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Original Article

Dentistry Section

Assessment of Mechanical Properties and Antimicrobial Effects of Hyaluronic Acid Coating on Absorbable Suture Material: An In-vitro Study

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ABSTRACT

Introduction: The use of absorbable suture materials in surgical procedures is a critical aspect of wound closure and tissue repair. In recent years, there has been growing interest in exploring the potential benefits of incorporating Hyaluronic Acid (HA), a naturally occurring biopolymer, into these sutures. By shedding light on the interactions between HA and absorbable sutures, the present study seeks to provide valuable insights that could enhance the quality of surgical wound management.

Aim: To assess the mechanical properties and antimicrobial effects of HA-coated and uncoated absorbable suture material.

Materials and Methods: The in-vitro study was conducted at Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India in a year 2022. A total of 70 samples of HA-coated and uncoated Vicryl 4-0 suture material were tested for tensile strength using an Instron machine. An anti-bacterial effect against *Pseudomonas aeruginosa* and *Streptococcus mutans* was assessed using a Mueller Hinton agar (MHA) plate, and the zone of inhibition was measured. Additionally, both the uncoated and HA-coated Vicryl 4-0 suture materials were analysed under the Scanning Electron Microscope (SEM) to determine the surface characteristics. An independent t-test was used to compare the tensile strength of coated and uncoated suture materials.

Results: The mean tensile strength of uncoated and HA-coated suture material was 6.47±0.23 MPa and 3.78±1.02 MPa, respectively. The difference between both groups was statistically significant ($p<0.05$). For *Pseudomonas aeruginosa* and *Streptococcus mutans*, the zone of inhibition was 8.0±0.5 mm in diameter and 9±0.5 mm in diameter, respectively, around coated Vicryl 4-0 suture material, whereas no zone of inhibition was observed for both *Pseudomonas aeruginosa* and *Streptococcus mutans* around uncoated suture material. SEM analysis showed superior surface characteristics of HA-coated suture thread when compared to uncoated suture thread.

Conclusion: HA-coated suture material does not exhibit superior tensile strength when compared to the uncoated suture material. However, HA-coated suture material has potent antibacterial effects against *Pseudomonas aeruginosa* and *Streptococcus mutans*. Additionally, SEM analysis showed improved surface characteristics in favour of the coated suture material.

Keywords: Antibacterial, Hyaluronan, Mechanical property, Multifilament suture

INTRODUCTION

Suturing has the critical goal of ensuring the appropriate and stable orientation of surgical flaps to enable optimal healing. This is determined by a variety of factors, including the optimal suturing method, thread type, diameter, and tension applied to the wound borders to promote healing via primary intention healing [1]. Accurate apposition of surgical flaps provides haemostasis, wound size reduction, patient comfort and prevention of bone destruction [2]. Otherwise, haemostasis occurs while blood and serum might accumulate under the flap, delaying the healing process by separating the flap from the underlying bone [3]. These necessary factors for successful wound healing can be achieved with the help of appropriate suture material [4].

Surgical flaps in various periodontal treatments were supposed to be positioned in an apical, coronal, or lateral position, based on the surgical purpose [5]. When tissues are positioned over hard or soft-tissue, autologous or allograft material, or regenerative Guided Tissue Regeneration (GTR) membranes, it is expository to choose the proper suturing methods, thread type, thread diameter, surgical needle, and use of the appropriate surgical knot for each respective suture material chosen in periodontal plastic, aesthetic, and reconstructive procedures to achieve requisitioned wound healing. As a result, the suture material utilised for wound closure should be chosen based on the biologic features of the specific wound [6].

Suture materials are classified into two types: absorbable and non absorbable suture materials. Non absorbable multifilament sutures are more likely to cause wound infection and sinusitis [6,7].

Absorbable suture material is utilised to surmise tissues when suture removal is not required. The two key properties desired in absorbable suture material are: i) retaining maximal tensile strength needed for wound healing and wound tensile strength; and ii) disappearance as soon as the suture material has lost its firmness [8]. Absorbable sutures dissolve in two phases. Tensile strength is lost first, followed by increasing loss until the suture is totally absorbed. It is critical that the suture retain adequate tensile strength to sustain the wound until the healing process is completed [9].

Lactide and glycolide, which are cyclic byproducts produced from lactic and glycolic acids, are copolymerised to form polyglactin 910 (Vicryl). The intermediates are first converted into a fibre-forming polymer before being formed into uniform particles. The particles are melted and extruded into fibres under precisely regulated temperature and pressure settings. To enhance the strength of the fibres, they are sprawled to appropriately position the fibre molecules. Following additional treatment, the filamentous exudate is braided into sutures and stretched while heated to boost suture tensile strength [10].

