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Evaluation of the Tissue Reaction of Five Different Suture Materials in Rabbit Palatal Mucosa

Tavşan Palatal Mukozasında Beş Farklı Sutur Materyaline Doku Reaksiyonunun Değerlendirilmesi

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Abstract

The objective of this study was to evaluate local tissue reactions at silk, chromic gut, polypropylene, polyester, and polyglactin 910 suture materials for intraoral applications. One hundred eighteen sutures were placed into the palatal mucosa of 26 male New Zealand rabbits so that each animal included all five biomaterials. The animals were fed a soft diet and decapitated 2, 4, or 8 days after suture placement. Soft tissue specimens including suture materials were prepared for light microscopy to determine the inflammatory zones including eosinophil infiltration on the suture tract. No significant differences were observed in the sutural zone diameter (Z1) between the suture materials at the 2nd day. At the 4th day, polypropylene and catgut had a lesser Z1 diameter compared to polyglactin 910. Dacron presented the widest mean Z1 diameter compared to polyglactin 910 ($p<0.01$), catgut ($p<0.01$), polypropylene ($p<0.05$) and silk ($p<0.05$) at the 8th day. On the day 8, the largest mean Z2 diameter was observed in dacron group compared to the mean Z2 values of catgut ($p<0.05$) and polyglactin 910 ($p<0.01$). Also the mean Z2 values of silk were significantly wider compared to polyglactin 910 ($p<0.05$). There was no difference between the eosinophil scores of the suture materials ($p>0.05$). Within the limitations of the present study, it may be said that silk and dakron sutures apparently induced more severe inflammatory reactions. When selecting a suture material for intraoral use the surgeons should take into consideration the tissue reaction caused by materials.

Keywords: Sutures/adverse effects; eosinophils; inflammatory response

Özet

Bu çalışmada, intraoral uygulamalar için ipek, kromik gut, polipropilen, polyester ve poliglaktin 910 sütür materyallerine karşın lokal doku reaksiyonunun değerlendirilmesi amaçlandı. Yirmi altı adet erkek Yeni Zelanda tavşanının palatal mukozasına her hayvanda beş biyomateryalin hepsi olacak şekilde 118 sütür konuldu. Hayvanlar yumuşak bir diyetle beslendi. Histolojik incelemeler yapılmak üzere hayvanların yaşamları 2, 4 ve 8. günlerde sonlandırıldı. Sütür materyallerini de içeren yumuşak doku örnekleri ışık mikroskopisi için hazırlanarak sütür traktusu üzerindeki eozinofil infiltrasyonu dahil enflamatuvar sahalar değerlendirildi. Sütür sahasında (Z1) ikinci günde, sütür materyalleri arasında anlamlı farklılık gözlenmedi. Dördüncü günde, polipropilen ve katgütte Z1 çapı poliglaktin 910'a göre daha küçük gözlendi. İpek, katgüt, polipropilen ve poliglaktin 910'a kıyasla sekizinci günde dakron da daha geniş Z1 çapı bulundu. Dakron grubunda sekizinci günde ortalama Z2 çapı, katgüt ($p<0,05$) ve poliglaktin 910'a ($p<0,01$) göre daha geniş bulundu. Ayrıca ipek materyalin Z2 çapı poliglaktin 910'a göre de anlamlı şekilde geniş gözlendi ($p<0,05$). Kullanılan sütür materyallerinin eozinofil değerleri arasında anlamlı farklılık bulunmadı ($p>0,05$). Bu çalışmanın sınırları içerisinde, ipek ve dakron sutur materyallerinin dokuda daha şiddetli enflamatuvar reaksiyon oluşturduğu söylenebilir. Cerrahi uygulamalarda cerrahlar, intraoral kullanım için sutur materyalleri seçerken bölgede oluşacak doku reaksiyonlarını da dikkate alması gerekmektedir.

Anahtar sözcükler: *Sütür/yan etkiler; eozinofil; enflamatuvar yanıt*

Introduction

Proper closure and stabilization of wound margins by sutures are critical events that may influence the success of any surgical procedure that favors hemostasis, nutrition and regeneration.[1-3]. However, the presence of foreign materials in a

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wound significantly enhances the susceptibility of host tissue's infection.[4,5] Moreover, the number of bacteria needed to establish infection is increased 10,000-fold by the presence of a silk suture.[6] Thus, the ultimate consequence of suturing can be postoperative infection resulting in compromised wound healing.

