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Living cellular constructs for keratinized tissue augmentation: A 13-year follow-up from a split-mouth randomized, controlled, clinical trial

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One sentence summary: Over the long-term, both living cellular construct and free gingival graft procedures augmented keratinized tissue width and attached tissue width; however superior clinical outcomes were found for free gingival graft, while living cellular construct showed better esthetics and patient-reported outcomes than the autogenous graft.

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Abstract

Background: to evaluate the long term outcomes of living cellular construct (LCC) compared to free gingival graft (FGG) for keratinized tissue width (KTW) augmentation in the natural dentition.

Methods: Twenty-four subjects out of the original 29 enrolled participants were available at the 13-year follow-up. The primary endpoint was the number of sites demonstrating stable clinical outcomes from 6 months to 13 years (defined as KTW gain, stability or ≤ 0.5 mm of KTW loss, together with reduction, stability or increase of PD and REC ≤ 0.5 mm). Secondary outcomes included the assessment of KTW, attached gingiva width (AGW), recession depth (REC), clinical attachment level (CAL), esthetics and patient-reported outcomes (PROMs) at the 13-year visit, assessing the changes from baseline to 6 months.

Results: Nine sites per group (42.9%) were found to have maintained stable (≤ 0.5 mm or improved) clinical outcomes from 6 months to 13 years. No significant differences were observed for the clinical parameters between LCC and FGG from 6 months to 13 years. However, the longitudinal mixed model analysis showed that FGG delivered significantly better clinical outcomes over 13 years ($p<0.01$). LCC-treated sites exhibited superior esthetic outcomes compared to FGG-treated sites at 6 months and 13 years ($p<0.01$). Patient-esthetics was significantly higher for LCC over FGG ($p<0.01$). Patient overall treatment preference was also in favor of LCC ($p<0.01$).

Conclusions: A similar stability of the treatment outcomes from 6 months to 13 years was found for LCC- and FGG-treated sites, with both approaches shown to be effective in augmenting KTW and AGW. However, superior clinical outcomes were found for FGG over 13 years, while LCC was associated with better esthetics and patient-reported outcomes than FGG.

1. Introduction

The role of keratinized tissue and attached gingiva on periodontal health has been extensively debated.[1] Expert opinions during the '60's and '70's advocated that lack of an adequate keratinized tissue width (KTW) could not protect periodontal tissues from injuries associated with mastication or muscle pulls, [2, 3] and could facilitate the formation of pockets, gingival recessions or clinical attachment loss.[3, 4] Lang & Loe found an increased gingival index and exudate scores at teeth with minimal KTW, leading the authors to conclude that the presence of at least 2 mm of KTW and 1 mm of attached gingiva width (AGW) was crucial for maintaining periodontal health.[5] Although some clinical studies did not support these findings [3, 6, 7], it seems that an adequate band of KTW and AGW may be beneficial

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in clinical situations, such as in the presence of subgingival restorations[8] or in subjects non-compliant with regular periodontal maintenance.[9] In a longitudinal study, Kennedy and coworkers demonstrated that while periodontal health could be maintained at sites with minimal KTW and AGW in presence of optimal plaque control, patients who were non-compliant with the recall appointments exhibited re-establishment of inflammation and further gingival recessions at the non-augmented sites.[9] The long-term effects of FGG on periodontal health were further demonstrated in a series of studies by Agudio and coworkers.[1, 10-12] The authors observed that teeth previously treated with FGG due to inadequate KTW and AGW maintained the stability of the gingival margin and prevented the progression/development of non-carious cervical lesions (NCCLs) over time, as opposed to contralateral non-augmented sites that displayed progressive increase of gingival recessions, developing of new recession defects, or non-carious cervical lesions.[1, 10-12]

A recent systematic review and network meta-analysis from our group assessed the available evidence on the efficacy of gingival phenotype (comprising KTW and gingival thickness) modification on periodontal health. [13] Soft tissue grafting materials that have been combined with apically positioned flap (APF)-based approaches, primarily aimed at increasing the amount of KTW and AGW without root coverage purposes, included autogenous FGG, human acellular dermal matrix, xenogeneic collagen matrix and tissue-engineered living cellular construct (LCC). [13] The network meta-analysis demonstrated that APF + any graft obtained significantly higher KTW than APF alone and untreated sites, in addition to a significant reduction of plaque scores. [13] This study also confirmed that FGG was the graft associated with the highest treatment effect for KTW gain, in line with previous investigations. [14-16]

Nevertheless, the concept of “gold standard” based on clinical efficacy has been often challenged in the literature. [15, 17-21] The relatively high patient morbidity, the need for a second surgical site, and patient anxiety towards palatal harvesting that characterize autogenous grafts [22, 23], led clinicians to investigate several graft substitutes for KTW augmentation, including tissue engineered grafts possessing living cells. [15, 17, 24-27] In a split-mouth clinical trial, McGuire and coworkers demonstrated that a LCC containing allogeneic fibroblasts and keratinocytes regenerated at least 2 mm of KTW in 95.3% of patients after 6 months, although conventional FGG obtained significantly higher KTW gain (4.57 vs 3.21 mm, on average, respectively). [17] Interestingly, LCC-grafted sites achieved a better color match and texture compared to FGG-treated teeth, with most of the patients indicating a preference for LCC over FGG. Nevertheless, long-term (≥10 years) follow-up of randomized controlled trials evaluating clinical and patient-reported outcomes of FGGs, tissue engineered grafts, and overall graft substitutes, are lacking.

