ORIGINAL ARTICLE

Visualization of Zone of Inhibition using Sutures Treated with 0.2% Hyaluronic Acid against *Porphyromonas gingivalis* – An *In Vitro* Microbiological Study

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ABSTRACT

Context: Sutures at the surgical site can act as a reservoir for microbes. Coating suture material with 0.2% hyaluronic acid (HA) could be one of the possible alternatives to reduce the microbial load. **Aims and Objectives:** The aim of the present study was to assess the antibacterial efficacy of black braided silk and vicryl sutures coated with 0.2% HA by visualizing the zone of inhibition against *Porphyromonas gingivalis* microorganism in comparison to non-coated black braided silk and vicryl sutures. **Settings and Study Design:** The present *in vitro* study evaluated zone of inhibition of *P. gingivalis* in agar media for 48 h and 1 week. Sutures were divided into two groups: Group 1 (Test) – vicryl and black braided silk sutures coated with 0.2 % HA and Group 2 (Control) – vicryl and black braided silk sutures. **Materials and Methods:** 10 cm length sutures were placed in two Petri dishes. After completing the inoculation of agar plate with *P. gingivalis*, the Group 1 (Test) and Group 2 (Control) from Petri dishes were placed in their respective inoculated plates. These plates were inverted and placed in an anaerobic jar and incubated at 37°C. Anaerobic bacterial growth around each suture was evaluated after 48 h and at 1 week. Inoculated plates were read by the Kirby–Bauer test. **Results:** Zone of inhibition was more in HA-coated sutures (Group 1) after 48 h and at 1 week compared to non-coated sutures (Group 2). Vicryl and black braided silk sutures showed 8 mm and 14 mm zone of inhibition after 48 h, respectively, and 13 mm and 15 mm zone of inhibition after 1 week. Zone of inhibition was resistant in Group 2. **Conclusion:** 0.2 % HA-coated sutures can be used to reduce the bacterial load of *P. gingivalis*.

Key words: Antibacterial sutures, black braided silk sutures, hyaluronic acid-coated sutures, *Porphyromonas gingivalis*, vicryl sutures

INTRODUCTION

The oral cavity is a source of different microorganisms that cause infections and inflammation. So far, more than 700 bacterial taxa have been identified in samples taken from oral cavities. Of the bacteria believed to be pathogenic in periodontal disease, *Porphyromonas gingivalis* has been extensively studied due to its unique ability to evade the immune response. *P. gingivalis* is a Gram-negative oral anaerobe and considered as a main etiological factor in periodontal diseases by producing a number of virulence factors and extracellular proteases such as lipopolysaccharide, fimbria, and gingipain, resulting in destruction of periodontal tissues.[1] To restore tissues back to normal, pocket elimination or pocket reduction procedures are carried out. After periodontal surgery flaps are approximated together with sutures. Sutures play a critical part in case of surgeries and have an effective role. The

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purpose of suture is to hold the opposing tissues together to facilitate and hasten healing process with minimal or no scar formation followed by an injury or surgical procedure.[2] However, suture material is known to be a potential agent of infection as well. To prevent microbial colonization of suture material in an operative wound, many antibacterial sutures such as triclosan-coated sutures and chlorhexidine-coated sutures have been developed. Triclosan-coated sutures reduced surgical site infections when compared to standard sutures.[3] Chlorhexidine diacetate-impregnated polyglactin sutures showed reduced infection rates, erythema, and trismus.[4] Apart from these two, sutures are also coated with tetracycline, but it kills benign organisms associated with health and also develop antibiotic resistance. To overcome disadvantages of above-mentioned antibacterial suture coatings, more recently hyaluronic acid (HA) is being used, not only for anti-bacterial effect but also for an anti-inflammatory effect. Other actions of HA are moisture balance, cell proliferation, and immune modulation, to maintain the networking effect of structures to prevent bacterial proliferation. It is also produced naturally as part of the wound healing process to promote proliferation and differentiation of fibroblasts and epithelial cells, leading to decreased levels of inflammatory mediators, which have an anti-inflammatory effect.[5] It also reduced wicking in both PGA and silk sutures.[6] Hence to know efficacy of antibacterial property of HA especially against *P. gingivalis*, the present study was designed to evaluate the visualization of zone of inhibition using different sutures treated with 0.2% HA against *P. gingivalis* which is first of its kind. In this present study, *P. gingivalis* organism was chosen because it is considered as one of the putative periodontal pathogens, and also, we have considered using black silk and vicryl sutures as they are commonly used in periodontal surgical procedures.

