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MICROBIOLOGICAL EVALUATION OF BACTERIAL PLAQUE IN SUTURE MATERIAL USED POST-EXTRACTION.

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Introduction:

Suturing, the final act of a surgical procedure is used to approximate tissues, reattach the removed tissue, control hemorrhage, and allow for primary healing (1). Commercially available sutures are composed of different materials and can be classified under different criteria: tridimensional structure (monofilament and multifilament), stability into tissues (absorbable and non-absorbable), materials (natural, artificial and synthetic) (2).In intraoral surgeries, a variety of suture types have been used, but silk has been widely accepted owing to its advantages of easy manipulation and capability of maintaining knot security (1)

Due to the oral conditions of moisture and susceptibility to infection by saliva, ingested food, microorganisms, past dental treatments, plaque etc together with the functions of speech, mastication and swallowing, makes a suture placed in an oral cavity affect differently than a suture in extraoral conditions [1]. Intraoral sutures are continuously bathed in saliva that contain microorganisms, resulting a continuous wicking along the suture material at the surgical site. This can cause chances for prolonged inflammatory reaction leading to surgical site infections (SSI) (3). In the oral cavity SSI is commonly seen as a postoperative local complication after surgical extraction of the impacted

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third molar that accounts for around 5% of cases. SSI is the third most common cause of nosocomial infections, and is the most among surgical patients. The incidence of SSI is related to intrinsic patient factors like immune-depression, diabetes mellitus, local or systemic infections, etc. and extrinsic factors such as smoking, surgical antiseptic measures, wound contamination or unsterile surgeries. Apart from the use of sutures, implantation of other devices such as joint prostheses, coronary stents also act as risk factors for SSI (4).Hence, the aim of this study is to evaluate the presence of aerobic and facultative anaerobic bacterial colonisation on silk suture material.

Materials and methods:

Before the commencement of the study, approval was obtained from the institutional ethics committee, and informed consent was taken from the participants.

The study included a total of 30 patients between the age group of 22-35 years who were posted for simple extraction procedure under LA. Patients with systemic diseases and active infections were excluded from the study. Natural black silk sutures were placed for all patients who underwent extraction. These patients were divided into three groups (group A, B and C), each group containing ten patients.

Suture removal

The black silk sutures were obtained postoperatively. Group A patients were called for suture removal on 3^{rd} day after the extraction, group B patients on 7^{th} day and group C patients on 10^{th} day after the extraction. After removal, the suture lines was taken in a size of 15 mm using a sterile endodontic millimetre ruler. This was then transferred aseptically to a test tube containing 2 ml sterile 0.1 M phosphate-buffered saline (0.9% NaCl), pH 7.2.

Microbiology

The test tube containing the suture was vortexed for 2 min to obtain a homogenous suspension (**1**). Dilution (10^{-1}) was prepared from this suspension using sterile saline. Aliquots of this dilution were taken in triplicates and were inoculated into Petri-plates with the following culture media: blood agar prepared with brain heart infusion agar (Sigma-Aldrich) supplemented with 5% sheep blood for total count of aerobic and facultative anaerobic microorganisms; mannitol agar (Himedia) for the growth of *Staphylococcus* spp (**2**); Mitis Salivarius bacitracin sucrose (MSBS) agar prepared with Mitis Salivarius agar (Himedia) supplemented with 0.2 IU/mL bacitracin and 15% sucrose for the growth of mutans group *streptococci*; LBS agar (Rogosa agar) for *lactobacillus spp.*

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The blood agar and mannitol agar plates were incubated at 37ºC for 24-48 h. The mitis salivarius agar plates were incubated at 37ºC for 48-72 h and LBS agar plate at 35 ºC for 24-72 h. The plates containing colonies were counted after incubation, and the number of colonies obtained was expressed in colony-forming units per millilitre (CFU/mL).

Results:

Table 1: Comparison of microbial count in blood agar media using ANOVA followed by post hoc analysis:

(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group A	2.00	-237.00000*	17.96602	.000	-284.2705	-189.7295
	3.00	-837.00000*	15.22060	.000	-877.7184	-796.2816
Group B	1.00	237.00000*	17.96602	.000	189.7295	284.2705
	3.00	-600.00000*	15.13642	.000	-640.4711	-559.5289
Group C	1.00	837.00000*	15.22060	.000	796.2816	877.7184
	2.00	600.00000*	15.13642	.000	559.5289	640.4711

*. The mean difference is significant at the 0.05 level.

