the test group were sutured in direct contact with the membranes. However, from a methodological perspective, we consider it plausible that the potential effect of the membranes will be observed not only in areas in direct contact with the membrane but throughout the entire flap extension.

3.5.1.2 CONTROL GROUP (CAF)

The intervention and remain protocol post-surgical are similar except for the use of L-PRF membranes during surgery.

3.5.2 SIMILARITY OF INTERVENTIONS

The similarity of interventions in this randomized controlled trial is highlighted by the common utilization of the Coronal Advanced Flap (CAF) technique in both the control and test groups. Both groups aim to achieve root coverage and improve tissue thickness, targeting similar patient demographics, including gender and age at surgery. The type of tooth treated and initial clinical characteristics, such as recession and keratinized tissue width (KTW) at baseline, are comparable across both groups. The control group employs the CAF technique alone, serving as an intervention benchmark, while the test group combines CAF with Leukocyte-Platelet Rich Fibrin (L-PRF) to potentially enhance outcomes. Both groups undergo identical evaluations, including changes in KTW, percentage of root coverage at six months, and gains in tissue thickness and volume. These similarities in patient selection, surgical approach, and evaluation criteria ensure a robust comparison between CAF and CAF + L-PRF, allowing for a clear assessment of the additional benefits provided by L-PRF in enhancing root coverage and tissue regeneration.

3.5.3 POST-OPERATIVE MONITORIZATION

At the day of surgery each patient received a written document with detailed post-operative instructions (Appendix V).

Participants were monitored at 5 days, 14 days, 6 weeks, 3 months and 6 months.

3.5.3.1 POSTSURGICAL INFECTION CONTROL AND PLAQUE CONTROL

Post-operative pain and edema were controlled with ibuprofen. Patients were instructed not to brush their teeth in the treated area but to rinse their mouths with chlorhexidine solution (0.12%) twice daily for 1 minute.

Fourteen days after the surgical treatment, the sutures were removed.

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Plaque control in the surgically treated area were maintained by chlorhexidine in the first two weeks. After this period, the patients were again instructed in mechanical tooth cleaning of the treated region using a post-surgical toothbrush and a roll technique.

All patients were recalled for prophylaxis, with an air-polishing device, at 6 weeks and three months after intervention. At 6 months data collection was performed according to protocol

3.6 VARIABLES

3.6.1 PERCENTAGE (%) OF ROOT COVERAGE (%RC) AT SIX MONTHS (PRIMARY OUTCOME)

The primary outcome of this trial was the percentage of root coverage achieved at six months post-surgery. This outcome was chosen due to its high clinical relevance and direct impact on patient care. Clinically, the percentage of root coverage is a crucial measure of success in periodontal surgical procedures aimed at treating gingival recession. It indicates how effectively the exposed root surface has been covered by gingival tissue.

The objective is to evaluate whether L-PRF, combined with the coronal advancement flap for root coverage in Miller Class 1 recessions in upper front teeth, will show any significant clinical difference in the root coverage percentage compared to the coronal advancement flap alone. We used two methods to measure the percentual of root coverage: one based on the changes in recession depth (standard), and one based on the changes in recession area for comparison.

This metric represents the exact proportion of the exposed root surface successfully covered by gingival tissue after surgery, expressed as a percentage, standardizing the results in different patients with varying initial recession depths, making it easier to compare the results. Provides a clear and easily understandable metric for both clinicians and patients. However, it ignores absolute values: It does not provide information on the absolute amount of root coverage achieved, which can be clinically significant.

% OF ROOT COVERAGE IN TERMS OF RECESSION DEPTH (RE)

$$\%\mathrm{RC} = \left(\frac{\mathrm{Re(T0)} - \mathrm{Re(Tf)}}{\mathrm{Re(T0)}}\right) \mathrm{x}100$$

This measure is widely accepted in scientific community as a valid and important outcome in root coverage research, forming a robust basis for evaluating new treatments and techniques in clinical trials

% OF ROOT COVERAGE IN TERMS OF RECESSION AREA (REAREA)

The percentage of root coverage in relation to recession area (ReArea) served as a supplementary metric. This parameter considers the total area of recession not just the depth (vertical) or the width (horizontal) of recession. Utilizing digital imaging and software analysis, we can go beyond linear measurements and achieve detailed assessments of area, volume, shape, texture, and temporal changes, enhancing the depth of analytical capabilities.

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$$= \%\text{RCArea} = \left(\frac{\text{ReArea(T0)} - \text{ReArea(Tf)}}{\text{ReArea(T0)}}\right)\text{x}100$$

Although area of root coverage can provide additional information, the use of vertical recession depth was favored as the primary result for its simplicity, clinical relevance, practicality and consistency in the reporting results. This approach ensures that the results are easily interpreted and comparable in different studies. The depth and area of the recession were measured digitally, as discussed next in material and methods.

3.6.2 COMPLETE AND MEAN ROOT COVERAGE AT SIX MONTHS

3.6.2.1 % OF COMPLETE ROOT COVERAGE (%CRC)

Complete root coverage indicates that the gingival margin has been successfully repositioned to cover the previously exposed root surface, which is often the primary goal of these treatments. Provides a clear binary outcome (complete vs. incomplete), making it easy to interpret. This measurement is directly correlated with patient satisfaction. Patients typically seek treatment for gingival recession to address aesthetic and sensitivity issues. Patients may only be satisfied when they achieve total coverage of the root surface. It's expressed as a frequency percentage, describing the relative occurrence of complete root coverage within each group.

$$CRC = \left(\frac{Frequency\ of\ 100\%\ RC}{total\ number\ of\ observations}\right)x100$$

It represents the "perfect" result in terms of root coverage

3.6.2.2 MEAN ROOT COVERAGE (MRC)

This metric represents the average root coverage achieved in a sample or study group, expressed in absolute terms (e.g., millimeters). It is an absolute measurement that provides information about the amount of root coverage achieved, which can be clinically important. It allows a detailed insight to become useful for understanding the magnitude of improvement in root coverage. However, variability in the depths of the initial recession can affect his results, making comparisons between patients or studies more difficult without additional context.

$$\text{MRC} = \bar{\text{x}}\ \text{Re (T0)} - \bar{\text{x}}\ \text{Re (Tf)}$$

3.6.3 KERATINIZED TISSUE WIDTH GAIN (ΔKTW)

The gain of keratinized tissue (ΔKTW) is a critical outcome in periodontal surgical procedures aimed at recession coverage due to its substantial impact on periodontal health, treatment stability, and patient satisfaction. Keratinized tissue, characterized by its dense and fibrous nature, plays a crucial role in maintaining gingival health and function. Keratinized tissue provides a more robust barrier against mechanical trauma from brushing and chewing, as well as bacterial invasion (Lang & Löe, 1972). This variable was obtained by a difference keratinized width at T_0 and keratinized tissue width at 6 months. The measurements were obtained by digital measurement (Lee et al., 2020).

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$$\Delta KTW = (KTW\ (T_f)) - (KTW\ (T_0))$$

3.6.4 THICKNESS GAIN (Δ GT)

The gain (Δ) of gingival tissue thickness (GT) is crucial in periodontal surgical procedures aimed at root coverage due to its significant impact on the long-term stability of the gingival tissues, patient comfort, and aesthetic outcomes. Thicker gingival tissues enhance the stability and resilience of the gingival margin, reducing susceptibility to mechanical trauma and bacterial invasion, thus preventing future recession and ensuring the longevity of treatment outcomes (G. Zucchelli & Sanctis, 2000)

This metrics were obtained by superimpose presurgical and postoperative digital models and posterior 3D comparison (Fons-Badal et al., 2020; T. Marques et al., 2021), as will be discussed in material and methods. In terms of Gingival thickness gain (ΔGT) we evaluate :

- Maximum gain in gingival thickness (maxΔGT) – at one region of interest ($ROI_{(1)}$)
- Mean gain in gingival thickness (meanΔGT)- at one region of interest ($ROI_{(1)}$)
- Maximum tissue thickness at recover recession ($maxΔGT_{(r)}$)-($ROI_{(2)}$)
- Mean thickness of tissue at recover recession ($meanΔGT_{(r)}$ -$ROI_{(2)}$

$$\Delta Vol = meanΔGT_{(R)} X\ ROI_{(2)}\ Area$$

This last variable was obtained by indirectly multiplying the newly recovered area of recession ($ROI_{(2)}$ Area), at 6 months, by the mean thickness in the same area.

SUMMARIZING TABLE

Table 13- Abbreviation of variables, formulas and Units used
(Data from author)

ABBREV	DEFINITION	FORMULA	UNITS	OUTPUT
%RC	% of root coverage (depth)	$\%RC = \left(\frac{Re(T0) - Re(Tf)}{Re(T0)}\right) x100$	%	Recession cover outcomes (Different measures for the same)
%RC AREA	% of root coverage (Area)	$\%RCArea = \left(\frac{ReArea(T0) - ReArea(Tf)}{ReArea(T0)}\right) x100$	%	
CRC OR %CRC	Complete root coverage frequency	$CRC = \left(\frac{Frequency\ of\ 100\%\ RC}{total\ number\ of\ observations}\right) x100$	frequency %	
MRC	Mean Root Coverage (expressed in mm)	$MRC = \bar{x}\ Re\ (T0) - \bar{x}\ Re\ (Tf)$	mm	
$ROI_{(2)}$ AREA	Area of Recession covered	Digital Direct measurement	mm^2	Volumetric evaluations. Thickness increase in different regions
MAXΔGT	Maximum gingival thickness gain (at $ROI_{(1)}$)	Digital Direct measurement	mm	
MEANΔGT	Mean thickness of tissue at ($ROI_{(1)}$)	Digital Direct measurement	mm	

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$MAX\Delta GT_{(R)}$	Maximum thickness of tissue over the denuded root ($ROI_{(2)}$)	Digital Direct measurement	mm	of interest from T_0 to T_f
$MEAN\Delta GT_{(R)}$	Mean thickness of tissue over the denuded root ($ROI_{(2)}$)	Digital Direct measurement	mm	
ΔVOL	Volume gain (at $ROI_{(2)}$) expressed in mm^3	$\Delta Vol= \bar{x}\ \Delta GT_{(R)} X\ ROI_{(2)}$ Area	mm^3	
ΔKTW	Keratinized tissue width gains between T_F and T_0 (expressed in mm)	$\Delta KTW =(KTW\ (T_f))- (KTW\ (T_0))$	mm	Gingival "quality" outcome

3.6.5 ANALYZE OF PAIN AND HEALING

3.6.5.1 HEALING (IPR SCALE)

Different measures have been applied to assess soft tissue wound recovery after surgery (Pippi, 2017). An examination of existing wound healing scales in oral surgery shows that they have low use, and their structure does not match the stages of the wound healing process (Hamzani & Chaushu, 2018). Because of that, we intend to use a recently proposed novel scale: the IPR (I = inflammatory, P = proliferative, R = remodeling) wound healing scale. This newer scale, already used in implant dentistry, is better for clinical research to evaluate new products that can affect wound healing results. (Hamzani & Chaushu, 2018). Pain is subjectively assessed by the patient and all other parameters are evaluated by an oral surgeon who is not the operating surgeon.

The inflammatory phase is evaluated 3-5 days after tissue injury based on eight parameters, measured on a 9-point scale (0-8): bleeding (spontaneously or in palpation), granulation tissue, hematoma, tissue color, incision margins, suppuration, edema, and pain. A score of 5-8 indicates a successful inflammatory phase.

The proliferative phase is evaluated 14 days after tissue injury based on five parameters, measured on a 6-point scale (0-5): re-epithelialization, tissue color, scar, suppuration, and pain. A score of 3-5 indicates successful healing.

The remodeling phase is evaluated six weeks after tissue injury based on three parameters, measured on a 4-point scale (0-3): scar, tissue color, and pain. A score of 2-3 indicates successful healing. The total score on the IPR scale ranges from 0 to 16:0-4, indicating poor healing; 5-10, acceptable healing; and 11-16, excellent healing.

The inflammatory phase has the most significant weight in the final score because its impairment can jeopardize the entire series of biological events that lead to early wound healing. The remodeling phase carries the lowest weight because it can spread over several years and involves maintaining a biological balance between matrix degradation and formation, which occurs after wound closure.

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Table 14 - Inflammatory, proliferative, and remodeling scale (IPR).
(Adapted from Hamzami (Hamzani & Chaushu, 2018))

PHASE	TIME	PARAMETER	SCORE 0	SCORE 1	SCORE/TOTAL
INFLAMMATORY	T: 3–5 days	Bleeding	Yes	No	0-8/16
		Granulation tissue	Yes	No	
		Hematoma	Yes	No	
		Tissue color	Redder or whiter than opposite side tissue	Like the opposite side tissue	
		Incision margins	Incomplete flap closure/ fibrin clot/ partial necrosis / complete necrosis	Complete flap closure/ fine fibrin line	
		Suppuration	Yes	No	
		Edema VAS (1-10)	6-10	1-5	
		Pain VAS (1-10)	VAS 6-10	VAS 1-5	
PROLIFERATIVE	T: 14 days	Re-epithelialization	Partial	Complete	0-5/16
		Tissue color	Redder or whiter than opposite side tissue	Like the opposite side tissue	
		Scar	Scar wider than 2 mm/ contour irregularity	No scar/ scar lesser than 2 mm/contour regularity	
		Suppuration	Yes	No	
		Pain VAS (1–10)	VAS 6–10	VAS 1–5	
REMODELING	T: 6 weeks	Scar	Scar wider than 2 mm/ contour irregularity	No scar/ scar lesser than 2 mm/contour regularity	0-3/16
		Tissue color	Redder or whiter than opposite side tissue	Like the opposite side tissue	
		Pain VAS (1–10)	VAS 6-10	VAS 1-5	

Table 15-IPR SCALE.
(Adapted from Hamzami (Hamzani & Chaushu, 2018)

IPR SCALE TOTAL	HEALING QUALITY
0-4	Poor healing
5-10	Acceptable healing
11-16	Excellent healing

3.6.6 PAIN, INDIRECT EVALUATION

Postoperative pain was evaluated based on, mean consumption (in mg) of analgesics (ibuprofen).

According to protocol, patients received 600 mg at the beginning of the surgical procedure. Subsequent doses were taken only, if necessary, to control pain. The number of pills was recorded by the patient at home. The number of pills taken was reported to us at 5 days follow-up and 14 days follow-up (Giovanni Zucchelli et al., 2010)

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PAIN, SELF-REPORTED (VAS QUESTIONNAIRE)

To measure the experience of self-reported pain, we used a questionnaire. The questionnaire asks about the strength of the pain event on a visual analog scale (VAS) of 10 (McCormack et al.,(McCormack et al., 1998; Giovanni Zucchelli et al., 2010) .

The VAS score was recorded at 5 days.

3.6.7 (PRO AND PROMS) SUBJECTIVE EVALUATION OF RESULTS BY THE PATIENT

A way to measure the quality of care and the effectiveness of root coverage procedures from the patient's point of view is to use PROs (patient-reported outcomes) by means of patient-reported outcome measures PROMs. PROMs can be questionnaires that ask the patient about their health status, experience, satisfaction, and expectations before and after treatment. Using PROMS, clinicians can understand the patient's needs, preferences, and values and assess the effect of their interventions on oral health and quality of life. PROMs can also help improve clinician and patient communication and increase shared decision making and patient participation in their care. There is a discrepancy between how the clinician and the patient perceive the success of a root coverage procedure. The patient's satisfaction with the aesthetic outcome did not always match the professional scoring (S. Kim et al., 2014). By collecting data directly from patients, healthcare professionals can find areas to improve quality of care and make changes based on actual patient feedback.

3.6.7.1 PATIENT-CENTERED AESTHETIC AND OVERALL SATISFACTION EVALUATION

Various objective and reproducible parameters (complete root coverage, root coverage, recession reduction, keratinized tissue increase) have been used to evaluate different surgical techniques, but are frequently ignored patient concerns and, hence, the true indications for treatment (Mounssif et al., 2018). The most recent consensus of the European Federation of Periodontology (Tonetti & Jepsen, 2014) pointed out the need to include patient perception and requests in future research to align better professional (surrogate) endpoints with patient-centered outcomes (true endpoints) (Hujoel, 2004).

Participants were asked to score their results among 10 (0 indicating very bad, five average and ten indicating excellent) on a VAS scale in terms of color match, amount of root coverage, and overall satisfaction (Stefanini et al., 2016, 2021; Giovanni Zucchelli, Mounssif, Mazzotti, Montebugnoli, et al., 2014).

Table 16-Patient Related Outcomes (PROMs) in esthetic and overall experience
(Adapted from Zuchelli, (Stefanini et al., 2016, 2021; Giovanni Zucchelli, Mounssif, Mazzotti, Montebugnoli, et al., 2014))

ASPECT	SCALE	MEANING
Color match	0-10	0: very bad, 5: average, 10: Excellent
Amount of root coverage	0-10	
Overall satisfaction	0-10	
Willingness to repeat	Yes, vs. No	

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WILLINGNESS TO REPEAT SURGERY

We evaluate the 'willingness to repeat surgery. 'Willingness to repeat' in the context of surgical evaluation means how ready the patient is to have the same surgery again based on their first surgical experience. This metric is important because it shows how satisfied the patient is with the whole surgical process, the results, pain control, recovery time, and the effectiveness of the procedure (Yuan et al., 2022).

3.6.7.2 COMPARISON OF RESULTS IN TERMS OF THE TREATMENT OF CERVICAL DENTIN HYPERSENSITIVITY

Cervical dentin hypersensitivity (CDH) is characterized by tooth pain that arises from exposure to the roots. Dentin hypersensitivity is a persistent clinical problem that poses a significant challenge for clinicians and affects the quality of life of patients (Zeola et al., 2019). CDH is often associated with gingival recession and is a common reason for treatment. However, there is a lack of reported evaluation after periodontal surgery.

A decrease in CDH was observed after periodontal surgery for root coverage, but there is not enough evidence to conclude that surgical root coverage procedures predictably reduce CDH (Oliveira et al., 2013). We evaluated the difference between the two groups, in terms of CDH, using a generic, self-reported patient evaluation from a PROM perspective (Appendix 10). We used a dichotomic question, increased/maintained versus Decreased/absent.

Table 17-PROMs in terms of Cervical Dentin Hypersensitivity (CDH)
(Data from author, 2024)

ASPECT	SCALE
CERVICAL DENTIN HYPERSENSITIVITY	Increased/maintained vs Decreased/absent

3.6.8 DATA COLLECTION

3.6.8.1 CLINICAL MEASUREMENTS METHODS

As referred before, clinical measurement were part of assessment of eligibility and selection data form before intervention.

At time T_0 the necessary clinical measurements were performed to verify the eligibility of the participants in the clinical trial. Pocket depth (PD) ≤ 3 mm, CAL = 0, KTW>1,5mm, PCR (plaque)/BOP <25% were accessed.

Every patient with interproximal CAL, PD>3mm, KTW<1,5 was excluded from the study. A periodontal probe (PCP UNC 15, LM, Finland) was used, and measurements were rounded to the nearest mm.

Surface defects and gingival recession were classified according to Pini-Prato and Miller's classifications (G. Prato et al., 2010)(Miller, 1985). In case of necessity, CEJ was restored with composite resin prior to intervention, placed at a "maximum level of root coverage prediction", according to Zucchelli (Stefanini et al., 2018; G Zucchelli et al., 2006; Giovanni Zucchelli et al., 2011). De Sanctis, in the literature, suggests that this restorative treatment modality does not produce a negative effect on periodontal conditions and the amount of recession recover

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(M. de Sanctis et al., 2020). The apical margin of the restoration was considered the “new CEJ” for future recession measurements.

At baseline, we record the de gingival phenotype using the 'Probe transparency method'. This technique involves inserting a periodontal probe into the gingival sulcus and observing whether the probe is visible through the gingival tissue. If the probe is visible, the gingiva is classified as thin; if not visible, it is classified as thick (thin (<1 mm) or thick (>1 mm) (Cortellini & Bissada, 2018; Wang et al., 2022) .

3.6.8.2 DIGITAL MEASUREMENTS METHODS - DIGITAL WORKFLOW

OVERVIEW

We implemented a digital evaluation protocol using advanced digital tools and software (Medit Link and Geomagic control X) for precise measurement and analysis.

Data collection was conducted through digital imaging, capturing accurate measurements directly into the software. Analysis involved specialized algorithms and automated processes to ensure precision. The discussion of validation is included in the description of protocol, and comparison with traditional methods to confirm reliability is also discussed.

This protocol provided advantages such as reduced human error and faster data processing, though it required initial setup and training and faced limitations in complex scenarios.

CLINICAL MEASUREMENTS VS DIGITAL MEASUREMENTS

Recent studies have shown that digital measurements of gingival recession and other periodontal parameters generally exhibit higher accuracy and reliability than conventional

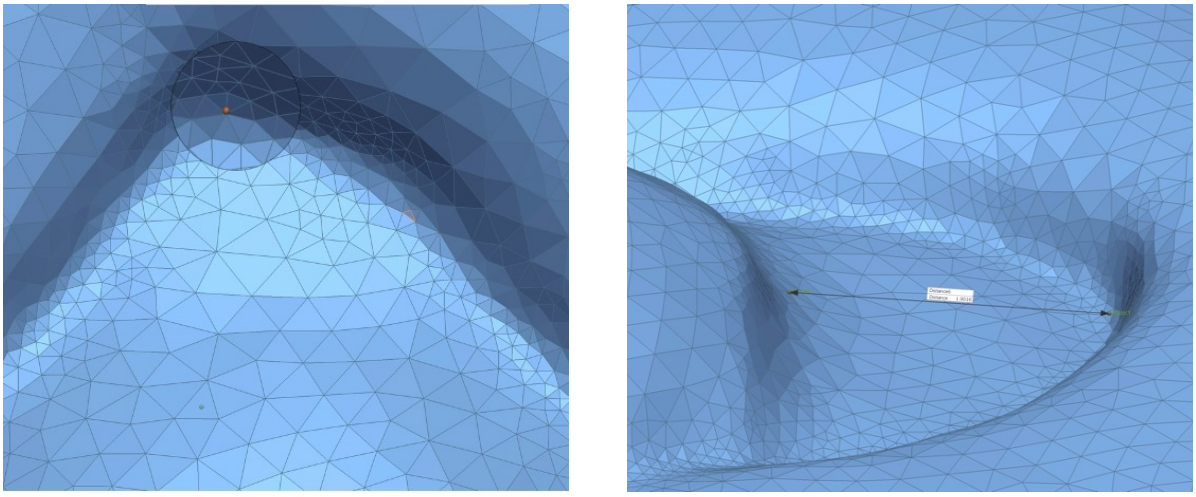


Figure 9 - Magnification and multi-angle view in definition of reference points and measurements. (Screenshot of software Magic Control X, taken by the author, 2024)

methods (Fageeh et al., 2019). Digital methods, including intraoral scanning and digital model analysis, offer significant advantages, namely, enhanced visualization, higher precision, and consequently greater reproducibility. Digital technology in addition to magnification also provides multi-angle viewing, improving the quality and repeatability of measurements (Fig 11).

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In terms of precision, measurement tools in digital software use a dimensional scale that can be 10 to 100 higher than periodontal probes (that use a mm scale) (Fig 12).

Traditionally, the evaluation of recession cover is made with clinical measurements made with a periodontal probe. However, the sensitivity of such evaluation is low because of the limitations of the periodontal probe, namely, difficult visualization and low resolution, since

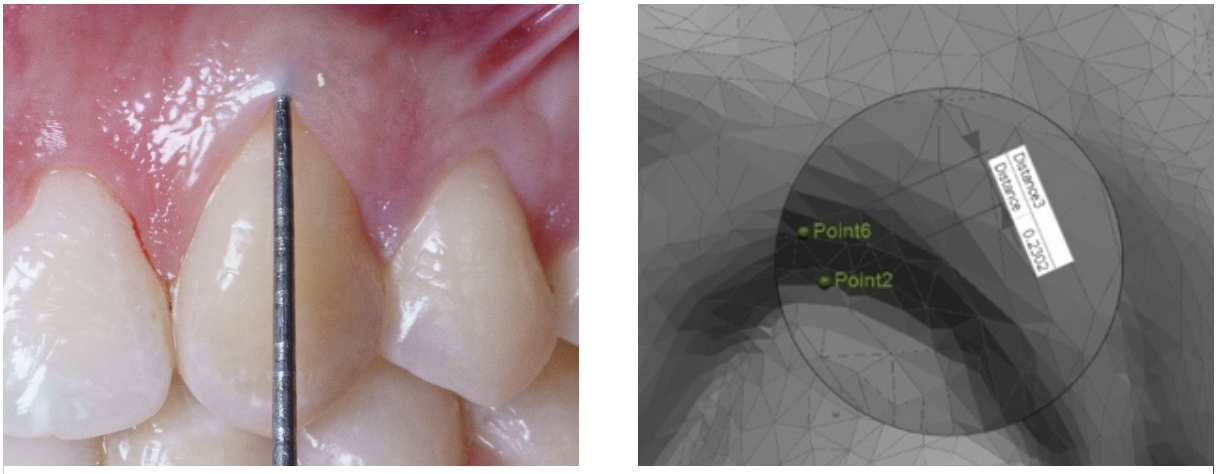


Figure 10- Periodontal probe scale vs Digital measurement scale (Created and screenshot by the author, 2024)

the measurements are rounded to the next millimeter. (Kuralt et al., 2021; Kuralt, Gašperšič, et al., 2022; Kuralt, Kučič, et al., 2022; Kuralt & Fidler, 2022).

Another interesting finding reported by Zuhr (Zuhr et al., 2021), was that when they compare clinical results with digital results, they found that clinical methods, tended to overestimate treatment outcomes. Indeed, within the same patient sample, employing the clinical method resulted in a higher percentage of root coverage compared to the digital method (Abrahamian et al., 2024; Zuhr et al., 2021).

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Furthermore, with digital method, measurements can easily discriminate minor differences.

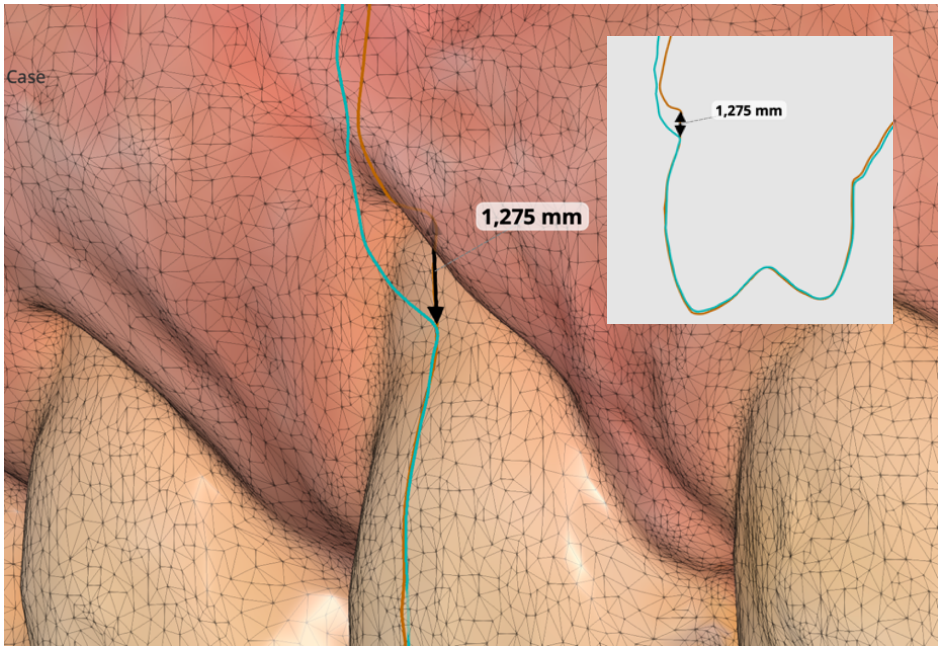


Figure 11-Measurements with superimposed digital models.
(Screenshot of software Medit Design, taken by the author, 2024)

Digital measurements enable a level of precision that is impossible with the traditional periodontal probe method. In terms of statistics, for the same sample, a scale with hundredths also gives more strength to a study than a scale with units. In summary, digital analyze allows finer resolution, lower variability, and improved ability to detect minor impacts. This increased power can be vital for drawing accurate and valid conclusions from the data.

