Coronally Advanced Flap in the Treatment of Multiple Adjacent Gingival Recessions along with a Connective Tissue Graft Harvested from Augmented or Nonaugmented Palatal Mucous Membrane: A Two-Year Comparative Clinical Evaluation

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Abstract: Achieving the coverage of multiple adjacent gingival recessions (MAGRs) in a single surgical procedure poses a major clinical challenge. The gold standard procedure involves the collection of autogenous connective tissue from the palatal mucosa. In case of reduced palatal tissue thickness, augmentation using a collagen sponge can be performed. The aim of this study was to compare the treatment outcome of MAGR coverage by a coronally advanced flap (CAF) along with a connective tissue graft (CTG) harvested from augmented or nonaugmented palatal mucous membrane. Thirty-five patients with 148 MAGRs were enrolled in the study. The recessions were covered with CTGs collected from 26 augmented- (test group) and from 24 nonaugmented (control group) palatal donor sites followed by a CAF. Clinical parameters were measured at baseline, 6, 12 and 24 months after intervention. Clinical results for both, the test and the control group were steady and similar with the exception of buccal gingival thickness (BGT 1) after 24 months, statistically greater BGT 1 and complete root coverage (CRC) was observed in the test group. The augmented CTG+CAF method achieves good and predictable clinical results in the coverage of MAGRs. It leads to the increase of gingival thickness in comparison to the nonaugmented intervention.

Keywords: gingival recession; connective tissue; graft; flap; augmentation

1. Introduction

Gingival recessions are single or multiple, partially exposed root surfaces of teeth connected with an apical shift of marginal gingiva in relation to the cemento-enamel junction [1].

The etiology of gingival recession is multifactorial. Possible morphological factors are the anatomy of bone structure as well as the size, shape and position of teeth and the surrounding soft tissues including gingiva, mucous membranes and muscles. Concerning functional factors the most significant one is mechanical damage, which most commonly is caused by improper and excessive tooth brushing. Furthermore, iatrogenic factors that occur during dental treatment, parafunctions or even chemical damage may result in the development of gingival recession. Another important etiological factor is the excessive accumulation of dental plaque, which in result will lead to the inflammation of periodontal tissue and hence to the loss of clinical attachment. Female sex and age have also been stated as possible risk factors [2].

Comprehensive treatment of gingival recession requires the elimination of most etiological factors favoring the development of gingival recession, as well as surgical root
coverage and long-term maintenance of the results. Various surgical techniques are used today in the treatment of gingival recession but the most favorable and greatest documented method is the coverage of gingival recessions with the aid of an autogenous connective tissue graft taken from the hard palate together with the coronally advanced flap (CAF) technique [3–6].

Despite certain differences in structure between the gingiva and the masticatory mucosa of the hard palate, the connective tissue graft collected from the hard palate is most commonly used in the treatment of gingival recession [7]. Gingival epithelial tissue shows more pronounced rete pegs, projecting locally and deeply into the underlying connective tissue layer. The lamina propria, being the first layer of connective tissue below the epithelium, shows a large number of fibroblasts and immune cells, mainly lymphocytes. On the other hand, the epithelial tissue of the mucous membrane of the hard palate is more regularly arranged, with shorter rete pegs and the lamina propria presents a smaller number of fibroblasts and a large amount of extracellular material, mainly filled with collagen fibers. There are no immune cells [8,9].

The main component of fibrous connective tissue, constituting the lamina propria of the palatal mucosa and keratinized gingiva, are collagen fibers formed by fibroblasts [10,11]. Fibroblasts participate not only in the formation of various fibers, mainly collagen, but also perform a role in the matrix synthesis [12].

The use of a graft increases the amount of connective tissue at the recipient site and the coronally advanced flap provides vascularization and thus nutrition for the graft. For anatomic and histological reasons, the palatal donor site is located between the canine and the second molar, bounded by the course of the greater palatine nerve and artery, resulting in a limited available graft size [13].

Generally, in case of multiple gingival recessions there is need for multistage proceeding. The minimal mucosal thickness of the donor site should be at least 2.5mm for an optimal connective tissue graft. Given a reduced tissue thickness, the epithelial tissue is proximate to the bone and the amount of underlying connective tissue is limited. Hence, these grafts present a large number of epithelial cells [11]. The presence of a large amount of epithelial tissue in a connective tissue graft increases the risk of its rejection or even the formation of a surgical cyst and thus resulting in procedural failure [14]. Therefore, in the event of a thin palatal mucous membrane at the potential donor site, its extension and thickening (augmentation) is accomplished by biostimulation of connective tissue cells—fibroblasts. It is carried out by intramucosal implantation of collagenous biomaterial.

Collagen is a chemotactic factor for the periodontal fibroblasts and the gingiva. It serves as a scaffold, allowing greater fibroblast migration in the early stages of healing. Its chemotactic effect on fibroblasts causes their build-up and therefore leads to the increase in the amount of collagen fibers and consequently to the thickening of the gingival tissue. Moreover, it is a haemostatic factor, causing the initial formation of a blood clot and stabilization of the wound [15].

Eight to ten weeks after the augmentation procedure the graft with an increased tissue volume and quality is harvested and used in the following coverage of multiple gingival recessions [15,16].

Another alternative is the use of a connective tissue substitute—a biostatic graft of human (allogenic) or animal (xenogeneic) origin. The most common and has been used the longest as a substitute for connective tissue grafts in allogeneic gingival recession coverage procedures is the acellular dermal matrix allograft [17,18]. Certain xenogeneic biomaterials that have been used in the coverage of gingival recessions are -Mucograft®, [16,19,20], Mucoderm® [21], DynaMatrix® [22], Enamel Matrix Derivative (Emdogain) and other biomaterials [23–25].

