

Living Cellular Construct for Increasing the Width of Keratinized Gingiva: Results From a Randomized, Within-Patient, Controlled Trial

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Background: The standard of care for increasing keratinized gingiva adjacent to teeth that do not require root coverage is the free gingival graft (FGG). A pilot study indicated that the use of a living cellular construct (LCC) could be effective in this clinical scenario.

Methods: A pivotal, multicenter, randomized, within-patient, controlled, open-label trial was conducted (N = 96 patients). After removing the mucosa and keratinized gingiva from the test site, either an LCC or FGG was applied. The primary efficacy endpoint was the ability of the LCC to regenerate ≥ 2 mm keratinized gingiva at 6 months. Secondary measures were the same color and texture as the adjacent tissue, a 1-mm width of keratinized gingiva at 6 months, patient treatment preference, surgical site sensitivity at 1 week, and patient-reported pain after 3 days. Safety was assessed by reports of adverse events.

Results: At 6 months, the LCC regenerated ≥ 2 mm of keratinized gingiva in 95.3% of patients (81 of 85 patients; $P < 0.001$ versus a 50% predefined standard). As expected, the FGG generated more keratinized gingiva than the LCC (4.57 ± 1.0 mm versus 3.2 ± 1.1 mm, respectively). The gingiva regenerated with the LCC matched the color and texture of the adjacent gingiva. All patients achieved ≥ 1 mm keratinized gingiva with the LCC treatment by 6 months, and more patients preferred treatment with the LCC than with the FGG. No difference in sensitivity or pain was noted between the treatments. The treatments were well tolerated, and reported adverse events were typical for this type of periodontal surgery.

Conclusion: The use of an LCC may provide a safe and effective therapy for augmenting the zone of keratinized gingiva. *J Periodontol* 2011;82:1414-1423.

KEY WORDS

Gingival recession; mouth mucosa; oral surgery; periodontal diseases; regenerative medicine; wound healing.

Most clinicians agree that it is preferable to have keratinized gingiva adjacent to teeth. The presence of keratinized gingiva may make homecare more effective, resist frenum pull, and potentially reduce further recession.^{1,2} The absence of an adequate keratinized mucosa has been associated with high plaque accumulation and gingival inflammation.³ If a decision is made to increase the zone of the keratinized gingiva, the standard of care for non-root-coverage techniques has been the free gingival graft (FGG). As a graft, the palatal tissue provides keratinized gingiva, but it retains its palatal phenotype and may be noticeably different in color and texture from the surrounding gingival tissue. Most importantly, the need for a donor site adds to the morbidity (e.g., pain and bleeding) of the procedure for the patient.

A living cellular construct (LCC) comprised of human allogenic fibroblasts, keratinocytes, bovine collagen, and human extracellular matrix proteins has been used for >10 years to treat patients with cutaneous wounds, including venous leg⁴ and diabetic foot ulcers,^{5,6} excised burn wounds,⁷ and excisional surgery.⁸⁻¹² Because of the similarities of healing between cutaneous and oral mucosal wounds, it was thought that LCC may deliver better results than the standard FGG in terms of the color and texture

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of the gingiva while providing an adequate amount of new keratinized gingiva.

Although the mechanism of action of the LCC has not been fully elucidated, it has been postulated that it improves the wound environment through growth factor interactions, matrix deposition and degradation, wound coverage, and the provision of responsive cells.¹³ It has been established that LCC does not function as an autograft,¹⁴ which normally vascularizes, integrates, and persists in situ, retaining the characteristics of the tissue of origin; instead, the LCC appears to stimulate the patient's own cells to regenerate site-appropriate tissue through the modulation and improvement of secondary intention healing.^{7,9,10,12,15} The LCC produces cytokines and cell growth factors involved in tissue development and wound healing (e.g., vascular endothelial growth factor, platelet-derived growth factor, bone morphogenetic protein 2, and transforming growth factor β).^{6,16} Despite being comprised of allogenic cells, numerous studies^{4,5,7-12,14,15,17} have determined that the LCC does not elicit an immune reaction.

Results from a single-center, randomized, within-patient, controlled pilot study¹⁵ with an aim similar to this randomized controlled trial suggested that treatment with the LCC was not statistically significantly different from FGG in terms of recession, clinical attachment level (CAL), bleeding on probing (BOP), resistance to muscle pull, or inflammation. At 6 months, the LCC generated 2.4 ± 1.0 mm of keratinized gingiva, without a donor site. However, the LCC provided a statistically significantly better color match and texture than the FGG ($P < 0.001$), and patients preferred the LCC more than FGG ($P = 0.041$). None of the data from the pilot study¹⁵ was included in the present report.