INTER AND INTRA-EXAMINER RELIABILITY OF DIGITAL MEASUREMENTS

Digital measurements can be more reliable than conventional measurements, both within and between examiners.

Van der Velden (Velden & Vries, 1980) reported that the standard deviation of periodontal probing depths for the same examiner can be around 0.5 mm. This value may be lower for measuring dental recessions, but it is still significant.

This methodology is still evolving and progressing. The possibility to automate digital comparison will help us minimize the use of inaccurate references and improve comparability between studies. Besides, these methods, being already more precise, and with few errors, in the future, they can be improved by automation. Automation of the measurement process can reduce human mistakes, such as interpreting CEJ landmarks subjectively (Kuralt & Fidler, 2022). For instance, a major source of error and variability in "traditional "gingival recession measurements are the unreliable detection of CEJ, which can be difficult to observe visually or even a reason for exclusion if invisible (Abrahamian et al., 2024; Kuralt & Fidler, 2022; Rebele et al., 2014). In future, we can have references calculated automatically with digital approach (Kuralt & Fidler, 2022).

To address the problem of variability, in dental research, examiners undergo appropriate training and calibration, and the intraclass correlation coefficient is computed to verify reproducibility within the study. However, this process does not solve the difficulty of

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comparing measurements between different studies, with different training and calibration programs.

DIGITAL EVALUATION OF VOLUME DYNAMICS

One of the advantages of using digital approach with optical scanners for volume evaluation is that they can capture the three-dimensional shape of the gingiva with high accuracy and resolution.

Traditionally, the classical method used to measure gingival thickness was transgingival probing (Cortellini & Bissada, 2018; Wang et al., 2022). It involves inserting a periodontal probe or endodontic file through the gingival tissue until it reaches resistance from the underlying tooth or bone surface. Considered the current gold standard for measuring gingival thickness, transgingival probing is invasive and can cause patient discomfort, requiring local anesthesia. The procedure also carries a risk of damaging gingival tissue, which can lead to bleeding and potential infection. This risk is particularly concerning for patients with thin gingival phenotypes. Also, there is a potential for variability in measurements due to differences in the force applied during probing and the angle of insertion and volume increase after anesthetic injection. At the same time, caliper measurement only provides a linear approximation of tissue thickness. This probing only provides measurements at specific points, rather than a comprehensive assessment of the entire gingival area. This limitation can result in an incomplete understanding of gingival thickness in different regions. The measurement accuracy of transgingival probing is estimated to be the nearest 0.5mm (Wang et al., 2022) .

While these classical methods are still widely used, newer technologies, such as ultrasound and 3D imaging, are emerging as more accurate and less invasive alternatives for evaluating gingival thickness in recession and implants sites (Fons-Badal et al., 2020; Gil et al., 2019; Lorenzo Tavelli et al., 2021; Zuhr et al., 2020, 2021).

The 3D images obtained with optical dental scanners can be a valuable alternative to volume evaluation. This technology can superimpose 3D images with CBCT DICOM images and measure the thickness at a defined time (Couso-Queiruga et al., 2021), or superimpose two 3D images obtained at two time points and compare variations. This last option allows us to measure tissue dynamics in terms of volume, obtaining 2D (section) or 3d measurements (Alves et al., 2019; T. Marques et al., 2021, 2022), without the inconvenience of the radiation associated with CBCT scans.

Optical scanners can also avoid the risk of damaging or compressing soft tissue during the measurement process, which can affect the reliability of the results. Moreover, optical scanners can reduce intra-operator and inter-operator variability, as they do not require manual manipulation or subjective interpretation of the data (Abrahamian et al., 2024).

This digital approach can also facilitate the digital storage and post-analysis of measurements and different treatment modalities at any time in future. Therefore, optical scanners can offer a more precise, reliable, and convenient method of evaluating volume than caliper measurement.

Concluding, digital evaluation based on 3D model superimposition shows great promise in accurately quantifying volume dynamics in recession coverage studies. Although some standardization is still needed, this approach can offer improved accuracy, and novel 3D assessment capabilities, compared to traditional methods.(Abrahamian et al., 2024; Lorenzo Tavelli et al., 2021)

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In this study, we subjectively classify the thickness of the gingival in "Thin" and "thick" by probe transparency (Cortellini & Bissada, 2018) in T_0 and objectively evaluate the volumetric changes by superimposition of dental scanners in T_0 and Tf as we will describe next.

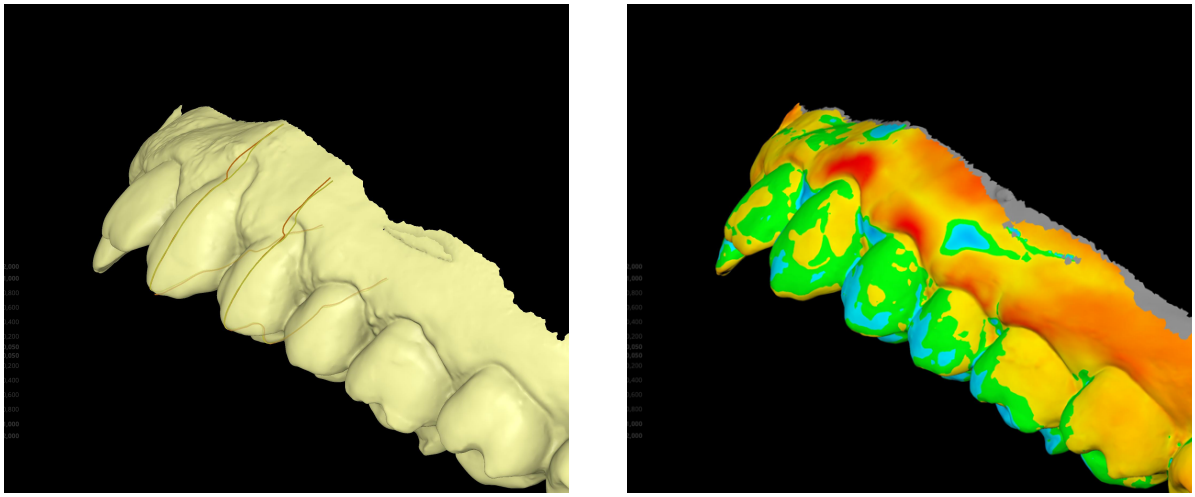


Figure 12 - Volume dinamics in terms of 2D (seccion) and 3 D evaluation (Screenshot of software Medit Design, taken by the author, 2024)

STL/PLY ACQUISITION (DIGITAL MODEL)

The STLS files or PLY were obtained directly from digital impressions using intraoral scanners (direct technique). An intra-oral scanner (MEDIT i700 ®, Seoul, South Korea) was used, and the files were acquired in Medit software, Medit Link® at T_0 and six months.

In the context of 3D files, STL stands for “Standard Tessellation Language. It is a file format native to the stereolithography CAD software created by 3D Systems. STL files describe the surface geometry of a 3D object without representing color, texture, or other common CAD model attributes. The format is widely used for 3D printing and computer-aided design (CAD). In the same context of 3D files, PLY stands for “Polygon File Format” or “Stanford Triangle Format.” It stores three-dimensional data from 3D scanners, particularly mesh models.

The format supports storing data in both ASCII and binary forms and can include details about vertices, faces, colors, standards, and other attributes. The PLY format is widely used for 3D printing, modeling, and visualization.

REPRODUCIBILITY OF DENTAL SCAN ACQUISITION

Repeated intraoral scan errors typically range from 20 to 50 micrometers (0.02 to 0.05 millimeters). This range reflects the precision and consistency that modern intraoral scanners can achieve under typical clinical conditions. However, accuracy and consequent reproducibility of intraoral scanning are influenced by various factors such as scanner selection, operator skill, calibration, patient’s oral anatomy, ambient conditions, and scanning aids (Alkadi, 2023)

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We used direct technique with intra-oral scanner, in opposition to the scan of conventional impression and posterior scan of models. This technique has been reported with high accuracy and reproducibility.

Regarding the size of the scanned area, this study includes scanning the entire maxillary arch or hemimaxilla arch with at least one tooth adjacent to each side of the recession's teeth.

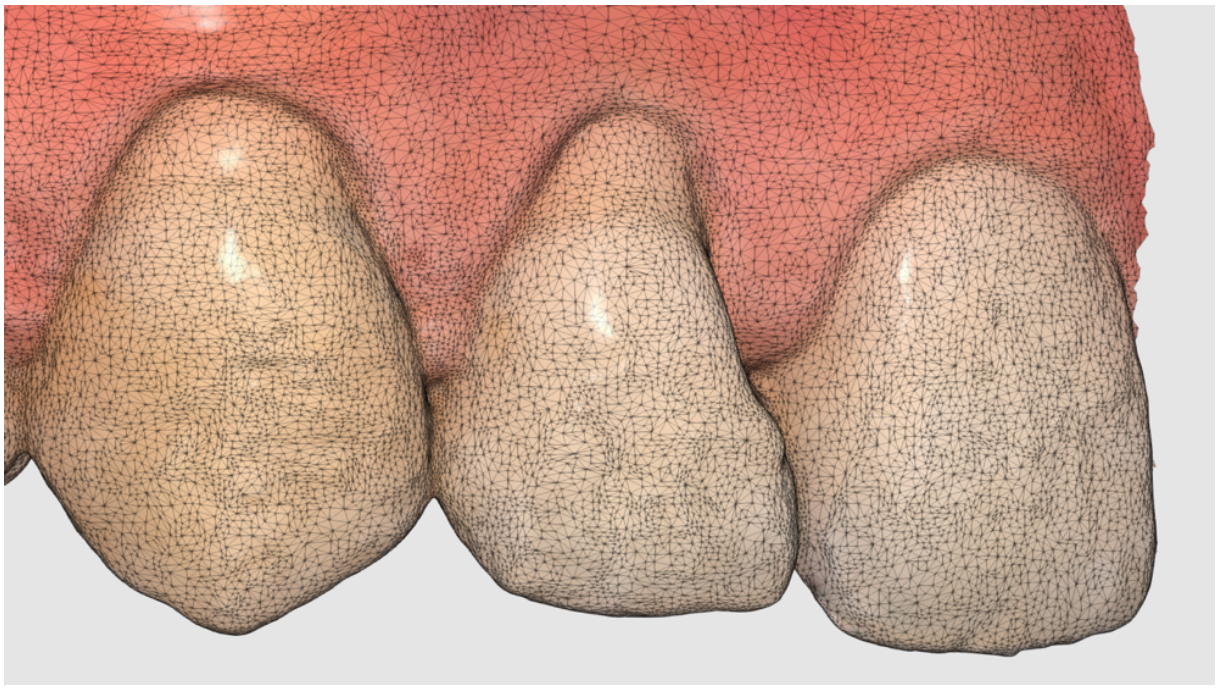


Figure 13 - Mesh structure of a PLY file, with vertices, faces and colors (Screenshot of software Geomagic control X, taken by the author, 2024)

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3.6.9 DIGITAL ASSESSMENT PROTOCOLS

The protocol for evaluation of periodontal surgery used in this work is the evolution of the protocol developed in an anterior publication by the team where the author was included and presented as a doctoral thesis in University of Salamanca by the first author Tiago Marques (T. M. S. Marques, 2022) and published an article by the same team (T. Marques et al., 2021).This protocol was recently used to measure treatment of Peri-Implant mucosa defects (Quispe-López et al., 2024)**.**

The computer program Medit Design® (Medit, Seoul, South Korea) and Geomagic Control X® (3D systems-South Carolina, USA), a widely used software for digital analyses in this domain, were used to perform the three-dimensional digital analysis and measurements of the areas that underwent intervention, based on the STL file for each case (Abrahamian et al., 2024).

For convenience, we made the measurements of Re and ReArea in Medit Design, a software for the dental field that allows compare STLs simply and intuitively. For the volumetric analysis, we use more complex software (Geomagic control X®), 3D metrology, and quality control software developed by 3D Systems. This software is designed to inspect and analyze 3D scan data to ensure the quality and accuracy of manufactured parts. In a recent review of digital methods to evaluate the healing dynamics in root coverage, Abrahamian found this software to be the most reported (Abrahamian et al., 2024).

The main ways to report how volumes change is by volume change (in mm^3), the average distance between the surfaces, and different changes in size at different planes along the root.

In this study we perform the measurement of mean difference between volumes, the max difference between volumes and calculation of volume gain, according to the follow protocol

3.6.9.1 DIGITAL PROTOCOL FOR THE LINEAR 2D MEASUREMENTS (MEDIT DESIGN).

Variables analyzed in Medit Design

The following variables were analyzed entirely digitally at T_f:

1. Initial (T_0) recession depth measured from CEJ to the gingival margin at the central buccal site. (Re T_0)
2. Area of the initial (T_0) gingival recession. Measured area between gingiva margin and CEJ (Re Area T_0)
3. Final (T 6 months) recession depth. (Re Area T_f)
4. Final (T 6 months) area reduction; ((Re Area T_f)
5. Complete root coverage for linear measurement and area measurements

STL FILE ALIGNMENT AND SUPERIMPOSITION IN MEDIT DESIGN

Imported Ply files to Medit Design, “automatic alignment,” and refinement of the alignment with “aligned select areas” > “smart teeth selection”>” apply”

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MEASUREMENTS IN MEDIT DESIGN

"Measurement mode" with the function "create sections" created a section of the tooth and tissues that intersects the zenith of the teeth, changed to sagittal view, and measured the linear variables using the function "measure between two points." The recurrence to sagittal view allows a complementary view to help identify CEJ based on curvatures (Dritsas et al., 2022;

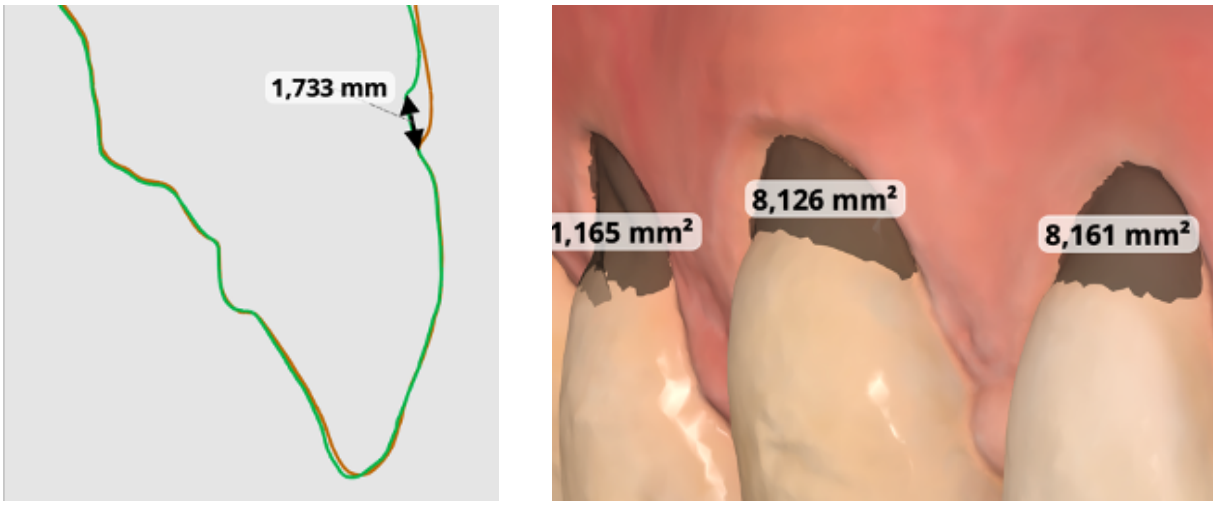


Figure 14 - Recession coverage measurements with superimposed models
(Screenshot of software Medit Design, taken by the author, 2024)

Kuralt et al., 2021). Similar methods have been reported to demonstrated accuracy and high reproducibility to record in vitro imitated gingival recessions to the nearest 0.01 mm (Lehmann et al., 2012; Zuhr et al., 2021)

For measurement of recessions area, used the function "calculate area by section" and selected the area between CEJ and marginal gingiva

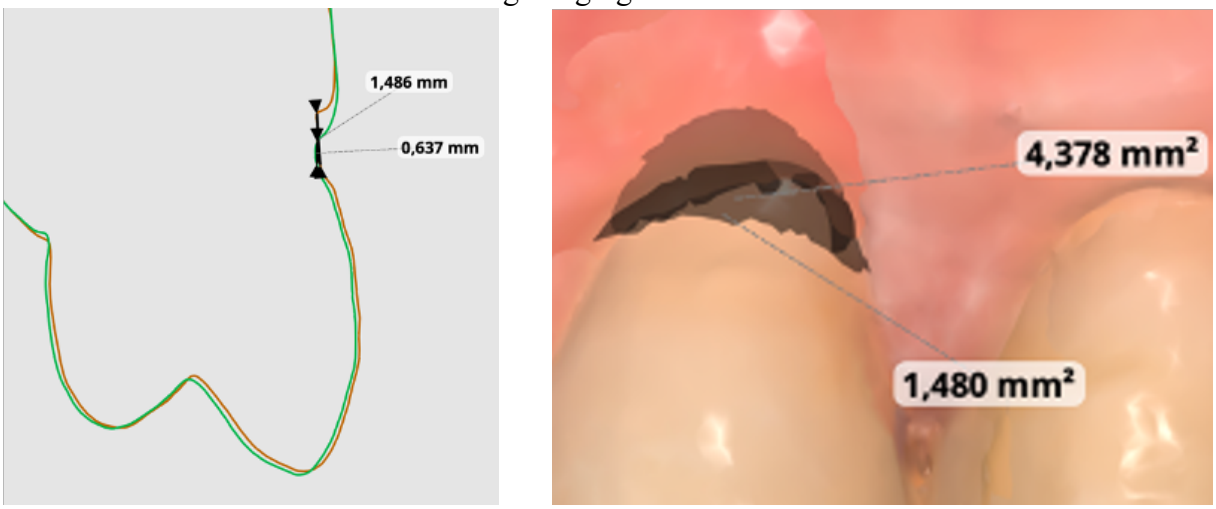


Figure 15 - Recession coverage measurements, with superimposed models, in an incomplete coverage
(Screenshot of software Medit Design, taken by the author, 2024)

All the measurements were made simultaneously (T_f) with both STL s (T_0 and T_f) already superposed. This protocol should allow us to use the same point of CEJ as a reference for both measurements, enabling more reliability between the two measurements (T_0/T_f).

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3.6.9.2 DIGITAL PROTOCOL FOR THE LINEAR 3D MEASUREMENTS (GEOMAGIC® SOFTWARE)

The following variables were analyzed entirely digitally with the Geomagic Control X software according to the protocol mentioned earlier (T. Marques et al., 2021):

1. mean gingival thickness gain at six months (meanΔGT);
2. maximum gingival thickness gain at six months (maxΔGT);
3. mean gingival thickness gain over the denuded root at six months (meanΔGT$_{(R)}$)
4. maximum thickness gain over the denuded root at six months (maxΔGT$_{(R)}$)
5. Calculation of volume over the denuded root (ROI$_{(2)}$)at six months.

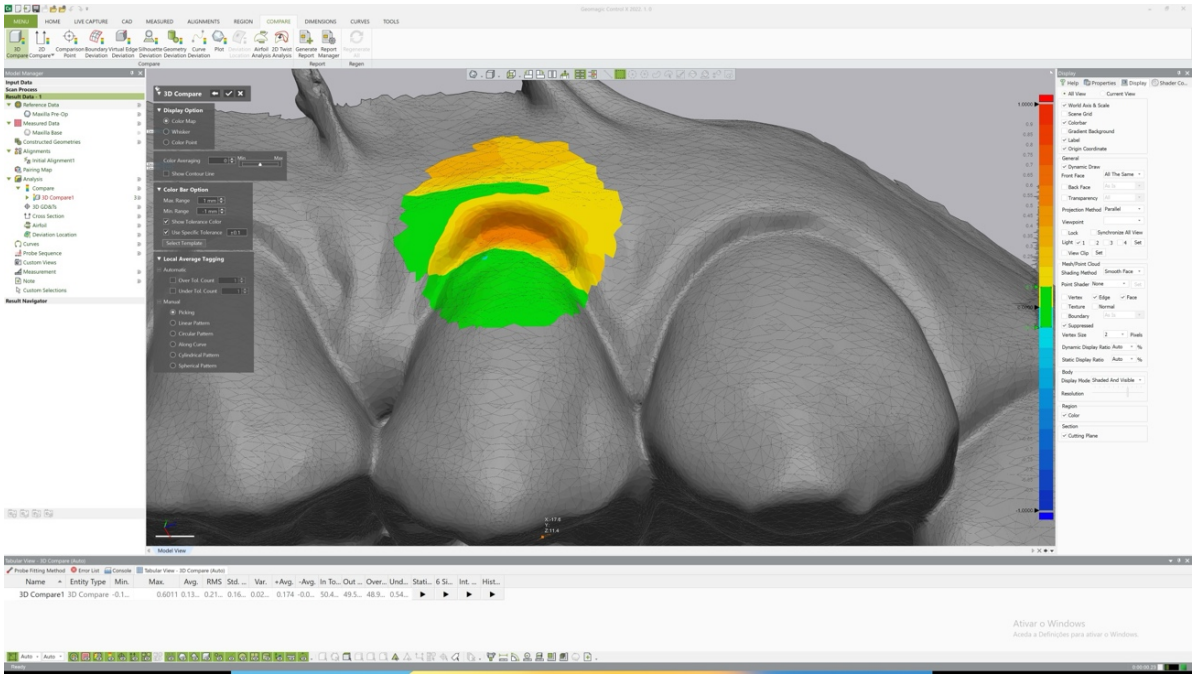


Figure 16 -Graphic display of the 3D compare function and work area in Geomagic control X (Screenshot of software Geomagic control X, taken by the author, 2024)

STL FILE ALIGNMENT AND SUPERIMPOSITION IN GEOMAGIC CONTROL X

The T_0 and T1 STL files were aligned using several functions:

1. "Align Between Measured Data Auto guess" – "Local Based on Auto Guess". Define the STL T_0 as "Reference" and the STL Tf as "Moving," respectively.
2. "Align Between Measured Data" – "Global and Fine."

"Sampling Ratio": 25%; "Max. Iteration Count": 20; "Max Average Deviation": Auto Best Fit Alignment": "Sampling Ratio": 25%, "Max. Iteration Count": 20, "

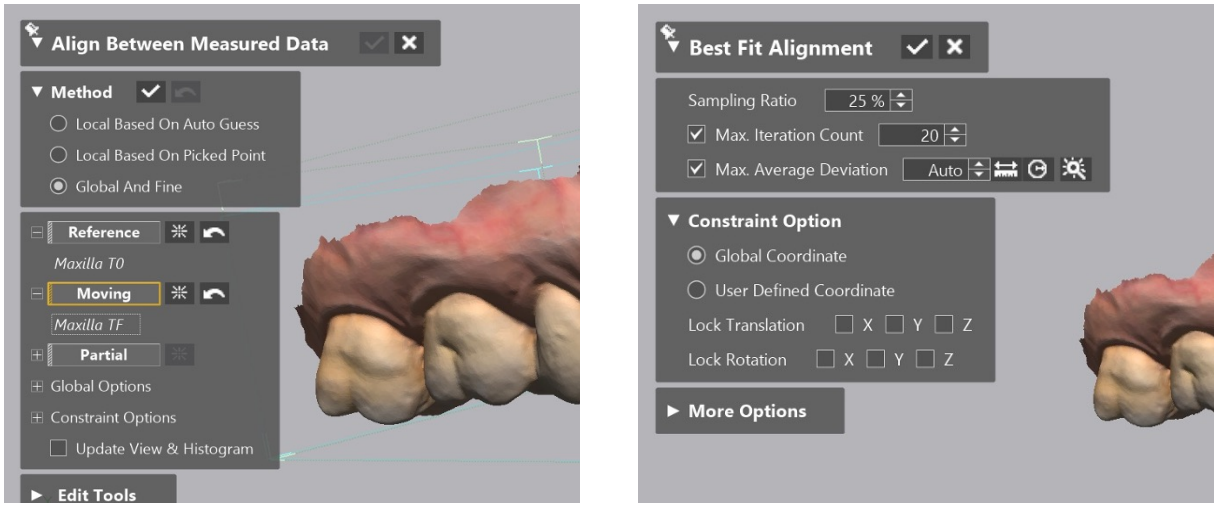


Figure 17- STL-Alignment
(Screenshot of software Geomagic control X, taken by the author, 2024)

DEFINITION OF ROI

To ensure the highest comparability between different studies, we identify two distinct ROIs that have been used before in previous research.

According to Lorenzo Tavelli and colleagues (Lorenzo Tavelli et al., 2021, 2022), the Region of Interest (ROI) for evaluating root coverage procedures should be carefully defined to ensure accuracy and consistency.

$ROI_{(1)}$

Therefore, we designated the first ROI as having a rectangular shape. The coronal border of the ROI is the final post-operative gingival margin (TF). The mesiodistal borders are the lines perpendicular to the occlusal plane and the adjacent teeth' cementoenamel junction (CEJ). These lines should pass through the midpoint of the mesial and distal papillae. The apical border extends 3 mm apically from the gingival margin (Table 25).

Table 18- Rectangular ROI -$ROI_{(1)}$1limits

TYPE	SHAPE	BORDER	DEFINITION
$ROI_{(1)}$	Rectangular	Coronal	The final post-operative gingival margin at 6 months
		Mesiodistal	The lines perpendicular to the occlusal plane and to the cementoenamel junction (CEJ) of the adjacent teeth passing through the midpoint of the mesial and distal papillae
		Apical	Extend 3 mm apically from the gingival margin

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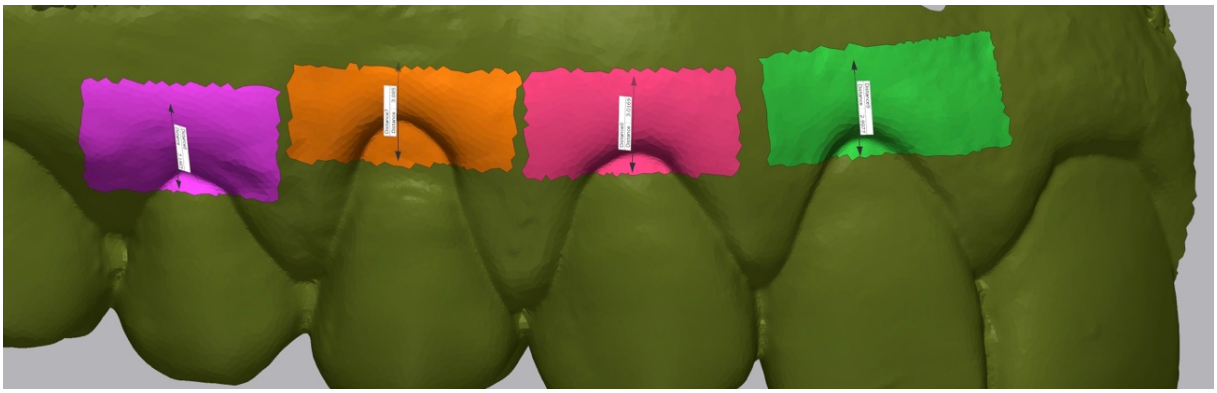


Figure 18-Region of interest ($ROI_{(1)}$) for a group of teeth
(Screenshot of software Geomagic control X, taken by the author, 2024)

Some authors suggest extending 6mm (Abrahamian et al., 2024; Lorenzo Tavelli et al., 2021), but we found that dimension can make some extent of ROI coincide with mobile mucosa and frenum, especially in the maxillary pre-molar region as we can see in the picture (Fig 18).