There was a statistical significant difference observed between the three groups of patients in blood agar media (p=.00) and the intra group comparison showed that group C patients showed an increased bacterial count in blood agar media compared to group B and then followed by group A patients

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Table 2: Comparison of microbial count in Mannitol agar media using ANOVA followed by post hoc analysis:

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group A	2.00	-38.00000*	6.71648	.000	-55.6927	-20.3073
	3.00	-383.00000*	6.33333	.000	-399.7665	-366.2335
Group B	1.00	38.00000*	6.71648	.000	20.3073	55.6927
	3.00	-345.00000*	5.93483	.000	-360.6469	-329.3531
Group C	1.00	383.00000*	6.33333	.000	366.2335	399.7665
	2.00	345.00000*	5.93483	.000	329.3531	360.6469

*. The mean difference is significant at the 0.05 level.

There was a statistical significant difference observed between the three groups of patients in mannitol agar media(p=.00). Following the post hoc analysis, group C patients showed an increased bacterial count in mannitol agar media (p=.00) when compared to group B and group A respectively.

Table 3: Comparison of microbial count in Mitis salivarius bacitracin sucrose agar media using

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group A	2.00	-55.00000*	4.53382	.000	-66.9509	-43.0491
	3.00	-141.00000*	8.62168	.000	-164.8113	-117.1887
Group B	1.00	55.00000*	4.53382	.000	43.0491	66.9509
	3.00	-86.00000*	8.45905	.000	-109.5948	-62.4052
Group C	1.00	141.00000*	8.62168	.000	117.1887	164.8113
	2.00	86.00000*	8.45905	.000	62.4052	109.5948

ANOVA followed by post hoc analysis:

*. The mean difference is significant at the 0.05 level.

There was a statistical significant difference observed between the three groups of patients in mitis salivarius bacitracin sucrose agar media(p=.00) and the post hoc analysis, explained the group C patients had an increased bacterial count in mitis salivarius bacitracin sucrose agar media (p=.00) when compared to group B and group A respectively

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Table 4: Comparison of microbial count in LBS media using ANOVA followed by post hoc analysis:

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group A	2.00	-58.00000*	7.65942	.000	-79.3850	-36.6150
	3.00	-162.00000*	8.56349	.000	-184.9125	-139.0875
Group B	1.00	58.00000*	7.65942	.000	36.6150	79.3850
	3.00	-104.00000*	5.37484	.000	-118.5189	-89.4811
Group C	1.00	162.00000*	8.56349	.000	139.0875	184.9125
	2.00	104.00000*	5.37484	.000	89.4811	118.5189

*. The mean difference is significant at the 0.05 level.

There was a statistical significant difference between the three groups of patients in LBS media(p.00), and the intra group comparison shows increased bacterial count in group C patients in LBS agar media.(p=.00), followed by group B and group A patients respectively

Table 5: Comparison of bacterial count in four different media on the third day of suture removal

(I) VAR00002	(J) VAR00002	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Blood agar media	2.00	211.00000*	13.69915	.000	167.7596	254.2404
	3.00	281.00000*	13.20353	.000	238.1769	323.8231
	4.00	270.00000*	14.63633	.000	225.3336	314.6664
LBS agar media	1.00	-211.00000*	13.69915	.000	-254.2404	-167.7596
	3.00	70.00000*	6.05530	.000	51.8293	88.1707
	4.00	59.00000*	8.74960	.000	32.7824	85.2176
Mitis salivarius bacitracin sucrose agar media	1.00	-281.00000*	13.20353	.000	-323.8231	-238.1769
	2.00	-70.00000*	6.05530	.000	-88.1707	-51.8293
	4.00	-11.00000	7.95124	.718	-35.6584	13.6584
Mannitol agar	1.00	-270.00000*	14.63633	.000	-314.6664	-225.3336
	2.00	-59.00000*	8.74960	.000	-85.2176	-32.7824
	3.00	11.00000	7.95124	.718	-13.6584	35.6584

*. The mean difference is significant at the 0.05 level.

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There was a statistical significant difference noted in the bacterial count among the four media on the third day of suture removal (p=.00)

When the post hoc analysis was made the mitis salivarius agar media showed the maximum bacterial count compared to other media on the third day of suture removal, followed by mannitol media , LBS agar media and blood agar respectively.

Table 6: Comparison of bacterial count in four different media on the seventh day of suture removal

(I) VAR00002	(J) VAR00002	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Blood agar media	2.00	410.00000*	13.42469	.000	367.2724	452.7276
	3.00	463.00000*	13.00000	.000	420.5608	505.4392
	4.00	449.00000*	12.93144	.000	406.5841	491.4159
LBS agar media	1.00	-410.00000*	13.42469	.000	-452.7276	-367.2724
	3.00	53.00000*	5.38516	.000	36.8055	69.1945
	4.00	39.00000*	5.21749	.000	23.1580	54.8420
Mitis salivarius bacitracin sucrose agar media	1.00	-463.00000*	13.00000	.000	-505.4392	-420.5608
	2.00	-53.00000*	5.38516	.000	-69.1945	-36.8055
	4.00	-14.00000*	4.00000	.015	-25.8294	-2.1706
Mannitol agar	1.00	-449.00000*	12.93144	.000	-491.4159	-406.5841
	2.00	-39.00000*	5.21749	.000	-54.8420	-23.1580
	3.00	14.00000*	4.00000	.015	2.1706	25.8294

*. The mean difference is significant at the 0.05 level.

