The influence of tissue adhesive to palatal donor site healing after de-epithelialized gingival graft harvesting: a randomized clinical trial

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Abstract

Aim: De-epithelialized gingival graft (DGG) is recommended due to its simplicity and successful outcomes. However, donor site morbidity still poses a problem. The aim of this trial was to evaluate the effectiveness of gelatin sponge-cyanoacrylate (GS-CY) combination at donor site healing.

Materials and Methods: DGG donor sites were treated with either GS (control) (n=21) or GS+CY (test) (n=21). Palatal tissue thickness, graft dimensions, working time (WT), primary bleeding time were recorded. Reported quantity of analgesics (QA), secondary bleeding (SB), and pain perception (PP) during the first week. Sensation loss (SL), color match (CM) and epithelization level (EL) at donor site were evaluated on days 7, 14, 21, and 28.

Results: SB was lower in GS+CY during the first three days ($p<0.05$). GS+CY showed less PP throughout the seven days period ($p<0.004$). QA was lower in GS+CY group but the inter-group difference reached to statistical significance at only day 1 ($p=0.003$). EL and CM did not exhibit any inter-group difference ($p>0.05$).

Conclusions: GS-CY combination is a strong candidate to improve donor site healing by showing less WT, PP, and SB findings. However, profits seem to be associated with its isolation property rather than increased EL. Further studies are needed to understand its efficacy on healing.

Keywords: Cyanoacrylate; gelatin sponge; graft harvesting; donor site morbidity.

Introduction

Bilaminar connective tissue graft (CTG) procedure is considered as 'gold standard' amongst the root coverage procedures due to its highest predictability on treatment outcomes (Zucchelli & Mounssif, 2015). Authors recommend different CTG harvesting techniques such as trap-door, double-incision, single-incision, and de-epithelialized gingival graft (DGG) as a free gingival graft (FGG) depending on the indication (Zuhr *et al*., 2014). In FGG and DGG harvesting methods, donor site heals with the secondary intention that may cause higher postoperative discomfort (Del Pizzo *et al*., 2002; Zucchelli *et al*., 2010). Nevertheless, recent meta-analysis reported that better root coverage and keratinized tissue gain outcomes can be obtained by using DGG compared to other CTG harvesting techniques (Tavelli *et al*., 2019b).

Despite better clinician- and patient-based outcomes, CTG harvesting still causes significant morbidity that needs further management (Tavelli *et al*., 2018). For this aim, various materials/methods such as periodontal dressing (Tavelli *et al*., 2018), acrylic stents (Keceli *et al*., 2015a), low-level laser therapy (Ustaoglu *et al*., 2017), photobiomodulation (Heidari *et al*., 2017), platelet-rich fibrin (PRF) (Femminella *et al*., 2016), hemostatics (Keceli *et al*., 2015a), tissue adhesives (Tavelli *et al*., 2018), hyaluronic

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acid (Yildirim *et al*., 2017) or flurbiprofen spray (Isler *et al*., 2018) have been utilized. From those, absorbable gelatin sponge (GS) is a low-cost hemostatic that provides bleeding control and clot stabilization in extraction sockets and other secondary healing wounds (Tavelli *et al*., 2018). Despite its limited benefit on pain reduction and recovery rate, GS is still one of the most frequently used materials/methods due to its low-cost, rapid bleeding control and unproblematic degradation properties (Saroff *et al*., 1982; Zucchelli *et al*., 2010). Another material with adhesive, hemostatic, biodegradable, and non-toxic properties, is cyanoacrylate (CY) (Borie *et al*., 2019). CYs are being used as tissue adhesive and dressing material in emergency departments, in surgical operations, and in dentistry on open or sutured wounds (Gumus & Buduneli, 2014; Singer *et al*., 2008). Gumus & Buduneli (2014) revealed that the perceived pain in the recipient site is significantly less when CY adhesive is used instead of suture materials. During the conduction of the present trial, Tavelli *et al*. (2018; 2019c) also evaluated the impact of various hemostatic agents/methods by mainly focusing on pain perception and reported that the GS+CY combination gave the best pain reduction result during palatal donor site healing.

The present trial aimed to evaluate the effectiveness of this combination through palatal wound healing, pain, sensation, and bleeding parameters by comparing with GS only.