Suture materials can be classified as biodegradable or non-absorbable and may be manufactured as either mono- or multifilament fibers.[7] The advantage of absorbable sutures is that they generally do not require removal. Polyglactin 910 (Vicryl), a synthetic absorbable suture, is prepared from a synthetic absorbable copolymer of glycolide and lactide and is totally absorbed by day 42.[8] Chromic catgut is composed of collagen which is derived from the serosal layer of beef or fibrous submucosal layer of sheep, and is coated with chromic salt solution. Its degradation results from the enzymatic collagen digestion, which is completed in 60 days.[9] These materials incite varying degrees of tissue response depending on their degradation by hydrolysis, enzymatic digestion or phagocytosis.[10]

Non-absorbable suture materials have advantages in terms of tensile strength, resistance to contamination and bio-compatibility with living tissues.[11] Braided polyester (Dacron), a synthetic non-absorbable suture, has a high tensile strength and low tissue reactivity but the coating may crack after the knot has been tied.[12] Monofilament polypropylene (Surgipro), a non-absorbable polypropylene surgical suture, is made from braided single polypropylene filaments, thus making it softer and resistant to wrinkling.[13] Silk has been a favored non-absorbable suture material in oral surgery due to the ease of its handling.[14]

The healing of an incision wound comprises several sequential and overlapping phases beginning with the inflammatory stage, progressing through the remodeling stage.[15,16] The mechanism by which suturing and choice selection of suture material may influence surgical outcome is not totally clear. Exudative foreign body reactions to sutures may give rise to inflammatory responses, decrease resistance to infection, and ultimately impair wound healing.[17] Sutures may also serve as a pathway for bacteria into a surgical wound, a physical process likely enhanced by the capillary action of the suture material.[18] The physical configuration of some suture materials may protect contaminating bacteria and enable microorganisms to multiply beyond the access of the body's defense system[19]. Therefore tissue reaction to these materials is a crucial factor in choosing the best suture material. Tissue reactions associated with suture materials have been reported in the past.[20-22] Experimental and clinical data indicate that most tissue reactions begin around suture material left within the wound[7,23] and that the infection rate in contaminated tissues containing sutures is significantly greater than that in contaminated needle puncture tracts without sutures.[7] Furthermore, allergic reactions and reactions to the chemical structure of the suture material have been reported and may contribute to less than optimal wound healing.[7,24] A thorough understanding of the physical, mechanical and chemical properties of the commonly used suture materials is essential to the clinical practice of dentistry. Thus, the purpose of the present study was to investigate the histological alterations of the tissues surrounding the five different suture materials after their implantation to rabbit palatal mucosa.

Materials and Methods

Animals

26 white male New Zealand adult rabbits, weighing about 2-2.5 kg were included in the study. All animals were fed a soft-consistency laboratory diet supplemented with vitamins throughout the experimental period. The research protocol was approved by The Animal Ethics Committee of Ege University (No: 2006-18).

Experimental suture materials

Every animal received 5 sutures, each with 4-0, EP 1.5; polyglactin 910 (Coated Vicryl, Ethicon, Edinburg, UK), chromic gut (chromic surgical

gut suture, Boz, Ankara), silk (Silk, Vomel, Istanbul), monofilament polypropylene (Surgipro, Tyco Healthcare, Mechelen, Belgium), coated braided polyester dacron (Surgidac, Syneture, Connecticut, USA).

Placement of experimental sutures

Five experimental interrupted sutures were randomly placed by one investigator within the palatal soft tissue of each rabbit using the same needle style and keeping a minimum 0.5 cm distance between each suture (Figure 1).

