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Histopathologic study of rat connective tissue responses to maxillofacial silicone elastomers

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Abstract The aim of this histopathologic study was to assess and compare the subcutaneous connective tissue reaction to three different maxillofacial silicone elastomers (Cosmesil, Multisil, Episil). The test materials were directly inserted subcutaneously into the dorsal subcutaneous tissue of Wistar albino rats. Histopathological examinations were done at 7, 30, and 90 days after the implantation procedure. The presence of inflammation, presence of inflammatory giant cells, and the thickness of fibrous connective tissue adjacent to each inserted sample were recorded. Data was evaluated by analysis of variance, Wilcoxon signed ranks test and Kruskal Wallis test. Cosmesil, Multisil and Episil silicone elastomers at 7 days elicited a severe inflammatory reaction. However, these reactions decreased by the 30 and 90 days. All silicone elastomers elicited a moderate inflammatory reaction at 30 and 90 days. There were no significant differences in tissue reaction between the materials at 7, 30, and 90 days ($P > 0.05$). All the maxillofacial silicone elastomers evaluated can not be assigned a favorable biocompatibility level based on this study's histologic findings.

1 Introduction

Maxillofacial prostheses are used to transform congenital, developmental, and acquired defects of the head and neck into natural-appearing reproductions of the missing parts, thus providing an acceptable appearance and improved function [1]. Over the past 60 years, many materials have been tried and found to be suitable for clinical use and applications of maxillofacial defects. These materials include polyvinylchloride, hard on plasticized acrylic resins, latex rubber, polyurethane, silicone elastomers, silphenylene elastomers, chlorinated polyethylene, and terpolymer acrylic latex. Of all these materials, silicone elastomers currently seem to be the most popular and most widely used in the manufacture of maxillofacial prostheses (facial prostheses, flexible obturators, implants, combined orofacial prosthetic devices) [2–4]. There are two basic types, namely, room temperature vulcanizing (RTV) and high temperature vulcanizing (HTV) silicone elastomers [3]. The preference for silicone, especially the RTV type has been overwhelming [2, 5]. Scientific investigations have demonstrated the superiority of HTV silicones, which are generally stronger, tougher and stiffer than RTV materials [3, 4, 6]. The major limitation of the HTV silicone is in fabrication. The material requires a milling machine and fabrication of metal molds, although stone molds have also been used [7].

With regard to ideal quality features (chemical, physical, or mechanical), the material should be compatible with human tissues (skin, oral and nasal mucosa) and should cause no irritation. The material must not be capable of initiating an inflammatory or foreign body reaction, and it should be noncarcinogenic [8].

Biocompatibility is the ability of a material to elicit an appropriate biological response in a given application in the body [9, 10]. It is an essential step and important requirement for dental materials toward the acceptance of the material in addition to testing of physical and mechanical properties, because the toxic components

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present in these materials could produce irritation or even degeneration of the surrounding tissues [11, 12]. In the literature various test methods have been proposed to determine the biocompatibility of dental materials [7, 8, 13–15] The principal ones are cytotoxicity test conducted in vitro on cell or tissue cultures [7, 8, 16], and subcutaneous connective tissue or bone implantation methods in experimental animals [10, 15]. Data from cell culture or implantation tests can provide information on basic biological properties. The subcutaneous connective tissue implantation in animals is one of the reliable methods of evaluating biocompatibility of dental materials [10, 15, 17] because inflammatory reactions are a characteristic features for all connective tissues [17, 18]. The mechanical and physical properties of maxillofacial silicones have studied previously [19, 20], however, a review of the available literature reveals that little information exists on the in vivo biocompatibility of external maxillofacial materials [15]. The little information about the biocompatibility of maxillofacial prosthetic materials is all the more surprising since these materials may have contact with intra-tissue spaces via compromised tissue surfaces. In a previous study [16], in vitro cytotoxicity of the silicone elastomers used in the current study (Cosmesil, Episil, Multisil) were evaluated and it was reported that all test materials had no toxic effect on the cells in MTT assay. However, it must be emphasized that even the most elaborate and specific test systems in vitro do not obviate the need to perform subsequent tests in experimental models in vivo [8].