MATERIALS AND METHODS

0.2% HA, vicryl braided sutures were purchased from (Ethicon), black braided silk sutures were purchased from (Sutures India), Petri dishes, inoculating loop, laminar air flow cabinet, 0.5 McFarland turbidity standard, cotton swabs, agar plates, and anaerobic jar. The study was approved by the Institutional Review Board.

0.2% HA Preparation

Hyalgan prefilled syringe (20 mg Sodium Hyaluronate/2 mL) was obtained. In the syringe for 1 mL of solution – 10 mg of sodium hyaluronate was present. To obtain 0.2% of HA, 2 ml solution present in the prefilled syringe has to be diluted. Commercially available hyalgan injection solution (20 mg Sodium Hyaluronate/2mL) was dispensed in a sterile container. Later, 8 mL of distilled water was taken in 10 mL syringe and dispensed in the same sterile container. Injection solution and distilled water were mixed well. Total amount of prepared solution present in sterile container after mixing is 10 mL, which has 0.2% of HA.

Pharmacy Laboratory Procedure

The present *in vitro* study was designed to measure *P. gingivalis* zone of inhibition on vicryl and black braided silk sutures. Two groups were taken (Group 1 and Group 2). In Group 1, vicryl and black braided silk sutures were coated with 0.2% HA and in Group 2 uncoated vicryl and black braided silk sutures were taken.

The sterile suture materials were cut into 10 cm (each). Group 1 sutures were placed in Petri dishes filled with 0.2 % HA, in laminar air flow cabinet. Group 2 sutures were kept in sterile Petri dishes without 0.2% HA. They were incubated for 24 h.

1. Using an inoculating loop, the *P. gingivalis* strain ATCC no 33277 bacterial colonies were transferred to broth.
2. Visually turbidity was adjusted with broth to equal that of a 0.5 McFarland turbidity standard that has been vortexed.
3. Within 15 min of adjusting the inoculum to a McFarland 0.5 turbidity standard, a sterile cotton swab was dipped into the inoculum and rotated it against the wall of the tube above the liquid to remove excess inoculum.
4. With the same cotton swab the entire surface of agar plate was swabbed 3 times, rotating plates approximately 60 between streaking to ensure even distribution. Avoid the cotton swab hitting sides of Petri plate and creating aerosols.
5. Allow inoculated plate to stand for at least 3 min but no longer than 15 min before placing the sutures.
6. All the above procedures were performed in a laminar air flow cabinet.
7. After completing the inoculation, the test group and control group sutures were placed in their respective inoculated plates.
8. These plates were inverted and placed in anaerobic jar and kept for incubation at 37°C in incubator for 48 h and for 1 week.

Reading the Plates

The inoculated plates were read by the Kirby–Bauer test.[7] First, this test was developed in the 1950s, it was refined by Kirby and Bauer, then standardized by the World Health Organization in 1961. If the organism is killed or inhibited by the concentration of the drug, there will be no growth in the immediate area. This is called the zone of inhibition. Place the metric ruler across the zone of inhibition, at the widest diameter, and measure from one edge of the zone to the other edge. Holding the plate up to the light might help. Use millimeter measurements. The suture diameter will actually be part of that number.

RESULTS

Results from the present study were obtained based on zone of inhibition against *P. gingivalis* microorganism in the Group 1 [Table 1 and Figure 1] and in Group 2 after 48 h and 1 week [Table 2 and Figure 2]. Zone of inhibition of black braided silk sutures coated with HA after 48 h was 14 mm and after 1 week, it was 15 mm. Zone of inhibition of vicryl sutures coated with HA after 48 h was 8 mm and after 1 week, it was 13 mm. Zone of inhibition was resistant in Group 2.

DISCUSSION

The present study assessed the effect of 0.2% HA treatment on two different types of sutures-black braided silk and vicryl. It was revealed that sutures coated with 0.2% HA significantly reduced the number of *P. gingivalis* following 48 h and at 1 week of incubation, respectively. These results suggest that treating suture materials with enzymatic solutions, such as 0.2% HA, significantly increased the zone of inhibition. When comparing the presence of *P. gingivalis* between 0.2% HA-coated silk suture and vicryl suture, it was found that a zone of inhibition was 15 and 13 mm, respectively [Table 1 and Figure 1], and zone of inhibition was resistant between uncoated black braided silk sutures and vicryl sutures [Table 2 and Figure 2]. Similar structure of two braided sutures in this study, eliminated selection bias, and wicking effect was also nullified.