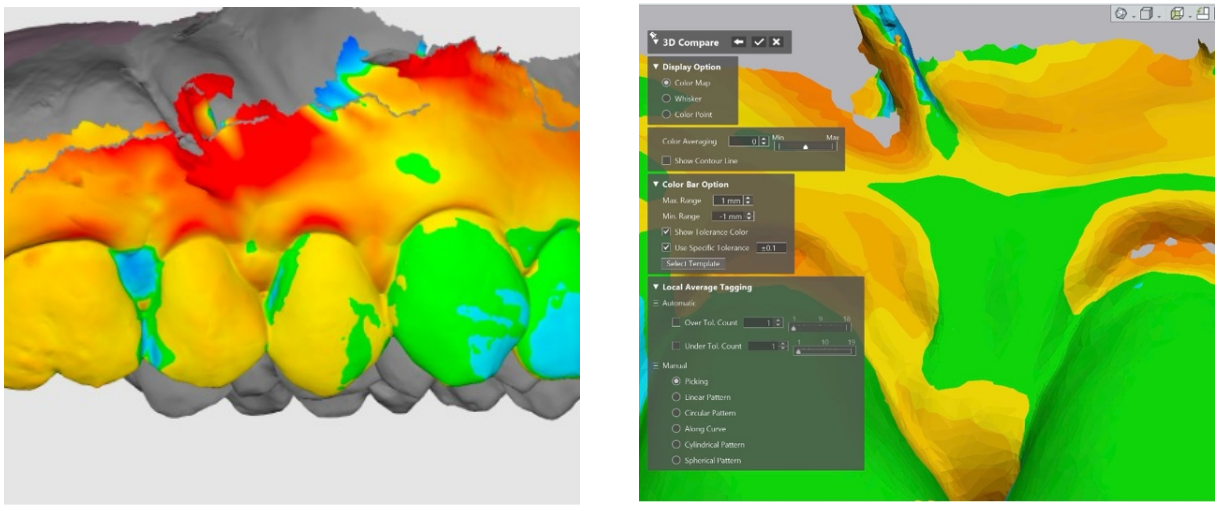


Figure 19- Volume increasing decorrent from artfacts
(Screenshot of software Geomagic control X, taken by the author, 2024)

Considering the medium values for the width of the attached gingiva and the fact that the relevant thickness is in keratinized mucosa and not in the free mucosa, we opted for 3mm as the apical dimension of the ROI.

$ROI_{(2)}$

The second ROI, defined by us, has a polygonal shape (semi-lunar) and is defined by the area of the newly covered at six months, corresponding to the new tissue formed between the pre-operative and post-operative gingival margins (Zuhr et al., 2020, 2021) (Table 24).

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Table 19- Poligonal ROI-ROI$_{(2)}$

TYPE	SHAPE	BORDER	DEFINITION
ROI$_{(2)}$	Polygonal (semi-lunar)	Area between gingival margin at 0 months and 6 months	new tissue formed between the pre-operative and post-operative gingival margins

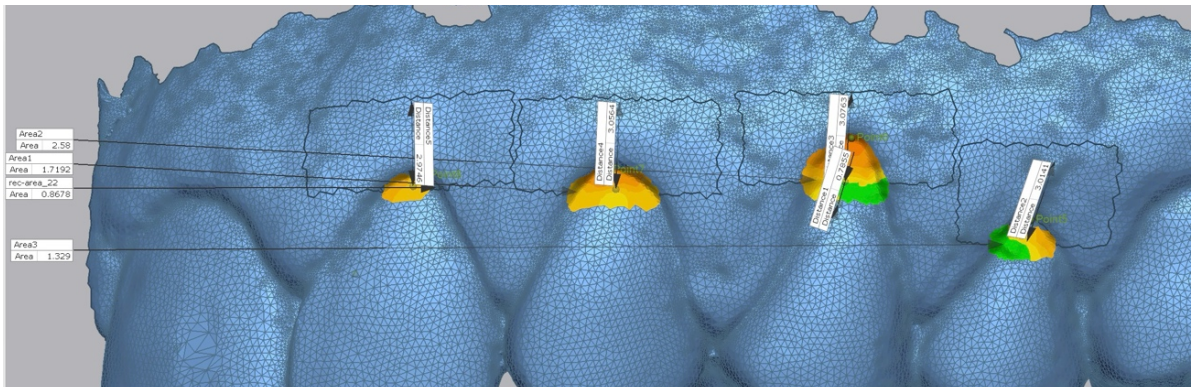


Figure 20 - Region of interest ROI $_{(2)}$ for a group of teeth
(Screenshot of software Geomagic control X, taken by the author, 2024)

We used both regions of interest (ROI) to measure de deviation from the final STL (Tf) to the starting STL (T_0), obtaining the mean gingival thickness gained at each ROI.

Table 20-ROI/variables

VARIABLE	USED ROI
MEANΔGT	Mean gingival thickness gained in the area of Rectangular ROI (ROI$_{(1)}$)
MAXΔGT	Maximum gingival thickness gained in the area of Rectangular ROI (ROI$_{(1)}$)
MEANΔGT$_{(R)}$	Mean gingival thickness gained in the area of Semi-lunar ROI over the covered root (ROI$_{(2)}$)
MAXΔGT$_{(R)}$	Maximum gingival thickness gained in the area of Semi-lunar ROI over the covered root (ROI$_{(2)}$)

3D MEASUREMENTS IN GEOMAGIC® SOFTWARE

The software generates a deviation map by calculating the distance between surfaces of the reference mesh (T_0 virtual model) and the "measurement" mesh (TF virtual model) on the defined ROI. This method involves complex surface fitting and distance calculations. The 3D Compare tool allows for a complete analysis of the entire surface of an object, providing a more thorough inspection compared to the 2D Compare tool, which is limited to cross-sectional analysis.

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The software also allows the visualization of the deviations using a color-coded scheme, where different colors represent different magnitudes. This color scale provides an intuitive understanding of where and how much the part deviates from the reference model.

Besides the visual information, the software generates a complete report of the deviation found. From that report, we use the mean and max values found in the defined ROI as the

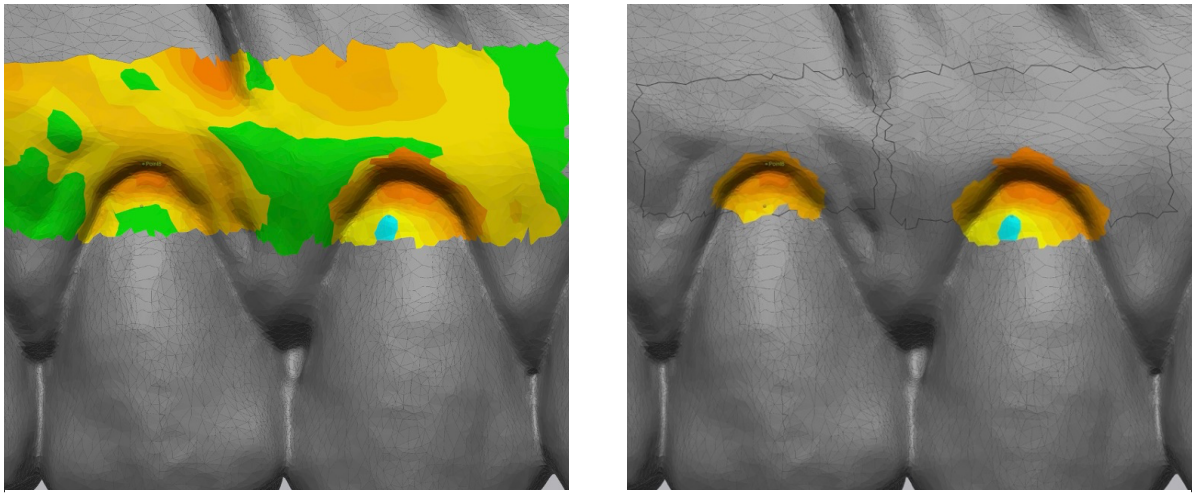


Figure 21 - Visual evaluation of volume difference for RO(I) and ROI(2), respectively (Screenshot of software Geomagic control X, taken by the author, 2024)

variables in the study (meanΔGT, maxΔGT for $ROI_{(1)}$, mean$\Delta GT_{(r)}$, max$\Delta GT_{(r)}$ for $ROI_{(2)}$).

The mean gingival thickness and maximum gingival thickness in each ROI were this way direct obtained without human intervention, besides the definition of ROI for each recession. The volume gain, at $ROI_{(2)}$ (in mm^3) was calculated by multiplication of the area of ROI_2 (mm^2) by the mean thickness on $ROI_{(2)}$ (mm).

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3.7 TEMPORAL MAP

3.7.1 STUDY TIME MAP

Table 21-Time map

YEAR	2019	2020	2021	2022	2023	2024
LITERATURE REVIEW/WRITING						
RECRUITMENT						
INTERVENTION						
DATA COLLECTION						
DATA ANALYSIS						
PRESENTATION/PUBLICATION						

3.7.2 DATA COLLECTION

3.7.2.1 TIMES OF DATA COLLECTION

BASELINE EXAMINATION (T_0)

Intra-oral scanning
Clinical Measurements

- Plaque index
- Bleed on probing (BOP)
- Pocket Depth (PD)
- Clinical attachment level (CAL)

Phenotype
Complementary data
Standardized Photos

FOLLOW-UP (TIME 5 DAYS)

IPR scale, mean consumption (in mg) of analgesics (ibuprofen) and pain visual analog scales (VAS)

FOLLOW-UP (TIME 14 DAYS)

IPR scale, mean consumption (in mg) of analgesics (ibuprofen) and pain visual analog scales (VAS)

FOLLOW-UP (TIME 6 WEEK)

IPR scale, mean consumption (in mg) of analgesics (ibuprofen) and pain visual analog scales (VAS)

FOLLOW-UP (TIME 3MONTHS)

Middle-term control, (Prophylaxis)

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FOLLOW-UP T_F (TIME 6 MONTHS)

Intra-oral scanning
Clinical Measurements

- Plaque index
- Bleed on probing (BOP)
- Pocket Depth (PD)
- Clinical attachment level (CAL)

Complementary data (photos)
Prophylaxis

3.7.2.2 RESUME OF DATA COLLECTION, EVALUATION AND EXAMINER

Table 22-Data collection and examiner
(Data from author, 2024)

VARIABLES	TIME OF DATA COLLECTION	EVALUATION METHOD	EXAMINER
ROOT COVERAGE (%)	6 months	Indirect calculation by digital measurement (Medit Design)	TM
RECESSION DEPTH (RE	T_0, 6 months	Scan at T_0, Digital measurement (Medit Design) at T_0 and Tf	TM
RECESSION AREA (REAREA)	6 months	Digital measurement (Medit Design)	TM
GINGIVAL THICKNESS (ΔGT)	6 months	Digital measurement (Geomagic control X)	TM
KERATINIZED TISSUE (KT	6 months	Digital measurement (Medit Design)	TM
CAL	T_0, six months	Analogical measurement with periodontal probe	TM
POS-OPERATIVE PAIN	5,14 days	Intake of pain medication (mean consumption (in mg) of analgesics)	GF
POS-OPERATIVE PAIN (SELF-REPORTED)	5,14 days	VAS form	GF
HEALING	5 days,14 days, 6 weeks	IPR wound healing scale	GF
PATIENT-CENTERED ESTHETIC EVALUATION	6 months	Form- Self-evaluation	GF
CERVICAL DENTIN HYPERSENSITIVITY	T_0, 6 months	Form-Self-evaluation	GF

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3.8 STATISTICS

In this study, after data collection, the data were coded and entered into a database using the IBM Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM, Armonk, New York, United States), where statistical analysis was performed considering the study objectives.

For descriptive analysis, frequency distributions for qualitative data and median, mean, and standard deviation for quantitative data.

In inferential analysis, as the comparison between evaluation moments and between groups was intended, mixed ANOVA was used, allowing for the comparison between repeated measures as well as between the test and control groups.

Thus, this method was used considering the group (test versus control) for the analysis of KTW, Re, and ReArea results at the two evaluation moments.

The chi-square test of independence was also used to investigate the possible association between characteristics: Sensitivity, Phenotype, CEJ, and STEP, and the group.

The distribution analysis was conducted using the Shapiro-Wilk Test, which revealed that the distributions were not normal. Since the data deviates from a normal distribution, it was deemed more suitable to employ a non-parametric test, as elaborated in the results and discussion chapter.

The non-parametric Mann-Whitney test was used to compare two independent groups of small size when considering the patients (nine in the control group and ten in the test group).

The non-parametric Kruskal-Wallis test was also used to compare three or more independent groups.

In constructing the patient database, the number of recessions per patient and the mean results of these recessions for the variables of interest as well as variables directly related to the patients were considered.

To identify the possible predictors of the variables GT mean, GT max, ROI area, and volume, multiple linear regression was used.

This is a technique that allows modeling the relationship between a continuous dependent variable and a set of independent variables, allowing the identification of each predictor variable's contribution to the dependent variable's variation.

In selecting the multiple linear regression method, the stepwise approach was chosen as it is an automatic approach that combines exclusion and inclusion techniques to identify the most relevant subset of predictor variables.

Additional, mixed-effects models were used to account for the hierarchical structure of the data, controlling for intra-patient variability and providing more accurate estimates of the treatment effects.

The significance level was set at 5%.

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4 RESULTS AND DISCUSSION

4.1 DEMOGRAPHIC AND CLINICAL CHARACTERIZATION OF THE TOTAL SAMPLE

4.1.1.1 PATIENT-RELATED CHARACTERIZATION OF THE TOTAL SAMPLE

Table 23- Continuous variables related to patients
(Data collected and analyzed by the author, 2024)

CONTINUOUS VARIABLES			
		MEAN	SD (±)
AGE		33.32 years	10.08 years
	Minimum	19	
	Maximum	51	
N RE		3.68	1.20
RE (T_0)		1.28 mm	0.55
REAREA (T_0)		3.64 mm^2	1.07
KTW(T_0)		2.81mm	0.64

Table 24-Patient-related nominal variables
(Data collected and analyzed by the author, 2024)

NOMINAL VARIABLES			
	VALUE	COUNT	PERCENTAGE
GENDER	Female	14	73.7%
	Male	5	35.7%
PHENOTYPE	Thin	13	68.4%
	Thick	6	31.6%
CERVICAL DENTIN HYPERSENSITIVITY	(+)(at least 1)	12	63.7
	(-)	7	36.9%
CEJ (PINI-PRATO)	A	9	42,1.%
	B (at least 1)	10	57.9%
STEP (PINI-PRATO)	(+) (at least 1)	11	57.9%
	(-)	8	42.1%

Table 25- Recession, patient-related
(Data collected and analyzed by the author, 2024)

RECESSIONS BY PATIENT			
PATIENT NUMBER	AVERAGE STARTING RECESSION (T_0) mm	SD (±)	No. RECESSIONS
1	1.84	0.64	3
2	1.00	0.70	4
3	1.33	1.11	4
4	1.41	0.84	5
5	1.83	1.03	3
6	0.89	0.56	4
7	1.06	0.13	2
8	0.57	0.28	2
9	1.04	0.48	5
10	1.07	0.63	5

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11	2.98	0.37	1
12	0.98	0.50	3
13	1.50	0.97	5
14	1.53	0.65	3
15	0.86	0.94	5
16	1.59	0.60	4
17	1.30	0.44	4
18	0.78	0.24	3
19	0.73	0.64	5
AVERAGE	3.68	1.20	3,7

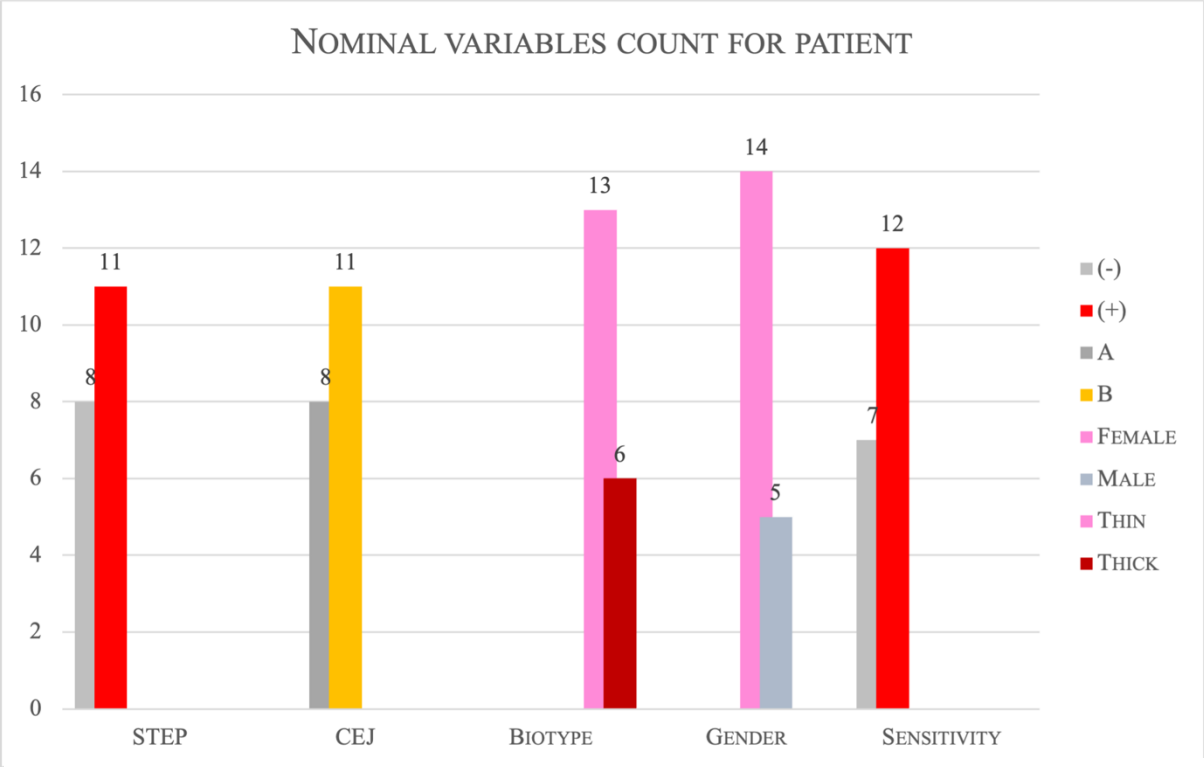


Figure 22- Patient-related -nominal variables
(Data collected and analyzed by the author, 2024)

PATIENT RELATED TOTAL SAMPLE OVERVIEW

The demographic and clinical characteristics of the study participants provide a comprehensive overview of the sample population. The gender imbalance, with a predominance of female participants, is noteworthy and should be considered when generalizing the study results. The age distribution indicates a moderately young to middle-aged group, which is typical for periodontal studies.

The clinical characteristics, including sensitivity, Phenotype, CEJ, and STEP, suggest that the study includes a diverse group of patients in terms of periodontal health and anatomical features. The predominance of thin phenotypes and the significant presence of positive STEP cases imply that the study will offer valuable insights into the effectiveness of the surgical techniques for patients with more challenging periodontal conditions.

Overall, the study sample appears to be well-suited for evaluating the effectiveness of the two surgical techniques for root coverage, but the results should be interpreted with consideration of the sample characteristics, especially the gender imbalance

SITE-RELATED CHARACTERIZATION OF THE TOTAL SAMPLE

Table 26- Continuous variables site-related
(Data collected and analyzed by the author, 2024)

CONTINUOUS VARIABLES			
VARIABLE	MEAN	SD	PERCENTAGE OF FREQUENCY
AGE AT SURGERY	33.75714 years	10.50526	25.7%
REA(T_0)	3.687529 mm^2	2.332645	21.4%
RE(T_0)	1.208653 mm	0.711817	20.6%
KTW(T_0)	2.847043 mm	0.912281	9.3%

Table 27-Categorical variables site- related
(Data collected and analyzed by the author, 2024)

NOMINAL VARIABLES			
VARIABLE	VALUE	COUNT	PERCENTAGE
GENDER	Male	22	31.43%
	Female	48	68.57%
CEJ (PINI-PRATO)	A	54	77.14%
	B	16	22.86%
STEP (PINI-PRATO)	(+)	21	30%
	(-)	49	70%
CERVICAL DENTIN HYPERSENSITIVITY	(+)	20	28.57%
	(-)	50	71.43%

Table 28- Tooth/recession distribution
(Data collected and analyzed by the author, 2024)

RECESSION DISTRIBUTION BY TYPE OF TOOTH			
TYPE OF TOOTH	AVERAGE FREQUENCY (n)	STANDARD DEVIATION	PERCENTAGE OF FREQUENCY
1ST PREMOLAR	1.05	0.91	25.7%
2ND PREMOLAR	0.79	0.92	21.4%
CANINE	0.84	0.85	20.6%
CENTRAL	0.37	0.67	9.3%
LATERAL	0.74	0.87	22.9%

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Table 29- Sensitivity, CEJ B and Step(+) by type of tooth
(Data collected and analyzed by the author, 2024)

Metric		1st Premolar	2nd Premolar	Canine	Central	Lateral
Sensitivity	Count	7	6	6	3	5
	%	38.89%	40.00%	37.50%	42.86%	35.71%
CEJ B	Count	10	5	0	0	1
	%	55.56%	33.33%	0.00%	0.00%	7.14%
STEP (+)	Count	9	6	2	1	3
	%	50.00%	40.00%	12.50%	14.29%	21.43%
Total	Total	18	15	16	7	14

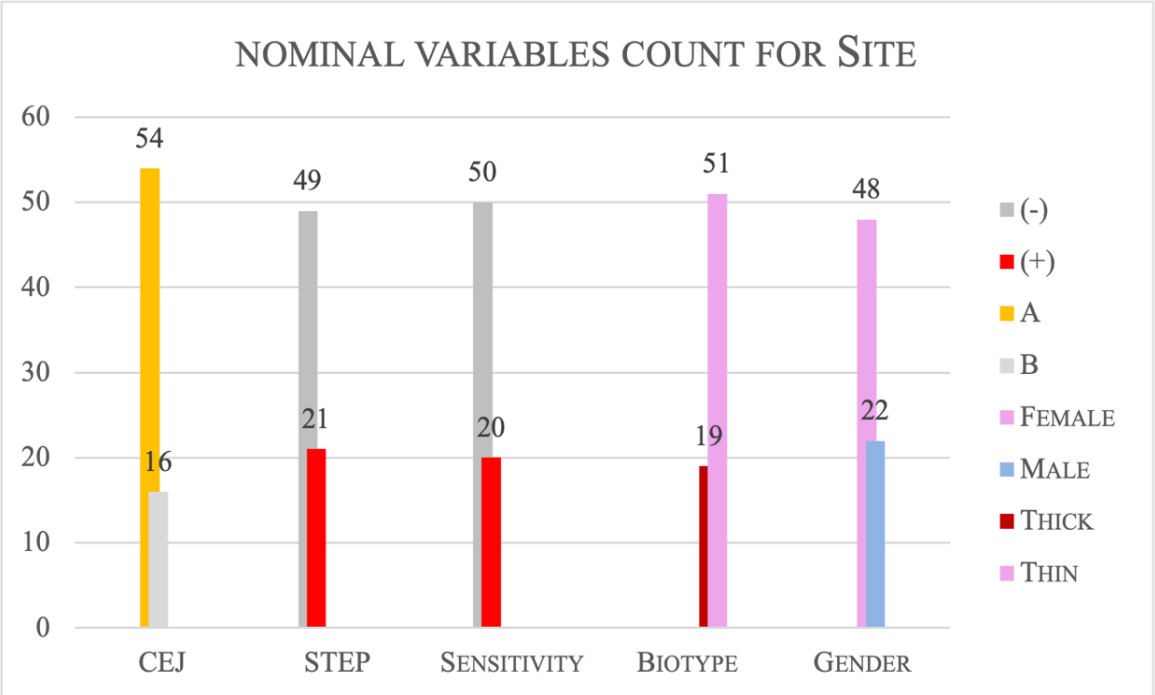


Figure 23 Frequency of nominal variables for the entire sample population
(Data collected and analyzed by the author, 2024)

4.2 SAMPLE CHARACTERIZATION RELATED TO GROUP DISTRIBUTION

We conducted a comprehensive comparative analysis of the sample distribution between the two study groups. The primary objective of this analysis was to assess the effectiveness of the randomization process. Specifically, we aimed to determine whether randomization successfully distributed known variables in a similar manner across both groups. By evaluating the distribution of these variables, we could ensure that the randomization process minimized potential biases and ensured comparability between the groups, thereby enhancing the validity and reliability of the study's findings. This analysis is crucial for confirming that any observed differences between the groups can be attributed to the interventions or treatments being studied, rather than to pre-existing differences in the sample populations

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4.2.1 PATIENT- CENTERED SAMPLE CHARACTERIZATION

Table 30-Patient-Centered Baseline continouos varialbles Comparison
(Data collected and analyzed by the author, 2024)

CHARACTERISTIC	CONTROL GROUP (MEAN ± SD)	TEST GROUP (MEAN ± SD)	T-STATISTIC	P-VALUE
NUMBER OF PATIENTS (N)	9	10		
AGE AT SURGERY (YEARS)	34.00 ± 10.23	33.60 ± 10.80	0,156804	0,875864
KTW(T_0) (MM)	3.00 ± 1.01	2.74 ± 0.84	1,151828	0,253425
(RE)T_0 (MM)	1.25 ± 0.79	1.18 ± 0.66	0,403831	0,687605
REAREA(T_0) (MM^2)	3.91 ± 2.74	3.54 ± 2.03	0,659714	0,511666
AVERAGE NUMBER OF RECESSIONS (N)	3.11 ± 1.36	4.20 ± 0.79		

Table 31-Patient-Centered Baseline nominal variables Comparison
(Data collected and analyzed by the author, 2024)

CHARACTERISTIC		CONTROL GROUP N	CONTROL GROUP %	TEST GROUP N	TEST GROUP %	ODDS RATIO	P-VALUE
NUMBER OF PATIENTS		9		10			
GENDER	Female	7	77,8	7	70	1,5	1
	Male	2	22,2	3	30		
PHENOTYPE	Thin	6	66,7	7	70	0,857143	1
	Thick	3	33,3	3	30		
SENSITIVITY	Sensitivity (+)(At least 1)	3	33,3	4	40	0,75	1
	Sensitivity (-)	6	66,7	6	60		
CEJ (PINI-PRATO)	B (At least 1)	4	44,4	7	70	0,342857	0,36985
	A	5	55,6	3	30		
STEP (PINI-PRATO)	STEP (+)(At least 1)	4	44,4	7	70	0,342857	0,36985
	STEP (-)	5	55,6	3	30		

4.2.1.1 SAMPLE DISTRIBUTION BY GROUPS, PATIENT -CENTERED

GENDER

Proportion of males to females is slightly higher in the test group compared to the control group, but this difference is not statistically significant

BASELINE CHARACTERISTICS

The t-tests for continuous variables confirm there are no significant differences in age, KTW(T_0), (Re)0, ReArea (T_0), and average number of recessions between the groups.

CATEGORICAL CHARACTERISTICS

Fisher's Exact Test shows no significant differences in Thin Phenotype, Sensitive, CEJ B, and STEP characteristics between the control and test groups.

4.2.1.2 CONCLUSION:

There are no statistically significant differences in the baseline characteristics between the control and test groups for both continuous and categorical variables, suggesting that the

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randomization process was effective. This supports the validity of the study's comparisons between the two surgical techniques.

