MACROSCOPIC AND MICROSCOPIC CHANGES IN THE WOUND AFTER INTRADERMAL CLOSURE USING BURIED KNOT AND PULLEY KNOT-FREE PATTERNS FOLLOWING OVARIECTOMY IN CATS

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ABSTRACT

Two methods of anchoring continuous intradermal suture (buried knot and pulley knot-free patterns) in abdominal skin incisions in cats were compared to determine which method is more cosmetically-acceptable. Nine female cats were subjected to routine ovariectomy with two ventral midline incisions 2 cm apart. The abdominal layer was closed routinely, while the cranial and caudal skin incisions were closed using the continuous intradermal suture pattern anchored alternately with the buried knot or pulley knot-free pattern. The wounds were observed daily and skin biopsy was done at 7, 14 and 21 days post-operation. Aesthetically, the pulley knot-free anchor showed a lesser degree of pus formation and less elevation than buried knot, while the buried knot had less dehiscence than pulley-free anchor. The two methods were found equal in degree of scab formation, hyperemia and scar formation. Microscopically, no differences between the two methods were observed with regards to the presence of hemorrhage, wound gap, inflammation and fibroblastic proliferation. Although there were no significant differences between the two anchor methods, the novel pulley knot-free anchor provides a more adequate wound apposition, especially at the end of the incision than the buried knot.

Key words: buried knot, cat, intradermal suture pattern, pulley knot-free anchor

INTRODUCTION

The buried knot has been traditionally used as the anchor method to initiate and end the continuous intradermal suture pattern (Fossum, 2007). However, burying the knot adequately at the end of the incision is the most difficult aspect of this pattern to master, leading to slight dermal separation or malapposition, particularly over the area of the knot (Smeak, 1992). Moreover, if a large knot remains immediately below or within the suture line, complications will arise, including excess pressure (resulting in local skin necrosis), suture extrusion, wound infection, and poor aesthetic result (Stashak and Theoret, 2008). The size and type

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of the knot are determined by the size and structure of the suture material and the surgeon's skill (*i.e.* how many throws used). Furthermore, should the knot protrude from the wound edges, it can interfere with healing, irritate the patient, act as a nidus for bacterial growth ultimately leading to infection, and become untied, resulting in wound dehiscence (Campbell, 2004).

The pulley knot-free anchor is currently used in human surgical closure, particularly in the field of plastic surgery (Campbell, 2004). Closure of the skin for primary intention healing following celiotomy procedures is standard practice to keep the wound closed during healing. As pet animals will be returned to their owners for home care, the closure method should produce results where post-surgical wound maintenance should be minimized and the healing should result in a cosmetically acceptable scar.

The continuous intradermal suture pattern has long been considered ideal when lack of scar tissue is important or when protruding sutures may irritate the patient (Archibald, 1974). Intradermal patterns result in complete wound apposition without creating skin irritation or tract infection into an incision (Slatter, 2002). When absorbable suture materials, which are broken down by the body through hydrolysis (Fossum, 2007) are used, there is no need for suture removal later on, which is especially useful when patients are intractable.

The main difference between the buried knot pattern and pulley-knot free anchor pattern is the knot used in initiation and termination of the intradermal suture. In the buried knot method, knots are placed at the beginning and end of the suture which are hidden under the skin. In contrast, the pulley knot-free anchor uses interlocking bites of suture at both ends of the incision (Campbell, 2004). A biomechanical test using skin from fresh canine cadavers found the pulley knot-free anchor to have good tensile strength, skin apposition, and used less suture material (Campbell, 2004).

This study was conducted to compare the resulting wound healing (macroscopic and microscopic changes) following the use of either the buried knot or the pulley knot-free anchor methods in the continuous intradermal ventral abdominal closure of ovariectomized cats.

MATERIALS AND METHODS

The protocol used in this study was approved by the University of the Philippines College of Veterinary Medicine's Institutional Animal Care and Use Committee.

Nine apparently healthy intact adult female domestic short-haired cats between the ages of eight months and three years of age were used in the study. The cats were randomly divided into three groups (A, B, and C) of three animals in each group. Each group corresponded to the post-operative period when wound observations were conducted (A = 7 days post-operation; B = 14 days post-operation; C = 21 days post-operation).

