Clinical Outcomes and Gingival Blood Flowmetry of Two Types of Subepithelial Connective Tissue Graft for Root Coverage in Multiple Gingival Recessions: A Preliminary Study

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This randomized split-mouth preliminary clinical trial aimed to evaluate periodontal parameters and gingival blood flowmetry, comparing sites that received subepithelial connective tissue graft from the palate after deepithelialization (DE) or obtained with parallel incision (PI). Periodontal parameters were evaluated at baseline and 6 months postoperative. Gingival blood flows were analyzed by laser Doppler flowmetry (LDF) at baseline and 2, 7, and 14 days postoperative. Statistical and LDF analyses were performed with R version 3.5.1 and MATLAB software, and clinical parameters through ANOVA and Wilcoxon signed-rank tests. LDF showed superior decrease in power spectral density (PSD) for DE after 2 days. After 7 days, PSD returned to initial values only for PI, and DE had not returned to the initial values by day 14. Despite major initial revascularization challenges for DE sites, both grafts promoted satisfactory root coverage in the treatment of multiple gingival recessions. Int J Periodontics Restorative Dent 2021;41:285–293. doi: 10.11607/prd.4353

Subepithelial connective tissue graft (SCTG) associated with coronally advanced flap (CAF) is considered the gold standard treatment for root coverage in Miller Class I and II gingival recessions (GRs). The term “gold standard” implies a well-defined and consistent technique standardization of the SCTG procedure. Nevertheless, different techniques and areas can be used for graft harvesting, resulting in SCTG with different anatomical characteristics, geometric shapes, and distinct histologic compositions. It may be speculated that these differences not only influence volume stability but also the physiologic process of graft revascularization. From clinical experience, it can be inferred that denser, firmer SCTG is therefore less susceptible to postoperative shrinkage. However, this type of tissue seems to undergo necrosis more easily than tissues from the anterior palate. The hypothesis is that denser tissue could present inferior requirements for graft survival, considering plasmatic circulation and revascularization during the initial postoperative phase. An early and predictable process of initial revascularization of the graft and flap to the recipient site should increase the success rate of root coverage. The clinical success of the root coverage procedures should ensure not only the esthetic but also the...

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biologic integration between the
graft and the surrounding tissues.8

One noninvasive method to
evaluate microcirculation and gin-
gival blood flow is laser Doppler
flowmetry (LDF).9–12 Although some
studies13,14 have already analyzed
the influence of SCTG type on root
coverage, flowmetry analysis by
LDF evidence is limited. Thus, the
aim of this preliminary study was to
evaluate clinical outcomes and LDF
after root-coverage procedures with
SCTG harvested with different tech-
niques: deepithelialized graft (DE)
and parallel incision graft (PI). The
study hypotheses were that (1) the
6-month clinical outcomes would be
greater than baseline values, as ex-
pected after root-coverage proce-
dures, and (2) the clinical outcomes
of the DE group would be superior
to those of the PI group.

Materials and Methods

The study protocol was approved
by the Institutional Ethics Commit-
tee of the Bauru School of Dentistry,
University of Sao Paulo (1.292.438)
and registered in ClinicalTrials.gov
(NCT04093674). All subjects includ-
ed in the study signed an informed
consent form. The patient inclusion
criteria were as follows: good sys-
temic health patients; having maxil-
lary bilateral Miller Class I or II mul-
tiple recession defects (≥ 2 mm in
depth) involving canine and/or pre-
molar teeth; visible cementoenamel
junction (CEJ); and acceptable peri-
odontal health. Patients with reces-
sion defects associated with caries
or restorations, pulpal pathology,
mobility, smoking habits, occlusal
interferences and use of any medi-
cation known to interfere with peri-
odontal condition were excluded.

Each patient received both
treatments (split-mouth) and sites
were randomly assigned to a DE or PI
group using a computer sequence.
Periodontal therapy consisted of
oral hygiene instructions as well as
scaling and root planing prior to
surgery. Bleeding on probing and
Plaque Index15 were both < 20% about
throughout the study.