There was a statistical significant difference noted in the bacterial count among the four media on the seventh day of suture removal (p=.00). The post hoc analysis showed that the mitis salivarius agar media showed the maximum bacterial count on the seventh day of suture removal, subsequent was mannitol agar media then LBS agar media, and lastly to blood agar media.

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Table 7: Comparison of bacterial count in four different media on the tenth day of suture removal

(I) VAR00002	(J) VAR00002	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Blood agar media	2.00	665.00000*	9.17121	.000	636.5140	693.4860
	3.00	977.00000*	11.47461	.000	943.1068	1010.8932
	4.00	945.00000*	9.52774	.000	915.9181	974.0819
LBS agar media	1.00	-665.00000*	9.17121	.000	-693.4860	-636.5140
	3.00	312.00000*	8.81917	.000	284.7328	339.2672
	4.00	280.00000*	6.07362	.000	261.9940	298.0060
Mitis salivarius bacitracin sucrose agar media	1.00	-977.00000*	11.47461	.000	-1010.8932	-943.1068
	2.00	-312.00000*	8.81917	.000	-339.2672	-284.7328
	4.00	-32.00000*	9.18937	.021	-59.9240	-4.0760
Mannitol agar	1.00	-945.00000*	9.52774	.000	-974.0819	-915.9181
	2.00	-280.00000*	6.07362	.000	-298.0060	-261.9940
	3.00	32.00000*	9.18937	.021	4.0760	59.9240

*. The mean difference is significant at the 0.05 level.

There was a statistical significant difference noted in the bacterial count among the four media on the tenth day of suture removal (p=.00). The intragroup analysis showed the mitis salivarius agar media with the maximum bacterial count compared to other three media on the tenth day of suture removal, where mannitol agar showed more bacterial colonies than LBS agar media and blood agar media.

Discussion:

Wound site closure by suture for better adaptation and maintenance are critical for the success of a surgical procedure. (1) Oral cavity is comprised of a plethora of bacteria, forming the proverbial bacterial biofilm. Some of these bacteria have been implicated in oral diseases such as caries and periodontitis, which are among the most common bacterial infections in humans while, some specific oral bacterial species have also been implicated in several systemic diseases, such as bacterial endocarditis, aspiration pneumonia, osteomyelitis in children, preterm low birth weight, and cardiovascular disease. Surprisingly, little is known about the microflora of the healthy oral cavity. (5)

It has long been accepted that the presence of suture material increases the risk of infection, and SSI, which are likely associated with bacterial growth as a biofilm on to the suture thread. (6). Sutures

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placed in gingiva and oral mucosa after surgery are partly embedded in tissue and partly bathed in saliva with a mean concentration of approximately 750 million bacteria per milliliter, This results in a continual influx of microbial contamination along the suture channel. These bacteria produces inflammation causing erythema surrounding the puncture wounds and leads clinicians to suspect that the suture could wick the bacteria into the surgical site itself . Moreover, studies have also shown that intraoral sutures placed produce a tissue response that is distinctly different from the response observed at other experimental sites, mainly due to the presence of moisture and infectious potential with a consequent tendency towards rapid epithelial invagination (effect of different and oral tissue reaction to suture materials). Chu and Williams studied the adherence of radiolabeled S. aureus and Escherichia coli to ten suture materials and summarized their study by saying that bacterial adherence depended on a number of factors, including filament configuration (monofilament vs. braided) and the chemical nature of the suture, as well as the suture coating material, with the coating likely to be more influential (with absorbable but not nonabsorbable suture) than physical configuration. (7)