The advantage of this alternative is the need for a single surgical site and the lack of volume limitation in the biomaterial used for gingival recession coverage. The disadvantage however, is the lack of cells in comparison to an autogenous graft, which results in a reduced regenerative potential and an extended period of incorporation [14,15].
Not all biomaterials, including the acellular dermal matrix allograft, have been approved for use in Poland, and the use of others are usually associated with high costs, which makes patients more willing to consent to the use of an autogenous connective tissue graft in the coverage of multiple gingival recessions. Furthermore, the fear of using foreign biomaterial in relation to the possibility of using the patient’s own tissue is significant. Taking into account all treatment options, patients most commonly choose the treatment protocol with the use of autogenous, nonaugmented or augmented connective tissue, regardless of the additional discomfort.

The aim of the study was to compare clinical results after the coverage of multiple adjacent gingival recessions using an autogenous connective tissue graft with or without prior augmentation of the palatal mucosal donor site using a Biokol® lyophilized xenogeneic collagen sponge (Ravimed Sp. z o.o., Poland).

2. Materials and Methods

The study comprised of 35 adults of both sexes, 27 women and 8 men, all of whom provided written consent for participation in the study. All clinical measurements as well as the surgical procedures were performed in the Specialist Outpatient Medical Clinic MEDIDENT in Gorlice, Poland between March 2008 and October 2011. The study was conducted in accordance with the recommendations of the Helsinki Declaration of 1975 and amendments introduced in the year 2000, and consent was obtained from the Bioethics Committee of Wroclaw Medical University (No. KB—284/2008).

2.1. Patient Selection

In each patient with multiple gingival recessions, oral hygiene instructions were given, followed by professional plaque removal. At the subsequent appointments, the correctness in tooth brushing was evaluated.

The inclusion criteria for the research project were as follows: (1) age group in the region of 18–60 years of age; (2) presence of ≥2 adjacent gingival recessions of class I, II and III according to Miller classification [26]; (3) no contraindications for dental surgery; (4) good oral hygiene (API < 15%); (5) BOP index for the entire oral cavity < 10%; (6) clinical attachment level measured mid-buccally on teeth with gingival recessions CAL ≥2 mm; (7) Gingival recession depth (GRD) ≥1 mm; (8) undamaged cemento-enamel junction (CEJ) in teeth with gingival recessions.

The exclusion criteria for the research project were as follows: (1) pregnancy, breastfeeding; (2) use of medication that may affect the structure of periodontal tissue (e.g., anti-convulsants, calcium channel blockers, immunosuppressants); (3) nicotinism (>10 cigarettes a day); (4) dentures or orthodontic braces; (5) systemic diseases (diabetes, autoimmune diseases, diseases with primary and secondary impairment of the immune system, acute infectious diseases); (6) previous coverage of gingival recessions in the past; (7) antibiotic therapy in the last three months before the scheduled surgery.

Patients meeting the inclusion criteria were divided into two groups depending on the mucous membrane thickness of the hard palate at the potential site for connective tissue graft collection. The palatal gingival thickness (PGT) was measured at points localized 4 mm apical from the marginal gingiva of each tooth from canine to second molar in places with greatest convexities of palatal roots:

- Control Group—patients not requiring augmentation of the palatal mucous membrane before harvesting the graft (PGT ≥ 2.5 mm in at least 4 out of 5 sites).
- Test Group—patients requiring augmentation (PGT < 2.5 mm in at least 2 out of the 5 examined places).

2.2. Clinical Parameters

Clinical measurements were made noninvasively using a periodontal probe (UNC15, Hu Friedy, Chicago, IL, USA) calibrated every 1 mm. For each examined tooth, probing depth (PD), clinical attachment level (CAL), gingival recession depth (GRD), gingival
recession width (GRW) and attached gingiva width (AG) were recorded. In addition, the class of each gingival recession was determined, according to Miller [26].

Gingival thickness (GT) measurements were taken at the gingival recession sites, using an endodontic instrument after local anesthesia. This invasive method was earlier described by Bednarz et al. [27]. In places with a present zone of attached gingiva, gingival thickness was measured mid-buccally at two points: BGT1 (buccal gingival thickness) -point located in the middle of keratinized tissue and BGT2—located 2 mm apically from the mucogingival junction. In the event that the attached gingiva was absent, the gingival thickness was measured mid-buccally at one point, which was localized 2 mm in apical direction from the clinical attachment level (BGT3).

Furthermore, after 6, 12 and 24 months: (ARC) percentage of average root (gingival recession) coverage according to the pattern (e.g., for 24 months of observation) were evaluated: \( \frac{GRT_2 - GRT_{24}}{GRT_0} \times 100% \); (CRC) percentage of complete root (gingival recession) coverage: \( \frac{cCRC}{2all \ GR} \times 100\% \); (%CAL) percentage of clinical attachment level gain: \( \frac{CAL_0 - CAL_{24}}{CAL_0} \times 100\% \); and (%GTI) percentage of gingival thickness increase: 

\[ \frac{BGT_{24} - BGT_0}{BGT_0} \times 100\% \], separately for BGT1, BGT2, BGT3.

The consort flowchart of the study is demonstrated in Figure 1.

![Consort flowchart of the study](image)

**Figure 1.** Consort flowchart of the study CAF, coronally advanced flap; A – CTG, augmented connective tissue graft; NA – CTG, nonaugmented connective tissue graft; n—number of subjects, N—number of gingival recessions.