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Therefore, the aim of the present study is to evaluate the 13-year results of gingival phenotype modification following LCC and FGG, and to assess the changes occurring from 6 months to 13 years.

2. Materials and Methods

2.1 Study design

The present study was designed as a 13-year follow-up of a short-term (6 months follow-up) multicenter randomized controlled trial (RCT) conducted from October 2007 to December 2008 investigating the efficacy of a LCC to regenerate KG in the natural dentition as compared to FGG [17] (ClinicalTrials.gov ID NCT00587834). The protocol of this follow-up study was approved by the Institutional Review Board of the University of Michigan Medical School (HUM00158319), and is in accordance with the Declaration of Helsinki of 1975, revised in Fortaleza in 2013. All participants were informed and understood the objectives and the details of the study and signed a written informed consent document. The CONSORT statement for improving the quality of parallel group randomized trials reports [28] was utilized for preparing the present manuscript.

Original inclusion criteria and intervention

The study protocol is described in detail in the original study.[17] Twenty-nine subjects with age ≥ 18 and ≤ 70 years, at least 2 non-adjacent teeth in contralateral quadrants of the same jaw with ≤ 1 mm attached gingiva requiring soft tissue grafting not for root coverage purposes were recruited between November 2007 and June 2008 at the Michigan Center for Oral Health Research, Ann Arbor, USA. The exclusion requirements were: i) Miller Class III recession in the presence of a shallow vestibule or Miller class IV recession, [29] ii) Vestibule depth < 7 mm from the base of recession, iii) Systemic condition, such as diabetes mellitus, HIV, cancer, or bone metabolic disease that could compromise wound healing, iv) Treatment with systemic corticosteroids, immunosuppressive agents, radiation therapy, or chemotherapy within 2 months of enrollment in the study, v) Acute infection lesions in intended surgical areas, vi) diagnosis of periodontal disease[30], vii) Received intravenous or intramuscular bisphosphonates, viii) Tobacco use within 3 months of enrollment in the study, ix) Only molar teeth suitable for soft tissue grafting, x) Miller grade ≥ 2 mobility,[31] xi) Known hypersensitivity to bovine collagen or iodine (shellfish allergy), xii) Previous treatment with a living cellular construct (LCC) or any skin graft product at the target site or immediately adjacent to it, and xiii) Pregnancy.

Each patient contributed to one test (LCC) and one control site (FGG), based on a computer-generated randomization scheme. The LCC* is a tissue engineering graft approved by the Food and Drug Administration for the treatment of mucogingival deformities, and it consists of a bovine collagen

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matrix seeded with allogeneic keratinocytes and dermal fibroblasts derived from neonatal foreskin.[17, 32]

Although as many as three teeth per quadrant were treated, only one tooth per quadrant was identified – prior to the surgical procedure - as the study tooth. The main criteria for selecting target sites were the relative matching of opposing sites in terms of tooth type, baseline clinical parameters (recession depth [REC], keratinized tissue width [KTW], bleeding on probing [BOP], probing depth [PD] and clinical attachment level [CAL]). The operator (R.N.) opened the sealed envelope containing the treatment allocation (as well as the sequence of operation, e.g. lower left first [FGG], then lower right [LCC]) only at the time of the surgical procedure. Due to the nature of the surgical intervention, with FGG requiring palatal harvesting, investigators and patients could not be masked regarding the order of the surgical procedure.

Control and test sites received the same flap approach, which consisted of a split-thickness apically positioned flap starting with a coronal incision performed at the level of the mucogingival junction (MGJ). The flap was designed in a way that the tooth identified as study site was always located in the middle portion of the flap and not at the mesial/distal regions. Vertical incisions were performed on mesial and distal aspects of study sites aiming at apically repositioning the flap of at least 7 mm. After removal of muscle fibers, the recipient bed was measured to determine the dimension of the graft. Both FGG and LCC were undersized in the apical portion compared to the recipient bed. FGG of approximately 1-2 mm in thickness was harvested from the palate, and sutured to the recipient bed with simple interrupted and periosteal sutures. LCC* was trimmed to the dimension of the recipient site and prepared in a Z-fold with the keratinocytes layer as more external and superficial layer. The LCC was stabilized with simple interrupted sutures at each papilla and with anchoring periosteal sutures (Figure 1). An additional single layer of the LCC – bigger than the recipient site in a mesio-distal direction – was utilized and stabilized over the surgical site with the goal of delivering an increased dose of cells and cytokines to the recipient bed and maximizing the synergistic interactions between the allogeneic cells of the LCC and the cells of the host.[17, 25] Periodontal dressing† was applied on both grafted sites and maintained until it was lost or removed at the 1- or 2-week follow-up appointment.[17]

In terms of post-operative regimen, patients were prescribed painkillers, antibiotics (Amoxicillin 500 mg tid for 1 week) and a 0.12% solution of chlorhexidine mouthrinse. Oral hygiene instructions were also provided. The subjects were contacted in the following post-operative days. Follow-up visits occurred at 1 week, 4 weeks, 3 months, and 6 months.[17]

