

Tunnel technique with connective tissue graft versus coronally advanced flap with enamel matrix derivate for root coverage: 5-year results of an RCT using 3D digital measurement technology for volumetric comparison of soft tissue changes

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Abstract

Aim: Comparison of the clinical efficacy (digitally volumetric, aesthetic, patient-centred outcomes) of tunnel technique (TUN) with subepithelial connective tissue graft (CTG) versus coronally advanced flap (CAF) with enamel matrix derivate (EMD) 5 years after gingival recession therapy.

Materials and methods: In 18 patients contributing 36 RT1 recessions, study models were collected at baseline and follow-ups. Optical scans assessed recessions computer-assisted [recession depth, recession reduction (RECR), complete root coverage (CRC), percentage of root coverage (RC), pointwise (pTHK) and mean areal (aTHK) marginal soft tissue thickness]. Root coverage aesthetic Score (RES) was used for aesthetic evaluation and visual analogue scales for patient-centred data collection applied.

Results: Sixty months after surgery, 50.0% (TUN+CTG) and 0.0% (CAF+EMD) of sites showed CRC ($p = 0.0118$), 82.2% (TUN+CTG) and 32.0% (CAF+EMD) achieved RC, respectively ($p = 0.0023$). CTG achieved significantly better RECR (TUN+CTG: 1.75 ± 0.74 mm; CAF+EMD: 0.50 ± 0.39 mm; $p = 0.0009$) and aTHK (TUN+CTG: 0.95 ± 0.41 mm; CAF+EMD: 0.26 ± 0.28 mm; $p = 0.0013$). RES showed superior outcomes ($p = 0.0533$) for TUN+CTG (6.86 ± 2.31) compared to CAF+EMD (4.63 ± 1.99). The study failed to find significant differences related to patient-centred outcomes (TUN+CTG: 8.30 ± 2.21 ; CAF+EMD: 7.50 ± 1.51 ; $p = 0.1136$).

Conclusions: Five years after treatment, CTG resulted in better clinical and aesthetic outcomes than CAF+EMD. Increased THK was associated with improved outcomes for RECR and RC.

KEYWORDS

coronally advanced flap, gingival recession therapy, randomized controlled trial, tunnel technique, volumetric measurement technology

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Clinical relevance

Scientific rationale for the study: Long-term data on volumetric soft tissue alterations after root coverage procedures do hardly exist. Thus, this study compared clinical efficacy and aesthetic as well as patient-centred outcomes of TUN+CTG versus CAF+EMD over a 5-year period for the first time using 3D digital data.

Principal findings: Application of CTG improved clinical (HKT), digitally assessed volumetric and aesthetic results. Higher THK values were associated with better outcomes regarding RECCred and RC.

Practical implication: Achieving sufficient soft tissue thickness seems to be a relevant prognostic factor for long-term stability and aesthetic success after gingival recession treatment.

1 | INTRODUCTION

Besides aesthetics and patient-centred criteria regarding root coverage procedures, complete root coverage (CRC), recession reduction (RECCred) and increased keratinized tissue height (HKT) are broadly recognized endpoints (Tonetti & Jepsen, 2014). With this respect, the coronally advanced flap (CAF) and its modifications combined with autologous connective tissue graft (CTG) offer the best prognosis to treat single (Cairo et al., 2014; Cairo, 2017; Chambrone et al., 2018) and multiple gingival recessions (Graziani et al., 2014; Cairo, 2017; Chambrone et al., 2018). True nature of the CTG effect is not fully understood (Zuhr et al., 2014). However, recent publications indicate a positive impact of the post-operative soft tissue thickness on the previously exposed root surface for mid- and long-term stability of the gingival margin (Tavelli, Barootchi, Cairo, et al., 2019; Tavelli, Barootchi, Di Gianfilippo, et al., 2019; Zuhr et al., 2020).

Connective tissue graft harvesting adds additional morbidity and pain to any root coverage procedure (Tonetti et al., 2018; Zucchelli et al., 2020). Thus, it is essential to clarify the clinical relevance and true long-term benefit of the combined procedure. Better understanding in this context will influence future research and clinical developments (e.g. biomaterials for substitution of autologous soft tissue grafts) (Zuhr, Baumer, et al., 2014). Some fundamental issues require clarification: How long takes healing regarding soft tissue volume? Is there any correlation between marginal soft tissue thickness and the long-term stability? Is there any critical soft tissue thickness required for stability?

Long-term evaluations performed in university (Jepsen et al., 2017; Pini Prato et al., 2018; Pini Prato et al., 2018; Barootchi et al., 2019; Bhatavadekar et al., 2019; Petsos et al., 2020) and in private office settings (Wessels et al., 2019; Petsos, Eickholz, Raetzke, et al., 2020) have contributed to better understanding of the wound healing dynamics after root coverage procedures. However, published research combining long-term outcomes after gingival recession therapy and precise measurement technologies is currently unknown to the authors.

To the best of the authors' knowledge, this is the first long-term investigation evaluating clinical and three-dimensional volumetric data. The aim of this 5-year follow-up (Rebele et al., 2014; Zuhr et al., 2014; Zuhr et al., 2020) randomized clinical trial (RCT)

was (a) to compare the efficacy of root coverage with TUN+CTG and CAF+EMD by means of clinical, digital, aesthetic and patient-centred outcomes, (b) to record three-dimensional soft tissue alterations with and without the use of CTG over time and evaluate possible clinical consequences, and (c) to relate volumetric findings to stability of root coverage in general.

