Evaluation of Bacterial Colonization Following Aesthetic Thread Implantation In Guinea Pigs Using Mannitol Salt Agar Culture

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Abstract.
Background: Infections following aesthetic thread implantation may cause abscesses, tissue damage, and the need for thread removal. Microbiological evaluation under standardized implantation conditions may complement clinical complication reports and inform assessments of procedural safety.Objective: To assess bacterial growth on mannitol salt agar (MSA) in tissue swab samples collected around thread implantation sites in guinea pigs. Methods: This descriptive microbiological analysis presents three thread implantation groups from a parent laboratory experiment, comprising nine female guinea pigs receiving polydioxanone (PDO), polycaprolactone (PCL), or APTOS NAMICA. All study thread products held marketing authorization from the Indonesian Ministry of Health and were implanted aseptically. Each group comprised three animals, with one animal euthanized on each of days 3, 7, and 21. Four sites per animal yielded 36 subcutaneous tissue swab samples. MSA cultures were incubated at 37°C for 24–48 hours. Findings were summarized as the number of samples showing growth relative to the number examined. Results: No colonies grew in any of the 36 samples obtained from nine animals. At each sampling time, all 12 samples from three animals were culture-negative. The same finding was observed in all three groups.Conclusion: No bacterial growth was detected on MSA in tissue swab samples collected around implantation sites through day 21 under the study conditions. These findings provide culture data following aseptic implantation of authorized thread products, with interpretation limited to the sampling sites, observation period, and methods used. Selection of authorized thread products and consistent aseptic technique remain important considerations for procedural safety.
Keywords: *aesthetic threads; thread lifting; bacterial colonization; mannitol salt agar; aseptic technique and guinea pigs.*

I. INTRODUCTION

Aesthetic thread implantation introduces biomaterials into tissue through skin penetration. Contamination control is therefore essential because microorganisms may enter through puncture sites, instruments, or implanted materials. Although thread lifting is minimally invasive, infection remains a clinically important complication. A meta-analysis by Niu et al. encompassing 26 studies reported a pooled infection incidence of 2% after facial thread lifting.[1] In a referral series by Li et al., infection was identified in 17 of 190 patients presenting with complications (8.9%).[2] The latter figure represents the proportion of infections among patients with complications rather than the incidence among all patients undergoing thread lifting.

The consequences of infection may extend beyond transient erythema or swelling. Surowiak reported abscesses along the tracks of four PDO threads, requiring thread removal and leaving residual deformity after the inflammation had subsided.[3] Joethy et al. isolated Staphylococcus aureus from wound cultures in a patient with facial abscesses and preseptal cellulitis following thread placement in a nonsterile setting.[4] These reports highlight the importance of preventing contamination from the preparation stage onward and assessing signs of infection after implantation.

The microbiological rationale for contamination control is supported by a 2026 study by de Oliveira Rabelo et al. All six bacterial strains tested adhered to fragments of PDO thread cannulas within 30 minutes of direct exposure to bacterial suspensions.[5] These in vitro findings indicate that instrument surfaces can support bacterial adhesion when exposed to microorganisms. Aseptic technique, including skin preparation

and maintenance of instrument sterility, is a fundamental component of infection prevention during invasive procedures.[6]

Product selection is another consideration in implantation safety. Indonesian medical device regulations establish product requirements and conformity assessment procedures, including those applicable to imported devices.[7] Device identity and marketing authorization status can be checked through official Ministry of Health services.[8] Product identity, packaging integrity, expiration dates, and handling after opening should be considered alongside procedural technique.

Culture assessment under standardized implantation conditions may complement evidence from clinical infection reports and bacterial exposure experiments. MSA is a selective medium primarily used to cultivate staphylococci, making it relevant to one of the organisms previously identified in infections following thread placement.[4,9] This study assessed growth on MSA from tissue swabs collected around PDO, PCL, and APTOS NAMICA implantation sites in guinea pigs on days 3, 7, and 21.

II. METHODS

Study design, setting, and period

This article presents a descriptive microbiological evaluation of three aesthetic thread implantation groups from a parent laboratory experiment. The groups included PDO, PCL, and APTOS NAMICA; other groups in the parent experiment were outside the scope of this article. The analysis was limited to postimplantation culture findings without comparison with a control group. The study was conducted in October–November 2025. Animal housing, implantation, euthanasia, and sample collection took place at the Laboratory Animal Facility, Faculty of Veterinary Medicine, Universitas Wijaya Kusuma Surabaya (UWKS). Cultures were examined at the UWKS microbiology laboratory in Surabaya, Indonesia. Animal numbers and sampling units were reported separately, with reference to the ARRIVE 2.0 reporting principles.[10]

Animals and study groups

The data presented were obtained from nine healthy female guinea pigs (Cavia porcellus), aged 4–6 months and weighing 300–500 g, that met the study eligibility criteria. Physical examination showed no skin abnormalities at the implantation sites or signs of systemic disease. Animals were acclimatized for seven days in individual cages at approximately 25°C, with food and drinking water available ad libitum. General condition, activity, and food and water intake were monitored throughout the housing period.