Clinical parameters were re-
corded to the nearest millimeter
with a North Carolina periodon-
tal probe (PCPUNC, Hu-Friedy) at
baseline and 6 months postop-
eratively: (1) Recession depth (RD),
measured as the distance from the
CEJ to the gingival margin (GM);
(2) width of keratinized tissue (WKT),
measured as the distance from the
mucogingival junction to the GM;
and (3) soft tissue thickness (STT),
determined 1.5 mm apically to GM
using an anesthesia needle with a
rubber endodontic stop inserted
perpendicularly, measured with a
caliper to the nearest 0.1 mm. A
formula was used to calculate the
mean percentage of root coverage
(%RC)12. Patient-centered outcome
analysis was assessed through a
visual analogue scale (VAS) after 7
and 14 days (pain) and after 3 and 6
months (esthetics).

Laser Doppler Flowmetry

A dual-channel laser Doppler blood
flow and temperature monitor
(moorVMS-LDF2, Moor Instruments)
was used to evaluate blood flow on
the recipient sites. The LDF ma-
chine is equipped with a laser diode
that emits in the infrared spectrum
range (maximum power: 2.5 mW,
wavelength 785 ± 10 nm). Measure-
ments with LDF were performed
with two probes for 1 minute and
30 seconds. These measurements
were recorded three times for each
site and 1-minute intervals. Dur-
ing the recordings, the LDF probes
were stabilized by individual stents
supported on teeth and mucosa,
providing repeatable positioning at
each control visit.9,12,16 Blood perfu-
sions in the recipient site were mea-
sured by LDF at baseline (prior to lo-
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surgical papilla was positioned on the anatomical deepithelialized papilla with nylon 5.0 sling sutures. The flap was positioned coronal to the CEJ (1 to 2 mm) in both PI (Fig 2) and DE groups (Fig 3).

Patients received nonsteroidal drugs (100 mg nimesulide twice a day) for 3 days and analgesics (750 mg paracetamol) for pain if needed. Sutures were removed after 15 days. Patients were instructed to carefully rinse with chlorhexidine solution (0.12%) twice a day for 4 weeks to avoid mechanical trauma and excessive mobilization. Patients were recalled for professional supragingival biofilm control weekly for the first 4 weeks and monthly thereafter.

Statistical Analysis

Descriptive analysis was expressed as mean ± SD. Statistical analyses of this study were performed with R version 3.5.1 (The R Foundation) with the R library nonparametric longitudinal data. Analysis of variance (ANOVA) and Wilcoxon signed-rank tests were used to evaluate RD, WKT, and STT. For LDF, the main goal was to analyze fundamental frequencies and a power spectral density (PSD). Adjustments were performed using a Savitzky-Golay filter to remove linear and nonlinear trends. The overlapped segment in the Welch method was used as an averaging method with following specifications: 1,024-point Fourier transform, a Hamming window length of 512, and an overlap of 256 samples. Additionally, to analyze the dynamic flux, Poincaré plots and SD1/SD2 ratios were used to compare both interventions during the different periods of evaluation.

Results

Nine healthy subjects (four men and five women; age range: 25 to 54 years), were enrolled in this preliminary study. Patients presented with Miller Class I and/or Miller Class II GRs, and each recession site was randomly assigned to the DE group (23 lesions) or the PI group (24 lesions). The mean %RCs for PI and DE sites were 67.44 ± 29.06 and 65.8 ± 27.48, respectively. Table 1 demonstrates clinical parameters of both treatment groups at baseline.
and after 6 months. A total of 47 recession defects were treated, with an average of 5.22 recessions per patient. The ANOVA results for RD suggest that the interaction effect of treatment and follow-up time is not significant ($P = .942$), while the effect between follow-up periods (baseline vs 6 months) is significant for RD ($P < .0001$). Borderline significance ($P = .055$) was obtained for RD in the test of treatment effect. When considering WKT, no interaction effect of treatment and follow-up time was observed ($P = .841$). The results suggest that neither PI nor DE treatment influences WKT ($P = .726$), but follow-up times are statistically significant for this variable ($P < .0001$). The STT results indicate a nonsignificant interaction effect of treatment and follow-up time ($P = .713$). Both follow-up times and treatment effects were statistically significant for STT ($P = .000042$ and $P = .0102$, respectively).