Each cat underwent a pre-operative examination including general physical examination, hematological examination and direct fecal smear examination. Only

cats that were considered fit for the surgical procedures were included in the study (*i.e.* normal hematology, negative for intestinal parasites, and apparently healthy) (Kahn, 2011).

The cats were food-fasted 12 hours prior to surgery. Drugs administered at 5 min-intervals consisted of atropine sulfate (0.65 mg/ml at 0.04 mg/kg, SC), xylazine (20 mg/ml at 1 mg/kg, IM) and tiletamine 25 mg/ml with zolazepam 25 mg/ml (Zoletil 50®; Virbac Laboratories, Carros, France) at 5 mg/kg, IM. The surgical site was prepared by shaving and disinfecting with 10% povidone-iodine and alcohol. Asepsis was observed throughout the procedure.

Two ventral midline incisions through the skin and subcutaneous tissue were made at least 2 cm apart: 1) one centimeter caudal to the xiphoid; and 2) starting at the level of the umbilicus caudally. A spay hook was used during the ovariectomy procedure. Closure of the ovariectomy site (caudal site) was done in two layers using 4-0 Polyglactin 910 absorbable swaged suture with three-eighths (3/8) circle needle. The abdominal layer was closed using a simple interrupted pattern. Wound sites were closed using a continuous intradermal suture pattern and anchored using the buried knot (knot tying subcutaneously; Figures 1b and 1d) as described by Fossum (1997) or pulley knot-free anchor (suture material located intradermally; Figures 1a and 1c) as described by Campbell (2004). Following closure, wounds

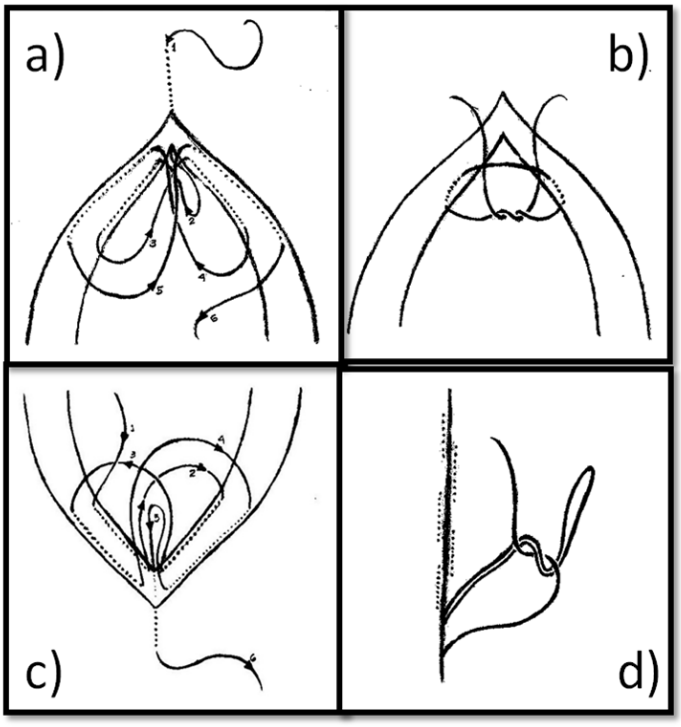

Figure 1. Two methods of anchoring the continuous intradermal suture pattern, showing the start (a) and end (c) of the pulley knot-free anchor method; and the start (b) and end (d) of the buried knot anchor method.

were disinfected with 10% povidone-iodine and bandaged using cotton gauze bandages (sterilized 9 cm squares) and surgical tape. Following surgery, all cats were given pain reliever (Tramadol HCl, 1/4 of a 50 mg tablet, PO) and antibiotic (150,000 IU procatin penicillin and 150,000 IU benzathine penicillin per mL, 0.5 ml/ 5 kg IM).

Wounds were observed on post-operative days 7 (group A), 14 (group B), and 21 (group C). Macroscopic changes were graded following the procedures of Pedrajas (2002). Following photo-documentation of the wound, two approximately 1 cm x 1 cm full-thickness skin sections of the terminal ends of each wound site were taken. The excised skin was attached flat to a wooden tongue depressor to prevent artifacts due to shrinkage, and preserved in 10% formalin for at least 72 hours. Routine paraffin processing for histopathological studies was done and cross sections (5-8 µm) of the samples were stained with hematoxylin and eosin. Microscopic changes were graded on a four-point scale following the procedures of Pedrajas (2002).