A member of the research team (J. P-B), who did not participate in the surgical procedure, was assigned the role of intra-examiner in order to record clinical parameters before treatment as well as 6, 12 and 24 months post-treatment.

Calibration of the research member (J. P-B) before the study, was performed in the following way: in three nonstudy patients, with multiple adjacent gingival recessions clinical parameters were measured in two visits two days apart. The measurements were performed with a periodontal probe (UNC15, Hu-Friedy, Chicago, IL, USA) with an accuracy of 1mm (PD, CAL, GRD, GRW, AG). The BGT1,2,3 measurements were determined
with an endodontic file together with a caliper (Iwanson) with an accuracy of 0.1 mm. The intraexaminer repeatability was recognized as adequate with the intraclass correlation coefficient (ICC) of more than 90%.

2.3. Surgical Procedures

All surgical procedures were performed by an experienced operator (W.B). Treatment of both groups was carried out under local infiltration anesthesia using 4% articaine with adrenaline at a dilution of 1:100,000 (Septodont®, Saint-Maur des Fosses, France).

In the control group (C), coverage of multiple gingival recessions was performed using an autogenous connective tissue graft taken from the palate (Figure 2).

![Figure 2. Multiple adjacent gingival recessions in control group patient before the surgical procedure.](image)

On each tooth, qualified for the procedure, an intrasulcular incision was made which joined in the interdental space an oblique incision to create a split thickness flap (Figure 3A,B).

![Figure 3. Flap design: (A) intrasulcular incisions join in the interdental space oblique incisions; two vertical releasing incisions, mesially and distally to the gingival recession defect; (B) flap is raised with a split-thickness approach.](image)

Medially and distally to the operating field, vertical releasing incisions were performed to obtain a trapezoidal flap shape (Figure 4). After the creation of surgical papillae, the coronal parts of the anatomical papillae were deepithelialized (Figure 5).

![Figure 4. Trapezoidal flap after vertical releasing incisions.](image)
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Figure 5. De-epithelization of anatomical papillae.

Next, a template was created with the dimension necessary to gain an adequate connective tissue graft (Figure 6).

Figure 6. Preparation of adequate template for connective tissue graft (CTG).

At the donor site, the first incision was carried out no closer than 1 mm from CAL, from canine to the second molar, perpendicular to the surface of the mucous membrane without touching the periosteum. The initial incision was performed longer than the template located at the donor site. During the advancement of the incision, the angulation of the microsurgical blade was changed until its position was parallel to the mucosal surface, in order to create a flap with a thickness of approx. 1 mm. Another incision was made parallel to the previous one with proximity to the periosteum. The following incisions—two parallel and two perpendicular to the dental arch, within the subepithelial connective tissue, not reaching the periosteum, resulted in the obtainment of a CTG with the desired size and thickness of about 1 mm. The surgical wound at the donor site was finished off with the cross mattress sling suture technique, with a 5/0 diameter nonabsorbable surgical thread and a 16 mm (3/8) taper point needle (Dafilon®, B. Braun Surgical, S.A. Rubi. Spain) (Figure 7A–C).

Figure 7. CTG collection: (A) Collection of CTG from the palatal masticatory mucosa, (B) Primary wound closure with mattress suturing, (C) Demonstration of harvested CTG of adequate size.

The graft was placed at the recipient site and immobilized with single knotted sutures using absorbable surgical thread material (Novosyn® Quick 6/0 DSMP11, 3/8c, B. Braun Surgical, S.A. Rubi. Spain) (Figure 8). The mucosal flap was coronally advanced, surgical papillae were positioned on the deep epithelialized anatomical papillae and stabilized with single sling mattress sutures (Dafilon 6/0, DSi2, 3/8c, B. Braun Surgical, S.A. Rubi. Spain). The sutures enlaced the crowns of the teeth to completely cover the CTG placed on the gingival recessions. The sites of the vertical releasing incisions were sutured using the “double O” technique to ensure primary closure of the surgical wound (Figure 9). Each patient of the Test Group (T) that participated in the study was treated using a Biokol®
lyophilized xenogeneic collagen sponge (Ravimed Sp. z o.o., Poland). The Biokol\textsuperscript{®} sponge comes in the shape of a cylinder with a diameter of 8 mm and a height of 15–18 mm, containing bovine type I collagen.

![Figure 8](image8.png)

**Figure 8.** Placement and immobilization of CTG graft at the recipient site.

![Figure 9](image9.png)

**Figure 9.** Coronal advancement of flap for recession coverage.

**Surgical Procedure Using Biokol\textsuperscript{®}, the Xenogeneic Lyophilized Collagen Sponge**

At the palatal site, the incision was carried out from canine to the second molar with a distance of at least 2 mm from the gingival margin, parallel to it and no closer than 1 mm from the clinical attachment level of the individual dentogingival unit. A split thickness flap was created adjacent to the periosteum, forming an envelope to which the properly shaped biomaterial was inserted. Primary surgical wound closure was assured by cross mattress suturing (Figure 10A,B).

![Figure 10](image10.png)

**Figure 10.** Augmentation of palatal mucous membrane using Biokol\textsuperscript{®} lyophilized collagen sponge: (A) Creation of split thickness flap and insertion of Biokol\textsuperscript{®} sponge (B) Donor site after augmentation procedure.