The removal of oral surgery sutures is routinely performed one week post-surgery. However, in the case of regeneration therapy, maintenance of the suture for at least two weeks is frequently allowed in clinical practice for the enhancement of tissue maturity. (1) Our study aimed to evaluate the presence of aerobic bacterial and facultative anaerobic bacterial colonisation on natural black silk suture material removed on three time intervals following extraction. As for the suture material black silk, in this study was selected based on their wide utilization in intraoral surgical practice. Microbiological evaluation of the suture threads were done to assess the microbial counts. We observed that the patients who underwent suture removal on the 10th day (Group C) showed an increased slimy accumulation surrounding the mass of silk suture material clinically; which is a typical characteristic of bacterial biofilms (8). Study done by Sergi Sala-Pérez on suture material obtained after removal of impacted third molar has observed, a larger number of bacterial colonies on suture material obtained after 3 days than after 7 days of suture placement. Apparently due to the inability to maintain adequate oral hygiene, as a result of the limitation in mouth opening, pain and swelling in the surgical zone still present on the third postoperative day. However, our study was not in agreement with this finding, the microbiological evaluation of the suture thread in our study showed the maximum number of colonies in all the 4 culture medias (blood agar media, mannitol agar media, mitis salivarius bacitracin sucrose agar media and LBS media) on the 10^{th} day than the patients who underwent suture removal on 7^{th} and 3rd day respectively (table1- 4), as our case selection was confined to simple extractions where post operative pain and swelling was very minimal. The findings in this study is in accordance to the widely accepted fact that demonstrate

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directly the ability of bacterial biofilms to situate on implanted suture material, serving as a nidus for microbial accumulation and wound contamination leading to infection acutely. Early literatures have documented that bacterial adherence to suture depends on the microbial species and the suture composition. (6). Moreover, the structural characteristic of natural black silk suture braiding also probably would explain this observation. Silk is a multifilament material that shows more wicking effect contributed by the wide interstitial spaces between suture material. (3) However, contrasting literatures are also noted stating that silk suture material manifests smaller affinity towards bacterial adhesion and lower wicking effect as it produces less fluid movement by capillary action than is produced by other braided sutures and it seems to transmit bacteria mechanically less often than do other suture materials.(9,10)

An attempt was made to isolate aerobic and facultative anaerobic bacteria from the bacterial plaque on the suture thread, aerobic bacteria are seen more supra gingivally, than anaerobic bacteria which are predominant in subgingival plaque (11). The suture material obtained postoperatively was subjected for microbiological evaluation. A homogenous suspension was prepared from the suture thread and was incubated in four petri dishes; blood agar evaluated the total count of aerobic and facultative anaerobic bacteria, mannitol agar evaluated the staphylococcus spp, mitis salivarius agar evaluated the growth of streptococcus spp and LBS agar evaluated the lactobacillus spp. Among the other aerobic and facultative anaerobic bacteria isolated from blood agar, streptococcus mutants, staphylococcus and lactobacillus were found to be more prevalent on 3^{rd}, 7^{th} and 10^{th} day after extraction (table5-7). They form the normal flora of the oral cavity and show a great affinity to adhere to sutures. These bacteria consequently act as a focus for odontogenic infection. In fact streptococcus spp are usually identified in odontogenic infections possing a potential risk for wound healing. These spp are well known as colonisers of the normal flora and are described as endocarditis pathogens.(9)

More than one-half of the microorganisms isolated from SSIs are gram-positive cocci, with *Staphylococcus aureus*, coagulase-negative staphylococci, and *Enterococcus* species being the three most common isolates from serious SSIs.(bacterial contamination)S. Yilmas has reported a Case of deep neck infection (DNI) which is a polymicrobial bacterial infection, that usually occurs following preceding infections such as tonsillitis/pharyngitis, dental caries or procedures, surgery or trauma to the head and neck region, or in intravenous drug abusers. Odontogenic infection is the most common cause of DNI that accounts for approximately 43% of the cases. Gram positive Staphylococcus haemolyticus and gram negative Klebsiella pneumonia, which are mainly facultative anaerobes were isolated from the drainage fluid in this case report and these organisms form the normal flora of the mouth, skin, and intestines.(12) Elek and cowen injected human volunteers with staphylococcus pyogenes and showed that a dose of 10^{6} bacteria was required to elicit a pus forming

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clinical infection, however in the presence of a braided silk suture ,this dose was reduced to only 100 bacteria, therefore explaining that the presence of a susceptible suture can enhance the risk of infection 10000 times. It should be hence, always kept in mind that all types of dental treatment could be a potential risk for severe, life threating infections (13). We could isolate lactobacillus from the suture material, though they were found to be less in number (table5-7). They belong to facultative anaerobic rod shaped bacteria forming the normal flora and are infrequent human pathogens but can induce several infections such as bacteremia and infective endocarditis. (14,15).

To conclude with, we observed that natural black silk being a multifilament material shows a greater wicking effect that can promote the adhesion of microorganism to the suture material. Common commensals like streptococcus aureus, staphalococcus and lactobacillus spp were isolated more frequently than the pathogenic organisms. The introduction of chemically modified suture materials to produce anti-bacterial effect can reduce the incidence of post-surgical infection and healing. However, it would be advisable to carry out a clinical study with a larger sample of patients for longer duration in order to evaluate the effectiveness of natural black silk as a suture material.

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