

## **Topical Effect of Propolis in the Repair of Sulcoplasties by the Modified Kazanjian Technique**

### **Cytological and Clinical Evaluation**

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#### **Abstract**

A study was conducted to analyze the effects of propolis mouth rinse on the repair of surgical wounds after sulcoplasty by the modified Kazanjian technique. Twenty-seven patients who underwent sulcoplasty were divided into three groups:

C1— patients who did not use the mouth rinse

C2— patients who used a mouth rinse containing 5% aqueous alcohol

T— patients who used a mouth rinse containing 5% propolis in aqueous alcohol solution.

The patients returned 7, 14, 30, and 45 days after surgery for cytological and clinical evaluation. It was concluded that: 1) the mouth rinse containing propolis in aqueous alcohol solution aids repair of intra-buccal surgical wounds and exerts a small pain-killing and anti-inflammatory effect; 2) the vehicle employed has a minor irritant effect on intra-buccal surgical wounds; 3) exfoliative cytology allows epithelization of intrabuccal surgical wounds.

#### **Introduction**

Increasing attention has been focused on the use of propolis in various fields of research in medicine and dentistry. Propolis is used for the treatment of aphthae<sup>[1]</sup>, moniliasis<sup>[2]</sup>, periodontitis<sup>[3]</sup>, and as a delayed-action curative in endodontics<sup>[4]</sup>.

Magro-Filho et al.<sup>[5]</sup> and Magro Filho and Carvalho<sup>[6]</sup> analyzed the action of propolis in the subcutis, in rats with experimental cutaneous wounds and in the repair of dental alveoli. They concluded that in both cases propolis accelerated the development of scarring.

In view of these results, and previous clinical and cytological studies<sup>[7]</sup> of sulcoplasty by the modified Kazanjian technique, we planned this investigation to analyze the effect of a mouth rinse containing propolis in aqueous alcohol solution on the repair of intrabuccal surgical wounds in patients after sulcoplasty by the above technique.

#### **Materials and Methods**

Twenty-seven edentulous patients with atrophy of the inferior alveolar ridge were studied at the Surgical Clinic of the Dental School of Aracatuba, UNESP (State University of São Paulo).

In patients for whom sulcoplasty was indicated (Fig. 1), the depth of the anterior inferior vestibular sulcus was measured and smears of the labial and alveolar ridge mucosa were collected.

Next, under local anesthesia, deepening of the sulcus by the modified Kazanjian technique (Fig. 2) (Edlan and Mejchar)<sup>[8]</sup> was carried out. Initially, a semi-elliptical superficial incision with the concavity turned towards the alveolar ridge was made in the labial mucosa, with the mucosa forming a pediculate rem-

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nant in the crista of the alveolar ridge. At the same time, an incision of the periosteum below the remnant, just below the alveolar ridge, was made and the periosteum was pulled back to the desired apical level. At this stage, inversion of the remnant was made, suturing the periosteum to the labial mucosa and to the mucosa of the remnant near the periosteum in the apical region (Figs. 2 and 3).



Fig. 1 Clinical preoperative view of alveolar ridge atrophy



Fig. 2 Immediate postoperative view following the modified Kazanjian technique

After surgery, the patients were divided into three groups:

C1 (control), nine patients who did not use any mouth rinse; C2, nine patients who used a mouth rinse containing 5% aqueous alcohol solution (the vehicle for propolis) five times a day for one week after surgery; T, nine patients who used a mouth rinse containing 5% propolis in aqueous alcohol solution five times a day for one week after surgery.