2 | MATERIALS AND METHODS

The present article complies with the CONSORT 2010 statement (Moher et al., 2010; Schulz et al., 2010).

The study reports the 5-year follow-up data of an RCT on the treatment of single and multiple gingival recessions. Corresponding papers describe the study protocol in detail (Rebele et al., 2014; Zuhr, Rebele, et al., 2014; Zuhr et al., 2020). The study protocol was approved by the University of Freiburg Ethics Committee (approval number: 148/09) and registered in the German Clinical Trials Register (ID: DRKS00003285).

2.1 | Participants

Patients were enrolled, treated and examined at the Private Dental Office Huerzeler/Zuhr (Munich, Germany) between July 2009 and June 2011 according to the following inclusion criteria (Rebele et al., 2014; Zuhr, Rebele, et al., 2014):

- Age ≥ 21 years
- Non-smokers
- No systemic diseases or pregnancy
- No active periodontal disease, full-mouth plaque and bleeding scores $\leq 25\%$
- No medication interfering with periodontal tissue health or healing
- No contraindication for periodontal surgery
- Presence of at least one RT1 recession defect (Cairo et al., 2011; Cortellini & Bissada, 2018) not exceeding 5 mm in depth with a clearly identifiable natural cemento-enamel-junction (CEJ).



FIGURE 1 (a) Single gingival recession defect on a right lateral incisor at baseline, which was randomly assigned to the TUN+CTG group. (b) 12-month follow-up, (c) 24-month follow-up, (d) and 60-month follow-up of the corresponding case. (e) Multiple gingival recession defect on both upper right premolars at baseline, which were randomly assigned to the CAF+EMD group. (f) 12-month follow-up, (g) 24-month follow-up, (h) and 60-month follow-up of the corresponding case

In patients exhibiting multiple adjacent gingival recessions, all defects contributed to data collection. Patients with appropriate defects in anatomically separated locations were allocated to both treatment approaches, resulting in independently treated sites.

2.2 | Study settings

Surgery was performed by the same operator (O.Z.) with long-standing experience in periodontal plastic surgery. Three

investigators performed clinical (D.A., Stephan Rebele, Kilian Hansen) and one of them (D.A.) digital assessments. D.A. was blinded with respect to surgery. All examiners were trained to optimize clinical recordings measured to the nearest 0.5 mm using a periodontal probe (PCP-UNC 15, Hu-Friedy; Zuhr, Rebele, et al., 2014).

Digital measurements of dimensional soft tissue alterations at baseline and follow-up examinations after 12, 24 and 60 months include measurements of recession depth as well as mean marginal soft tissue thickness (THK).

2.3 | Surgical procedure

Randomization and allocation concealment, surgery and post-operative care were comprehensively depicted before (Zuhr et al., 2020).

2.4 | TUN+CTG

TUN was basically performed according to the modified tunnel technique (Zuhr et al., 2007). A continuous split-thickness tunnel was created by undermining the buccal mucosa of the involved teeth followed by detachment of the adjacent papillary tissues allowing for coronal displacement of the soft tissue complex. CTG was harvested according to the single-incision technique (Hürzeler & Weng, 1999). The graft was trimmed to a thickness of 1–1.5 mm and inserted into the tunnel. The soft tissue complex was stabilized 1–2 mm coronally to the CEJ with double-crossed sutures (Seralene® 6.0; Serag-Wiessner KG) (Zuhr et al., 2009; Figure 1a–d).

2.5 | CAF+EMD

After split-thickness flap preparation, remaining papillae were de-epithelialized. The root surfaces were pre-conditioned with ethylenediaminetetraacetic acid (Straumann® PrefGel) before EMD (Straumann® Emdogain) was applied (Institute Straumann AG). Tension-free flap adaption was ensured coronally to the CEJ using sling sutures (Seralene® 6.0; Serag-Wiessner KG) and single interrupted sutures to close vertical releasing incisions (Seralene® 7.0; Serag-Wiessner KG) (Figure 1e–h).

During the first year, patients were scheduled at 1, 3, 6 and 12 months for professional dental hygiene and afterwards discharged from study-related recalls (Rebele et al., 2014; Zuhr, Rebele, et al., 2014; Zuhr et al., 2020). For this reason, tooth- and site-specific plaque and bleeding scores were not recorded any longer.

2.6 | Clinical measurements

Clinical parameters were assessed to the nearest 0.5 mm using a PCP-UNC 15 periodontal probe (Hu-Friedy):

- Periodontal probing depth (PPD) at the central buccal site.
- Height of keratinized tissue (HKT) mid-buccally from the most apical extension of the gingival margin to the mucogingival junction.

2.7 | Digital measurements

The preparation and superimposing/matching of scans (Imetric D103®, Imetric 3D GmbH; Swissmedia software/Smop, Swissmeda) for three-dimensional measurement of soft tissue alterations over time (Figure 2a–d) were described previously (Rebele et al., 2014). All digital parameters were recorded to the nearest 0.01 mm. REcred was measured according to Zuhr, Rebele, et al. (2014). THK was assessed in two different ways by defining an area of interest (AOI) according to the explanations given in the 2-year follow-up publication (Zuhr et al., 2020) (Figure 2d).

