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ORIGINAL ARTICLE

Histopathological evaluation of the effects of fiber reinforced acrylic resins on living tissues

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Abstract

Objective. The aim of this study was the histopathological evaluation of the effects of the fiber reinforced acrylic resins on living tissues. ***Materials and methods.*** The study was performed on 21 rabbits. Three groups, each including seven subjects, were formed. There was no applied plate in the control group. For the second group, heat-polymerized acrylic resin plates were inserted. For the third group, heat-polymerized acrylic resin plates containing proportionally 5% chopped silanated E type glass fiber were inserted. Plates were fixed to the palatine bone of the rabbits with titanium screws. Before the implementation of the plates and 1 month after the plates were applied, soft tissue samples were taken from the buccal mucosa of the rabbits. Also, tissue samples were taken from the control group. All samples were evaluated histopathologically. ***Results.*** In the control group, only a focal atrophy was observed. In the acrylic group, large decomposition containing erythrocytes under the parahyperkeratotic region and micro-vesicle like spongiotic tissue reactions were observed. In the fiber reinforced acrylic group, widespread focal atrophy, bulgy look of the epithelium cells similar to apoptosis, over-distension and sub-corneal decomposition had been observed. In terms of atrophy and hyperkeratosis there were no statistically significant differences among groups. However, in respect to sub-corneal decomposition, there was a statistically significant difference in the fiber reinforced group ($p < 0.01$). ***Conclusions.*** The statistically significant difference in the sub-corneal decomposition of the fiber reinforced group had made us think that fiber edges had a traumatic effect on the reaction.

Key Words: *fiber reinforced PMMA, acrylic resin, rabbit, histopathologic evaluation*

Introduction

Polymethylmethacrylate (PMMA) is a commonly used denture base (acrylic resin) material. However, it has some weak mechanical and physical properties. There are a lot of studies in the literature aiming to improve these weak properties [1-8]. One of the procedures to reinforce the PMMA is to use various fibers, mainly glass fibers [1–4,8–16].

Glass fibers are high-strength materials and do not wear out in water and alkaline [17]. They are esthetic [4,14–16] and transparent [14]. Glass fibers' improvement of acrylic resins' transverse strength [3,8,9,18,19], elastic modulus [3,14,18], fatigue strength [19,20] and impact strength [4,8,14,21] are reported in a great number of studies.

Özdemir and Polat [22] had reported that the fiber edges were released out of the polished surfaces, especially after the finishing and polishing procedures, in chopped fiber reinforced acrylic resins. In the literature review, there were no studies about the evaluation of pathologic effects of released fiber tips on the oral mucosa.

Atrophy, parahyperkerathosis or sub-corneal decomposition could be observed in the oral mucosa by trauma. Atrophy is the thinning and decreasing of the normal width of the epithelium [23].

Parahyperkeratosis is the retention of nuclei in the cells of the stratum corneum (corneocites) of the epidermis. In parahyperkertosis, the nuclei in the cells were intact with the thick keratin layer. This is a reaction to trauma [23]. Sub-corneal decomposition is the

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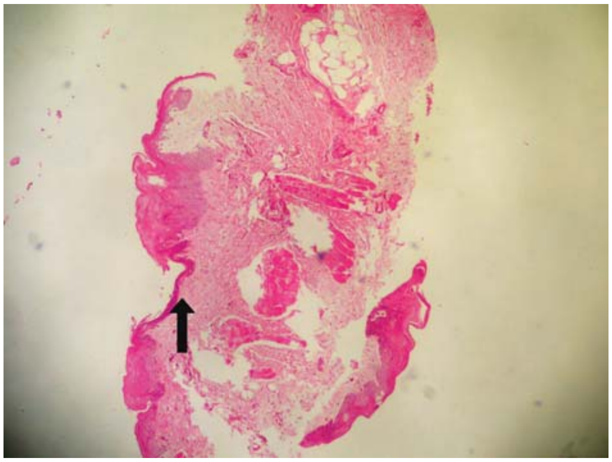

Figure 1. Mild focal atrophy seen in the control group (⇑) (HE × 40).

decomposition of the stratum corneum from the stratified squamous epithelium beneath, which protects the epidermis from the environment. It can be seen as a result of inflamed accumulation and exudates in the dermatologic and various inflammatory diseases (dermatosis) and also with the mechanical trauma. This decomposition can cause sub-corneal separations. Furthermore, this case can occur also on the bases of edema and inflammation or perhaps in the deficiency of structural cell adhesion. Minor trauma can also cause the same effects and decomposition can occur [23].

