Early postoperative healing following guided tissue regeneration in aggressive periodontitis patients

Wczesne gojenie pozabiegowe po sterowanej regeneracji tkanek u pacjentów z agresywnym zapaleniem przyzębia

Bartłomiej Górski^{A–D}, Tomasz Kaczyński^{B,C}, Andrzej Miskiewicz^{B,C}, Renata Górka^{E,F}

Department of Periodontology and Oral Diseases, Medical University of Warsaw, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

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Address for correspondence

Bartłomiej Górski
E-mail: bartek_g3@tlen.pl

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Abstract

Background. In order to regenerate periodontal tissues, necessary conditions for this process must be created during surgery, primarily by allowing uninfected adhesion of the clot to the root surface, and then its stable position in the defect.

Objectives. The aim of this secondary analysis was to evaluate early postoperative healing of papillary incisions and its correlations with patient-, site- and technique-related factors following guided tissue regeneration (GTR) in treatment of intrabony defects in patients with aggressive periodontitis (AgP).

Material and methods. The analysis included the data from 25 patients and 59 treatment sites. Surgical treatment consisted of using grafts together with collagen membranes. Post-operative healing was assessed 1 week and 2 weeks after GTR using the Early Wound-Healing Index (EHI).

Results. Early Wound-Healing Index values ranged from 1 (complete flap closure and primary healing) to 4 (incomplete flap closure, partial tissue necrosis, secondary healing). After 1 week, primary healing ($EHI \leq 3$) was observed in 55 sites, and secondary healing ($EHI = 4$) in 4 sites. After 2 weeks, the values were 45 and 14, respectively. No correlations between EHI and patient-related factors were found. However, 1-rooted teeth, sites with thin phenotype and the presence of gingival recessions were associated with impaired healing (higher EHI recordings and secondary healing), as was analyzed in a multiple regression model.

Conclusions. Site-related factors may impinge on the early postoperative healing of papillary incisions succeeding GTR in AgP patients.

Key words: wound healing, aggressive periodontitis, guided tissue regeneration, papilla preservation flap

Słowa kluczowe: gojenie się ran, agresywne zapalenie przyzębia, sterowana regeneracja tkanek, płat oszczędzający brodawkę

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Introduction

The main features of aggressive periodontitis (AgP) are rapid loss of connective tissue attachment and alveolar bone, the presence of dental plaque disproportionate to the severity of the disease, occurrence in individuals without coexisting general diseases, and familial aggregation of the disease.¹ There is genetic susceptibility depending on many genes, and only its interaction with environmental factors (periopathogens and other acquired risk factors) determines the occurrence of symptoms of periodontitis and/or the pace of its clinical course. Aggressive periodontitis, as any complex disease, is characterized by the interaction of multiple gene loci with various alleles, and only the whole complex interacts with environmental factors.² Its prevalence in the population is estimated at 0.5–2.5%.³ Treatment of AgP depends on non-surgical treatment; however, vertical intrabony defects in patients with AgP are indications for the implementation of a chosen regenerative strategy.

At the core of tissue regeneration are complex processes and interactions at the molecular and cellular level. Appropriate preprocedural management, a proper surgical technique and follow-up care are prerequisites that can promote proper healing. For the periodontal regeneration to take place, necessary conditions must be created during the surgery, primarily by allowing uninfected adhesion of the clot to the root surface, and then its stable position in the defect.⁴ Dehiscence of the wound within the first weeks after surgical treatment may disturb the cascade of reactions guiding the course of regenerative processes. In addition, in the case of the use of biomaterials and barrier membranes, impaired healing may lead to partial or total loss of the implant material and even to infection of the barrier membrane. In this context, the role of non-disturbed primary healing is well-known, especially in the first few weeks of guided tissue regeneration (GTR).^{5,6}

The most frequently highlighted factors, directly correlated with the level of obtained results, are the selection of surgical technique for individual clinical situation and performing the procedure in accordance with the principles of minimally invasive surgery.⁷ Conducting a regenerative procedure requires attention at every stage, starting with the appropriate design of incisions and flap preparation range, and ending with the right stitching technique. In recent years, there has been a significant change in understanding of the influence of surgical techniques on tissue healing, and some flap properties, such as its thickness and vascular sources, achieved recognition.⁸ Current treatment techniques are based on tissue healing biology, which excludes formation of extensive surgical wounds that can worsen the conditions for natural regeneration of tissues.⁹ The principles of minimally invasive periodontal surgery have been modified by Cortellini et al., who proposed novel techniques for performing incisions that save interdental papilla to further improve and

maintain flap closure during wound healing.^{10,11} If the interproximal space is wider than 2 mm, a horizontal incision should be performed at the base of the papilla, which was described as modified papilla preservation technique (MPPT).¹⁰ The interproximal space with a width up to 2 mm obliges to make oblique incision maximally close to the tip of the papilla, but not through its peak; this technique is called simplified papilla preservation flap (SPPF).¹¹ Blood flow tests showed that tissue vascularization after SPPF is faster than after incisions typical for Modified Widman Flap (MWF), i.e., through papilla peaks. The technical aspect of minimally invasive surgical methods that promote tissue healing has evident effect on the clinical effects of treatment.

