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Comparative evaluation of antibacterial activity of silk sutures coated with 0.2% hyaluronic acid gel and 0.2% chlorhexidine gel against Porphyromonas gingivalis: An Invitro study

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Abstract

Introduction: Sutures at the surgical site provides a favourable environment for the growth of microorganisms leading to surgical site infections (SSI).Sutures coated with antibacterial agents can reduce the microbial load. Presently, there are no studies which compared the antibacterial efficacy of 0.2% hyaluronic acid gel and 0.2% chlorhexidine gel coated silk sutures against Porphyromonas gingivalis.

Aim : The aim of present study is to evaluate the antibacterial activity of 3-0 silk sutures coated with 0.2% hyaluronic acid gel and 0.2% chlorhexidine gel in comparison to uncoated sutures against P.gingivalis.

Materials And Methodology: Equal segments of hyaluronic acid, chlorhexidine and normal saline coated 3-0 silk sutures are to be incubated in blood agar and incubated at 37°C for 48-72 hrs in anaerobic jar. Sutures coated with normal saline acts as control group. Assessment was done using disc diffusion test.

Results: The zone of inhibition around chlorhexidine coated suture is nearly double than that of hyaluronic acid coated suture. Thus indicating better antibacterial efficacy of chlorhexidine coated sutures. The saline coated sutures show no inhibition zone indicating that that it has no significant antibacterial activity.

Conclusion: It can be concluded that chlorhexidine and hyaluronic acid coated sutures have significant antibacterial activity against P. gingival is. So the antibacterial coated sutures have a promising potential in

preventing the colonization of periodontal pathogens around it thereby inhibiting biofilm formation.

Keywords: antibacterial suture, chlorhexidine, hyaluronic acid, P. gingivalis.

Introduction

Periodontitis is as an inflammatory disease of supporting tissues of teeth caused by microorganisms resulting in progressive destruction of the periodontal ligament and alveolar bone with periodontal pocket formation, gingival recession or both.The periodontal therapy aims at arresting the progression of periodontal disease and maintaining the natural dentition in health and comfortable function. In patients with mild to moderate periodontitis, this can be achieved by nonsurgical therapy whereas in advanced cases, open flap debridement results in greater success[1].

Once a surgical procedure is completed, the flaps are approximated with the help of sutures. Sutures provide multiple functions. They mainly helps to hold the tissue in position. In addition, they also enhance the primary healing and control hemorrhage. There are many types of suture materials available depending upon their diameter, resorbability, source and filament type (monofilament and polyfilament). Silk sutures are multifilament sutures that are commonly used in periodontal surgical procedures due to their low-cost availability.

Sutures that are used for wound closure can act as a reservoir of microbes at the surgical site, leading to increased chances of surgical site infection (SSI). Sutures used in oral cavity are continuously bathed in saliva containing 7.5×10^8 microorganisms/mL. This results in continuous wicking of microorganisms along the suture at the surgical site which results in a prolonged inflammatory response and surgical site infections[2]. P. gingivalis is one of the several etiological agents in chronic periodontitis and peri-implantitis. This species is a Gram-negative anaerobe identified in the bloodstream and atherosclerotic plaques, most recently, inside migratory antigen presenting cells. The introduction of chemically modified suture materials reduced the incidence of postsurgical infection and healing time.

The positive effect of Hyaluronic acid on periodontal wound healing following nonsurgical and surgical therapy has been reported earlier. There is significant improvement in gingival and periodontal health after subgingival application of hyaluronic acid as an adjunct. 0.2% Hyaluronan containing gel has a beneficial effect in the treatment of plaque induced gingivitis. Subgingival application of 0.8% Hyaluronic acid gel following scaling and root planing has shown anti-inflammatory effect and has a beneficial effect on clinical periodontal parameter in moderate to severe chronic periodontitis patients.[3] The high concentration of medium and lower molecular weight Hyaluronic acid has the greatest bacteriostatic effect, particularly on Aggregatibacter actinomycetemcomitans, Prevotella oris and Staphylococcus aureus strains, which are commonly found in oral gingival lesions and periodontal wounds. Hyaluronic acid has many properties which include anti-inflammatory, bacteriostatic, anti-oedematous, antioxidant, biocompatible and non-immunogenic[4]. Porphyromonas gingivalis acts as a strong inhibitor of wound closure[5,6].