Following additional processing, the sutures are fumigated with ethylene oxide and loaded in an inert atmosphere to prevent the suture from being altered by ambient moisture [11]. Hyaluronic Acid (HA) has been commonly used to treat wounds and repair tissues [12]. Past clinical trials have shown that HA has anti-inflammatory, anti-oedematous, and antibacterial properties in periodontal disease, which is mainly caused by microorganisms present in subgingival plaque [13]. Due to its great biocompatibility and non immunogenicity, HA has been used in a variety of therapeutic applications, including augmenting joint fluid in arthritis, acting as a surgical assist in eye surgery, and promoting bone, surgical wound, and periodontal tissue healing and regeneration [1].

The aim of present study was to comparatively evaluate the mechanical and antibacterial properties of Vicryl suture material coated with HA. The primary objective was to compare the tensile strength of Vicryl suture material coated with HA to uncoated Vicryl suture material. The secondary objective was to assess the effect of HA against microbial colonisation by *Pseudomonas aeruginosa* and *Streptococcus mutans* and to evaluate the surface characteristics of the coated and uncoated suture material using SEM.

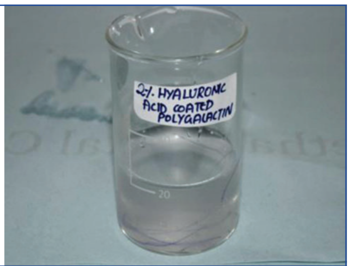
MATERIALS AND METHODS

The in-vitro study was conducted at Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences in the year 2022. A total of 70 samples (35 for HA coating and 35 for uncoated suture) were considered for the study.

Sample size calculation: The sample size was calculated based on the inferences obtained from a pilot study with three samples in each group. With a significance level of 5% and power of 20%, the final size was estimated to be 70 samples (35 per group) for further analysis.

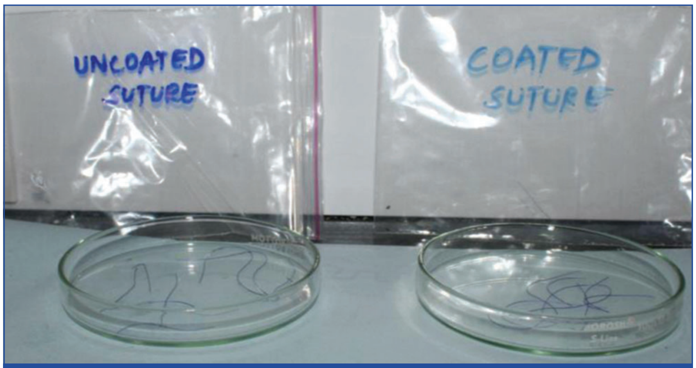
Study Proceudre

Coating of Vicryl 4-0 with Hyaluronic Acid (HA) and characterisation: Coating of Vicryl 4-0 with Hyaluronic Acid (HA) and characterisation: Vicryl 4-0 suture threads were initially cut into 70 pieces of uniform length of 10 cm. A total of 35 samples of suture threads were treated under ultraviolet radiation in a spectrophotometer for 30 minutes in a petridish [Table/Fig-1]. This was done to enhance the mechanical properties of the suture material. Then, after treating under Ultraviolet (UV) radiation, these suture threads of Vicryl 4-0 were dipped into HA and were immersed in it for the next 24 hours [Table/Fig-2]. The other 35 samples were not coated with HA, and these were the uncoated samples. Both the coated and uncoated samples were immersed in artificial saliva to simulate the dynamic and complex environment within the oral cavity, enabling a comprehensive analysis of the sutures' behavior over time [Table/Fig-3].



[Table/Fig-1]: Suture thread treated under UV light.
[Table/Fig-2]: Suture thread immersed into Hyaluronic Acid (HA). (Images from left to right)

Assessment of tensile strength of Vicryl 4-0 coated versus uncoated: The HA-coated Vicryl 4-0 suture material and uncoated Vicryl 4-0 suture material were evaluated for tensile strength using the Universal Testing Machine, Instron. The tensile strength was measured in Newtons. The suture threads were placed in the arms of the universal testing machine. The evaluation of tensile strength was performed in a wet environment. The suture threads were immersed in artificial saliva to mimic the oral environment. The



[Table/Fig-3]: Coated and uncoated suture samples.

artificial saliva provided a realistic medium for assessing the suture material's response to moisture, enzymes, and other components present in natural saliva. Each sample was stretched until the point of breakage, and the values were recorded.