The present study is designed to determine whether the LCC could achieve a clinically acceptable, safe, and effective amount of keratinized gingiva (≥ 2 mm) 6 months after surgery in patients who had an insufficient zone of attached gingiva (defined as < 1 mm attached gingiva) associated with ≥ 2 non-adjacent teeth. Although ≥ 2 mm keratinized gingiva (generally corresponding to 1 mm attached gingiva) has been suggested to be required to maintain gingival health,¹⁸ no single clinical measures are universally accepted to assess periodontal treatment outcomes.¹⁹ Thus, we designed a study with one primary and six secondary endpoints that reflected the most commonly used factors that periodontists consider when determining the clinical effectiveness of mucogingival treatment.

MATERIALS AND METHODS

Patients

The study protocol was approved by institutional review boards of participating sites (Perio Health

Professionals, Houston, TX; Boston Periodontics and Dental Implants, Boston, Massachusetts; Michigan Center for Oral Research, University of Michigan School of Dentistry, Ann Arbor, MI; and University of Texas Health Science Center, San Antonio, Texas), and all patients gave written informed consent before any study-related procedures were done. Patients were eligible for inclusion in the study if they had: 1) ≥ 18 and ≤ 70 years of age, and 2) ≥ 2 non-adjacent teeth in contralateral quadrants of the same jaw with ≤ 1 mm attached gingiva that required soft tissue grafting without the need for root coverage. Other inclusion and exclusion criteria are provided in Table 1.

Study Product

The LCC[#] is comprised of a device component (collagen matrix) and biologic components (cells and their products) supplied as a single circular construct ≈ 75 mm in diameter and ≈ 0.75 mm thick on a semi-permeable polycarbonate membrane on top of an agarose-rich nutrient medium. The LCC was stored at room temperature.

Study Design

This study was a pivotal, multicenter, randomized, within-patient, controlled, open-label, treatment-comparison study designed to establish the superiority for predefined efficacy endpoints. After screening, two contralateral teeth were randomly assigned to treatment with the LCC or an FGG as indicated by a predetermined computer-generated randomization scheme. Treatment assignments were stored in sealed envelopes for each patient and opened only at the time of the procedure. As many as three teeth per quadrant were treated, but only one tooth in each quadrant was identified as the study tooth. When two teeth were selected, the study tooth was identified before surgery; when three teeth were selected, the study tooth was the middle tooth. The criteria for selecting target teeth were teeth relatively matched in terms of recession, BOP, width of keratinized tissue, probing depth (PD), and CAL. Baseline measurements were taken for standard periodontal treatment (e.g., plaque score, BOP, PD [using a standardized periodontal probe], keratinized gingiva width, and inflammation). Because of the nature of the control treatment (i.e., FGG), investigators (MKM, ETS, MN, RN, DLC, JTM) and patients could not be masked to the study-tooth treatment assignment or order of surgical procedures. Thus, all clinical measurements were taken by calibrated independent examiners (not the surgeons) at each treatment center in an attempt to reduce the bias. Before study sites began enrolling patients, periodontal examiners were identified and participated in a calibration training session

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Table 1. Inclusion and Exclusion Criteria for Enrollment in the Study

Inclusion requirements
≥18 and ≤70 years of age
≥2 non-adjacent teeth in contralateral quadrants of the same jaw with ≤1 mm attached gingiva that required soft tissue grafting
Root coverage not desired
Exclusion requirements
Class III recession in the presence of a shallow vestibule or Class IV recession
Vestibule depth <7 mm from the base of recession
Systemic condition, such as diabetes mellitus, HIV, cancer, or bone metabolic disease that could compromise wound healing
Treatment with systemic corticosteroids, immunosuppressive agents, radiation therapy, or chemotherapy within 2 months of enrollment in the study
Acute infectious lesions in intended surgical areas
Received intravenous or intramuscular bisphosphonates
Tobacco use within 3 months of enrollment in the study
Only molar teeth suitable for soft-tissue grafting
Miller grade ≥2 mobility
Known hypersensitivity to bovine collagen or iodine (shellfish allergy)
Previous treatment with an LCC or any skin graft product at the target site or immediately adjacent to it
Pregnancy

HIV = human immunodeficiency virus.

to evaluate the intra- and interexaminer variability for PD, recession depth, and identification of the mucogingival junction. Levels of both intra- and interexaminer variability were found to be within prespecified limits ($\kappa \geq 0.7$ for interexaminer comparisons and $\kappa \geq 0.9$ for intraexaminer comparisons).