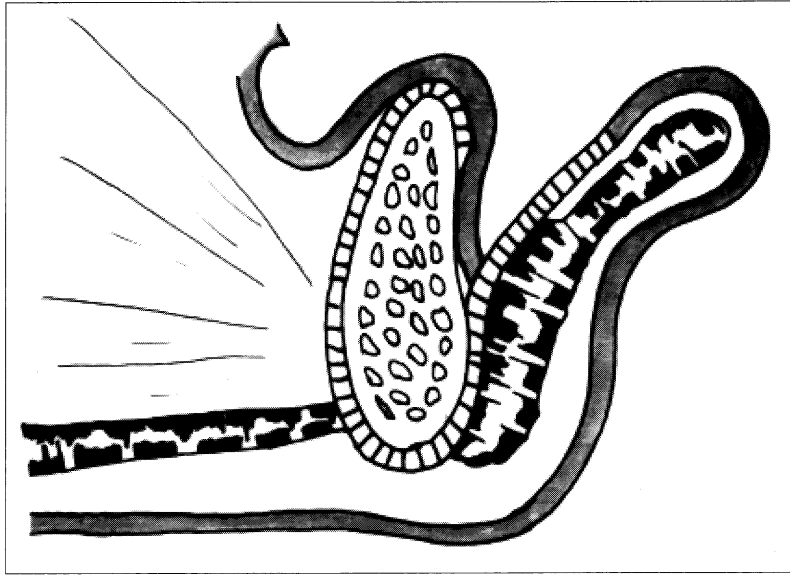


Fig. 3 Schematic drawing in the transverse plane of the periosteum sutured to the labial mucosa and mucosa of the ridge sutured near the periosteum in the apical region

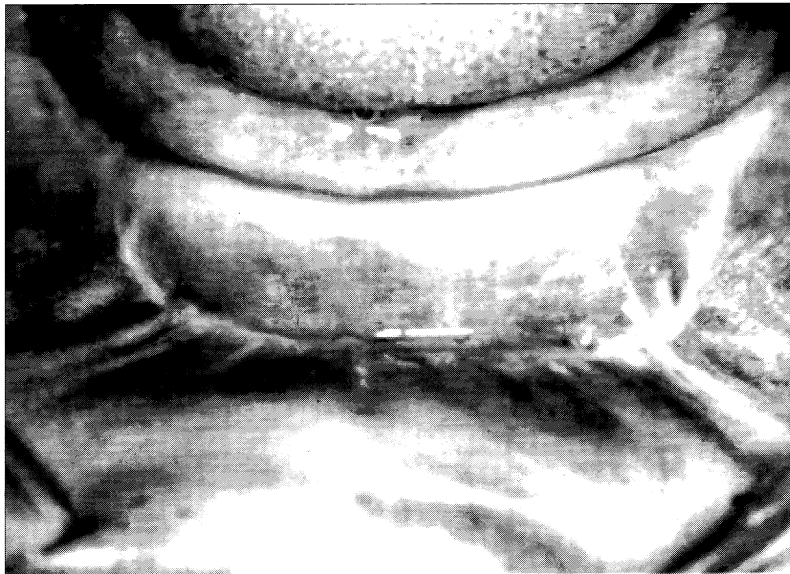


Fig. 4 Postoperative view after 45 days

To prepare the mouth rinse, 40 g of propolis *in natura* was mixed with 100 ml of grain alcohol. This mother liquor was then diluted to 5% in double-distilled water.

The patients returned at 7, 14, 30 and 45 days after surgery (Fig. 4) for collection of smears from the region of the inverted periosteum and from the mucosa of the repositioned alveolar ridge, and for measurement of sulcal depth. Clinical analysis using the criteria adopted by Lima et al.<sup>[9]</sup> was then performed. Parameters evaluated were: pain—absent, moderate (managed without the use of pain-killers), intense (use of pain-killers was necessary); secondary hemorrhage—absent, moderate (blood clot in the area of the sutures or in the extremities of the remnant); suppuration—absent, moderate (presence of small points of pus in the area of the sutures or in the extremities of the remnant), severe (spontaneous pus drainage through the sutures or the extremities of the remnant); inflammation—absent, moderate (small area of erythema and/or edema adjacent to the remnants); adhesion to the remnant—absent (remnant separated), moderate (slight loosening in the extremities), severe (remnants showing strong adhesion).

