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The rabbit gingival tissue response to retraction liquids and tetrahydrozoline

Odgovor gingivalnog tkiva kunića na retrakciona sredstva i tetrahidrozolin

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Abstract

Backround/Aim. Retraction agents for temporary vertical and lateral suppression of gingival tissue as well as bleeding control and fluid flow in the gingival sulcus are expected to have maximal efficiency without irreversible damage of local tissue and adverse systemic effects. The research started from the assumption that tetrahydrozoline is a biologically more acceptable means of gingival retraction than commercially available preparations. The aim of the study was to comparatively analyse the inflammatory effects of different retraction materials and tetrahydrozoline. **Methods.** The effect of retraction liquid on the basis of aluminum chloride and epinephrine and tetrahydrozoline hydrochloride on gingival tissue of rabbits was investigated. The application time in the rabbit's gingival sulcus was 7 minutes. Tissue biopsy was performed after an hour, a day, and 7 and 30 days. Tissue preparations were analyzed under a microscope. **Results.** The obtained results indicate a reversible damage of gingival tissues as a result of local application of aluminum chloride- and epinephrine-based retraction agents. Their use led to acute inflammatory response after an observation period of 1 and 7 days. After 30 days reparation of damaged tissue was observed. The use of tetrahydrozoline resulted in a visibly weaker inflammatory response. **Conclusion.** Retraction liquids insertion led to an acute inflammatory response of gingival tissue which in time assumed a chronic character. The inflammatory response to the administered tetrahydrozoline was significantly lower with complete reparation of gingival tissue. Taking this fact into account it is recommended as a potential retraction agent.

Key words:
gingivitis; tissues; inflammation; animals, laboratory.

Apstrakt

Uvod/Cilj. Retrakciona sredstva za privremeno vertikalno i lateralno povlačenje gingivalnog tkiva i kontrolu krvarenja i protoka tečnosti u gingivalnom sulkusu trebalo bi da imaju maksimalnu efikasnost bez ireverzibilnog oštećenja lokalnog tkiva i neželjenih sistemskih efekata. U istraživanju se krenulo od pretpostavke da je tetrahidrozolin biološki prihvatljivije sredstvo za gingivalnu retrakciju od komercijalno dostupnih preparata. Cilj rada bio je komparativna analiza inflamatornog odgovora na dejstvo retrakcionih materijala i tetrahidrozolina. **Metode.** Istraživan je efekat retrakcionih sredstava na bazi aluminijum-hlorida i epinefrina, kao i tetrahidrozolin-hidrohlorida na gingivalno tkivo kunića. Vreme primene ispitivanog materijala u gingivalni sulkus kunića iznosilo je 7 min. Nakon opservacionog perioda od 1 h, jednog, sedam i 30 dana vršena je biopsija gingivalnog tkiva i dobijeni preparati su mikroskopski analizirani. **Rezultati.** Utvrđeno je reverzibilno oštećenje tkiva gingive izazvanog lokalnom aplikacijom retrakcionih sredstava na bazi aluminijum hlorida i epinefrina. Njihova upotreba, nakon 1-dnevnog i 7-dnevnog opservacionog perioda, dovela je do akutnog inflamatornog odgovora. Nakon 30 dana uočena je reparacija oštećenog tkiva. Primena tetrahidrozolina imala je za rezultat značajno slabiji inflamatorni odgovor. **Zaključak.** Upotreba retrakcionih rastvora dovela je do akutnog inflamatornog odgovora tkiva gingive, koji je vremenom poprimio hronični karakter. Inflamatorni odgovor sa primenom tetrahidrozolina bio je značajno slabiji sa potpunim oporavkom gingivalnog tkiva. S obzirom na to tetrahidrozolin se može preporučiti kao potencijalni retrakcioni agens.

Ključne reči:
gingivitis; tkiva; zapaljenje; životinje, laboratorijske.

Introduction

Regular impression taking is a prerequisite for construction of high-quality fixed prosthetic appliance, thus allowing maximum accuracy possible at the contact site of biological tissue and restoration margin and ensuring integrity of periodontal structures. If a preparation margin is set at the level of or below the gingival margin, it is necessary to make it accessible to impression material by reversible temporary shift in apical direction.

