A scanning electron microscope study of plaque accumulation on silk and PVDF suture materials in oral mucosa

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Abstract

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Aim To examine plaque accumulation on silk and polyvinylidene fluoride (PVDF) sutures at different time intervals.

Methodology Twenty-one male albino rabbits received sutures under general and local anaesthesia. After 3, 5 and 7 days sutures were removed and processed for scanning electron microscope (SEM) observation. The Friedman and the Wilcoxon tests were used to compare contamination on PVDF and silk suture materials at different time intervals.

Results At all time intervals, the whole surface of silk sutures was covered with a thick layer of bacterial plaque and debris. Microorganisms and blood cells on the surface and between the filaments of the silk suture material were observed. Light debris appeared around the knot area of PVDF sutures after 3 days. At 5 and 7 days, contamination could be seen in scattered areas along the suture material. The average contaminated area was smaller on PVDF suture materials, which were removed at 5 than at 7 days after insertion. At 3 days, PVDF sutures showed significantly less contamination than at 5 and 7 days ($P = 0.002$). There were statistically significant differences between silk and PVDF sutures at 3, 5 and 7 days.

Conclusion SEM observation showed that PVDF sutures were contaminated less than silk sutures at 3, 5 and 7 days.

Keywords: contamination, plaque, PVDF, silk, sutures.

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Introduction

Sutures are probably the material most frequently implanted in humans, and are used widely in all fields of surgery (Yu & Cavaliere 1983). Sutures serve to maintain tissue approximation until a wound attains sufficient tensile strength to prevent dehiscence during normal physiological activity (Wallace *et al.* 1970). Suture materials should cause minimal tissue damage, minimal tissue reaction, encourage primary wound healing and induce minimal scar formation (Pinheiro *et al.* 1997). The use of sutures in general surgery has been extensively studied and reviewed (Selvig *et al.* 1998), but in recent years concern over suture type, tissue reaction and plaque accumulation has increased (Selvig *et al.* 1998, Kim 2002, Morrow & Rubinstein 2002).

A variety of suture materials are currently used in surgery within the mouth, including organic and synthetic non-resorbable and resorbable materials (Selvig *et al.* 1998, Kim 2002, Morrow & Rubinstein 2002).

Silk has been a favoured suture material in oral, periodontal and endodontic surgery because of its ease of handling (Selvig *et al.* 1998). However, many studies have reported that silk causes a more intense and

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prolonged inflammatory response in gingiva and oral mucosa than synthetic materials (Wallace *et al.* 1970, Selvig *et al.* 1998).

Non-resorbable monofilament suture materials have advantages in terms of tensile strength, resistance to contamination and bio-compatibility with living tissues. However, they are difficult to handle, especially during suturing and knotting, because of their rigidity (Urban *et al.* 1994).

Polyvinylidene fluoride (PVDF) has a monofilament structure and represents an attractive suture material for vascular surgery because of its satisfactory physico-chemical properties, good bio-compatibility, excellent bending properties and low surface friction. These characteristics of PVDF sutures make them easy for surgeons to manipulate, particularly in terms of making a knot and sliding it into position (Urban *et al.* 1994, Laroche *et al.* 1995, Mary *et al.* 1998). The intrinsic elastic properties of PVDF under tension bring an additional handling advantage. Because the PVDF suture is easy to stretch, once tightened a knot will stay in place with little tendency to move or become untied (Urban *et al.* 1994, Laroche *et al.* 1995). The PVDF suture has been shown to provide greater creep resistance and improved dimensional stability (Urban *et al.* 1994).

For many years, surgeons relied on clinical experience or histological results obtained from animal research on extraoral structures to evaluate different suture materials (Wallace *et al.* 1970). More recently, information has become available from intraoral studies (Wallace *et al.* 1970, Giray *et al.* 1997, Selvig *et al.* 1998). It has been shown that sutures placed intraorally produce a tissue response that is distinctly different from the response observed at other experimental sites; this response is a result of the presence of moisture and infectious potential with a consequent tendency towards rapid epithelial invagination (Selvig *et al.* 1998).