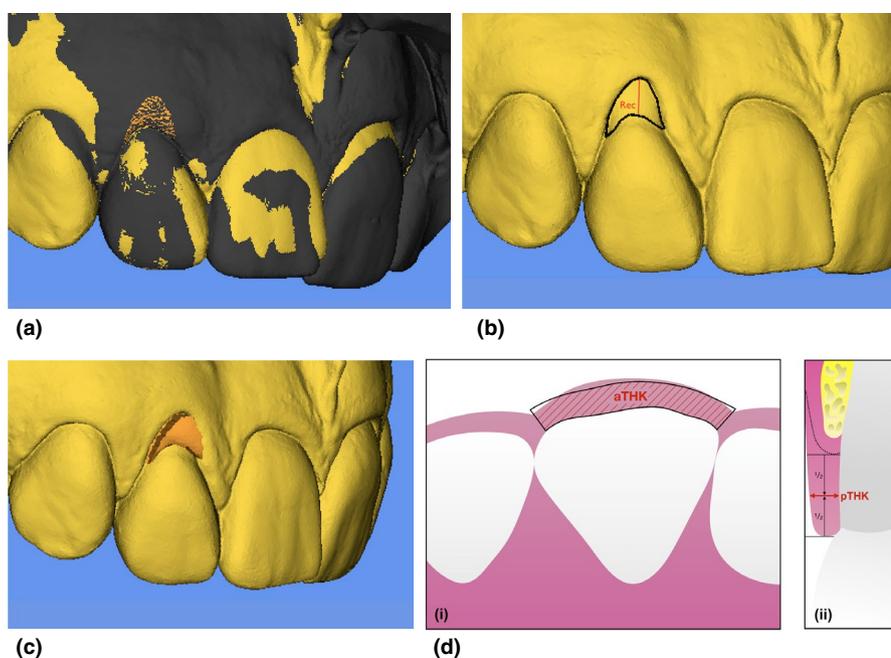


FIGURE 2 (a) Frontal view of the digital model gained through virtual superimposing of baseline and 60-month follow-up scans. (b) Frontal view of a digital model with the outlined AOI derived from superimposition. (c) Areal measurement of aTHK above the formerly exposed root. (d) (i) Areal, three-dimensional measurement of aTHK as mean thickness of the marginal soft tissue on the formerly exposed root surface. aTHK was exhibited as a polygonal shape and was bordered by the pre- and post-operative lining of the margo gingivae. (ii) Point-like, two-dimensional measurement of pTHK. Measurement was performed in a rectangular cross section at the central buccal site in the middle of the vertically augmented soft tissue

2.8 | areal THK

Area of interest was defined as the entire area of the newly formed soft tissue on the previously denuded root surface (Zuhr et al., 2020). Thus, aTHK was defined as mean thickness of this marginal soft tissue (aTHK [mm] = vol [mm³]/area [mm²]). In cases of recurrence of the gingival recession, reaching or even exceeding the baseline value, aTHK was defined as "0." Accordingly, data collection included all models collected from baseline, 6-month, 1-year, 2-year and 5-year follow-up [Figure 2d (i)].

2.9 | pointwise THK

pTHK was defined as pointwise, two-dimensional measurement evaluating the distance from the formerly exposed root surface at baseline to the soft tissue surface at follow-ups. It was performed in a cross section rectangular at the central buccal site in the middle of the newly formed soft tissue [Figure 2d (ii)].

2.10 | Aesthetic evaluation

Three experts in plastic periodontal surgery (Ali Daouk, Ronny Jung, Giulio Rasperini), all blinded with respect to the surgical procedures, evaluated the aesthetic outcomes using root coverage aesthetic

score [RES (Cairo et al., 2009)]. The evaluation was based on baseline and 60-month photographs paired in a file for presentation. RES includes five outcome variables leading to a final score ranging from 0 to 10 points (Cairo et al., 2009).

2.11 | Patient questionnaire

At the 60-month follow-up, questionnaires according to those from the 12-month evaluation (Zuhr, Rebele, et al., 2014) were answered by the patients. The questionnaires collected subjective data on willingness to repeat surgery (yes or no) and aesthetic satisfaction using a Visual Analogue Scale from 0 to 10.

2.12 | Statistical analysis

Information on sample size calculation has been described before (Zuhr, Rebele, et al., 2014). Statistical analysis was conducted in accordance with previous publications (Rebele et al., 2014; Zuhr, Rebele, et al., 2014).

Patient-level analysis was performed regarding the experimental site as the statistical unit. Means for each patient and treatment method were calculated for all measured parameters at different follow-up time points. Clinical attachment level (CAL) was calculated by summing up PPD and REcred. Descriptive data have been analysed for

TABLE 1 Patient-/site-specific characteristics

Patient ID	Sites	Tooth/teeth [FDI]	Surgical approach	Gender	Age at surgery [years]
01	01	12	TUN+CTG	Female	21
02	02	21	TUN+CTG	Female	25
03	03	13	CAF+EMD	Female	47
05	06	21, 22	CAF+EMD	Female	41
07	08	22	TUN+CTG	Female	43
08	09	11, 21	TUN+CTG	Female	34
09	10	13	CAF+EMD	Male	54
	11	22, 23, 24	TUN+CTG		
10	12	14	TUN+CTG	Female	36
11	13	22, 23	TUN+CTG	Female	40
12	14	14, 15	CAF+EMD	Female	38
	15	24, 25	TUN+CTG		
13	16	12	TUN+CTG	Female	55
14	17	22, 23	TUN+CTG	Male	46
16	20	12, 11, 21, 22	CAF+EMD	Female	43
17	21	12, 13	CAF+EMD	Female	44
19	24	14	CAF+EMD	Male	34
21	27	15	CAF+EMD	Female	40
22	28	13	CAF+EMD	Male	44
23	29	13, 14, 15	CAF+EMD	Female	25

Abbreviations: CAF+EMD, coronal advanced flap with enamel matrix derivative; FDI, Fédération Dentaire Internationale (French); TUN+CTG, tunnel with connective tissue graft.