4.2.2 SITE-SPECIFIC SAMPLE CHARACTERIZATION

Table 32-Site especific-Sample caractherization (continuous)
(Data collected and analyzed by the author, 2024)

VARIABLE	CONTROL GROUP (MEAN ± SD)	CONTROL GROUP (MEDIAN)	TEST GROUP (MEAN ± SD)	TEST GROUP (MEDIAN)	T-STATISTIC	P-VALUE
NUMBER OF RECESSIONS/SITES	28		42			
AGE AT SURGERY (YEARS)	34.00 ± 10.23	31.0	33.60 ± 10.80	36.0	0.157	0.876
KTW(T_0) (MM)	3.00 ± 1.01	2.90	2.74 ± 0.84	2.63	1.152	0.253
RE(T_0) (MM)	1.25 ± 0.79	0.94	1.18 ± 0.66	0.91	0.404	0.688
REAREA(T_0) (MM^2)	3.91 ± 2.74	3.63	3.54 ± 2.03	3.01	0.660	0.512

Table 33 - Site-especific sample caractherization (nominals)
(Data collected and analyzed by the author, 2024)

VARIABLES	VALUE	CONTROL N	CONTROL %	TEST N	TEST %	TOTAL N	TOTAL %	X2 (P)	FISHER (P)
SITES		28		42					
SENSITIVITY	(-)	21	75	29	69	50	71,4	0.29 (0.59)	1.34 (0.79)
	(+)	7	25	13	31	20	28,6		
PHENOTYPE	Thin	21	75	30	71,4	51	72,9	0.11 (0.74)	1.20 (0.79)
	Thick	7	25	12	28,6	19	27,1		
CEJ	A	22	78,6	32	76,2	54	77,1	0.05 (0.82)	1.15 (1.00)
	B	6	21,4	10	23,8	16	22,9		
STEP	(-)	21	75	28	66,7	49	70	0.56 (0.46)	1.50 (0.60)
	(+)	7	25	14	33,3	21	30		

Table 34-Distribution of Re by type of tooth
(Data collected and analyzed by the author, 2024)

TYPE OF TOOTH	GROUP	MEDIAN (MM)	STANDARD DEVIATION (MM)	MEDIAN (MM)	KRUSKAL-WALLIS TEST RESULT
PREMOLAR	control	0.88	0.60	0.72	Test Statistic (K): 6.976 p-value: 0.073
	test	0.94	0.48	0.85	
	Total	0.91	0.53	0.76	
CANINE	control	1.10	0.66	1.09	
	test	1.42	0.81	1.16	
	Total	1.30	0.75	1.16	
CENTRAL	control	0.38	0.14	0.38	
	test	0.74	0.41	0.70	
	Total	0.64	0.38	0.50	
LATERAL	control	1.29	1.09	1.03	
	test	0.84	0.41	0.72	
	Total	0.97	0.66	0.72	

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INTERPRETATION

Test group presents higher mean and median values for Re, except for the lateral tooth type. Overall, the central tooth type shows the lowest results in terms of recession, with 0.64±0.38, and the canine shows the highest, with 1.30±0.75.

4.2.3 SAMPLE CHARACTERIZATION CONCLUSIONS

AGE AT SURGERY

There is no statistically significant difference in the age at surgery between the control and test groups, indicating that the age distribution is similar across both groups.

KTW(T_0)- (KERATINIZED TISSUE WIDTH AT BASELINE):

There is no statistically significant difference in the keratinized tissue width at baseline between the control and test groups, indicating that the initial keratinized tissue width is comparable across both groups.

RE(T_0)- (RECESSION AT BASELINE):

There is no statistically significant difference in the initial recession between the control and test groups, indicating that the recession at baseline is similar across both groups.

REAREA(T_0)- (RECESSION AREA AT BASELINE):

There is no statistically significant difference in the recession area at baseline between the control and test groups, indicating that the recession area is comparable across both groups.

NUMBER OF RECESSIONS/SITES

The number of recession sites differs between the control and test groups, but this variable alone does not indicate a significant difference without further context.

SENSITIVITY

There is no statistically significant difference in sensitivity between the control and test groups.

PHENOTYPE

There is no statistically significant difference in Phenotype between the control and test groups.

*CEJ (CEMENTO-ENAMEL JUNCTION CLASSIFICATION)**

There is no statistically significant difference in CEJ types between the control and test groups.

STEP (ROOT STEP CLASSIFICATION)

There is no statistically significant difference in STEP between the control and test groups.

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TYPE OF TOOTH DISTRIBUTION

Application of the non-parametric Kruskal-Wallis test concluded that the differences between the tooth types are not statistically significant (K=6.016; p=0.111).

4.2.3.1 Overall Conclusion:

Both the continuous and nominal variables indicate no statistically significant differences between the control and test groups. This suggests that the randomization process was effective in creating comparable groups for the study. Consequently, any differences observed in the outcomes of the study can be attributed to the interventions being tested rather than differences in baseline characteristics.

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4.3 ROOT COVERAGE RESULTS

4.3.1 PRIMARY OUTCOME % ROOT COVERAGE LENGTH

4.3.1.1 NORMALITY AND VARIANCE HOMOGENEITY TESTS FOR THE % RECESSION COVER DEPTH

Table 35-Normality tests (Shapiro-Wilk Test)
(Data collected and analyzed by the author, 2024)

GROUP	SHAPIRO-WILK STATISTIC	SHAPIRO-WILK P-VALUE
Control	0.805	0.000001
Test	0.835	0.000008

Table 36Variance Homogeneity Test (Levene's Test)-
(Data collected and analyzed by the author, 2024)

LEVENE STATISTIC	LEVENE P-VALUE
1.78	0.19

INTERPRETATION

Normality Test: The p-values for both groups are very low (below 0.05), indicating that the data for both groups significantly deviates from a normal distribution.

Variance Homogeneity Test: The p-value is 0.19, which is above 0.05, indicating that there is no significant difference in the variances between the two groups.

CONCLUSION

Given that the data does not follow a normal distribution, it is more appropriate to use a non-parametric test instead of a t-test.

TESTS USED

Mann-Whitney U Test: This test is a non-parametric alternative to the t-test and can be used to compare the medians of the two groups.

Mixed-Effects Models: In clinical studies like recession coverage studies, it's essential to account for the clustering effect, particularly when the data might be clustered within individuals (e.g., multiple teeth treated in the same patient). Ignoring the clustering can lead to incorrect conclusions due to the potential correlation within clusters. We consider the use of Mixed-Effects Models: These models can account for both fixed effects (e.g., treatment) and random effects (e.g., patient variability). They are suitable for handling data where measurements are nested within clusters.

Table 37- Descriptive Statistics and Mann-Whitney U Test Results for % Recession Cover Length
(Data collected and analyzed by the author, 2024)

GROUP	CONTROL	TEST	TOTAL
MINIMUM (%)	11.17	24.47	11.17
MAXIMUM (%)	100.00	100.00	100.00
MEAN (%)	81.60	89.30	84.00
STD DEV (%)	27.93	20.33	24.00

Median (%)	100.00	100.00	100.00
Mann-Whitney U Statistic	475.0	-	-
p-value (Mann-Whitney)	0.15	-	-
Mixed-Effects Model Coef	0.000	0.082	-
p-value (Mixed-Effects)	-	0.310	-

Table 38 - Descriptive Statistics, Mann-Whitney U Test, and Mixed-Effects Model Results for % Recession Cover Area (Data collected and analyzed by the author, 2024)

Group	Control	Test	Total
Minimum (%)	8.00	22.00	8.00
Maximum (%)	100.00	100.00	100.00
Mean (%)	79.46	87.69	84.00
Std Dev (%)	30.27	23.48	26.00
Median (%)	100.00	100.00	100.00
Mann-Whitney U Statistic	487.0	-	-
p-value (Mann-Whitney)	0.18	-	-
Mixed-Effects Model Coef	0.000	0.036	-
p-value (Mixed-Effects)	-	0.631	-

4.3.1.2 Interpretation

Descriptive Statistics:

The mean and median percentages of root coverage (both length and area) are higher in the test group compared to the control group.

The high median values (100%) in both groups suggest that at least half of the samples in each group achieved complete root coverage.

Mann-Whitney U Test

The p-values for % cover length (0.15) and % cover area (0.18) are both greater than the common significance threshold of 0.05. This indicates that the differences in the percentages of root coverage between the test and control groups are not statistically significant according to this test.

Mixed-Effects Model

The coefficients for the test group in both % cover length (0.082) and % cover area (0.036) are positive, suggesting a trend towards better outcomes in the test group. However, the p-values (0.310 for length and 0.631 for area) indicate that these differences are not statistically significant when accounting for the clustering effect.

Conclusion

The study found no statistically significant difference in the percentages of root coverage (both length and area) between the control group (CAF alone) and the test group (CAF combined with L-PRF).

Although the descriptive statistics show a trend towards higher mean coverage in the test group, this difference was not confirmed by the Mann-Whitney U test or the mixed-effects model.

These results suggest that combining CAF with L-PRF does not significantly improve root coverage compared to CAF alone, at least within the scope and sample size of this study. Further research with larger sample sizes and perhaps different methodologies might be needed to fully explore the potential benefits of L-PRF in root coverage procedures.

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4.4 SECONDARY OUTCOMES RESULTS

4.4.1 COMPLETE ROOT COVERAGE (CRC)

Table 39 - Sample-Descriptive statistics for complete root coverage (CRC)
(Data collected and analyzed by the author, 2024)

METRIC	TEST	CONTROL	TOTAL
COUNT (SAMPLE)	42	28	70
PROPORTION (%)	74	57	67
STD (%)	45	50	47
MIN (%)	0.00	0.00	0.00
50% (MEDIAN) (%)	100	100	100
MAX (%)	100	100	100

Table 40 -CRC Results
(Data collected and analyzed by the author, 2024)

GRUPO	Nº %COVER(DEPTH) 100%	CRC	BINOMIAL TEST	Z TEST (PROPORTION COMPARISON)
CONTROL (N=28)	16	57.14%	Sig=0.572	ET=1.45 Sig=0.072
TEST (N=42)	31	73.81%	Sig.=0.003	
TOTAL (N=70)	47	67.14%		

Table 41- Complementary Statistics test for complete root coverage (CRC)
(Data collected and analyzed by the author, 2024)

METRIC	VALUE
CRC PERCENTAGE (TEST)	73.81%
CRC PERCENTAGE (CONTROL)	57.14%
MIXED-EFFECTS INTERCEPT	0.577
MIXED-EFFECTS GROUP COEFFICIENT	0.162
MIXED-EFFECTS P-VALUE (GROUP)	0.193
VARIANCE (GROUP)	0.014
CHI-SQUARE TEST STATISTIC	1.651
CHI-SQUARE TEST P-VALUE	0.199

4.4.2 MEAN ROOT COVERAGE (MRC)

Table 42- Descriptive Statistics for mean root coverage (MRC)
(Data collected and analyzed by the author, 2024)

MEAN ROOT COVERAGE			
METRIC	TEST	CONTROL	TOTAL
COUNT (N)	42.000	28.000	70.000
MEAN (MM)	1.005	0.949	0.983
MEDIAN (MM)	0.835	0.685	0.753
STD (MM)	0.587	0.680	0.622
MIN (MM)	0.308	0.228	0.228
25% (MM)	0.630	0.410	0.535
50% (MM)	0.835	0.685	0.753
75% (MM)	1.344	1.486	1.414
MAX (MM)	2.760	2.750	2.760

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Table 43- Statistical Tests for MRC.
(Data collected and analyzed by the author, 2024)

Metric	Value
Mixed-Effects Intercept	0.98
Mixed-Effects Group Coefficient	0.05
Mixed-Effects p-value (Group)	0.81
Mann-Whitney U statistic	683.00
Mann-Whitney U p-value	0.26

4.4.2.1 Interpretation for CRC and MRC

CRC

The test group showed a higher percentage of complete root coverage compared to the control group. However, the statistical tests (both mixed-effects model and chi-square test) did not find this difference to be statistically significant.

MRC

The test group had a higher mean and median MRC compared to the control group. Statistical tests (both mixed-effects model and Mann-Whitney U test) also indicated no statistically significant difference between the groups.

4.4.3 Results in terms of keratinized tissue width gain (ΔKTW)

4.4.3.1 Descriptive analysis

Table 44 - Sample- Descriptive analysis for KTW
(Data collected and analyzed by the author, 2024)

Metric	KTW at T_0 (mm)	KTW at 6 months (mm)
Mean (mm)	2.85	3.04
Standard Deviation (mm)	0.91	0.89
Minimum (mm)	1.14	1.39
25th Percentile (mm)	2.18	2.45
Median (50th Percentile) (mm)	2.72	2.95
75th Percentile (mm)	3.48	3.71
Maximum (mm)	5.20	5.09

Table 45.-.Sample-Descriptive analysis for Keratinized tissue gain (ΔKTW)
(Data collected and analyzed by the author, 2024)

Metric	(ΔKTW) Difference (mm)
Count (n)	70
Mean (mm)	0.20
Standard Deviation (mm)	0.69
Minimum (mm)	-1.57
25th Percentile (Q1) (mm)	-0.12
Median (50th Percentile) (mm)	0.00
75th Percentile (Q3) (mm)	0.55
Maximum (mm)	2.01

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4.4.3.2 COMPARATIVE ANALYSIS

Table 46-Comparison of ΔKTW descriptive statistics
(Data collected and analyzed by the author, 2024)

METRIC	CONTROL (ΔKTW)	TEST (ΔKTW)
COUNT (N)	28	42
MEAN (MM)	0.06	0.29
STANDARD DEVIATION (MM)	0.58	0.74
MINIMUM (MM)	-1.20	-1.57
25TH PERCENTILE (Q1) (MM)	-0.22	-0.08
MEDIAN (50TH PERCENTILE) (MM)	-0.001	0.21
75TH PERCENTILE (Q3) (MM)	0.41	0.66
MAXIMUM (MM)	1.15	2.01

Table 47- Statistical tests for ΔKTW
(Data collected and analyzed by the author, 2024)

METHOD	T-STATISTIC	COEFFICIENT	STD. ERROR	Z-VALUE	P-VALUE	INTERPRETATION
NORMALITY TEST (CONTROL DIFFERENCE)	W=0.9530				0.2351	Normally distributed (p > 0.05)
NORMALITY TEST (TEST DIFFERENCE)	W=0.9630				0.1893	Normally distributed (p > 0.05)
INDEPENDENT T-TEST	-1.38	-	-	-	0.1714	No significant difference (p > 0.05)
MIXED-EFFECTS MODEL	-	0.230	0.225	1.022	0.307	No significant difference (p > 0.05)

4.4.3.3 INTERPRETATION

Although the differences are not statistically significant, there is a trend suggesting that the test group (CAF with L-PRF) tends to have a greater KTW improvement compared to the control group (CAF alone). Both the independent t-test and the mixed-effects model indicate that there is no statistically significant difference in the KTW difference between the control group (CAF alone) and the test group (CAF with L-PRF).

The addition of L-PRF to CAF does not result in a statistically significant improvement in KTW compared to CAF alone, based on the results of this study.

4.4.4 EFFICACY OF RESULTS (TIME/RESULTS)

Table 48 - Characterization of Clinical Measurements by Time and Group
(Data collected and analyzed by the author, 2024)

VARIABLE	STATISTIC	CONTROL GROUP		TEST GROUP		MIXED ANOVA RESULTS
		Baseline (T0)	Final (Tf)	Baseline (T0)	Final (Tf)	
KTW (MM)	Mean ± SD	3.00 ± 1.01	3.06 ± 0.86	2.75 ± 0.84	3.03 ± 0.91	Time: F=4.30; p=0.042; η^2=0.059 Time*Group: F=1.91; p=0.171; η^2=0.027 Group: F=0.47; p=0.474; η^2=0.007
RE (MM)	Mean ± SD	1.25 ± 0.79	0.30 ± 0.49	1.18 ± 0.66	0.18 ± 0.36	Time: F=163.86; p=0.000; η^2=0.707 Time*Group: F=0.133; p=0.716; η^2=0.002 Group: F=0.67; p=0.416; η^2=0.010
REAREA (MM2)	Mean ± SD	3.91 ± 2.75	0.99 ± 0.61	3.54 ± 2.03	0.61 ± 1.19	Time: F=134.50; p=0.000; η^2=0.664 Time*Group: F=0.01; p=0.996; η^2=0.001 Group: F=0.93; p=0.34; η^2=0.014

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4.4.4.1 INTERPRETATION

KTW (KERATINIZED TISSUE WIDTH):

The mean values increase slightly in both groups: from 3.00 mm ± 1.01 to 3.06 mm ± 0.86 in the control group and from 2.75 mmm ± 0.84 to 3.03 ± 0.91 in the test group.

Mixed ANOVA shows that the differences over time are statistically significant (p=0.042), indicating a significant increase in KTW over time.

There are no statistically significant differences between groups (p=0.474) or in the time/group interaction (p=0.171).

RE (RECESSION):

The results decrease considerably over time in both groups: from 1.25mm ± 0.79 to 0.30 mm ± 0.49 in the control group and from 1.18 mm ± 0.66 to 0.18 mm ± 0.36 in the test group.

Mixed ANOVA shows that the differences over time are statistically significant (p=0.000), indicating a significant reduction in recession.

There are no statistically significant differences between groups (p=0.416) or in the time*group interaction (p=0.716).

REAREA (RECESSION AREA):

The results decrease considerably over time in both groups: from 3.91mm ± 2.75 to 0.99 mm ± 0.61 in the control group and from 3.54 mm ± 2.03 to 0.61mm ± 1.19 in the test group.

Mixed ANOVA shows that the differences over time are statistically significant (p=0.000), indicating a significant reduction in recession area.

There are no statistically significant differences between groups (p=0.34) or in the time*group interaction (p=0.996).

OVERALL CONCLUSION

The analysis of this table using mixed ANOVA indicates that while there are significant improvements over time in KTW, Re, and ReArea, these improvements are consistent across both the test and control groups. There are no significant differences between the groups or in the interaction between time and group, suggesting that both treatments are similarly effective over the study period.

4.4.5 THICKNESS/VOLUME RESULTS

Table 49 - Descriptive statisics for variables related to diference in Gingival thickness(ΔGT) (Data collected and analyzed by the author, 2024)

VARIABLE/STATISTIC	MEAN	STANDARD DEVIATION	MINIMUM	25TH PERCENTILE	MEDIAN (50TH PERCENTILE)	75TH PERCENTILE	MAXIMUM
MEANΔGT ($ROI_{(1)}$) (MM)	0.15	0.13	-0.14	0.04	0.15	0.21	0.37
MAXΔGT ($ROI_{(1)}$) (MM)	0.46	0.23	0.23	0.41	0.58	0.63	1.20
MEANΔGT ($ROI_{(2)}$) (MM)	0.273	0.16	0.05	0.17	0.28	0.37	0.65
MAXΔGT ($ROI_{(2)}$) (MM)	0.65	0.88	0.15	0.42	0.55	0.64	6.40

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VOLUME GAIN ($ROI_{(2)}$) (MM^3)	1.29	1.37	0.05	0.28	0.72	1.31	6.52

Table 50 - Shapiro-Wilk Test Results for normality of data
(Data collected and analyzed by the author, 2024)

VARIABLE	TEST STATISTIC	P-VALUE	INTERPRETATION
MEANΔGT ($ROI_{(1)}$)	0.9565	0.0884	normally distributed
MAXΔGT ($ROI_{(1)}$)	0.9431	0.0237	not normally distributed
MEANΔGT ($ROI_{(2)}$)	0.9612	0.1217	normally distributed
MAXΔGT ($ROI_{(2)}$)	0.8221	0.0000	not normally distributed
VOLUME GAIN ($ROI_{(2)}$)	0.6514	0.0000	not normally distributed

Table 51 - Statistical test for descriptive statistical ΔGT
(Data collected and analyzed by the author, 2024)

COMPARISON	TEST	TEST STATISTIC	P-VALUE
MEANΔGT ($ROI_{(1)}$) VS MEANΔGT ($ROI_{(2)}$)	Independent Samples t-test	-4.23	0.00005
MAXΔGT ($ROI_{(1)}$) VS MAXΔGT ($ROI_{(2)}$)	Mann-Whitney U Test	744.0	0.02176
VOLUME GAIN ($ROI_{(2)}$)	Mann-Whitney U Test	1102.0	0.00006

Table 52 - Comparative table between groups for ΔGT
(Data collected and analyzed by the author, 2024)

VARIABLE	GROUP	MIN - MA	$\bar{X}\pm S$	MEDIAN	MANN-WHITNEY TEST
MEANΔGT (ROI_1)(MM)	Control	-0.14 – 0.28	0.11±0.10	0.12	-0.78 (0.075)
	test	-0.05 – 0.37	0.16±0.11	0.15	
MAXΔGT ($ROI_{(1)}$(MM)	Control	0.25 – 1.11	0.55±0.20	0.55	-0.18 (0.857)
	test	0 .19– 1.46	0.58±0.27	0.55	
MEANΔGT ($ROI_{(2)}$)(MM)	Control	0 .01– 0.61	0.30±0.16	0.30	-0.43 (0.670)
	test	0.02 – 0.77	0.33±0.17	0.28	
MAXΔGT ($ROI_{(2)}$ (MM)	Control	0.11– 1.11	0.51±0.23	0.54	-0.11 (0.914)
	test	0.15 – 6.40	0.67±0.93	0.49	
$ROI_{(2)}$AREA(MM^2)	Control	0.41 – 5.27	2.38±1.46	2.24	-0.89 (0.375)
	test	0.63 – 10.21	2.83±1.91	2.41	
VOLUME IN $ROI_{(2)}$ (MM^3)	Control	0.00 -3.05	0.86±0.84	0.71	-0.82 (0.415)
	test	0.05 -6.52	1.13±1.25	0.64	

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Additional, mixed-effects models were used to account for the hierarchical structure of the data, controlling for intra-patient variability and providing more accurate estimates of the treatment effects

Table 53 - Statistical mixed-effects models test for ΔGT
(Data collected and analyzed by the author, 2024)

Variable	Intercept (Coef.)	Group (Coef.)	p-value (Group)	Group Variance	Residual Variance	Log-Likelihood
meanΔGT ($ROI_{(1)}$)	0.111 (p < 0.001)	0.050	0.106	0.002	0.0089	55.546
maxΔGT (ROI)1	0.547 (p < 0.001)	0.040	0.536	0.003	0.0561	-3.785
meanΔGT (ROI)2	0.297 (p < 0.001)	0.040	0.482	0.010	0.0182	27.029
meanΔGT ($ROI_{(2)}$)	0.509 (p = 0.001)	0.165	0.414	0.048	0.5018	-79.107
Volume in (ROI)2	0.841 (p < 0.001)	0.314	0.346	0.228	1.0006	-105.089

4.4.5.1 Interpretation

$mean\Delta GT\ (ROI_{(1)})$

The mean thickness gain for ROI 1 is higher in the test group compared to the control group. The p-values from both the Mann-Whitney U test and the mixed-effects model indicate that this difference is not statistically significant.

$max\Delta GT\ (ROI_{(1)})$

The maximum thickness gain for ROI 1 is slightly higher in the test group compared to the control group. Both p-values indicate no statistically significant difference between the groups

meanΔGT (ROI)2

The mean thickness gain for ROI 2 is slightly higher in the test group compared to the control group. Both p-values indicate no statistically significant difference between the groups.

meanΔGT (ROI)2

The maximum thickness gain for ROI 2 is higher in the test group compared to the control group. However, the high standard deviation in the test group indicates greater variability. Both p-values indicate no statistically significant difference between the groups.

Overall interpretation

The volume gain is higher in the test group compared to the control group. Both p-values indicate no statistically significant difference between the groups.

The test group shows higher mean and maximum thickness gains for both ROI 1 and ROI 2 compared to the control group.

The p-values from the Mann-Whitney U tests and the mixed-effects models consistently indicate no statistically significant differences between the test and control groups.

The closest to significance is the mean thickness gain for ROI 1 (meanΔGT) with a p-value of 0.075 from the Mann-Whitney U test and 0.106 from the mixed-effects model.

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This analysis confirms that while there are observable differences in thickness gains between the test and control groups, these differences are not statistically significant. The use of mixed-effects models further accounts for patient variability, strengthening the robustness of these findings

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4.4.6 RESULTS IN TERMS OF HEALING (IPR SCALE)

The healing results were obtained by implementing a IPR scale defined in material and methods (Table 10 and 11). The detailed results found can be find in the table 53.

The healing is scored, up to, 8 points in IPR Inflammatory, 5 points in IPR Proliferative, and 3 points on IPR Remodeling. Maximum score is 16 in total.

Table 54 - Healing results
(Data collected and analyzed by the author, 2024)

VARIABLE	GROUP	MIN – MAX (SCORE)	$\bar{X}$±S (SCORE)	MEDIAN(SCORE)	MAX (SCORE)	TESTE MANN-WHITNEY
IPR INFLAMMATORY	Control	6 – 8	7.00±0.71	7.00	8	-1.48 (0.182)
	test	4 – 8	6.10±1.37	6.50		
PROLIFERATIVE	Control	3 – 5	4.00±0.71	4.00	3	-1.30 (0.278)
	test	4 – 5	4.40±0.52	4.00		
REMODELING	Control	2 – 3	2.89±0.33	3.00	3	-1.05 (0.720)
	test	3 – 3	3.00±0.00	3.00		
IPR TOTAL	Control	9 – 12	10.11±1.05	10.00	16	-0.72 (0.497)
	test	7 – 12	9.50±1.58	10.00		

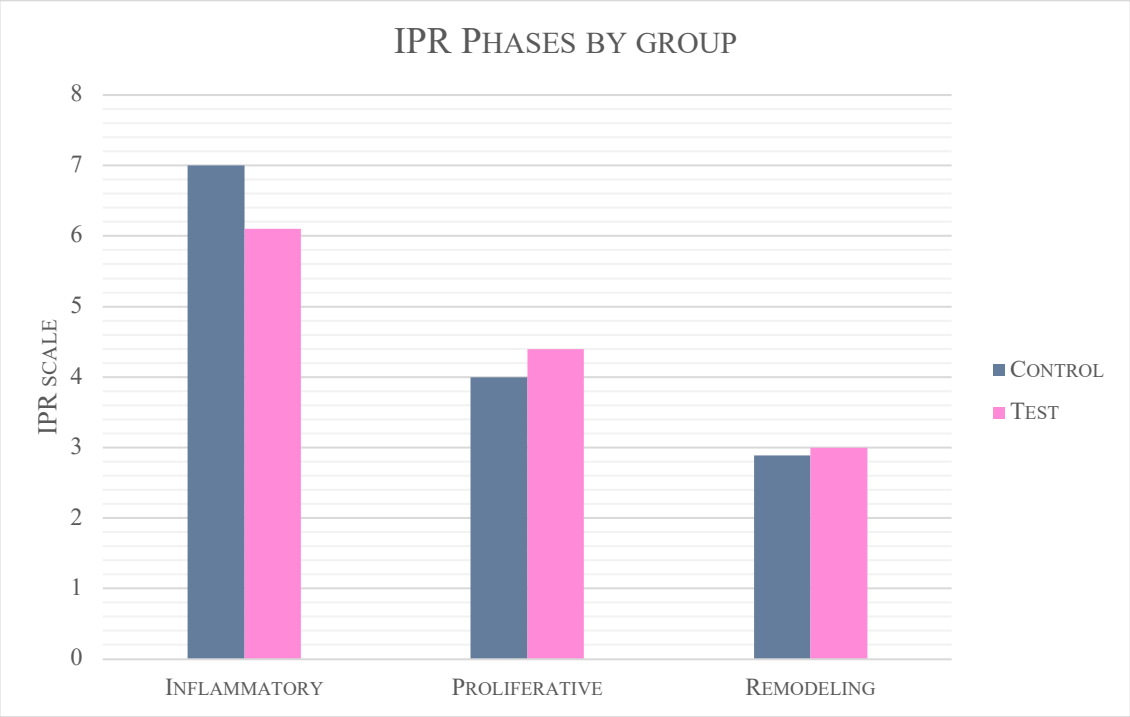


Figure 24- Healing Scores for IPR.
(Data collected and analyzed by the author, 2024)

INTERPRETATION

The total scores are quite similar between the two groups, with the control group having slightly higher mean and median values.