Surgery

On day 0, patients were treated with the LCC on one study tooth and the FGG on the contralateral tooth. Recipient beds were created according to standard procedures that ensured no muscle tension on the bed.^{20,21} The size of the LCC was determined by the investigator and was based upon the number of teeth being treated with the LCC. The LCC was Z-folded (Fig. 1) with minimum dimensions of 6 mm wide and 10 mm long. The size of the FGG applied was 4 mm wide, with the length dictated by the number of teeth being treated. The details of the surgical procedure have been previously reported.¹⁵ In brief, after the patients received anesthesia with 2% lidocaine hydrochloride, a partial-thickness dissection was performed to remove any keratinized gingival mucosa and non-keratinized alveolar mucosa from the site.

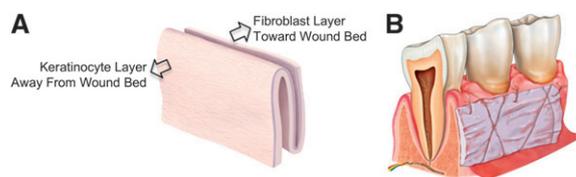


Figure 1.

The LCC arranged in a Z-fold fashion (A) and placed over an oral defect in which the mucosal and keratinized gingiva was removed (B). (1A: figure prepared for, and exclusively licensed to, Organogenesis by Mark Lefkowitz; 1B: figure prepared for, and exclusively licensed to, Organogenesis by Levent Efe.)

A coronal incision was made at the height of the existing mucosa that extended at least to the line angle of adjacent teeth, and from that point, vertical incisions were made on mesial and distal aspects of study sites to allow ≥7 mm apical to the base of the recession. Mesial and distal incisions were connected apically, and any muscle fibers were removed to create a clean periosteal bed. If feasible, a full-thickness horizontal incision was made just apical to the planned level of the LCC or FGG placement. In this way, the separation of the apical periosteum from the coronal periosteum was accomplished to reduce the risk of the retraction of the treated area during healing.

The graft was harvested from the palatal donor site according to standard practices.²¹ A partial-thickness (i.e., ≈1 to 2 mm deep) incision was used to harvest a graft the size of the recipient bed. The palatal donor site was covered with surgical dressing** which remained until it fell off on its own or was removed at the 1- or 2-week follow-up visit.

Coverage of Wound Bed

The LCC was prepared in a Z-fold with the keratinocyte cell layer facing out and was trimmed to the size needed and applied to the wound bed within 15 minutes after removal from the storage bag. The LCC was sutured in place at each papilla (Fig. 1). An additional single layer of the LCC that extended laterally beyond the wound margins was placed over the entire preparation and sutured at the four corners, when possible. The surgical area was placed under tension to confirm that it was free of movement.

The harvested FGG was placed directly on the recipient wound bed and sutured in place using standard FGG techniques.²¹ Both treatment areas were covered with surgical dressing until it fell off on its own or was removed at the 1- or 2-week follow-up visit.

Follow-Up Care

Patients were prescribed antibiotics in accordance with local standards of care and were provided with a 0.12% solution of chlorhexidine mouthrinse and

** Coe-Pak, GC America, Alsip, IL.

postoperative oral hygiene instructions. Patients were to contact the investigational site if the surgical dressing fell off before the first follow-up visit (at 1 week). Patients maintained a diary for 1 to 14 days, noting the adherence of the surgical dressing at surgical sites and at the site of the palatal tissue donation, assessing pain, and recording any medications taken for pain.

Follow-up visits occurred at 1 and 4 weeks and 3 and 6 months after surgery. A visit at week 2 was required if the surgical dressing remained at any site at the week-1 visit. Changes in medications and adverse events were recorded at all visits; photographs of treatment sites were taken, clinical measurements were obtained, and the color and texture of test sites were evaluated. An oral exam was done at 4 weeks and 6 months, and dental prophylaxis was provided at 3 and 6 months. At the 6-month visit, a patient treatment-preference questionnaire was completed, radiographs of study teeth were taken, and a pregnancy test was given to women of childbearing potential.

Study Endpoints

The primary efficacy endpoint was the ability of the LCC treatment to regenerate ≥ 2 mm keratinized gingiva at 6 months. Secondary measures of efficacy were: color the same as the adjacent tissue (visually evaluated), texture (i.e., firmness) the same as adjacent tissue (tactually evaluated), width of keratinized gingiva at 6 months ≥ 1 mm, patient treatment preference which was related to the procedure and outcome, report of surgical site sensitivity (in response to a puff of air) at 1 week, and pain after 3 days. The primary measures of safety included adverse events (overall and mouth-specific and treatment-specific events). Adverse events were reported using a medical dictionary.^{††} Additional 6-month endpoints that were prospectively measured included the width (in millimeters) of keratinized gingiva and attached gingiva regenerated.

Statistical Analyses

Statistical analyses were prospectively defined. The power was calculated based on achieving 85% power for the primary endpoint. A sample size of 74 patients was required to achieve this power. No interim analysis was done. The treatment randomization was determined using a computer-generated randomization scheme that included the type of treatment used on each side and the order of the treatment implementation.