2.3 Study outcomes

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The number of sites demonstrating stable clinical outcomes from 6 months to 13 years was set as the primary outcome of the study. Sites were defined stable in presence of the following conditions at the 13-year time point, compared to the 6-month outcomes: i) KTW gain, no changes of KTW, or KTW loss ≤ 0.5 mm, ii) PD reduction, no changes of PD, or PD increase ≤ 0.5 mm, and ii) REC reduction, no changes of REC, or REC increase ≤ 0.5 mm.[33]

Secondary outcomes included changes within KTW, AGW, PD, REC, and CAL over time, as well as the evaluation of presence of plaque, BOP, inflammatory score, professional esthetic outcomes, and patient-reported outcome measures (PROMs) at 6 months and 13 years. Factors associated with patient treatment preference and discomfort during brushing at the grafted sites were also set as secondary outcomes.

2.4 Clinical parameters, esthetic outcomes, and PROMs

The following clinical parameters were measured at the midbuccal aspect of the study sites at baseline, 6 months and 13 years by the same calibrated examiner (T.J.O.) using a periodontal probe‡ and rounding down to the nearest 0.5 millimeter: REC (measured from the cemento-enamel junction [CEJ] to gingival margin at the level of deepest recession, PD (measured as the distance from the gingival margin to the base of the sulcus), CAL (measured by adding REC to PD), KTW (determined as the distance from the gingival margin to the MGJ after applying Lugol's Iodine Solution§), and AGW (obtained by subtracting the PD from the KTW). [17] The single calibrated independent examiner attended a calibration training session prior to participants enrollment. The intra-examiner reproducibility for KTW, PD, and REC, was found to be within prespecified limits (k≥ 0.9). [17] Similarly, the operator was re-calibrated prior to the 13-year follow-up visit (intra-examiner reproducibility for the same parameters ≥0.9).

In addition, muscle pull resistance (yes/no), plaque score (assessed at the midbuccal and midlingual aspect and reported as presence/absence), BOP (assessed at the mid-buccal, mesio-buccal, disto-buccal aspect and reported as presence/absence) and the inflammation score (assessed at the mid-buccal aspect and reported as normal [score 0], localized mild inflammation [score 1], mild inflammation of the entire gingival unit [score 2], moderate inflammation [score 3] and severe inflammation [score 4])[17, 25] were evaluated at each study site. Gingival phenotype was assessed at the 13-year follow-up using color-coded probes‖. [34]

The presence of NCCLs [35] was also recorded as previously described.[12]

Professional esthetic assessment included the appraisal of color match with the adjacent soft tissue (more, equal or less red), scar or keloid-like formation (presence/absence), MGJ alignment compared

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to the MGJ of the adjacent dentition (aligned/not aligned), and marginal tissue contour (following the CEJ or not).[17, 36, 37] This assessment was performed at the time of the follow-up visit by the same examiner that collected the clinical parameters (T.J.O.).

The following PROMs were assessed at 6 months and 13 years using the same questionnaire, where subjects were asked to indicate their esthetic preference (right vs left site), and their overall treatment preference (LCC vs FGG, after disclosing site/treatment allocation). In addition, at the 13-year visit, patient-reported esthetics, and discomfort during brushing were assessed using a 100-mm visual analogue scale (VAS). The assessment of PROMs, patient-reported esthetics, and discomfort during brushing was completed before recording the clinical measures of interest.

Lastly, patients returning to the 13-year recall were classified as compliant or non-compliant based on the average number of maintenance appointments/year following the surgical procedure.[33]

2.6 Data and statistical analysis

Means and standard deviations (SD) were calculated for continuous measures (KTW, PD, AGW, REC, CAL, etc.). Presence of plaque, BOP, inflammatory score, gingival phenotype, and incidence of new NCCL were calculated as the percentage of sites showing the condition of interest, and were expressed as a binary outcome. Patient preference towards the appearance of the sites and the overall treatment were also reported as a percentage and expressed as a binary outcome. A multilevel longitudinal mixed model was used to assess the effect of two treatments on KTW, PD, AGW, REC, and CAL over 13 years, accounting for repeated measures with random effects for time and subject, and fixed effects for potential confounders (sex, age, previous smoking habit and history of periodontal treatment). Additionally, a paired T-test was performed to compare means of continuous measures across single time-points across a single binary variable (treatment). A chi-squared test was used for comparing the outcomes of categorical variables, while patient preference in terms of appearance and overall treatment was investigated with a binomial test. Lastly, a linear regression was used to test for correlation between PROMs and continuous variables at the 13 year time point. Confidence intervals (CI) were produced and a *p* value of 0.05 was set for statistical significance. The analyses were performed by an independent biostatistician, who had not taken part in the clinical portion of the study or data collection, using a statistical software¶.

3. Results

3.1 Participant flow and descriptive analysis

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From the original cohort of 29 subjects that completed the 6-month study, 21 subjects (12 male and 9 females, mean age at the 13-year visit 58.8 ± 11.0 years) were available for the 13-year recall appointment (response rate of 72.4%). In addition, three subjects that had moved to other states and could not travel to our clinical center, agreed to participate to the study by filling the questionnaire. Therefore, the clinical outcomes of the present investigation are based on 21 subjects, while 24 patients contributed to the PROMs (Figure 2). Additional information of patient and site characteristics at baseline are reported in the Supplementary Table 1 (see Table S1 in online Journal of Periodontology).