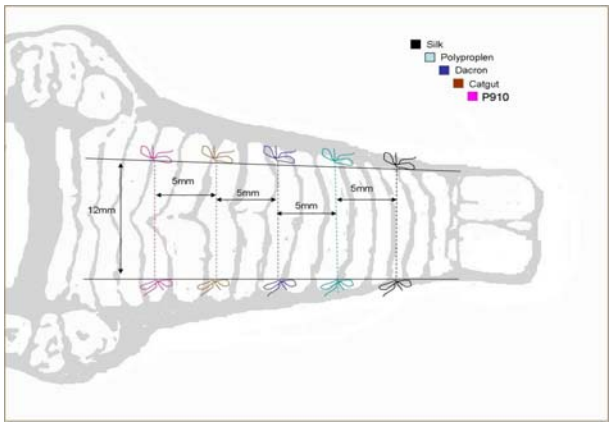

Figure 1. Placement of five suture specimens on the palatal mucosa of rabbits

Suture materials were placed in turn of polyglactin 910, chromic gut, silk, monofilament polypropylene, coated braided polyester Dacron by shifting the suture materials to the anterior part of the rabbit palatina. This data was recorded for each rabbit. The mesio-distal direction and bite (12 mm) and depth of the sutures were kept as standard as possible and the sutures were tied with two surgical knots at each end, keeping the tissue tension at minimum. The animals were randomly divided into three experimental groups and they were decapitated on days 2, 4 and 8. The loop suture form was avoided to prevent suture loss while feeding. Despite this attempt, some sutures were lost during the experimental period (Table 1); therefore at the end of the study each decapitation day had 6 sutures for each suture materials.

Specimen handling and histological analysis

Experimental sutures together with their surrounding tissues were removed and subsequently fixed in 10% formaldehyde solution. The specimens underwent routine histological processing and evaluation. The specimens were embedded in paraffin and oriented to perform serial slices of 4 μm thicknesses parallel to the suture in situ.

Consequently, each suture had a cross sectioned profile in the serial section. Starting 1 mm away from each knot, every third slide was selected. A total of six slides, three for the left and three for the right were stained with hematoxylin and eosin (H&E). The average of six histometric evaluations was recorded as a single value for each suture line. A total of 90 sutures were processed for histologic and histometric examination by an examiner blinded to the experimental protocol. However, all of the sutures were identifiable because of their microscopic characteristic.

A light microscope (Model:BX50F4, Olympus, Optical Co. Ltd., Japan) and an ocular micrometer were used to assess the following parameters (Figure 2):

Table 1. Number of sutures inserted and lost

Experimental Suture Materials	Silk	Polyprolene	Catgut	Dacron	P910	Total
Inserted	24	24	26	23	21	118
Lost	6	6	8	5	3	28
Evaluated	18	18	18	18	18	90

Diameter of the "sutural zone" (Zone 1), which is the dense cellular infiltrate surrounding the suture (Z1) (μm)

Diameter of the "perisutural zone" (Zone 2), which is the infiltration of scattered inflammatory cells and elements of immature granulation tissue (Z2) (μm)

Eosinophil intensity in the area of perisutural zone minus sutural zone = Eo: Eosinophil distribution is assessed as 1= mild, 2= moderate and 3= severe aggregation. A modification of the method described by Racey et al. was used to evaluate the inflammatory reaction.[25]

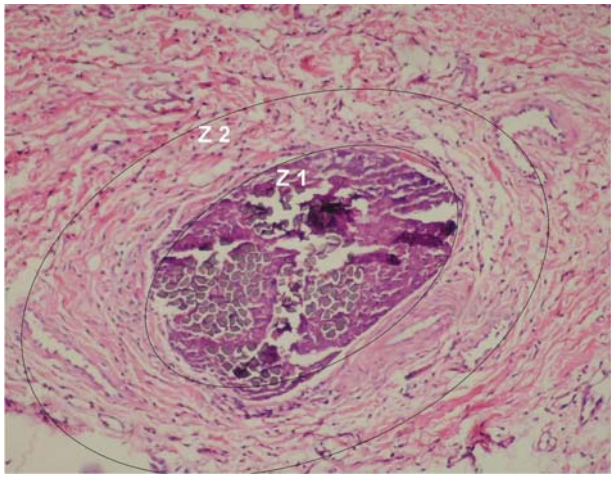

Figure 2. Illustration of Zone 1 (Z1) and Zone 2 (Z2)

Statistical analysis

The analysis of variance (ANOVA) was performed to test the differences between sutures for each decapitation day and to reveal significant differences between them. If significance was achieved, analysis of Tukey was used as a post-hoc test to detect the significant differences. In all statistical analyses suture material was the experimental unit and a significance level (α) of 0.05 was used.

Results

Histological findings

The 2nd day specimens of all types of suture materials revealed a polymorphonuclear leucocytes (PNL) infiltration at the perisutural area. Just adjacent to the sutural zone, a dense aggregate of inflammatory cells were frequently present. Early tissue response for silk suture at day 2 includes fine zone of dense cellular infiltrate and zone of inflammatory cells. Polyprolene sutures were undergoing degradation during the histological processes and were seen with complete absence of suture thread. Eosinophils were striking in Z1 and among the filaments of the suture materials. At day 4, perisutural connective tissue response became evident due to the accumulation of inflammatory cells. Generally the tissue response varied from a dense inflammatory infiltrate to a predominance of granulation tissue. At day 8, fibroblastic and angioblastic proliferation has started and, except the silk sutures, there was an apparent decrease in the intensity of inflammatory cells (Z2). Furthermore, there was a dense eosinophil infiltration on day 8 for silk and dacron sutures (Figure 3).