It was observed that the tissue reactions were not sufficiently mentioned in the studies of mostly *in vitro* mechanical properties of glass fiber reinforced acrylic resins [3,4,8,9,14,18–21]. Therefore, the aim of this study is to investigate the pathologic effects of chopped glass fiber reinforced acrylic resin in the oral mucosa of the rabbits.

Materials and methods

This study was supported by the Cumhuriyet University, Scientific Research Projects Commission Presidency. Cumhuriyet University, Faculty of Dentistry, Department of Prosthodontics, Cumhuriyet University, Experimental Animal Laboratory and Cumhuriyet University, Faculty of Medicine, Department of Pathology resources were used in the study. The study was approved by the local ethics committee.

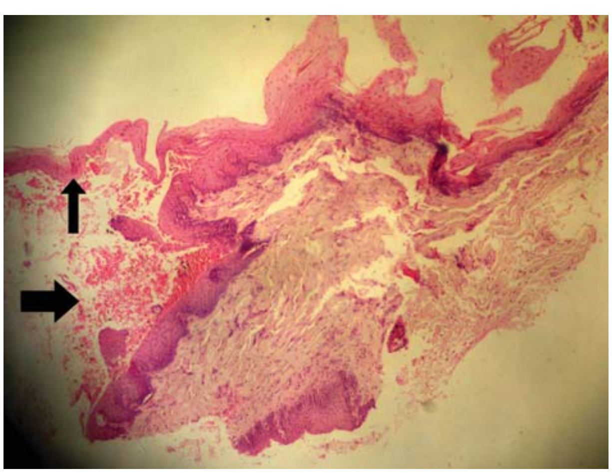

Figure 2. Wide decomposition erythrocyte under the parahyperkeratosis (⇑) in acrylic group (⇒) (HE × 40).

Generating the experimental groups

New Zealand rabbits weighing above 3 kg were used as experimental animals. Three groups, each including seven subjects, were formed. There was no applied plate in the control group. For the second group, heat-polymerized acrylic resin (Meliodent, Bayer UK Ltd Newbury, Berkshire, UK) plates were inserted. For the third group, heat-polymerized acrylic resin plates containing proportionally 5% chopped silanated (γMPS, HÜLS-Veba GmbH, Germany) E type glass fiber (Ahlstrom, Karhula, Finland) were inserted.

Construction of appliances

Before the construction of the appliance, an impression of the maxilla was taken from a rabbit out of the experiment under general anesthesia (subcutane 10 mg/kg Xylazin, Rompun, Bayer Vet. 50 cc and 50 mg/kg Ketamine HCL, Ketalar 5%, Pfizer, İstanbul, Turkey) with a putty elastomeric impression material (Durosil L, PD, Munich, Germany). The borders of the appliance were drawn on the cast obtained from this impression. Appliances were formed in the borders of; palatinal sides of the incisors in the anterior, the base of the vestibular sulcus on both right and left sides and ~ 15 mm from the palatines of the incisors in the posterior. One layer modeling wax (Modelling Wax, Toughened Dental, Hampshire, UK) was placed on these borders and an autopolymerizing acrylic resin (Panacryl, Imicryl, Konya, Turkey) individual impression tray was constructed. By the help of this impression tray, first impressions were taken under general anesthesia. Second impressions were taken with putty (Durosil L, PD) and light body (Ekoflow Light, Tıssı Dental, Milan, Italy) elastomeric impression materials with putty-wash technique by the individual impression trays for each subject. A layer of modeling wax was placed on the obtained models in the borders of the appliance and the surface was trimmed. Then the models were flasked. After the wax elimination procedure, heat-polymerized acrylic resin was prepared according to the manufacturer’s instructions. In the fiber reinforced group, chopped 5 mm in length silanated E type glass fiber were added proportionally 5% to each heat-polymerized acrylic resin plate. The obtained mixture was polymerized according to the manufacturer’s instructions. During the preparation of the acrylic resin dough in this group the addition of monomer (0.7 ml monomer for 1 g fiber) for the

Table I. Existence of focal atrophy, parahyperkeratoz and sub-corneal in study groups.

