<PE-AT>Gingival phenotype modification therapies on natural teeth: a network meta-analysis

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ABSTRACT

Background. The periodontal phenotype consists of the bone morphotype, the keratinized tissue (KT) and gingival thickness (GT). The latter two components, overlying the bone, constitute the gingival phenotype (GP). Several techniques have been proposed for enhancing or augmenting KT or GT. However, how phenotype modification therapy (PMT) affects periodontal health and whether the obtained outcomes are maintained over time have not been elucidated.

Aim. The aim of the present review was to summarize the available evidence in regard to the utilized approaches for gingival PMT and assess their comparative efficacy in augmenting KT, GT and in improving periodontal health using autogenous, allogeneic and xenogenic grafting approaches.

Materials and Methods. A detailed systematic search was performed to identify eligible randomized clinical trials (RCTs) reporting on the changes in GT and KT (primary outcomes). The selected articles were segregated into the type of approach based on having performed a root coverage, or non-root coverage procedure. A network meta-analysis was conducted for each approach to assess and compare the outcomes among different treatment arms for the primary outcomes.

Results. A total of 105 eligible RCTs were included. 95 pertaining to root coverage (3,539 treated gingival recessions), and 10 for non-root coverage procedures (699 total treated sites). The analysis on root coverage procedures showed that all investigated techniques (the acellular dermal matrix (ADM), collagen matrix (CM), connective tissue graft (CTG)) are able to significantly increase the GT, compared to treatment with flap alone. However, KT was only significantly increased with the use of CTG or ADM. Early post-treatment GT was found to inversely predict future gingival recession. For non-root coverage procedures, only the changes in KT could be analyzed; all investigated treatment groups (ADM, CM, free gingival graft (FGG), living cellular constructs (LCC), in combination with an apically positioned flap (APF)), resulted in significantly more KT than treatment with APF alone. Additionally, the augmented GT was shown to be sustained, and KT displayed an incremental increase overtime.

Conclusions. Within its limitations, it was observed that any graft material was able to significantly enhance GT, while KT in root coverage procedures was significantly enhanced with CTG and ADM, and in non-root coverage procedures, with ADM, CM, FGG and LCC compared to APF alone. The autogenous soft tissue graft (CTG/FGG) proved to be superior in all comparisons for both outcomes of GT and KT. <PE-FRONTEND>

Introduction

The term periodontal phenotype refers to the phenotypic characteristics of the bone and its overlying gingival phenotype, that includes keratinized tissue (KT), and gingival thickness (GT) [1]. While, the bone morphotype can only be assessed via cone-beam computed tomography (CBCT), the gingival phenotype (GP) can be assessed in a more consistent and standardized way [1]. It has been demonstrated that the transparency of the periodontal probe through the gingival margin can effectively differentiate a thin, from a medium or thick gingiva in a reproducible way [2]. A variety of different methods have also been employed for evaluating the GT that include transgingival probing, utilization of ultrasound, or a color-coded probe [3-5]. The presence of a thin GP has been associated with a greater risk for developing gingival recessions (GRs) [1, 6] not only throughout a patient's lifetime, but also as a consequence of specific dental therapeutics such as orthodontic treatment[7].

Despite a lacking consensus of what treatment is truly superior for GT augmentation, or if the augmented thickness is sustainable over time, it has been shown that periodontal plastic procedures with the use of autogenous grafts (whether free gingival grafts [FGG] or connective tissue grafts [CTG]) or substitutes (acellular dermal matrix [ADM] or collagen matrices [CM]) can significantly increase the amount of GT [8-13]. While previous reports have documented the relapse of recession treatment over time and the factors associated with it [11, 12, 14], studies concerning the associated role of GT, its potential benefits with regard to gingival margin stability, periodontal/gingival health are scarce in the literature.

Whether a specific amount of KT is required around teeth for maintaining periodontal health, has always been a topic controversy and debate. It has been reported an inflammatory reaction associated with GRs in 67% of sites when there was an inadequate KT width (< 2 mm) [15]. Lang & Löe found an increase in gingival and exudate index when KT was minimal, thus suggesting that at least 2 mm of KT and 1 mm of attached gingiva are crucial for maintaining gingival health [16]. Similarly, it was shown that having ≥ 2 mm of KT was beneficial for the maintenance of gingival health in the presence of subgingival restorations [17]. In a longitudinal study, it was demonstrated that while FGG could be effective in preventing recurrent inflammation and future GRs (as opposed to non-grafted sites), in the presence of optimal plaque control there was no difference in terms of periodontal health between the treated and non-treated sites [18].

Therefore, the need for a certain amount of gingival thickness for maintaining periodontal health, especially when optimal oral hygiene can be obtained remains unclear [19, 20]. In this scenario, an evidence-based review is needed for conceptualizing a model to underpin the research focused on the changes in gingival phenotype, otherwise known as phenotype modification therapy (PMT), alongside a complex and comprehensive statistical methodology. At present, one of the ways to explore the interrelationships between several treatment arms, regardless of their pre-existing comparison is to utilize a network meta-analysis (NMA). The NMA is a powerful statistical tool that allows for the integration of all suitably comparable evidence for conducting robust simultaneous testing of all direct and indirect correlations between different treatment arms, while accounting for and investigating different variables [14].

The aim of the present review was therefore to utilize the above-mentioned methodology for investigating the effect of phenotype modification therapy (PMT) as it relates to root coverage and non-root coverage techniques on the periodontal conditions of natural teeth through the following focused questions:

1. What are the currently available and effective approaches for PMT in root coverage and non-root coverage procedures, as reflected in the changes in GT, and KT width; and how do treatments compare to each other, or when assessed relative to no treatment or non-intervention?

2. What are the effects of PMT on: gingival margin stability overtime, and health-related parameters as defined by changes in: Pocket Depth (PD), Plaque index (PI), and Gingival index (GI)?