	TUN+CTG		CAF+EMD		p-value
	N	Mean ± SD [mm]	n	Mean ± SD [mm]	
Recession depth					
Baseline	15	1.93 ± 0.57	15	1.69 ± 0.63	0.41
6 months	15	0.02 ± 0.05 ^A	13	0.53 ± 0.47 ^A	<0.0001
12 months	14	0.04 ± 0.07 ^A	14	0.55 ± 0.49 ^A	<0.0001
24 months	15	0.13 ± 0.20 ^{A,B,C}	14	0.72 ± 0.48 ^A	<0.0001
60 months	10	0.36 ± 0.56 ^{A,B,D}	10	1.28 ± 0.67 ^{A,B,C}	<0.0001
aTHK					
6 months	15	1.30 ± 0.32 ^A	13	0.66 ± 0.22 ^A	<0.0001
12 months	14	1.32 ± 0.26	14	0.72 ± 0.19	<0.0001
24 months	15	1.11 ± 0.26	14	0.60 ± 0.26 ^B	<0.0001
60 months	10	0.95 ± 0.41 ^A	10	0.26 ± 0.28 ^{A,B}	<0.0001
pTHK					
6 months	15	1.74 ± 0.38 ^A	13	0.90 ± 0.19 ^A	<0.0001
12 months	14	1.62 ± 0.31	14	0.98 ± 0.22	<0.0001
24 months	15	1.41 ± 0.35 ^B	14	0.78 ± 0.32 ^B	<0.0001
60 months	10	1.24 ± 0.52 ^{A,B}	10	0.50 ± 0.39 ^{A,B}	<0.0001

TABLE 2 Recession depth (REC, mm) and marginal soft tissue thickness (aTHK, pTHK, mm) at baseline, 6, 12, 24 and 60 months

Note: Superscript letters indicate significant differences within the respective group (A: compared to baseline, B: compared to 6 months, C: compared to 12 months, D: compared to 24 months).

Abbreviations: aTHK, areal marginal soft tissue thickness; CAF+EMD, coronal advanced flap with enamel matrix derivate; n, number of patients; pTHK, point-like marginal soft tissue thickness; SD, standard deviation; TUN+CTG, tunnel technique with subepithelial connective tissue graft.

all sites examined per follow-up examination. The differences described in the text are based on the sites that were available at the respective time points. To comply with the split-mouth design, linear mixed models were used to analyse differences between and within the groups. For additional pairwise comparisons, a Bonferroni correction was used.

Pearson's correlation coefficient was calculated with the complete 5-year data set to analyse the relationship between THK and RC/RECRD within each group. Linear mixed models were used for standardized variables to compute the corresponding correlation coefficients for all data. Mixed linear regression analysis explored the relationship between THK and RC/RECRD, and a mixed logistic model was calculated to analyse the influence of THK on CRC. To compare VAS and RES for each timepoint between the groups, Wilcoxon rank-sum test was used, while answering the questions about repeating the surgical procedure Fisher's exact test was applied. All tests were based on a significance level of 5%.

Statistical analysis was performed by K.V. with STATA 15.1. (StataCorp LT).

3 | RESULTS

3.1 | Experimental population

Originally 24 patients with a mean age of 37.9 ± 9.8 years contributing 47 gingival recessions (30 study sites) were recruited for the study (Rebele et al., 2014; Zühr, Rebele, et al., 2014; Zühr et al.,

2020). One patient in CAF+EMD received a new suprastructure at the study site and was excluded from further analysis. Two more patients moved out of reach, and additional three patients were not able to keep the 60-month follow-up. Therefore, 18 patients were available for the 5-year follow-up contributing 10 study sites to each group (Figure S1). Further patient- and site-specific characteristics are shown in Table 1.

3.2 | Defect characteristics at baseline, 6, 12, 24 and 60 months

Table 2 shows descriptive data for mean RECRD and mean THK at baseline, 6, 12, 24 and 60 months. Both, aTHK and pTHK show significantly higher values in TUN+CTG than in CAF+EMD at all follow-up timepoints ($p < 0.005$). Twice as many patients in CAF+EMD showed an increase in REC ($n = 10$) in at least one site between 12 and 60 months compared to TUN+CTG ($n = 5$; decrease: $n = 1$; unchanged: $n = 4$). For CAF+EMD, REC increased ≥ 0.5 mm in 2 and ≥ 1.0 mm in 4 patients. In case of TUN+CTG, one patient showed an increase of ≥ 0.5 mm and two patients of ≥ 1.0 mm. In all other cases, only a slight increase in REC of < 0.5 mm was detected.