Consequently, the purpose of this investigation was to assess and compare the subcutaneous connective tissue reaction to commercially available HTV maxillofacial silicone elastomers. The hypothesis was, that the commercially available silicone elastomers that may have contact with intra-tissue spaces via compromised tissue surfaces could present good biocompatibility.

2 Materials and methods

Twenty-one adult male Wistar albino rats, weighing between 200 and 250 g were used for the present study. Specimens were distributed in three groups of seven animals each, to be examined after 7, 30, 90 days from the surgical procedure. Test specimens were obtained by converting disk shaped wax specimens (10 mm in diameter and 2 mm in thickness) to silicone. The silicone materials were processed in the mold according to the manufacturers' instructions. The specimens were specially produced in disk shaped form in order to avoid mechanical irritation. Table 1 shows the materials tested in the present study. All procedures in this experiment were conducted according to the guidelines approved by the Animal Etchical Committee of Gazi University (06.051).

After the animals had been anesthetized by the administration of ketamine and xylazine (40–80 mg/kg) intraperitoneally, the dorsal skin was shaved and disinfected. Four incisions were made through the skin and subcutaneous pockets were carefully prepared by a blunt dissection. Specimens were previously autoclaved at 120°C for 15 min and each rat was implanted with of three different maxillofacial silicones. The forth incision without any implanted material was used as a control. Finally the incisions were closed with surgical gut sutures. The animals maintained in cages on regular diet and water ad libitum.

After the experimental periods, all animals from the group were killed by an overdose of anesthetic. The dorsal skin was shaved and disinfected and the implants together with their surrounding tissues were removed and fixed in 10% formalin solution (37% formaldehyde, Merck Darmstadt, Germany) for 24 h. Soft tissue material containing the area of insertion of silicone material was sampled with a cut right in the middle of the material. Silicone materials were then extracted and tissue samples were embedded in paraffin. Each tissue in paraffin block was cut with a standard thickness of 4 μm. Sections were then deparaffinized at 65°C for 1 h, washed in xylene and alcohol. Tissue slides were stained with routine hematoxylin eosin stain. Evaluation of slides was performed under Leica DM4000-B light microscope. Quantitative evaluation of inflammatory cells was performed in 4 high power field by Leica DC-500 camera and Leica QWin 3.3 image analyzer software system. The type of inflammatory cells was also noted. The inflammatory reactions were scored and evaluated as: 0, none or few inflammatory cells and no reaction; 1, $n < 25$ cells and mild reaction; 2, between 25 and 125 cells and moderate reaction; 3, $n \geq 125$ cells and severe reaction [21, 22]. Fibrous capsule thickness was measured by Leica DC-500 camera attached to mentioned microscope and Leica QWin 3.3 image analyzer software

Table 1 Maxillofacial silicone elastomers

Material	Processing procedure	Type	Manufacturer
Cosmesil	1 h at 100°C in mold	Addition curing	Principality Medical Ltd, Newport, UK
Multisil	30 min at 60°C in mold	Addition curing	Bredent, Senden, Germany
Episil	1 h at 60°C in mold	Addition curing	Dreve-Dentamid GmbH, Unna, Germany

was used. Five separate walls from each capsule of every sample were photographed and the thickest cross section length of the capsule was measured. Areas of artificial separation of the collagen bundles were disregarded.

Quantitative data of the fibrous capsule thickness were tested for statistical significance using analysis of variance and multiple comparison (Duncan's test, $P < 0.05$). Wilcoxon signed ranks test was performed to compare the intensity of inflammation between the materials on each of the experimental period at a significance level of $P < 0.05$. Furthermore the statistical significance between the periods (7, 30, 90) was analyzed with Kruskal Wallis Test ($P < 0.01$).