3. How are the outcomes of PMT maintained over time?

Materials and Methods:

Protocol Registration and Reporting Format:

The protocol of the present study was prepared and registered prior to the study, and allocated the identification number CRD42019147343 on the PROSPERO International Prospective Register of Systematic reviews database [21] . This manuscript has been prepared following the Cochrane Collaboration guidelines [22] and is reported in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analysis Extension (PRISMA) statement for Systematic Reviews incorporating network meta-analyses for health care interventions [23, 24].

Objectives:

The goal of this review was to address the previously stated focused questions in regard to PMT in natural dentition.

Population, Intervention, Comparison, Outcome, Time (PICOT) question[25]:

The following PICOT framework was used to guide the inclusion and exclusion of studies for the aforementioned focused questions:

Population (P): Patients undergoing gingival augmentation procedures with/without initial GR defects, whether intended for root coverage or non-root coverage purposes.

Intervention (I): Surgical treatment for PMT with harvesting of a soft tissue graft, or utilization of a substitute material, whether aimed at correction of the GR defect, or change in the gingival phenotype.

Comparison (C): All possible comparisons among the included interventions were explored, including non-intervention or treatment with the use of a flap alone.

Outcome (O): The change in phenotype as conveyed by changes in the clinical parameters of GT, and KT.

Time (T): Any study duration or follow-up after the surgical intervention of at least 12 months for root coverage, and 6 months for non-root coverage procedures. Data at every follow-up time point were recorded.

Eligibility Criteria and Transitivity:

Only randomized controlled trials (RCTs) with a well-defined clinical protocol were considered for this study. In order to assure transitivity and methodological strength, a series of strict inclusion and exclusion criteria were set *a priori* to address and observe for significant heterogeneity in selection of the trials in terms of their design-driven differences, selection criteria, interventions, and overall approach. Please refer to the Supplementary Appendix in online Journal of Periodontology for explicit details in this regard.

Information sources and Search Strategy:

Briefly, a detailed computerized systematic search was conducted in the literature to identify eligible RCTs, followed by additional manual searching in relevant journals, past reviews [14, 26-50], and cross-reference checks in the retrieved articles. Details about the search strategy and the development of the search key terms for every database, are elsewhere, see Supplementary Appendix in online Journal of Periodontology.

Study Selection and data retrieval:

Two pre-calibrated review authors (SB, LT) performed the selection process of the RCTs. First by titles and abstracts, followed by a through full read of the studies that remained for careful assessment and alignment with the set inclusion criteria. Refer to the Supplementary Appendix in online Journal of Periodontology for the undertaken process, with the subsequent data retrieval from the selected final RCTs.

Data analysis and statistical methodology:

For studies published more than once (i.e., duplicates), or those that utilized the same patient population, only one report with the most informative and complete data was included in the analyses. The goal was to assess and compare the changes in GT and KT as the two constituents of the gingival phenotype.

To evaluate the transitivity assumption underlying NMA, the distribution of clinical and methodological variables that could act as effect modifiers across treatment comparisons (such as the study design/approach, and baseline measures of the relevant variable) were compared [51]. We investigated if these variables were similarly distributed across our included trials grouped by comparison.

Consequently, two sets of NMA were conducted, based on the utilized approach among the selected RCTs; the first being for root coverage procedures (the bilaminar approach where the flap is coronally advanced), and the second for non-root coverage approaches (APF-based approach).

See Supplementary Appendix in online Journal of Periodontology for details in regard to the construction of the model, its mathematical representation and the utilized fixed- and random-effects.

For each approach (root coverage or non-root coverage), changes in GT and KT among different treatment arms served as the primary outcome. For the NMA on root coverage procedures, the four treatment arms of ADM, CM, CTG, and Flap alone were considered, with Flap alone serving as the reference for the initial comparisons. For the second NMA on non-root coverage procedures, the following treatment arms were assessed: ADM, APF, CM, FGG, living cellular constructs (LCC), and untreated sites (UTS) which served as the reference category.

The robustness of the relationships between changes in the primary outcomes (KT, GT), and health-related parameters (PI, GI, PD) were evaluated through subgroup analyses and network meta-regression. Additionally, to evaluate the influence of PMT on gingival margin stability, we created a lagging variable for KT and GT by choosing the early treatment outcomes (6 or 12 months KT/GT after initial therapy, and assuming a negligible time effect from 6 to 12 months) to predict the quantitative level of recession at all points in the future that were covered by the original studies (the conditional relationship between the initial post-treatment KT/GT with changes in recession at the cohort level).

Baseline characteristics (i.e., GT baseline and KT baseline) were accounted for in each corresponding model and controlled for along with the type of treatment approach (single/multiple site treatment) for capturing potential heterogeneity and possible confounders. The arms were weighted according to the treated sample size. Smoking was taken into account by calculating the percentage of smoking individuals among the study arms (as a continuous variable) and controlled for in the models. The analyses accounted for correlations induced by multi-group studies, by using multivariate distributions. The variance in the random-affects distribution (heterogeneity variance) was considered to measure the extent of across-study and within-comparison variability on the treatment effects.

To obtain direct and indirect pairwise comparisons for all treatment arms, different reference levels were specified in the model and all contrasts were recorded along with their standard errors, and *p* values. Confidence intervals were produced, and a *p* value threshold of 0.05 was set for statistical significance. The results of the pairwise comparisons were presented in tabular form and network plots were produced to visualize the formulated relationships for both sets of NMAs.

Finally, to assess the effect of different treatments on the changes in gingival phenotype as a composite outcome for the analysis on root coverage procedures (due to data availability), the data was merged to contain both outcomes of GT and KT in the same dataset. Thus, GT and KT were interleaved into the same model to contrast the impact of different arms on both outcomes of GT and KT through multi-regression modeling by allowing the treatment effects to vary across the outcomes. Ecological correlations were also tested in the model via interactions between KT and GT with other available co-variates. The mathematical representation of this model is brought in the Supplementary Appendix in online Journal of Periodontology.