Analysis regarding HKT, RECRD, RC and CRC changes after 6, 12, and 60 months compared to baseline is shown in Table 3. Intra-group changes of RC comparing the 60-month results to the 12- and 24-month data were decreasing in both groups (TUN+CTG: $-16.79 \pm 25.25\%$, $p = 0.002$; CAF+EMD: $-43.86 \pm 25.98\%$,

TABLE 3 Comparison of TUN+CTG and CAF+EMD for variables of height of keratinized tissue (HKT, mm), recession depth reduction (RECRed, mm), percentage of root coverage (RC, %) and percentage of defects with complete root coverage (CRC, %) 6, 12, 24 and 60 months after surgery

	Height of keratinized tissue (HKT)		Recession depth reduction (RECRed)		% Root coverage (RC)		% Defects with complete root coverage (CRC)	
	N	Mean ± SD [mm]	n	Mean ± SD [mm]	n	Mean ± SD [%]	n	Mean ± SD [%]
Baseline – 6 months								
TUN+CTG	15	0.58 ± 0.82	15	1.91 ± 0.56	15	99.2 ± 1.6	15	80.0 ± 41.4
CAF+EMD	13	-0.36 ± 0.47	13	1.21 ± 0.46	13	72.2 ± 18.4	13	15.4 ± 37.5
<i>p</i> -value		<0.0001		<0.0001		0.0001		<0.0001
Baseline – 12 months								
TUN+CTG	14	0.62 ± 0.83	14	1.94 ± 0.57	14	98.4 ± 3.6	14	78.6 ± 42.6
CAF+EMD	14	-0.34 ± 0.51	14	1.17 ± 0.42	14	71.8 ± 20.3	14	21.4 ± 42.6
<i>p</i> -value		<0.0001		<0.0001		<0.0001		<0.0001
Baseline – 24 months								
TUN+CTG	15	0.54 ± 0.99	15	1.81 ± 0.56	15	94.0 ± 10.0	15	60.0 ± 50.7
CAF+EMD	14	-0.28 ± 0.61	14	0.90 ± 0.45	14	57.3 ± 25.5 ^A	14	0.0 ^B
<i>p</i> -value		0.001		<0.0001		<0.0001		<0.0001
Baseline – 60 months								
TUN+CTG	10	0.42 ± 2.04	10	1.75 ± 0.74 ^{A,B}	10	82.2 ± 27.0 ^{A,B,C}	10	50.0
CAF+EMD	10	-0.32 ± 1.13	10	0.50 ± 0.39 ^{A,B,C}	10	32.0 ± 26.5 ^{A,B,C}	10	0.0
<i>p</i> -value		0.825		<0.0001		<0.0001		0.002

Note: Superscript letters indicate significant differences within the respective group (A: compared to 6 months, B: compared to 12 months, C: compared to 24 months).

Abbreviations: CAF+EMD, coronal advanced flap with enamel matrix derivate; n, number of patients; SD, standard deviation; TUN+CTG, Tunnel technique with subepithelial connective tissue graft.

$p < 0.001$; TUN+CTG: $-9.7 \pm 17.84\%$, $p=0.049$; CAF+EMD: $-31.1 \pm 30.81\%$, $p < 0.0001$). Intra-group changes of RECRed increased significantly from 12 to 60 months ($p = 0.0001$) in both groups, and from 24 to 60 months ($p = 0.001$) in CAF+EMD (Table 3). Comparing RECRed, RC and CRC at all follow-ups with the baseline situation, significant improvements ($p < 0.005$) could be determined favouring TUN+CTG. Descriptive values for HKT, CAL and PPD are shown in Table 4.

3.3 | The relationship of marginal soft tissue thickness to recession reduction and root coverage

Regarding complete data set, Pearson's correlation coefficient indicated a significant positive correlation between aTHK and RECRed after 60 months in the TUN+CTG group ($r = 0.7444$, $p = 0.0135$) as well as in the CAF+EMD group ($r = 0.7286$, $p = 0.0169$) (Figure 3a). The same correlation was found for pTHK and RECRed (TUN+CTG: $r = 0.600$, $p = 0.0666$; CAF+EMD: $r = 0.848$, $p = 0.0019$).

RC and aTHK correlate significantly after 60 months in TUN+CTG ($r = 0.7901$, $p = 0.0065$), but not for CAF+EMD ($r = 0.5056$, $p = 0.1360$). However, Pearson's correlation coefficient revealed a significantly positive correlation between aTHK ($r = 0.972$, $p < 0.0001$) as well as pTHK and RC ($r = 0.910$, $p < 0.0001$). Data indicate a mean aTHK

after 60 months above which no further benefit was seen with regard to RC (Figure 3b). A logistic model analysis assumed a mean aTHK of 1.26 mm and a mean pTHK of 1.68 mm being maintained as minimum thickness over 60 months to predict CRC with a confidence of 95%.

3.4 | Patient questionnaires, RES

Patient-centred outcomes and RES results are presented in Table 5. Five years after root coverage, there was no significant difference between TUN+CTG and CAF+EMD with regard to the patients' aesthetic satisfaction ($p = 0.1136$). RES assessment revealed a trend to superior outcomes ($p = 0.0533$) for TUN+CTG (6.86 ± 2.31) compared to CAF+EMD (4.63 ± 1.99). 90% of patients from both groups would undergo surgery again.

Supplementary tables provide descriptive data limited to the patients who were available for the 5-year follow-up (Tables S1–S4).