3 Results

All animals remained in good health during the whole implantation periods. No postoperative complications were observed and the surgical sites healed with no objective signs of infection. The mean and standard error mean of inflammatory cell numbers are shown in Table 2. The statistical comparisons among the experimental periods (7, 30, 90) are shown in Table 3. According to analysis of variance, there was no two factor interaction amongst the material and period factor of fibrous capsule thickness, thus the statistical comparisons were performed by general mean values of fibrous capsule thickness of materials or periods and the significant differences were determined by Duncan's test ($P < 0.05$). The statistical comparisons of the fibrous capsule thickness values between the test materials were presented in Table 4. A clear fibrous capsule was observed beginning from day 7 in all of the groups. Thickness of fibrous capsule increased with time and the individual comparisons revealed that the differences between 7, 30, and 90 days were statistically significant ($P < 0.05$) (Table 5). An incision without any implanted material served as a control for the technique and showed no inflammatory reaction in all experimental periods.

Table 2 The mean and standard error of mean of inflammatory cell numbers

Materials	7 days Mean ± SE	30 days Mean ± SE	90 days Mean ± SE
Cosmesil	235.1 ± 17.4	107.3 ± 14.7	73.9 ± 10.06
Episil	258 ± 23.1	101.71 ± 8.57	65 ± 11.3
Multisil	206.3 ± 28.5	115.9 ± 14.1	84.14 ± 4.46

Table 3 Statistical comparisons of tissue reactions between the experimental periods for each material

Days	Cosmesil Median	Episil Median	Multisil Median
7	3A	3A	3A
30	2B	2B	2B
90	2B	2B	2B

Vertically, medians with identical capital letters were not significantly different ($P > 0.01$)

Table 4 Statistical comparisons of fibrous capsule thickness values (μm) according to materials

Materials	Mean	SE Mean	SD
Cosmesil	47.65A	3.83	17.54
Episil	58.14B	5.76	26.39
Multisil	56.70AB	4.27	19.57

Vertically, medians with identical capital letters were not significantly different ($P > 0.05$)

Table 5 Statistical comparisons of fibrous capsule thickness values (μm) according to experimental periods

Periods	Mean	SE Mean	SD
7	33.34C	1.36	6.21
30	59.12B	4.84	22.16
90	70.02A	2.93	13.41

Vertically, medians with identical capital letters were not significantly different ($P > 0.05$)

3.1 7 Days

Histological evaluation of tissue response at 7 days revealed that the inflammation was dominated by mononuclear cells, mainly lymphocytes. Plasma cells, macrophages and less neutrophils were admixed (Fig. 1a–c). Multinucleated giant cell was observed only in 1 sample of Cosmesil group. There was not any statistical significance between the amounts of inflammatory infiltration of tested materials. The median inflammatory reaction scores of both groups were 3 ($P > 0.05$). Mean values of fibrous tissue thicknesses were 33.73, 28.82, and 37.46 μm in Cosmesil, Episil and Multisil, respectively.

3.2 30 Days

Histological evaluation of tissue response at 30 days showed that the amount of inflammation was decreased according to 7 day; however, it was still dominated by lymphocytes in all groups (Fig. 2a–c). Although lessen in number, macrophages and plasma cells were also present in