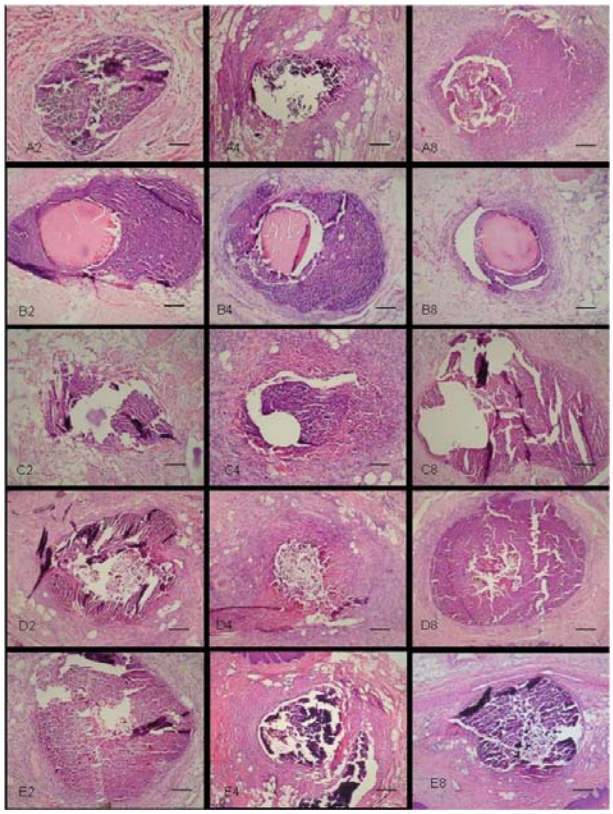

Figure 3. Five suture specimen at 2, 4, 8 days illustrating areas with inflammatory infiltrate in the connective tissue. A, Silk; B, Catgut; C, Polypropylene; D, Dacron; E, P 910 (Hematoxylin and eosin stain; bar=0.1 mm).

Histometric findings

Table 2 shows the inflammatory reaction in terms of the diameter of sutural zone (Z1) of all suture materials.

Z1-day 2: There were no statistically significant difference between polyglactin 910 / dacron as well as silk / polypropylene / catgut. Polyglactin 910 and dacron sutures presented a widest Z1 diameter compared to silk, polypropylene and catgut, however these differences were not significant.

Z1- day 4: The difference between the diameters decreased. Polyglactin 910 presented a wider Z1 diameter, where the difference was also not significant.

Z1- day 8: Dacron presented the widest mean sutural zone diameter compared to polyglactin 910 ($p<0.01$), catgut ($p<0.01$), polypropylene ($p<0.05$) and silk ($p<0.05$).

Table 3 shows the diameter of perisutural zone (Z2) of the suture materials. There were no significant statistical differences between the suture materials on the days 2 and 4. On the day 8, the largest mean Z2 diameter was observed in dacron group and the difference was statistically significant when the mean Z2 value of dacron was compared to the mean Z2 values of catgut ($p<0.05$) and polyglactin 910 ($p<0.01$). Also the mean Z2 values of silk were significantly wider compared to polyglactin 910 ($p<0.05$).

The Z1 and Z2 mean value differences of each suture material between day 2 and day 8 were tested with one way ANOVA and compared with post-hoc Tukey test.

Table 2. Inflammatory reaction in terms of the diameter of sutural zone (Z1) (μm) of all the suture materials on 2, 4, and 8 days (n=6).

Experimental Suture Materials	Day 2 μm (Mean ± SD)	Day 4 μm (Mean ± SD)	Day 8 μm (Mean ± SD)
Silk	650.60 ± 175.24	736.00 ± 166.05	886.40 ± 275.04 *
Polypropylene	639.00 ± 191.06	631.80 ± 269.14	835.80 ± 369.44 ¶
Catgut	697.00 ± 176.84	638.00 ± 180.38	637.20 ± 205.81 ¥
Dacron	879.80 ± 201.01	753.14 ± 253.85	1533.20 ± 261.37 *,¶, ¥, #
P910	789.00 ± 103.82	938.40 ± 82.94	538.66 ± 118.68 #

*: Significant differences between Silk and Dacron (P<0.05).
¶: Significant differences between Polyproylene and Dacron (P<0.05).
¥: Significant differences between Catgut and Dacron (P<0.001).
#: Significant differences between P910 and Dacron (P<0.001).