4 | DISCUSSION

The results of this 5-year follow-up RCT reveal that both root coverage procedures with TUN+CTG and CAF+EMD exhibit deterioration

	TUN+CTG		CAF+EMD		p-value
	n	Mean ± SD [mm]	n	Mean ± SD [mm]	
Height of keratinized tissue (HKT)					
Baseline	15	3.26 ± 1.25	15	2.95 ± 1.06	<0.0001
6 months	14	3.83 ± 1.58	13	2.52 ± 0.99	<0.0001
12 months	14	3.86 ± 1.67	14	2.61 ± 1.14	<0.0001
24 months	15	3.79 ± 1.68	14	2.71 ± 1.25	0.002
60 months	10	3.80 ± 2.11	10	2.91 ± 0.40	0.247
Clinical attachment level (CAL)					
Baseline	14	3.28 ± 0.71	15	2.98 ± 0.62	0.720
6 months	14	1.27 ± 0.54 ^A	13	1.89 ± 0.64 ^A	<0.0001
12 months	14	0.99 ± 0.59 ^A	14	1.55 ± 0.70 ^A	0.001
24 months	15	1.54 ± 0.38 ^{A,C}	14	2.04 ± 0.66 ^{A,C}	0.001
60 months	10	2.53 ± 0.85 ^{B,C,D}	10	3.07 ± 0.75 ^{A,B,C,D}	0.194
Periodontal probing depths (PPD)					
Baseline	14	1.34 ± 0.37	15	1.31 ± 0.42	0.847
6 months	14	1.76 ± 0.47	13	1.44 ± 0.46 ^A	0.049
12 months	14	1.45 ± 0.50 ^B	14	1.12 ± 0.19 ^B	0.022
24 months	15	1.31 ± 0.26	14	1.22 ± 0.31 ^B	0.389
60 months	10	1.89 ± 0.44 ^C	10	1.48 ± 0.69 ^{A,C,D}	0.066

Note: Superscript letters indicate significant differences within the respective group (A: compared to baseline, B: compared to 6 months, C: compared to 12 months, D: compared to 24 months).

Abbreviations: CAF+EMD, coronal advanced flap with enamel matrix derivate; n, number of patients; SD, standard deviation; TUN+CTG, Tunnel technique with subepithelial connective tissue graft.

of RC and CRC. TUN+CTG was found to be significantly superior ($p < 0.005$) to CAF+EMD in terms of RC, CRC and REcred. HKT as well as pTHK and aTHK changes failed to be significantly different. A significant correlation between RC as well as REcred and aTHK as well as pTHK was detected ($p < 0.0001$). After 5 years, minimal aTHK of 1.26 mm and pTHK of 1.68 mm correlated with CRC. Differences related to patient-centred and aesthetic outcomes failed to be significant, but tended to do better for TUN+CTG.

Systematic reviews have analysed the predictability of both investigated procedures (Cairo et al., 2014; Graziani et al., 2014; Chambrone & Tatakis, 2015; Chambrone et al., 2018; Tavelli et al., 2018; Dai et al., 2019). However, there is only little data on soft tissue stability after surgical root coverage procedures with and follow-up of 5 years or longer. The relapse of the gingival margin in the test as well as in the control group with a decrease of RC and CRC between 12 and 60 months was one of the major findings in our investigation.

Only one prospective clinical study considering long-term efficacy of CAF+EMD has been published so far. Ten years after treatment, the authors reported in a split-mouth comparison of 9 patients similar clinical results after surgical root coverage using CAF+EMD or CAF+CTG. RC was $89.8 \pm 22.7\%$ for CAF+CTG and $83.3 \pm 21.7\%$ for CAF+EMD. CRC could be detected in 77.8% of the teeth treated with CAF+CTG and in 55.6% of the teeth treated with CAF+EMD (McGuire et al., 2012). Mid-term data on TUN+CTG show comparable

TABLE 4 Clinical parameters at baseline, 6, 12, 24 and 60 months after surgery

results to the present findings. Salem and coworkers examined 24 patients with single and multiple maxillary gingival recession defects 4 years after root coverage with CAF+CTG or TUN+CTG. While there was a significant superiority in favour of TUN+CTG related to THK (TUN+CTG: 2.06 mm, CAF+CTG: 1.23 mm; $p = 0.0012$) and HKT (TUN+CTG: 5.0 mm, CAF+CTG: 3.62 mm; $p = 0.0014$), no significant differences between both groups were noticed for RC (TUN+CTG: 90.1%, CAF+CTG: 95.9%, $p = 0.32$) and CRC (TUN+CTG: 81.3%, CAF+CTG: 100%, $p = 0.52$) (Salem et al., 2020). Comparable mid-term root coverage outcomes for TUN+CTG could not be maintained over a 5-year period in the present investigation (RC 12–60 months: $-16.79 \pm 25.25\%$, REcred 12–60 months: -0.3 ± 0.55 mm). Due to the lack of long-term evidence for TUN+CTG, a comparison with other studies is currently not possible.

Success rates of CAF-based procedures for the treatment of single (Cairo et al., 2014; Cairo, 2017; Chambrone et al., 2018) and multiple gingival recessions (Graziani et al., 2014; Cairo, 2017; Chambrone et al., 2018) are primarily based on a combined split-full-split flap design mostly without additional vertical releasing incisions (Zucchelli et al., 2009; Cortellini & Prato, 2012). In the present study, a consistent split-thickness flap preparation including vertical releasing incisions (Zuhr, Rebele, et al., 2014) was applied. Based on the fact that McGuire et al. applied a combined split-full-split flap design (McGuire et al., 2012), the divergent flap preparation may explain their superior results compared to our RCT.