For cytologic analysis, the collected smears were stained by the Papanicolau method, 100 epithelial cells were counted per field, and the percentages of cyanophilic, eosinophilic and orange cells were determined according to the criteria adopted by Miller et al.<sup>[10]</sup> and Montgomery<sup>[11]</sup>. The smears obtained before surgery were taken to represent the normal cytologic standard and were compared with the post-surgery smears. This permitted analysis of the development of the epithelium and its maturation in the three study groups.

Furthermore, in the cytological analysis, the presence of cocci and inflammatory cells was observed and classified as absent, moderate or intense according to the criteria adopted by Perri de Carvalho et al.<sup>[7]</sup>.

The data obtained were subjected to statistical analysis comprising, fundamentally, the Kruskal-Wallis test as a non-parametric test for verification of significant differences between the groups, and the Mann-Whitney U test as a non-parametric test for characterization of differences between the groups, the level of significance being 5%.

## Results

### Cytologic Analysis

#### a) Epithelial Cells

Table 1 shows the relationship between cyanophilic and eosinophilic cells originating from the inverted periosteum, allowing its comparison with the pre-surgery standard desired. Where there is no difference between each of the C1, C2 and T groups in relation to the pre-surgery standard, the epithelium is scarred and mature. The Kruskal-Wallis test indicated that the groups showed no significant differences at 30 and 45 days after surgery. In the initial period, C1 and C2 showed significant differences from the pre-surgery standard, group T showed greater numeric proximity, and a hypothesis of equality from a statistical viewpoint was accepted.

Table 2 shows data for collected mucosal smears from the alveolar ridge. It is evident that even after 45 days, the groups did not reach the desired cytological standard, either quantitatively or statistically. However, for orange cells, group T at 45 days was equal to the pre-surgery standard when compared using the Mann-Whitney U test. According to the same statistical analysis for the C1 and T groups, at 7, 14, 30, and 45 days after surgery, group T was significantly different from C1 and numerically closer to the standard for orange cells. At 14 days, with regard to eosinophilic cells, groups C1, C2 and T, and for cyanophilic cells, groups C2 and T also presented significant differences among themselves, with greater proximity to group T in relation to the cytologic standard desired.

#### b) Inflammatory Cells

As shown in Table 3, at 7 and 14 days after surgery, all of the groups started to present significant differences from the pre-surgery standard (absence of inflammation). Nevertheless, at 7 days, while C1 and C2 showed intense neutrophil infiltration, group T also showed histiolympo-plasmocyte infiltration, which differed significantly from the pre-surgery standard and from the remaining groups (Mann-Whitney

U test). However, at 14 days, by the same analysis, only group C2 showed a significant difference in relation to the standard, while the remaining groups were already closer to the desired pre-surgery standard.

Table 4 shows data for mucosal smears collected from the alveolar ridge. At 7 days, although the three groups presented significant differences (Mann-Whitney U test) from the pre-surgery standard, group T appeared different from the C1 and C2 groups, with a predominantly moderate inflammatory infiltrate, whereas the rest showed intense inflammatory infiltration. At 14 days, with regard to acute and chronic inflammatory infiltration, the groups were significantly different from the standard (Mann-Whitney U test). However, in terms of percentages, group T showed a tendency for moderate, light or absent inflammation, while the remaining groups showed only intense inflammation.

#### c) Bacterial Flora

The smears taken from the area of the inverted periosteum and those collected near the mucosa of the alveolar ridge showed no significant differences (Kruskal-Wallis test) among the groups.

### Clinical Analysis

#### a) Inflammation

The results of clinical analysis of inflammation are shown in Table 5. The Kruskal-Wallis test indicated that at 30 and 45 days the groups showed no significant differences. At 7 days, the Mann-Whitney U test showed that only group T did not possess intense inflammation. At 14 days, only group C2 showed a significant difference from the pre-surgery standard.