Table 3. The diameter of perisutural zone (Z2) (μm) of all the suture materials on 2, 4 and 8 days

Experimental Suture Materials	Day 2 n:6 μm (Mean ± SD)	Day 4 n:6 μm (Mean ± SD)	Day 8 n:6 μm (Mean ± SD)
Silk	945.40 ± 104.11	1120.00 ± 328.99	1473.40 ± 350.60 *
Polypropylene	840.00 ± 236.40	1033.20 ± 188.84	1086.40 ± 126.12
Catgut	1060.00 ± 214.21	966.60 ± 302.03	1033.40 ± 343.75 ¶
Dacron	900.00 ± 105.30	1104.70 ± 252.75	1873.20 ± 158.66 ¶,¥
P910	989.20 ± 136.07	1045.00 ± 164.35	646.60 ± 127.68 *,¥

*: Significant differences between Silk and P910 (P<0.05).
¶: Significant differences between Catgut and Dacron (P<0.05).
¥: Significant differences between Dacron and P910 (P<0.001).

Table 4. The density scores of eosinophils for each material on 2, 4 and 8 days

Experimental Suture Materials	Day 2 n:6 (Mean ± SD)	Day 4 n:6 (Mean ± SD)	Day 8 n:6 (Mean ± SD)
Silk	1.00 ± 1.22	2.20 ± 0.83	1.8 ± 0.44
Polyprolene	1.60 ± 1.51	0.20 ± 0.44	0.60 ± 0.89
Catgut	0.80 ± 0.44	1.80 ± 0.83	0.60 ± 0.89
Dacron	1.20 ± 0.44	1.40 ± 0.89	2.20 ± 0.44
P910	1.40 ± 0.54	1.00 ± 1.41	1.50 ± 0.83

* No significant differences between suture materials on 2, 4 and 8 days ($p>0.05$).

The increase in mean Z1 values of dacron from day 2 to day 8 *(Z1 diameter on day 8 – Z1 diameter on day 2)* was significantly higher than the silk ($p<0.01$), polypropylene ($p<0.05$) and polyglactin 910 ($p<0.01$). Likewise, the Z2 diameters (*Z2 diameter on day 8 – Z2 diameter on day 2)* of dacron presented a higher value when compared to catgut ($p<0.05$) and polyglactin 910 ($p<0.01$). There was also significant statistical difference between silk and polyglactin 910, silk had the larger diameter than the polyglactin 910 ($p<0.05$).

Eosinophil intensity (Eo): There was no difference between the eosinophil scores of the suture materials at day 2, but on day 4 silk had highest eosinophil score (Table 4). On day 8, it has been found that polypropylene and catgut presented a similar eosinophil score. On day 8, dacron had higher eosinophil score compared to other suture materials ($p<0.05$).

Discussion

Tissue reaction to suture materials is a crucial factor in choosing the best suture material. A thorough understanding of the physical, mechanical and chemical properties of the commonly used suture materials is essential to the clinical practice of dentistry. Sutures used in oral surgery behave differently from other parts of the body due to the quality of the tissues involved, presence of saliva and specific microbiota.[26] They represent a pathway communicating the internal and external regions of the tissues, influencing the quality of wound healing. A good suture avoids that the displacing forces generated by the muscular insertions, functional movements and by the external agents destabilize or cause the surgical wound dehiscence. It has been mentioned that rather than factors related to suture materials and different surgical techniques, and with the exception of surgeon experience, general characteristics of the patients (i.e., sex and age) and of the wounds (i.e., length and site) seemed to be primarily responsible for local wound complications (Biological behavior must be considered during the selection of the suture material to be used in oral surgery.[26,27] Although, there was no surgical sites were prepared to the palatal area, in the present study we investigated the local inflammatory effect and the infiltration of eosinophils around the different absorbable and non-absorbable mono- and multifilamented suture materials.