### Table 1 Clinical Parameters of Both Groups at Baseline and 6 Months Postsurgery

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PI</th>
<th>DE</th>
<th>$P^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>RD, mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>$2.64 \pm 0.80^{\text{A}}$</td>
<td>$2.61 \pm 1.11^{\text{A}}$</td>
<td>.932</td>
</tr>
<tr>
<td>6 mo</td>
<td>$0.94 \pm 0.81^{\text{A}}$</td>
<td>$0.83 \pm 0.79^{\text{A}}$</td>
<td>.942</td>
</tr>
<tr>
<td>WKT, mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>$2.67 \pm 1.20^{\text{A}}$</td>
<td>$2.72 \pm 0.87^{\text{A}}$</td>
<td>.726</td>
</tr>
<tr>
<td>6 mo</td>
<td>$4.00 \pm 1.41^{\text{A}}$</td>
<td>$3.72 \pm 1.09^{\text{A}}$</td>
<td>.841</td>
</tr>
<tr>
<td>STT, mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>$0.92 \pm 0.34^{\text{A}}$</td>
<td>$1.20 \pm 0.43^{\text{A}}$</td>
<td>.713</td>
</tr>
<tr>
<td>6 mo</td>
<td>$1.40 \pm 0.55^{\text{A}}$</td>
<td>$1.79 \pm 0.73^{\text{B}}$</td>
<td>.0102</td>
</tr>
</tbody>
</table>

$P^a$: Interaction effect between treatment and follow-up time.

$P^b$: Comparison between follow-up times (baseline vs 6 months).

PI = parallel incision treatment; DE = de-epithelialized graft treatment; RD = recession depth; WKT = width of keratinized tissue; STT = soft tissue thickness. Values are shown as mean ± SD. Nine patients comprised each group. Different superscript capital letters indicate statistical difference by groups/treatments.

*aComparison effect between treatment and follow-up time.

*bComparison between follow-up times (baseline vs 6 months).
As the ANOVA rejected the null hypothesis of interaction effect, moments and treatments were compared using one-tailed Wilcoxon test. The null hypothesis that the 6-month RD would be less than the baseline RD was not rejected for either treatment (PI: \( P = .9963 \); DE: \( P = .998 \)). Consequently, the results suggest a reduction in RD after 6 months, independent of treatment protocol (Figs 4 and 5). When analyzing WKT, the null hypothesis that the 6-month value would be greater than the baseline value was not rejected for either treatment (PI: \( P = .9942 \); DE: \( P = .9921 \)), suggesting an increase in WKT after 6 months for both groups. For STT tests, the null hypothesis that the 6-month value would be greater than the baseline value was not rejected for either treatment (PI: \( P = .9809 \); DE: \( P = .9924 \)), indicating an increase in STT after 6 months, independent of the treatment used.

Corroborating with the ANOVA results, a two-sided test (Wilcoxon signed-rank test) did not reject the null hypothesis of no significant difference for RD when treatments were compared (baseline: \( P = .8918 \); 6 months: \( P = .8539 \)), suggesting that group effect is not significant to this variable. For WKT, the null hypothesis that values observed for DE were greater than that observed for PI was not rejected for either follow-up time (baseline: \( P = .3889 \); 6 months: \( P = .6612 \)). This result indicated no difference on the WKT outcomes between protocols. When considering STT, the null hypothesis that the values observed for DE were greater than PI
was not rejected (baseline: $P = .918$; 6 months: $P = .972$), suggesting an increase in STT when using the DE protocol.

Patient-centered outcomes showed no significant differences between techniques regarding pain/discomfort after 7 (PI: 8 ± 2.12; DE: 6.55 ± 3.32) and 14 days (PI: 8.55 ± 1.74; DE: 7.66 ± 1.73). Esthetic evaluation demonstrated no statistically significant difference comparing groups after 3 (PI: 9.33 ± 0.86; DE: 9.44 ± 0.72) and 6 months (PI: 9.22 ± 0.66; DE: 9.33 ± 0.70). PI and DE wound-healing outcomes are shown in Figs 6 and 7.

Mean blood-flow values for absolute Welch PSD (Fig 8) suggest that the absolute value decreased for both sites after 2 days, with a more pronounced decrease for DE sites. After 7 days, absolute PSD returned to initial values for PI, but not for DE, which were lower than the initial values, even 14 days after the surgical procedure. The typical Poincaré plot for both PI and DE sites and the mean SD1/SD2 ratio are shown in Figs 9 and 10. On typical Poincaré plots, the greater axis of the ellipse represents long-term variability while a smaller axis represents short-term variability. At baseline, the ellipses are elongated, and thus the short-term variability is smaller than the long-term variability. Therefore, 2 days after surgery, a greater reduction in long-term variability was observed for the DE graft, causing an increase in SD1/SD2 ratios. After 7 and 14 days, DE and PI sites presented similar characteristics.