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IPR INFLAMMATORY

The control group shows slightly higher mean IPR Inflammatory scores compared to the test group. However, the difference is not statistically significant (p = 0.182). This suggests that there is no substantial difference in inflammatory responses between the two groups.

PROLIFERATIVE

The test group has a slightly higher mean proliferative score compared to the control group. However, this difference is not statistically significant (p = 0.278). This indicates that the proliferative response is comparable between the two groups.

REMODELING

Both groups have similar remodeling scores with no significant difference (p = 0.720). This implies that the remodeling phase of healing is consistent across both groups.

IPR TOTAL

The IPR total scores are similar between the two groups with no significant difference (p = 0.497). This indicates that the overall inflammatory and proliferative response, as measured by the IPR total, does not differ significantly between the control and test groups.

OVERALL INTERPRETATION

The results indicate that there are no statistically significant differences between the control and test groups in terms of the IPR scale outcomes (inflammatory, proliferative, remodeling, and total scores). Both groups show similar mean and median values across all phases, suggesting comparable healing responses in both groups based on the IPR scale.

4.4.7 RESULTS IN TERMS OF INDIRECT MEASUREMENT OF PAIN

Results in terms of indirect measurement of pain as described in material and methods, ibuprofene consumption pos operatory and VAS at 5 days (scale 1-10, with 0 corresponding to no pain and 10 corresponding to a maximum pain).

Table 55-Ibuprofen consumption (number of pills consumption) and VAS Score at 5 days (Data collected and analyzed by the author, 2024)

VARIABLE	GROUP	MIN - MAX	$\bar{X}$±S	MEDIAN	TESTE MANN-WHITNEY
VAS 5 DAYS (MAX SCORE 10)	Control	0 – 3	1.22±1.09	1.00	0.01 (0.999)
	test	0 – 5	1.40±1.51	1.00	
TOTAL IBUPROFEN (MG)	Control	0 – 4800	1800.00±1558.85	1200.00	-1.09 (0.315)
	test	0 – 2800	1030.00±813.84	600.00	
PILLS	Control	0 – 8	3.11±2.57	3.00	-1.22 (0.243
	test	0 – 4	1.70±1.25	1.00	

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4.4.7.1 Interpretation of Results:

Pills consummation

The mean number of pills taken by the control group is 3.11 with a standard deviation of 2.57, indicating moderate variability in pill consumption within this group. The median is 3.00, suggesting that half of the control group took fewer than 3 pills.

The mean number of pills taken by the test group is 1.70 with a standard deviation of 1.25, indicating less variability compared to the control group. The median is 1.00, indicating that half of the test group took 1 or fewer pills.

Statistical Significance: The Mann-Whitney test result (-1.22, p = 0.243) indicates that the difference in the number of pills taken between the control and test groups is not statistically significant at the 5% significance level.

VAS at 5 Days:

Control Group: The mean VAS score for pain at 5 days in the control group is 1.22 with a standard deviation of 1.09, showing low pain levels within this group. The median is 1.00, suggesting that half of the control group reported pain levels of 1 or lower.

Test Group: The mean VAS score for pain at 5 days in the test group is 1.40 with a standard deviation of 1.51, indicating higher variability in pain levels compared to the control group. The median is also 1.00, indicating that half of the test group reported pain levels of 1 or lower.

Statistical Significance: The Mann-Whitney test result (0.01, p = 0.999) indicates that the

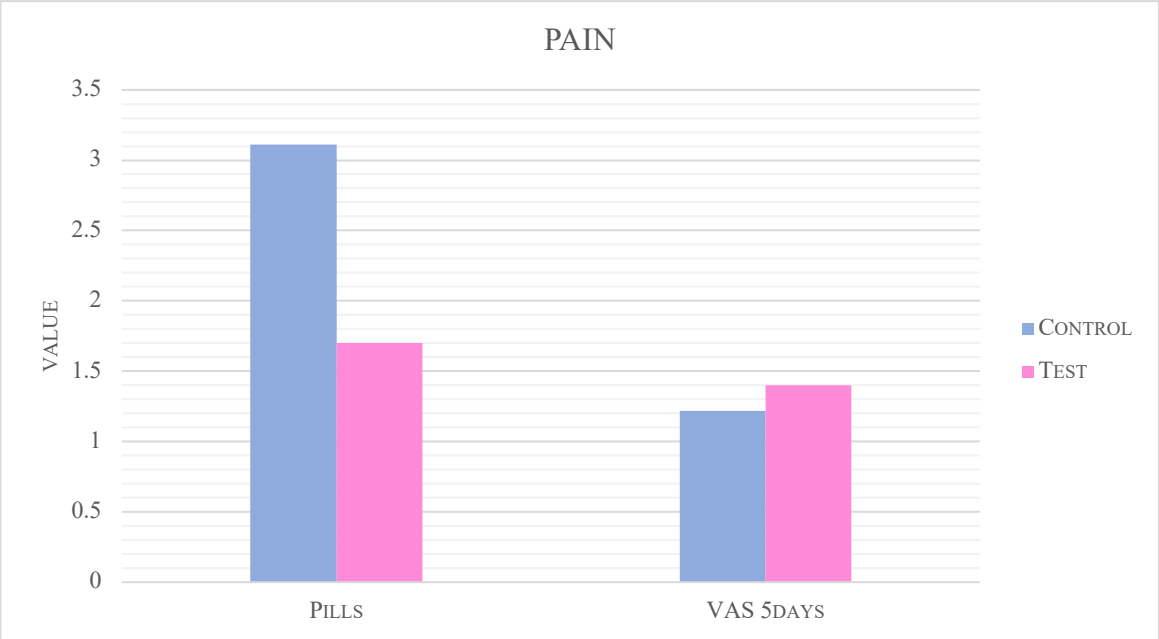


Figure 25 - Pills consumption and VAS score at 5 days (Pills-n,VAS-score).
(Data collected and analyzed by the author, 2024)

difference in pain levels between the control and test groups is not statistically significant at the 5% significance level.

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4.4.8 RESULTS IN TERMS OF PROMS

As discussed in material and methods, patient scored the satisfaction in a VAS scale, from 1-10, with 10 corresponding to maximum satisfaction, related to gingiva color satisfaction, recession coverage and overall satisfaction.

4.4.8.1 RECESSION COVERAGE SATISFACTION

Table 56-PROMs results in recession coverage satisfaction (Score,1-10)
(Data collected and analyzed by the author, 2024)

VARIABLE	GROUP	MIN – MAX	$\bar{X}$±S	MEDIAN	MAX SCORE	TESTE MANN-WHITNEY
GINGIVA COLOR (SCORE)	Control	8 – 10	9.78±0.67	10.00	10	-0.45 (0.780)
	test	8 – 10	9.70±0.67	10.00	10	
RE COVER (SCORE)	Control	3 – 10	7.44±2.74	8.00	10	-.051 (0.661)
	test	2 - 10	8.00±2.67	9.00	10	
OVERALL SATISFACTION (SCORE)	Control	2-10	8.22±2.86	10	10	-0.09 (0.968)
	test	2-10	8.30±2.75	10	10	

$\bar{X}$±s - média± desvio padrão;

4.4.8.2 SENSITIVITY SATISFACTION AND WILLINGNESS TO REPEAT SURGERY

Sensitivity and willingness to repeat the treatment were assessed on a binary response scale.

Table 57- PROMs results in sensitivity and willingness to repeat treatment (%)
(Data collected and analyzed by the author, 2024)

VARIABLES	RESPONSE	GROUP				TOTAL		CHI-SQUARE VALUE	P-VALUE (CHI-SQUARE)
		Control		Test					
		n	%	n	%	n	%		
SENSITIVITY (%)	Decrease/ Absent	7	77.8	10	100.0	17	89.5	2.59	0.11
	Increase	2	22.2	0	0.0	2	10.5		
	Total	9	100.0	10	100.0	19	100.0		
WILLINGNESS TO REPEAT (%)	No	0	0.0	1	10.0	1	5.3	1.98	0.16
	Yes	9	100.0	9	90.0	18	94.7		
	Total	9	100.0	10	100.0	19	100.0		

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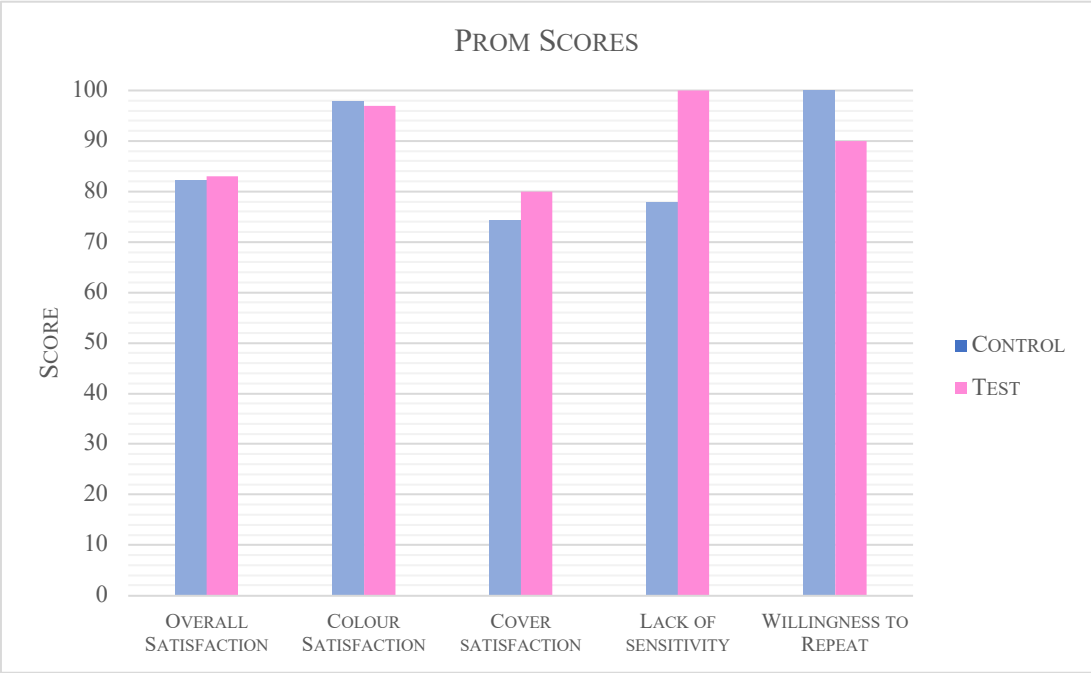


Figure 26- PROM scores (%).
(Data collected and analyzed by the author, 2024)

4.4.8.3 INTERPRETATION OF RESULTS

GINGIVA COLOR (1-10)

Both the control and test groups reported very high scores for gingiva color, with means close to 10 and low standard deviations indicating consistent responses. The Mann-Whitney test result ($p = 0.780$) indicates no statistically significant difference between the groups. Therefore, the intervention did not significantly affect patients' perception of gingiva color.

QUANTITY COVERAGE SATISFACTION (1-10)

Satisfaction was moderately high in both groups, with the test group showing slightly higher mean and median scores.

OVERALL SATISFACTION (1-10)

Overall satisfaction scores were high for both groups, with medians at the maximum score of 10. The Mann-Whitney test result ($p = 0.968$) indicates no statistically significant difference between the groups. This suggests that overall satisfaction with the treatment outcomes was not significantly different between the control and test groups.

ABSENCE OF SENSITIVITY (%)

The control group had 77.8% of patients reporting decreased or absent sensitivity, while the test group had 100% reporting decreased or absent sensitivity. Despite this apparent difference, the Chi-Square test result ($p = 0.11$) indicates no statistically significant association

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between the group and sensitivity. This suggests that while the intervention might appear to reduce sensitivity more effectively, this difference is not statistically significant.

WILLINGNESS TO REPEAT TREATMENT (%)

All patients in the control group (100%) would undergo the treatment again, compared to 90% in the test group. The Chi-Square test result ($p = 0.16$) indicates no statistically significant association between the group and the willingness to undergo the treatment again. Thus, the intervention does not significantly impact patients' willingness to repeat the treatment.

These interpretations suggest that the intervention did not produce significant differences in the patient-reported outcomes measured.

4.4.8.4 EXPLORATORY DATA ANALYSIS

In addition to addressing the primary objectives and hypotheses of our study, we recognize the importance of leveraging the full potential of our dataset through Exploratory Data Analysis (EDA). EDA serves as a vital step in uncovering hidden patterns, relationships, and anomalies that may not have been considered initially. This approach allows us to delve deeper into the data, offering a more comprehensive understanding of the variables at play and their interactions.

Table 58-Regression models result for relation between predictors and %RC (Data collected and analyzed by the author, 2024)

DEPENDENT VARIABLE	PREDICTORS	ADJUSTED R^2	F-STATISTIC (P-VALUE)	STANDARDIZED COEFFICIENTS	T-STATISTIC (P-VALUE)
%RC (%)	Constant	0.410	16.952 (0.000)	118.905***	15.894 (0.000)
	CEJ(B)			-0.303**	-3.155 (0.002)
	Vol(ROI_2) (mm^2)			0.470***	4.279 (0.000)
	ReT_0 (mm)			-0.548***	-4.972 (0.000)
MRC (MM)	Constant	0.787	85.953 (0.000)	0.375**	3.189 (0.002)
	ReT_0 (mm)			0.657***	9.922 (0.000)
	Vol(ROI_2) (mm^2)			0.351***	5.325 (0.000)
	CEJ(B)			-0.160**	-2.769 (0.007)

Table 58 presents the results of applying multiple linear regression models using the stepwise method to predict two dependent variables: %RC and MRC

4.4.8.5 MODEL STATISTICS

%RC(DEPTH)

ADJUSTED $R^2 = 0.410$

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The model explains approximately 41.0% of the variation in %RC This is a reasonable value, indicating that almost half of the variability in %Cover (depth) can be explained by the variables included in the model.

F-STATISTIC = 16.952 (P < 0.1%)

The overall significance of the model is very high, with a p-value less than 0.1%, indicating that at least one of the independent variables in the model is a significant predictor of %Cover (depth).

MRC

ADJUSTED R^2 = 0.787

The model explains approximately 78.7% of the variation in MRC. This is a very high value, indicating that most of the variability in MRC can be explained by the variables included in the model.

F-STATISTIC = 85.953 (P < 0.1%)

The overall significance of the model is extremely high, with a p-value less than 0.1%, indicating that at least one of the independent variables in the model is a significant predictor of MRC.

4.4.8.6 COMPARATIVE AND JOINT INTERPRETATION

EFFECT OF CEJ (B)

In both models, an increase in CEJ results in a decrease in the dependent variables (%Cover (depth) and MRC). This suggests a consistent negative impact of CEJ on both measures.

EFFECT OF VOL(ROI_2)

An increase in volume is associated with an increase in both dependent variables. This indicates that volume is a positive factor influencing both %Cover (depth) and MRC.

EFFECT OF RET_0

Interestingly, an increase in Re0 results in a decrease in %Cover (depth) but an increase in MRC. This suggests a bidirectional effect of Re0, depending on the specific dependent variable considered.

4.4.8.7 EXCLUDED VARIABLES

The variables group, KTW(T_0), meanΔGT, mean$\Delta GT_{(R)}$, mean$\Delta GT_{(R)}$ biotype, and STEP(+) were excluded from the models as they did not show significant effects on the variation of %RC or MRC. This indicates that, within the context of the data and the stepwise method used, these variables did not contribute significantly to the prediction of the dependent variables.

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4.4.8.8 INFLUENCE OF TYPE OF TOOTH IN %RC

Table 59-Influence of type of toot in %RC
(Data collected and analyzed by the author, 2024)

TOOTH TYPE	GROUP	MEAN	STANDARD DEVIATION	MEDIAN	KRUSKAL-WALLIS
PREMOLAR	Control	74.62	32.18	89.79	(K=9.619; p=0.022).
	Test	78.21	26.64	100.00	
	Total	76.47	29.05	100.00	
CANINE	Control	95.83	10.21	100.00	
	Test	96.54	10.93	100.00	
	Total	96.28	10.32	100.00	
CENTRAL	Control	100.00	0.00	100.00	
	Test	97.22	6.22	100.00	
	Total	98.01	5.25	100.00	
LATERAL	Control	78.97	27.76	88.79	
	Test	96.94	9.68	100.00	
	Total	91.81	17.71	100.00	

Considering the results from the previous table, it is observed that the mean values in the test group are higher, except for the central tooth type, although the differences between groups are small. However, overall, it is noted that for premolars, the mean value was 76.47±29.05, while for other tooth types, the mean values were higher, exceeding 90%. The application of the non-parametric Kruskal-Wallis test concluded that, overall, the results are statistically different between tooth types (K=9.619; p=0.022).

4.4.8.9 INFLUENCE OF TYPE OF TOOTH IN MRC

Table 60 - Influence of type of tooth in MRC
(Data collected and analyzed by the author, 2024)

TOOTH TYPE	GRUPO	MEAN	STANDARD DEVIATION	MEDIAN	KRUSKAL-WALLIS
PREMOLAR	control	0.88	0.60	0.72	(K=6.016; p=0.111)
	test	0.94	0.48	0.85	
	Total	0.91	0.53	0.76	
CANINE	control	1.10	0.66	1.09	
	test	1.42	0.81	1.16	
	Total	1.30	0.75	1.16	
CENTRAL	control	0.38	0.14	0.38	
	test	0.74	0.41	0.70	
	Total	0.64	0.38	0.50	
LATERAL	control	1.29	1.09	1.03	

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test	0.84	0.41	0.72
Total	0.97	0.66	0.72

Considering the previous results, it is observed that the test group has higher mean and median values, except for the lateral tooth type. Overall, the central tooth type presents the lowest results, 0.64±0.38, and the canine the highest, 1.30±0.75. However, the application of the non-parametric Kruskal-Wallis test concluded that the differences between the tooth types are not statistically significant (K=6.016; p=0.111).

4.4.8.10 INFLUENCE OF AGE

We test also the possible influence of age in outcomes.

Table 61- Spearman correlation test for age and outcomes
(Data collected and analyzed by the author, 2024)

VARIABLE	CONTROL	TESTE	GLOBAL
Δ KTW	0.224 (0.251)	-0.209 (0.185)	-0.039 (0.749)
%COVER (DEPTH)	-0.149 (0.449)	-0.065 (0.682)	-0.103 (0.395)
MRC	-0.250 (0.199)	0.096 (0.544)	0.156 (0.198)
%COVER (AREA)	-0.142 (0.472)	0.205 (0.193)	0.159 (0.189)
MEANΔGT	0.153 (0.438)	-0.142 (0.371)	0.043 (0.727)
MAXΔGT	-0.182 (0.353)	0.039 (0.804)	0.104 (0.391)
$MEAN\Delta GT_{(R)}$(	-0.357 (0.063)	-0.036 (0.822)	-0.041 (0.739)
$MAX\Delta GT_{(R)}$	-0.175 (0.373)	-0.046 (0.771)	0.038 (0.752)
$ROI_{(2)}$)	0.088 (0.657)	-0.096 (0.545)	0.115 (0.342)
VOL AT $ROI_{(2)}$	-0.097 (0.623)	-0.043 (0.785)	0.056 (0.646)

We didn't find any statistical significance for these correlations.

4.5 KEY FINDINGS FROM THE STATISTICAL RESULTS

4.5.1.1 SAMPLE CHARACTERIZATION

GROUP DISTRIBUTION

The randomization process effectively created comparable groups. No significant differences were found in baseline characteristics (age, KTW, recession, recession area, average number of recessions, gender, phenotype, sensitivity, CEJ classification, STEP classification) between the control and test groups.

SITE-SPECIFIC CHARACTERIZATION

Similarly, there were no significant differences in the site-specific continuous variables (age, KTW, recession, recession area) and categorical variables (sensitivity, phenotype, CEJ classification, STEP classification) between the groups.

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4.5.1.2 EXPLORATORY DATA ANALYSIS

Regression Models: The variables CEJ(B), Vol($ROI_{(2)}$), and ReT_0 are consistently significant, though with different effects depending on the dependent variable. The high adjusted R^2 values, especially for the MRC model, indicate that the models are robust and explain a substantial portion of the variability in the dependent variables, %RC and MRC.

Tooth type: For premolars, the mean value was 76.47±29.05, while for other tooth types, the mean values were higher, exceeding 90%. The application of the non-parametric Kruskal-Wallis test concluded that, overall, the results in terms of %RC are statistically different between tooth types (K=9.619; p=0.022).

Age Influence: No statistically significant correlations between age and the various outcomes (ΔKTW, %Cover depth, MRC, %Cover area, meanΔGT, maxΔGT, meanΔGT(R), maxΔGT(R), Volume at ROI(2)) were found.

4.5.1.3 OVERALL CONCLUSION

The study's randomization process was effective, resulting in comparable control and test groups.

The intervention (CAF combined with L-PRF) did not show statistically significant improvements in root coverage, keratinized tissue width gain, gingival thickness, volume gain, healing, pain measurement, or patient-reported outcomes compared to the control group (CAF alone). Despite trends suggesting potential benefits of the test treatment, these were not confirmed statistically within the scope and sample size of this study.

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4.5.2 SUMMARY TABLE AND GRAPHIC

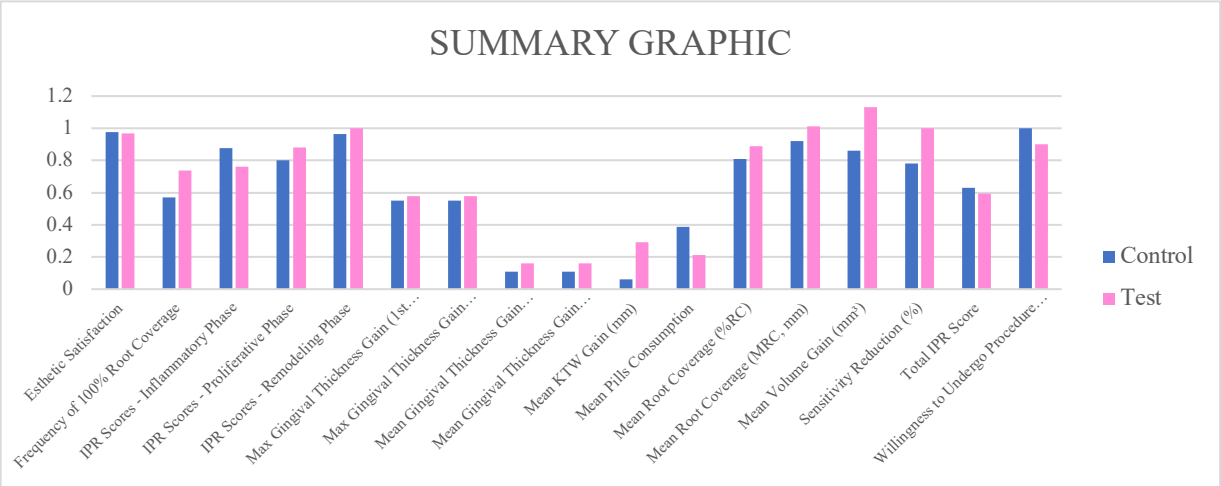


Figure 27 - Sumary Graphic (see table 62 for values and units)
(Data collected and analyzed by the author, 2024)

Table 62-Summary Table: Treatment of Miller Class I Gingival Recessions by CAF with or without Platelet-Rich Fibrin
(Data collected and analyzed by the author, 2024)

PARAMETER	TEST GROUP (CAF+L-PRF)	CONTROL GROUP (CAF ALONE)	P-VALUE	GRAPHIC PRESENTATION UNITS (FIG 28)
NUMBER OF SITES	42	28		
MEAN ROOT COVERAGE (%RC)	89.30% ± 20.33%	81.60% ± 27.93%	0.168	(%)
MEAN ROOT COVERAGE (MRC, MM)	1.01 ± 0.59	0.92 ± 0.68	0.260	mm
FREQUENCY OF 100% ROOT COVERAGE (CRC,%)	73.81%	57.14%	0.193	(%)
MEAN KTW GAIN (ΔKTW, MM)	0.29 ± 0.74	0.06 ± 0.58	0.307	mm
MEAN GINGIVAL THICKNESS GAIN (1ST $ROI_{(1)}$) ($_{(MEAN}\Delta GT$,MM)	0.16 ± 0.11	0.11 ± 0.10	0.075	mm
MAX GINGIVAL THICKNESS GAIN ($ROI_{(1)}$) ($_{(MAX}\Delta GT$, MM)	0.58 ± 0.27	0.55 ± 0.20	0.857	mm
MEAN GINGIVAL THICKNESS GAIN $ROI_{(2)}$ ($_{(MEAN}\Delta GT_{(R)}$, MM)	0.33±0.17	0.30±0.16	0.670	mm
MAX GINGIVAL THICKNESS GAIN ($ROI_{(2)}$) ($_{(MAX}\Delta GT_{(R)}$, MM)	0.58 ± 0.27	0.55 ± 0.20	0.857	mm
MEAN VOLUME GAIN AT $ROI_{(2)}$ (ΔVOL, MM^3)	1.13 ± 1.25	0.86 ± 0.84	0.415	mm^3
IPR SCORES - INFLAMMATORY PHASE (SCORE)	6.10 ± 1.37	7.00 ± 0.71	0.182	(%)/max
IPR SCORES - PROLIFERATIVE PHASE (SCORE)	4.40 ± 0.52	4.00 ± 0.71	0.278	(%)/max
IPR SCORES - REMODELING PHASE (SCORE)	3.00 ± 0.00	2.89 ± 0.33	0.720	(%)/max
TOTAL IPR SCORE (SCORE)	9.50 ± 1.58	10.11 ± 1.05	0.497	(%)/max

MEAN PILLS CONSUMPTION (N)	1.70 ± 1.25	3.11 ± 2.57	0.243	n
ESTHETIC SATISFACTION	9.70 ± 0.67	9.78 ± 0.67	0.780	(%)
WILLINGNESS TO UNDERGO PROCEDURE AGAIN	90%	100%	0.16	(%)
SENSITIVITY REDUCTION	100%	77.8%	0.11	(%)

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4.6 DISCUSSION

4.6.1 METHODS AND OVERVIEW

Optical scanning-based digital technologies that measure volumetric changes after root coverage therapy are increasingly preferred as the best way to assess the results of periodontal plastic surgery. However, this technology still faces the challenges of having no standardized method and reporting guidelines for volumetric outcomes, which makes it hard to compare different studies and treatments.