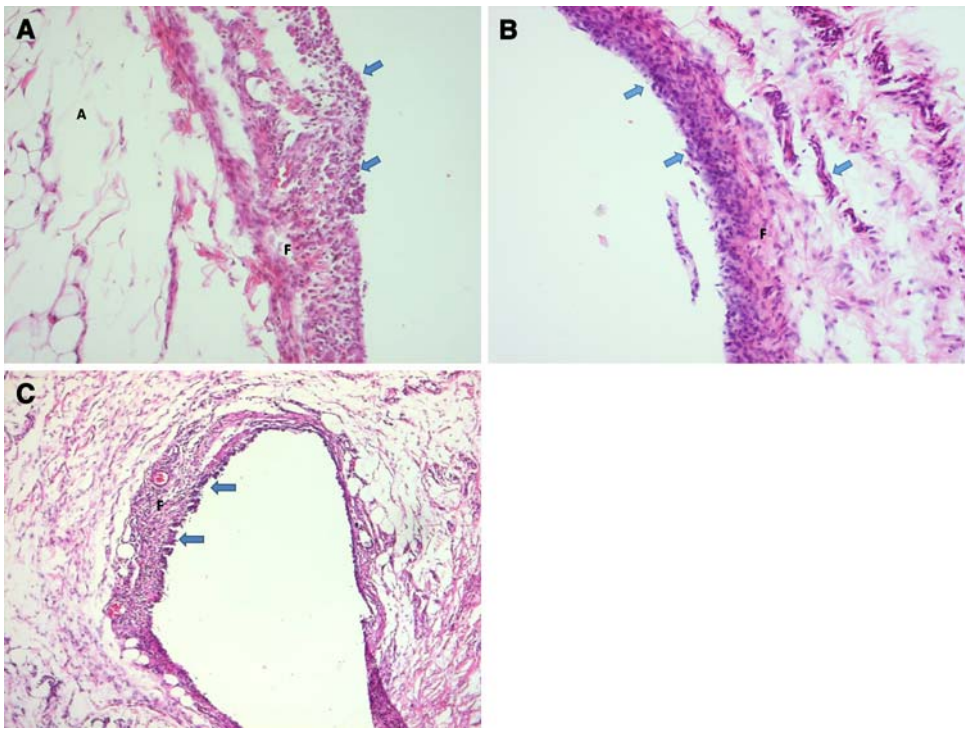

Fig. 1 **a** Cosmesil implants after 7 days. Note severe inflammation around the implant space. Lymphocytes, plasma cells, macrophages and neutrophils compose the inflammatory infiltration. Vascularization is relatively high (×200, H&E). **b** Episil implants after 7 days. The severe inflammation is dominated by mononuclear cells, mainly lymphocytes. Plasma cells, macrophages and less neutrophils are admixed. A thin fibrous capsule is observed (×100, H&E). **c** Multisil implants after 7 days. Note severe inflammation and thin fibrous capsule at the periphery of the implant space (×100, H&E)