The differences in macroscopic and microscopic wound healing were analyzed using the Friedman's Test with a 5% degree of significance.

RESULTS AND DISCUSSION

For all wound closures following surgery, complete apposition of wound edges was achieved with no suture material exposed to the surface. This was more easily achieved using the pulley knot-free anchor method as there is no mass of suture material to hide, which is a difficult aspect to master (Smeak, 1992). For all wound sites, erythema and slight swelling were noticed several hours after closure generally lasting for 3-4 days post-operatively. This is considered a normal inflammatory response leading to healing of the wound (Fossum, 2007).

Macroscopic changes

Macroscopic characteristics (pus, scab formation, hyperemia, elevation, dehiscence, and scar formation) (Figure 2) of closures anchored with the buried knot and the pulley knot-free methods grade values from all observation days showed no differences (Table 1, $P>0.05$).

Scab formation was only seen on post-operative day 7 observations with both anchors having only slight scab formation (Grade 1). Scabs form when the blood clot dries. The scabs protect the wound, prevent further hemorrhage and allow healing to progress beneath their surface (Fossum, 2007). At post-operative day 7, the stage of wound healing would be in the third/repair phase during which scab formation is on the decline.

Only observations on post-operative day 7 wounds that used the buried knot showed pus (Grade 1; Figure 2a). This may be attributed to the position of the buried knot (*i.e.* sub-dermal) and the space occupied by the knot, resulting in the formation of a small pocket where pus formation and pooling can occur.

The degree of hyperemia (Figure 2c) was slight in both anchor methods (Grade 1). Hyperemia is expected to abate over time as the wound heals and blood supply to the area returns to normal.

Table 1. Macroscopic characteristic grade (mode) of the wound in buried knot and pulley-knot free anchor in intradermal wound closure.

	Day 7 Post-op		Day 14 Post-op		Day 21 Post-op	
Parameters	Buried	PKF	Buried	PKF	Buried	PKF
Scab	1	1	0	0	0	0
Pus	1	0	0	0	0	0
Hyperemia	1	1	1	1	1	1
Elevation	1	0	1	1	0	0
Dehiscence	0	1	0	0	0	0
Scar	1	1	1	1	1	1

Post-op: post-operative; Buried: buried knot; PKF: pulley knot-free.
Macroscopic changes were graded following the procedures of Pedrajas (2002).