Many scientific papers have described the importance and role of various factors affecting early healing after regenerative procedures on bone defects in patients with periodontitis.^{12,13} Variables were assessed at the level of the patient or the tooth (the treated site). However, none of the papers assessed factors related to the occurrence of complications, such as wound dehiscence after GTR in patients with AgP.

This study aimed at evaluating the early postoperative healing of the papillary incisions, as described by the Early Wound-Healing Index (EHI), and its correlations with patient-, site- and technique-related factors following GTR in the treatment of intrabony defects in patients with AgP.¹⁴

Material and methods

The study is a secondary analysis of data extracted from 3 randomized clinical trials conducted at the Department of Periodontology and Oral Diseases of Medical University of Warsaw, Poland, which assessed different regenerative strategies in patients with AgP. The study received the approval by the institutional review board (KB/135/2014; KB/37/2016; KB/209/2017). All clinical procedures were carried out in accordance with the Helsinki Declaration of 1975, as revised in Tokyo in 2004. Every patient signed a written informed consent form.

Patient and defect eligibility

The inclusion criteria were as follows: 1. diagnosis of AgP in line with definition of American Academy of Periodontology¹; 2. no systemic diseases; 3. taking no medications affecting periodontal status; 4. no pregnancy or lactation; 5. no cigarette smoking; 6. history of periodontitis in parents or siblings; 7. presence of at least 1 tooth with probing pocket depth (PPD) ≥ 6 mm, clinical attachment level (CAL) ≥ 5 mm and intrabony defect ≥ 3 mm as detected in periapical radiographs; 8. full-mouth plaque index (FMPI) $\leq 20\%$; 9. bleeding on probing index (BoP) $\leq 20\%$; 10. tooth had to be vi-

tal or properly treated; 11. no furcation involvement; 12. the width of keratinized tissue on the labial/buccal site of the tooth ≥ 2 mm.

Presurgery procedures

For each patient, a full-mouth disinfection (FMD) protocol was implemented, which involved simultaneous non-surgical treatment of all the pockets during 1 visit (scaling and root debridement) using hand and ultrasonic tools. Mechanical debridement treatments were combined with the use of antiseptics in the form of rinses containing 0.2% chlorhexidine or a gel with 1% chlorhexidine. In addition, all patients received antibiotics (amoxicillin 500 mg + metronidazole 250 mg 3 times per day for 1 week). Individual instructions were also given on maintaining optimal oral hygiene.

Clinical and radiographic recordings

After 6–9 weeks from scaling and root debridement, clinical parameters were carefully evaluated and they included the following: 1. dichotomous (yes/no) FMPI according to O’Leary et al. on 4 tooth surfaces (i.e., distal, buccal, mesial, and lingual).¹⁵ The index was determined by dividing the number of surfaces with plaque by the number of all studied surfaces; 2. dichotomous (yes/no) BoP index according to Ainamo and Bay.¹⁶ Bleeding was assessed at 6 points for each tooth (i.e., distobuccal, buccal, mesiobuccal, distolingual, lingual, and mesiolingual). The index was determined by dividing the number of bleeding points by the number of all assessed points; 3. PPD in 6 points of each tooth as a distance from the gingival margin to the bottom of the pocket; 4. CAL in 6 points of each tooth as a distance from the cemento-enamel junction (CEJ) to the bottom of the pocket; 5. gingival recession (GR) in buccal point of each tooth as a distance from CEJ to the gingival margin; 6. width of keratinized tissue (WKT) was evaluated as a distance from the gingival margin to the mucogingival junction evaluated mid-buccally. Mucogingival junction was demarcated by staining the mucogingival complex with iodine solution; 7. gingival phenotype was categorized as thin if gingival thickness was ≤ 1 mm, and as thick if gingival thickness was > 1 mm. Gingival thickness was measured 2 mm apically to the gingival margin by perpendicularly inserting a 10-millimeter endodontic spreader with a silicone stopper until the alveolar bone or root surface was reached. An electronic caliper (YATO® YT-7201; Toya, Wrocław, Poland) was used to assess gingival thickness indicated on the endodontic instrument with a rubber stop; 8. interdental contact point was recorded as present or absent. All the abovementioned clinical measurements were registered by 1 calibrated examiner using a graded periodontal probe (UNC probe 15 mm; Hu-Friedy, Chicago, USA).

Standardized intraoral radiographs were taken for each site with film holders and paralleling cone technique using an X-ray unit operating at 70 kV, 4 mA and 0.1-second exposure time. The radiographs were evaluated using Planmeca Romexis® Viewer software (Planmeca, Helsinki, Finland). Some anatomical landmarks, such as CEJ, alveolar crest (AC) and base of the defect (BD) were selected and 2 auxiliary lines were drawn, 1st in the tooth axis (AUX1) and 2nd (AUX2) from AC, perpendicularly to AUX1. The following measurements were obtained: 1. radiographic defect depth (DD) as a distance from the spot where AUX2 crossed the CEJ–BD line to the base of the defect; 2. radiographic defect angle (RVG angle) between the intersection of CEJ–BD line of the tooth and the delimitation of the wall of the defect; 3. distance from CEJ to AC (alveolar crest position – ACP). Subjects who fulfilled all of the inclusion criteria were enrolled into surgical treatment.