Chlorhexidine, to date is the most potent antiplaque agent. It is considered gold standard antiplaque agent, against which efficacy of other antiplaque and antigingivitis agents is measured[7]. Its efficacy is due to its bacteriostatic and bactericidal properties and its substantivity within the oral cavity. Chlorhexidine gel are available in different concentrations as 1%, 0.2%, 0.12%. When 0.2% and 0.12% rinses are used in similar doses, shows equal efficacy. Chlorhexidine usage after periodontal surgery enhances wound healing. Thus, the use chlorhexidine-coated suture (CCS) can be a possible

alternative to conventional noncoated sutures (NCSs) in preventing or reducing the incidence of SSIs.

In the present study we assess the non absorbable black braided silk 3-0 sutures. There is no available study that has compared antibacterial activity of 0.2% hyaluronic acid gel and 0.2% chlorhexidine gel against Porphyromonas gingivalis. Hence the aim of the study is to evaluate the antibacteriall efficacy of 3-0 silk sutures coated with 0.2% hyaluronic acid gel and 0.2% chlorhexidine gel in comparison to uncoated sutures against P.gingivalis.

Materials and methods

The study protocol was reviewed and approved by the institutional review board of Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, Belgaum, Karnataka. The study used an in vitro experimental design.

Study Groups

Group A- Sutures coated with 0.2% hyaluronic acid (HA) gel

Group B- Sutures coated with 0.2% Chlorhexidine (CHX) gel

Group C (Control group)- Sutures coated with normal saline (NS)

Preparation Of 0.2% Hyaluronic acid Gel

Carbopol 934 (1 w/v) was weighed and soaked overnight in the Milli-Q water at 4 °C. Specified amount of Hyaluronic acid (0.2 % w/v) was added to the carbopol solution under magnetic stirring. Methyl paraben (0.18% w/v) and propyl paraben (0.02% w/v) was added to the above dispersion as preservative. Finally 1N NaOH solution was added drop wise to the above dispersion until it forms the gel. The obtained gel was stored in the air tight containers in refrigerator for further studies (Figure 1).

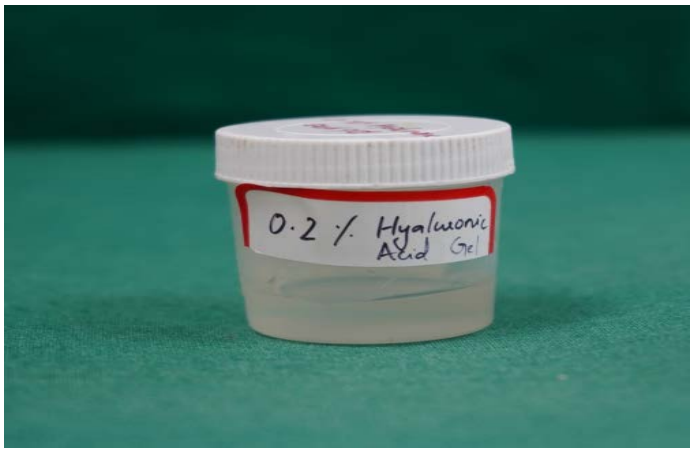

Figure 1: 0.2% Hyaluronic acid gel

Preparation Of 0.2% Chlorhexidine Gel

Carbopol 934 (1 w/v) was weighed and soaked overnight in the Milli-Q water at 4 °C. Specified amount of Chlorhexidine (0.2 % w/v) was added to the carbopol solution under magnetic stirring. Methyl paraben (0.18% w/v) and propyl paraben (0.02% w/v) was added to the above dispersion as preservative. Finally 1N NaOH solution was added drop wise to the above dispersion until it forms the gel. The obtained gel was stored in the air tight containers in refrigerator for further studies (Figure 2).