The linearity assumption was tested for all models by including quadratic terms, however no evidence of non-linearity was observed. All analyses were performed by an author with expertise in biostatistics (SB) using the lme4 [52], lmerTest [53], dplyr [54], and tidyr [55] statistical packages in Rstudio (version 1.2.1335). The igraph [56] and ggplot2 [57] packages were used to make the network plots, and the plot for representation of the outcomes from the multi-regression model.

Quality assessment and risk of bias:

All included studies were evaluated according to the Cochrane collaboration group [58] independently and in duplicate by two examiners (SB, LT). See Supplementary Appendix in online Journal of Periodontology for details in this regard.

Results:

Search results and study selection

Figure 1 displays the search process for selection of the included trials. More details in regard to the search and selection strategy, the characteristics of the included RCTs, their interventions and approach as it relates to PMT are presented in the Supplementary Appendix in online Journal of Periodontology.

Synthesis of mixed-modeling based Network Meta-Analysis:

Figure 2 illustrates the generated direct and indirect comparisons from the two NMAs performed on root coverage, and non-root coverage procedures, based on data from 95 and 10 RCTs, respectively.

1) **Network Meta-Analysis on root coverage procedures:**

Results of the pairwise comparisons from the network model, investigating the changes in KT and GT as individual outcomes, among the included treatment arms are presented in Figure 3.

The variances of the included random effects are displayed in the Supplementary Appendix in online Journal of Periodontology (Supplementary tables 4, 5, and 6).

Keratinized Tissue as an individual component of gingival phenotype:

When flap alone served as the reference, both treatment groups of CTG (1.09 mm (95% CI [0.78, 1.38], p<0.001)), and ADM (0.47 mm (95% CI [0.05, 0.89], p=0.02)) showed a statistically significant higher final KT, while this was not observed for the CM-treated sites (0.25 mm (95% CI [-0.22, 0.73], p=0.28). Nevertheless, while the treatment groups of CTG and ADM, both showed significantly higher final KT than flap (as reference), and CM did not, in the generated pairwise comparisons, when CM served as the reference, the difference between ADM- and CM-treated arms did not reach statistical significance in terms of the final obtained KT (ADM estimate with CM as reference: 0.22 mm (95% CI [-0.33, 0.78], p=0.43)). However, treatment with CTG remained its superiority in terms of KT gain, with a statistically significance estimate of 0.84 mm (95% CI [0.36, 1.31], p<0.001).

Time itself revealed to be a significant factor in the model, showing an increase of 0.006 mm/month (95% CI [0.002, 0.0009], p=0.002). However, without a statistically significant interrelationship when interacted with different treatment arms. Additionally, when the effect of KT on gingival margin stability was explored (via change in recession after the obtained 12-month results), it was observed that an increase in KT may lead to reduction in the progression of recession overtime with a p value approaching significance (-0.18 (95% CI [-0.34, -0.02], p=0.05).

In regard to its effect on health-related parameters, no significant relationship could be observed between changes in KT with PI (0.41 (95% CI [-1.49, 2.31], p=0.67)), GI (-2.397 (95% CI [-4.66, -0.12], p=0.04)), or PD (-0.007 (95% CI [-0.4, 0.38], p=0.65)).

Gingival Thickness as an independent variable of gingival phenotype:

It was shown that all treatment groups significantly increased GT after the root coverage procedure compared to flap alone, with CTG presenting the highest estimate (0.66 mm (95% CI [0.51, 0.81], p<0.001)), followed by ADM (0.48 mm (95% CI [0.31, 0.65], p<0.001)), and CM (0.35 mm (95% CI [0.18, 0.52], p<0.001)). Nevertheless, when either ADM, or CM served as the reference, no statistically significant differences could be observed between the two treatments (figure 3).

While GT at baseline was found to be positively correlated to the final GT gain (0.58 mm (95% CI [0.29, 0.86], p<0.001)), individually, this effect lacked statistical significance when interacted with different treatment arms. Additionally, no significant time effect could be observed in regard to GT, among the treatment groups (-0.002 (95% CI [-0.004, 0.0009], p=0.24)).

Lastly, when the stability of the gingival margin was assessed, it was found that the early post-treatment GT (6/12 months) significantly and inversely predicts future recession (-0.71 mm (95% CI [-1.02, -0.4], p=0.01)), indicating that every 1 mm of increase in GT, leads to 0.71 mm less future recession.

Also, the model demonstrated that an increase in GT was significantly associated with a reduction in PI scores (-0.61 (95% CI [-1.15, -0.05], p=0.03)), however failed to show a significant associations with GI scores (-0.008 (95% CI [-0.16, 0.14], p=0.76) or changes in PD (0.02 (95% CI [-0.17, 0.21], p=0.83)).

Assessment of KT and GT as the two constituents of gingival phenotype as a composite outcome

The results of the multi-regression model, simultaneously assessing the influence of different treatments on GT and KT, showed that in regard to gingival PMT in root coverage procedures, the treatment effect with CTG, boosts the KT component significantly more than GT (0.402 (94% CI [0.03, 0.76], p=0.02), while there was no other evidence in the model suggesting that either KT or GT would respond differently with changing the treatment arms.

Additionally, age was shown to be a significant factor in relation to KT (-0.038 (95% CI [-0.07, -0.001], p=0.03)). As such that studies which had recruited older individuals compared to younger patients, obtained lower final KTs.

Another significant result from the combined model was in regard to KT at baseline that was shown to be significantly and positively correlated to the outcomes of the combined model (0.32 (95% CI [0.16, 0.48], p<0.01)).

Figure 4 plots the outcomes of the multi-regression model from the NMA up to 10 years.

2) Network Meta-Analysis on non-root coverage procedures:

As only one publication group reported the outcome of GT[13, 59], the NMA on non-root coverage procedures could only be performed in regard to changes in KT. The results of the NMA, and the pairwise comparisons are presented in Figure 5. The variances of the included random effects are presented in the Supplementary Appendix in online Journal of Periodontology (see Supplementary table 7).