Surgical treatment and intrasurgery evaluation

All defects were treated in line with guidelines of minimally invasive surgery. The procedure was started with incisions in the interdental spaces. The choice of incision was dependent on the width of the interdental space. In the case of narrow spaces, an SPPF incision was used, and in the case of wide spaces, MPPT. In the spaces between molars and teeth with difficult access, SPPF incisions were made regardless of the width of the space. These cuts went further into intrasulcular incisions. The extent of the incision depended on the extent of the defect. If necessary, vertical cuts were prepared. The full-thickness buccal flaps were then reflected. Subsequently, the interdental papilla were separated from the bone base using a scalpel and the palatal flap was prepared. The next step was to remove the granulation from cavities and to perform scaling and root planing (SRP) using hand and ultrasonic instruments.

After surgical debridement, the following evaluation was made: 1. depth of the defect as the distance from the alveolar crest to the deepest point in the defect; 2. width of the defect as the distance from the alveolar crest to the root surface; 3. the number of remaining walls of the defects. Defects were categorized as 1-, 2- or 3-wall.

Intrabony defects were filled with biomaterials (Bio-Oss®; Geistlich Biomaterials, Princeton, USA / Gen-Os®; Tecno, Turin, Italy / allogenic bone grafts; Department of Transplantology and Cell Tissue Bank, Medical University of Warsaw, Poland) and covered with collagen membrane (Bio-Gide®; Geistlich Biomaterials). In order to advance the flap coronally without tension, a periosteal incision was performed at its base. The interdental spaces were closed with vertical modified mattress sutures (5/0 polypropylene monofilament suture, Prolene® 5/0 16 mm 3/8; Ethicon, Somerville, USA), and vertical incisions with simple sutures. Primary flap closure was obtained in all sites.

Postsurgery procedures and assessments

After the procedure, patients were administered 600 mg of ibuprofen; the next the same dose was taken by patients after 8 h. Patients were then given postoperative recommendations that included: 1. rinsing the mouth with 0.2% chlorhexidine solution (Curasept® ADS 220; Curaden Polska Sp. z o.o., Wrocław, Poland) 3 times daily for 1 min; 2. no brushing of the treatment area; 3. a soft, mild diet; 4. limiting physical effort; 5. control visit after 7 and 14 days. During the follow-up visits, wound healing in the interdental spaces was evaluated and the supragingival plaque was removed from the entire dentition using prophylaxis brushes and gel containing 1% chlorhexidine (Curasept ADS 100; Curaden Polska Sp. z o.o.). The sutures were removed after 2 weeks.

Healing assessment was based on EHI, as categorized in the following grades: 1. EHI = 1 – complete flap closure, no fibrin line in the interproximal area; 2. EHI = 2 – complete flap closure, thin fibrin line in the interproximal area; 3. EHI = 3 – complete flap closure, fibrin clot in the interproximal area; 4. EHI = 4 – incomplete flap closure, partial necrosis of the interproximal tissue; 5. EHI = 5 – incomplete flap closure, complete necrosis of the interproximal tissue (Fig. 1).¹⁴ EHI $\leq$ 3 was regarded as primary healing, while EHI $\geq$ 4 as secondary healing.

Fig. 1. Early Wound-Healing Index (EHI). A – EHI = 1 of the papilla between teeth 35 and 34: complete flap closure and no fibrin line in the interproximal area; B – EHI = 2 of the papilla between teeth 36 and 35: complete flap closure and thin fibrin line in the interproximal area; C – EHI = 3 of papilla between teeth 46 and 45: complete flap closure and fibrin clot in the interproximal area; D – EHI = 4 of papilla between teeth 37 and 36: incomplete flap closure and partial necrosis of the interproximal tissue

Statistical analysis

For the statistical analysis, measurements at the site with the greatest presurgical CAL value were used. Data was expressed as mean $\pm$ standard deviation (SD).

Descriptive analyses on early postoperative healing were based on the entire defect population ($n = 59$). The EHI was the primary outcome variable. The patient-related (age, gender, FMPI, and BoP) and site-related parameters (tooth type, tooth position, PPD, CAL, GR, presence of interdental contact point, width of interdental space, WKT, phenotype, DD, RVG angle, ACP, defect configuration, defect depth and width), together with technical aspects (presence of vertical incision, papilla preservation technique) were considered to be independent variables. Patients were divided according to papilla preservation technique (MPPT or SPPF) to evaluate which parameters were associated with wound healing after 1 and 2 weeks. Analyses were carried out for sites being categorized using the EHI. The EHI was treated as a binomial variable. Analyses were carried out for sites that were divided as EHI $\leq$ 2 or EHI $\geq$ 3, as well as by comparing sites with primary healing (EHI $\leq$ 3) with sites that presented secondary healing (EHI $\geq$ 4).