One of the most important aspects of oral tissue response to suture materials is the tendency for microbial attachment and accumulation. Bacteria and necrotic debris that attach or lodge between the filaments of suture materials could delay repair and maintain infection (Kim 2002, Morrow & Rubinstein 2002).

This study was carried out to compare plaque accumulation on PVDF and silk sutures at different time intervals, using scanning electron microscopy (SEM).

Materials and methods

The research protocol was approved by the Research Ethics Committee of Kerman University of Medical Sciences and experiment was carried out in accordance with the European Economics Community's directive of 24 November 1986 (86/609/EEC). Twenty-one adult male albino rabbits weighting 2.5–3 kg were used. All experimental procedures were completed after intraperitoneal injection of 7.5 mg kg^{-1} ketamine HCl (Alfasan, Woerden, the Netherlands) and 0.1 mg kg^{-1} xylazine (Bayer, Munich, Germany). After anaesthesia, the head and neck area of the animals were scrubbed with betadine iodine (Daropakhsh, Tehran, Iran) and their mouths rinsed with 2% chlorhexidine gluconate mouthwash.

An infiltration injection of 2% lidocaine (Daropakhsh) with 1:80 000 epinephrine was then made in the posterior areas of each rabbit jaws where suturing was performed. Then one suture of 4/0 PVDF (CG, Tehran, Iran) was inserted at buccal vestibular area in one side of the jaw and one suture of silk (Supa, Tehran, Iran) size 4/0 in the other, alternating the sides on which the suture types were inserted.

Animals were randomly divided into three experimental groups of seven animals each. After 3, 5 or 7 days the sutures were removed and kept in 70% alcohol. Unused silk and PVDF sutures of the same size were used as controls.

For SEM processing, the material was critical-point dried (Balzers, Liechtenstein) using the dry ice method (Tanaka & Iino 1974), sputter-coated with gold in a Polaron E5000 (Polaron, Watford, UK) and were observed in a field emission Hitachi 4500 SEM (Hitachi Ltd, Tokyo, Japan). Digital SEM micrographs were taken along the whole length of every suture at 200×. This provided a complete picture of the debris on the one half of the circumference of the sutures visible in the SEM. The proportion of this length showing some contamination by debris (length per cent of debris contamination) was measured while unaware of the time intervals. Because of the very different appearance of the silk and PVDF suture materials it was impossible for the observer to be blind to the suture types (Figs 1 and 2). For statistical analysis, contamination of the suture surface was scored as follows: 0 = no contamination; 1 = 1–25% of suture length contaminated with debris; 2 = 26–50% of suture length contaminated; 3 = 51–75% of suture length contaminated; 4 = 76–100% of suture length contaminated.

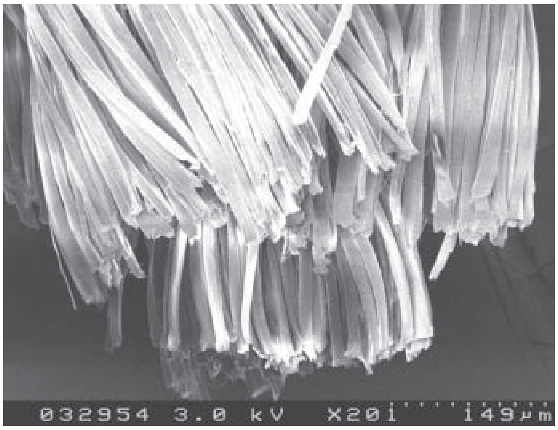

Figure 1 Scanning electron micrograph of a control silk suture showing multi-filamental structure (201×).