Materials and Methods

Trial design

The prospective, randomized controlled trial was conducted between July 2018 and February 2019 at Hacettepe University Periodontology Department after ethical approval from the local ethics committee (Date: 21.06.2018, No: GO-18/578-28) in accordance with Helsinki Declaration (1975, revised at Tokyo in 2004) and CONSORT statement. All volunteers signed informed consent after understanding the objectives and methods.

Inclusion criteria

- Age ≥18 years.
- Periodontal plastic surgery indication that needs CTG in the mandible to be able to differentiate the subjective symptoms of donor and recipient sites.
- Clinically healthy gingiva after phase I therapy (full-mouth plaque and bleeding scores <15%).

Exclusion criteria

- Previous palatal harvesting history.
- Unstable endodontic conditions.
- Tooth mobility at the surgical site.
- Systemic disease.
- Pregnancy.
- Use of medications with potentially adverse effects on periodontal tissues.

Phase I therapy, randomization, allocation concealment, and blinding

Full-mouth supragingival scaling-polishing was performed and oral hygiene instructions were given at least three weeks before the surgical phase. One author randomly assigned the patients, with 1:1 allocation ratio, into GS and GS+CY groups by making simple randomization without stratification (computer-generated randomization scheme). Number-labeled opaque envelopes containing the name of the assigned method were used for allocation concealment. The surgical procedures were carried out by one author whereas another author acquired the intra- and postoperative data.

Surgical procedure and intraoperative measurements

After local anesthesia (2% articaine HCl with epinephrine 1:100,000), palatal tissue thickness (PTT) was measured by perpendicularly inserting a Michigan-O periodontal probe from the corners of the rectangular donor area and recorded as their mean value. Epithelialized FGG was harvested from the palate with the method described by Zucchelli *et al*. (2010). After a rectangular initial incision, the graft with 1-1.5 mm thickness was harvested approximately 1.5-3 mm away from gingival margins of upper teeth. The donor site was closed either with GS (Spongostan®, Ethicon, Somerville) (GS group) or GS was covered with high viscosity CY (PeriAcryl®, Glustitch Inc., Delta, Canada) (GS+CY group) and in both groups, the applied materials were fixed with suspending palatal sutures. In the GS+CY group, CY was applied with a pipette as small drops until completely covering the GS over the wound.

Post-operative care

Analgesic (flurbiprofen, 100 mg, twice-a-day) was prescribed and started immediately after the surgery. Subsequent doses were taken only if necessary. Patients were suggested to abstain from brushing and other traumatic applications until suture removal. Antiseptic spray (Kloroben®, Drogsan, Ankara, Turkey, 0.12%, twice-a-day) application to the donor site was recommended. Seven days after surgery, sutures were removed and the region was irrigated with sterile saline.

Demographic and intra-operative data

Gender and age data were included in the assessment.

Intra-operative data

Immediately after harvesting, sterile gauze was compressed to the palatal wound for 2 minutes, blood wiped once every 30 seconds until bleeding stops. The duration was recorded as primary bleeding time (PBT). Dimensions of the graft were measured with a periodontal probe and the values were recorded as graft height (GH), graft width (GW), and graft thickness (GT). Working time (WT) was also measured and noted.

Post-operative data

Patients recorded quantity of analgesics (QA) taken, presence/absence of secondary bleeding (SB) and also instructed to score their daily donor site pain perception (PP) level (primary outcome measure) by using the visual analog scale (VAS) (0: no pain, 1: minimal pain, 10: severe pain) (Price *et al*., 1983) during the first postoperative week. Sensation loss (SL) at the donor site was scored as none, medium, or severe by testing with the method described by Del Pizzo *et al*. (2002) 7, 14, 21, and 28 days following surgery. Donor site color match (CM) was determined with a VAS scale (0: the absence of harmony, 10: excellent harmony) (Lektemur & Torumtay, 2020). At days 7, 14, 21, and 28, donor site epithelization level (EL) was scored as none, partial or full epithelization by means of bubble formation after dripping hydrogen peroxide (3%) to the wound surface (Marucha, 1998; Keceli *et al*., 2015a). Except for these parameters, all patients were instructed to give feed-backs about any extraordinary developments such as CY detachment, systemic infection findings, excessive edema, bad taste, or swelling.