Keratinized Tissue as an independent variable of gingival phenotype:

All the treatment groups of FGG (4.72 mm (95% CI [4.24, 5.21], p<0.001)), CM (3.14 mm (95% CI [2.30, 3.99], p=0.005)), LCC (3.13 mm (95% CI [2.52, 3.75], p=0.003)), ADM (2.54 mm (95% CI [1.67, 3.41], p=0.007)), and APF (1.27 mm (95% CI [-2.08, -0.45], p=0.02)) showed a significant increase in KT compared to untreated sites (reference category).

Time in relation to KT was also proved to be a significant factor in this model with a positive intercept of 0.01 mm/month (95% CI [0.006, 0.014], p<0.01). Additionally, no time by treatment arm interaction could be observed, confirming that KT progressively increases overtime regardless of the initial intervention.

With APF as reference, while untreated sites showed significantly less final KT (-1.27 mm (95% CI [-2.08, -0.45], p=0.02)), all other grafted sites displayed a significantly higher KT gain. However, compared to treatment with FGG, all treatments showed to be inferior (figure 5). Additionally, with ADM as the reference, no statistical differences were observed with CM (0.60 (95% CI [-0.39, 1.59], p=0.3)), or LCC (0.59 (95% CI [-0.22, 1.4], p=0.22)), while APF (-1.26 (95% CI [-2.24, -0.29], p=0.01)) and untreated sites (-2.52 (95% CI [-3.4, -1.67], p=0.01)) presented with significantly less final KT. Similarly with CM as the reference, while FGG (1.58 mm (95% CI [0.88, 2.27], p=0.01) proved superior, APF (-1.87 (95% CI [-2.83, -0.90], p=0.02)), and untreated sites (-3.14 (95% CI [-3.98, -2.29], p=0.005) showed significantly less KT gain, and no significant differences were observed for ADM (-0.60 (95% CI[-1.59, 0.39], p=0.3)), and LCC (-0.009 (95% CI [-0.80, 0.78], p=0.98)).

An interesting finding of this analysis was the interaction between KT at baseline with the treatment arms. Despite its overall significant effect in the model (0.63 mm (95% CI [0.31, 0.94], p=0.001)), when interacted with different treatment arms, it was observed that LCC is significantly affected by KT at baseline (2.22 mm (95% CI[0.90, 3.53], p=0.006); the higher the initial KT, the greater the benefit of treatment with LCC for KT augmentation. However, this did not hold true for FGG or other groups.

Regarding the associated changes the gingival margin, the only significant correlation was found with FGG (-1.34 (95% CI [-2.60, -0.08], p=0.02)), demonstrating a decrease in recession for the FGG-treated sites overtime as a result of the FGG treatment.

Finally, it was observed that KT augmentation resulted in a significant reduction in PI scores when compared with untreated sites (-0.59 (95% CI [-0.86, -0.32], p=0.03)), and treatment with APF alone (-0.56 (95% CI[-0.98, -0.14], p=0.02)). Nonetheless, PD (0.57 (95% CI [-0.04, 1.19], p=0.11)), and GI (-0.55 (95% CI [-1.22, 0.11], p=0.21)) failed to be statistically significant in this model.

Assessment of the risk of bias:

Most of the included trials (59 studies) were considered to have a low risk of bias, followed by a moderate risk for some of the articles (24), mainly due to unclear allocation concealment or blinding of patients. Lastly a high risk of bias was noticed for some, mainly due to absence of blinded examiners (19 studies). Detailed can be found in the Supplementary Appendix in online Journal of Periodontology (Supplementary Table 7).

Discussion

The importance of gingival PMT in natural dentition has been largely investigated[8, 60-64]. While previous reports have found initial KT and GT to play a role in the likelihood of obtaining complete root coverage [65-68], recent studies have shown that these parameters can also affect the stability of the gingival margin over time [11, 12, 69, 70]. Nonetheless, little is known in regard to which technique is more effective in increasing KT or GT and whether PMT is maintained over time. Data from clinical studies suggest that autogenous grafts result in an increased GT [11, 71-73], and graft substitutes, such as ADM and CM, are more effective in increasing the GT than KT [12, 74, 75]. However, a definitive conclusion has not been, and cannot be drawn individually from independent studies due to a limited sample size and lack of comparisons between all possible treatment approaches (e.g., ADM vs. CM). Hence the preface for basing the foundation of this review on a network methodology. Nevertheless, it should be noted that despite our rigorous efforts towards having homogeneous comparisons and treatment groups, as aforementioned, the exact method of measuring KT and GT varied slightly among articles, a notion which may have indeed influenced the outcomes of the present manuscript.

NMAs have been previously performed in the dental and periodontal literature to examine regeneration therapies in terms of their outcomes[76], root coverage procedures for their immediate results[38], esthetic appearance [45], and sustainability[14], and to a significantly greater degree in the medical field. The aim of the network approach is to integrate all suitably comparable evidence in the literature, to form and assess direct and indirect comparisons, and perform powerful meta-regressions to reach conclusions which would otherwise have been untenable to reach. In the current review, we utilized a linear mixed-model approach to NMA, for assessing the two components that constitute the gingival phenotype; GT and KT. We performed two separate analyses, each for the type of approach towards gingival PMT; one for root coverage procedures, and the second for non-root coverage approaches.

Additionally, for the analysis on root coverage procedure as our data allowed, we went further to conceptualize a joint model for evaluating both GT and KT simultaneously as two inter-related outcomes, as they make up the gingival phenotype, a concept that to the best of our knowledge is novel to the periodontal literature.