All patients randomly assigned to treatments, treated, and followed for ≥ 1 week were included in the safety assessment. The first two patients treated by each surgeon (11 patients) were treated for training in material handling and standardization purposes only. Five surgeons completed training with two patients, and one surgeon performed one training case. Data from these patients were included in safety-related analyses but

omitted from efficacy analyses. The efficacy population (modified intention-to-treat [mITT] population) included all patients with the exception of those included in the study for training purposes. Continuous variables were summarized using descriptive statistics (i.e., the mean, median, SD, minimum, and maximum) and categorical variables were summarized using frequencies and percentages.

The primary endpoint tested whether $\geq 50\%$ of patients achieved a ≥ 2 -mm keratinized gingiva threshold at 6 months by using an exact binomial test with a type I error rate of 0.05. The success threshold of 50% of patients was prespecified during discussions with the United States Food and Drug Administration, Silver Spring, Maryland. Secondary endpoints were tested using a closed testing strategy in which the order of testing was prespecified. Each test was conducted sequentially at the 0.05 level until a test was found not to be statistically significant. Endpoints for color and texture compared the tissue at the surgical site with the adjacent non-treated tissue (superiority at 6 months). For a band of keratinized gingiva ≥ 1 mm, the endpoint was the superiority versus a prespecified standard empirically chosen of 80%. The patient preference for treatment and endpoints of surgical site sensitivity of mild or absent after 1 week and pain absent after 3 days were superiority tests between treatments. The patient questionnaire was not validated; however, the study staff was trained to ensure the uniform questioning and recording of responses.

RESULTS

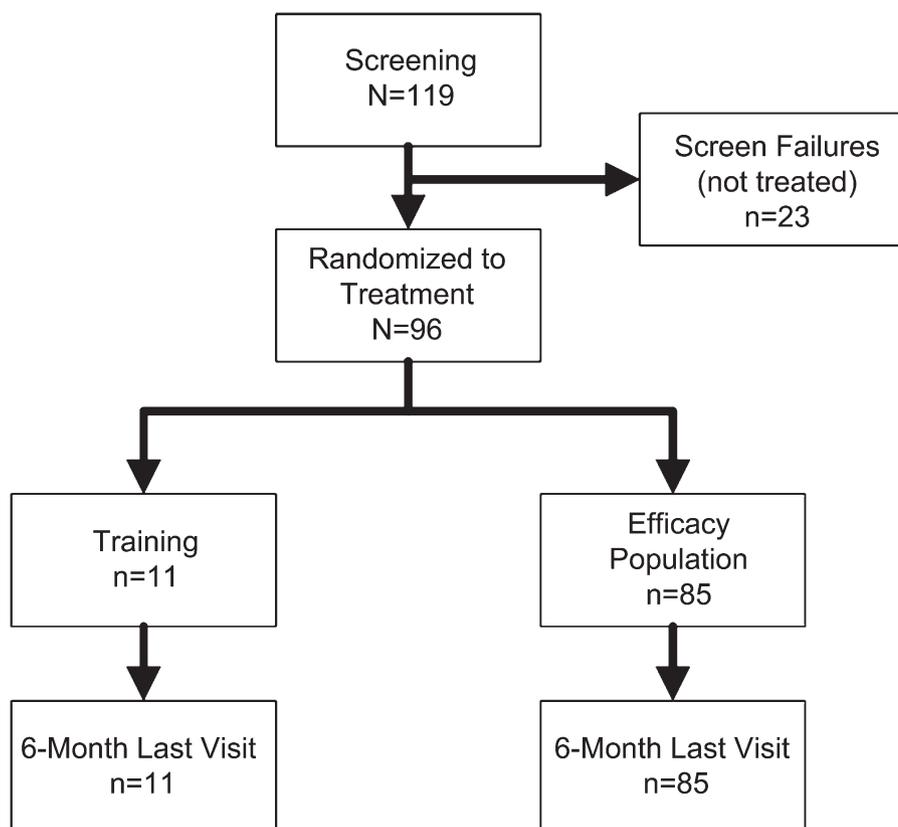
Patients

The study commenced in October 2007, and the last patient visit was in December 2008. A total of 119 patients at four sites in the United States were screened, with 96 patients randomly assigned and treated (Fig. 2). Of the 96 patients assigned to treatment, 11 patients were considered training patients and included in the safety data analyses only. The numbers of patients enrolled from investigative sites were as follows: Perio Health Professionals ($n = 34$), Boston Periodontics and Dental Implants ($n = 30$), Michigan Center for Oral Research ($n = 29$), and University of Texas Health Science Center ($n = 3$). Consistent with study inclusion criteria, all patients had mucogingival deformities at the time of surgery, with no other clinically remarkable findings upon oral examinations (Table 2). All 96 patients completed the study (to month 6).