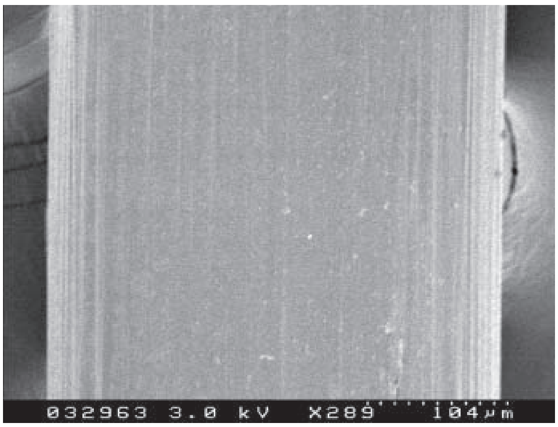

Figure 2 Scanning electron micrograph of control PVDF suture showing the monofilamental structure (289×).

The Friedman and Wilcoxon tests were used to compare the contamination of silk and PVDF suture materials along their length at different time intervals.

Results

Control sutures

SEM images of control silk sutures showed a clean braided structure without debris or microorganisms on the surface or between the filaments (Fig. 1).

The surface of unused PVDF sutures under SEM showed a smooth, monofilamental surface without debris or microorganisms (Fig. 2).

Silk suture material

At all time intervals in experimental animals, SEM observation showed that all the silk sutures were encrusted with plaque attachment on the surface (Table 1) and in the interfilamental spaces (Fig. 3). The surface of silk sutures at all time intervals was completely contaminated by debris along the whole surface. There were no obvious differences in the amount of plaque and debris accumulated at different time intervals. Higher magnification of SEM showed some blood cells, and numerous microorganisms which were predominantly rod-shaped bacteria (Fig. 4). No significant difference was found in the amount of contamination at 3, 5 and 7 days (Table 2) in silk sutures ($P = 1$).

Table 1 The percentage length of PVDF and silk suture materials that was contaminated at different time intervals

	TI					
	3 days		5 days		7 days	
No.	PVDF	Silk	PVDF	Silk	PVDF	Silk
1	6	100	37	100	48	100
2	8	100	38	100	47	100
3	5	100	42	100	50	100
4	9	100	42	100	54	100
5	6	100	40	100	52	100
6	4	100	45	100	50	100
7	7	100	43	100	56	100
Mean	6.4	100	41	100	51	100
STD	1.71	0	2.82	0	3.21	0
Significance[a]	$P = 0.008$		$P = 0.008$		$P = 0.015$	

TI, time intervals.
[a]The Wilcoxon test.

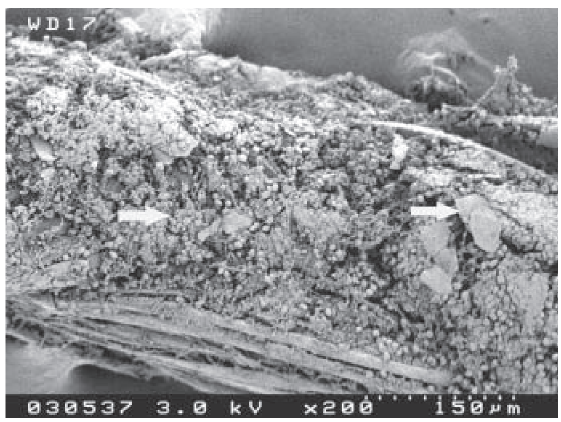

Figure 3 Scanning electron micrograph of silk suture 5 days after suturing. A large amount of debris (white arrows) is seen among filaments (200×).

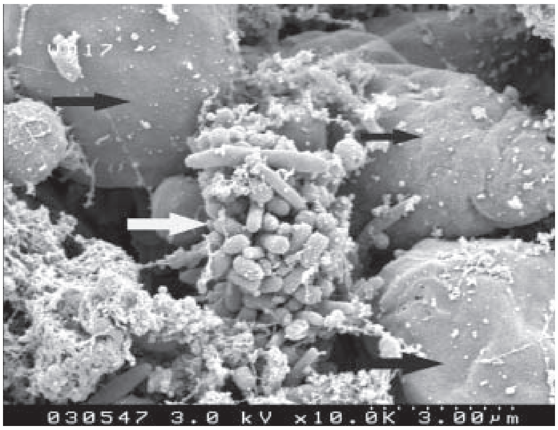

Figure 4 Scanning electron micrograph with higher magnification (10 000×) from the surface of silk suture material (5 days after suturing). A colony of rods (white arrow) among the PMNs (black arrows) could be seen.