Phenotype modification as a result of root coverage procedure

It has been established that the CTG is the treatment of choice in terms of providing the greatest probability of achieving complete root coverage, increasing KT, better esthetics and maintaining the root coverage outcomes over time [14, 62, 77, 78]. On the other hand, it has been observed that soft tissue graft substitutes while effective in GT augmentation, provide limited potential for increasing KT [12, 74, 75]. Our results confirmed that the CTG is the superior treatment in terms of KT gain. Interestingly, the ADM was also found to be significantly associated with a greater KT gain (0.47 mm) than treatment with flap alone, whereas no significant differences were noted between CM and flap in terms of KT gain. Although the lack of living cells has been suggested to account for the limited efficacy of soft tissue graft substitutes in inducing keratinization of the overlying epithelium [79, 80], it can be speculated that ADM serves as a scaffold promoting the migration of keratinocytes from the surrounding tissue and thus resulting in increased KT compared to flap alone. This property may not hold true as much for CM and may be due to the different origin (human vs. animal), processing methods and resorption rate compared to ADM. However, these results have to be interpreted with caution as no randomized studies have yet directly compared ADM with CM in periodontal plastic literature. These findings could have also been due to the nature of this analysis. Integrating data from nearly 100 RCTs provides a significant power for detection of details which would not be discernable otherwise.

Nevertheless, and in line with our results, several authors have reported an increase in KT when using ADM [81-83]. Interestingly, KT was found to have a negative correlation with age, according to which, studies which had included older individuals had a tendency to obtain lower KT gain than younger patients. It is important to highlight that the KT was found to increase over time with a similar pattern among all treatment groups, supporting the theory that the mucogingival line tends to regain its genetically determined position as a slow but determined rate, a notion that was consistent throughout our analyses[84]. The importance of the obtained KT has been demonstrated in a recent article from our group, in which KT ≥ 2 mm at 6 months was found to be a predictor for stability of the gingival margin 12 years following treatment with ADM [12].

The importance of PMT while treating GR is based on the fact that thin phenotypes are at greater risk for developing GRs [85, 86] and may also be more prone to recession recurrence after the root coverage treatment, as a thicker marginal soft tissue may better tolerate traumatic toothbrushing in patients that cannot correct their brushing technique [11, 12]. An observational study has confirmed a correlation between thin GT and higher incidence of GRs in young adults as an important etiologic factor for GRs, and their recurrence [87]. In a 12-year follow-up from a RCT, it was observed that sites treated with a CTG resulted in an increased GT that was maintained over time, compared to their adjacent sites that were included in the flap but had not received a graft[11].

Despite the advantages of PMT, no study exists regarding the efficacy of different treatments for GT augmentation, and whether the increased soft tissue thickness is maintained over time (the possibility of the shrinkage of the grafted materials). Our results showed that CTG, ADM and CM provide significantly higher GT gain compared to flap alone, with CTG showing the highest results compared to treatment with flap. While the CTG maintained its superiority in all comparisons, ADM showed a higher estimate in the model than CM when flap was the reference. Nevertheless, generated pairwise comparisons between the two latter treatments failed to reach a statistically significant difference. It can be speculated that this increased GT obtained with ADM, may be due to its preserved extracellular matrix that enhances/supports cellular migration and revascularization from the surrounding host tissue [80]. On the other hand, CM is a non-cross-linked, resorbable, xenogeneic collagenous matrix that was designed with different layers (compact and porous) for facilitating tissue integration and angiogenesis[80]. Nevertheless, the preserved intact extracellular skin matrix of the ADM may better mimic the native tissue microenvironment than CM.

In agreement with our findings, several authors have reported a significant increase in GT following CTG and ADM [12, 73, 82, 83, 88]. Chambrone et al. had concluded that ADM is the graft substitute that can provide the most similar results to that of the gold standard CTG [89]. Nevertheless, it has to be mentioned that a recent NMA from our group addressing the effect of time on root coverage outcomes, demonstrated that ADM has a tendency to have an apical relapse of the gingival margin over time, while CTG is the only material that shows a trend towards stability [14]. In addition, it has been found that when the obtained GT at 6 months is ≥ 1.2 mm, the mean root coverage with ADM is more likely to remain stable [12] . Interestingly, our results showed that the obtained GT is maintained over time with a similar slope for each treatment, suggesting that PMT is effective not only in the short-term but is also maintained in the long-term.

Phenotype modification as a result of soft tissue non-root coverage procedure

The 2015 American Academy of Periodontology Regeneration Workshop concluded that there is not a threshold amount of KT that is required around teeth in the presence of optimal plaque control [43]. However, in the presence of an inadequate plaque control, KT ≥ 2 mm appears to be beneficial for preventing progressive attachment loss [60]. While this consensus highlighted that FGG is the most predictable technique for increasing KT, it was also concluded that additional research is necessary given the limited available data regarding alternative approaches [60]. Through the NMA, we were able to investigate the outcomes of soft tissue augmentation following APF alone, ADM, LCC and CM, and compare each treatment with no intervention/therapy as well. The results confirmed that FGG stands true as the gold standard treatment for increasing KT [77] . When the arms were compared to untreated sites, all treatments resulted in significantly greater KT gain. When APF was used as reference, the grafted sites (ADM, CM, FGG and LCC) showed significantly better outcomes.

The benefits of soft tissue augmentation versus no intervention has been addressed [18, 20, 90]. No significant differences were found for attachment loss and gingival inflammation between experimental and control sites. However, in patients that discontinued participation in the study, gingival inflammation and further recession were noticed, as opposed to those allocated to the experimental sites (treated with FGG) which showed recession reduction and CAL gain. In a series of long-term studies, Agudio et al. corroborated the efficacy of FGG in maintaining the stability of the soft tissue, observing a tendency for the coronal migration of the gingival margin as well (creeping attachment). Similarly, the untreated sites were found to be prone for an increase in their existing recessions or developing new ones [8, 71, 72].