Five subjects of this cohort conveyed at baseline to have previously used tobacco, while none of the 21 patients available for the 13-year visit reported to currently smoke, nor to have smoked within the past 13 years. Based on the average number of maintenance appointments after the surgical procedure, nineteen patients were defined compliant (90.5%), while two were considered non-compliant (9.5%). At the 13-year follow-up, all the treated teeth were maintained and periodontally stable (no PD ≥ 5 mm, presence of suppuration, or mobility). No new crowns or restoration at the level of the cervical margin have been performed at the study sites between the 6 month and the 13-year visits.

3.2 Stability of the clinical outcomes from 6 months to 13 years

Nine sites per group (42.9%) were found to have maintained stable (≤ 0.5 mm or improved) clinical outcomes from 6 months to 13 years. No statistically significant differences were observed between LCC and FGG for the long-term changes within the clinical outcomes obtained at 6 months, including KTW gain, PD change, AGW gain, REC change and CAL change. Both groups showed a stable KTW from 6 months to 13 years (-0.05 mm and -0.17 mm for LCC and FGG sites, respectively, $p>0.05$). A mean PD increase of 0.38 mm was observed between 6 months to 13 years in both groups, while LCC- and FGG-treated sites showed an average increase in REC of 0.55 mm and 0.45 mm, respectively ($p>0.05$). From 6 months to the 13-year recall, the two groups exhibited a CAL loss of almost 1 mm (0.93 mm at LCC-treated sites, and 0.95 mm at FGG-treated sites, $p>0.05$) (Figure 3).

3.3 Long-term clinical performance of FGG and LCC

The longitudinal mixed model analysis showed that FGG provided significantly higher KTW (estimated coefficient of 0.440 (95% CI [0.347, 0.534] $p<0.01$) and AGW (estimated coefficient of 0.440 (95% CI [0.344, 0.537] $p<0.01$) than LCC over 13 years. FGG-treated sites displayed also better outcomes in terms of REC (estimated coefficient of -0.218 (95% CI [-0.291, -0.146] $p<0.01$) and CAL (estimated

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coefficient of -0.234 (95% CI [-0.319, -0.149] p<0.01) over time. No differences in PD were observed between the groups (Tables 1 and 2).

In addition, no differences were observed in terms of presence of plaque, BOP, and inflammatory score between the two groups, with most of the sites that showed a normal appearance (85.7% of the LCC-treated sites and 66.7% of the FGG-treated sites were rated with an inflammatory score of 0, p>0.05) (see Table S2 in online Journal of Periodontology).

When gingival phenotype was assessed with color-coded probes †† at the 13-year follow-up, FGG-treated sites exhibited more sites with very thick phenotype (p<0.01). No differences between the two groups were observed in terms of incidence of new NCCLs (Table 1).

3.4 Long-term Professional Esthetic Outcomes

The sites allocated to LCC exhibited statistically significant better esthetic outcomes than FGG-treated sites, in terms of professionally-assessed texture, color, MGJ alignment, marginal tissue contour and overall esthetic score, both at 6 months and 13 years (Table 3). The final esthetic scores at 6 months for LCC and FGG were 3.33 and 1.10 points, respectively (p<0.01). At the 13-year recall, the overall esthetic scores for LCC- and FGG-treated sites were 3.81 and 0.95 points (p<0.01). Between 6 months and 13 years, the LCC group showed an improvement of the scores associated with texture compared to the FGG group (p<0.01), together with a significantly higher stability of the marginal tissue contour (p=0.04). Overall, LCC-treated sites showed a mean improvement of the final esthetic score from 6 months to 13 years of 0.48 points, while FGG-treated sites displayed a mean loss of 0.14 points (Table 3).

3.5 PROMs

At 6 months, 21 subjects (87.5%) preferred the esthetic appearance of the site treated with LCC over the site that received FGG (p<0.01). The preference for the overall treatment was in favor of LCC over FGG (66.7% vs 33.3%, respectively, p=0.03) (see Table S3 in online Journal of Periodontology). At the 13-year follow-up, 23 subjects (95.8%) indicated their preference in terms of esthetic appearance for LCC over FGG (p<0.01). In terms of overall treatment, LCC was preferred to FGG by 20 subjects (83.3%) (p<0.01). Minimal discomfort during brushing was reported by patients at the grafted areas (VAS 4.5 and 5.9 points for LCC and FGG sites, respectively, p>0.05), while the subjective rating of esthetics using a VAS was significantly higher at LCC-treated sites than FGG-treated sites (80.9 vs 47.2, p<0.01) (see Table S3 in online Journal of Periodontology). The regression analysis demonstrated a significant correlation between patient-reported discomfort during brushing with AGW (estimated coefficient of

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-0.266 (95% CI [-0.460, -0.071] p=0.01) and REC (estimated coefficient of 0.271 (95% CI [0.076, 0.467] p=0.01) at 13 years.