Statistical analysis

The sample size was determined by considering PP as the main outcome variable with the assistance of previous data (Keceli *et al*., 2015a). Considering α=0.05 for independent groups in t-test, 20 patients for each group was found necessary to reach 80% power and regarding the drop-out possibility, the trial was started with 25 patients per group. The data collected in the trial were analyzed with IBM-SPSS v.25 (IBM, Chicago, IL) with a significance level of 5%. The quantitative variable analysis was made by t-test (displayed as mean±SD) whereas the chi-square test was performed during quantitative variable analysis (displayed as n(%)). To analyze the effect of multiple independent variables, multiple regression analysis was performed with the backward method. At the first step, group, age, gender, PTT, GT, WT, GH, and GW variables were included in the model. The final model was reached in step 4, and group, gender, age, GT, and GH variables were evaluated for the first day PP score ($p < 0.05$).

Results

The trial started with 50 patients, and 8 patients (4 from CY+GS and 4 from CS) who did not attend the appointments or notified personal reasons to give up just before the surgery were removed from the trial (Figure 1). Except for CY detachment noticed by two patients on day 5, no extra-ordinary local/systemic complications were reported. The trial ended with the completion of follow-up visits and analysis of the data. A clinical view of the healing steps is shown in Figure 2.

Demographic data

Patients comprised of 27 females and 15 males between 19 and 58 years old (39.02±10.61). No inter-group difference was present in terms of mean age (p=0.830) and gender (p=0.099) (Table 1).

Intra-operative data

No remarkable difference was present between GS+CY and GS regarding PTT (p=0.284) and GW (p=0.730). Mean GT were 1.60±0.49 and 1.97±0.25 for GS and GS+CY, respectively and the difference was statistically significant (p=0.005). WT was significantly lower (p<0.001) and PBT was significantly higher (p<0.001) at GS+CY compared to GS (Table 1).

Post-operative data

Both groups demonstrated SB. However, patient quantity with SB at the first three days was significantly less in GS+CY compared to GS (days 1 and 2, p=0.001; day 3, p=0.045) (Table 2) (Figure 3a).

Overall PP scores were less in GS+CY compared to GS throughout the seven days period and the difference was statistically significant at the first five days (p<0.004). Overall PP scores showed a reduction trend for seven days in GS. In GS+CY, a slight rise was seen after four days of steady decline (Table 2) (Figure 3B). Parallel to PP scores, QA was lower in GS+CY group but the inter-group difference reached to statistical significance at only day 1 (p=0.003) (Table 2).

None of the patients showed complete epithelization in the first week. Epithelization was completed on day 28 at all donor sites and there was no significant difference between the two groups throughout the trial (p>0.05) (Table 3).

Although the inter-group CM difference was in favor of the CY + GS group, the differences were not statistically significant (p>0.05) (Table 3).

Severe SL was detected at one patient in the GS group while moderate SL was present in both groups 7, 14, and 21 days after surgery. As examined on day 28, no sequel remained in any patient regarding SL. No inter-group difference was observed in terms of SL (p>0.05) (Table 3).

Multiple regression analysis with the backward method was performed for the day 1, PP score. Accordingly, variables of study group, gender, age, GT and GH were found significant (p<0.001, p=0.042, p=0.002, p=0.002 and p=0.039, respectively). The interpretation of the non-standardized coefficient (B) calculated from the model was as follows; while other variables in the model were under control, the PP score of a patient in the GS+CY group was, on average, 6.17 points lower than that of a patient in GS group (according to the univariate analysis result) this average difference was found as 4.76, and females had 1.41 lower points than males.