Eight to ten weeks after the palatal augmentation procedure the subepithelial connective tissue graft was harvested and used for the treatment of multiple gingival recessions as described in the surgical protocol of the control group and shown in Figure 7A–C. No clinical difference can be determined by the naked eye between the augmented and nonaugmented harvested graft. Treatment was finished up by an adhesive periodontal dressing, based on cellulose, (Reso-pac\textsuperscript{®}, Hager Werken, Duisburg, Germany) which was applied on all surgical wounds at the donor and recipient site. Additionally, cold compresses were used for 1 h immediately after the procedure.
2.4. Post-Surgical Management

After the surgical procedure, the patients controlled plaque levels chemically with a 0.1% chlorhexidine oral mouthwash, 3 times a day. Patients were instructed to discontinue tooth brushing at the surgical site for 7 days and to withhold from dental flossing for 2 months. Subsequently, the patients were guided to use an ultra-soft post-operative toothbrush and 3 weeks post-treatment the usage of a soft toothbrush was directed. Initially, every 7 days and then every 14 days between the 4th and the 8th week the patients’ plaque was removed professionally. The patients adhered to a liquid diet on the first day, a semi-liquid diet for the next 3 days, followed by a soft diet up to the 14th day. The sutures were removed after 2 weeks. Figure 11 shows the clinical situation 24 months after multiple adjacent gingival recessions coverage in a control group patient. Figure 12 shows the clinical situation in a test group patient.

![Figure 11](image1.png)

**Figure 11.** Control group patient: Clinical situation 24 months after treatment of multiple adjacent gingival recessions.

![Figure 12](image2.png)

**Figure 12.** Test group patient: (A) Multiple adjacent gingival recessions at baseline (B) 24 months post-surgery.

2.5. Statistics

Data was collected in a standardized manner and recorded in the research card. After every single scheduled check-up appointment, it was completed by a member of the research team. The study results underwent statistical analysis.

The normality of distribution was tested with the Shapiro–Wilk test.

For all groups, the mean values and standard deviation (SD) of the examined continuous parameters were calculated. Hypothesis testing for equality of mean parameters in independent groups was carried out with the ANOVA variance analysis method; homogeneity of variances was checked with the Bartlett’s test. Hypothesis testing for equality of mean parameters in matched pairs (successive time intervals) was carried out with the Student’s t-test for dependent variables. The frequency of distribution of discrete parameters in groups was analyzed with the chi-square test. For each test, \( p < 0.05 \) was considered statistically significant. Statistical analysis was performed using Statistica 13.1.

3. Results

Patients presented multiple gingival recession types according to Miller Class I—70 sites, II—60 sites and III—18 sites. A number of 120 gingival recessions were located in the maxilla and 28 gingival recessions in the mandible. A total of 148 gingival recessions were covered, 77 in the test group (T) and 71 in the control group (C) with the collection of
connective tissue grafts from 24 nonaugmented and 26 augmented palatal donor sites. The distribution in both groups is presented in Table 1.

**Table 1.** Patient related records.

|                          | Test Group (CAF + A−CTG) | Control Group (CAF + NA−CTG) |
|--------------------------|--------------------------|------------------------------|
| Number enrolled patients | 16                       | 19                           |
| (12 females, 4 males)    | (15 females, 4 males)    |
| Number covered recessions| 77                       | 71                           |
| Number type of recession (Miller) |                     |
| I                        | 34                       | 36                           |
| II                       | 30                       | 30                           |
| III                      | 13                       | 5                            |
| Number treated teeth per patient |                  |
| Mean value ± SD          | 4.81 ± 2.40              | 3.74 ± 2.08                  |
| Range                    | 2–10                     | 2–9                          |
| Number localisation of recessions |                |
| Incisor                  | 19                       | 24                           |
| Canine                   | 22                       | 13                           |
| Premolar                 | 30                       | 28                           |
| Molar                    | 6                        | 6                            |
| Maxilla                  | 66                       | 54                           |
| Mandible                 | 11                       | 17                           |
| Number surgical sessions per patient |
| Mean value ± SD          | 1.63 ± 0.62              | 1.26 ± 0.56                  |
| Range                    | 1–3                      | 1–3                          |

CAF, Coronally advanced flap; A−CTG, Augmented connective tissue graft; NA−CTG, nonaugmented connective tissue graft; SD—standard deviation.

Tables 2 and 3 provide an intergroup and intragroup comparison of the clinical parameters during different observation periods.

### 3.1. Clinical Measurements for CAL and GRD

Apart from CAL (p = 0.029) and GRD (p = 0.044), the mean baseline values of the clinical parameters did not differ statistically significantly in the intergroup comparison (Table 2). The initial mean CAL0 value for the test group was 4.19 ± 1.07 mm, while the corresponding mean value for the control group was 4.62 ± 1.26 mm. No statistically significant differences in terms of CAL were observed between the groups at 6, 12 and 24 months after the procedure. The mean values after 24 months of observation were equal to 1.14 ± 0.77 mm and 1.31 ± 0.81 mm respectively. In both groups, a statistically significant improvement was noted in the position of CAL after 6, 12 and 24 months in comparison to baseline.

The mean percentage of clinical attachment level gain (%CAL) for the test group, 6 months after the surgery was 71.96 ± 15.89%, 12 months—73.48 ± 16.72% and 2 years—73.56 ± 16.77% and for the control group 72.93 ± 15.38%, 73.12 ± 14.56%, 71.98 ± 13.96% respectively. The differences between both groups were insignificant for each stage of the observation period.