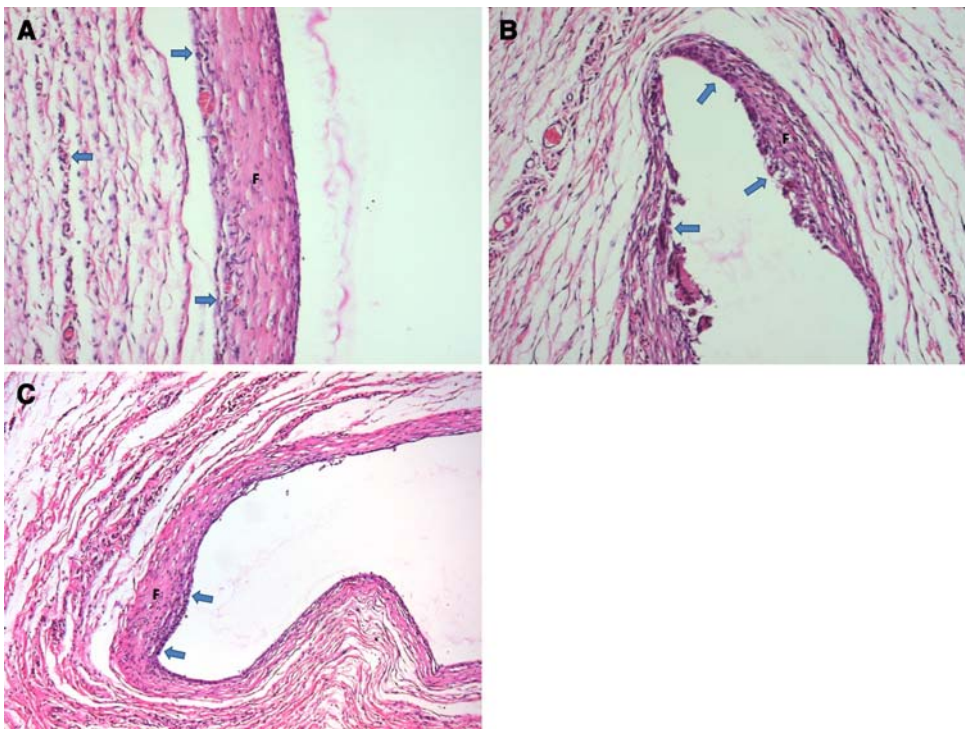

Fig. 2 **a** Cosmesil implants after 30 days. The amount of inflammatory cells is decreased, but the inflammation is still moderate and dominated by lymphocytes. Although lessen in number, macrophages and plasma cells are also present in the inflammatory infiltration. Note the thick fibrous capsule at the periphery of the implant space. *F* fibrous capsule, *arrows* inflammation (×200, H&E). **b** Episil implants after 30 days. Thick fibrous capsule formation as well as moderate inflammation is present (×200, H&E). **c** Multisil implants after 30 days. Note the thick fibrous capsule and moderate inflammation around the implant space (×200, H&E)

the inflammatory infiltration of each group. It was noted that the decrease was much evident in Episil group. Multinucleated giant cell was observed only in one sample of Episil group. There was not any statistical significance between the amounts of inflammatory infiltration of tested materials. The median inflammatory reaction scores of both groups were 2 ($P > 0.05$). Mean values of fibrous tissue thicknesses were 46.62, 65.19 and 65.57 μm in Cosmesil, Episil and Multisil, respectively.

3.3 90 Days

At 90 days, the number of inflammatory cells was decreased in all groups. Decrease of inflammation was