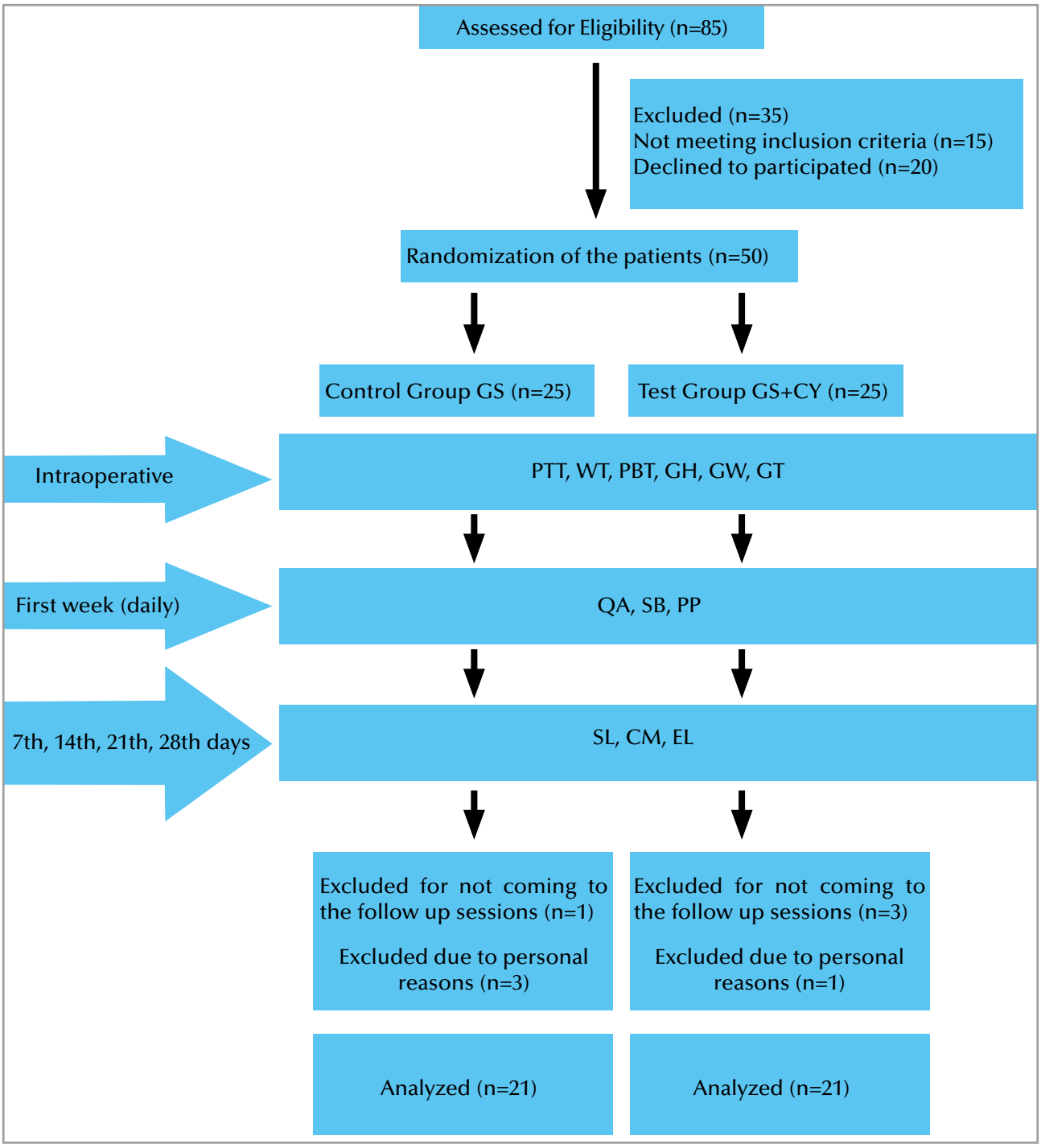

Figure 1. Flowchart of the trial (GS = gelatin sponge; CY = cyanoacrylate).