The best results are achieved in the Z1 and Z2 values of polyglactin 910. In both of these zones, a slight increase was found on day 4 and a decrease on day 8 which had a lower value when compared to day 2. Polyglactin 910 also enabled the organization of fibrous connective tissue around itself at late phases, showing a similar behavior as reported by Nary Filho et al.[28]

With regard to polyglactin 910, Andrade et al. observed that chromic gut had induced tissue necrosis and more granulation tissue formation.[3] According to Racey et al., catgut sutures started to disappear by the fifth day and were totally

absorbed after 7 days, but the inflammation reaction did not completely vanish.[25] Yaltirik et al.,[29] compared the tissue reaction effects of different suture materials in the soft tissues of rats and stated that polyglactin 910 had produced the mildest tissue reaction among the test materials. At this point, the results of the present study agree with Yaltirik et al.[29] According to their analysis, catgut suture, which is the natural absorbable material investigated, had incited a greater degree of inflammatory response with large cellular infiltration compared to polypropylene. In the present study, Z1 values of chromic catgut on days 2, 4 and 8 did not present a significant difference compared to polyglactin 910 and polypropylene.

In our study the response of the tissues to the silk material resembles with most of the observations made on animals or humans, in which silk has been considered to be a material inducing unwanted tissue reactions.[16,29,30-32] There was no significant statistical difference between days 2, 4 and 8 regarding the Z1 values of silk; but in Z2, the diameter significantly increased from day 2 to day 8. Most of the inflammatory reactions observed with multifilament materials have been attributed to the presence of bacteria within the interstices of the suture.[33] In Leknes et al.'s study, the braided silk sutures displayed a pronounced bacterial invasion in the interstices of the suture material.[16,31] In some specimens, parakeratotic epithelialization of the suture channel was evident both in the presence and absence of anti-infective therapy. Even though the bacterial invasion to silk material has not been investigated in this study, it may be the reason of increase in Z2 diameters towards day 8.

Monofilament polypropylene sutures are very popular in cardiac surgery because they are not subject to degradation or weakening by tissue enzymes; they are extremely inert in tissues and have been found to retain tensile strength for as long as 2 years *in vivo*.[13,33] Z1 and Z2 scores of polypropylene in this study revealed not significant diameter changes between days.

Braided polyester materials have been reported to allow capillary penetration of bacteria and fluids into their interstices, favoring the occurrence of tissue reactivity and infection.[30,34] Results of the present study indicate that dacron suture material presented a significant increase from day 2 to day 8 in both Z1 and Z2, concordant with the results of the studies above.

When the values of the suture materials on days 2, 4 and 8 were compared for both Z1 and Z2, the only significant difference was found on day 8 for both zones (Tables 2 and 3). Polyglactin 910 presented the best, that is the least diameter score, which is in concordance with the results of Andrade et al., Nary Filho et al. and Yaltirik et al.[3,28,29] Even though no significant differences were found between the Z1 and Z2 values of catgut between the days 2, 4 and 8, catgut ranks the second among other suture materials with its mean diameter values. Dacron suture presented the highest diameter scores for both Z1 and Z2 and is followed by silk. A dense population of inflammatory cells in the surrounding infiltrate was evident for these two suture materials. These results conclusion supports the idea that bacterial invasion may cause the increase in the diameters for both sutures.

In a recent study, Yılmaz et al.[35] reported that monocryl which is a monofilament suture, created less reaction compared with catgut and silk which are in polyfilament character. In accordance with that study, our results agree with most of the observations made on animals or humans, where silk has been considered as a suture material that induces unwanted tissue reactions.[7,14,16,31,32,36] In a case report, Kurosaki et al. investigated a patient who had an inflammatory response associated with Type I allergy.[21] They concluded that the inflammatory response was probably a result of a late-phase reaction to silk fibroin which was used as the suture material in the operation. They also added that the pathological findings of the granulomatous inflammation around the sutures

had shown a remarkable infiltration of eosinophils. In the present study, the eosinophil counts of each suture material on days 2, 4 and 8 did not reveal a significant difference. The possible reason might be the artifacts in the histological sections. Nevermore, eosinophil scores increase from day 2 to day 8 for dacron and polyglactin 910. The reason of this increase may be that dacron which is a multifilament suture material like silk and polyglactin 910 may have caused a tissue reaction during the resorption of the suture material itself.

Conclusion

Within the limitations of this study, polyglactin 910 has produced the most favorable response compared to natural absorbable and non-absorbable mono and multifilamented materials in terms of Z1 and Z2 diameters. Chromic gut and polypropylene presented very similar results and provoked a less inflammatory reaction compared to dacron and silk.

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