4. Discussion

Previous authors advocated that an adequate band of KTW and AGW could be beneficial for preserving periodontal health, facilitating patient's oral hygiene, and maintaining the gingival margin stable over time. [1, 5, 8-10, 12]

The present study aimed at investigating the long-term stability of the 6-month outcomes obtained in patients that had been treated with LCC and FGG at contralateral sites within the same arch exhibiting ≤ 1 mm of AGW. Clinical data from the 21 subjects available at the 13-year follow-up revealed that there was no difference between LCC- and FGG-treated sites in terms of number of sites remaining stable from 6 months to 13 years, and overall, in terms of changes within KTW, AGW, REC, PD and CAL. Nevertheless, the longitudinal analysis demonstrated that FGG promoted a greater KTW and AGW over 13 years, together with better REC and CAL, compared to LCC.

The long-term stability of periodontal plastic surgical outcomes remains of keen interest in the field of periodontics and implant dentistry. [33, 38-43] Previous studies demonstrated that coronally advanced flap alone for root coverage procedures had a tendency towards relapse and recession reoccurrence over time. [13, 41-45], which may be explained by the lack of phenotype modification promoted at the treated sites. It has been advocated that KTW and gingival thickness (GT), as well as patient compliance with regular maintenance/ follow-up visits and atraumatic toothbrushing, are the main factors affecting the stability of the gingival margin over time. [13, 33, 41, 42, 45] A recent study from our group demonstrated the key role of gingival phenotype on the long-term stability of the gingival margin, showing that in a compliant patient cohort, irrespective of the rendered treatment, KTW ≥ 1.5 mm and GT ≥ 1.46 mm at 6 months were the main predictors for the stability of the root coverage outcomes over 10 years. [33] Although these studies assessed sites treated for gingival recessions, it can be assumed that the same concepts are also valid for teeth that received KTW augmentation with an APF. In the present study, FGG-treated sites exhibited a mean KTW and AGW of 5.12 mm and 4.12 mm, respectively, while the correspondent mean values for LCC-treated sites were 3.74 mm and 2.69 mm. The augmented KTW and AGW were more likely the main determinant for the overall stability of the clinical parameters observed from 6 months to 13 years. Interestingly, when applying a relatively strict definition of outcome stability,[33] involving either improved, stable or loss ≤ 0.5 mm of KTW, PD, and REC, nine sites per group (42.9%) were considered stable from 6 months to 13 years. However, it should be noticed that significant differences were not found when comparing 6 month- and 13-year

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clinical outcomes. Although GT was not assessed in the original study, the use of a color-coded probe at the 13-year recall allowed us to observe that most of the sites had either a thick or very thick phenotype, with FGG showing a higher number of sites with very thick phenotype compared to LCC. It can be speculated that the GT of the treated sites at 6 months also contributed to the stability of the surgical outcomes. In addition, most of the patients (90.5%) were defined as complaint with the follow-up/hygiene appointments, and this aspect has also probably played a significant role in the 13-year results. Indeed, in a 5-year clinical trial, Moslemi et al. showed a significant relapse of the gingival margin in non-compliant patients that had resumed horizontal toothbrushing habit. [46]

Our findings demonstrate that both LCC and FGG are effective in promoting an increase in KTW and AGW, in maintaining periodontal health (lack of pockets, inflammation, nor substantial attachment loss at the treated sites) over time. The two groups also showed an overall stability of the gingival margin from 6 months to 13 years (mean REC increase of 0.55 mm and 0.45 mm at the LCC- and FGG-treated sites, respectively). The greater KTW gain and AGW gain obtained by FGG over the tissue engineered construct are obtained during the initial 6 months, in line with previous studies by McGuire and coworkers. [15, 25] The differing nature of the two procedures can explain these findings, with the LCC containing allogeneic fibroblasts and keratinocytes that is considered a wound healing promoter, able to regenerate an adequate band of KTW and AGW, that histologically and esthetically more closely resemble the adjacent tissue, while the FGG retains the original characteristics of the palate. [17, 32, 36, 47, 48] Therefore, it is not surprising that professional- and patient-assessed esthetic outcomes were in favor of LCC-treated sites at 6 months and 13 years. FGG is overall considered a very predictable approach, but due to its esthetic outcome, it is seldom performed in the esthetic zone. [49] Interestingly, an improvement of the professional esthetic outcomes was observed from 6 months to 13 years at the LCC-treated sites, which exhibited higher scores in terms of texture, color, and overall outcome. On the other hand, FGG-treated sites displayed a slight worsening of the esthetic outcomes. Some FGG-treated sites displayed a change in the shape of the graft from 6 months to 13 years, while in most of the cases the color difference between FGG-grafted sites and adjacent areas became more pronounced over time.

It should be noted that one of the strengths of the present study is the fact that all the measurements (at baseline, 6 months, and 13 years) were performed by the same pre-calibrated examiner (T.J.O.). Another interesting finding, which can be due to the changes within the esthetic appearance of the two grafts as observed in the professional esthetic evaluation, is related to PROMs and their changes from 6 months to 13 years. While LCC-treated sites were already found to be associated with significantly higher esthetic preference and overall treatment preference over FGG at 6 months, 2

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subjects that previously indicated esthetic preference for FGG and 4 patients that previously preferred FGG over LCC as overall treatment, changed their answer at the 13-year recall. The patient-reported esthetic scores measured with a 0-100 VAS at the 13-year visit were, on average, 80.9 and 47.2 for LCC and FGG, respectively. Aside from the esthetics, it should be highlighted that the regression analysis showed a significant correlation between patient reported discomfort during brushing and the clinical parameters AGW and REC, which further supports the long-term benefits of soft tissue augmentation at sites with gingival recessions lacking AGW.