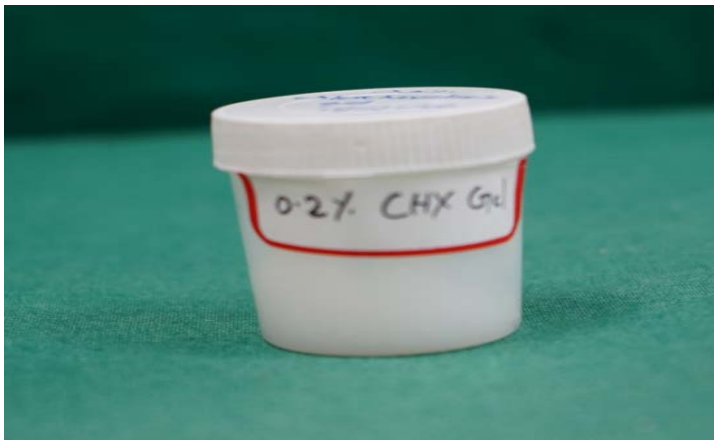

Figure 2: 0.2% Chlorhexidine gel

Pretreatment of sutures

Non absorbable black braided 3-0 silk sutures were used in the study. In order to remove any traces of impurities from the surface of silk sutures, it was scoured in 1%

NaOH solution for 1 minute at room temperature and washed with distilled water[8].

Preparation of sutures coated with hyaluronic acid (HA), chlorhexidine and saline

The scoured sutures was cut into a piece of 5cm length and autoclaved at 121°C for 15 minutes to ensure a complete sterilization of the suture for the study. The silk suture was then placed in a petridish for treating with 0.2% hyaluronic acid gel which was left to stand for overnight. Later, the suture was dried in a desiccator to remove the solvent adsorbed on to the surface. The same procedures were repeated for coating the sutures with chlorhexidine and saline.

Bacterial Strain

The P.gingivalis strains (ATCC 33277) in the lyophilized form is procured and revived in thioglycollate broth .It was then incubated for 3-4 days at 37ºC in anaerobic jar. After incubation the increase in turbidity was checked. Sub culturing was done on blood agar and the plate was incubated in the anaerobic jar for 3-4 days .The growth on blood agar plate was checked after incubation and was confirmed by performing Gram's staining and basic biochemical test. This confirmed strain was used for the further study.

Disc Diffusion Test

The zone of inhibition is an area around the coated suture in which the bacterial growth in inhibited. In this study the standard strains of P. gingivalis bacterial suspension at a concentration of 1×10^8 colony-forming unit per milliliter lawn culture was made on blood agar plate . Then, the sutures coated with hyaluronic acid, chlorhexidine and normal saline were placed on this inoculated blood agar plate and gently pressed which was incubated at 37°C for 48-72hrs in anaerobic jar. After incubation the zone of inhibition was checked surrounding the three sutures and was measured using standard callipers (Figure 3). This procedure was repeated for nine times according to sample statistics

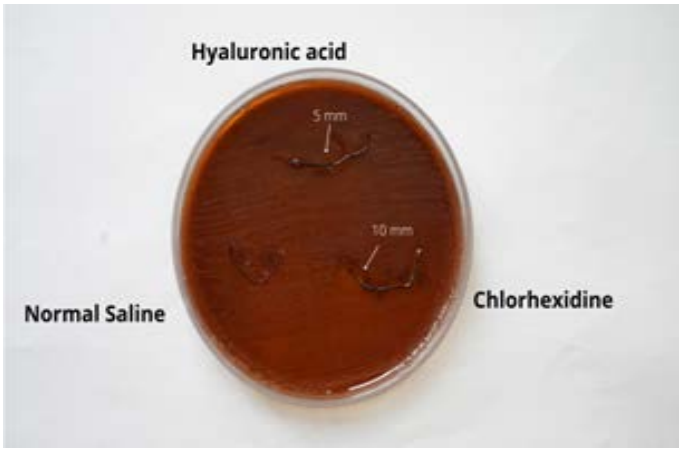

Figure 3: Zone of inhibition around sutures indicated by arrows in mm

Results

Among the three groups, the zone of inhibition is significantly more in chlorhexidine coated sutures followed by hyaluronic acid coated sutures. The sutures coated with normal saline shows the least zone of inhibition (Table 1).