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One of the most commonly used clinical methods for gingival retraction is a mechanical-chemical method which involves the use of special cotton cords of different thickness, impregnated with a solution (retraction agent) [1]. The role of retraction agent implies temporary vertical and lateral suppression of gingival tissue as well as bleeding control and fluid flow in gingival sulcus [2, 3]. For this purpose, vasoconstrictors (epinephrine) and astringents (aluminum chloride, aluminum sulfate, zinc chloride) are currently used. The applied retraction agent is expected to have maximal efficiency without irreversible damage of local tissue and adverse systemic effects [4].

On the other hand, some literature findings suggest that these gingival retraction agents may cause systemic reactions and local damage to periodontal tissues [5–7]. Systemic effect is related to epinephrine, especially if it is applied to damaged marginal gingiva and greater number of teeth, because it is contraindicated in patients with cardiovascular diseases, hyperthyroidism and diabetes [8, 9]. Since astringents act by precipitation of proteins and show very low cellular permeability they cause no systemic effects. Astringents of moderate concentrations cause irritation of surrounding tissue, and those of high concentrations cause caustic effect, which is especially important if one takes into account the fact that there is inadequate dose control [4].

Sympathomimetic vasoconstrictors also show retraction activity and are commercially available as nasal and olfactory decongestives [7]. Thus, these preparations having tetrahydrozoline as active component are also advantageous in dental prosthetics. In this study tetrahydrozoline was assumed to represent biologically more acceptable retraction agent when compared to commercially available products.

The aim of the study was to compare the effects of different commercially available retraction agents and tetrahydrozoline-based preparation on gingival tissue of rabbits.

Methods

The study included 3 commercialy available retraction agents and tetrahydrozoline-based agent (Table 1).

Experimental design was based on the following parameters: investigations were performed on gingival tissue of all 4 incisors of rabbits from each group; gingiva of the right incisors in all rabbits were the controls; gingiva of the left incisors were used for application of tested materials. Application was performed in gingival sulcus for 7 min using a retraction cord (Retracto®, Roeko) to enable even distribution of retraction agents. The impregnated cord was carefully placed along the whole tooth surface using plastic instrument and dental forceps. Gingival tissue of the upper right incisors in all the rabbits served as the negative control, *ie* it was intact tissue in the experiment. Gingival tissue of the lower right incisor in all the rabbits served as the control of the false treatment type, representing application of the retraction cord without tested material in the same way as it was used in the application of material. Simultaneous gingival biopsy of the left and right incisors of the jaw was done to avoid the influence of gingival biopsy injury of adjacent incisor.

The application plan, as well as observation period duration for each of the investigated retraction agents is shown in Table 2.

After 1 h, 1, 7 and 30 days of the treatment, gingival tissue samples were taken for histopathological analysis. Using a scalpel, 2 vertical incisions were made on the labial gingiva and one horizontal incision at the level of the alveolar ridge. Tissue samples of 2 × 2 mm were carefully separated in vertical direction by a raspatory. Upon biopsy tissue samples were fixed in 10% formaline. The material was further dehydrated in increasing concentrations of ethanol (from 50% to absolute). The material was illuminated by xylene and then put in paraffin molds. Tissue blocks embedded in periplast were cut on microtome (LKB Bromma, Sweden) (1.5 μL) and stained by hematoxylin & eosin (HE) method. Stained preparations were analyzed histopathologically on a NU-2 microscope (Carl Zeiss, Germany). The presence of collagen fibers was analyzed under polarizing light. Intensity of inflammation reaction was estimated semiquantitatively.

Table 1

Tested materials

Agent	Chemical content	Manufacturer
Retrargin®	25% aluminium chloride hexahydrate, pH = 0.8	Galenika, Serbia
Gingiva Liquid®	10% aluminium chloride hexahydrate, pH = 1.8	Roeko, Italy
Surgident® retraction solution	8% epinephrine -HCl, pH=2.5	Sigma Dental Systems Emasdi GmblH, Germany
Visine® Original	0.05% tetrahydrozoline hydrochloride, pH = 5.6	Pfizer, USA

Experimental studies were carried out in accordance with the Helsinki Declaration (Approval of the Ethics Committee of the Faculty of Medicine in Niš, No. 01-2066-2).