Relationships between the abovementioned variables and EHI depending on papilla preservation technique were evaluated using Pearson's correlation coefficient. Attributes of patients (such as age and sex) were assigned to teeth, but patients were not treated as experimental units. All analyses were conducted at site level, not at patient level. For some patients, only 1 site was assessed, but the general assumption was that analyses are at site level. Subsequently, statistically significant factors were entered into a logistic regression model and odds ratios (ORs) were calculated. The valuation of predictive power of independent variables in logistic regression was based only on ORs and p-values.

Statistical analysis was performed using STATISTICA v. 13 (StatSoft Inc., Tulsa, USA). Any p-values of less than 0.05 were considered statistically significant. The strength of correlations was determined by R value; if it was >0.70 , the correlation was considered strong; if it was 0.40 – 0.70 , the correlation was considered moderate; and if it was <0.40 , the correlation was considered weak.

Results

The data and information on 25 patients (18 females, mean age 35.1 ± 11.07 years, and 7 males, mean age 39.8 ± 4.5) extracted from 3 previous studies were included in this secondary analysis. Patients presented with 59 intraosseous defects and 45 surgical procedures were carried out. Four patients presented with 1 defect, 14 patients presented with 2 defects, 4 patients presented with 3 defects, and 3 patients presented with 5 defects. Defect characteristics are shown in Table 1.

After 1 week, the EHI was 2.18 ± 0.97 , while after 2 weeks the EHI was 1.92 ± 1.39 and ranged from 1.0 to 4.0. Fifty-five sites demonstrated primary healing (EHI: 1–3) after 1 week and 45 sites exhibited primary healing after

2 weeks. Four sites after 1 week and 14 sites after 2 weeks showed secondary healing (EHI = 4). None of the defects demonstrated EHI = 5.

Table 1. Baseline clinical features for the study group

Variables	Sites (n = 59)
Tooth type (n)	
molars	34
premolars	14
upper incisors, canines	11
Tooth position (n)	
maxillary teeth	18
mandibular teeth	41
Clinical measurements	
FMPI (%)	9.30 (7.31–11.30) $\pm$ 6.87
BoP (%)	13.17 (11.45–14.90) $\pm$ 5.94
PPD [mm]	7.31 (6.98–7.63) $\pm$ 1.24
CAL [mm]	8.46 (8.07–8.85) $\pm$ 1.50
GR [mm]	1.27 (1.03–1.52) $\pm$ 0.94
Radiographic measurements	
DD [mm]	5.08 (4.68–5.49) $\pm$ 1.56
ACP [mm]	3.99 (3.55–4.42) $\pm$ 1.67
RVG angle [°]	27.96 (25.62–30.31) $\pm$ 8.99
Sites-specific characteristics	
interdental contact point (present/absent)	50/9
interdental space width [mm]	2.66 (2.47–2.86) $\pm$ 0.75
WKT [mm]	2.78 (2.55–3.01) $\pm$ 0.89
phenotype (thin/thick)	13/46
Technical aspects	
vertical incision (yes/no)	23/36
papilla preparation	
MPPT (n)	24
SPPF (n)	35
Intrabony defect characteristics	
depth [mm]	4.92 (4.40–5.43) $\pm$ 1.77
width [mm]	3.01 (2.68–3.34) $\pm$ 1.13
Defect configuration (n)	
1-wall	12
2-wall	19
3-wall	28
Healing after 1 week	
primary/secondary (n)	55/4
EHI	
1 (n)	22
2 (n)	17
3 (n)	16
4 (n)	4
5 (n)	0
Healing after 2 weeks	
primary/secondary (n)	45/14
EHI	
1 (n)	43
2 (n)	2
3 (n)	0
4 (n)	14
5 (n)	0

The means with 95% CI (in brackets) and $\pm$ SD of probing values and radiographic measurements of the defects before surgery.

FMPI – full-mouth plaque index; BoP – bleeding on probing index; PPD – probing pocket depth; CAL – clinical attachment level; GR – gingival recession; DD – radiographic defect depth; ACP – alveolar crest position; RVG angle – radiographic defect angle; WKT – width of keratinized tissue; MPPT – modified papilla preservation technique; SPPF – simplified papilla preservation flap; EHI – Early Wound-Healing Index; n – number of defects; SD – standard deviation; CI – confidence interval.

Correlations between patient-, site- and technique-related factors and postoperative healing depending on papilla preservation technique (SPPF/MPPT/both) are summarized in Tables 2–4, which present correlation coefficients. No associations between age, gender, BoP, PPD, CAL, DD, ACP, defect depth, presence of vertical incision, interdental space width, and WKT were observed. Weak correlations ($R < 0.40$) were found with tooth position, tooth type, FMPI, RVG angle, defect width, and phenotype. When analysis distinguished between SPPF sites and MPPT sites, weak correlations between postoperative healing and tooth position / GR at SPPF sites were detected, while moderate correlations ($R = 0.40$ – 0.70) with tooth type / phenotype at SPPF sites, as well as moderate correlations with FMPI / presence of interdental space at MPPT sites were noted.