The study aimed to compare the efficacy of two techniques for gingival recession coverage: the control group using Coronally Advanced Flap (CAF) and the test group using CAF with Leukocyte-Platelet Rich Fibrin (L-PRF). Various parameters were measured, including keratinized tissue width (KTW), recession coverage (% cover depth and area), mean root coverage (MRC), volume gain, and patient-related outcomes (PROMs). The study involved 19 patients with 70 recessions, and the follow-up period was 6 months. The data was analyzed

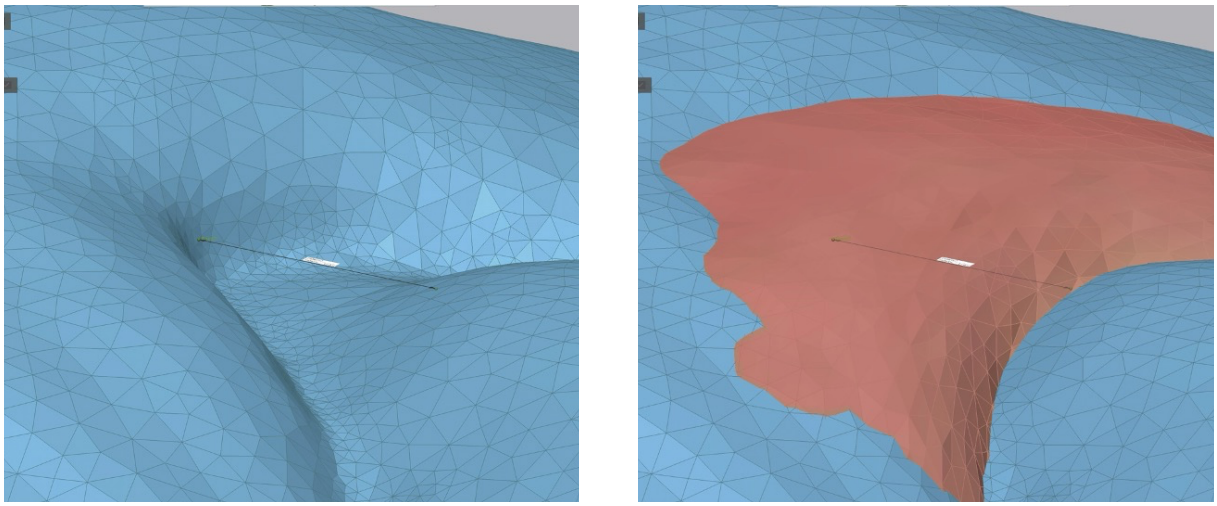


Figure 28 - Root coverage visualization obtained by superimpositon of STL (Screenshot of software Geomagic control X, taken by the author, 2024)

using appropriate statistical tests to determine the significance of the differences between the two groups. For continuous variables like age, KTW at baseline (T_0), recession (Re0), and recession area at T_0 (ReArea T_0), the mean and standard deviations were comparable between the control and test groups.

4.6.2 ROOT COVERAGE

Full root coverage is often considered the ideal outcome from an aesthetic point of view; however, partial coverage can be clinically significant when it solves hypersensitivity or improves aesthetics. Patient perception of improvement may also be important, not just clinical measurements.

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This study aimed to compare the effectiveness of the two surgical techniques for root coverage. Coronal Advanced Flap (CAF) alone (control) and CAF combined with Leukocyte-Platelet Rich Fibrin (L-PRF) (test). The primary outcome was the percentage of root coverage, in terms of length (% cover length) and area (% cover area).

The results indicated that the test group achieved a mean root coverage percentage (%RC) of 89.30% (±20.33%) compared with 81.60% (±27.93%) in the control group, although this difference was not statistically significant (p=0.168). Similarly, the mean root coverage (MRC) in millimeters was slightly higher in the test group (1.01 mm ±0.59 mm) than in the control group (0.92 mm ±0.68 mm), with a p-value of 0.260, indicating that there were no significant differences. Furthermore, the frequency of achieving 100% root coverage was higher in the test group (73.81%) than in the control group (57.14%); however, this difference was not statistically significant (p=0.193).

Related to various recession coverage techniques, in multiple recessions, overall results report (in the absence of loss of interdental attachment (Miller class I&II)), different frequencies of complete root coverage (ranging from 25% to 90%) and high levels of percent root coverage (ranging from 57% to 97%) (Tonetti & Jepsen, 2014).

The frequency of achieving full root coverage and the mean root coverage obtained globally in the total sample can be considered consistent with previous findings in the literature.

According to Miron, the use of PRF in conjunction with CAF statistically significantly improved relative root coverage (%RC) and clinical attachment level (CAL) compared with CAF alone. Specifically, the meta-analysis reported a mean difference in relative root coverage of 9.60% (95% CI: 0.00 to 19.1) that favored CAF + PRF; however, this was borderline significant (p=0.05). Miron reported that apart from the clinical trial by Sofia Aroca (Aroca et al., 2009) that found lower root coverage in the PRF group than in the control group, most of the trials found positive results in root coverage in the PRF groups. In general, the meta-analysis demonstrated an approximately 10 to 15% higher improvement in relative root coverage when PRF was combined with CAF compared to the use of CAF alone (Miron et al., 2020). Miron suggested that the differences in results in some studies can be explained by the lack of guidelines for treatment recession in terms of the thickness of PRF membranes, the number of membranes needed per site, and the ideal surgical technique for its use. In accordance with the suggested protocols for centrifugation (as recommended by Miron), this research employs these procedures. Quirynen et al., 2024) and a technique and number of membranes (4 a 6) suggested by his recent reviews to minimize the constraints referred to by Miron. Although we followed the recommended protocols, we found no significant differences between groups with or without L-PRF.

This referred meta-analysis (Miron et al., 2020) have shown significant results but the heterogeneity between studies and the absence of distinction for multiple or single recession compels to a cautious interpretation. Only two studies, with opposite results, studied the effect of L-PRF vs CAF alone in multiple recessions (Aroca et al., 2009; Kuka et al., 2018; Miron et al., 2020). Kuka finds better outcomes in root coverage parameters (%RC, CRC) for PRF group, but no statistical significance. Arouca find statistical significance in favor of control group for.

Most single recession studies do not show clinical significance or have borderline significance, usually finding better outcomes, but having difficulty obtaining statistical significance, this can be due to lack of statistical power to prove the superiority of L-PRF or can be due to real "no effect" or very "small effect". A probable explanation, for a real lack of effect, can be, according to Tavelli, an insignificant enrichment of growth factors (Lorenzo Tavelli, McGuire, et al., 2019). All other limitations of L- PRF discussed early in the chapter 1, should also be considered.

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Our study also showed a trend towards better root coverage in the PRF group but did not find statistically significant differences (p-0.168), which may be due to differences in sample size or study design.

Mancini (Mancini et al., 2021) recently analyze root coverage outcomes, on multiple adjacent gingival recessions (MAGR), with CAF + L-PRF vs. CAF alone. He finds no statistically significant differences between groups for the treatment of MAGRs in terms of %RC. In a systematic review, Castro (Castro et al., 2017) resumed their finds; “For periodontal plastic surgery, the comparison of CAF + L-PRF versus CAF led to controversial results. Although most studies did not show statistically significant differences, L-PRF was superior for all parameters recorded"

This aligns with the trend observed in our study, better outcomes without statistical significance.

As verified in our study, CAF alone has shown excellent clinical results for root coverage, with mean root coverage percentages often exceeding 80% in controlled studies at short-term and longer follow-ups (K. Jepsen et al., 2017; Giovanni Zucchelli, Mounssif, Mazzotti, Stefanini et al., 2014; Giovanni Zucchelli et al., 2019). This high baseline also created a "ceiling effect,” making it difficult to show statistically and clinically significant improvements with additional interventions. Given the already favorable results with CAF alone, any improvements from the addition of PRF or other adjuncts need to be sufficiently substantial to be statistically or clinically significant, especially in studies with limited sample sizes, such as ours.

The addition of L-PRF to the CAF technique was hypothesized to enhance gingival recession coverage by promoting healing and tissue regeneration. However, our results showed that both techniques were similarly effective in terms of% RC (depth and area), MRC, and CRC. The recessions decrease considerably over time in both groups: from 1.25 ± 0.79 to 0.30 ± 0.49 in the control group and from 1.18 ± 0.66 to 0.18 ± 0.36 in the test group. Mixed ANOVA showed that the differences over time are statistically significant (p=0.000), indicating a significant reduction in recession and effectiveness of treatments.

While L-PRF may have theoretical advantages due to its growth factors, its clinical benefits in our study context are not statistical significantly superior to those of CAF alone. This, "no significant" results, can be due to a "real" no addictive effect, or to result of methodological or statistics limitations in our study.

CAF shows promise but does not significantly outperform CAF alone in terms of root coverage in our study.

4.6.3 KTW INCREASE

The amount of KTW measured at baseline and one month after the surgery has been consistently associated with the short- and long-term clinical outcomes: the more significant the KTW, the more favorable the outcomes (Bertoldi et al., 2024)

In a recent meta-analysis, Miron et al. found that adding PRF did not improve KMW (Miron et al., 2020) related to control.

In this study, the baseline KTW was comparable between the control group (CAF alone) and the test group (CAF with L-PRF). This indicates that both groups started under similar conditions, allowing for a fair comparison of the effectiveness of the treatments.

Both groups exhibited an increase in KTW after six months, but residual in control group (0,06±0,589. Mixed ANOVA showed that the differences over time were statistically

significant (p=0.042), indicating a significant increase in the KTW over time, overall. The mean KTW gain (ΔKTW) was higher in the test group (0,29±0,74) than in the control group (0,06±0,58) , and the mean values increased slightly in both groups from baseline: from 3.00 ± 1.01 to 3.06 ± 0.86 in the control group and from 2.75 ± 0.84 to 3.03 ± 0.91 in the test group, and there were no statistically significant differences between groups (p=0.474) or in the time/group interaction (p=0.171). These findings are in line with the meta-review from Miron (Miron et al., 2020) and from Mancini (Mancini et al., 2021) that not found statistical significance between CAF + PRF and CAF alone in terms of KTW gain in the treatment of MAGRs.

The test for KTW gain (between the control (0.06 ± 0.58 mm) and test group (0.29 ± 0.74 mm) showed no significant difference (p-value > 0.05). This referred statistical tests suggests that both treatments were similarly effective in increasing KTW, but the addition of L-PRF to CAF did not result in a statistically significant improvement in KTW compared with CAF alone, based on the results of this study.

Our KTW gain results are inline than those typically reported in the literature for similar procedures that reported (0,08 mm at 6 months (Giovanni Zucchelli, Mounssif, Mazzotti, Stefanini, et al., 2014)) and inferior at reported values at 1 year (0,6-1,8 mm (G. Zucchelli & Sanctis, 2000; G Zucchelli & Sanctis, 2005)).

During Coronally Advanced Flap (CAF) surgery, there is coronal movement of the mucogingival line along with the flap. So theoretical, shouldn't occur a increase in KTW, however, as the tissue maturation process evolves after surgery, mucogingival line tends to return to its initial position. This movement result in an increase in the band of attached gingiva. The authors empirically observed this phenomenon (fig 30). When overlapping the T_0 and T_F models, there was coincidence in the vertical position of the mucogingival line in some cases.

These findings are consistent with the observation of Zucchelli and De Sanctis (G Zucchelli

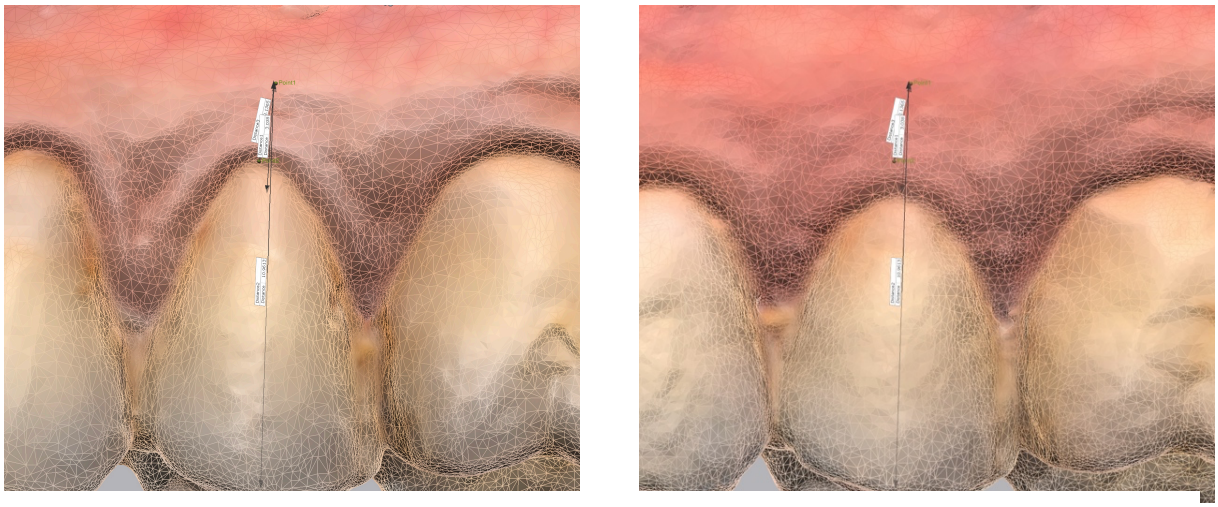


Figure 29 - Keratinized tissue measurements
(Screenshot of software Geomagic control X, taken by the author, 2024)

& Sanctis, 2005), who found a progressive increase in keratinized tissue from 6 months to 5 years), which was greater when, before surgery, there was a greater recession depth and narrower residual band of attached gingiva apical to the defects, which were the clinical situations in which a greater coronal displacement of the mucogingival line was performed during the surgery. The apical shift was proportional to the coronal positioning, and vice versa. The increase in keratinized tissue height following the coronally advanced flap procedure may

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be attributed to the tendency of the mucogingival line to regain its genetically determined position and relationship with the subjacent bone (Ainamo et al., 1981).

4.6.4 VOLUME INCREASING

The baseline gingival thickness can be a predictor of the outcomes of gingival recession. The study by Rebele (Rebele et al., 2014) also identified a critical soft tissue thickness necessary to achieve complete root cover (CRC). A mean marginal soft tissue thickness of 1.44 mm was calculated as necessary to achieve CRC with a confidence of 95%. These results suggest a threshold thickness above which the likelihood of successful root coverage increases significantly. A positive association exists between weighted flap thickness and mean and complete root coverage (Hwang & Wang, 2006).

Thick gingival tissue resists trauma and subsequent recession, allows better tissue manipulation, can promote creeping attachment, and exhibits less clinical inflammation.

Techniques that increase thickness (and KTW) tend to have better stability over time (Holtzman et al., 2020; L. Tavelli et al., 2019). It has been speculated that one of the reasons connective tissue graft techniques have better long-term results is due to increased gingival thickness (Bertoldi et al., 2024). When a technique does not increase gingival thickness, the initial GT becomes an important predictor of the long-term success of the technique. That is, with thick phenotypes, all the techniques tend to result, or at least in the short term. However, in thin phenotypes, an increase in thickness is mandatory. GT is considered a relevant component of the gingival phenotype, and its assessment is considered in the selection of the recession cover procedure (Cortellini & Bissada, 2018; Jepsen et al., 2018). In a long-term follow-up RCT (twelve years) in root coverage, Tavelli (Lorenzo Tavelli, Barootchi, et al., 2019) found that the presence of gingival thickness ≥ 1.2 mm at 6 months was found to be a predictor of the stability of the gingival margin throughout the 12 years. On the contrary, GT at baseline was not associated with changes in the gingival margin throughout the follow-up period. Therefore, surgical techniques that improve gingival thickness should be prioritized in the treatment of gingival recession defects. In the long term, CAF alone can be used, but is expected to have a partial recurrence (Bertl et al., 2021; G. P. Prato et al., 2011). We can speculate that if we capacitate the CAF to increase gingival thickness, it will tend to have better long-term results.

The studies by Aroca and Kuka (Aroca et al., 2009; Kuka et al., 2018) in multiple recessions both reported statistical significance for the gain in gingival thickness (GT). This difference, in addition to other factors, can be attributed to the difference measurement methods, as we use the full digital protocol for ΔGT measurements. In our RCT, volume-related outcomes showed a tendency for better outcomes favoring the test group, but without statistical significance. However, ΔGT thickness in $ROI_{(1)}$ almost had statistical significance (p-0,0759. This indicates that L-PRF may contribute to better gingival thickness, which is crucial for the long-term stability of the treated sites, as discussed above. However, the lack of significance in our results suggested, in our limitations, that the impact of L-PRF on volume gain might be limited.

In addition to the comparison between groups, the absolute value of the increase in thickness should also be considered for clinical relevance. The results in $ROI_{1,}$ in meanΔGT, were 0.16±0.11mm in the test group and 0.11±0.10mm in the control group (p-0.075). For $ROI_{(2)}$ 0.33±0.17mm in the test group and 0.30±0.16mm in the control group (p-0.670). The Δvolume results in $ROI_{(2)}$ were 0.86±0.84mm^3 for the control group and 1.13±1.25mm^3 for the

test group (p-0.415). The clinical relevance of an increase of this magnitude should be considered for each patient, pondering the previously discussed need to have a thickness of 1.2 mm at 6 months for long-term stability (Lorenzo Tavelli, Barootchi, et al., 2019). We can speculate that, for patients with a GT thickness of ±1mm this increase can be relevant, but for inferior GT, techniques with a great potential for a GT increase (i.e. CTG) should be preferable.

Possible reasons to explain this increased thickness phenomenon, with PRF, can be the composition and biological effects of L-PRF. L-PRF has high levels of growth factors such as Transforming Growth Factor-Beta (TGF-β) and Platelet-derived Growth Factor (PDGF). These growth factors activate fibroblasts, essential cells in connective tissue. Fibroblasts make collagen, a key structural protein that gives thickness and strength to the tissue. More collagen production leads to thicker and stronger tissue.

In terms of fibroblast proliferation and activity, growth factors in L-PRF, especially TGF-β, promote the multiplication and activity of fibroblasts. Active fibroblasts produce collagen and other components of the extracellular matrix that add to the overall thickness of the tissue.

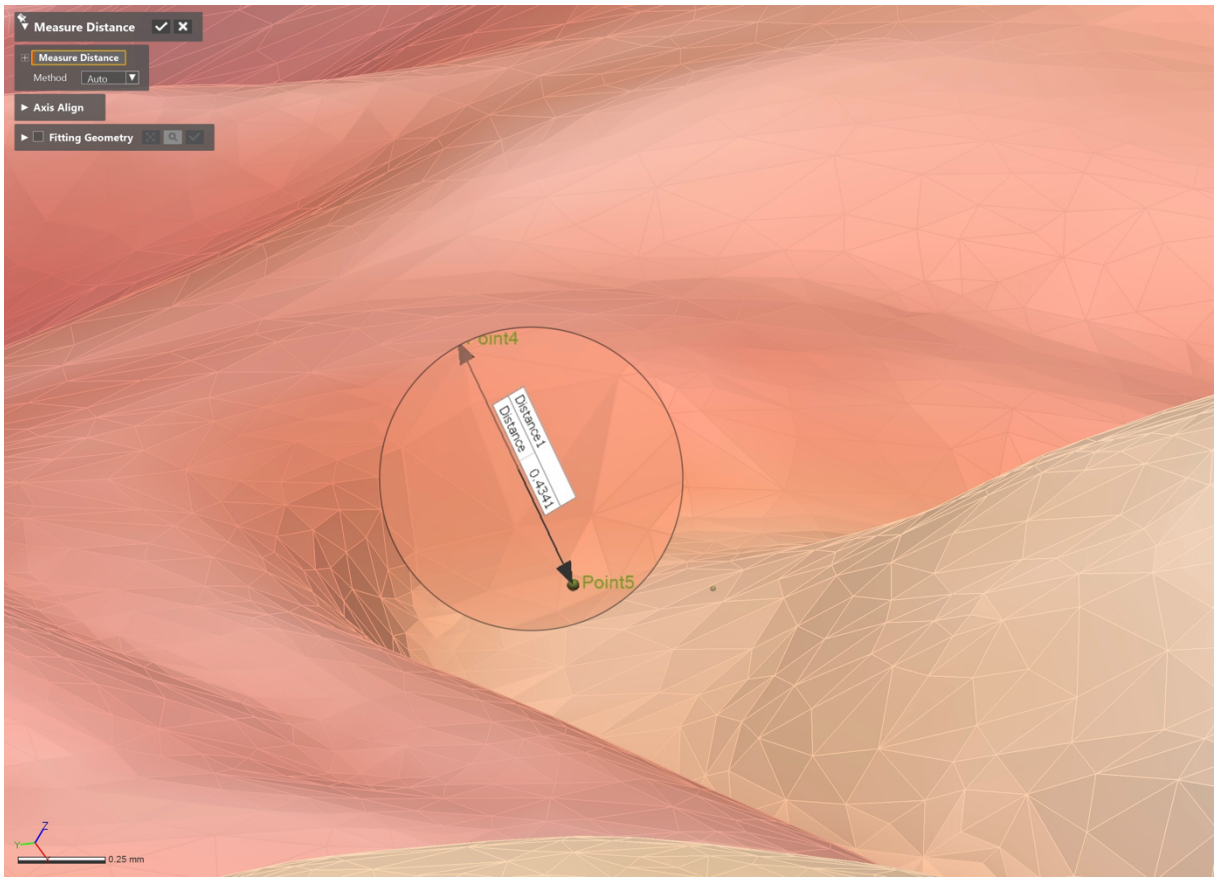


Figure 30- Digital measurement of thicknes of marginal gingiva
(Screenshot of software Geomagic control X, taken by the author, 2024)

The increased number and activity of fibroblasts cause more significant tissue regeneration and increased thickness.

In addition, L-PRF creates a dense fibrin matrix that supports cell movement and tissue regeneration. This matrix provides a structural base that supports the growth of new tissue layers. As cells move into the fibrin scaffold, they multiply and form new tissue, increasing the thickness.

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Growth factors such as Vascular Endothelial Growth Factor (VEGF) in L-PRF stimulate angiogenesis, creating new blood vessels. Better blood flow ensures that the regenerating tissue receives enough oxygen and nutrients. Better vascularization supports healthier and thicker tissue formation, as new vessels allow for more extensive tissue growth and maintenance.

The sustained release of growth factors from PRF provides a slow and steady release of growth factors over time. This long-lasting exposure ensures ongoing stimulation of cellular activities essential for tissue growth. Sustained release helps maintain an ongoing regenerative process, which increases the gradual increase in tissue thickness. The fibrin matrix of L-PRF allows infiltration and integration of different cell types, including endothelial cells, fibroblasts, and keratinocytes. These cells are vital in the formation of connective tissue and epithelial layers. Their increased presence and activity within the grafted area led to thicker, more integrated tissue. Additionally, PRF contains leukocytes and cytokines that help control inflammation. By reducing excessive inflammation, L-PRF creates a more favorable healing environment. Controlled inflammation prevents tissue breakdown and supports the preservation and growth of new tissue, which increases overall thickness. Resuming, we can speculate that the increase in tissue thickness when using L-PRF in recession coverage is primarily due to improved collagen production, increased fibroblast activity, the formation of a supportive fibrin matrix, improved angiogenesis, sustained release of growth factors, and better cellular infiltration and integration. These combined effects can create a supportive environment for more thick and robust tissue regeneration.

4.6.5 HEALING AND PAIN

In line with the reported results for anterior studies, we found that this kind of surgery has a low impact on quality of life as measured with the VAS scale (Mounssif et al., 2023). We found low scores for medication intake and VAS pain scores for both groups.

4.6.5.1 HEALING (IPR SCALE)

The total scores are quite similar between the two groups, with the control group having slightly higher mean and median values. (Test-9.50±1.58, Control-10.11±1.05). The control group shows slightly higher mean IPR Inflammatory scores compared to the test group. In the proliferative phase, the test group has a slightly higher mean proliferative score compared to the control group. Both groups have similar remodeling scores without significant differences, which implies that the healing remodeling phase is consistent between both groups (Table 53 and Fig 24).There is no statistically significant difference in IPR responses between the two groups. We did not find significant differences in healing between the two groups. This lack of significance could be due to a small sample size or limited measurement sensitivity. The tools and methods used, such as visual examinations and the Visual Analog Scale (VAS), might not be sensitive enough to detect subtle differences between the groups.

Furthermore, CAF results in terms of healing and reported pain have been very positive (Cairo et al., 2016, 2020; Tonetti & Jepsen, 2014). This high baseline also creates a "limiter effect", making it difficult to show significant statistical and clinical improvements with additional interventions.

Interesting, we have better inflammatory phase in the control group and better proliferative and remodeling phase in test group (Table 53 and Fig 24). These results can be related to the effects of L-PRF. L-PRF have been suggested having significant benefits across the inflammation, proliferation, and remodeling phases of healing. During the initial inflammation

phase (3-7 days), L-PRF's enriched leukocyte and platelet content plays a pivotal role in modulating the immune response by releasing cytokines such as IL-1β, IL-6, and TNF-α, which effectively reduce excessive inflammation and promote a balanced healing environment. These anti-inflammatory properties of L-PRF help control bleeding and prevent infection, ensuring a smoother transition to the subsequent proliferation phase.

In the proliferation phase (1-3 weeks), L-PRF continues to support healing by providing a sustained release of crucial growth factors, including TGF-β, PDGF, and VEGF (J. Blanco et al., 2024). These factors are essential for stimulating cell proliferation, angiogenesis, and tissue regeneration, with the fibrin matrix serving as a scaffold for new tissue growth.

As healing progresses into the remodeling phase, lasting several months, the continuous release of growth factors from L-PRF aids in the maturation and reorganization of newly formed tissue. This ongoing support minimizes chronic inflammation and enhances tissue integration and stability, thereby improving the expected healing outcomes. However, as Blanco acknowledged (J. Blanco et al., 2024), there is controversy on the role that leukocytes play in these autologous platelet concentrates. There are some groups that suggest the possible negative effects of using platelet-rich plasma with leukocytes in tissue regeneration because a high concentration of proinflammatory cytokines would increase the adverse effects of the inflammatory process, such as pain and swelling. This can explain the worst performance of test group in inflammatory phase of healing and consequent result in IPR scale. Another explanation can be the necessity of a more aggressive releasing of the flap during the surgery to accommodate the volume of membranes. The augmented time of surgery in group test could also influence this inflammatory result.

4.6.5.2 Post-Surgery Outcomes: Pills Taken and VAS at 5 days Inquiry discussion

The mean number of pills taken by the control group is 3.11 with a standard deviation of 2.57, indicating moderate variability in pill consumption within this group. The median is 3.00, suggesting that half of the control group took fewer than 3 pills. The mean number of pills taken by the test group is 1.70 with a standard deviation of 1.25, indicating less variability compared to the control group. The median is 1.00, indicating that half of the test group took one or fewer pills. This result can be explained by the reported analgesic and anti-inflammatory effect of L-PRF. L-PRF significantly reduces postoperative pain and swelling in procedures such as tooth extractions and recession cover surgeries (J. Blanco et al., 2024).

Both groups have a very low consumption of ibuprofene confirming the minimum invasiveness of booth procedures, with lower consumption in test group but without statistical significance.

L-PRF have been reported has having statistically significant better results in terms of pain reported when compared to CTG, but the literature sometimes is omissive to differentiate if the reported pain is related do surgical site or the existence of a second donor site. It could be easily understood that the use of autologous substitutes minimizes patient morbidity by avoiding a second surgery site. CAF alone has also been reported to have better results when compared with CTG (Giovanni Zucchelli, Mounssif, Mazzotti, Stefanini, et al., 2014). Aroca and Kuka in similar studies didn't report healing outcomes (Aroca et al., 2009; Kuka et al., 2018).

Pain and healing discussion conclusion

In our study, we observed contradictory results. While we experienced less pain in the test group, we also noted increased initial inflammation in the same group, followed by an improved proliferative phase. This may be related to the dual effects of leukocytes present in L-PRF,

which can exhibit both pro-inflammatory activities, important for initiating the healing process, and anti-inflammatory activities, which help modulate the inflammatory response and promote tissue regeneration. The sustained release of growth factors and cytokines by leukocytes may explain this dynamic, resulting in a better proliferative phase following the initial inflammation. However, we cannot exclude the possibility that the observed results could be due to other causes, such as surgical trauma or mere chance

4.6.6 PROs

PROs are essential for a patient-centered approach, enhancing the quality of care, treatment effectiveness, and overall patient experience in the healthcare system. In clinical research, PROMs are used to measure the effectiveness of treatments from the patient's perspective, complementing traditional clinical measures and providing a more comprehensive view of the benefits and risks of treatments. Pain, discomfort, and success perception are mandatory in evaluating the techniques. Pain can alter the willingness to undergo future surgical procedures. Both groups reported high overall satisfaction and willingness to undergo the procedure again, indicating that both techniques are well-received. The decrease in sensitivity reported by most patients further supports the effectiveness of these techniques in improving patient comfort.