	Control (n = 6)		Acrylic (n = 6)		Fiber reinforced acrylic (n = 7)	
	existing	absent	existing	absent	existing	absent
Focal atrophy	6	—	6	—	6	1
Parahyperkeratosis	4	2	4	2	6	1
Sub-corneal decomposition[a]	1	5	1	5	6	1

Data were expressed as a number.
[a] $p < 0.05$ fiber reinforced acrylic resin.

fiber were done. The finishing and polishing procedures of the polymerized appliances were done in standard ways.

Application of the appliances to the rabbits

Appliances were stored in distilled water for 1 week at room temperature and sterilized in ethylene oxide before they were applied to the rabbits. Soft tissue samples were obtained from the rabbits' buccal mucosa in contact with the appliances with a punch biopsy before they were applied. Then the appliances were fixed to the maxilla of the rabbits with titanium screws; 7 mm titanium screws (Tıpmed Tıbbi-Medikal Ürünler San. Tic. Ltd. Şti., İzmir, Turkey) were used to place the plates to the palatine bone.

Pathological evaluation

Routine pathologic procedures were processed in the pathology laboratory on the samples taken from the experimental zones. The samples taken were first fixed in the 10% formalin solution for 24 h. Suitable samples (1–2 mm thick) were taken for cross-section, after the fixation. These pieces were traced in Shandon Pathcentre (Thermo Electron Corporation, Cambridge, UK) auto-tracking device. These tracing procedures consisted of 14 steps (seven alcohols, three xylen and four paraffin) and last 16 h. Pieces are then embedded in paraffin blocks; 3 μ thick cross-sections were taken from these blocks in a full-automatic rotary microtome (Finesse, Thermo Electron Corporation). Then the sections were stained with hematoxylin and eosin method.

Sections were examined with a digital camera attached (Nikon FDX-35) light microscope (Nicon E clipse E 600) and photographed by 100 × objectives.

The same procedures were applied to the tissue samples taken from the adjacent soft tissues with a punch biopsy at the end of the first month. One rabbit from each control and acrylic groups had died before the completion of a month.

Statistical analysis

The data of pathologic values were analyzed with the chi-square test in SPSS (10.0, SPSS Inc., Chicago, IL) program. A p-value of less than 0.05 was considered as significant.

Results

The experimental groups were evaluated pathologically in terms of sub-corneal decomposition,

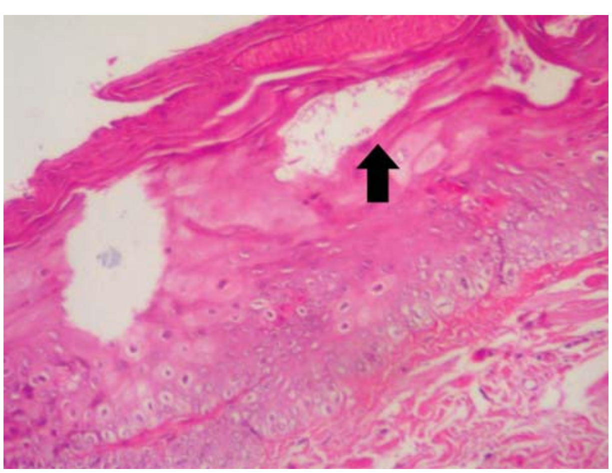

Figure 3. Close image of the microvesicule pattemed spongiotic tissue reaction in the acrylic group (⇑) (HE × 100).