When site-related factors were put into a multiple regression analysis, the model was statistically significant ($p < 0.05$) for tooth type and phenotype for all sites; tooth type, phenotype and gingival recessions in cases of SPPF sites; and presence of interdental contact point in cases of MPPT sites (Tables 5,6). Tooth type and GR were significantly correlated ($p = 0.029$ and $p = 0.032$, respectively) with EHI after 1 week only in SPPF sites, whereas tooth type and phenotype determined healing 2 weeks postoperatively in all sites ($p = 0.033$ and $p = 0.004$, respectively). At MPPT sites, the presence of the interdental contact point affected the healing after 2 weeks ($p = 0.053$). Generally speaking, the likelihood of better healing was higher for molars than 1-rooted teeth (incisors / canines / premolars) ($OR = 0.24$) and sites with a thick phenotype ($OR = 0.13$). On the other hand, the presence of gingival recession or interdental contact points increased the probability of impaired healing (higher grades of the EHI; $OR = 32.78$ and $OR = 0.08$, respectively).

Discussion

In the present analysis, we evaluated the early healing phase after GTR treatment of intrabony defects in patients with AgP. To achieve this goal, the EHI was used. To the best of the authors' knowledge, the importance of the EHI has never been investigated in any study to express healing phase of periodontal wounds in patients with AgP. In all cases, 1 type of resorbable collagen membrane was used, whereas intraosseous defects were filled using various biomaterials. The use of barrier membranes was associated with the preparation of more extensive flaps and with periosteal incisions to obtain coronal flap mobilization without tension. In many cases, vertical cuts were also made. Recent research on blood clot formation during early wound healing revealed that even minimal tensile forces on the blood clot affect its morphology and nature.¹⁷ Albeit primary tension-free flap closure was obtained at suturing in all treated defects, some sites presented

Table 2. Correlations between patient-, site- and technique-related parameters and healing p-values in brackets

Variables	1 week		2 weeks	
	EHl ≤ 2 or EHl ≥ 3	EHl ≤ 3 or EHl = 4	EHl ≤ 2 or EHl ≥ 3	EHl ≤ 3 or EHl = 4
Age [years]	0.20 (0.183)	0.05 (0.754)	−0.07 (0.636)	−0.11 (0.450)
Gender (females/males)	−0.12 (0.372)	−0.01 (0.952)	0.00 (0.993)	0.05 (0.700)
Tooth position (maxillary teeth / mandibular teeth)	−0.07 (0.614)	−0.26* (0.046)	−0.04 (0.790)	−0.12 (0.364)
Tooth type (incisor/canines/premolars/molars)	−0.29* (0.026)	−0.13 (0.310)	−0.29* (0.027)	−0.24 (0.065)
FMPI [%]	0.32* (0.028)	−0.13 (0.383)	0.14 (0.338)	0.12 (0.405)
BoP [%]	0.26 (0.076)	0.19 (0.187)	0.16 (0.270)	0.17 (0.243)
PPD [mm]	0.04 (0.763)	0.10 (0.460)	0.08 (0.545)	0.17 (0.192)
CAL [mm]	0.03 (0.815)	0.23 (0.074)	0.09 (0.497)	0.11 (0.415)
GR [mm]	−0.06 (0.632)	0.21 (0.110)	−0.04 (0.792)	−0.09 (0.517)
DD [mm]	−0.03 (0.806)	0.09 (0.499)	−0.14 (0.286)	−0.09 (0.479)
RVG angle [°]	−0.01 (0.921)	0.05 (0.724)	0.26* (0.046)	0.23 (0.082)
ACP [mm]	0.02 (0.855)	−0.06 (0.659)	0.00 (0.994)	−0.04 (0.751)
Vertical incision (present/absent)	0.16 (0.214)	0.22 (0.101)	−0.07 (0.578)	−0.01 (0.927)
Defect morphology (1-/2-/3-wall)	−0.08 (0.564)	0.08 (0.550)	0.08 (0.540)	0.15 (0.267)
Defect depth [mm]	0.07 (0.632)	0.06 (0.679)	−0.16 (0.264)	−0.13 (0.373)
Defect width [mm]	−0.32* (0.027)	−0.16 (0.290)	−0.03 (0.856)	−0.03 (0.858)
Papilla preservation technique (SPPF/MPPT)	0.16 (0.216)	−0.05 (0.700)	0.14 (0.283)	0.17 (0.207)
Interdental contact point (present/absent)	0.01 (0.954)	−0.07 (0.582)	−0.10 (0.456)	−0.08 (0.562)
Interdental space width [mm]	−0.16 (0.217)	−0.01 (0.922)	−0.17 (0.196)	−0.18 (0.176)
WKT [mm]	0.03 (0.850)	−0.09 (0.521)	−0.06 (0.662)	−0.03 (0.818)
Phenotype (thin/thick)	−0.17 (0.208)	−0.02 (0.885)	−0.39* (0.002)	−0.35* (0.007)