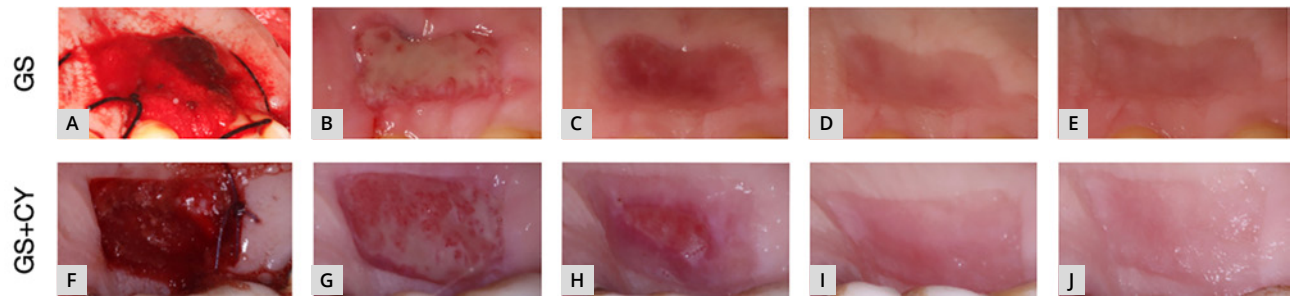

Figure 2. Clinical images of GS group, during surgery (A), day 7 (B), day 14 (C), day 21 (D), day 28 (E) and GS+CY group, during surgery (F), day 7 (G), day 14 (H), day 21 (I), day 28 (J) (GS = gelatin sponge; CY = cyanoacrylate).

Table 1. Demographic and intra-operative data.

Variable/Group	GS	GS+CY	Total	p
	n = 21	n = 21	n = 42	
Age	39.38±9.56	38.67±11.78	39.02±10.61	0.830
Gender				
Female	16 (76.2)	11 (52.4)	27 (64.3)	0.099
Male	5 (23.8)	10 (47.6)	15 (35.7)	
PTT (mm)	3.56±0.77	3.3±0.82	3.43±0.8	0.283
GT (mm)	1.6±0.49	1.97±0.25	1.79±0.43	**0.005**
GH (mm)	6.48±0.75	6.52±0.75	6.5±0.74	0.838
GW (mm)	11.05±0.92	11.14±0.85	11.1±0.88	0.730
WT (min)	8.61±4.55	4.67±1.1	6.64±3.83	**0.001**
PBT (sec)	230.95±81.42	312.86±65.66	271.90±83.99	**0.001**

Age, PTT, GT, GH, GW, WT, and PBT values are shown as mean±SD while gender values are shown as n (%); GS = gelatin sponge; CY = cyanoacrylate; PTT = palatal tissue thickness; GT = graft thickness; GH = graft height; GW = graft width; WT = working time; PBT = primary bleeding time.

Table 2. Secondary bleeding (SB), pain perception (PP), and quantity of analgesics (QA) scores.

Variable	Days/Group		GS	GS+CY	Total	p
			n=21	n=21	n=42	
SB	Day 1	Absent	8 (38.1)	19 (90.5)	27 (64.3)	**0.001**
		Present	13 (61.9)	2 (9.5)	15 (35.7)	
	Day 2	Absent	8 (38.1)	19 (90.5)	27 (64.3)	**0.001**
		Present	13 (61.9)	2 (9.5)	15 (35.7)	
	Day 3	Absent	14 (66.7)	20 (95.2)	34 (81.0)	**0.045**
		Present	7 (33.3)	1 (4.8)	8 (19.0)	
	Day 4	Absent	20 (95.2)	19 (90.5)	39 (92.9)	1.000
		Present	1 (4.8)	2 (9.5)	3 (7.1)	
	Day 5	Absent	19 (90.5)	17 (81)	36 (85.7)	0.663
		Present	2 (9.5)	4 (19.0)	6 (14.3)	
	Day 6	Absent	19 (90.5)	19 (90.5)	38 (90.5)	1.000
		Present	2 (9.5)	2 (9.5)	4 (9.5)	
	Day 7	Absent	19 (90.5)	19 (90.5)	38 (90.5)	1.000
		Present	2 (9.5)	2 (9.5)	4 (9.5)	
PP	Day 1		6.90±2.53	2.14±2.2	4.52±3.36	**<0.001**
	Day 2		5.90±2.34	1.86±2.15	3.88±3.02	**<0.001**
	Day 3		4.90±2.84	1.38±1.75	3.14±2.93	**<0.001**
	Day 4		4.19±3.19	1.19±1.44	2.69±2.88	**0.001**
	Day 5		3.81±3.14	1.43±1.47	2.62±2.7	**0.004**
	Day 6		3.05±3.09	1.52±2.06	2.29±2.71	0.069
	Day 7		2.81±3.25	1.19±1.47	2.00±2.62	**0.047**
QA	Day 1		2.19±0.4	1.67±0.66	1.93±0.6	**0.003**
	Day 2		1.90±0.44	1.57±0.81	1.74±0.66	0.107
	Day 3		1.62±0.59	1.57±0.87	1.60±0.73	0.837
	Day 4		1.38±0.86	1.43±0.93	1.40±0.89	0.864
	Day 5		1.33±1.02	1.19±0.93	1.26±0.96	0.637
	Day 6		1.10±1.14	0.95±1.07	1.02±1.09	0.677
	Day 7		1.00±1.14	0.62±0.92	0.81±1.04	0.241

SB values are given as n (%) whereas PP and QA are given as mean±SD; PP, pain perception; QA, the quantity of analgesics; GS, gelatin sponge; CY, cyanoacrylate.