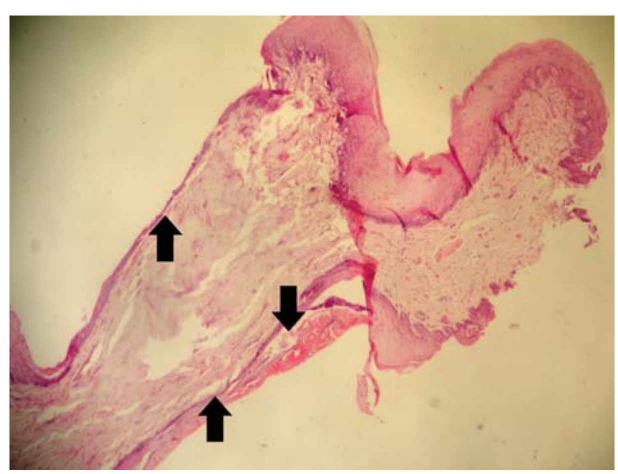

Figure 4. Focal atrophy formed on both sides of the tissue in fiber acrylic group (⇑) . Bleeding sub-corneal decomposition on the right side (⇓) (HE × 40).

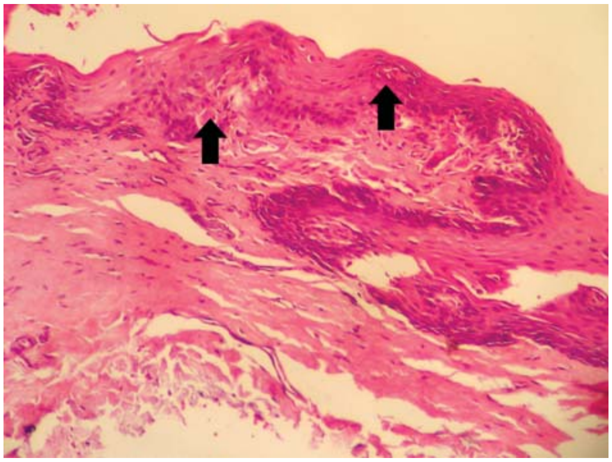

Figure 5. In the fiber reinforced acrylic group, bulgy look of the epithelium cells similar to apoptosis (⇑) (HE × 100).

parahyperkeratosis and focal atrophy. There were no statistically significant difference among the groups in terms of atrophy and parahyperkeratosis, however sub-corneal decomposition was observed in six of the seven fiber reinforced plates applied subjects ($p < 0.01$) (Table I) .

Although, only mild focal atrophy was observed in the histopathological examination of the cross-sections of the control group (Figure 1), a wide decomposition containing erythrocyte under the parahyperkeratotic zone and microvesicule like spongiotic tissue reactions in the acrylic group (Figures 1, 2).

In the fiber group, common focal atrophy due to trauma, swollen appearance of epithelial cells similar to apoptosis, ballooning and sub-corneal decomposition was observed (Figures 4, 5, 6).

Discussion

In the fiber reinforced acrylics, additional monomer usage is necessary to increase the bonding between fiber and acrylic. This procedure increases the residual monomer release [24]. The prolongation of the test samples immersion in water decreases the residual monomer in the acrylic resin [25]. It was shown that the maximum monomer release was in the first 5 days [26]. Therefore, plates were immersed in water for 1 week before they were applied to the rabbits.

Some researchers reported that random placement of fibers into the acrylic resin is not a time-consuming method, has good results and is easy to apply [3,4,13]. For these reasons, the fibers were mixed into the polymer powder. Kanie et al. [14] showed that the small pieced, randomly mixed, 1% in weigh, glass-fiber reinforced acrylic resin increase the resistance to fracture strength. Belvedere [27] had expressed that as the amount of fiber in the resin increases, the fracture strength gets higher. However, Ladizesky et al. [28] did not recommend fiber addition higher than 4% in weight. Stipho [15] had reported that higher than 5% in weight addition of glass-fiber does not bring significant mechanical advantage. For these reasons, glass-fiber of 5% in weight was added in the study.

Fibers placed appropriately to the weakest parts of the denture make a very good reinforcement [3]. In clinical conditions, randomly placed, short fiber reinforcement was recommended in cases of deep labial frenum notch, torus palatinus and resilient alveolar ridges which cause midsagittal fractures of the dentures.