The experiment involved 32 experimental male rabbits, 8 weeks of age and 1.8–2.2 kg of weight. The animals were divided into 4 experimental groups, 8 rabbits for each tested material. Each animal was administered 10 mg/kg intramuscular anesthesia Zoletil® (Virbac).

Results

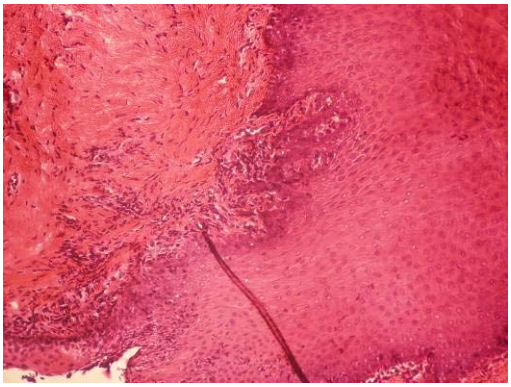
The tissue samples of clinically healthy gingiva of the negative controls that underwent no surgical procedures showed normal histological image of the gingiva (Figure 1a). On histological preparations of the control gingiva of a false treatment type, 1 h and a day after the treatment mild inflammatory reaction was visible (Figure 1b). After 7 and 30

Table 2

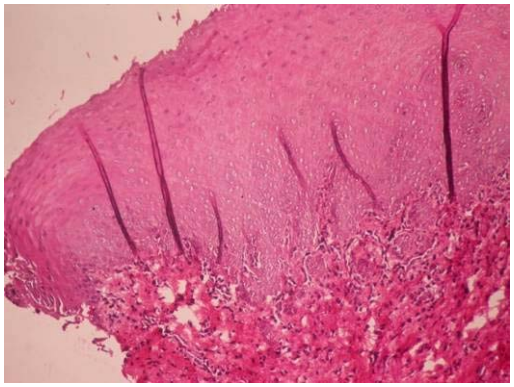
Experimental design of the study

Rabitt	Treatment		Observation period [hour(s) - h; day(s) - d]
	right incisor (upper – u; lower - l)	left incisor (upper – u; lower - l)	
1st	NC(u)	TA (u)	1d
	FT (l)	TA (l)	1h
2nd	NC(u)	TA (u)	1h
	FT (l)	TA (l)	1d
3rd	NC(u)	TA (u)	1d
	FT (l)	TA (l)	1h
4th	NC(u)	TA (u)	1h
	FT (l)	TA (l)	1d
5th	NC(u)	TA (u)	30d
	FT (l)	TA (l)	7d
6th	NC(u)	TA (u)	7d
	FT (l)	TA (l)	30d
7th	NC(u)	TA (u)	30d
	FT (l)	TA (l)	7d
8th	NC(u)	TA (u)	7d
	FT (l)	TA (l)	30d

TA - tested retraction agent; NC - negative control (intact control); FT – false treatment.



a)



b)

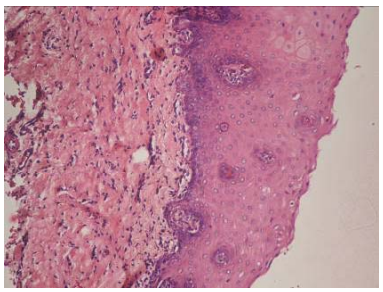
Fig. 1 – a) The tissue samples of the intact controls (the negative controls) had normal amount of collagen fibres; b) Negative control preparations after one day biopsy showed mild focal inflammatory infiltrates that were later replaced with healthy connective tissue (HE, ×200).

days from the false treatment examined gingivae were not histologically different from the negative controls.

The gingival samples treated with different retraction agents showed different degrees of inflammatory reaction. One hour after removal of a retraction cord, gingival tissue showed slight inflammatory changes compared to the controls. All the tissue samples had foci of inflammatory infiltrate with a reduced amount of collagen fibers (Figure 2).

A day after removal of a retraction cord, inflammatory infiltration was more prominent in the tissue samples treated with retraction agents in relation to those treated with tetrahydrozoline (Visine®) (Figure 3).