An interesting finding from our results is the efficacy of FGG substitutes in regenerating a band of KT when combined with APF. Although histological analyses have shown that the KT following APF + ADM is closer in resemblance to a scar tissue than regenerated KT [91], better esthetic and color match with the surrounding tissues have been reported for ADM, compared to FGG[13, 59]. Similar esthetic outcomes were found by McGuire et al. when CM was compared to the gold standard FGG. The authors also reported a strong patient preference towards CM [92]. The lower morbidity, unlimited blood supply and regeneration of a site-appropriate tissue compared to the autogenous gingiva have been considered as the main advantages of graft alternatives [93, 94]. In particular, the cultivation of human living keratinocytes and fibroblasts into scaffold matrices have also been investigated for creating an autogenous soft tissue graft equivalent, showing promising outcomes however inferior to FGG [93, 95, 96]. Our results demonstrated the efficacy of LCC in KT augmentation compared to untreated sites and APF. It is important to highlight that KT baseline significantly affected the KT gain for LCC treatment. This finding indicates that LCC significantly benefits from a certain amount of baseline KT for achieving optimum KT gain. This is in line with the hypothesis that LCC acts more as a local wound healing agent (than a graft), stimulating patients' own cells for regenerating KT from the surrounding gingivae [93, 97]. An important consideration should be the expected outcome of the soft tissue augmentation. According to McGuire et al., and as also suggested by Lang and Loe[16], achieving a minimum of 2 mm keratinized gingiva is generally accepted as an endpoint of soft tissue augmentation [95]. LCC was found capable of regenerating ≥ 2 mm KT in 95.3% of patients [95], which can lead to a debate of whether it is better to obtain greater KT (with the gold standard FGG) or to regenerate a smaller, however arguably sufficient amount of KT in favor of patient related and esthetic outcomes.

Phenotype modification therapy on periodontal health

The present NMA showed that an increase in GT can positively influence the stability of the level of the gingival margin. In other words, an increased soft tissue thickness is more likely to prevent the relapse of the gingival margin over time. The tendency towards progression of GRs and development of new recession defects in untreated sites [8, 11, 12, 71, 72, 98] may be due to the persistence of thin phenotype, which is one of the main predisposing factors for gingival recessions[85]. PMT by using autogenous grafts or substitutes has been shown to effectively increase the gingival phenotype and be able to reduce the tendency towards relapse of the gingival margin [8, 11, 12, 14, 71]. In addition, our results corroborate that GT gained after the surgery can act as a predictor of gingival margin stability over time [12]. Indeed, a thickened gingival margin can not only protect from the resume of traumatic tooth brushing, but can also result in the coronal migration of the gingival margin over time [12, 14, 77]. Similarly, our results showed that FGG was the only gingival augmentation treatment that had a tendency for recession reduction over time.

The analysis on root coverage procedures demonstrated that an increase in GT was significantly associated with a reduction in PI scores. This is in line with the findings of a 5-year RCT that compared ADM with CTG [99]. Additionally, the result from the NMA showed that soft tissue augmentation using an autogenous graft or substitute (FGG, ADM, CM and LCC) may reduce PI as compared to untreated sites, flap treatment alone or APF. This finding is crucial as gingival augmentation procedures are performed not for esthetic purposes but for re-establishing an adequate band of attached and keratinized gingival that can facilitate the maintenance of oral hygiene, even in presence of a suboptimal plaque control [18, 71].

Refer to Supplementary Appendix in online Journal of Periodontology for discussion of limitations.

Conclusions

Based on the currently available evidence, and the limitations within this research, the following conclusions can be drawn:

1. With regard to root coverage procedures, the following graft materials in combination with flap advancement are all capable of significantly increasing the GT (as compared to treatment with flap alone) and in an increasing benefit from: CM, ADM, to CTG.
2. With regard to KT augmentation in root coverage procedures, compared to treatment with flap alone, only ADM and CTG (in increasing order) were demonstrated to significantly increase the KT.
3. Both outcomes of GT and KT augmentation are stable overtime with KT showing a minimal incremental increase over time (of approximately 0.01 mm per year).
4. Early post treatment GT (6/12 months) significantly and inversely predicts future gingival recession overtime, and an increase in KT may also contribute to enhance stability of the gingival margin as well. An increase in GT was also significantly associated with decreased PI scores.
5. While treatment with a CTG during root coverage procedures enhances both GT and KT, its effect on increasing KT is significantly greater than GT.
6. For non-root coverage procedures, while treatment with APF alone was able to show significant KT increase compared to no treatment (untreated sites), the utilization of a graft material (i.e., ADM, CM, FGG, or LCC) significantly enhanced the outcomes in terms of KT gain compared to non-grafted APF sites.
7. Soft tissue grafting in the APF-based approach for phenotype modification therapy showed to significantly reduce PI scores, as compared to untreated sites or APF alone.

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Figure legends:

Figure 1. The study search process and the screening of the articles for identifying the included randomized clinical trials. NMA, network meta-analysis; GT, gingival thickness; KT, keratinized tissue. * refers to search in the grey literature.