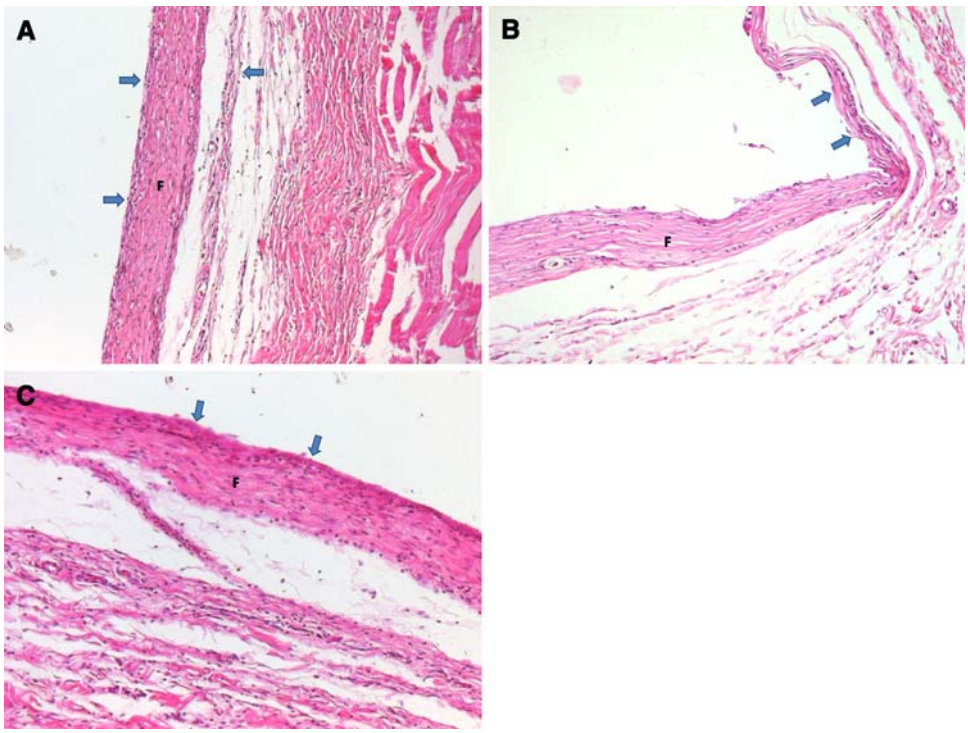

Fig. 3 **a** Cosmesil implants after 90 days. Thick fibrous capsule formation as well as moderate inflammation is present. Inflammation is spread to the adjacent connective tissue. *Arrows* fibrous capsule (×200, H&E). **b** Episil implants after 90 days. Although the decrease in inflammation is prominent, moderate inflammation is present (×200, H&E). **c** Multisil implants after 90 days. Thick fibrous capsule formation as well as a moderate inflammation is present. Macrophages and plasma cells are also present in the inflammatory infiltration (×200, H&E)

prominent in 3 samples of Episil group. The remaining inflammation was mainly consisting of lymphocytes. A few macrophages and plasma cells were present only in 2 samples. In Multisil group lymphocytes were the dominant cells of inflammation. Approximately all samples comprised scattered macrophages, and 4 samples also comprised few plasma cells. In Cosmosil group the dominant inflammatory cells were also lymphocytes. In 4 samples few macrophages and plasma cells were also present (Fig. 3a–c). There was not any multinucleated giant cell in any group. There was not any statistical significance between the amounts of inflammatory infiltration of tested materials. The median inflammatory reaction scores of both groups were 2 ($P > 0.05$). Mean values of fibrous tissue thicknesses were 62.60, 80.40 and 67.06 μm in Cosmesil, Episil and Multisil, respectively.

4 Discussion

Various methods to evaluate the biocompatibility of dental materials have been used, including cell culture tests [8, 16], and subcutaneous implantation tests [13–15, 23]. Assessment of the cytotoxic potential of silicone elastomers can not provide a definitive answer as to whether the materials are acceptable when they are used in the construction of maxillofacial prostheses. Correlative studies between cell culture and in vivo testing have been carried out, and in general poor correlation between the two methods has been observed [24–26]. Hensten-Pettersen and Hulterström [27], evaluated the cytotoxicity of four room temperature vulcanizing silicone elastomers used for maxillofacial prosthesis and the results of their study indicate that all materials tested were cytotoxic. In 1994, Polyzois et al. [8] reported a cell culture study of five room temperature cross-linking (RTC) silicone elastomers and they indicate that the RTC-silicone elastomers tested adversely affected cells in culture and concluded that the composition and type of polymerization (addition or condensation) of silicones contribute the effect on cell cultures. In another study the authors [7], evaluated the cytotoxic profiles of RTV and HTV silicones and they reported that all materials demonstrated no cytotoxic effects with the agarose overlay test. In 2009, Bal et al. [16] studied the in vitro cytotoxicity of three commercially available HTV maxillofacial silicone elastomers (Cosmesil, Episil, Multisil) by MTT test. They also reported that all test materials had no toxic effect on the cells in MTT assay and they added that Episil silicone demonstrated higher cell survival rates than Cosmesil and Multisil silicones. In the present in vivo study, although it was not found to be statistically significant, Episil silicone demonstrated better tissue response than Cosmesil and Multisil silicones.

Both condensation-type polymers using a tin compound or an organic acis as a catalyst, and addition type polymers using a platinum compound as a catalyst are currently used for making maxillofacial prostheses [28]. The examined materials in the current study were addition type high temperature vulcanizing silicone. Their basic structure unit is siloxane and they are in clinical use today. A review of

the international literature reveals that little work on the in vivo biocompatibility of maxillofacial prosthetic materials has been conducted.