Surgery

The study surgery was completed under appropriate surgical conditions at all sites. The surgical

†† Medical Dictionary for Regulatory Activities (MedDRA) v.10.0 coding program, Cerner Corporation, Kansas City, MO.

**Figure 2.**

Disposition of patients.

procedures, including training cases, were performed on the mandible in 89 (93%) patients, with the LCC being applied to the right side in 50 (52%) patients. All patients tolerated surgery well. Most surgical procedures used the cemento-enamel junction as the reference point (LCC: 72%; FGG: 66%). Mean and median alveolar bone levels and surgical position margins were similar between both sides of the mouth for any given patient. Additional baseline characteristics of surgical sites are provided in Table 3.

Lengths of palatal grafts ranged from 8 to 30 mm, with widths \approx 4 mm in all but two patients who had grafts with widths \approx 5 mm. Lengths of LCC were similar to those reported for palatal grafts, but widths varied from 5 to 20 mm. No patient required a reapplication of LCC or surgical dressing during the study. In one patient, the palatal graft was taken from the side of the mouth treated with the LCC.

Efficacy

Regeneration of keratinized gingiva at 6 months. At the 6-month visit in 81 of 85 (95.3%) patients, the sites that were treated with the LCC had regenerated a clinically relevant width of keratinized gingiva (i.e., \geq 2 mm) compared to the 50% standard ($P < 0.001$; exact binomial test; 95% confidence interval [CI]:

88.4% to 98.7%). As expected, the FGG generated statistically significantly ($P < 0.001$) more keratinized gingiva than the LCC (4.57 ± 1.0 mm versus 3.21 ± 1.1 mm, respectively). The four (4.7%) patients who did not achieve keratinized gingiva \geq 2 mm achieved a width \geq 1 mm (Table 4). All patients in the mITT population achieved \geq 1 mm keratinized gingiva with the LCC treatment by 6 months, significantly exceeding the standard established at the beginning of the study, which stipulated that \geq 80% of patients should have achieved \geq 1 mm keratinized gingiva (LCC: $P < 0.001$, exact binomial test; 95% CI: 95.8% to 100.0%). Figure 3 provides a photographic depiction of the results achieved with both treatments.

Although not defined as a primary or secondary endpoint, the amount of attached gingiva generated was captured. At 6 months, sites treated with the LCC generated 1.77 ± 1.32 mm attached gingiva, whereas the FGG sites had

3.17 ± 1.17 mm attached gingiva.

Color. The color of both treated recipient sites was compared to the adjacent, non-treated gingiva at 4 weeks and 3 and 6 months, and the endpoint assessed whether the color was the same as adjacent tissue at 6 months. At all time points, the gingiva of the tooth treated with the LCC was scored as equally red (i.e., the same) as the adjacent gingiva more often than the gingiva of the tooth treated with the FGG (Table 5). At month 6, a significantly larger proportion of patients treated with the LCC, compared to the FGG, achieved a color that was comparable with the color of the adjacent tissue ($P < 0.001$; McNemar test).

Texture. The texture of both treated recipient sites was compared to the adjacent, non-treated tissue at 4 weeks and 3 and 6 months and assessed whether the firmness was equivalent to the adjacent tissue at 6 months. At all time points, the gingiva of the tooth treated with the LCC was scored as equally firm as the adjacent gingiva more often than the gingiva of the tooth treated with the FGG (Table 5). At 6 months, a significantly larger proportion of patients treated with the LCC, compared to the FGG, achieved a texture comparable to the adjacent tissue ($P < 0.001$; McNemar test).

Table 2.
Demographic and Clinical Characteristics of Patients at Baseline by Cohort

Characteristic	Efficacy* (n = 85)	Training (n = 11)	Total (n = 96)
Sex (n [%])			
Female	46 (54)	6 (55)	52 (54)
Male	39 (46)	5 (45)	44 (46)
Age (years) (n [%])			
Mean (SD)	46.9 (12.7)	49.4 (16.7)	47.1 (13.1)
Median	48.3	53.6	48.8
Minimum, maximum	18.0, 70.8	21.2, 70.3	18.0, 70.8
Race (n [%])			
White	77 (91)	10 (91)	87 (91)
Black	1 (1)	0 (0)	1 (1)
Asian	4 (5)	1 (9)	5 (5)
Other	3 (3)	0 (0)	3 (3)
Previous tobacco use (n [%])			
Yes	34 (40)	4 (36)	38 (40)
No	51 (60)	7 (64)	58 (60)
Selected dental history (n [%]) [†]			
None	12 (14.1)	0 (0)	12 (12.5)
Loose teeth	14 (16.5)	4 (36.4)	18 (18.8)
Bleeding gums	29 (34.1)	3 (27.3)	32 (33.3)
Grinding/clenching	42 (49.4)	5 (45.5)	47 (49.0)
Sores in mouth	17 (20.0)	2 (18.2)	19 (19.8)
Previous periodontal work (n [%])			
Yes	58 (68)	9 (82)	67 (70)
No	27 (32)	2 (18)	29 (30)

* Included all patients except those included in study training.