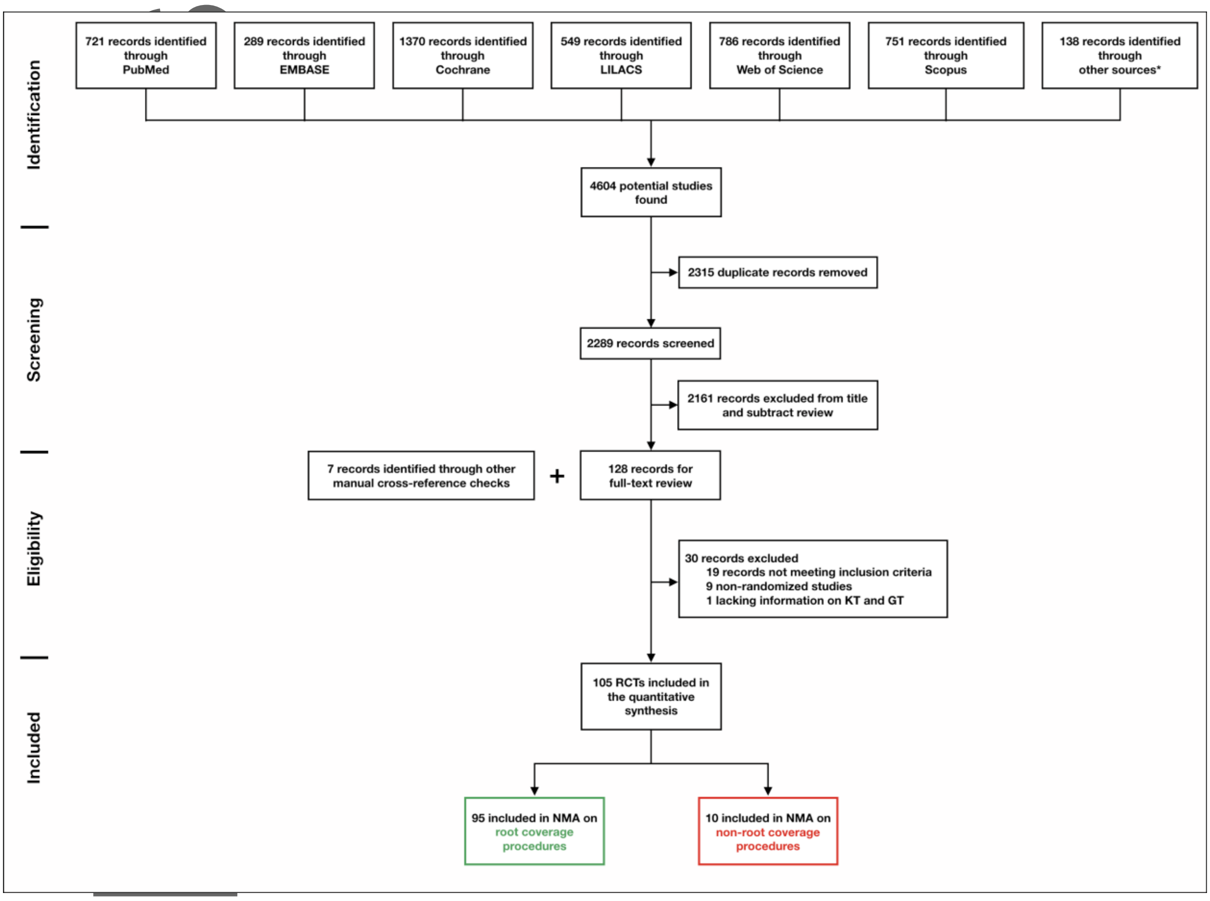


Figure 2. Network meta-analysis of eligible comparisons for A) root coverage, and B) non-root coverage procedures.

Gray solid lines connect treatments that are directly compared in at least 1 study. Studies contributing with only one arm are not presented. Gray dotted lines display the indirect comparison of the treatments that have not been compared head-to-head previously and formed through the network model. Note that distances are for plot clarity alone. The node size is proportional to the number of treated sites. ADM, Acellular dermal Matrix; APF, Apically positioned flap; CM, Collagen Matrix; CTG, Connective tissue graft; FGG, free gingival graft; LCC, Living cellular constructs; UTS, Untreated sites.

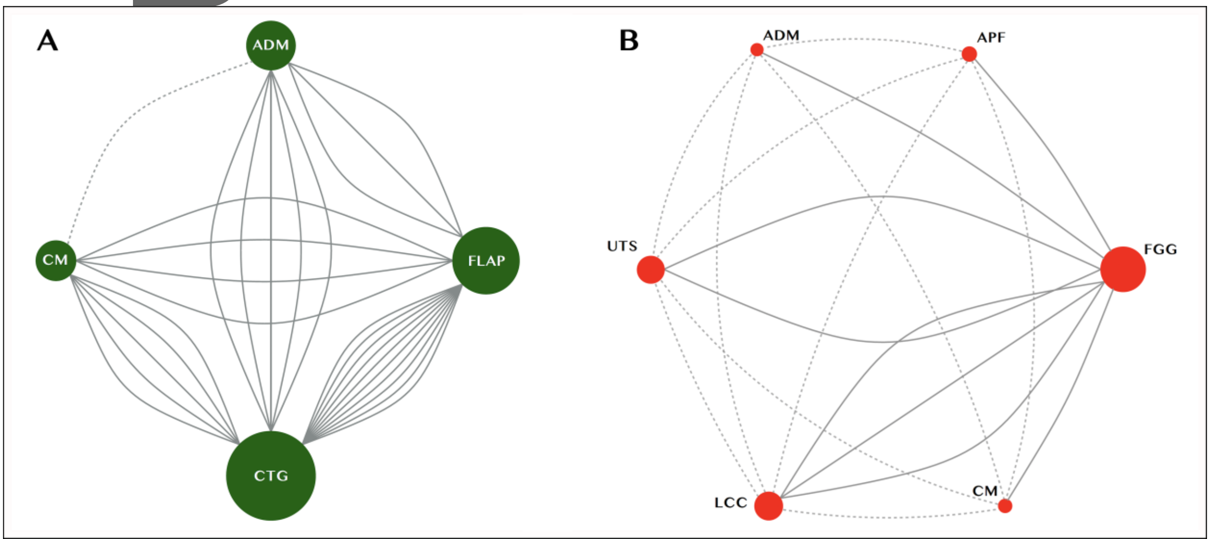
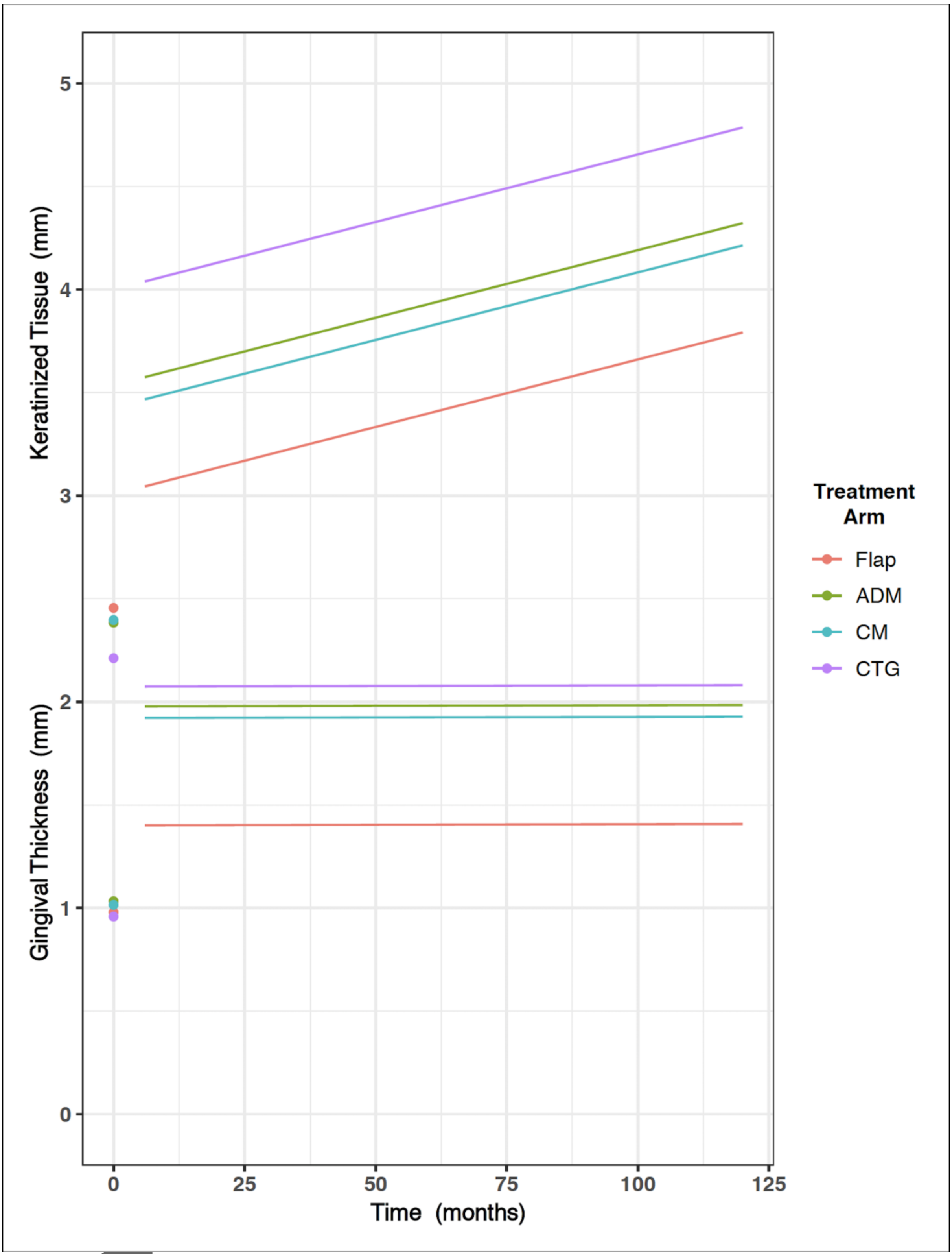