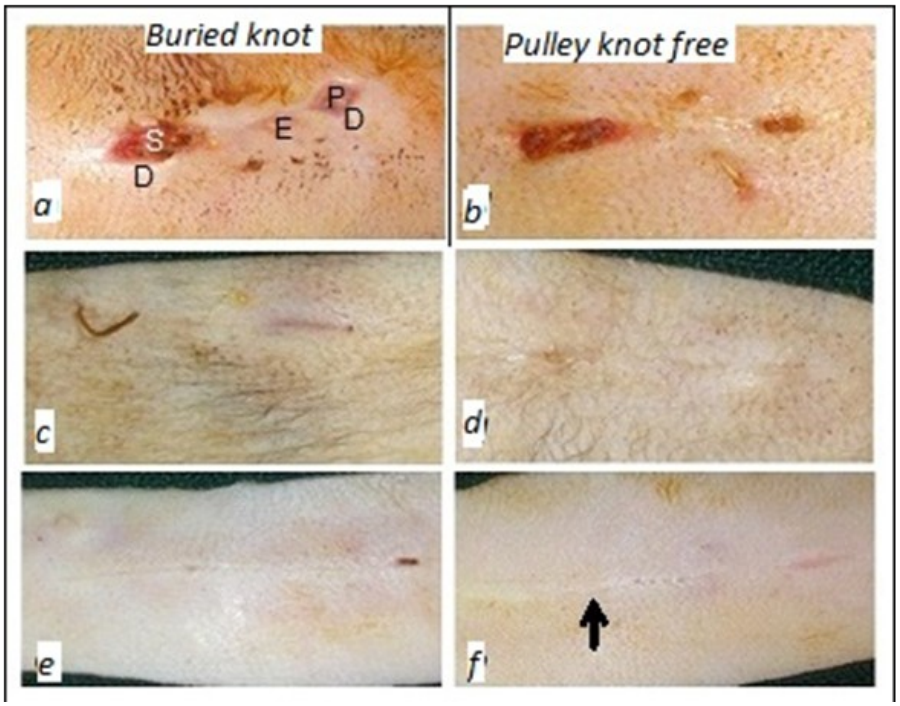

Figure 2. Post-operative appearance of buried knot and pulley knot-free anchor methods at day 7 (*a* and *b*), day 14 (*c* and *d),* day 21 (*e* and *f)*. Grading criteria shown in *a*: presence of 40% scab formation (Grade 2; S); moderate levels of pus (Grade 2; P); slight elevation (Grade 1; E); *b* shows Grade 1 dehiscence; *d* shows absence of scab formation (Grade 0), absence of pus (Grade 0), absence of dehiscence (Grade 0); *f* shows slight scar formation (Grade 1; black arrow).

Swelling was minimally noted across all groups (Grade 0-1). Fewer observations of elevation (Figure 2d) were noted when using the pulley knot-free anchor (Grade 1 in the 14-day post-operative group) than when using the buried knot (Grade 1 in the 7 and 14 day post-operative groups). This may be due to the presence of suture material beneath the skin, in the case of the buried knot, causing some mechanical irritation.

There were some observations of slight dehiscence (Grade 1, Figure 2b) noted in the 7 day pulley knot-free group. The buried knot may result to fewer incidences of dehiscence because the knot holds the suture material firmly in place, making it more difficult for the wound edges to pull apart.

Scarring (Figure 2f) was minimally seen across both anchor methods and all groups (Grade 1). Continuous intradermal suture pattern produces minimal scar without suture tracts since no sutures are placed in the skin and there are fewer avenues for contamination to enter the wound since no suture is exposed and dermal edges are better apposed (Smeak, 1992). If scars are present, they do not tend to enlarge over time.

Microscopic changes

Microscopic characteristics of closures anchored with the buried knot and the pulley knot-free anchor methods grade values from post-operation days 7, 14, and 21 are shown in Table 2. The degree of invagination was higher at 7-day post-operative groups (Grade 2; Figure 3) than it was later on for both methods of anchorage, which may be due to early epidermal bridging. The pulley knot-free at 14 day post-operative group had a slightly elevated level (Grade 1). This may be due to the extra manipulation needed to place the double figure-of-eight for this suture. Since two loops (one shallower than the other) are holding together the same area of skin, there is greater chance for misalignment leading to epidermal invagination (Fossum, 2007).

Degree of epidermal thickening was noted at day 7 post-operative (Grade 2; Figure 3) for both anchor methods. Slight epidermal thickening was noted in the day

Table 2. Microscopic characteristic grade (mode) of the wound in buried knot and pulley knot free anchor in intradermal wound closure.

	Day 7 Post-op		Day 14 Post-op		Day 21 Post-op	
Parameter	Buried	PKF	Buried	PKF	Buried	PKF
Invagination	2	2	0	1	0	0
Thickening	2	2	0	1	0	1
Hemorrhage	0	0	0	0	0	0
Wound Gap	0	0	0	0	0	0
Inflammation	3	3	3	3	3	3
Fibroblasts	3	3	3	3	3	3

Post-op: post-operative; Buried: buried knot; PKF: pulley knot-free.
Microscopic changes were graded following the procedures of Pedrajas (2002).

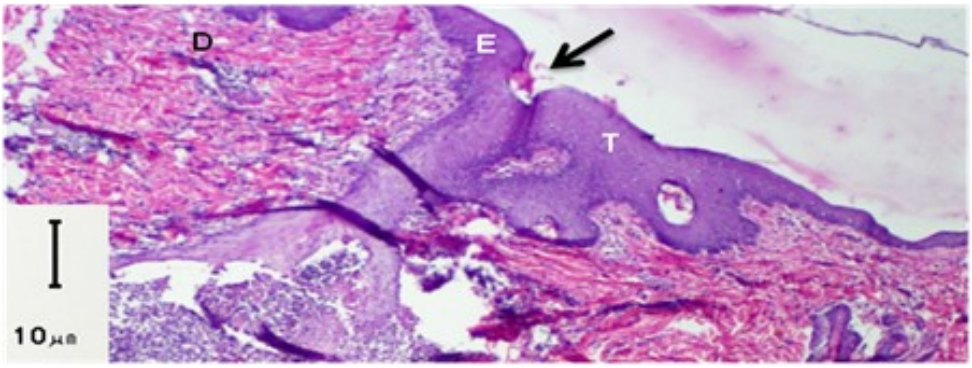

Figure 3. Cross section of the skin from cat with buried knot anchor day 7 post-operative; showing the presence of moderate epidermal invagination (Grade 2; black arrow) and moderate epidermal thickening (Grade 2, T). E (epidermis), D (dermis). Hematoxylin-eosin stain.