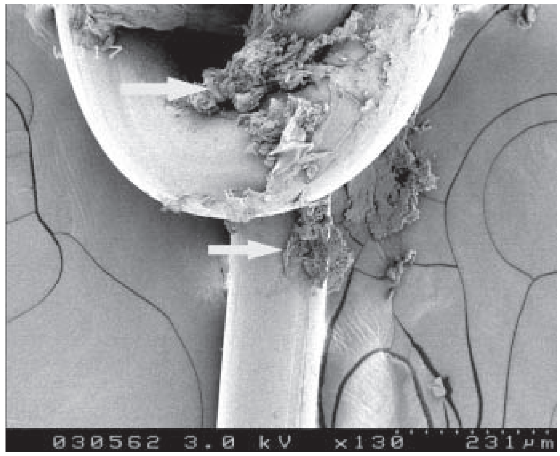

Figure 5 Scanning electron micrograph of PVDF suture 3 days after suturing showed some debris (white arrows) around the knot area (130×).

Table 2 Comparison of plaque accumulation on silk and PVDF suture material at 3, 5 and 7 days

	Group	
Time	PVDF (mean rank)	Silk (mean rank)
3 days	1	2
5 days	2.71	2
7 days	2.29	2
Significance[a]	$P = 0.002$	$P = 1$

[a]The Friedman test.

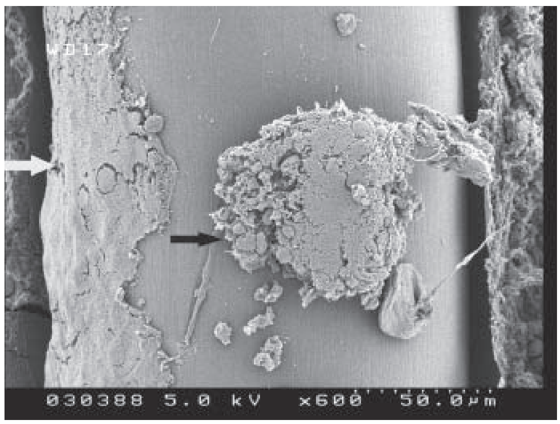

Figure 6 Scanning electron micrograph of PVDF suture showed scattered debris (black and white arrows) in some part of material at 5 days interval (600×).

PVDF suture material

PVDF sutures 3 days after insertion showed minor contamination only at the knot and adjacent areas (Fig. 5). The debris consisted of bacteria and amorphous organic material. Most of the suture surface was free of debris; the monofilament surface remained uncontaminated even where small flaws in the smooth surface were evident.

Evaluation of the proportion of contamination along PVDF sutures (measuring the percentage length of each suture that was contaminated) showed that the contaminated area at 3 days was minor, averaging 6.4 ± 1.71% of each suture. However, the average length of contamination on each suture 5 and 7 days after insertion was 41 ± 2.82 and 51 ± 3.21%, respectively (Table 1). At 5 and 7 days, there were scattered areas of debris attached to the surface (Fig. 6). SEM evaluation showed contamination at both ends of PVDF sutures, progressing inwards towards the middle of the sutures (that is, the part of the suture placed deeper in the connective tissue) at the longer time interval. The Friedman test showed a statistically significant difference between PVDF suture materials at 3 days than 5 and 7 days ($P = 0.002$). There was no significant difference in contamination at 5 and 7 days.

Higher magnification showed this debris to be composed mainly of clusters of spherical sub-micron organisms with some filamentous structures (Fig. 7).