Assessment of antimicrobial activity: The antimicrobial activities of HA-coated Vicryl 4-0 suture material and uncoated Vicryl 4-0 suture material were assessed against clinical isolates of *Pseudomonas aeruginosa* and *Streptococcus mutans* using MHA plates. Inoculum containing 106 Colony Forming Unit (CFU)/mL of each bacterial culture was spread onto the prepared MHA plates with a sterile cotton swab moistened with each of the bacterial suspension. Subsequently, 10 mm of the samples (both coated and uncoated suture materials) were placed on MHA and allowed to diffuse at room temperature for two hours. Then, the culture plates were incubated at 37°C for 24 hours. After incubation, the diameters (mm) of the bacterial (*Pseudomonas aeruginosa* and *Streptococcus mutans*) growth inhibition zones were recorded. The zone of inhibition of both coated and uncoated samples was measured.

Assessment of surface characteristics by Scanning Electron Microscopy (SEM) analysis: The morphological characteristics of both coated and uncoated suture materials were evaluated by SEM. At room temperature, the samples were initially coated with platinum using a sputter coater. SEM was used to evaluate the overall morphology of the suture samples after plating with platinum on a stub (JEOL JSM-IT800) [Table/Fig-4]. The SEM used 10 μm resolution and a power of 3.00 kV for analysing the samples.

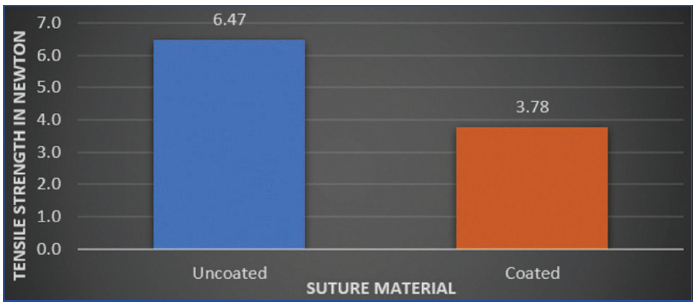
[Table/Fig-4]: Scanning Electron Microscope (SEM) analysis.

STATISTICAL ANALYSIS

An independent t-test was conducted to compare the tensile strength between coated and uncoated suture material.

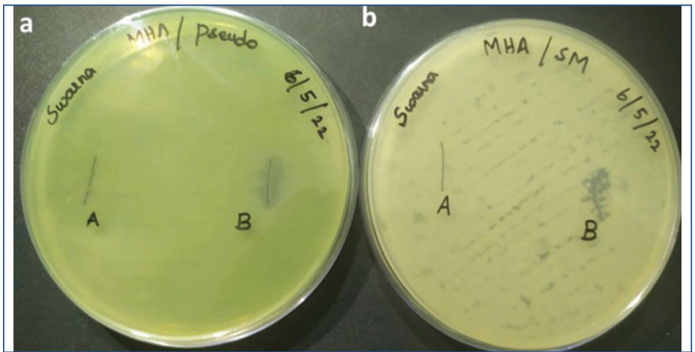
RESULTS

Tensile strength: The independent t-test showed that there was a statistically significant difference between the coated and uncoated Vicryl 4-0 sutures. The uncoated suture thread (6.47±0.23 MPa) showed higher tensile strength when compared to the coated suture thread (3.78±1.02 MPa). This difference was statistically significant (p<0.0154) [Table/Fig-5].



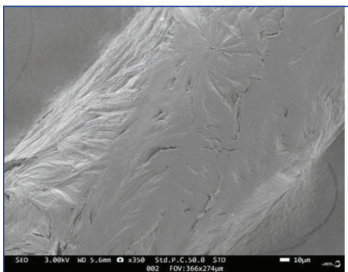
[Table/Fig-5]: Comparison of tensile strength between coated and uncoated Vicryl suture material.

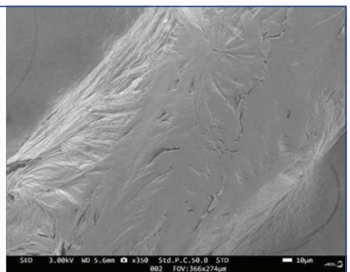
Antimicrobial activity: The coated sample exhibited zones of inhibition against *Pseudomonas aeruginosa* (8.0±0.5 mm) and *Streptococcus mutans* (9±0.5 mm). However, the uncoated sample exhibited a negative zone of inhibition against *Pseudomonas aeruginosa* and *Streptococcus mutans* [Table/Fig-6]. This revealed the antimicrobial effect of HA.