A study by Choukroun et al. (2006) highlighted the role of L-PRF in reducing postoperative discomfort and promoting faster healing. The current study did not find significant differences in postoperative pain or medication use, suggesting that while L-PRF may have theoretical benefits, its impact on immediate postoperative recovery may not be substantial.

Since the patient's perception of aesthetics has changed dramatically in recent years, only achieving CRC can even be enough to guarantee the successful result of the treatment and patient satisfaction. Color/textural integration with adjacent tissue, gingival contour, absence of keloids/scars and alignment of the muco-gingival junction must also be taken into account.

For gingiva color, Re Cover, and overall satisfaction, both groups showed similar mean and median values, with very high satisfaction levels (control group-9,7+-0,67/test group -9,78+-0,67 in a scale of maximum 10). With no significant differences observed in the statistical tests.

Sensitivity and willingness to undergo the procedure again, were analyzed. Most patients reporting decreased sensitivity (with all patients referring absence of sensitivity in test group and 77% absence in the control group), without statistical difference between the groups.

Regarding willingness to repeat the procedure the results were very high again with 18 in 19 referring the willingness of repeat the treatment. This outcome is very interesting because the patient for the answer considers all the parameters (cost/benefit). The high results in this confirm the minimum invasiveness and effectiveness of the treatments.

While patient satisfaction in the current study was high for both groups, no significant differences were found, indicating that both techniques are well-received by patients.

In terms of PROMS results we have a very high score of satisfaction for all parameters, we can speculate that could exist a positive bias arising from the Hawthorne effect. This effect could influence the study results, as participants might over-report favorable outcomes, mitigating possibles differences among groups. Considering the small sample the results should be interpreted with caution.

4.6.7 TECHNICAL CONSIDERATIONS FROM A SURGEON PERSPECTIVE

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It is interesting to consider the influence of surgeon skills on the outcomes of procedures. Theoretically, the same technique can achieve different outcomes by different operators. Ideal, the predictability of the one technique should not depend on the operator. In other words, one technique that achieved 100 percent results in 10 percent of operators can be disfavored for a technique that achieved 90 percent results in 90 percent of operators. So, the influence of the operator on the results should be considered and the results should be possible to generalize.

Proper preparation and application of L-PRF require specific training and expertise. The fibrin matrix can be fragile and difficult to handle, posing challenges during surgical procedures. In surgeon perspective we found the CAF+L-PRF more challenging in technical terms. The suture of four to six L-PRF membranes together and his stabilization in the pretended surgery site was, sometimes challenging. The increased volume of L-PRF membranes under surgical flap implies a vast release and carefully suture to assure the absence of tension that could influence outcomes. These circumstances dictated longer surgeries, that could influence healing and pain outcomes.



Figure 31 - Before and after intervention, in a test subject (Created by author, 2024)

4.6.8 EXPLORATORY DATA ANALYSIS

We applied multiple linear regression models using the stepwise method to predict the% RC MRC outcomes. Among the various correlations tested, we found that CEJ (B), ReT0 and Vol(ROI2) act as predictors of the% RC MRC influencing the outcomes of these variables.

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These variables are consistently significant, though with different effects depending on the dependent variable. The high adjusted R^2 values, especially for the MRC model, indicate that the models are robust and explain a substantial portion of the variability in the dependent variables.

An increase in $Vol(ROI_2)$ is associated with an increase in both dependent variables. This relationship can be visualized as a pyramid effect: just as the size of a pyramid determines its height, increasing the volume at the base of recession increases de recession cover length. This analogy helps to understand how improving a volumetric measure translates into substantial linear gains in recession coverage. Clinically interesting is the concept that if we increase the thickness of gingival tissues, we improve the linear recession coverage; this can explain why techniques like CTG (that improve substantially the thickness of tissue), have better results in linear results of root coverage.

The effect of ReT_0 was expected, a greater recession tends to have worst results in terms of % root coverage but tends to have better results in terms of MRC. It results from the different nature of the outcomes. MRC is a linear (absolute) measure, deeper recessions have more mms to cover, so it's natural that the amount of mm covered will be greater, but the relative cover (% RC) tends to diminish.

Related to CEJ (B), this can be explained with the lack of stabilization of the blood clot. A defined margin can help stabilize the flap and theoretically improve outcomes (G Zucchelli et al., 2006). Even after the CEJ were reconstructed we have the problem of the margin of restauration and the retention of plaque.

Relatively to the influence of tooth type, we find that there is a statistical difference in the % RC. It is noted that for premolars, the mean value was 76.47±29.05, while for other tooth types, the mean values were higher, exceeding 90%. This is in line with previous publications that found similar results (Giovanni Zucchelli et al., 2018, 2019). In a similar conclusion, Zucchelli found that the treated teeth in the distal position were found to be related to a statistically significant lower %RC. The lack of vertical release incisions may pose a challenge in achieving a tension-free flap in these areas. Additionally, other anatomical conditions, including root prominence, limited KTW and reduced vestibule depth, which are commonly found in posterior areas, also negatively affect the predictability of root coverage procedures (Giovanni Zucchelli et al., 2019).

In summary, although we tested several correlations, we did not find significant correlations. This fact was a surprise as we expected correlations between $KTW(T_0)$ and phenotype with the recession coverage outcomes, as discussed previously.

4.6.9 Overall discussion and Study limitations

When we analyze the systematic review and meta-analysis by Miron (Miron et al., 2020) while there is a trend suggesting L-PRF + CAF may provide an improvement in root coverage compared to CAF alone, with mean differences of 3-14%, the evidence is not strong enough to definitively state this as a clinically relevant effect size. While there is a general trend favoring L-PRF + CAF, the results are not entirely consistent across studies, with some showing larger effects than others. So, we can consider that our results are in general terms is in line with previous reported results in literature as discussed previous. Nonetheless, some constraints and limitations could influence our results and should be addressed in this discussion. The failure to find a statistical difference between groups, resulting in a negative clinical trial, does not provide evidence that two products are equivalent. This is because there are two possibilities when a statistical test fails to reject a null hypothesis. The failure maybe due to no real

difference between products or a failure of finding a real difference between products due to inadequate statistical power (Gunsolley, 1998).

In this work we have two types of outcomes, patient related, and site related. Relatively to the patient related outcomes the findings of no statistical relevance, in all outcomes, was expected considering the size of sample (n=19).

Regarding the site related outcomes, the lack of statistical significance in our study may be, also, attributed to the small sample size and variability in the measured outcomes. We based our sample calculations in previous studies (Kuka et al., 2018). We calculated an effect size (Cohen's d) of approximately 1.12. We aim for a large effect size that could represent a clinically meaningful difference between the treatment groups. In practical terms, this corresponds to a 13.73% greater mean root coverage for the CAF + PRF group compared to the CAF alone group. With this value for effect size , we calculate a minimum sample size of sites/recessions of 40. We manage to recruit and analyze in the study 70 sites/recessions. This number is in line of the reported literature in this field. Miron in a recent systematic review and meta-analyze evaluated 17 studies, the average sample size was 49 treated gingival recessions per study. We could consider a smaller effect in our sample calculation, but we risk to lost clinical relevancy considering the small size of recessions in our study (1,28±0,55mm).The clinical relevance threshold for gingival recession typically relates to the measurement at which gingival recession concerns the patient and in recession coverage field is considered to be 0,5mm. Never less, a large sample would be preferable to have more conclusive results.

Another limitation we have that could influence the study results is the small size of the recessions in the sample. We can hypothesize that with biggest recession the effect can be more pronounced. This was result of our inclusion criteria where we determine the necessity to have at least 1,5 mm of keratinized tissue. Normally, sites with largest recession tend to not fulfill that requisite, especially in pre-molar area. A large population could be necessary to get a sample with large recessions that fulfill both requisites, deepest recession and keratinized tissue. New studies, without that limitation, and can be necessary to clarify this aspect.

One of the challenges we have in study was in terms of patient recruitment. With small recessions, patient and clinicians tend to postpone the treatment and control evolution. In our sample the main reasons for willingness to undergo treatment was cervical dentin hypersensitivity and esthetics concern. This difficulty in recruitment, and the coincidence with the COVID pandemic, make us to considerably extend the timeline of study, and restrain us to have a longer follow-up.

In our study all the surgeries were performed by the same surgeon. Using the same surgeon makes easier to control variability, consistency and cofounding variables but add several limitations. The outcomes can be affected by a surgeon-specific bias, as surgeons have different levels of skill and experience. Including multiple surgeons can increase the generalizability of the study findings. If only one surgeon is used, the results may be specific to that surgeon's technique and experience. Provided, that the study is carefully designed to control for variability its preferably to have multi-surgeons and be multicentric.

Although we follow a restrict protocol in the production of LPRF membranes, and in patient selection, we find a subjective difference in macroscopic characteristics of L-PRF membranes between patients. This variability, originated from differences in blood composition among patients, could influence the obtained results.

In terms of patient-related outcomes (IPR, Pills consumption, and PROMs) the results are extremely conditioned by the small sample, and no conclusion should be drawn. They should be analyzed with extreme caution. The findings should be viewed as preliminary and exploratory in nature; they serve as supplementary information rather than the main focus of

this study. Regarding the evaluation of healing, we employ a recent (IPR) scale that distinguishes the various stages of healing. However, due to its novelty and low adoption, this scale did not enable us to conduct an analytical comparison with previous studies that used comparable indices, as it only permitted qualitative comparisons.".

As for patient-related outcomes, we have attained an exceptionally high level of satisfaction across all parameters, and it is essential to consider the potential influence of the Hawthorne effect, as previously mentioned in this document.

Large sample would be preferable to increase the power of study in both patient-related and site-related outcomes.

4.6.10 Clinical Implications and Future Research

Numerous studies have suggested the potential benefits of L-PRF in enhancing wound healing and tissue regeneration. The current study contributes to this body of knowledge by confirming a trend to some of these benefits, particularly in terms of gingival thickness, but fails to confirm overall statistics difference in these outcomes in relatively small recessions (1,21+-0,71) with more dan 1,5 mm of keratinized tissue remaining. The study's findings suggest that while L-PRF may offer some benefits in terms of gingival thickness, its overall impact on recession coverage is comparable to CAF alone. Clinicians can consider using L-PRF in cases where enhanced gingival thickness is desired but should also weigh the cost and additional steps involved in preparing and applying L-PRF.

We want to highlight the need for further research to understand the clinical advantages of L-PRF at long-term follow up. Although this technique is used for more than 20 years, literature still lacks long-term follows to provide clinicians evidence-based decisions in different scenarios. At our knowledge the longest follow-up is 18 months for a study that compare the use of L-PRF with CTG (Carrera et al., 2023)

Future research, besides concentrate on longer follow-up periods to assess the long-term benefits of L-PRF, could, as well, explore its impact on different types of recessions, and patient related variability on L-PRF production . Additionally, larger sample sizes and multi-center trials could provide more robust data to support clinical decision-making. The understanding of the benefits and limits of L-PRF seems of outmost importance to avoid controversial results and to provide the best treatment to our patient

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5 CONCLUSIONS

5.1 CONCLUSION FOR THE PRIMARY OBJECTIVE (% ROOT COVERAGE LENGTH)

Despite the statistics suggesting a tendency towards higher average coverage in the experimental group, the Mann-Whitney U test and mixed-effects model did not confirm statistical significance. Given the absence of a statistically significant difference between the groups, we are unable to reject the null hypothesis, which posits that incorporating L-PRF into CAF will not yield significantly greater root coverage percentage compared to CAF alone at the six-month post-operative mark. Therefore, we cannot assert that the addition of L-PRF to CAF leads to a significantly higher percentage of root coverage length.

5.2 COMPLETE ROOT COVERAGE (CRC) AND MEAN ROOT COVERAGE (MRC)

No statistically significant difference was found in root coverage between the CAF combined with L-PRF group and the CAF alone group. Thus, we fail to support the alternative hypothesis that CAF combined with L-PRF results in significantly greater root coverage at six months post-surgery.

5.3 KERATINIZED TISSUE WIDTH (KTW) GAIN AND THICKNESS GAIN (ΔGT)

No statistically significant difference in gingival tissue thickness DGT and quality (ΔKTW) was observed between the groups. Therefore, we fail to support the alternative hypothesis that CAF combined with L-PRF leads to thicker gingival tissue and better tissue quality compared to CAF alone.

Relative to gingival thickness variations, in general the test group perform better in all variables, with better mean, but never achieving statistical significance. However, our analysis found a potential trend for mean thickness gain (meanΔGT at $ROI_{(1)}$) in favor of the test group, almost achieving statistical significance. Better studies, with larger samples can, confirm this trend. However, the clinical relevancy of the difference should also be addressed.

5.4 HEALING AND INDIRECT MEASUREMENT OF PAIN

The results of the implement of a healing scale indicates that there are no statistically significant differences between the control and test groups in terms of the IPR scale outcomes (inflammatory, proliferative, remodeling, and total scores). Both groups show similar mean and median values across all phases, suggesting comparable healing responses in both groups based on the IPR scale. In terms of Ibuprofen consumption and VAS Scores: The test group showed lower mean ibuprofen consumption and slightly higher VAS scores at 5 days, but these differences were not statistically significant the analysis showed no statistically significant difference in healing time or complications between the CAF with L-PRF group and the CAF

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alone group. Thus, we fail to support the alternative hypothesis that CAF combined with L-PRF results in faster healing and recovery with fewer complications.

5.5 PATIENT-REPORTED OUTCOMES S (PROS)

There were no statistically significant differences between the groups regarding gingiva color, coverage satisfaction, overall satisfaction, sensitivity, and willingness to repeat treatment. Both groups indicated high levels of satisfaction and a willingness to undergo the treatment again. Consequently, due to the lack of statistical significance, we cannot uphold the alternative hypothesis suggesting that the combination of CAF with L-PRF leads to greater satisfaction with treatment outcomes compared to CAF alone.

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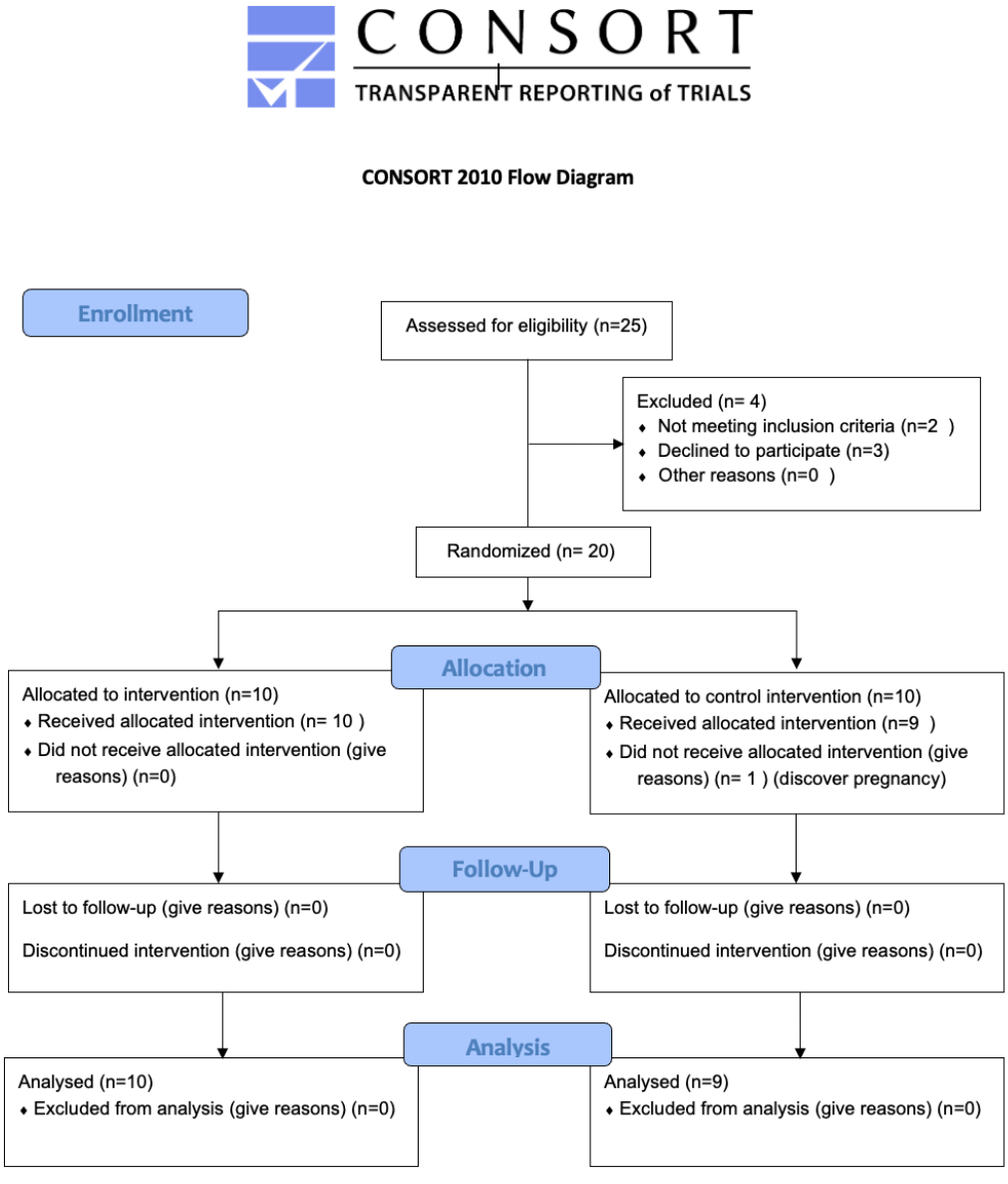
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APPENDICES

APPENDIX I: CONSORT FLOW DIAGRAM



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APPENDIX II: SELECTION DATA FORM

4.3. PERSONAL DATA FORM

Data: / /

NOME:

BI:

MORADA:

TELEFONE:

CODE

29

4.4. SELECTION DATA FORM

Data ____/____/____

CODE

Tem ou teve alguma condição de saúde ou enfermidade?

1.
2.
3.
4.
5.

Faz alguma medicação ?

1.
2.
3.
4.
5.

INCLUSION CRITERIA	EXCLUSION CRITERIA
☐ Maior de 18 anos	☐ Gravidez
☐ Não Fumador	☐ Fumador >10 cigarros/dia
☐ Fumador <10 no_____	
☐ Sem doenças sistemicas condicionante	Doenças/condições sistémicas
☐ Sem medicação condicionante	☐ Déficit vitaminico ou proteico
☐ BOP/IP<25%	☐ Radiação terapeutica
	☐ Transtornos metabolicos
	☐ Transtornos medicamentosos

ESPECIFIC INCLUSION CRITERIA

- ☐ Anterior, maxillary gingival recession
- ☐ Multiple gingival recessions
- ☐ Miller Class I
- ☐ No occlusal trauma
- ☐ IP<25%

30

4.5. BASELINE DATA FORM

Data ____/____/____

CODE

Reasons for treatment

- ☐ Esthetics
- ☐ Fear of losing teeth
- ☐ Hypersensitivity
- ☐ Other _____

Buccal

tooth	17	16	15	14	13	12	11	21	22	23	24	25	26	27
PD														
BOP														
CAL														
plaque														

Palatal

tooth	17	16	15	14	13	12	11	21	22	23	24	25	26	27
PD														
BOP														
CAL														
plaque														

RECESSION TABLE				
Tooth (FDI)				
Recession height (RH)				
Recession width (RW)				
Gingival thickness (GT)				
Keratinized Tissue (KT)				
Miller Class				
Cairo Class				
Pini-Prato Class				
Sensitivity (+/-)				

Others

- ☐ Oral hygiene instructions
- ☐ Photos
- ☐ Analogical impressions, scan of cast
- ☐ Digital impressions
- ☐ CT scan

4.6. DAY 5 , DATA FORM

Data ____/____/____ | Week _____ | CODE

Changes in general health or habits:

- ☐ Yes _____
- ☐ No

IPR INFLAMATORY PHASE

1. Bleeding:
   - ☐ yes
   - ☐ no
2. Granulation tissue
   - ☐ yes
   - ☐ no
3. Hematoma
   - ☐ yes
   - ☐ no
4. Tissue color
   - ☐ Redder or whiter than opposite side tissue
   - ☐ Like the opposite side tissue
5. Incision margins
   - ☐ Incomplete flap closure/ fibrin clot/ partial necrosis / complete necrosis
   - ☐ Complete flap closure/ fine fibrin line
6. Suppuration
   - ☐ yes
   - ☐ no
7. Edema (surgeon VAS 1-10)
   - ☐ 6-10 VAS
   - ☐ 1-5
8. Pain (Patient self-reported-VAS 1-10)
   - ☐ 6-10 VAS
   - ☐ 1-5

TOTAL SCORE _____

32

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Medication (painkiller, first week):

Days _____
Mg _____

Complications:

- ☐ No
- ☐ Pain
- ☐ Infection
- ☐ Wound dehiscence
- ☐ Others _____

4.9. TIME 3 AND 6 MONTH DATA FORM

Data ____/____/____ | Month _____ | CODE

Buccal

tooth	17	16	15	14	13	12	11	21	22	23	24	25	26	27
PD														
BOP														
CAL														
plaque														

Palatal

tooth	17	16	15	14	13	12	11	21	22	23	24	25	26	27
PD														
BOP														
CAL														
plaque														

RECESSION TABLE				
Tooth (FDI)				
Recession height (RH)				
Recession width (RW)				
Gingival thickness (GT)				
Keratinized Tissue (KT)				
Miller Class				
Cairo Class				
Pini-Prato Class				
Sensitivity (+/-)				

Other

- ☐ Photos
- ☐ Analogical impressions, scan of cast
- ☐ Digital impressions
- ☐ CBCT scan

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APPENDIX III: PROMS EVALUATION FORM

Paciente_____

Idade na altura da cirurgia_____

Grupo

❑ Com PRF

❑ Sem PRF

Satisfação em relação ao resultado estético:

Cor da gengiva

0	1	2	3	4	5	6	7	8	9	10



Recobrimento da Gengiva

0	1	2	3	4	5	6	7	8	9	10



Estética Geral

0	1	2	3	4	5	6	7	8	9	10



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Paciente_____

Idade na altura da cirurgia_____

Grupo

❑ Com PRF

❑ Sem PRF

Satisfação em relação ao resultado estético:

Cor da gengiva

0	1	2	3	4	5	6	7	8	9	10



Recobrimento da Gengiva

0	1	2	3	4	5	6	7	8	9	10



Estética Geral

0	1	2	3	4	5	6	7	8	9	10



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Satisfação em relação à sensibilidade

❑ Aumentou

❑ Diminuiu

❑ Desapareceu

Disponibilidade para fazer novamente, na mesma situação.

Voltava a fazer?

❑ Sim

❑ Não

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APPENDIX IV: INFORMED CONSENT

CONSENTIMENTO INFORMADO

-Informação ao paciente-

TÍTULO DO ESTUDO

TREATMENT OF MILLER CLASS 1 GINGIVAL RECESSIONS BY CAF WITH OR WITHOUT PLATELET-RICH FIBRIN

A RANDOMIZED CLINICAL CONTROL TRIAL

OBJECTIVO

O objetivo deste estudo é avaliar os resultados clínicos obtidos pela associação de fibrina rica em plaquetas ao procedimento cirúrgico de recobrimento radicular de recessões gengivais.

DESCRIÇÃO DO ESTUDO E PROCEDIMENTOS

Existirão dois grupos de pacientes no qual o participante pode ser incluído.

A escolha do grupo a que pertencerá será totalmente aleatória, não sendo dependente de quaisquer factores ou elementos da investigação.

1) No primeiro grupo (controlo) proceder-se-á ao tratamento pela técnica cirúrgica que é opção de referência para o tratamento da condição em causa (tratamento cirúrgico de recessões gengivais por retalho de reposição coronal tipo Zucchelli).

2) No segundo grupo utilizar-se-á a mesma técnica, mas associada ao uso de fibrina rica em plaquetas e leucócitos.

VANTAGENS

Em termos de resultados, para qualquer dos grupos, espera-se uma melhoria significativa da condição a tratar.

Efeitos do plasma autologo:

Investigações realizadas nos últimos anos têm revelado evidência experimental e clínica que demonstram que os factores de crescimento contidos na fibrina rica em plaquetas e leucócitos, favorecem a regeneração tecidular. As plaquetas possuem em seu interior factores de crescimento, proteínas que podem acelerar os processos de cicatrização dos tecidos.

PROCEDIMENTOS E RISCOS

Este estudo não envolve procedimentos cirúrgicos que não se enquadrem na prática clínica normal desta especialidade e que não tenham sido já objeto de estudos prévios, sugestivos dos resultados expectados.

Procedimento cirúrgico.

O procedimento consiste na libertação cirúrgica dos tecidos gengivais e recolocação numa posição em que recobre as recessões gengivais.

Caso esteja englobado no grupo de estudo, é necessário a obtenção de fibrina rica em plaquetas e leucócitos. A fibrina rica em plaquetas e leucócitos é obtida pela colheita de sangue. Posteriormente o sangue é sujeito a centrifugação para isolamento dos constituintes sanguíneos pretendidos obtendo-se assim uma membrana. Essa membrana será interposta entre a gengiva e os tecidos subjacentes durante o procedimento cirúrgico atrás descrito. A obtenção e aplicação do plasma sanguíneo realiza-se sobre estritas condições de assepsia, minimizando o risco de contaminação e a possibilidade de infecção zona de aplicação.

Riscos

Os riscos associados ao procedimento cirúrgico incluem; risco de infecção da zona cirúrgica, hemorragia da zona cirúrgica e hematoma peri-cirúrgico entre outros.

Riscos associados a colheita e utilização de plasma autólogo; ainda que não se possam, em termos absolutos, descartar totalmente efeitos adversos na utilização de plasma autólogo, estudos prévios têm demonstrado a segurança do procedimento e ausência desses mesmos efeitos indesejáveis nos estudos realizados. Com esta técnica, como se utiliza plasma do próprio paciente, não existe risco de reacções imunológicas à sua aplicação.

Riscos relacionados com medicação associada; posteriormente ao procedimento; é necessário proceder a medicação com anti-inflamatórios ocorrendo os riscos normais para essa situação.

Seguro

O investigador principal possui seguro de responsabilidade social

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RECOLHA DE DADOS

Serão recolhidos dados clínicos, fotografias, exames radiológicos e questionários pós-operatórios para possibilitar a análise de resultados. Serão recolhidos dados por um período de 6 meses, correspondente à duração do estudo.

Todos os dados fornecidos serão apenas utilizados pelos responsáveis do estudo.

A informação recolhida será tratada com a máxima confidencialidade, sendo o seu nome codificado e tendo apenas os investigadores deste estudo acesso a essa mesma relação.

Todos os elementos recolhidos passarão apenas a ser identificados a partir daí apenas pelo código anónimo.

Mediante a vontade do participante, após o término do estudo, os dados poderão; 1) ser destruídos, 2) conservados, mas sem possibilidade de identificação (anónimo), 3) conservados mantendo a codificação apenas acessível aos investigadores.

Na ausência de informação da vontade do participante os dados serão eliminados após termino do estudo

ESCLARECIMENTOS ADICIONAIS

Pode efetuar todas as questões que achar necessárias para o seu esclarecimento ou pedir informações aos responsáveis do estudo, em qualquer etapa do mesmo.

O participante é livre para interromper a sua colaboração no estudo, ou no esquema terapêutico instituído, em qualquer momento, sem necessidade de explicações, sem que com isso seja prejudicado no seu tratamento e acompanhamento clínico.