#### b) Suppuration, Adhesion to the Remnant, and Secondary Hemorrhage

The groups showed no significant differences among themselves (Kruskal-Wallis test).

#### c) Pain

The C1, C2 and T groups differed significantly from the pre-surgery standard (absence of pain) only at 7 days after surgery (Kruskal-Wallis and Mann-Whitney U tests). These results, analyzed in terms of percentage, demonstrated that while groups C1 and C2 presented intense, moderate or absence of pain, group T showed only moderate pain or absence of pain (Table 6).

#### d) Sulcal depth

There were no significant inter-group differences (Kruskal-Wallis test).

Table 1 Percentages of epithelial cells in the three study groups, in the area of the inverted periosteum, in the postoperative and standard preoperative periods

postoperative period						
group		7 days		14 days	30 days	45 days
C1	cell color	absent cells				
	eosinophilic	73.34	22.22	80.89	53.56	44.62
	cyanophilic	4.44		19.11	46.44	55.38
C2	eosinophilic	65.00	25.00	68.25	66.56	50.12
	cyanophilic	10.00		31.75	33.44	49.88
T	eosinophilic	77.50	12.50	61.67	46.86	45.67
	cyanophilic	10.00		38.33	53.14	54.33
preoperative	eosinophilic	44.36		44.36	44.36	44.36
	cyanophilic	55.64		55.64	55.64	55.64

Table 2 Percentages of epithelial cells in the repositioned alveolar ridge mucosa, in the three study groups in the postoperative and standard preoperative periods

postoperative period		7 days	14 days	30 days	45 days
group					
C1	cell color				
	orange		15.00	30.00	44.29
	eosinophilic	79.44	61.25	40.50	25.00
	cyanophilic	20.56	23.75	29.50	30.71
C2	orange	7.14	26.88	33.56	51.29
	eosinophilic	60.57	33.87	42.44	28.57
	cyanophilic	32.29	39.25	24.00	20.14
T	orange	17.14	33.38	44.57	61.55
	eosinophilic	65.72	41.87	37.29	20.89
	cyanophilic	17.14	24.75	18.14	17.56
preoperative	orange	76.66	76.66	76.66	76.66
	eosinophilic	14.47	14.47	14.47	14.47
	cyanophilic	8.87	8.87	8.87	8.87

Table 3 Percentages of acute and chronic inflammatory cells in the three study groups in the area of the inverted periosteum in the postoperative and standard preoperative periods

postoperative period		7 days		14 days		30 days		45 days	
group		acute	chronic	acute	chronic	acute	chronic	acute	chronic
C1	inflammation degree								
	absent		100.00	62.50	25.00		44.44		87.50
	low			12.50	62.50		55.56		12.50
	moderate				12.50				
	intense	100.00		25.00					
C2	absent		100.00	33.33	11.11		55.56		100.00
	low				11.11		33.33		
	moderate			33.33	44.44		11.11		
	intense	100.00		33.33	33.33				
T	absent		55.56	55.56	22.22		57.14		100.00
	low				44.44		42.86		
	moderate			33.33	33.33				
	intense	100.00	44.44	11.11					
preoperative	absent	100.00	93.33	100.00	93.33		93.33		93.33
	low		6.67		6.67		6.67		6.67
	moderate								
	intense								

Table 4 Percentages of acute and chronic inflammatory cells in the three study groups in the repositioned alveolar ridge mucosa in the postoperative and standard preoperative periods

postoperative period		7 days		14 days		30 days		45 days	
group	inflammation	acute	chronic	acute	chronic	acute	chronic	acute	chronic
C1	inflammation degree								
	absent					88.89	88.89	85.71	
	low					11.11	11.11	14.29	
	moderate			33.33	33.33				
C2	intense	100.00		66.67	66.67				
	absent					100.00	55.56	100.00	
	low						44.44		
	moderate			88.89	88.89				
T	intense	100.00		11.11	11.11				
	absent			12.50	12.50	100.00	71.43	100.00	
	low			37.50	37.50		28.57		
	moderate	71.43		50.00	50.00				
preoperative	intense	28.57							
	absent	100.00		100.00	100.00	100.00	100.00	100.00	
	low								
	moderate								
	intense								