The groups presented were PDO, PCL, and APTOS NAMICA. Each group comprised three animals, with one animal euthanized on each of days 3, 7, and 21. Four sites were established on the dorsal region of each animal, approximately 2 cm apart. All sites on a given animal received the same treatment.

The individual animal was the biological unit, whereas the implantation site was the sampling unit. Each site was swabbed separately, yielding 36 samples from nine animals. For each group–time combination, four samples were obtained from the same animal. Samples collected from different sites on one animal were not treated as independent biological replicates.[10,11]

Biomaterials and implantation procedure

The biomaterials were USP 2-0 barbed PDO, USP 2-0 barbed PCL, and USP 2-0 barbed APTOS NAMICA threads. APTOS NAMICA is based on a lactide–caprolactone copolymer incorporating hyaluronic acid encapsulation technology. All study thread products held marketing authorization from the Ministry of Health of the Republic of Indonesia. Threads were removed from their sterile packaging immediately before implantation.

Hair was removed from the dorsal region using electric clippers and depilatory cream. Anesthesia was induced with ketamine at 50 mg/kg body weight, administered intramuscularly into the thigh, and anesthetic depth was assessed before the procedure. The skin was prepared with 70% isopropyl alcohol. Implantation was performed aseptically using sterile gloves and instruments and a single-use 18-gauge cannula.

Threads were placed linearly in the subcutaneous layer over a length of 2 cm. Implantation depth and direction were standardized. The biomaterial was secured using simple interrupted silk sutures (GEA,

Indonesia). After the procedure, the implantation area was treated with 5% povidone-iodine. Animals were housed individually until their scheduled euthanasia.

Sample collection and MSA culture

Animals were euthanized on day 3, 7, or 21 according to the assigned schedule. Euthanasia was performed using intraperitoneal ketamine at 150 mg/kg body weight, in accordance with the laboratory protocol approved by the ethics committee. After death was confirmed, the skin was incised approximately 2 cm from the outermost implantation point. The skin was excised while preserving the position of the thread within the subcutaneous tissue, and its inner surface was exposed for sampling. The subcutaneous area on the medial side of the implantation site was sampled using a disposable cotton swab (Onemed, Indonesia). Sampling was performed before histological processing and formalin exposure. Each site was swabbed separately, yielding one sample labeled with the group, animal, site, and sampling day.

Swabs were placed in plastic breast milk storage bags (Bunny, Indonesia), stored at 0℃, and transported to the UWKS microbiology laboratory. Culture samples were handled separately from tissue fixed for histology. Sample suspensions were incubated for 18–24 hours before plating. The inoculum was then streaked onto MSA using the quadrant streak technique with a sterile inoculating loop. Plates were labeled and incubated in an inverted position at 37℃ for 24–48 hours. Colony growth and changes in medium color were assessed after incubation.

The primary outcome was the presence or absence of colony growth on MSA. Samples were classified as positive if colonies were observed and negative if no growth was detected. A yellow color change in the medium was interpreted as an indication of mannitol fermentation. Neither growth nor mannitol fermentation on MSA was used as the sole basis for bacterial species identification.[9]

Data analysis

Data were summarized descriptively as the number of samples showing growth relative to the number examined in each group at each sampling time. Animal numbers were reported separately from the numbers of implantation-site samples. Negative results were reported as the absence of detectable growth on MSA. Because all samples yielded the same result, no inferential comparisons were performed. The data were not used to estimate differences in infection risk between materials.

Ethical approval

The study was approved by the Animal Care and Use Committee of the Faculty of Veterinary Medicine, Universitas Wijaya Kusuma Surabaya (approval no. 222-KKE, dated October 8, 2025). Animal care and procedures followed the approved protocol and the principles of replacement, reduction, and refinement.

III. RESULTS

A total of 36 subcutaneous tissue swab samples from nine animals were examined. At each sampling time, three animals provided 12 samples. Each group contributed 12 samples from three animals euthanized at different time points.

No colony growth was detected in any sample from the PDO, PCL, or APTOS NAMICA group. All samples collected on days 3, 7, and 21 were negative on MSA culture. Results by group and sampling time are presented in Table 1.

Table 1. MSA culture results by group and sampling time

Group	Animals (n)	Day 3	Day 7	Day 21	Total samples
PDO	3	0/4	0/4	0/4	0/12
PCL	3	0/4	0/4	0/4	0/12
APTOS NAMICA	3	0/4	0/4	0/4	0/12
Total	**9**	**0/12**	**0/12**	**0/12**	**0/36**

Values indicate the number of samples showing growth/number examined. Within each group at each time point, four samples were obtained from four sites on a single animal. The 36 samples were obtained from nine animals. PDO, polydioxanone; PCL, polycaprolactone.