Figure 3. Pairwise comparisons from the Network Meta-analysis on root coverage procedures, for changes in KT and GT. Treatments are reported in alphabetical order. Results are the estimates in millimeter (95% CIs) from the NMA model in the cell in common between the column-defining treatment (defined-treatment 1), and the row-defining treatment (defined-treatment 2). Statistically significant results are in bold. *(p<0.05), **(p<0.001). CI, Confidence interval; NMA, network meta-analysis; ADM, Acellular dermal matrix; CM, Collagen matrix; CTG, Connective tissue graft.

KT (95% CI) | Treatment group | GT (95% CI)

ADM	-0.12 (-0.32, 0.07)	**0.17* (0.01, 0.34)**	**-0.48** (-0.65, -0.31)**
-0.22 (-0.78, 0.34)	CM	**0.31** (0.13, 0.47)**	**-0.35** (-0.52, -0.18)**
0.61* (0.21, 1.01)	**0.84** (0.37, 1.30)**	CTG	**-0.66** (-0.81, -0.51)**
-0.47* (-0.89, -0.05)	-0.25 (-0.73, 0.21)	**-1.08** (-1.38, -0.78)**	FLAP

Figure 4. The multi-regression model outcomes based on the Network Meta-analysis for up to 10 years. The x-axis represents follow-up time among treatment arms within studies, and the y-axis displays the changes in treatment arms by the outcomes (KT and GT) and their mean baseline values (the colored dots). Each color consistently represents a treatment arm.



Keratinized Tissue (mm)
Gingival Thickness (mm)
5
4
3
2
1
0
0
25
50
75
100
125
Time (months)
Treatment Arm
Flap
ADM
CM
CTG

Figure 5. Pairwise comparisons from the Network Meta-Analysis on non-root coverage procedures, for changes in KT. Treatments are reported in alphabetical order. Results are the estimates (95% CIs) from the NMA model in the cell in common between the column-defining treatment (defined-treatment 1), and the row-defining treatment (defined-treatment 2). Statistically significant results are in bold. *(p<0.05), **(p<0.001).

NMA, network meta-analysis; ADM, Acellular dermal matrix; APF, Apically positioned flap; CM, Collagen matrix; CTG, Connective tissue graft; LCC, Living cellular constructs; UTS, Untreated sites

KT (95% CI) — Treatment group

ADM					
-1.26* **(-2.24, -0.29)**	APF				
0.61 (-0.38, 1.59)	**1.87*** **(0.91, 2.82)**	CM			
2.18* **(1.47, 2.89)**	**3.45*** **(2.78, 4.12)**	**1.58*** **(0.89, 2.27)**	FGG		
0.61 (-0.19, 1.41)	**1.87*** **(1.11, 2.64)**	0.004 (-0.77, 0.78)	**-1.57*** **(-1.95, -1.19)**	LCC	
-2.53* **(-3.40, -1.67)**	**-1.27*** **(-2.08, -0.45)**	**-3.14*** **(-3.98, -2.30)**	**-4.72*** **(-5.21, -4.23)**	**-3.14*** **(-3.76, -2.53)**	UTS