[Table/Fig-6]: Positive zone of inhibition around sample B (Coated Vicryl 4-0 suture) and negative zone of inhibition around sample A (Uncoated Vicryl 4-0 suture). 'a' represents MHA plate containing *Pseudomonas aeruginosa* and 'b' represents MHA plate containing *Streptococcus mutans*.

SEM analysis: SEM observations revealed a smooth surface morphology in the coated Vicryl 4-0 compared to the uncoated Vicryl 4-0 suture material. No aggregation or breakage of the suture material was observed in the coated suture thread compared to the uncoated suture material. The coating of HA was well exhibited in the SEM image of the coated suture sample. SEM observations, therefore, revealed that the coating of HA has enhanced the bonding of the suture material together and exhibited appreciable surface characteristics in the coated suture material [Table/Fig-7]. However, the surface characteristics were not much appreciable in the uncoated suture material [Table/Fig-8].





[Table/Fig-7]: SEM analysis of coated suture.
[Table/Fig-8]: SEM analysis of uncoated suture. (Images from left to right)

DISCUSSION

The present study compared the tensile strength of HA-coated and uncoated sutures as it is necessary for the absorbable suture material to withstand until appropriate wound healing to accomplish [13]. Additionally, the sutures used in oral procedures are continuously bathed in saliva, which contains mostly bacteria [14-16]. This results in continuous wicking along the suture material at the surgical site, which can cause a prolonged inflammatory reaction [17].

Monofilament sutures are composed of a single filament, whereas multifilament sutures are made of many filaments that are either entangled or twisted. Compared to braided suture materials, monofilament sutures offer reduced knot tie-down resistance, less tissue drag, and a lower risk of infection. There is less possibility of microbial infestation, and they are easier to knot. Their chopped edges, on the other hand, might irritate mucosa and induce ulcers. Because of their low bending stiffness and ease of formation of a secure knot, multifilament sutures are simple to handle and tie. Its braided form, on the other hand, frequently favours the buildup of food debris or microbes [18]. The interstitial spaces between filaments in multifilament sutures induce the capillary action, which acts as a wick, transferring fluid and bacteria along the entire length of the suture material. As a result, they should not be used on inflamed or diseased tissue. Multifilament sutures can be coated to avoid undesirable capillary action, which lowers the risk of bacterial colonisation [19].

The absorbable sutures break down and decompose following implantation, primarily as a result of enzymatic degradation coupled with subsequent hydrolysis or hydrolysis alone. Enzymatic degradation is employed for natural absorbable suture materials, whereas non enzymatic degradation is employed for synthetic absorbable suture materials [20]. The advantages of absorbable sutures include compatibility for conditions where suture support is required for a short duration or when removing the sutures is challenging.

These sutures also facilitate rapid re-epithelialisation, provide high tensile strength during the initial stages of healing, minimise foreign body reactions, and result in minimal scar formation. The drawbacks include the potential for these sutures to temporarily function as foreign bodies within the body, which may, in some cases, lead to local antigen-antibody reactions. Additionally, they can exacerbate existing infections. Another significant disadvantage to consider is the risk of wound dehiscence, especially when these sutures are used in areas that have the potential to expand, stretch, or undergo distension [21].

Vicryl is a synthetic absorbable suture material composed of 90% glycolide and 10% lactide. This heteropolymer is characterised by its braided, multifilament structure, and it is coated for added durability. These sutures can also be provided in antibiotic form by incorporating triclosan. They maintain roughly 75% of their strength after 14 days, retain about 49% of their strength at the end of the 21-day mark, and still hold around 27% of their strength at 28 days. Full absorption is achieved within a range of 60 to 70 days [22].

Antimicrobial sutures have been used to prevent wound infection [23]. In another study, where triclosan coating was impregnated with Vicryl suture and the number of positive bacterial cultures was calculated, the findings revealed that there was a decrease in the number of positive bacterial cultures [24]. Therefore, the present present study was done to assess the coating of HA against bacterial colonisation, especially against *Pseudomonas aeruginosa* and *Streptococcus mutans*.

Grigg TR et al., conducted an evaluation of the impact of HA on the healing of incisions within the oral cavity [25]. Their findings indicated that HA possesses the capability to expedite wound healing processes and mitigate inflammation. Furthermore, the investigation done by Leknes KN et al., also examined HA's effects on wound healing and its anti-inflammatory properties in surgical contexts [26].