After a 7 day observation period, the tissue samples treated with retraction agents showed signs of extensive acute inflammation. More intense degradation of collagen fibers was observed after application of epinephrine-based



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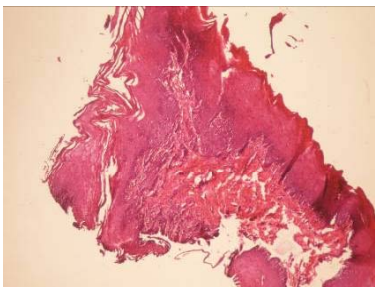

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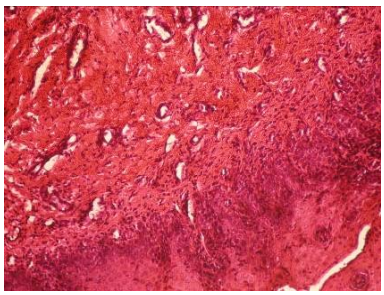

c)

Fig. 2 – Histopathological findings after a 1-hour biopsy.

a) Retrargin® (25% aluminium chloride hexohydrate); b) Surgident® (8% epinephrine-HCl); c) Visine® (0.05% tetrohydrozoline hidrochloride). All tissue samples presented foci of inflammatory infiltrate with reduced amounts of collagen fibres (HE ×100).

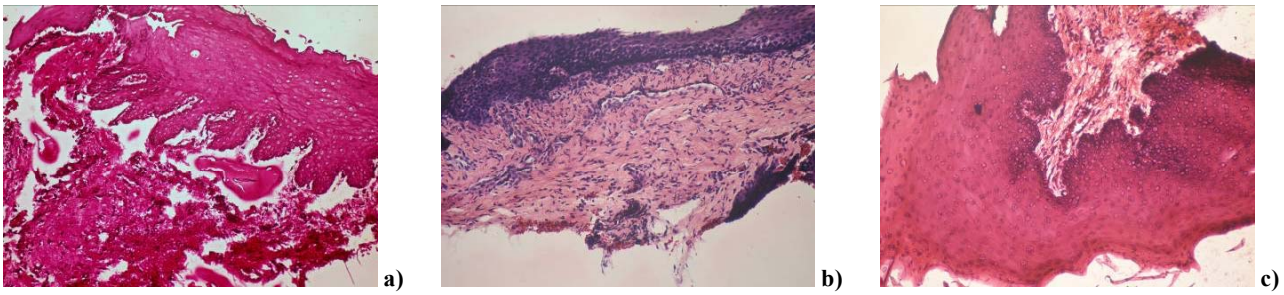

Fig. 3 – Histopathological findings after a 1-day biopsy (HE, ×100).
a) Retrargin® (25% aluminium chloride hexahydrate): tissue edema, with focal inflammatory infiltrate, b) Surgident® (8% epinephrine-HCl): tissue showed strong acute inflammatory response: c) Visine® (0.05% tetrahydrozoline hidrochloride): there was a mild inflammatory reaction, but noticeably lower than in the tissue after the removal of retraction cords.

agent. Tetrahydrozoline-based preparation showed the least inflammatory effect in this case, where tissue fibrosis was observed on a histopathological preparation (Figure 4).

With increasing duration of observation period, there occurred tissue fibrosis, and inflammation became chronic. The newly formed fibrous tissue was the sign of defect reparation in the treated tissue. Complete reparation occurred only in case of tetrahydrozoline application (Figure 5).

Figure 6 shows different amounts of collagen in the tissue structure observed under the polarization microscope after a 7-day observation period. A small amount of collagen observed after application of retraction agents was the sign of more intense acute inflammatory response.