Marei's [3] study showed that the different sized—short glass-fibers (approximate lengths were 88 μm)—and randomly spread fibers, increase the transverse strength of acrylic resins and had similar results with the reinforcement with thread-like fibers. In Keyf and Uzun's [18] study the chopped glass fibers had increased the transverse strength and elasticity modulus of the acrylic base plates. Karacaer et al. [29] had similar favorable results. For these reasons and also the ease of application, chopped glass-fiber was used in the study.

Non-silanated fibers added to the resin had weakened the acrylic instead of strengthening it [4,30]. Non-silanated fiber decomposed the homogen matrix formation. The silane agents bonding the glass-fiber to the resin had rendered the mixture more homogenous and strengthened the PMMA [14,30]. In the literature, AA h174 silane coated glass-fibers had significantly increased the strength of the acrylic resin [9,19]. Therefore, A 174 silanated E-type glass-fiber was used in the study. Heat-polymerized acrylic study of Vallittu [9] had shown that PMMA had sufficient adherence with E-type glass-fiber.

It is hard to increase the fiber concentration in the acrylic polymer matrix because the fibers are spread laterally during the pressure of the acrylic dough and disperse non-homogenously [7,19]. This event

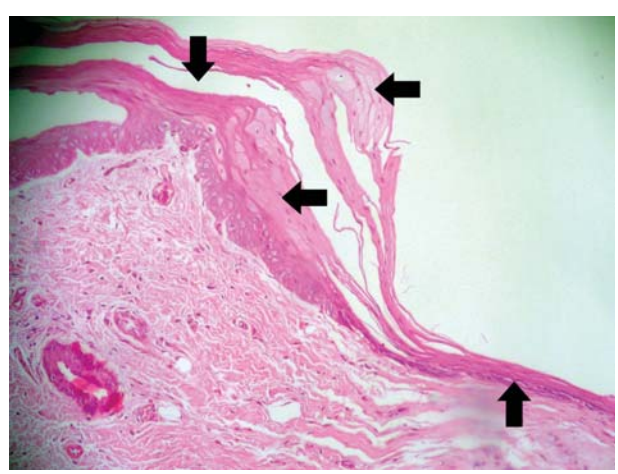

Figure 6. In the fiber acrylic group, focal atrophy due to trauma (⇑) , ballooning (⇐) and sub-corneal decomposition (⇓) was observed (HE ×100).

increases the risk of especially the chopped-fiber edges' spread out of the acrylic [13]. In one of Marei's [3] studies, it was shown with SEM analyses that, if the samples were prepared according to the standard feature, the fiber edges were not coming out of the acrylic resin. However, some researchers [18,31] had expressed that, when chopped-fibers were used, during the finishing procedure of the denture, the fiber edges would come out and get in contact with the oral mucosa.

In Özdemir and Polat's [22] study, it was determined by SEM that the chopped-fiber edges were coming out of the polished surfaces of the acrylic resin samples. This study is aimed to pathologically investigate the effects of fiber edges coming out of the acrylic plate's polished surface on the oral mucosa of the rabbits.

Focal atrophy develops depending on the pressure. Therefore, it is thought that the fibers were not responsible for the focal atrophy observed in each group.

Parakeratosis is an auxiliary histopathological finding in various dermatological diseases. It is notified that, in diseases showing spongiotic reaction pattern up to various leveled decomposition and vesiculation in tissues, parakeratosis formation on the spongiotic zone is related either to the deterioration of maturation or the increase in the movement of keratinocyt to the surface [32]. When this occurs with hyperkeratosis, developed as a mechanism to protection from trauma, it is called parahyperkeratosis. In the study, statistically significant parahyperkeratosis and sub-corneal decomposition seen in the fiber group are believed to be due to both mechanical response to trauma and contact allergy caused by the acrylic resin in touch with the tissue. Because the sub-corneal decomposition is statistically significantly higher in the fiber acrylic group, it is thought that the fiber edges are efficient in the reaction. However, there were no similar studies in the literature review so we couldn't find a comparison opportunity.

Conclusions

According to the limitations of the study, fiber edges on the acrylic surface had created pathological changes up to sub-corneal decomposition on the oral mucosa of the rabbits. Efforts should be made to make studies to prevent the spreading of the fibers to the surface during the strengthening procedures of the acrylics.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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