14 post-operative biopsies when the pulley knot-free anchor was used (Grade 1); however, for the most part of the wound, no epidermal thickening was observed. Thickening may be due to the misalignment of the wound edges due to the increased manipulation needed to place the double figure-of-eight of the pulley knot-free anchor (Fossum, 2007).

Hemorrhage was not observed at least 7 days post-operatively after using either anchor method (Figure 3). This is likely due to the rate of repair of damaged blood vessels in the skin, which may be damaged during the closing process, yet repaired before the seventh post-operative day.

Degree of wound gap was moderate, seen only in one 7 day post-operation animal (both buried knot and pulley knot-free anchor sites; Figure 4). This may have been due to the animal's behavior, reaction to the suture material, or the yet incomplete process of wound contraction, where there was not enough time for the wound edges to fully meet. It is suggested by Smeak (1992) that one or more split-thickness skin sutures may be required to achieve a finer approximation of skin; which may be removed as early as one or two days post surgery because epidermal bridging is rapid if skin is well approximated. This is supported by the finding that no wound gaps were noted in day 14 and 21 post-operative groups, although no split-thickness skin sutures were placed in the current study.

Histologically, degree of inflammation was observed as presence and infiltration of the surrounding tissue by neutrophils. Degree of inflammation (Figure 5) was high (Grade 3) in all groups regardless of anchor methods. Microscopically, wherever there was presence of suture material, it was surrounded by a mass of neutrophils. Suture material, being a foreign body, attracts neutrophils and is eventually broken down. Hydrolysis can also dissolve suture material providing there is contact with body fluids (Fossum, 2007).

Fibroblastic proliferation was present (Grade 1) in all wound sites regardless

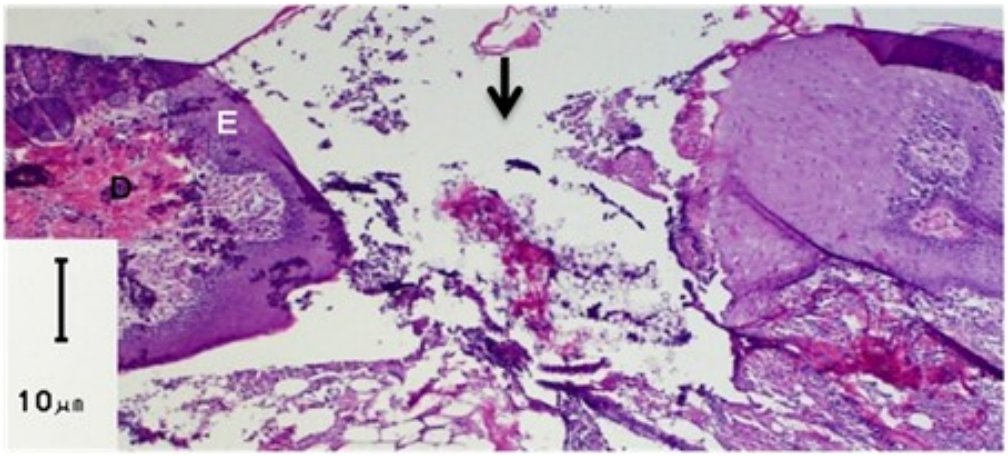

Figure 4. Cross section of the skin of cat with buried knot day 7 post-operative; showing the presence of moderate wound gap (Grade 2; black arrow). E (epidermis), D (dermis). Hematoxylin-eosin stain.

of which suture anchor method was used. This is part of the normal process of wound healing (Fossum, 2007).