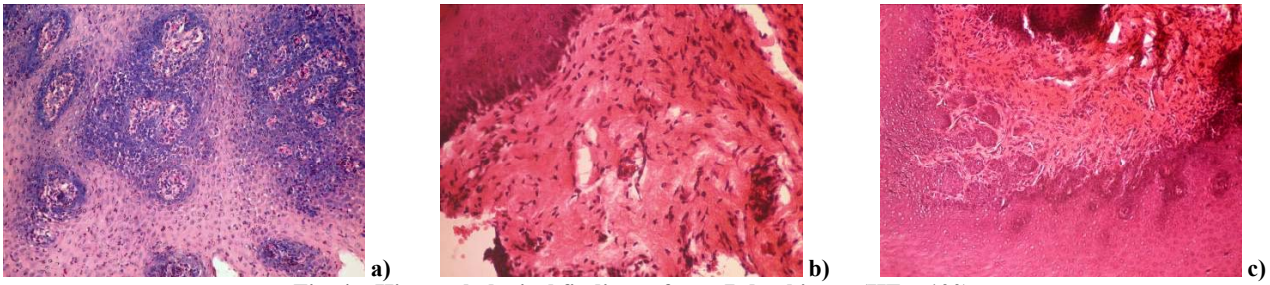

Fig. 4 – Histopathological findings after a 7-day biopsy (HE, ×100).
a) Gingival liquid: tissue samples after the removal of retraction cords with aluminum chloride showed signs of extensive inflammatory reactions; b) Surgident® (8% epinephrine-HCl): inflammatory response and the reduced amount of collagen fibers of small-scale than that presented in Fig. – 4a); c) Visine® (0.05% tetrahydrozoline hidrochloride): tissue fibrosis.

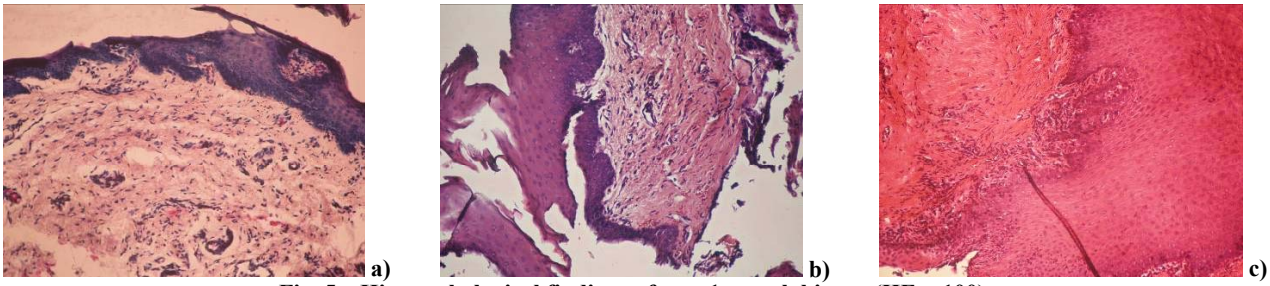

Fig. 5 – Histopathological findings after a 1-month biopsy (HE, ×100).
a) Retrargin® (25% aluminium chloride hexahydrate) and b) Surgident® (8% epinephrine-HCl): tissue preparations showed a reduced amount of collagen fibers and less focal inflammatory infiltrates, c) Visine® (0.05% tetrahydrozoline hidrochloride): a month after the removal of terahydrozoline, there was complete reparation of gingival tissue.

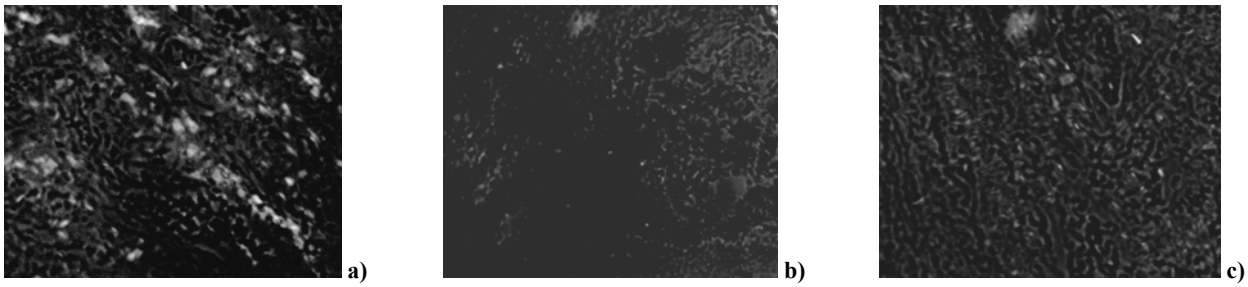

Fig. 6 – Amount of collagen fibers in the gingival tissue 7 days after the application of retraction agents (magnification ×100).
a) normal amount of collagen in the negative controls; b) reduced amount of collagen in the gingival tissue treated with Retrargin® (25% aluminium chloride hexahydrate); c) reduced amound of collagen in the gingival tissue treated with Visine® (0.05% tetrahydrozoline hidrochloride).