In addition, it should be highlighted that we observed 2 sites per group with new NCCLs at the 13-year follow-up. While the etiology of this condition is still controversial, [12, 50] the prevalence of NCCLs can be relatively high (11.4% to 62.2%) and tends to increase with age.[35]. These conditions may cause patients' esthetic concern, dental hypersensitivity, and suboptimal hygiene procedures.[12] Agudio et al. reported that 55.9% of the study sites developed NCCLs over a period of 25-30 years. In particular, NCCLs were found to be significantly more frequent at non-augmented sites compared with teeth that previously received FGG (58.3% vs 36.7%, respectively). [12] In our cohort we observed a smaller incidence of NCCLs (9.52% per site/group), which could be to the different patient population, the lower number of study sites, and the shorter follow-up period compared to the study of Agudio and coworkers. [12]Gingival phenotype modification promoted by LCC and FGG may have also contributed to the prevention of NCCLs in the majority of the sites in our cohort.

Lastly, it should also be mentioned that, although promising, LCC and overall living cell tissue-engineered constructs are currently unavailable in the market. Alternative biomaterials, devoid of cellular components, such as human acellular dermal matrix [16, 51] and xenogeneic bilayered collagen matrix, have been use as FGG substitutes in the natural dentition. [14, 52] Human acellular dermal matrix was originally introduced as an alternative to FGG [51, 53, 54]. However, due to the risk of necrosis when left exposed during the healing period, this graft is nowadays mainly utilized for root coverage purposes or for increasing soft tissue thickness around teeth and dental implants, in combination with bilaminar approaches. [55] A recent study from McGuire and coworkers described the 6-8 year outcomes of a xenogeneic collagen matrix vs FGG for increasing KTW. Although FGG outperformed the matrix in terms of KTW gain, the authors observed that the two treatments were equally effective in preventing future recession. More studies investigating the outcomes of long-term outcomes of soft tissue graft substitutes are needed.

The strengths of this long-term study include the split-mouth randomized design, the assessment of the outcome measures by the same operator both for the original study as well as for the 13-year follow-up, and the evaluation of PROMs. On the other hand, it would have been beneficial knowing

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the GT at baseline and at 6 months; nevertheless, this outcome was not assessed in the original study. In addition, using living cell tissue engineering constructs inevitably increases the cost of the treatment. Further studies are needed in the future to determine the economics of tissue-engineered technologies for clinical application.

5. Conclusions

This study demonstrates that teeth possessing limited zones of KTW augmented with either LCC or FGG maintained stable outcomes from 6 months to 13 years. Both approaches are effective in augmenting KTW and AGW, however, a superior gain was observed at FGG-treated sites. LCC resulted in significantly greater professional- and patient-assessed esthetic outcomes, as well as superior treatment preference compared to FGG.

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Conflict of interest

The authors do not have any financial interests, either directly or indirectly, in the products or information enclosed in this manuscript.

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Footnotes

* CelTx, Organogenesis, Canton, MA, USA

† Coe-Pak, GC America, Alsip, IL, USA

‡ PCP UNC 15, Hu-Friedy, Chicago, IL, USA

§ Lugol's Iodine Solution 2%, J. Crow's LLC, New Ipswich, NH, USA

‖ Colorvue biotype probes, Hu-Friedy, Chicago, IL, USA

¶ RStudio, Version 1.3.959, Rstudio, Inc., Boston, MA, USA

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Figure 1. Clinical case where KTW augmentation was performed with the LCC in the lower right area and with FGG in the lower left area. Clinical views at baseline (BL), immediate post-op after the surgical procedure (Sx), 1-month follow-up (1M), 6-month follow-up (6M) and 13-year recall (13Y) are presented. Note that iodine solution was applied at baseline, 6 months, and at the 13-year follow-up to better discriminate the keratinized tissue from the alveolar mucosa.