FMPI – full-mouth plaque index; BoP – bleeding on probing index; PPD – probing pocket depth; CAL – clinical attachment level; GR – gingival recession; DD – radiographic defect depth; ACP – alveolar crest position; RVG angle – radiographic defect angle; WKT – width of keratinized tissue; MPPT – modified papilla preservation technique; SPPF – simplified papilla preservation flap; EHl – Early Wound-Healing Index; * statistically significant ($p < 0.05$).

with secondary healing. The values of the EHl ranged from 1 to 4. After 7 days from the procedure, secondary healing was related to <7% of the examined sites, but after 2 weeks, this percentage increased to 23.73%. Most frequently, there was a loss of primary closure in places where the EHl values = 3. No correlations between the EHl and any patient-related factors were found. However, a multivariate analysis revealed that 1-rooted teeth (incisors, canines and premolars), as well as sites with thin phenotype and with the presence of gingival recessions were associated with impaired healing (higher EHl recordings and secondary healing).

Over the next 2–4 weeks, a complete epithelialization of exposed barrier membranes occurred in patients who were subject to secondary healing (data not shown). All patients were subject to a rigorous post-treatment protocol and used a mouth-rinsing solution containing 0.2% chlorhexidine. Due to the lack of mechanical plaque control in the treatment area, this seems to be a “golden standard”. However, Laugisch et al.¹³ found that a postoperative protocol including 0.05% chlorhexidine herbal extract might have the potential to improve patient compliance during postoperative period, in comparison with 0.1% chlorhexidine solution, as tooth staining and discomfort associated with irritation or taste were more frequent in the former.

The present findings might be associated with the relatively small dimensions of 1-rooted teeth and the small distances between them when compared to molars, which might be further complicated by dental malposition and crowding. The gingival vessels on the buccal side run in the apical-coronal direction; therefore, incisions in the papilla may lead to disturbances of blood flow within the interdental papilla.¹⁸ Vascularization of these gingival areas is mainly a network of vascular anastomoses and loops, so both the number of soft tissues in the interproximal spaces, and their structure may affect the regulation of blood flow after surgery.¹⁹ Furthermore, animal studies proved that the mandibular vasculature was characterized by arterial vessels transversing somewhat obliquely in a general posterior to anterior direction.²⁰ In addition, the collateral vessels from the periodontal ligament contribute to the marginal blood supply. The morphology of gingiva remains in close relation to the process of eruption, as well as to shape and position of the teeth, and to the shape of the alveolar ridge. The characteristics of gingival morphology, bone and teeth are referred to as gingival phenotype. Thin and thick phenotypes are most often distinguished. In the case of the thin phenotype, wherein the gum is thin and delicate, the course of the gingival margin is strongly arched and there are often gingival recessions

Table 3. Correlations between patient-, site- and technique-related parameters and healing at SPPF sites (p-values in brackets)

Variables	1 week		2 weeks	
	EHl ≤ 2 or EHl ≥ 3	EHl ≤ 3 or EHl = 4	EHl ≤ 2 or EHl ≥ 3	EHl ≤ 3 or EHl = 4
Age [years]	0.04 (0.862)	0.04 (0.857)	−0.17 (0.393)	−0.26 (0.189)
Gender (females/males)	−0.05 (0.775)	0.14 (0.406)	0.19 (0.279)	0.26 (0.135)
Tooth position (maxillary teeth / mandibular teeth)	−0.29 (0.088)	−0.36* (0.032)	−0.07 (0.669)	−0.20 (0.239)
Tooth type (incisors/canines/premolars/molars)	−0.46* (0.006)	−0.19 (0.266)	−0.43* (0.010)	−0.36* (0.033)
FMPI [%]	0.17 (0.393)	−0.17 (0.409)	0.13 (0.517)	0.10 (0.618)
BoP [%]	0.32 (0.099)	0.13 (0.532)	−0.01 (0.957)	0.00 (0.989)
PPD [mm]	−0.06 (0.716)	0.03 (0.866)	0.06 (0.720)	0.20 (0.252)
CAL [mm]	0.00 (0.986)	0.23 (0.178)	0.14 (0.423)	0.18 (0.300)
GR [mm]	−0.07 (0.674)	0.36* (0.032)	−0.04 (0.820)	−0.12 (0.499)
DD [mm]	0.11 (0.522)	0.12 (0.499)	−0.14 (0.421)	−0.04 (0.803)
RVG angle [°]	0.07 (0.675)	−0.09 (0.615)	0.32 (0.060)	0.27 (0.116)
ACP [mm]	0.04 (0.812)	−0.13 (0.459)	0.15 (0.387)	0.09 (0.625)
Vertical incision (present/absent)	0.11 (0.526)	0.18 (0.307)	−0.27 (0.122)	−0.16 (0.361)
Defect morphology (1-/2-/3-wall)	−0.06 (0.747)	0.23 (0.191)	0.13 (0.470)	0.23 (0.191)
Defect depth [mm]	−0.10 (0.602)	0.10 (0.626)	−0.21 (0.303)	−0.15 (0.452)
Defect width [mm]	−0.05 (0.801)	−0.28 (0.151)	−0.08 (0.704)	−0.04 (0.845)
Interdental contact point (present/absent)	0.30 (0.077)	0.06 (0.729)	0.16 (0.348)	0.17 (0.339)
Interdental space width [mm]	−0.28 (0.097)	−0.13 (0.447)	−0.29 (0.096)	−0.26 (0.125)
WKT [mm]	−0.06 (0.745)	−0.24 (0.166)	−0.20 (0.243)	−0.17 (0.342)
Phenotype (thin/thick)	−0.23 (0.175)	0.09 (0.613)	−0.60* (<0.001)	−0.53* (0.001)

All the explanations as in Table 2.