Table 1: Zone of inhibition against *P. gingivalis* after 48 h and 1 week in Group 1

Group 1	Zone of inhibition	
	After 48 h	After 1 week
Black braided silk sutures with 0.2% HA	14 mm	15 mm
Vicryl sutures with 0.2% HA	8 mm	13 mm

HA: Hyaluronic acid

Table 2: Zone of inhibition against *P. gingivalis* after 48 h and 1 week in Group 2

Group 2	Zone of inhibition	
	After 48 h	After 1 week
Black braided silk sutures without HA	Resistant	Resistant
Vicryl sutures without HA	Resistant	Resistant

HA: Hyaluronic acid

Anaerobic Gram-negative species *P. gingivalis* has been implicated as a major etiological agent in periodontitis. *P. gingivalis* interferes with normal wound healing by causing keratinocyte apoptosis and interfering with keratinocyte proliferation. *P. gingivalis*, a virulent pathogen, shows its impact on either wound healing locally or inflammation systemically.[8]

Hyaluronan has a multi-faceted role in the mediation of the tissue repair process.[9] Chen *et al.* have assessed the effect of various molecular weights of HA (30, 300, and 1300 kDa) on *P. gingivalis*-induced inflammatory and wound-healing responses in human

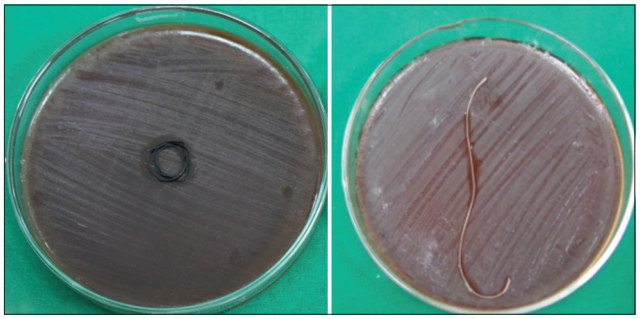

Figure 1: Group 1-Black braided silk sutures and vicryl sutures coated with 0.2% hyaluronic acid

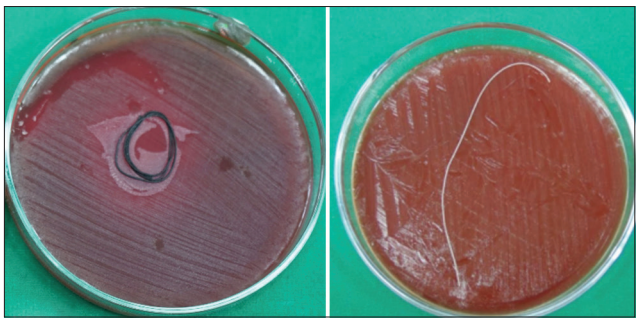

Figure 2: Group 2-Black braided silk sutures and vicryl sutures without hyaluronic acid

gingival fibroblasts and found that 1300 kDa HA inhibited *P. gingivalis*-induced IL-1β, IL-6, IL-8, IL-4, and IL-10 production in a dose-dependent manner.[10]

Clinical trials conducted by Pagnacco *et al.* revealed the anti-inflammatory, anti-edematous, and antibacterial properties of HA in periodontal disease.[11] Park *et al.* evaluated the effect of HA injection with systemic antibiotics for prevention and treatment of surgical site infection and found that HA showed significantly better wound healing and a lower bacterial count.[5] Pirnazar *et al.* investigated the potential bacteriostatic effect of HA in different concentrations and molecular weight on oral and non-oral microorganisms and concluded that the clinical application of hyaluronan in form of membranes, gels, or sponges during surgical therapy may reduce bacterial contamination of the surgical wound, thereby lessening the risk of postsurgical infection and promoting more predictable regeneration.[12]

Drago *et al.* found that HA is able to interfere with bacterial adhesion to a cellular substrate in a concentration-dependent manner.[13] Grigg *et al.* have assessed the effects of HA on incision healing in the oral cavity and found that it can accelerate wound healing and reduce inflammation.[14] The present study is also in accordance with above studies, where HA is having antibacterial property *in vitro*, which makes it superior to triclosan.[5,11-15]

Triclosan and chlorhexidine have their own disadvantages. Triclosan do not provide a sufficient antimicrobial effect to prevent *in vitro* colonization by oral bacteria and promotes multi drug resistance.[15] Chlorhexidine becomes inactive in the presence of saline solution, also has minimal activity against spores, and do not possess tissue dissolving property.[4] However, HA possesses anti-inflammatory property along with antibacterial property and may play an important role in healing response in gingival fibroblasts.[6]

CONCLUSION

This *in vitro* study demonstrated that suture materials can render *P. gingivalis* activity when coated with 0.2% HA. Further, testing is needed to confirm long-term efficacy of suture materials and research on HA should be done to know the immune mechanisms, as it can give better information about the role of specific suture materials in different conditions. Further studies are to be done with 0.2% HA to evaluate antibacterial efficacy against other putative periodontal pathogens.

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