Microscopic changes in continuous intradermal suture pattern would be rapid epithelial bridging, thus minimizing hypertrophic scar or keloid formation (Smeak, 1992). There is narrower wound gap and higher frequency of wound union at 7 days post-operation compared to simple interrupted suture pattern (Pedrajas, 2002).

Test statistics (Friedman's test) showed that the two anchor methods were not significantly different at $P>0.05$. The pulley knot-free anchor method is as good as the buried knot, and the two can be used interchangeably at the surgeon's preference. However, the pulley knot-free anchor was noted by the operator to be easier to learn and apply. Moreover, it resulted to a smoother finish, minimal bulge and with good wound apposition.

Further studies could be done using monofilament absorbable suture material with this anchorage method. Smeak (1992) suggested the use of this suture material as they are non-capillary and have low coefficient of friction compared to multifilament sutures (Bellenger, 1982). An extended observation period (until complete healing), other animal species, and varied anatomical sites could also be tested. While the effective strength between the buried knot and the pulley-knot free anchors can be considered comparable, the tensile strength should be directly measured in cats. Special staining techniques could also be done in order to identify the proliferation of collagen (Masson's trichome stain) and elastic fibers (Weigert's hematoxylin) involved in wound healing.

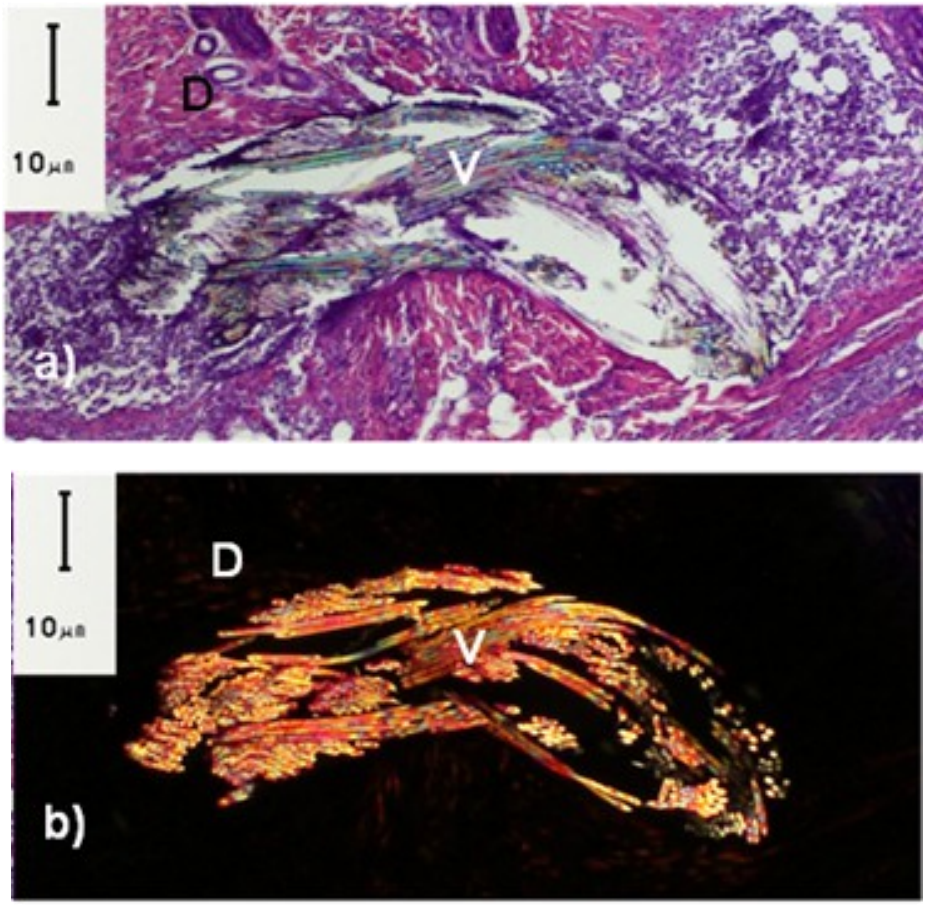

Figure 5. Cross section of the skin of cat with pulley knot-free day 21 post-operative in (a) normal histopathology view and (b) using polarized lens; showing the presence of abundant neutrophils (inflammatory cells) around the suture material (Grade 3). D (dermis), I (inflammatory cells), V (suture material). Hematoxylin-eosin stain.

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