Discussion

The main finding was the absence of detectable growth on MSA at all sampling sites through day 21. This result was consistent across the three thread groups presented in this article. These findings provide microbiological data from tissue surrounding implantation sites in an animal model using aseptic procedures and authorized materials. Interpretation centers on growth detectable by culture, within the scope of the sample type and examination conditions.

These negative culture findings complement clinical reports of infection after thread lifting. In Surowiak's report, abscesses along the threads complicated their removal and left residual tissue deformity.[3] In the series by Kim et al., three of seven patients with complications after PDO thread rhinoplasty developed open wounds, abscesses, and skin necrosis; six patients had a history of rhinoplasty.[12] These contextual differences matter because tissue conditions, previous procedures, and clinical settings may differ from those of an experimental model. The present results therefore cannot be directly translated into estimates of clinical infection risk.

The spectrum of infections after thread placement also includes microorganisms requiring specialized testing. In 2026, Nagase et al. reported Mycobacterium abscessus infection after thread lifting, with erythema, induration, and fistula formation developing several months after the procedure.[13] This report illustrates the importance of considering the causative organism and clinical course when postimplantation symptoms do not resolve promptly.

The relationship between bacterial exposure and materials helps explain the relevance of contamination control. De Oliveira Rabelo et al. demonstrated early bacterial adhesion to PDO cannulas, with the extent of adhesion varying among species.[5] Their experiment deliberately exposed instruments to bacterial suspensions, whereas the present study assessed culture findings after aseptic implantation without deliberate bacterial challenge. These approaches address different questions. Adhesion under exposure conditions demonstrates the potential for surface contamination, whereas the present findings describe growth detected under the implantation conditions used.

Aseptic technique provides a plausible context for these findings. Skin antisepsis, sterile instruments, and careful handling of threads from package opening through implantation are intended to limit microbial introduction. These measures are consistent with infection prevention principles for invasive procedures.[6] Joethy et al., who identified S. aureus after thread placement in a nonsterile setting, illustrated the clinical consequences of inadequate contamination control.[4] However, this study did not include a nonaseptic procedure group; the contribution of individual preventive measures therefore cannot be quantified from these culture findings.

All study thread products held marketing authorization. In clinical practice, authorized products allow material identity to be checked against regulatory documentation and facilitate traceability if problems arise. Requirements for imported medical device authorization also encompass information such as trade name, type, size, manufacturer, and labeling.[7] Verification through official Ministry of Health services should be accompanied by confirmation that the product packaging matches the registered identity.[8] Marketing authorization was a characteristic of the materials used in this study; the study design did not test its effect on culture outcomes.

Product selection and maintenance of aseptic conditions should be considered throughout the procedure. Sterile packaging must be handled according to the product instructions until the thread is placed in tissue. After placement, puncture sites and potential thread exposure also require attention. Li et al. reported that thread extrusion preceded 14 of the 17 infections in their complication series.[2] These clinical findings emphasize skin integrity and thread position as important aspects of postprocedural surveillance, alongside product and instrument preparation before implantation.

Interpretation of this study must account for the number of animals and the scope of testing. Each group–time combination was represented by one animal; four samples from different sites on the same

animal do not increase the number of independent biological replicates.[10,11] MSA primarily selects for staphylococci while inhibiting many other microorganisms.[9] Tissue swabs collected around the implantation track also do not assess the entire thread surface. Negative findings therefore apply to growth detectable by this method through day 21. Sample handling and culture quality control also influence bacterial recovery. Further studies with more biological replicates, direct examination of implanted materials, and a broader range of culture media and observation times could strengthen microbiological assessment after implantation.

This study provides culture data from tissue surrounding implants after placement of different thread types under the same experimental conditions. All groups yielded negative results at the specified sampling times. In practice, selection of threads with Indonesian Ministry of Health marketing authorization and traceable product identities should form part of procedural preparation. Verification of authorization numbers, sterile packaging integrity, and expiration dates should be accompanied by consistent aseptic technique from preparation through implantation.[6–8].

IV. CONCLUSION

No bacterial growth was detected on MSA in any of the 36 tissue swab samples collected around implantation sites in nine guinea pigs. Negative results were observed in the PDO, PCL, and APTOS NAMICA groups on days 3, 7, and 21. These findings provide culture data following aseptic implantation of authorized thread products, with conclusions limited to the examination method and observation period used. Selection of legally marketed threads authorized by the Indonesian Ministry of Health, together with verification of sterile packaging integrity and expiration dates, should accompany consistent aseptic technique to support procedural safety.

V. DECLARATIONS

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Competing interests

The study was conducted independently and funded personally by M. Hafid Ernanda. M. Hafid Ernanda has a professional relationship with APTOS as a trainer and speaker.

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