The initial mean value for GRD in the test group was 2.72 ± 1.00 mm, while the corresponding value for the control group was 3.08 ± 1.14 mm. In both groups the mean GRD values at 6, 12 and 24 months post-treatment had declined significantly in relation to the pre-operative state. Two years after surgery, the test group mean value was equal to 0.27 ± 0.44 mm and in the control group 0.37 ± 0.55 mm. The differences between both groups were not statistically significant.
### Table 2. Clinical parameters at baseline, 6, 12 and 24 months after intervention.

| Group/Time | CAL (mm) ± SD | PD (mm) ± SD | GRD (mm) ± SD | GRW (mm) ± SD | AG (mm) ± SD | BGT<sub>1</sub> (mm) ± SD | BGT<sub>2</sub> (mm) ± SD | BGT<sub>3</sub> (mm) ± SD |
|------------|---------------|--------------|--------------|--------------|------------|-----------------|-----------------|-----------------|
| Baseline   | 4.19 ± 1.07 **| 1.47 ± 0.48  | 2.72 ± 1.00 **| 3.47 ± 0.92  | 1.33 ± 1.41 | 1.04 ± 0.21     | 1.17 ± 0.20     | 1.02 ± 0.28     |
| Test group |               |              |              |              |            |                 |                 |                 |
| 6 months   | 1.17 ± 0.67 *  | 1.22 ± 0.33  | 0.21 ± 0.37 * | 0.69 ± 1.14 *| 3.73 ± 1.00 | 2.09 ± 0.25 ** | 1.93 ± 0.28 **  | 1.85 ± 0.22 **  |
| 12 months  | 1.12 ± 0.72 *  | 1.18 ± 0.31  | 0.22 ± 0.36 * | 0.73 ± 1.13 *| 3.70 ± 0.94 | 1.90 ± 0.27 ** | 1.74 ± 0.23     | 1.67 ± 0.18 *   |
| 24 months  | 1.14 ± 0.77 *  | 1.19 ± 0.34  | 0.27 ± 0.44 * | 0.80 ± 1.21 *| 3.69 ± 0.98 | 1.79 ± 0.28 ** | 1.60 ± 0.28 *   | 1.55 ± 0.19 *   |
| Control group |           |              |              |              |            |                 |                 |                 |
| Baseline   | 4.62 ± 1.26 **| 1.54 ± 0.48  | 3.08 ± 1.14 **| 3.64 ± 1.25  | 1.51 ± 1.78 | 1.11 ± 0.27     | 1.17 ± 0.29     | 0.86 ± 0.39     |
| 6 months   | 1.25 ± 0.75 *  | 1.13 ± 0.26  | 0.30 ± 0.49 * | 0.85 ± 1.24 *| 3.93 ± 1.33 | 1.63 ± 0.31 ** | 1.70 ± 0.33 **  | 1.60 ± 0.46 **  |
| 12 months  | 1.25 ± 0.77 *  | 1.08 ± 0.29  | 0.34 ± 0.49 * | 0.95 ± 1.16 *| 3.91 ± 1.29 | 1.63 ± 0.27 ** | 1.64 ± 0.34     | 1.55 ± 0.43 *   |
| 24 months  | 1.31 ± 0.81 *  | 1.11 ± 0.26  | 0.37 ± 0.55 * | 0.98 ± 1.21 *| 3.87 ± 1.33 | 1.51 ± 0.27 ** | 1.57 ± 0.33     | 1.42 ± 0.45     |

* statistically significant difference in comparison to baseline (Student’s t-test for dependent variables) ** between-groups statistically significant difference (ANOVA); CAF, coronally advanced flap; A<sub>−</sub>CTG, augmented connective tissue graft; NA<sub>−</sub>CTG, nonaugmented connective tissue graft; CAL, clinical attachment level; PD, probing depth; GRD, gingival recession depth; GRW, gingival recession width; AG, attached gingiva width; BGT<sub>1,2,3</sub>, buccal gingival thickness.

### Table 3. Descriptive statistics recorded between 6, 12 and 24 months post-surgery.

| Group/Time | ARC % ± SD | %CAL % ± SD | %BGT<sub>1</sub> % ± SD | %BGT<sub>2</sub> % ± SD | %BGT<sub>3</sub> % ± SD | CRC % ± SD |
|------------|------------|-------------|-----------------|-----------------|-----------------|-------------|
| Test group |            |             |                 |                 |                 |             |
| 6 months   | 93.22 ± 12.24 | 71.96 ± 15.89 | 49.99 ± 9.42 ** | 38.93 ± 10.91 ** | 44.21 ± 16.02 | 71.43       |
| 12 months  | 92.64 ± 12.29 | 73.48 ± 16.72 | 44.66 ± 10.76 **| 32.04 ± 13.25   | 38.74 ± 16.83 **| 68.83       |
| 24 months  | 91.76 ± 13.29 | 73.56 ± 16.77 | 42.15 ± 14.65 **| 25.67 ± 25.85   | 34.21 ± 16.10 | 67.53       |
| Control group |           |             |                 |                 |                 |             |
| 6 months   | 91.47 ± 15.50 | 72.93 ± 15.38 | 31.56 ± 10.61 **| 31.59 ± 11.02 **| 48.03 ± 18.31 | 66.20       |
| 12 months  | 90.48 ± 12.66 | 73.12 ± 14.56 | 31.60 ± 11.41 **| 28.92 ± 11.87   | 47.33 ± 16.51 **| 57.75       |
| 24 months  | 89.90 ± 13.36 | 71.98 ± 13.96 | 26.63 ± 9.55 **  | 26.07 ± 10.32   | 42.26 ± 16.91 | 57.75       |

** between-groups statistically significant difference (ANOVA) CAF, coronally advanced flap; A<sub>−</sub>CTG, augmented connective tissue graft; NA<sub>−</sub>CTG, nonaugmented connective tissue graft; ARC, percentage of average root coverage; %CAL, percentage of clinical attachment level gain; %BGT<sub>1,2,3</sub>, percentage of buccal gingival thickness increase; CRC, percentage of complete root coverage.
3.2. Clinical Measurements for GRW, PD and AG

The mean baseline GRW value was $3.47 \pm 0.92$ mm for the test group and $3.64 \pm 1.25$ mm for the control group and decreased significantly in both groups after 6 months—$0.69 \pm 1.14$ mm and $0.85 \pm 1.24$ mm respectively. The intragroup differences for the mean value after 12 and 24 months were statistically insignificant and similar for both groups at each stage of observation.