The result of present study showed that the HA possessed a considerable antibacterial effect against both *Pseudomonas aeruginosa* and *Streptococcus mutans*. This was in accordance with the result of the previous study by Varma S et al., who evaluated the effect of HA added to suture material and its effect on bacterial colonisation and found that HA has a significant antibacterial activity against *S. mutans* [27]. Another study by Mohammadi H et al., also showed that when HA is coated onto the suture material, it possesses a good antibacterial effect [28].

The present study also showed that the addition of HA has no effect on increasing the tensile strength of the Vicryl suture material, which is in line with the results of the previous study by Sudhir V et al., who evaluated the effect of HA in modifying the tensile strength of non absorbable suture materials and found that HA as a chemical adjunct did not alter the tensile strength [29]. In a study conducted by Blaker JJ et al., where antibacterial coatings of silver-doped bioactive glass were used on absorbable Vicryl suture and non-resorbable silk sutures and mechanical properties were compared. The results proved that the bioactive glass coating did not alter the mechanical properties of the suture materials [30]. Another study by Wu CS and Liao HT showed that HA increases the tensile strength only up to 20% in the preparation of biodegradable polymers [31]. Another study by Cawthorne DP et al., concluded that immersion of Vicryl suture material into a sodium chloride and povidone iodine solution decreases the tensile strength of the material [32]. Similar findings were observed by Bruner SC et al., where the tensile strength of Vicryl was decreased after seven days of exposure to saline [33]. In another study by Alnaqi A et al., where Vicryl suture material was immersed in chlorhexidine gluconate solution and sodium hypochlorite solution for about five minutes, the results revealed that there was a decrease in the tensile strength of the material [34].

A key factor influencing a suture's tensile strength is its diameter. Therefore, it is crucial to maintain consistent suture size and exposure duration to ensure a fair and unbiased comparison across different studies. Due to the variations in the methodologies used in the aforementioned studies, it is reasonable to expect inconsistent results. In the present study, we included sutures of the same size to abolish the astonishing effect of diameter on tensile strength. Despite variations in the research approaches, the outcomes of Cawthorne DP et al., and Bruner SC et al., appear to be consistent with our findings [32,33]. It reveals that subjecting Vicryl suture material to a 0.9% sodium chloride exposure is linked to a decrease in tensile strength. Additional investigations involving human subjects are needed to clarify and confirm the relevance of previous findings for our everyday clinical practice.

In addition, with the SEM images, it was clear in the present study that HA-coated Vicryl 4-0 suture material exhibited appreciable surface morphology without any deterioration when compared to uncoated suture material. The uncoated suture material revealed a rough surface morphology along with breakage of the suture material, indicating the deterioration of the material. Therefore, it's important to consider the interaction between viruses or bacteria and the materials used to close wounds. Viruses and bacteria can potentially attach to or grow on suture materials, which may affect the healing process or lead to infections [35,36]. Choosing the right suture material can influence how effectively a wound heals and whether it becomes susceptible to infection.

Limitation(s)

The main limitation of present study is its in-vitro study design. Another limitation is the shorter duration of HA immersion, which could potentially influence the tensile strength. However, within the limitations, the present study demonstrated that HA coating in the suture thread provides an antimicrobial effect against *Pseudomonas aeruginosa* and *Streptococcus mutans*. Additionally, artificial saliva was used to simulate the oral conditions, which is of paramount importance because the oral cavity is a complex and dynamic environment characterised by a variety of factors such as pH fluctuations, enzymatic activity, and microbial presence. Replicating these conditions accurately is crucial for obtaining meaningful insights into how suture materials interact within the oral environment. Artificial saliva serves as a sophisticated tool designed to mimic the composition and properties of natural saliva. Therefore, the primary objective of immersing suture materials in artificial saliva is to create a controlled and standardised environment that mirrors the challenge faced by sutures in clinical scenarios. Also, by immersing the sutures in artificial saliva, biocompatibility of the material can be assessed, ensuring that they do not elicit inflammatory responses or tissue irritation, thereby enhancing patient-related outcomes and reducing the complications in clinical scenarios. This makes the present study unbiased and reliable.

CONCLUSION(S)

The HA-coated absorbable suture material does not exhibit superior tensile strength when compared to the uncoated suture material. However, the HA-coated suture material exhibited an antibacterial effect against *Pseudomonas aeruginosa* and *Streptococcus mutans*. In addition, SEM analysis showed improved surface characteristics in favour of the coated suture material.

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