Table 4. Correlations between patient-, site- and technique-related parameters and healing at MMPT sites (p-values in brackets)

Variables	1 week		2 weeks	
	EHl ≤ 2 or EHl ≥ 3	EHl ≤ 3 or EHl = 4	EHl ≤ 2 or EHl ≥ 3	EHl ≤ 3 or EHl = 4
Age [years]	0.38 (0.090)	0.07 (0.762)	0.04 (0.854)	0.04 (0.854)
Gender (females/males)	−0.21 (0.326)	−0.22 (0.309)	−0.32 (0.126)	−0.32 (0.126)
Tooth position (maxillary teeth / mandibular teeth)	0.29 (0.170)	−0.14 (0.520)	0.04 (0.849)	0.04 (0.849)
Tooth type (incisors/canines/premolars/molars)	−0.21 (0.329)	−0.05 (0.823)	−0.21 (0.313)	−0.21 (0.313)
FMPI [%]	0.52* (0.016)	−0.08 (0.740)	0.15 (0.508)	0.15 (0.508)
BoP [%]	0.36 (0.122)	0.00 (0.985)	0.01 (0.972)	0.01 (0.972)
PPD [mm]	0.21 (0.318)	0.21 (0.331)	0.10 (0.633)	0.10 (0.633)
CAL [mm]	0.17 (0.429)	0.22 (0.291)	0.11 (0.605)	0.11 (0.605)
GR [mm]	0.09 (0.669)	0.07 (0.729)	0.11 (0.607)	0.11 (0.607)
DD [mm]	−0.11 (0.596)	0.04 (0.853)	−0.07 (0.751)	−0.07 (0.751)
RVG angle [°]	−0.11 (0.605)	0.19 (0.368)	0.20 (0.344)	0.20 (0.344)
ACP [mm]	0.15 (0.491)	−0.03 (0.881)	−0.09 (0.685)	−0.09 (0.685)
Vertical incision (present/absent)	0.20 (0.344)	0.28 (0.189)	0.19 (0.382)	0.19 (0.382)
Defect morphology (1-/2-/3-wall)	−0.12 (0.578)	−0.10 (0.652)	0.00 (1.000)	0.00 (1.000)
Defect depth [mm]	0.28 (0.223)	0.01 (0.961)	−0.12 (0.591)	−0.12 (0.591)
Defect width [mm]	−0.41 (0.065)	−0.07 (0.768)	0.17 (0.470)	0.17 (0.470)
Interdental contact point (present/absent)	−0.32 (0.123)	−0.14 (0.520)	−0.45* (0.027)	−0.45* (0.027)
Interdental space width [mm]	0.18 (0.390)	0.05 (0.823)	0.16 (0.464)	0.16 (0.464)
WKT [mm]	0.08 (0.709)	0.26 (0.218)	0.19 (0.365)	0.19 (0.365)
Phenotype (thin/thick)	−0.24 (0.259)	−0.08 (0.718)	−0.35 (0.097)	−0.35 (0.097)

All the explanations as in Table 2.

Table 5. Odds ratios (ORs) calculated for site-specific factors associated with wound healing at 1 week

Factor	OR	95% CI	p
EHl ≤ 2 or EHl ≥ 3			
Tooth type (SPPF sites)	0.17	0.03–0.83	0.029
EHl ≤ 3 or EHl = 4			
GR (SPPF sites)	32.78	1.34–799.6	0.032

SPPF – simplified papilla preservation flap; GR – gingival recession.

Table 6. Odds ratios (ORs) calculated for site-specific factors associated with wound healing at 2 weeks

Factor	OR	95% CI	p
EHl ≤ 2 or EHl ≥ 3			
Tooth type (all sites)	0.24	0.06–0.089	0.033
Phenotype (all sites)	0.13	0.03–0.52	0.004
Tooth type (SPPF sites)	0.09	0.02–0.52	0.007
Phenotype (SPPF sites)	0.02	0.00–0.49	0.016
Presence of interdental contact point (MPPT)	0.08	0.01–1.03	0.053 [†]
EHl ≤ 3 or EHl = 4			
Phenotype (all sites)	0.13	0.03–0.52	0.004
Tooth type (SPPF sites)	0.17	0.03–0.83	0.028
Phenotype (SPPF sites)	0.03	0.00–0.71	0.029
Presence of interdental contact point (MPPT)	0.08	0.01–1.03	0.053 [†]

SPPF – simplified papilla preservation flap; MPPT – modified papilla preservation technique; EHl – Early Wound-Healing Index; [†] borderline statistical significance.

and a narrow band of keratinized tissue. The shape of the teeth is close to the triangle, and the contact points are close to the incisal edges. Characteristic features of the thin phenotype are instability of tissues and their high sensitivity to all kinds of injuries, including those related to surgical treatment. Tissue phenotype might be a significant factor influencing surgical treatment outcomes, as a flap thickness ranging from 0.8 to 1.2 mm was associated with a more predictable prognosis.²¹ Consequently, flap thickness might be assumed to affect the vascularity of the pedicle.