The initial mean value for PD in the test group was $1.47 \pm 0.48$ mm, while the corresponding value for the control group was $1.54 \pm 0.48$ mm. At the end of the 24-month observation period, PD had increased insignificantly for both groups—$1.19 \pm 0.34$ mm and $1.11 \pm 0.26$ mm respectively, without differences between groups.

The mean AG value for the test group at baseline was $1.33 \pm 1.41$ mm and for the control group $1.51 \pm 1.78$ mm. The corresponding values after 24 months were $3.69 \pm 0.98$ mm and $3.87 \pm 1.33$ mm respectively. These were statistically significant for both groups, but insignificant between groups.

In the test group, 34 gingival recessions with the absence of a zone of attached gingiva were found, including 30 gingival recessions of class II and 4—of class III. In the control group, only 30 gingival recessions of class II acc. to Miller classification were identified.

3.3. Clinical Measurements for BGT$_1$, BGT$_2$, BGT$_3$

The mean BGT$_1$ value for the test group at baseline was $1.04 \pm 0.21$ mm and for the control group was $1.11 \pm 0.27$ mm and after 2 years $1.79 \pm 0.28$ mm and $1.51 \pm 0.27$ mm respectively.

The mean value of BGT$_2$ prior to treatment in the test group was $1.17 \pm 0.20$ mm and in the control group was $1.17 \pm 0.29$ mm and after 2 years adequately $-1.60 \pm 0.28$ mm and $1.57 \pm 0.33$ mm.

The initial mean value for BGT$_3$ was equal $1.02 \pm 0.28$ mm in the test group and $0.86 \pm 0.39$ mm in the control group and similarly after 24 months, $1.55 \pm 0.19$ mm in the test group and $1.42 \pm 0.45$ mm in the control group.

In both groups, a statistically significant improvement of BGT$_1$, BGT$_2$ and BGT$_3$ was noted after 6, 12 and 24 months in relation to baseline (Table 2). During the intergroup comparison, all analyzed GT parameters showed a statistically significant difference after 6 months, after 12 months in mean values of BGT$_1$ and BGT$_3$, and after 2 years only in mean values of BGT$_1$ ($p < 0.001$).

3.4. Clinical Values for %BGT$_1$, CRC and ARC

Percentage of buccal gingival thickness increase (%BGT$_1$) two years after surgery; for the test group it was equal to $42.15 \pm 14.65\%$, and in the control group, $6.63 \pm 9.55\%$. This difference was statistically significant ($p < 0.001$) (Table 3).

The complete root coverage (CRC) after 24 months was $67.53\%$ for the test group, while the corresponding value for the control group was $57.75\%$, and this difference was not statistically significant. However, when comparing the CRC values for the individual gingival recession classes according to the Miller classification, statistically significant differences were noted in class II.

In class III there was not a single case of complete root coverage in the control group, while in the test group the CRC was $30.77\%$. In Miller class I gingival recessions, complete root coverage was achieved in $85.29\%$ for the test group and $80.56\%$ in the control group (NS).

After 24 months, the ARC was $91.76 \pm 3.29\%$ in the test group and $89.90 \pm 13.36\%$ in the control group. No statistically significant differences were observed between the groups. Similarly, no statistically significant differences in terms of ARC were observed between the groups after 6 and 12 months.

4. Discussion

The main surgical methods used in the treatment of gingival recession are mucogingival procedures with flap preparation, tunnel techniques together with autogenous connective tissue grafts, free gingival grafts and partially de-epithelialized free gingival
grafts [28–31]. However, other alternatives have also been employed, including substitutes for autogenous connective tissue (allogeneic, xenogeneic, synthetic meshes), tissue cultures (fibroblasts, keratinocytes), guided tissue regeneration (resorbable and nonresorbable barrier membranes) as well as biologically active proteins (amelogenins, growth factors) [4,25,32]. The golden standard involves the usage of a connective tissue graft harvested from the mucous membrane of the hard palate combined with flap displacement or the creation of supraperiosteal tunnels. These methods show the best long-term clinical and aesthetic results [31,33–35].

According to Chambrone et al. [36] the outcome measures of this 24-month follow-up study can be classified as medium term (13–59 months). Most of the studies evaluating the clinical and aesthetic outcome of gingival recession coverage are of short term, i.e., 6–12 months [37–40], and only a few are of long term (≥60 months) [35,41–44].

No similar study assessing the use of augmented connective tissue in the coverage of MAGRs was found in the available literature. Only one study was focused on a two-year evaluation of multiple gingival recession coverage, but here the modified CAF/EMD method and CAF alone was performed [45]. Cordaro et al. [45] compared clinical outcomes after 6 and 24 months in 10 patients with at least two bilateral adjacent gingival recessions (58 recessions in total, 29 in each group) after the treatment with a CAF/EMD (Test) enamel matrix derivative and modified CAF alone (Control). Mean root coverage after six months in the test group was 82.8% and in the control group 80.7%, and after 24 months 74.8% and 71.0% respectively. After six months the authors obtained complete root coverage in 13 out of 29 sites (45%) in the control group and in 9 out of 29 sites (31%) in the test group but after 24 months CRC was equal 24% and 17%, respectively. Similarly, there were no statistically significant differences between the groups in terms of KTWs and CAL, but differences between the mean baseline values as well as between 6 and 24 months CRC were found. Two years after intervention CAL gain was 50% in the CAF alone group and 54.3% in the CAF/EMD group. Average PD values during the whole observation period did not change in comparison to baseline, which is consistent with our results.