Table 5 Percentage relationship between degree of clinical alleviation of inflammation in each group in the postoperative and standard preoperative periods

postoperative period		7 days		14 days		30 days		45 days	
group	inflammation degree								
C1	absent	40.00		80.00		100.00		100.00	
	moderate	40.00		20.00					
	intense	20.00							
C2	absent	22.22		22.22		100.00		100.00	
	moderate	66.67		77.78					
	intense	11.11							
T	absent	22.22		55.56		100.00		100.00	
	moderate	77.78		44.44					
	intense								
preoperative	absent	100.00		100.00		100.00		100.00	
	moderate								
	intense								

Table 6 Percentage relationship of pain symptomatology between the three study groups in the postoperative and standard preoperative periods

group	postoperative period	7 days	14 days	30 days	45 days
	degree of pain symptomatology				
C1	absent	40.00	80.00	100.00	100.00
	absent	20.00	80.00	100.00	100.00
	moderate	40.00	20.00		
C2	absent		77.78	100.00	100.00
	moderate	77.78	22.22		
	intense	22.22			
T	absent	33.33	100.00	100.00	100.00
	moderate	66.67			
	intense				
preoperative	absent	100.00	100.00	100.00	100.00
	moderate				
	intense				

## Discussion

Statistical and quantitative analysis of exfoliative cytology showed that the mouth rinse containing 5% propolis solution in aqueous alcohol enhanced epithelization of intrabuccal surgical wounds, thus confirming the results of previous studies in animals, which have emphasized the capacity of this apiotherapeutic to accelerate epithelization<sup>[7,12]</sup>.

The possibility that the vehicle, humidity, or the act of mouth-rinsing were factors that accelerated the epithelization can be ruled out, since comparison of the results for the three groups allowed these variables to be eliminated.

Some previous experiments have given some clues to the mode of action of propolis. Scheller et al.<sup>[13]</sup> noticed that propolis, in contact with embryonic cells, stimulated cellular metabolism. Scheller et al.<sup>[14]</sup> identified many chemical elements in propolis, among them iron and zinc, which are important for the synthesis of collagen<sup>[15]</sup>. Gabrys et al.<sup>[16]</sup> also demonstrated the presence of amino acids, such as arginine and proline, which are important for stimulating cellular mitosis and as raw materials for protein synthesis.

These results in association with cytologic and clinical studies of inflammation allow clearer interpretation of the possible implications. Consequently, it can be verified that propolis does have an anti-inflammatory effect. Other authors have also mentioned an anti-inflammatory effect of propolis<sup>[17,18]</sup>.

It has been stated that an antibiotic action of propolis is responsible for the accelerated scarring of cutaneous wounds, on the basis of results obtained *in vitro*<sup>[17,19,20]</sup>. In our study, we could not verify if the results obtained were due to an antibiotic action of propolis because there was no marked infection or differences between the analyzed smears in relation to the flora encountered.

In the analysis of painful symptomatology, we verified that deepening of the sulcus by the modified Kazanjian technique is tolerable for the patient from about 14 days after surgery onwards. The groups did not show differences when compared to the pre-surgery standard. These results demonstrate that propolis exerts an analgesic effect in the initial period after surgery. Atiasov et al.<sup>[19]</sup> and Orkin and Douzanski<sup>[21]</sup> also reported a topical analgesic effect of propolis in cutaneous wounds in patients.

Thus, we have verified that the scarring reaction occurred more quickly after use of propolis mouth rinse in the first month after surgery, and that at 45 days, the loss of sulcal depth in the three groups was 43%.



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