Guttuso [29] and Olsson et al. [18] indicated that the implantation in subcutaneous connective tissues of small experimental animals was one of the most suitable test method to determine the local effects of materials. The toxic and inflammatory reactions in subcutaneous connective tissues against materials are thought to be characteristic features for all connective tissues [18, 29]. Wistar albino rats were used in the current study as they have the least postoperative infection risk compared to other experimental animals and they are not influenced by infection under aseptic conditions [17, 29]. The inflammation symptoms occurred in the regions where the tested materials were implanted until the end of the second week, and this inflammatory infiltration subsides after the third week [30]. In the present study, the inflammatory reactions at 7 days were more severe than at 30 and 90 days. This condition may be explained as the result of trauma produced during implantation of the material. Furthermore, tissue reactions can be effected by the shape and size of the implanted material [17], thus test materials of the same size and shape were used in this study.

The results of the study revealed that inflammation cell number was less for the Multisil group compared to Cosmesil and Episil at 7 days. However, at 30 and 90 days, Multisil was found to be causing more inflammatory response compared to others. Although it was not found to be statistically significant, Episil silicone demonstrated better tissue response than the other materials. The difference in inflammatory response among the maxillofacial silicone materials could be related to the variations in their chemical composition and quantity of chemotoxic leachables migrating from these materials and may be related to the difference in curing procedures of the materials.

Materials led to severe inflammatory responses initially, but the response decreased in time with an increasingly thickening fibrous connective tissue capsule forming around the samples and the statistical comparisons revealed that there were significant differences between 7, 30, and 90 days. The capsule is derived from the stroma of the tissue as the parenchymal cells atrophy under the pressure of the foreign material [31]. The fibrous tissue capsule seen around the samples can be expected around all synthetic materials such as those investigated. Thus, it can be concluded that the degree of inflammatory response is of greater importance in determining the biocompatibility of the materials. In the current study, all of the tested materials elicited a moderate inflammatory reaction at 30 and 90 days. There was not any multinucleated giant cell in any group. However, lymphocytes, few macrophages and plasma cells were still present in some samples at 90 day. Additionally, the differences between 30 and 90 days in Cosmesil, Episil and Multisil groups were not found statistically significant, thus the present study revealed that all the silicone elastomers tested can not be considered biocompatible with the connective tissue of rats.

No information was found in the available literature relative to the in vivo biocompatibility of the Episil and Multisil silicone elastomers tested. In 1989, Schmalz and Hambrok [32], studied the biocompatibility of Mollomed silicone by intramuscular implantation in rabbits. They reported no reaction to macroscopic examination of the implant sites and no inflammatory response of fibrous tissue capsule around Mollomed specimens. Wolfaardt et al. [15] reported on the results of subperiosteal, submucosal, and intramuscular implantation in five Chacma baboons. They found that Cosmesil, Silastic 382 (implantable silicone), and heat-cured acrylic resin materials all provoked the same mild-to-moderate inflammatory response. Our results did not support the hypothesis since the current study has demonstrated that both silicone elastomers implanted into the dorsal connective tissue of rats promoted a severe inflammatory reaction at 7 days, and a moderate inflammatory reaction at 30 and 90 days. The adverse tissue reactions elicited by the elastomers tested in the current study may be related to the composition or type of polymerization of the materials. To evaluate the leachable components and determine which components are responsible for the adverse tissue reactions, further studies are needed.

5 Conclusion

Biocompatibility is as important as the physical and chemical features when selecting a material for maxillofacial therapy because of the contact with internal tissue spaces that are contiguous to external surfaces. Within the limitations of this in vivo study, it can be concluded that all the maxillofacial silicone elastomers evaluated can not be assigned a favorable biocompatibility level based on this study's histologic findings.

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