[†] Only clinically relevant parameters are presented.

Patient preference. At 6 months, more patients preferred the LCC treatment compared to the FGG treatment (71.8% versus 28.2%, respectively; $P < 0.001$; 95% CI: 61.0% to 81.0%). At 6 months, more patients in the mITT population preferred the appearance of the LCC compared to the FGG site (76.5% versus 23.5%, respectively; $P < 0.001$; exact binomial test; 95% CI: 66.0% to 85.0%).

Surgical site sensitivity. The sensitivity at the palatal graft site and both treatment sites after 1 week was not frequently reported in the study population. No sensitivity was noted in >70% of patients at any site after 1 week, and no patient reported a severe sensitivity at any site at any evaluation period. No difference in sensitivity was noted between the LCC site and FGG site (data not shown).

Pain. More than one-half of the patients reported some pain at one of the procedure sites (palatal graft, LCC, or FGG) for the first 6 days of self-assessment, as recorded in daily diaries. Although pain at the treatment site was reported more frequently by patients at the LCC site compared to the FGG site at 3 days

(70.6% versus 62.3%, respectively) and 7 days (45.9% versus 37.7%, respectively), differences between groups were not statistically significant. Most of the pain was reported as being mild or moderate; <5% of patients at any time point reported pain as severe. Pain was reported at the palatal graft site in 43.6% of patients on day 1, and >35% of patients continued to report pain at this site on days 2 through 4, with some patients reporting pain at day 12. Other measurements taken at baseline (e.g., plaque score, BOP, PD, and inflammation) were unremarkable (data not shown).

Safety. All treatments were well tolerated in this patient population, and events reported were typical of those in patients undergoing this type of periodontal surgery. Because of the localized application of the LCC, adverse events were summarized by the event location (i.e., LCC, FGG, or palatal graft, mouth, or other; Table 6).

A total of 24 of 96 (25%) patients reported ≥ 1 adverse event during the study, with a total of 43 events reported, with most events reported as mild or moderate in severity. No event was reported by >2 patients. The most commonly reported events were infections (10 events in eight [8.3%] patients) and included nasopharyngitis, respiratory tract infection, sinusitis, and upper respiratory tract infection ($n = 2$ each). Eight patients reported nine gastrointestinal events, including two reports each of aphthous stomatitis and dental caries. Three patients reported an adverse event occurring at the LCC-treated site. Two of these events were secondary to the inadvertent placement of the polycarbonate membrane on which the LCC was supplied, and the third was a mouth ulceration. Two patients experienced adverse events occurring at the palatal harvest site (a postprocedural hemorrhage and thrombosis), and two patients experienced adverse events occurring at the FGG-treated site (gingivitis and skin exfoliation).

Three serious adverse events were reported during the study and required hospitalization: one each for

Table 3.
Selected Baseline Characteristics of Surgical Sites of the mITT Cohort (n = 85)

Characteristic	LCC	FGG
Presence of plaque (n [%])		
Buccal	18 (21)	19 (22)
Lingual	27 (32)	29 (34)
BOP (n [%])	23 (27)	18 (21)
PD (mm)		
Mean (SD)	1.39 (0.49)	1.35 (0.49)
Keratinized gingiva width (mm)		
Mean (SD)	1.41 (0.72)	1.43 (0.69)
Attached gingiva width (mm)		
Mean (SD)	0.02 (0.76)	0.08 (0.79)
Inflammation (n [%])		
0 (normal)	71 (83.5)	70 (82.4)
1 (mild of any portion of the marginal unit)	12 (14.1)	14 (16.5)
2 (mild of the entire gingival unit)	2 (2.4)	1 (1.2)
3 (moderate)	0 (0)	0 (0)
4 (severe)	0 (0)	0 (0)
Treatment jaw (n [%])		
Mandible	80 (94.1)	80 (94.1)
Maxilla	5 (5.9)	5 (5.9)

Table 4.
Amount of Soft Tissue Regenerated at 6 Months

Tissue Type	LCC	FGG
Keratinized gingiva (mm; mean [SD] width)	3.21 (1.14)	4.57 (1.00)
Attached gingiva* (mm; mean [SD])	1.77 (1.32)	3.17 (1.17)

* Prospectively collected but not identified as a primary or secondary endpoint.

pneumonia, chest pain, and metastatic malignant fibrous histiocytoma. The investigator assessed these events to be unrelated or unlikely to be related (in the case of histiocytoma) to the treatment.