The Wilcoxon test (Table 1) showed significant differences in contamination between silk and PVDF

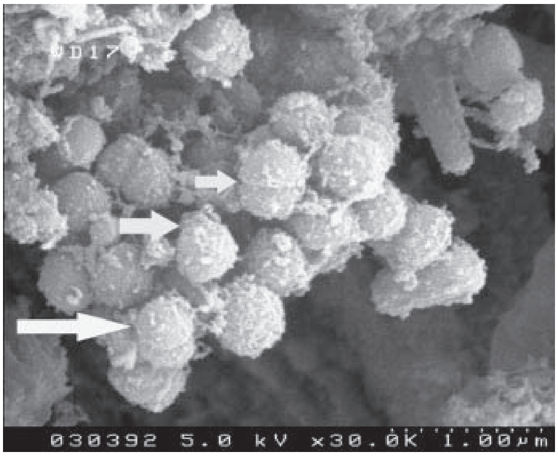

Figure 7 Scanning electron micrograph with higher magnification (30 000×) of debris on PVDF showed a colony of spherical submicron organisms (white arrows) at 5 days.

sutures at 3 ($P = 0.008$), 5 ($P = 0.008$) and 7 days ($P = 0.015$).

Discussion

The primary concern during surgery is to ensure that an operation is successful and that the patient is rehabilitated. Consequently, the biomaterials, devices and sutures used in the operation must not hinder, delay or impose additional risk in achieving these objectives (Urban *et al.* 1994).

Bacterial plaque accumulation on the surface of sutures has been infrequently studied. It has been shown that silk sutures, which are multi-filamentous, consistently produce greater inflammatory reactions than monofilament sutures in the oral mucosa (Lilly *et al.* 1973, Racey *et al.* 1978).

The greater inflammatory reaction observed with multi-filament materials has been attributed to the presence of bacteria within the interstices of the suture. Braided sutures seem to conduct bacterial migration to a great extent than monofilament sutures. Even immobile bacteria are transported inside multi-filament suture materials, where the cellular and immunological defence against them is considerably impaired (Selvig *et al.* 1998).

Lilly *et al.* (1968) postulated that multi-filament suture materials permitted a 'wicking phenomenon' that could advance oral fluids and bacteria along the suture filaments by capillary action. The wicking effect is evidenced by swelling and fragmentation with necrotic debris and exudate among the suture fibres. In the present study, a great number of particles that could be interpreted as bacteria and necrotic debris were observed between the filaments of silk sutures and over the outer surfaces even at 3 days. However, PVDF sutures after 3 days showed debris only on surfaces that were very close to the knot.

Previous histological studies showed that polymorphonuclear (PMN) cells could be seen after 3, 5 and 7 days between the filaments of silk sutures (Wallace *et al.* 1970, Selvig *et al.* 1998). In this study, SEM high magnification of silk sutures showed some round cells, which may be PMN, between filaments.

Selvig *et al.* (1998) reported that at 14 days bacterial plaque extended more than 1 mm into the suture channel regardless of the suture materials. In this study, silk sutures at 3 days showed that the whole surface had been covered with a thick layer of bacterial plaque and debris.

Previous SEM studies in vascular surgery (Urban *et al.* 1994, Mary *et al.* 1998) showed that even long-term PVDF implants had a clean and smooth surface. However, in this study plaque accumulation after 3 days in the knot area and at 5 days along PVDF suture's surface was noted. This probably reflects the unique nature of the oral environment compared to other sites in the human body; the oral microbial flora, humidity and rapid tendency to epithelial invagination which may have an important role in increasing plaque attachment (Selvig *et al.* 1998).

One SEM study (Mary *et al.* 1998) has shown evidence of occasional iatrogenic trauma on PVDF sutures. They concluded that it resulted from manipulation with the needle holder during suturing procedure. In the present study, such damage was not observed.

Conclusion

SEM observation showed that PVDF sutures had less contaminated surface area than silk sutures at 3, 5 and 7 days.

Further research is needed to compare various types of monofilament sutures such as coated, absorbable, non-absorbable and impregnated.

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