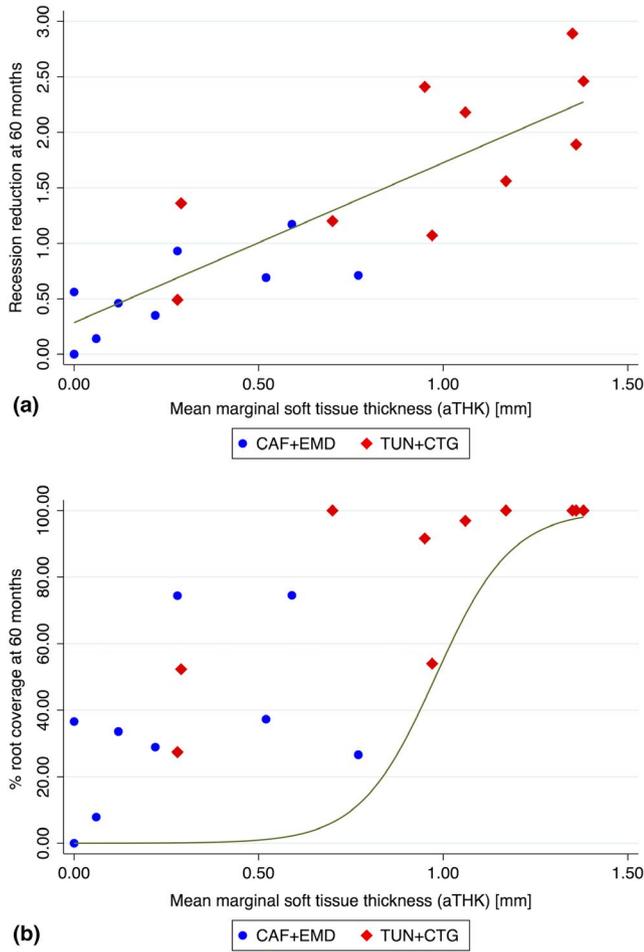


FIGURE 3 (a) Scatter plot of aTHK versus REcred at 60 months with fitted linear regression line: There is a positive, linear relationship between aTHK achieved on the previously exposed root surfaces and REcred. This leads to greater REcred in the TUN+CTG group compared to the CAF+EMD group. (b) Scatter plot of aTHK versus RC at 60 months with fitted logistic function (curve): There is a positive correlation between aTHK achieved on the on the previously exposed root surfaces and RC. This leads to greater relative RC in the TUN+CTG group compared to the CAF+EMD group

The use of three-dimensional measuring methods makes a comparison with other studies using rigid millimetre-scaled periodontal probes difficult (Leknes et al., 2005; Cordaro et al., 2012; McGuire et al., 2012; Garcés-McIntyre et al., 2017; Barootchi et al., 2019; Neves et al., 2019; Petsos, Eickholz, Raetzke, et al., 2020; Salem et al., 2020). The applied measuring technique has demonstrated accuracy and high reproducibility to record in vitro imitated gingival recessions to the nearest 0.01 mm (Lehmann et al., 2012). This method proved to improve measurement reproducibility, lowered intra- and inter-individual variance of measurement (Schneider et al., 2014; Fons-Badal et al., 2020; Lee et al., 2020), and set the threshold for CRC to 0.01 mm of remaining gingival recession. Therefore, no rounding errors must be accepted, and CRC is only recorded, when the gingival margin actually reaches or exceeds the CEJ. The same applies to THK recordings. Up to now, studies applied diverse

TABLE 5 Results for subjective (patients/VAS) and objective (professional examiners/RES) evaluation of the aesthetic outcomes and willingness for repetition of surgery according to patient questionnaires

	VAS (patients)		Mean RES (examiners 1–3)		Questionnaire (patients)					
	n	12 months Mean ± SD	Cases	12 months Mean ± SD	60 months Mean ± SD	n				
TUN+CTG	14	9.21 ± 1.41	10	9.06 ± 0.83	10	6.86 ± 2.31	14	13	10	9
CAF+EMD	14	9.07 ± 1.07	10	7.50 ± 1.51	10	4.63 ± 1.99	14	14	10	9
p-value	0.4568	0.1136	0.0034	0.0533	1.0	1.0				

Abbreviations: CAF+EMD, coronal advanced flap with enamel matrix derivate; n, number of patients/cases; RES, Root coverage aesthetic score; SD, standard deviation; TUN+CTG, Tunnel technique with subepithelial connective tissue graft; VAS, Visual analogue scale.

TABLE 6 Complete root coverage (CRC, %) and percentage of root coverage (RC, %) 60 months after surgery digitally measured and rounded to the nearest 0.01 mm as well as manually assessed using a periodontal probe and rounded to the nearest 0.5 and 1.0 mm

	% Defects with complete root coverage (CRC)					% Root coverage (RC)				
	TUN+CTG		CAF+EMD		p-value	TUN+CTG		CAF+EMD		p-value
	n	Mean ± SD [%]	n	Mean ± SD [%]		n	Mean ± SD [%]	n	Mean ± SD [%]	
Digital measurements to the nearest 0.01 mm	10	50.0 ± 52.70	10	0.0	0.0118	10	82.2 ± 27.04	10	32.0 ± 26.51	0.0023
Manual measurements rounded to the nearest 0.5 mm	10	60.0 ± 51.64	10	0.0	0.0062	10	80.2 ± 27.69	10	38.2 ± 29.71	0.0107
Manual measurements rounded to the nearest 1.0 mm	10	60.0 ± 51.64	10	10.0 ± 31.62	0.0223	10	75.0 ± 36.22	10	28.3 ± 34.29	0.0132