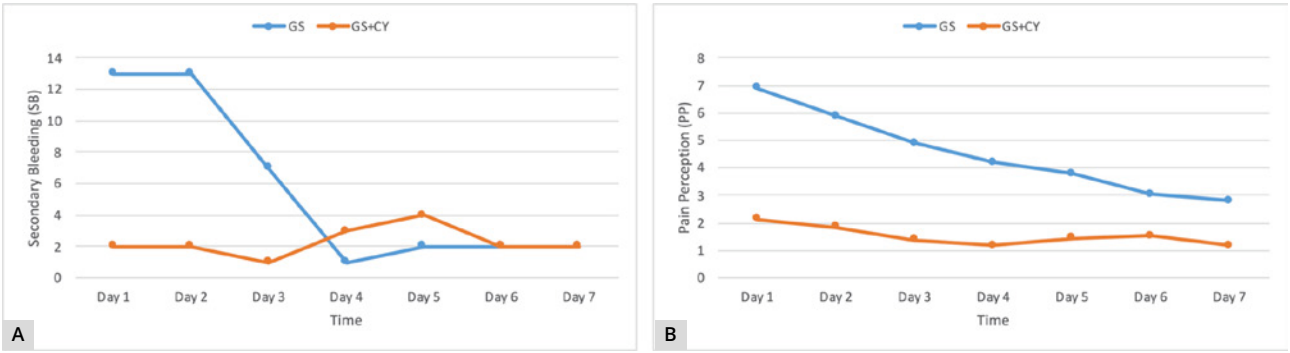

Figure 3. Secondary bleeding (A) and pain perception (PP) (B) scores at first seven postoperative days (GS = gelatin sponge; CY = cyanoacrylate).

Table 3. Epithelization level (EL), color match (CM), and sensation loss (SL) scores.

Variable	Days/group		GS	GS+CY	Total	p
			n=21	n=21	n=42	
EL	Day 7	none	20 (95.2)	21 (100)	41 (97.6)	0.999
		partial	1 (4.8)	0 (0.0)	1 (2.4)	
		total	0 (0.0)	0 (0.0)	0 (0.0)	
	Day 14	none	11 (52.4)	5 (23.8)	16 (38.1)	0.111
		partial	10 (47.6)	16 (76.2)	26 (61.9)	
		total	0 (0.0)	0 (0.0)	0 (0.0)	
	Day 21	none	1 (4.8)	0 (0.0)	1 (2.4)	0.488
		partial	19 (90.5)	21 (100)	40 (95.2)	
		total	1 (4.8)	0 (0.0)	1 (2.4)	
	Day 28	none	0 (0.0)	0 (0.0)	0 (0.0)	-
		partial	0 (0.0)	0 (0.0)	0 (0.0)	
		total	21 (100)	21 (100)	42 (100)	
CM	Day 7		2.71±1.35	2.95±0.80	2.83±1.10	0.492
	Day 14		4.57±1.57	4.90±1.04	4.74±1.33	0.422
	Day 21		7.10±1.48	7.24±1.18	7.17±1.32	0.731
SL	Day 7	none	15 (71.4)	17 (81.0)	32 (76.2)	0.719
		moderate	5 (23.8)	4 (19.0)	9 (21.4)	
		severe	1 (4.8)	0 (0.0)	1 (2.4)	
	Day 14	none	17 (81.0)	19 (90.5)	36 (85.7)	0.663
		moderate	4 (19.0)	2 (9.5)	6 (14.3)	
		severe	0 (0.0)	0 (0.0)	0 (0.0)	
	Day 21	none	20 (95.2)	20 (95.2)	40 (95.2)	1.000
		moderate	1 (4.8)	1 (4.8)	2 (4.8)	
		severe	0 (0.0)	0 (0.0)	0 (0.0)	
	Day 28	none	21 (100)	21 (100)	42 (100)	-

EL and SL values are given as n (%) whereas CM values are given as mean±SD; GS = gelatin sponge; CY = cyanoacrylate; EL = epithelization level; CM = color match; SL = sensation loss.