CONTACTOS

O investigador Principal é Nuno Bernardo Malta dos Santos (berna74@gmail.com) , OMD 3851, especialista em Periodontologia pela Ordem dos Médicos Dentistas, e este estudo está inserido nos estudos de Doutoramento do investigador, a decorrer na Universidade de Santiago Compostela, com supervisão de Prof. Dr. Juan Blanco Carrion (USC) e Prof. Dr. André Correia (UCP)

Contacto telefónico: 96887774 E-Mail: berna74@gmail.com

Clínica Dentaria Universitária da Universidade Católica, Telefone: 232 419 656

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DECLARAÇÃO DE CONSENTIMENTO INFORMADO

TÍTULO: TREATMENT OF CLASS 1 AND 2 GINGIVAL RECESSIONS BY CAF WITH OR WITHOUT PLATELET-RICH FIBRIN

1. Recebi informação sobre o estudo mencionado acima e foram respondidas todas as perguntas necessárias.
2. Entendo que a participação é voluntária e que é possível retirar-me do estudo quando desejar, sem ter que dar explicações, e sem isso afetar os meus cuidados médicos.
3. Autorizo o uso dos meus dados e exames diagnósticos, sendo eles arquivados com codificação de forma a assegurar o meu anonimato
4. Dou o meu consentimento informado para participação neste estudo.

Com relação à conservação e uso dos dados coletados neste estudo (Na ausência de informação da vontade do participante os dados serão eliminados):

- Não permito a conservação dos dados após o término do estudo.
- Permito a conservação e acesso aos dados sem identificação (anónimo), após o término do estudo
- Permito a conservação e acesso aos dados com codificação, após o término do estudo

O participante:

Nome _____

Data:_____ , ___/___/_____

O pesquisador:

Nome _____

Data:_____ , ___/___/_____

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APPENDIX V: POST-OPERATIVE CARE (PORTUGUESE)

CUIDADOS PÓS-OPERATÓRIOS

ANTI-INFLAMATÓRIO

1 comprimido ibuprofeno 600 mg -30 minutos antes da cirurgia (pré-operatorio)

1 comprimido ibuprofeno 600 mg 6 horas depois da cirurgia

1 comprimido 12 em 12 horas, **se necessário**, durante os dias seguintes

– ANOTE O NÚMERO DE COMPRIMIDOS UTILIZADOS-

HIGIENE

Não escovar a zona envolvida nas primeiras 3 semanas

Primeiras 3 semanas:

Irrigação com clorexidina 0,12% por 1 minuto, **3 vezes ao dia**

Semana 3 a 6:

Irrigação com clorexidina 0,12% por 1 minuto, **2x ao dia** e utilização de escova **ultra-suave** (cirúrgica) por mais 3 semanas

Semana 6 a 10

Irrigação com clorexidina 0,12% por 1 minuto,**1x dia** e utilização de escova **suave** (cirúrgica) por mais 3 a 4 semanas

2,5 meses em diante

Escova suave e fio dentário esponjoso

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OUTROS CUIDADOS PÓS-CIRÚRGICOS

Gelo durante as primeiras **24 horas** (aplicado por 10 minutos intervalando com 15 minutos de descanso

Repouso máximo da zona afectada nos primeiros dias. Evitar trauma mecânico e não pressionar ou distender a zona intervencionada.

Não praticar desporto ou esforços físicos na primeira semana

DIETA

Dieta semi-líquida, **fria,** no dia do procedimento.

Dieta semi-líquida no dia seguinte

Comida suave, nas duas primeiras semanas

CONSULTAS DE CONTROLO

5º Dia – Consulta de controlo

14º dia – Consulta de controlo e remoção de sutura

6 semanas – Consulta de controlo

3 meses - Consulta de controlo

6 meses – Consulta de controlo

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APPENDIX VI: DECLARATION OF APPROVAL BY THE RESEARCH ETHICS COMMITTEE

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Parecer sobre o projeto nº 123
Comissão de Ética para a Saúde da Universidade Católica Portuguesa
Mandato 2019/2023

Projeto de Investigação
Em reunião do dia 25 de Fevereiro de 2021 procedeu-se a nova apreciação de projeto já apreciado pela CES-UCP em reunião do dia 30 de abril de 2020, então designado Projeto nº 71-2020.
A ausência de resposta às objeções da Comissão por período superior a seis meses, determinou a necessidade de submissão formal de novo projeto que, no entanto, manteve o mesmo título, investigador principal, orientador e demais traços característicos do projeto originário. Os documentos apresentados procuram acomodar as objeções apresentadas.
No dia 25 de Fevereiro foi emitido parecer favorável condicional sujeito à resposta a esclarecimentos reproduzidos em baixo.
No dia 3 de Novembro é enviada resposta ao pedido de esclarecimentos assinada pelo investigador principal. Procede-se aqui a essa resposta, em sede da 35ª reunião da CES-UCP, no dia 16 de dezembro de 2021

Título: Treatment of Miller Class 1 Gingival Recessions by CAF with or without Platelet-Rich Fibrin a Randomized Clinical Control Trial", programa de doutoramento em Ciencias Odontolóxicas da Universidade de Santiago Compostela

Investigador Principal: Nuno Bernardo Malta dos Santos
Orientador: Juan Blanco-Carrion, do Departamento de Estomatologia, da Universidade de Santiago de Compostela e André Correia, da Faculdade de Medicina Dentária – UCP.

Resumo: Trata-se de um estudo clínico com intervenção, com a seguinte fundamentação: "As recessões gengivais constituem uma condição patológica caracterizada pela migração apical das gengivas, resultando na exposição da raiz dentária. Esta condição tende a ser progressiva, resultando em sensibilidade dentária, cáries radiculares e problemas estéticos." Tendo como **objetivo** a "verificação se a adição de membranas de L_PRF melhora os resultados clínicos do no tratamento cirúrgico desta condição". Conta, para o efeito, com a metodologia ". Conta, para o efeito, com a **metodologia** de "ensaio clínico, controlado, aleatorizado" que detalha adianta no formulário de submissão.

O **local onde decorre** é a Universidade Católica Portuguesa, Clínica Universitária – Centro Regional de Viseu. E abrange como **população** "pacientes da Clínica Universitária com indicação para recobrimento radicular que demonstraram interesse na participação do estudo. Alocação aleatória dos participantes". Não envolve menores, nem grupos vulneráveis. Exige a distinção de um grupo de controlo e de um grupo teste.
Identifica como **potenciais riscos**, "Risco de hemorragia local cirúrgica. Edema e dor pós-cirúrgico", embora destaque que "Os procedimentos clínicos, que serão comparados no estudo, constituem prática usual para o tratamento deste tipo de condição. Os procedimentos em estudo, têm o seu uso documentado clínica e experimentalmente. Não existem riscos suplementares ao que é a prática clínica para o tratamento cirúrgico desta condição.".
Destaca como **benefícios** associados ao estudo "Em qualquer dos grupos de estudo é expectável a melhoria da condição clínica de acordo com os standards atuais do tratamento. No grupo de estudo com L-PRF poderá ocorrer um potencial benefício em termos de melhoria do pós-operatório de acordo com os estudos prévios". Considera, por fim, que o estudo **prevê intervenção clínica** que não implica a existência de um seguro para os participantes.

Em reunião de 30 de abril de 2020 e no que concerne o projeto originário, então denominado, projeto nº 71-2020, deliberou assim a Comissão:
"Ouvido o Relator, e o plenário da reunião do dia 30 de abril de 2020, realizada por vídeo-conferência, esta CES delibera, por unanimidade, a emissão de **Parecer Favorável Condicional** aos esclarecimentos seguintes:

1. O formulário de consentimento é muito detalhado e informativo. Apenas se justifica:
   a. Menção expressa da possibilidade, a qualquer momento, de revogação de consentimento na participação no estudo; **b**. Indicação do método de arquivamento que garante as condições de

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anonimato; **c.** Indicação do destino dos dados em caso de ausência de manifestação de vontade por parte do participante;
2. Justificar-se-ia indicar no formulário de submissão, de modo sucinto, os termos em que a colaboração institucional e/ou pessoal entre orientadores se encontra protocolada neste programa de doutoramento;
3. Indica-se não se justificar a necessidade de um seguro específico para os participantes. Seria conveniente a verificação da solidez da opção e a sua justificação no formulário de submissão, tendo em conta tratar-se de um estudo com intervenção clínica, com inflição de riscos potenciais;
4. Atendendo aos riscos potenciais identificados solicita-se a clarificação sobre as medidas previstas para a mitigação desses riscos."

Analisados os elementos agora apresentados, cumpre considerar o seguinte, do ponto de vista ético:

Reproduzindo as objeções então expendidas, analisamos à luz de cada uma, as alterações propostas no novo projeto.

1. **O formulário de consentimento é muito detalhado e informativo.** Apenas se justifica:
   a. Menção expressa da possibilidade, a qualquer momento, de revogação de consentimento na participação no estudo; **b**. Indicação do método de arquivamento que garante as condições de anonimato; **c.** Indicação do destino dos dados em caso de ausência de manifestação de vontade por parte do participante;

Sobre este ponto as alterações constantes do novo formulário de consentimento parecem ser satisfatórias. ***Chama-se a atenção, no entanto, para o acrescento nesse formulário de informação relativa a existência a seguro referida em baixo, no ponto 3, nos termos aí indicados****.*

2. **Justificar-se-ia indicar no formulário de submissão, de modo sucinto, os termos em que a colaboração institucional e/ou pessoal entre orientadores se encontra protocolada neste programa de doutoramento;**

Sobre este ponto nada é dito no formulário de candidatura reformulado e ora apresentado. Não obstante, dos documentos apresentados, constam as pertinentes autorizações das instituições participantes, pelo que lhes cabe assegurar a colaboração institucional e pessoal que o projeto requeira.

3. **Indica-se não se justificar a necessidade de um seguro específico para os participantes. Seria conveniente a verificação da solidez da opção e a sua justificação no formulário de submissão, tendo em conta tratar-se de um estudo com intervenção clínica, com inflição de riscos potenciais;**

Opta, desta feita, pela indicação de seguro de responsabilidade civil contratual, subscrito pelo investigador principal, na qualidade de médico dentista, indicando a apólice respectiva no formulário de candidatura do projeto (ponto 7).
Não se trata, aparentemente, de um seguro subscrito para efeitos de atividade de investigação científica, de índole clínica e com potencial de inflição de danos.
A Comissão não informada se o seguro em causa assegura cobertura de danos realizados no exercício de atividade de investigação científica. ***Não pode, por isso, asseverar que as preocupações indicadas neste ponto tenham sido obviadas.***
Fica, por isso, à responsabilidade do investigador principal a garantia de que o seguro mencionado assegura a cobertura mencionada.
Deverá o investigador informar as entidades sob a égide das quais decorre o estudo da existência de seguro, da sua apólice, assim como das condições do contrato. Também deverá informar os participantes da sua existência e apólice, bem como, em traços gerais, dos termos em que se vê assegurada a cobertura de riscos potenciais. Esta informação dirigida aos participantes no estudo clínico deverá ainda, de modo sucinto, constar do formulário de consentimento.

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4. **Atendendo aos riscos potenciais identificados solicita-se a clarificação sobre as medidas previstas para a mitigação desses riscos.**

No formulário de candidatura (ponto 3.6.) consta descrição detalhada relativa a esta preocupação.
Citamos:
"Como forma de minimização de riscos, os participantes serão alvo de uma história clínica completa de forma a identificar potenciais riscos acrescidos, serão efetuados exames radiológicos e os procedimentos serão realizados com o recurso a técnica cirúrgica, e materiais, de acordo com o "gold standard" de controlo de infeção cirúrgica. A colheita de sangue será realizada por um enfermeiro experiente, a intervenção cirúrgica será executada por um especialista em Periodontologia, pela Ordem dos Médicos Dentistas, com mais de 10 anos de experiência na área. Será dado informação ao paciente, por escrito, dos cuidados pós-operatórios e medicação necessária. Os participantes terão acompanhamento pós cirúrgico de acordo com o protocolo de investigação."

As medidas parecerem, no essencial, suficientes, no entanto, entende-se que **deverá ser identificada e autonomizada cada procedimento clínico gerador de risco potencial, identificando em relação a cada uma o risco que potencial que pode gerar, assim como as medidas adoptadas para o prevenir**.

Do formulário do projeto e do formulário do consentimento, neste último caso em linguagem perceptível, **deve constar** essa autonomização de cada procedimento e risco respetivo, bem como as medidas adoptadas para os prevenir.

Deste modo, ouvido o Relator, e o plenário da reunião do dia 25 de fevereiro de 2021, realizada por videoconferência, esta CES delibera, por unanimidade, a emissão de **Parecer Favorável Condicionado** ao ajustamento do projeto, nos termos acima especificados, e do formulário de consentimento no sentido indicado.

Apreciação na 35ª reunião da CES-UCP
II. No dia 3 de Novembro foi enviada **resposta ao pedido de esclarecimentos assinada pelo investigador principal.**

Apreciada a resposta, assim como a reformulação do formulário de candidatura e ainda a nova redacção de consentimento informado, conclui-se no sentido de que as dúvidas levantadas se veem respondidas de forma satisfatória, de um ponto de vista ético.

Estiveram presentes na reunião nº 35 da CES-UCP
Presidente: Doutora Mara de Sousa Freitas
Vice-Presidente: Doutora Teresa Marques
Doutor Jerónimo Santos Trigo
Dr. Eugénio Fonseca
Doutora Ana Mineiro Zaky
Doutora Marta Brites

Conclusão
Ouvido o Relator, e o plenário da reunião de 16 de dezembro de 2021, realizada por videoconferência, esta CES delibera, por unanimidade, emitir P**arecer Favorável.**

Esta CES solicita ao Investigador Principal que, aquando da conclusão do estudo, lhe seja enviada uma síntese dos resultados obtidos e respetivas conclusões, via eletrónica, para o correio eletrónico da CES UCP.

A Presidente,

Mara de Sousa Freitas

Mara de Sousa Freitas
16/12/2021

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APPENDIX VII: CONFLICT OF INTEREST STATEMENT

I, Nuno Bernardo Malta dos Santos, declare that during the conduct of the research titled "Treatment of Miller Class I gingival recessions by CAF with or without platelet-rich fibrin - a randomized clinical control trial." or in preparation for any future article, no conflicts of interest could have influenced the results or interpretation of the data.

Funding Sources: The research was self-funded. No brand, Agency, or Institution influenced the study design, data collection, analysis, interpretation of data, manuscript writing, or decision to submit the article for publication.

Personal or Professional Relationships: I do not have any personal or professional relationships that could be considered a conflict of interest regarding the work presented.

Financial Interests: I do not receive any financial benefits or hold interests in commercial entities that could gain or lose financially from the publication of this work.

Aveiro, 1 Jul. 2024
Nuno Bernardo Malta dos Santos

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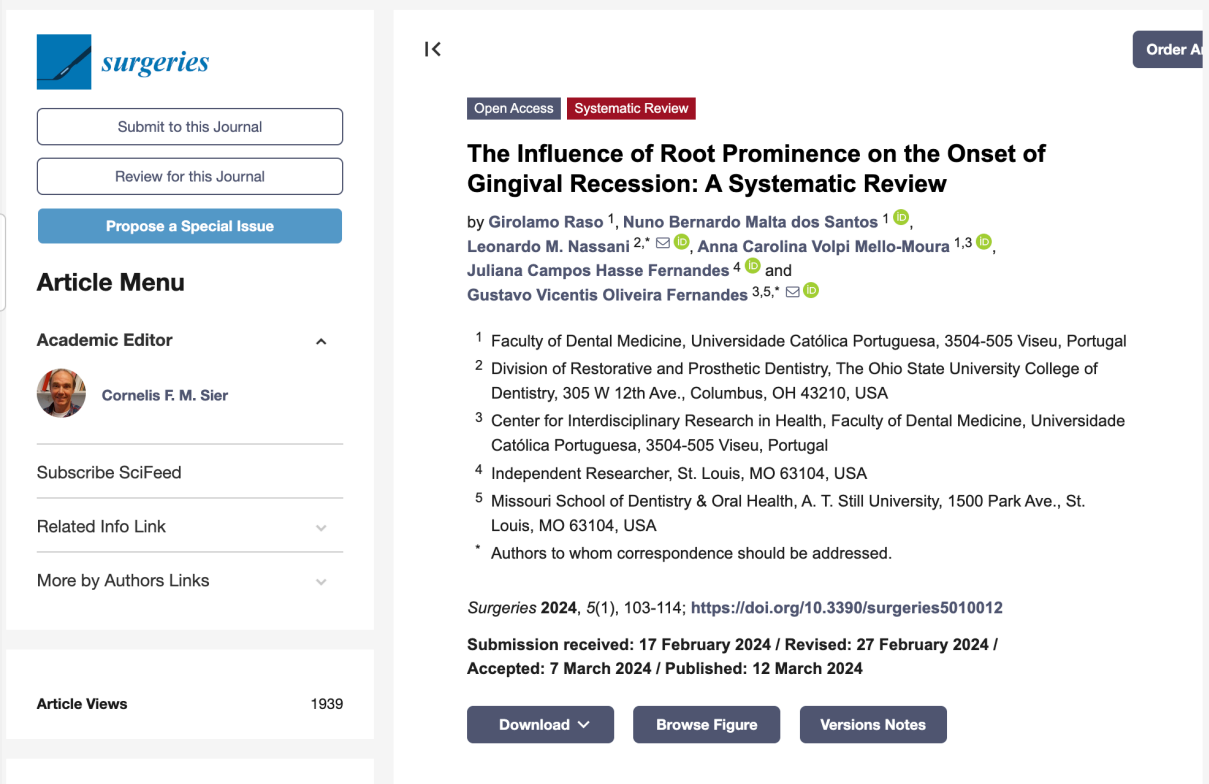
APPENDIX VIII: RIGHTS

Statement of Original Authorship and Content

All figures in this thesis were created by the author, who holds full rights over them, and they have not been used in previous publications. Hence, they constitute original content that does not require permission from any journal.

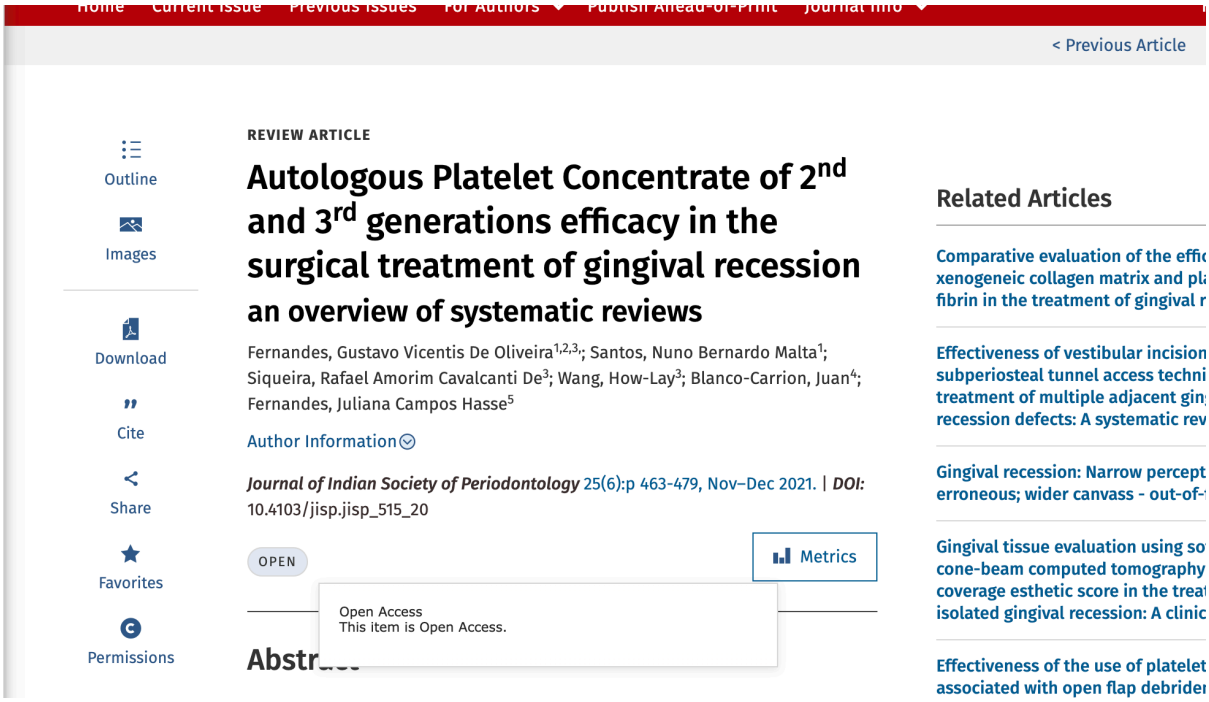
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Screenshots confirming the publications in open access

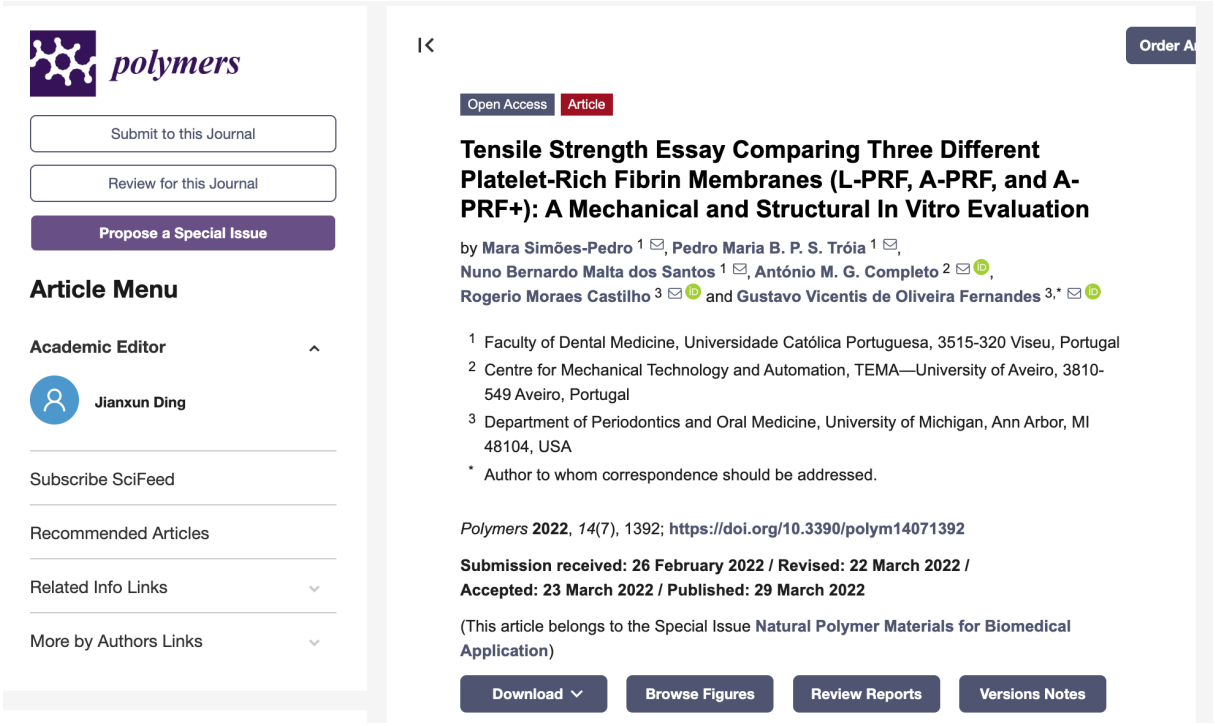


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Screenshot of the “Open Access” label on 19/02/2025 (https://journals.lww.com/ijdr/fulltext/2023/34010/the_effect_of_the_interproximal_creeping.19.aspx)

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APPENDIX XIX: PERMISSIONS

ESCOLA DE DOUTORAMENTO INTERNACIONAL (EDIUS)

INSTRUCIÓNS: Ao depositar unha tese monográfica con reprodución de artigos, o doutorando/a será responsable de recoller a autorización do resto de persoas coautoras doutoras e non doutoras dos diferentes artigos incluídos na tese. Deberá achegar en formato PDF o Documento 1, copias do Anexo 1 para cada unha das persoas coautoras non doutoras e copias do Anexo 2 para cada unha das coautoras doutoras. En caso de ser necesario, achegar Documento 2.

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Nome e Apelidos
Gustavo Fernandes
Juan Blanco-Carrion
Tensile Strength Essay Comparing Three Different Platelet-Rich Fibrin Membranes (L-PRF, A-PRF, and A-PRF+): A Mechanical and Structural In Vitro Evaluation
Nome e Apelidos
Gustavo Fernandes
Liquid Platelet-Rich Fibrin Coating Implant Surface to Enhance Osseointegration: A Double-Blinded, Randomized Split-Mouth Trial with 1-Year Follow-up
Nome e Apelidos
Gustavo Fernandes
The Influence of Root Prominence on the Onset of Gingival Recession: A Systematic Review
Nome e Apelidos
Gustavo Fernandes

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INTERNACIONAL (EDIUS)

A new digital evaluation protocol applied in a retrospective analysis of periodontal plastic surgery of gingival recessions
Nome e Apelidos
Tiago Marques
Andre Correia
A 3D Digital Analysis of the Hard Palate Wound Healing after Free Gingival Graft Harvest: A Pilot Study in the Short Term
Nome e Apelidos
Tiago Marques
André Correia
Gustavo Fernandes
The Effect of the Interproximal Creeping Attachment in Aesthetic Site After Root Coverage Surgery with VISTA Technique - A Case Report
Nome e Apelidos
Tiago Marques
André Correia
Gustavo Fernandes

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En Santiago de Compostela/Lugo, a *02/02/2025*

Nuno Bernardo Malta dos Santos

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Email: juan.blanco@usc.es

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D./Dna.: **Gustavo Fernandes**

Email: **gustfernandes@gmail.com**

MANIFESTO QUE:

Como coautor/a DOUTOR/A dos artigos/publicacións integrados na tese "Treatment of Miller Class I gingival recessions by CAF with or without Platelet-Rich Fibrin: A Randomized Clinical Trial " que presenta o doutorando/a **Nuno Bernardo Malta dos Santos**:

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Email: tiagomiguelmarques@gmail.com

MANIFESTO QUE:

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AUTORIZACIÓN COAUTOR/A DOUTOR/A

D./Dna.: **André Ricardo Maia Correia**

Email: **andrecorreia@ucp.pt**

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Rafael Siqueira
How-Lay Wang
Tensile Strength Essay Comparing Three Different Platelet-Rich Fibrin Membranes (L-PRF, A-PRF, and A-PRF+): A Mechanical and Structural In Vitro Evaluation
Nome e Apelidos
Antonio Completo
Rogerio Castilho
The Influence of Root Prominence on the Onset of Gingival Recession: A Systematic Review
Nome e Apelidos
Ana Mello-Moura
Rogerio Castilho
A new digital evaluation protocol applied in a retrospective analysis of periodontal plastic surgery of gingival recessions
Nome e Apelidos
Antonio Completo
Rogerio Castilho
A new digital evaluation protocol applied in a retrospective analysis of periodontal plastic surgery of gingival recessions
Nome e Apelidos

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Javier Montero
The Effect of the Interproximal Creeping Attachment in Aesthetic Site After Root Coverage Surgery with VISTA Technique - A Case Report
Nome e Apelidos
Javier Montero

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DECLARA RESPONSABLEMENTE:

3. Que a utilización destas publicacións/artigos cumpre cos requisitos establecidos no artigo 34.3e) o no artigo 34.3f) do Regulamento de Estudos de Doutoramento da USC.

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Nuno Bernardo Malta dos Santos
02/02/2025

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CHAVE MÓVEL

Gingival recession, characterized by gingival apical migration and subsequent root exposure, poses significant clinical challenges, including aesthetic concerns, hypersensitivity, and potential root caries. The coronally advanced flap (CAF) procedure, with or without the adjunctive use of platelet-rich fibrin (PRF), is used to achieve root coverage and promote periodontal regeneration. This study aims to evaluate the clinical outcomes of the treatment of Miller Class I gingival recessions using CAF alone versus CAF combined with leukocyte- and platelet-rich fibrin (L-PRF).