Group	N	Mean Rank
HA coated sutures	9	14.00
CHX coated sutures	9	23.00
NS coated sutures	9	5.00

Table 1: Mean distribution among 3 groups on zone of inhibition [Kruskal Wallis Test]

Table 2 depicts the intergroup comparison between 0.2% hyaluronic acid, 0.2% chlorhexidine and saline using Mann Whitney Test. Among the three groups, chlorhexidine is showing a sum rank of 126.Therefore, among the three interventions, maximum zone of inhibition is projected by 0.2% chlorhexidine.

SL NO	GROUP	MEAN RANK	SUM OF RANK
1			
	0.2% HAA	14	126
	Saline	5	45
2			
	0.2%CHX	14	126
	Saline	5	45
3			
	0.2% HAA	5	45
	0.2% CHX	14	126

Table 2: Comparison of 3 groups on zone of inhibition [Mann Whitney Test]

Discussion

A wide range of virulence factors of P. gingivalis contribute to the inflammatory process occurring around natural teeth and implants. P. gingivalis has been shown to interfere with the normal process of wound healing by causing degradation of integrin- related signaling proteins, Paxillin and FAK[9,10].

Wu et al (2017) in a systematic review stated that sutures coated with antimicrobials significantly reduced SSI risk[11]. Studies have shown that bacteremia can result from the removal of intraoral sutures, which is a potential risk factor for developing bacterial endocarditis in high-risk patients[12]. A study conducted by Obermeier A et al showed that sutures coated with chlorhexidine at three different antiseptic concentrations demonstrated their high anti-microbial efficacy against S. aureus in vitro[13]. Walker et al conducted a study using chlorhexidine as an antibacterial coating on suture against methicillin-resistant Staphylococcus aureus (MRSA), Staphylococcus epidermis, and E. coli, were Vicryl Plus serves as a positive control. The chlorhexidine-coated suture and Vicryl Plus showed statistically equivalent zone of inhibition when tested against MRSA and S. epidermis, whereas when chlorhexidine-coated suture was tested against E. coli , a statistically significant zone of inhibition was observed[14]. Sharma C et al concluded that chlorhexidine coated polyglactin 910 suture results in less adherence of anaerobic bacteria as compared to polyglactin 910 (non-coated) suture[15].This is in accordance with our present study which showed a significant zone of inhibition against P. gingivalis. An invitro study by Chen et al showed that hyaluronic acid inhibits P. gingivalis-induced IL-1β, IL-4, IL-6, IL-8, and IL-10 production in a dose-dependent manner. Hyaluronic acid also reduces inflammation and promotes wound healing[16]. Sudhir et al showed that hyaluronic acid -modified suture related to both silk and PGA has shown significant antibacterial action against both S. aureus and E. coli colonization. Moreover hyaluronic acid reduced wicking in both PGA and silk sutures[17]. The current study has also shown significant antibacterial effect against P. gingivalis. Further studies should be carried out in an invivo experimental design in a large scale to generalize the result of present study. The current study shows that the silk sutures coated with 0.2% chlorhexidine gel shows comparatively high antibacterial activity against Porphyromonas gingivalis than 0.2% hyaluronic acid gel. So it is evident that antibacterial agents have a promising potential in preventing the colonization of periodontal pathogens.

Conclusion

Chlorhexidine and hyaluronic acid are known antibacterial agents. So the use of antibacterial coated sutures significantly reduces the bacterial load and thereby reducing the surgical site infections. Thus within the limitations of our study it can be concluded that chlorhexidine and hyaluronic acid coated sutures have significant antibacterial activity against Porphyromonas gingivalis.

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