Table 4. Multiple regression analysis results for pain perception (PP) at day 1.

						95.0% Confidence Interval for B		Correlations		
	B	Std. Error	Beta	t	p	Lower Bound	Upper Bound	Zero-order	Partial	Part
(Constant)	9.508	3.305		2.877	**0.007**	2.805	16.212			
Group	-6.171	0.681	-0.930	-9.066	**0.000**	-7.551	-4.790	-0.718	-0.834	-0.814
Gender	-1.411	0.668	-0.204	-2.111	**0.042**	-2.767	-0.056	0.028	-0.332	-0.190
Age	-0.099	0.030	-0.313	-3.281	**0.002**	-0.160	-0.038	-0.149	-0.480	-0.295
GT (mm)	2.652	0.816	0.338	3.252	**0.002**	0.998	4.307	-0.074	0.477	0.292
GH (mm)	0.880	0.412	0.194	2.138	**0.039**	0.045	1.716	0.137	0.336	0.192

R-sqr = 0.71; Adj.R-sqr = 0,669; SE = 1.932; ($F_{(5,36)}$ = 17.593; $p < 0.001$).

The 1-year increase in the age variable caused the 0.099-point decrease, 1-mm increase in the GT variable caused the 2.65-point increase and 1-mm increase in the GH variable caused the 0.88-point increase in the PP variable. Standardized β-coefficients revealed that the study group variable showed the highest effect on PP (β = -0.930), and GT, age, gender, and GH parameters seemed to be effective, respectively (Table 4).

Discussion

According to the findings of this study, GS+CY revealed better outcomes compared to GS only in terms of WT, SB, and PP. However, findings did not exhibit any difference with regard to EL, CM, and SL and exhibited longer PBT for GS+CY when compared to GS only.

Increased GT may cause exposure of numerous nerve and vessel endings that is concluded with severe pain and bleeding especially during deep harvesting (Tavelli 2019a). Several studies showed a positive association between GT and PP of the patients after harvesting (Burkhardt *et al*. 2015). In the present trial, parallel to the idea that higher GT works as a morbidity-enhancing factor, GS+CY group experienced longer PBT and higher PP at day 1 with the higher GT values according to the regression analysis. However, SB, PP, and QA revealed opposite findings in the same group probably owing to the favoring effect of CY in the coagulation process during early healing (Borie *et al*., 2019). This finding was compatible with similar studies (Tavelli *et al*., 2018; 2019c). In those studies, CY not only minimized PP but also reduced the analgesics need that was not compatible with the present findings showing a mismatch between PP and QA can possibly be linked to the confounding effect of simultaneous analgesic need derived from recipient site discomfort.

SB is not a rare complication at donor site (Griffin *et al*., 2006). In a similar study, Ustaoglu *et al* (2016) detected SB at 100%, 66.7% of the empty donor sites on days 1 and 2, respectively. The wound sealers such as stent, PRF or stent+medicinal plant extract reduced the rate of SB (Keceli *et al*., 2015a; Ustaoglu *et al*., 2016; Keceli *et al*, 2015b). The data obtained with GS exhibited similarity with the stent group of Keceli *et al*. (2015a) while GS+CY was less than the others.

On day 4, only three patients remained with SB. In accordance with the relevant literature (Keceli *et al*., 2015a), this result might indicate that the critical period for the prevention of bleeding is the first four days of healing. However, in especially the GS+CY group, the number of individuals with SB increased on the next day and such an increase was not reported by any of the relevant papers. Owing to the patient feed-backs, the reoccurrence of bleeding was attributed to the detachment of the polymerized CY from the wound surface. The increasing PP in the CY group also supported this fact. Thus, follow-up is still strongly needed and detachment of CY seems to be a critical complication which was also reported by Tavelli *et al*. (2019c).