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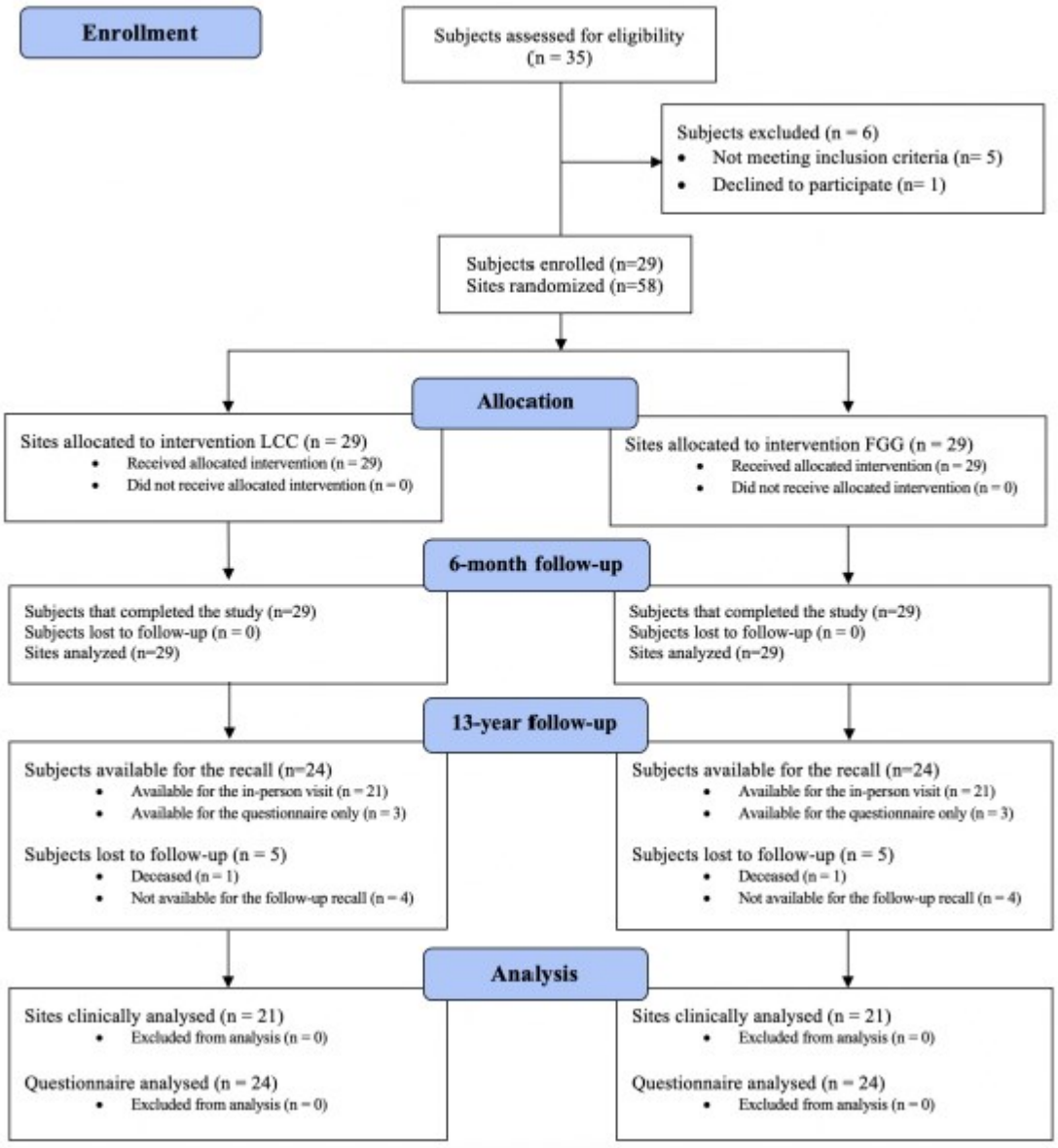


Figure 2. CONSORT flowchart

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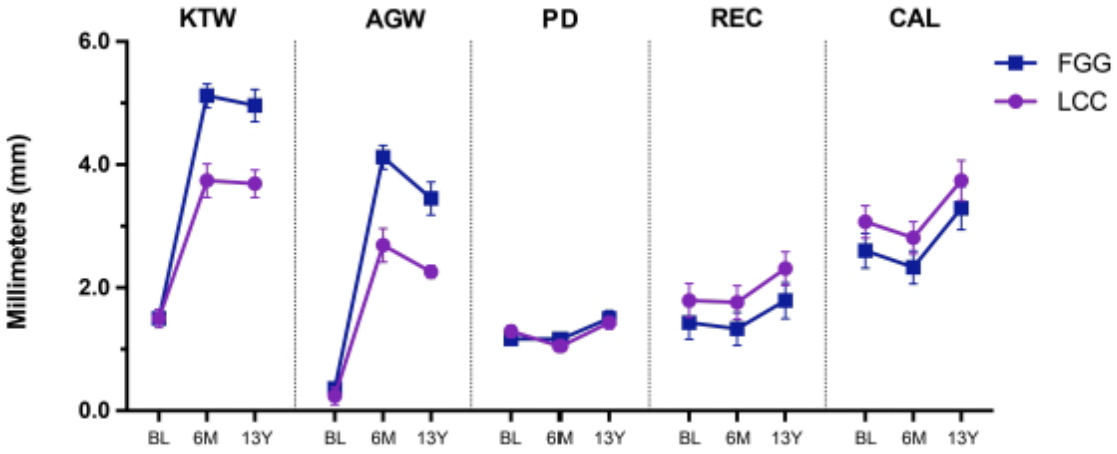


Figure 3. Plot depicting the changes within the keratinized tissue width (KTW), attached gingiva width (AGW), probing depth (PD), recession depth (REC) and clinical attachment level (CAL) at baseline (BL), 6 months (6M) and 13 years (13Y). Mean and standard errors are reported for each outcome of interest.

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TABLE 1 Clinical outcomes at baseline, 6 months, and 13 years.