When comparing the present results with those from other studies, it has to be underlined that different biomaterials and miscellaneous regenerative strategies were implemented. For instance, Wachtel et al.¹⁴ described a high ratio (89–96%) of sites showing EHl ≤ 2 in MPPT sites 2 weeks postoperatively. It has to be kept in mind that in the abovementioned study the authors used the microsurgical access flap and enamel matrix derivatives (Emdogain®; Straumann, Basel, Switzerland) alone. Similarly, in the study by Farina et al.¹² following the buccal single flap approach (SFA), highly predictable (>80%) complete flap closure and optimal healing of about 50% of sites at 2 weeks were observed. Some site-specific characteristics, such as the narrow base of the interdental papilla, the presence of an interdental contact point

and interdental soft tissue crater were noted in the group with either EHl > 1 or EHl > 3 compared to the group with EHl = 1. However, 2-week soft tissue healing was not associated with 6-month clinical results. Moreover, sites treated with reconstructive devices (graft, graft + Emdogain, graft + membrane) exhibited suboptimal healing (EHl > 1). On the other hand, Rakmanee et al.²² compared clinical outcomes between GTR and access flap (AF) in which SPPF were used in patients with AgP. While healing at the control sites seemed to be uneventful, in 13 out of 18 GTR-treated sites, the authors noted membrane exposure and secondary healing during the early postoperative phase. Moreover, CAL gain was found to be diminished at the sites with membrane exposure, compared to those with the non-exposed membrane. A meta-analysis that evaluated the effect of membrane exposure on the obtained clinical treatment results showed that the sites with an exposed membrane had a significantly reduced CAL gain (4.22 (0.15) mm) than the sites without membrane exposure (4.69 (0.13) mm) ($p < 0.05$).²³ Aside from this, exposing the barrier membrane increases the probability of bacterial colonization, and exposure to the oral environment can accelerate resorption of absorbable membranes.^{24,25}

All things considered, it may be speculated that the ancillary use of biomaterials may hinder obtaining favorable early healing of soft tissues. The use of a barrier membrane may be the reason for the temporary hindrance of mucoperiosteal flaps' revascularization.²⁶ Zanetta-Barbosa et al.²⁷ demonstrated the existence of a relationship between impaired blood flow in the flaps associated with the use of the barrier membrane and the incidence of wound dehiscences. Consequently, membranes might severely impinge on the microcirculation of flaps and jeopardize optimal physiological wound healing. Therefore, the selection of a regeneration strategy should be given careful consideration, especially in the case of extensive and non-supporting intrabony defects. In such cases, 2 options should be considered: GTR + biomaterial (this strategy was evaluated in our study) or Emdogain + biomaterial. The choice of technique depends on a detailed analysis of individual conditions and an assessment of likely outcomes of treatment after the application of a given technique in a specific clinical case. It should be emphasized that membrane-based surgical procedures are more demanding; therefore, technical errors can significantly worsen the effects of treatment. Thus, in a situation where the abovementioned techniques have the same indications to be performed, the choice of Emdogain + biomaterial seems to be a safer method.²⁸

When interpreting the findings of this study, it is important to understand that only early healing within 2 weeks of GTR was assessed. Various biomaterials were used in the treatment of intraosseous defects, but only 1 type of resorbable collagen membrane was used. Therefore, the impact of the type of material used for

defect filling on soft tissue healing cannot be ruled out, but it seems unlikely. On the other hand, due to the time of observation, it is not known whether early healing would affect the clinical outcome of the treatment after 6 and 12 months, and in a longer perspective. Moreover, some limitations of a secondary analysis, such as higher risk of bias, must be emphasized. However, all data of interest was collected from all patients, so it was available for the analysis. The researchers who were analyzing the data were involved in the data collection process. Consequently, they were aware of study-specific nuances that might have been important for understanding of variables in the dataset. It would definitely be advisable to perform a similar analysis based on a larger group of patients and treatment sites, because the limited number of points in the study could be too small for a statistical analysis to show a significant impact of other factors, related not only to the treatment site but also to the patient, on early healing of soft tissues.

Conclusions

Within the limitations of this secondary analysis, it can be said that GTR of intrabony defects in patients with AgP resulted in a complete wound closure in a great majority of sites, especially after 7 days. However, site-related factors may impinge on early postoperative healing, which should be taken into account when choosing a regenerative strategy. Thick gingival phenotype might be a prerequisite for optimal wound healing.

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