Abbreviations: CAF+EMD, coronal advanced flap with enamel matrix derivate; n, number of patients; SD, standard deviation; TUN+CTG, Tunnel technique with subepithelial connective tissue graft.

endodontic instruments, anaesthetic needles or calliper to measure THK (Paolantonio, 2002; da Silva et al., 2004; Zucchelli et al., 2010; Cardaropoli et al., 2012; Jepsen et al., 2013; Zucchelli et al., 2018; Pietruska et al., 2019; Tavelli, Barootchi, Di Gianfilippo, et al., 2019; Cairo et al., 2020; Salem et al., 2020). Other investigations used ultrasonic devices (Muller et al., 1998; Muller et al., 1999; Leknes et al., 2005). In the present study, a non-invasive three-dimensional digital measuring method has been used to measure soft tissue volume changes and to quantify the two-dimensional thickness of the marginal soft tissues that has been surgically established on the root surfaces. Table 6 shows CRC and RC values of the present investigation after fictitious rounding to the nearest 0.5 and 1.0 mm. Recalculation based on rounding values leads in both study groups to higher values concerning CRC and divergent results regarding RC. The latter finding clarifies that rounding values differ from the real situation and illustrates the impact of rounding errors on the final results. The application of a three-dimensional digital measurement technology provides precision in the evaluation of surgical root coverage outcomes.

The present investigation identified post-operative THK to be a significant long-term prognostic factor. Analysis of our 60-month data indicated that the overall relapse of the gingival margin in the test and the control group comes with a decrease of THK over time. Therefore, it is conclusive that REcred and RC were significantly better at CT-grafted sites (TUN+CTG: 1.75 mm and 82.2%) compared to non-augmented sites (CAF+EMD: 0.50 mm and 32.0%) confirming other long-term evaluations (Barootchi et al., 2019; Tavelli, Barootchi, Di Gianfilippo, et al., 2019). In contradiction to our findings, a recently published 2-year follow-up RCT on gingival recession treatment revealed stable THK with a tendency for increased HKT and REcred values from 1 to 2 years (Neves et al., 2019). A possible explanation might consist of the fact that the composition of the applied CTGs differs substantially from the ones used in our study. Hence, different to the CTGs in our study being composed of deeper portions of the lamina propria and submucosa, the CTGs used in the trial by Neves et al. mainly consisted of superficial layers of the lamina propria (Bertl et al., 2015). The results of a 5-year follow-up

examination on coverage of mucosal recessions at dental implants using epithelialized grafts after extraoral deepithelialization point to an influence of the harvesting technique (Zucchelli et al., 2018). In contrast to our findings, THK increased by 0.3 mm from 12 months to 5 years post-operatively. The results of Gil et al. also point to the graft composition as predictive factor for THK gain. The authors used different graft materials and revealed different THK values after 14.6 months using a similar digital measurement method. The greatest THK gain was achieved with the tuberosity graft (Gil et al., 2019). However, recent data point out that the obvious advantages of superficially harvested CTGs with respect to post-operative volume stability might to some extent be offset by aesthetic disadvantages (Zucchelli et al., 2014; Pietruska et al., 2019).

Is there a critical soft tissue thickness above which additional thickening does not improve prognosis of root coverage? This question can only be addressed indirectly, as no baseline THK values were recorded. Analysis of our 24-month data indicates that sites with aTHK of at least 1.6 mm and pTHK of 1.8 mm reached CRC with a confidence of 95%. The corresponding 60-month data are reduced to 1.3 mm and 1.7 mm, respectively. It can be derived from our results that especially sites of thinner gingival phenotypes do benefit even from minor gingival thickening during surgical root coverage, aiming 1 year post-operatively for THK values of about 2 mm at the central areas and 1.5 mm.

As limitations of this study, the evaluation of singular and multiple gingival recession defects, the incorporation of different investigators, the lack of a reliability assessment among the examiners and the fact that the majority of enrolled patients discontinued regular maintenance care in our office after the 12-month follow-up should be considered critically. Within these limitations, the following conclusions can be drawn:

- TUN+CTG revealed significantly superior long-term results compared to CAF+EMD in terms of REcred, CRC and RC.
- A notable retraction of the gingival margin over 5 years occurred in both groups.

- Increased THK correlated significantly with better results in terms of REcred and RC.
- A minimal remaining tissue thickness (aTHK pTHK) after 60 months correlated with CRC with high confidence. Thus, substantial augmentation of thickness seems to be a prerequisite of long-term CRC.
- For the best possible long-term stability after gingival recession therapy with CTG originating from deeper layers of palatal masticatory mucosa, clinicians should aim for 12-month THK values of around 2 mm at the centre and 1.5 mm at the peripheral areas of the formerly exposed root surfaces.

ETHICS STATEMENT

University of Freiburg Ethics Committee (approval number: 148/09) approved the protocol and consent form. It was conducted following the principles of the Declaration of Helsinki. All patients signed informed consent after receiving explanations on study objectives, design, risks and potential benefits.

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CONFLICT OF INTEREST

The study was self-funded by the authors and their institutions.

DATA AVAILABILITY STATEMENT

All data generated and analysed during this study are included in this published article. All data and materials are available upon request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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