Outcome measure	LCC (N=21)	FGG (N=21)	p-value
Baseline			
KTW (mean ± SD) (mm)	1.50 ± 0.67	1.50 ± 0.52	1.00
PD (mean ± SD) (mm)	1.29 ± 0.44	1.17 ± 0.37	0.60
AGW (mean ± SD) (mm)	0.24 ± 0.66	0.36 ± 0.39	0.84
REC (mean ± SD) (mm)	1.79 ± 1.28	1.43 ± 1.23	0.12
CAL (mean ± SD) (mm)	3.07 ± 1.21	2.60 ± 1.29	0.07
6 months			
KTW (mean ± SD) (mm)	3.74 ± 1.25	5.12 ± 0.89	<0.01
PD (mean ± SD) (mm)	1.05 ± 0.22	1.17 ± 0.37	0.09
AGW (mean ± SD) (mm)	2.69 ± 1.26	4.12 ± 0.89	<0.01
REC (mean ± SD) (mm)	1.76 ± 1.25	1.33 ± 1.23	0.01
CAL (mean ± SD) (mm)	2.81 ± 1.20	2.33 ± 1.23	<0.01
13 years			
KTW (mean ± SD) (mm)	3.69 ± 1.01	4.96 ± 1.21	<0.01
PD (mean ± SD) (mm)	1.43 ± 0.48	1.50 ± 0.58	0.44
AGW (mean ± SD) (mm)	2.26 ± 1.09	3.45 ± 1.23	<0.01
REC (mean ± SD) (mm)	2.31 ± 1.25	1.79 ± 1.34	<0.01
CAL (mean ± SD) (mm)	3.74 ± 1.51	3.29 ± 1.59	<0.01
Gingival phenotype (n [%])			<0.01
Thin	4 (19.05)	1 (4.76)	
Medium	6 (28.57)	1 (4.76)	
Thick	4 (19.05)	5 (23.81)	
Very thick	7 (33.33)	14 (66.67)	
Incidence of new NCCLs (n [%])	2 (9.52)	2 (9.52)	1.00

Abbreviations. AGW: attached gingiva width. CAL: clinical attachment level. FGG: free gingival graft. KTW: keratinized tissue width. LCC: living cellular construct. NCCL: non-carious cervical lesion. PD: probing depth. REC: recession depth. SD: standard deviation.

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TABLE 2 Initial outcomes and stability of the clinical parameters from the 6 months to 13 years.

Outcome measure	LCC (N=21)	FGG (N=21)	p-value
Baseline – 6 months			
KTW gain (mean ± SD) (mm)	2.24 ± 1.00	3.62 ± 0.84	<0.01
PD change (mean ± SD) (mm)	-0.24 ± 0.41	0 ± 0.37	0.11
AGW gain (mean ± SD) (mm)	2.45 ± 1.13	3.76 ± 0.70	<0.01
REC change (mean ± SD) (mm)	-0.02 ± 0.33	-0.10 ± 0.44	0.50
CAL change (mean ± SD) (mm)	-0.26 ± 0.34	-0.26 ± 0.41	1.00
6 months – 13 years			
KTW gain (mean ± SD) (mm)	-0.05 ± 0.76	-0.17 ± 1.00	0.64
PD change (mean ± SD) (mm)	0.38 ± 0.57	0.38 ± 0.67	1.00
AGW gain (mean ± SD) (mm)	-0.43 ± 1.11	-0.67 ± 0.91	0.39
REC change (mean ± SD) (mm)	0.55 ± 0.63	0.45 ± 0.55	0.57
CAL change (mean ± SD) (mm)	0.93 ± 0.94	0.95 ± 0.95	0.92

Abbreviations. AGW: attached gingiva width. CAL: clinical attachment level. FGG: free gingival graft. KTW: keratinized tissue width. LCC: living cellular construct. PD: probing depth. REC: recession depth. SD: standard deviation.

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TABLE 3 Esthetic outcomes at 6 months and 13 years.

Outcome measure	LCC (N=21)	FGG (N=21)	p-value
6 months			
Texture (mean ± SD) (points)	0.62 ± 0.50	0.05 ± 0.22	<0.01
Color (mean ± SD) (points)	0.81 ± 0.40	0.05 ± 0.22	<0.01
MGJ alignment (mean ± SD) (points)	0.95 ± 0.22	0.29 ± 0.47	<0.01
Marginal tissue contour (mean ± SD) (points)	0.95 ± 0.22	0.71 ± 0.47	0.10
Overall score (mean ± SD) (points)	3.33 ± 0.86	1.10 ± 0.89	<0.01
13 years			
Texture (mean ± SD) (points)	0.95 ± 0.22	0 ± 0	<0.01
Color (mean ± SD) (points)	0.95 ± 0.22	0 ± 0	<0.01
MGJ alignment (mean ± SD) (points)	0.90 ± 0.30	0.38 ± 0.50	<0.01
Marginal tissue contour (mean ± SD) (points)	1.00 ± 0.00	0.57 ± 0.51	<0.01
Overall score (mean ± SD) (points)	3.81 ± 0.51	0.95 ± 0.70	<0.01
6 months – 13 years			
Change in texture (mean ± SD) (points)	0.33 ± 0.48	-0.05 ± 0.22	<0.01
Change in Color (mean ± SD) (points)	0.14 ± 0.36	-0.05 ± 0.22	0.10
Change in MGJ alignment (mean ± SD) (points)	-0.05 ± 0.22	0.10 ± 0.54	0.33
Change in marginal tissue contour (mean ± SD) (points)	0.05 ± 0.22	-0.14 ± 0.48	0.04
Change in the overall score (mean ± SD) (points)	0.48 ± 0.75	-0.14 ± 0.85	0.02

Abbreviations. FGG: free gingival graft. LCC: living cellular construct. MGJ: mucogingival junction. SD: standard deviation.

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