Discussion

In order to make an adequate impression of the marginal line area located in or below the gingival level it is necessary to dilate and dry the gingival sulcus. The consequent ischaemia is reversible and is accompanied by reactive hyperemia limited to a 17-minute period upon cord removal [8]. Prolonged ischaemia might lead to tissue damage and necrosis. Changes that occur after retraction procedure usually last from 1 to 2 weeks [10]. Still, infection or serious tissue damage may develop within this period [5]. Changes occur at the junction line of gingiva and connective tissue and may result in periodontitis, apical migration of epithelial junction and alteration of cement surface. After a period of tissue reparation clinically acceptable apical migration of marginal gingiva must not exceed 0.1 mm [4, 11].

Jokstad [12] has shown that the retraction effect of epinephrine- and aluminum-salt-based astringents is almost equal. On the other hand, there is a wide range of adverse systemic reactions to absorption of epinephrine, which significantly reduces its indication area. Therapeutic effect of epinephrine is vasoconstriction of blood vessels, leading to increased blood pressure and heart rate. The risk increases if epinephrine in retraction agent is combined with local anaesthetic, endogenous secretion in a stressful situation, or at greater damage of gingival tissue during tooth preparation [13]. In this study, retraction agents were administered to healthy gingival sulci, without previous tooth preparation, so as not to damage the tissue during preparation and thus jeopardize the objectivity of the results. Retraction was the result of local absorption of a retraction agent and the degree of resorption depended on the degree of tissue damage as well [14].

Previous studies have shown that retraction agents damage epithelium, sulcus epithelium as well as connective tissue *in vitro* and *in vivo* conditions [6, 15]. Changes in the periodontal tissue may be the result of mechanical damage of epithelium during application of retraction cord, but are more often related to the effect of the applied retraction agent. From the clinical point of view, the use of retraction cord without retraction agent indicates lower therapeutic effect [11].

The obtained results show that careful application of retraction cord cause no inflammatory changes in gingival tissue. The study results indicate a reversible damage to gingival tissue as a result of local application of aluminum-chloride- and epinephrine-based retraction agents. There were no significant changes in tissue structure 1 h after retraction agents removal. However, their use led to acute inflammatory response after an observation period of one and seven days. After thirty days reparation of damaged tissue was observed. These results are consistent with the findings of Harrison [16] and Ramadan et al. [17].

Tetrahydrozoline belongs to the group of sympathomimetic vasoconstrictors or α-adrenergic agonists and is commercially available as nasal and olfactory decongestants. Systemic reactions to the use of these products are very rare, given that the maximum recommended doses are significantly higher than those required for effective gingival retraction [2]. Studies by Bowles et al. [2] showed a satisfactory clinical effect of tetrahydrozoline, strong local vasoconstrictive effect and absence of systemic reactions. Clinical study conducted by Tardy et al. [18] demonstrated greater retraction efficiency of tetrahydrozoline in relation to epinephrine without adverse effects.

An *in vitro* study by Kopač et al. [19, 20] found significantly lower damage of cell cultures treated by tetrahydrozoline compared to aluminum chloride. Retraction agents represent acidic solutions with pH values from 0.8 to 3, the parameter which is considered to be major cause of periodontal tissue damage [21]. Conversely, pH value of tetrahydrozoline is 5.6, so it is considered biologically acceptable from that point of view [20]. An *in vitro* study of Nowakowska et al. [22] showed high cell viability values of human gingival fibroblasts after treatment with tetrahydrozoline-HCl based gels. On the other hand, the authors demonstrated cytotoxic activity of astringent retraction agents [23].

Inflammatory changes occurred as the result of application of tetrahydrozoline were of significantly lower intensity compared to the retraction agents based on aluminum chloride and epinephrine, and resulted in a complete tissue reparation after a 1-month observation period. Tetrahydrozoline proved to be biologically acceptable in relation to the investigated retraction agents. As biocompatibility is considered to be an essential feature of dental materials, clinical use of terahydrozoline is recommended [24].

Conclusion

All the examined retraction agents led to an acute inflammatory infiltration of gingival tissue in rabbits, which eventually became chronic. The inflammatory response to the administered tetrahydrozoline was significantly lower with complete reparation of tissue. Taking this fact into account it is recommended as a potential retraction agent.

Acknowledgment

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