At the day 7, none of the patients had SB whose frequency significantly reduced to the range of 0-8.3% in the associated papers (Isler *et al*., 2018; Keceli *et al*., 2015a; Yildirim *et al*., 2017). In general, the comparison of SB data with the relevant literature had a consistency within each other except Del Pizzo *et al*. (2002) who reported remarkably higher (91.7%) SB probably due to leaving the empty wound in contact with silk sutures gathering the high amount of plaque onto its surface. Moreover, the possible effect of confounders such as GT, PTT, or methodology should be carefully considered in this comparison.

The easy handling and fast polymerization features of CY provided lower WT. That might have reduced the present morbidity findings (Borie *et al*., 2019; Keceli *et al*.,2015a). CY constitutes a mechanical barrier that preserves the wound site from trauma and increased bacterial accumulation, thus helps to shorten the healing time (Ilgenfritz *et al*., 2017; Prince, 2018). However, because of the insufficient histological data, the full epithelial behavior under CY could not be defined yet. Only in their review, Borie *et al*. (2019) speculated that accelerated keratinization might be a possible mechanism enhancing the EL of intra-oral wounds under CY. Contrary to the expectations, higher EL values did not accompany to reduced early PP and SB in the GS+CY group of this study.

Although, CY did not induce epithelization rate, pain reduction and prevention of bleeding could be provided by the isolating effect of the CY material. The increasing SB and PP values after detachment (4th day) also support this phenomenon.

The present findings of the GS group resided in between empty-left wounds and GS+CY was similar to PRF results (Yildirim *et al*. 2017; Ustaoglu *et al*. 2016). Although CM results were in favor of GS+CY, the inter-group difference was not statistically significant. Thus, in addition to EL, CM could not be improved with the contribution of CY. This finding may also support isolating effect of CY.

Persistent or temporary SL is a possible complication of CTG harvesting (Griffin *et al*., 2006; Tavelli, *et al*., 2019a). Former studies; Buff *et al*. (2009) and Yildirim *et al*. (2017) reported none, Del Pizzo *et al*. (2002) 14.3% persistent SL and Keceli *et al*. (2015a) 15.2% temporary SL. In this trial, temporary SL was detected at 9/42 (21.4%) patients and neither GS nor GS+CY application caused the difference. Technically, the variations in the relevant literature might depend on PTT, GT, and harvesting region selection (distance to gingival margin, premolar/molar site) (Zucchelli *et al*., 2010; 2019; Zuhr *et al*., 2014).

The subjective character of the evaluation parameters in donor site healing trials can be encountered as a common limitation and should be considered during interpretation of the results. The absence of standardization in the wound regions is also a common limitation of donor site healing studies. In the present trial, the similar baseline PTT in both groups and excessive care is taken to stay within a constant GT and furthermore, excessive care was taken to stay within a constant thickness (less than <2mm), which may histologically represent an FGG consisted of 0.5-1.0 mm epithelium and 1.0-1.5 mm connective tissue layers (Bertl *et al*., 2015; Zucchelli *et al*., 2014), were positive contributors to overcome this limitation. Although the absence of an empty-left wound group caused a limitation in the comparability of the findings with some of the similar papers, it was decided to use GS as the negative control group (Tavelli *et al*. 2019c) in terms of relieving the PP of the patients to an acceptable level. Moreover, Tavelli *et al* (2019c) reported that gender heterogeneity between groups may affected the pain perception. Contrary to the previous data (Wiesenfeld-Hallin, 2005), less PP was experienced by the females in the present study. This result might be attributed to the inter-individual differences or heterogeneity of the females in the study groups. Thus, absence of a stratification on the randomization of the study for balancing the groups in terms of gender can be considered as a limitation.

GS-CY combination is a good candidate to improve palatal donor site healing and associated patient discomfort after DGG harvesting with its easy handling, fast polymerization, effective wound sealing, antibacterial and non-toxic properties. The present trial mostly confirmed this hypothesis by showing fewer WT, PP, and SB findings achieved with GS+CY use. Therefore, the clinical use of GS-CY after DGG harvesting can be highly recommended. Accordingly, it may strengthen the preference of the DGG method compared to its alternatives such as single-incision and trap-door methods that should be tested by further comparative studies. However, profits from CY seem to be acquired with its isolation property rather than an accelerated epithelization and careful consideration is also necessary in terms of its detachment risk with the progression of healing. Thus, in order to understand the biological effects of CY in donor site healing, further studies carrying the perspective of improving its mechanical and chemical properties are needed.

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