In our study, after six months the ARC was 93.22% in the test group and 91.47% in the control group, and after 24 months, 91.76% and 89.90% respectively. The CRC after six months was 71.43% in the test group and 66.20% in the control group, and after two years 67.53% and 57.75% respectively. Furthermore, better results compared to Cordaro et al. [45] were recorded in the CAL gain values (%CAL), with an average value of 73.56% in the augmented CTG group and 71.98% in the nonaugmented CTG group.

Our collated results demonstrate postoperative stability during the two-year observation period, and the percental increase in CAL gain throughout time can possibly be related to the phenomenon of creeping attachment, especially among the augmented CTG group.

In another study, Pini Prato et al. [42] compared the results of the coverage of 93 Miller class I, II, III maxillary MAGRs in 13 patients, treated using the CAF/CTG method and CAF alone during a 5-year observation period. In both study groups, recessions were located in the area of incisors, canines and premolars. However, four recessions were located in the area of first molars, while 15 gingival recessions were of Miller class III (8 in the CAF/CTG group), which is comparable to our study (11 Miller class III gingival recessions in the test and 17 in the control group).

At the six-month follow-up, CRC was achieved in 52% in the CAF alone group and 37% in the CAF/CTG group. Although, there was a decrease in the percentage of CRC in the CAF alone and an increase in the CAF/CTG group after 12 months, 37% and 45%, respectively, and after 5 years, 35% and 52%, respectively. The authors also noted that the apical relapse of gingival margin was statistically significantly higher in the CAF alone group, which was explained by the lower keratinized gingival tissue thickness due to the lack of use of a connective tissue graft, similar to other studies [38].

Comparing the use of autogenous connective tissue and a substitute for CTG in covering gingival recessions, Gholami et al. [46] observed statistically greater increase in
keratinized tissue width (baseline—2.4 mm, after 6 months 4.1 ± 0.8 mm) in the CTG group than in the ADMA group (baseline—2.9 mm after 6 months 3.5 mm).

Tavelli et al. [47] underlined in a long-term 12-year study of MAGRs treatment with the use of an autogenous connective tissue substitute—Acellular Dermal Matrix and CAF or tunnel technique, a significant recurrence of gingival recession between 6 months and 12 years after the treatment. According to the authors, the factors determining the stability of results were KTW ≥ 2mm and GT ≥ 1.2mm at the 6 months period.

Multiple adjacent 48 Miller class I gingival recessions with a GT less than 0.8 mm were covered using the CAF/ADM and CAF alone method. The average GT value in the treated MAGRs sites with complete root coverage was more than or equal to 1.3mm. The values for ARC and CRC after 12 months were 94.84% and 83.33% in the CAF/ADM group, 74.99% and 50.00% in the CAF alone group, respectively. The increase in gingival thickness during the 12 months observation period was 0.69 mm, from 0.75 mm to 1.41 mm for the CAF/ADM group, namely the percentage of gingival thickness increase (GTI) was equal 88.00%. For the CAF group GT increase was 0.07 mm from 0.71 mm to 0.77 mm, namely the percentage of GTI was equal 8.45% and the intergroup difference was statistically significant.

In the Cairo et al. study [38], evaluating the efficacy of MAGRs coverage procedures using the CAF method with and without the use of a connective tissue graft, GT at baseline was 0.73 mm in the CAF/CTG group and after 12 months 1.39 mm, i.e., an increase of gingival thickness of about 90.41% was achieved.

In our study, at baseline the mean value for GT, measured in the center of the keratinized tissue (BGT1) in the test group was 1.04 mm and for the control group was 1.11 mm and after two years 1.79 mm and 1.51 mm respectively and therefore GT increase was 42.15 ± 14.65% in the test and 26.63% in the control group. The mean AG value for the test group at baseline was 1.33 mm and for the control group 1.51 mm, after 24 months they increased to 3.69 mm and 3.87 mm respectively. Despite the demonstrated lower percentage of GTI, GT values greater than 1.5 mm and well over 3 mm width of attached gingiva may contribute to the long-term stability of the clinical results obtained.

In order to determine the gingival thickness most of the authors apply the invasive bone sounding method using an endodontic file or periodontal probe together with a caliper [27,28,31,37,40,43,44]. The measuring point is located either 1.5 mm mid-buccally from the gingival margin [28,38,43] or 3mm from the gingival margin [40,44] or in the center of the keratinized tissue and marked as GT1 and 2mm apically from the mucogingival junction marked as GT2 [27,37]. Within this study during each observation stage, apart from GT1 and GT2, another measurement point was determined given the absence of attached gingiva, located 2mm apical from the clinical attachment level.

According to Huang et al. [48], the presence of vestibular keratinized gingiva with the thickness of at least 1.2 mm gives the possibility to raise a mucosal flap, which after repositioning guarantees complete gingival recession coverage without the need of a CTG. Different values for the minimum gingival thickness to achieve complete root coverage are presented by several authors; >1.1 mm [49] > 1.44 mm [50].