DISCUSSION

The absence of sufficient keratinized gingiva may lead to gingival recession, which in turn may result in the loss of a portion of the cortical plate and a worsen-

ing prognosis for the integrity of the affected tooth. Although a meta-analysis suggested that no single clinical measure was universally acceptable to assess periodontal treatment outcomes,¹⁹ the objective standard of ≥ 2 mm keratinized gingiva in mucogingival procedures is generally accepted. In this study, we evaluate the utility of an LCC to regenerate keratinized gingiva when applied to a wound bed in which the mucosa or keratinized gingiva are removed. The use of the LCC gave a 95.3% success rate in the primary effectiveness endpoint (e.g., ≥ 2 mm keratinized gingiva at month 6) ($P < 0.001$ versus 50% standard), and all patients achieved ≥ 1 mm keratinized gingiva ($P < 0.001$ versus 80% standard). The clinical significance of these findings was supported by the fact that a mean of 1.77 ± 1.32 mm attached gingiva was present at 6 months. Other endpoints, the color and texture compared to adjacent tissue and patient preferences, were statistically significant in favor of the LCC ($P < 0.001$ for all measures).

Statistically significant differences were not obtained for the secondary efficacy endpoints of the absence of pain by 3 days and a minimal surgical sensitivity at the week-1 time point. The harvesting of the FGG from the palate was associated with some morbidity; however, the within-patient study design may not have allowed for the accurate localization of pain and sensitivity because of multiple surgical sites within the mouth. It was also possible that the bed preparation for LCC was somewhat larger than the bed for FGG, which could have influenced pain-related endpoints.

Even though FGG yielded statistically significantly more keratinized tissue than LCC (4.57 ± 1.0 mm), the use of LCC may have several advantages compared to the use of FGG. First, a second surgical site, the palatal donor-site, is not required with its use, eliminating pain and bleeding at the donor site. Second, an unlimited supply of readily available material may prevent undertreatment in situations of limited autogenous tissue. The use of LCC provides an unlimited amount of material to treat demanding cases in one surgical visit. Furthermore, based on the unlimited availability of the LCC, the augmentation of keratinized gingiva could be indicated for extensive oral rehabilitation cases for which the palatal tissue would be insufficient. Third, the LCC is not a graft but affects healing by improving the rate and quality of wound repair by a secondary intention^{10,14} and, therefore, allows the regeneration of a clinically relevant width of site-appropriate (form and function) tissue that has the same color and texture of surrounding tissues. Although palatal tissue is a reliable means to generate additional keratinized gingiva, the tissue remains palatal, not gingival, and is often paler and of different texture than surrounding tissue. Shrinkage is often

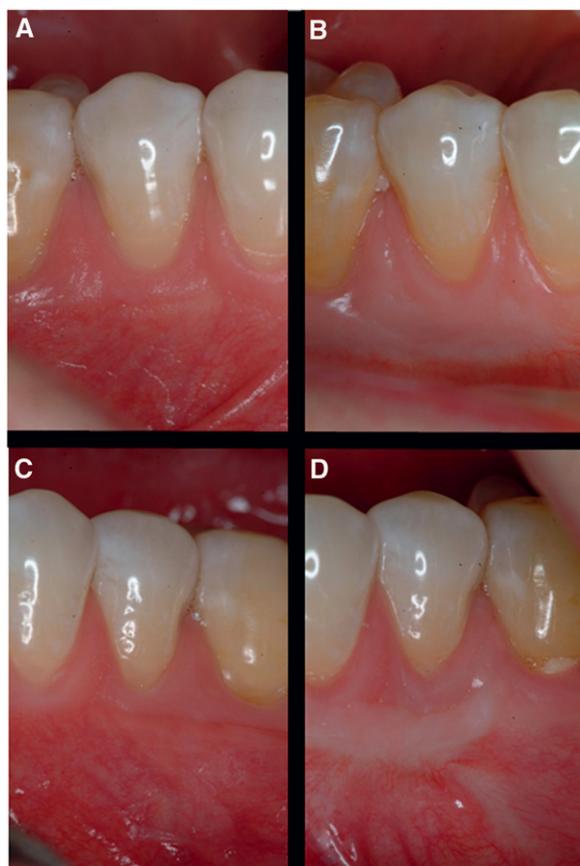


Figure 3. Photographs of treatment sites within a single patient taken at baseline (A and C) and 6 months after the application of LCC (B) or FGG (D).