Discussion

This preliminary study demonstrated that both DE and PI grafts promoted satisfactory clinical root-covering outcomes in multiple GR, with a tendency for greater STT gain at DE sites. No significant difference was observed in patient-centered outcomes. However, LDF results suggested that DE presented an inferior blood-flow frequency compared to PI. Thus, the DE method represents a major initial challenge for healing, but similarity in LDF values was observed between the techniques after 7 days.
One study compared patient morbidity and root coverage with SCTG harvested by trap-door and deepithelized graft, and the clinical parameters after 12 months were superior compared to the present outcomes. However, the study included single and multiple GRs, while only multiple GRs were included in the present sample. The literature indicates that multiple GRs are usually more challenging defects, implicating a larger surgical field with higher anatomical variability, such as prominent roots, shallow vestibules, and variation in keratinized tissue. The inferior gain in WKT in the present study could be associated with the short evaluation period (6 months). The progressive coronal migration of the gingival margin and consequent increase in root coverage could be observed due to creeping attachment, as described previously in long-term evaluations. The present study's STT gain was slightly inferior to the outcomes from another study, but with the same tendency for a superior gain in DE sites. This result could be explained by the nature of DE, which presents better quality (greater stability and less shrinkage) of a more superficial connective tissue. On the other hand, the trap-door approach and PI graft are composed of deeper and less-dense connective tissue, with a higher submucosal percentage.

Despite some limitations of this preliminary study, and even with inferior blood-flow frequency presented in the LDF results, the DE group presented a tendency for superior STT gain. Similar to other studies with LDF evaluation, blood flow varied among treatments, but only during the initial period of 7 days. However, LDF measurement protocols are highly variable in the literature. According to previous studies, LDF is an adequate method to record variations in gingival blood flow. Some investigations used LDF in free gingival grafts, modified Widman flap surgery, periodontal access flap surgery, and simplified papilla preservation. Other studies investigated LDF comparing DE grafts associated or not with ozone therapy, and xenogeneic collagen matrix compared to SCTG. Similar to a previous description, the results of the present preliminary evaluation should be interpreted with caution because of the limited sample size and descriptive statistics. The advantage of the present study is the split-mouth design, reducing the effect of individual healing variability.

The LDF technique presents significant clinical applicability and is a useful tool to evaluate gingival blood flow. However, the technique is sensitive and provides indirect evidence of the revascularization process. Furthermore, LDF evaluation could present discrepancies during recordings caused by tissue motion and intra- and inter-
individual variability, and depends on the scattering properties of surrounding tissues. Other imaging modalities or histologic markers offer a more accurate representation of the revascularization events, but these methods are difficult to apply in human studies.

SCTG could be obtained from palatal donor sites by different techniques. A DE graft generally is an option when palatal thickness is thin (1 mm). In spite of a large wound area, the morbidity associated with this technique presented no influence in patients’ pain perception. Conversely, the PI method promotes a smaller wound in the donor site, harvesting a deeper connective tissue, but requires a thicker palatal area. Selecting the harvesting technique in the decision-making process depends on operator experience and the palatal thickness. Although DE and PI grafts presented no differences in root coverage, DE had a tendency for greater gain in STT, probably because of the histologic and anatomical nature of this tissue. Despite the DE group’s inferior LDF results, the characteristics and qualities of this graft eventually overcame the initial revascularization difficulties.

Conclusions

According to the findings of this preliminary study, DE and PI grafts pro-
motivated satisfactory root coverage in the treatment of multiple GR, with a tendency for superior STT gain in the DE group. However, the LDF results suggest that DE represents a major initial challenge for revascularization. No difference between procedures was observed in patient-centered outcomes concerning pain and esthetics. Randomized controlled clinical trials with larger sample sizes are necessary to confirm these differences.

Acknowledgments

This work was supported in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. All procedures performed in this study on human participants were in accordance with the ethical standards of the authors’ institutional research committee, the Institutional Ethics Committee of the Bauru School of Dentistry - University of São Paulo (1.292.438) and registered in ClinicalTrials.gov (NCT04093674). Informed consent was obtained from all participants. The authors declare no conflicts of interest.

References