Before the collection of a CTG from the palatal masticatory mucosa, its thickness needs to be assessed. If the soft tissue at the donor site shows suitable thickness, an acceptable connective tissue graft can be prepared, without fragments of epithelial tissue, bearing in mind that its’ presence in the graft could lead to graft rejection [11]. When a thin mucosal phenotype is identified at the potential donor site, the free gingival grafting technique is used in combination with its extracorporeal de-epithelialisation [28]. Another solution involves preoperative augmentation of the palatal mucosa using a lyophilized xenogeneic collagen sponge, after which a suitable graft is harvested to cover the gingival recession [15,16]. Carnio et al. [15] used a lyophilized bovine collagen sponge to thicken the thin palatal mucosa, by placing it subperiosteally at the potential donor site. After 8 weeks, they collected a subepithelial connective tissue graft of appropriate thickness from this site, which ensured effective usage in the treatment of gingival recessions.
Harris [11] suggested a minimum mucosal thickness of 3 mm at the potential palatal donor site to assure the collection of an adequate connective tissue graft for utilization in periodontal plastic surgery.

Carnio et al. [51] performed in 26 patients an augmentation of the palatal mucosa with an initial thickness of less than 3 mm, measured at the mid-palatal points of teeth with a distance of 6 mm from the gingival margin. As a result, they achieved a thickening of the palatal mucosal tissue from 2.03 mm at baseline to 3.57 mm, 8 weeks after augmentation.

Bednarz et al. [16] performed in 10 patients a bilateral augmentation of thin palatal mucosa, using two collagen materials in a single procedure. From one side they used a lyophilized collagen sponge Biokol®. The average thickness of the mucous membrane was measured at two points, the first one placed 4 mm below (PGT1—palatal gingival thickness 1) and the second, 8 mm below the gingival margin (PGT2—palatal gingival thickness 2). PGT1 and PGT2 values were measured in this group at 86 sites, demonstrating the following values: PGT1 2.27 ± 0.25 mm, PGT2 2.53 ± 0.21 mm at baseline and 3.71 ± 0.41 mm and 4.08 ± 0.56 mm at 8–10 week after augmentation procedure respectively.

Significant thickening was achieved.

In order to enable a histological evaluation of the connective tissue, biopsies were taken at the potential donor sites with prior augmentation and without augmentation. The histological images of the grafts, taken from the augmented sites, exhibited characteristics of mature fibrous connective tissue. Compared with the biopsies taken from the nonaugmented sites, the biopsies from the augmented tissue with collagen material, did not reveal the presence of any adipose cells. Neither did any of the collagen-augmented sites present any xenogeneic collagen residues, concentrations of inflammatory cells, swelling, necrotic focus or the presence of epithelial cells.

An important parameter used to determine the predictability of gingival recession coverage is the percentage of complete root coverage achieved. The closer this value is to 100%, the more the assessed technique meets the modern clinical requirements and effectiveness for such treatment. Graziani et al. [33] evaluated in a systematic review the efficiency of periodontal plastic procedures in the treatment of multiple gingival recessions. In nine studies with a minimum 6 months observation period, a total of 858 multiple gingival recessions were treated in 208 patients, demonstrating a CRC of 24–89%. Zucchelli et al. [34] compared the clinical and aesthetic results in 50 patients for 5 years after gingival recession coverage, using a coronally positioned flap (CPF) together with a connective tissue graft and CPF alone. Zucchelli et al. [34] reported no difference between groups in mean values for RD reduction and CRC values after 6 and 12 months. However, after five years a statistically significant difference in CRC was noted as well as an increase in keratinized tissue width in the CPF/CTG group.

The authors underlined that after a five-year observation period, the probability of complete root coverage in multiple gingival recessions treated with a CTG is three times higher than in the treatment without a CTG. In the CPF alone group, a significant drop in CRC was noted throughout the five-year observation period.

Nevertheless, in other studies the authors underlined that the usage of a CTG in the coverage of gingival recessions increases treatment predictability expressed by CRC as well as the stability of results over a long observation period, connected to the increase of gingival thickness [35,42,47,50]. Furthermore, the connective tissue graft utilized in the CAF allows for better adaptation of the flap to the exposed root surface [38].

The results of our study confirm the predictability of this procedural protocol, where in case of a thin palatal mucosa at the potential donor site, augmentation is performed. The increase of keratinized tissue thickness after 24 months post-surgery assures stability of the achieved clinical results. Further long-term observations are planned and will follow in the same group of patients that participated in this study.
Limitation of the Study

Patients were qualified for the study and assigned to the test group based on the palatal mucosal thickness at the potential donor site. There are significant statistical differences between the test and the control group in the initial mean value of GRD ($p = 0.044$) and CAL ($p = 0.029$).

Furthermore, only a few of the procedures performed were located in the mandible. Eleven out of 77 treated sites in the test group and 17 out of 71 in the control group were located in the mandible. Due to the specific anatomical conditions in the mandible, treatment outcome of gingival recession coverage in the mandible presented less successful than in the maxilla [30,40,52,53].

An additional limitation appeared by including Miller class III gingival recessions in the study. All of these gingival recessions i.e., 18 (13 in the test and 5 in the control group) were classified as such due to an identified dental malposition (tooth rotation, protrusion, vestibular position) and were part of multiple adjacent gingival recessions [30,42].

5. Conclusions

Within the limits of this study, the palatal augmentation procedure together with a coronally advanced flap were seen to achieve good and predictable clinical results in this 24-month follow-up in terms of multiple adjacent gingival recessions coverage. In the event of a reduced palatal donor thickness, the earlier augmentation allows the collection of an acceptable graft. Its usage shows an overall greater increase of gingival thickness in comparison to the nonaugmented procedure.

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