Table 5. Measure (n [%]) of Color and Texture Compared With Tissue Adjacent to the Surgical Site in the mITT Cohort (n = 85) at 4 Weeks and 3 and 6 Months

Study Visit	LCC	FGG
Color		
4 weeks		
More red	28 (32.9)	6 (7.1)
Equally red	56 (65.9)	41 (48.2)
Less red	1 (1.2)	38 (44.7)
3 months		
More red	3 (3.5)	1 (1.2)
Equally red	80 (94.1)	29 (34.1)
Less red	2 (2.4)	55 (64.7)
6 months		
More red	4 (4.7)	0 (0)
Equally red	79 (92.9)	23 (27.1)
Less red	2 (2.4)	62 (72.9)
Texture		
4 weeks		
More firm	2 (2.4)	27 (31.8)
Equally firm	63 (74.1)	57 (67.1)
Less firm	20 (23.5)	1 (1.2)
3 months		
More firm	0 (0)	37 (43.5)
Equally firm	80 (94.1)	48 (56.5)
Less firm	5 (5.9)	0 (0)
6 months		
More firm	0 (0)	39 (45.9)
Equally firm	81 (95.3)	46 (54.1)
Less firm	4 (4.7)	0 (0)

All patients had ≥1 tooth treated with each therapy.

seen with an FGG, and overcorrection is sometimes needed, requiring a larger donor site with its inherent morbidities.^{22,23} Because the LCC does not function as a graft, and the cells of the device do not persist, it made no sense to standardize the width of LCC to the width of FGG. An effort was made to place as wide a piece of the LCC as the vestibule would allow. Patients with shallow vestibules may not be good candidates for this procedure.

The results from this study extend the findings from a pilot investigation.¹⁵ However, changes from the pilot study included the definition of a minimum size for the Z-folded LCC of 5 × 10 mm, with deeper vestibules permitted to have a wider treatment area. In an attempt to protect the LCC, an additional single layer of LCC was applied, which was not used in the pilot study. The FGG with a 4-mm width was selected to account for interpatient anatomic variability, which sometimes limits the width of harvested palatal tissue.²¹ Additionally, the 4-mm width was used, compared to a larger width of the FGG, to minimize the chance of a poor esthetic outcome (color and texture match) commonly seen with FGG proce-

dures while reliably generating a clinically relevant amount (i.e., ≥2 mm) of keratinized gingiva.

Nevertheless, the pilot study results showed the generation of 2.4 ± 1.02 mm of keratinized gingiva, whereas the present study results showed the generation of 3.2 ± 1.14 mm of keratinized gingiva. This amount of keratinized gingiva exceeded the proposed minimum keratinized gingiva necessary to maintain gingival health by >1 mm.¹⁸ Eighty-one of 85 (95.3%) patients treated with the LCC regenerated a clinically relevant width of keratinized gingiva (i.e., ≥2 mm).

As with any study, the interpretation of these results was limited by certain aspects of the study design. Although the duration of this study was the typical length for soft tissue studies,²⁴⁻²⁶ a longer follow-up would have allowed us to more thoroughly evaluate the durability of the tissue regenerated in the presence of the LCC and whether further creeping attachment occurred with time.²⁷ Additional studies need to be conducted to determine whether patients

Table 6.

Overall Incidence of Adverse Events by Location, Severity, and Relationship to Treatment by Patients Reporting ≥ 1 Event (Safety Population, n = 96)

Adverse Events	LCC	FGG	Donor Site	Mouth	Other
Overall (n [%])	3 (3.1)	2 (2.1)	2 (2.1)	5 (5.2)	17 (17.7)
By severity (n [%])					
Mild	3 (3.1)	1 (1.0)	1 (1.0)	4 (4.2)	14 (14.6)
Moderate	0	0	1 (1.0)	0	5 (5.2)
Severe	0	1 (1.0)	0	1 (1.0)	2 (2.1)
By relationship (n [%])					
Not related	1 (1.0)	1 (1.0)	2 (2.1)	3 (3.1)	14 (14.6)
Related*	2 (2.1)	1 (1.0)	0	2 (2.1)	3 (3.1)

* Includes unlikely, possible, probable, and definite.

find the benefit of not having a donor site offsets the additional cost of the tissue-engineered device. Additionally, because of the nature of the surgical procedures, we were unable to mask the investigators, and although we do not believe this to be a substantial influence, some bias in the performance of the procedures may have occurred.²⁸ Last, the use of an autograft may not have been the best control to evaluate a treatment for which the mode of healing was via a secondary intention. However, a more appropriate control procedure, such as the “push back technique”²⁹ or a gingivectomy,³⁰ is no longer considered a viable treatment option.

CONCLUSIONS

The results of this study suggest that LCC may be a safe and effective alternative to the standard of care, FGG, for restoring gingival tissue adjacent to teeth that do not require root coverage. The treatments were well tolerated, and the adverse events reported were events typical of this type of surgery. These positive results open the possibility of considering LCC as a treatment for oral mucosal surface defects for which the regeneration of site-appropriate tissue is required.

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