



## Evaluation Of the Degradation, Microbial Colonization, Sustainability of Physical and Mechanical Properties of Lycopene Coated Suture Materials - An In-Vitro Study

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### ABSTRACT

**Background:** Lycopene has biological properties and is widely used in medicine these days. The purpose of this study was to compare the effects of lycopene, on the physical and mechanical properties of the suture material, which is commonly used in periodontal, oral and maxillofacial surgeries.

**Material and Method:** The lycopene gel was prepared and the suture material was dipped in it for 24 hrs. Tensile strength, wound healing assay were evaluated. The presence of lycopene was confirmed using SEM analysis.

**Result:** The lycopene coating was well adhered to the suture and results obtained by the wound healing assay demonstrated that the presence of lycopene promoted cell migration and proliferation in the wound area suggesting an active role of lycopene in the wound healing process. The tensile strength was stable after 24 hrs.

**Conclusion:** This research showed that the antioxidant coatings developed on silk sutures may offer important advantages in terms of prevention from surgical infections and wound healing process, and suggested a novel approach towards antioxidant biomaterials for clinical practice.

**Keywords:** *lycopene, suture material, wound healing, periodontal surgery, antioxidants*

### INTRODUCTION

Despite the wide variety of suture materials present, there are numerous scenarios in which specialised suture materials are employed for repairing tissue and aid in the healing of oral wounds. Because of the relevance of special oral

cavity characteristics like the presence of saliva, distinct biota, high vascularisation, mastication, and swallowing, clinicians must understand the nature of suture materials. "The goal of wound closure aims to aid in efficient healing and return to function

while also maintaining the surgical site's aesthetics."1 As a result, careful selection of both the suture material, along with the needle diameter & technique used, is critical. These variables allow the surgical flaps to be properly stabilised, resulting in patient comfort.

In addition to high tensile strength and low tissue reactivity, ideal features of a suture material include sterility, uniform thickness, flexibility for simple handling, and the ability to retain knot security, as well as low inflammatory response to promote healing. Suture materials are broadly classified according to the degradability into absorbable and nonabsorbable; according to their source into natural or synthetic, their coating into coated or uncoated, dyed or undyed; and last, according to their structure into monofilament or polyfilament2. Absorbable sutures such as catgut and polyglycolic acid (PGA) are mostly used in internal tissues; absorption is usually caused by the enzymatic degradation of natural sutures or by hydrolysis of synthetic materials, as opposed to non-absorbable sutures, like nylon and silk, which are preferably used for tissues that need stabilization for a longer periods and must be removed by the operator. Monofilament suture material is made of a single strand which provides less tissue resistance and less likely to harbour microorganisms than multifilament sutures. However, crushing of the suture can lead to undesirable and premature suture failure. As reported, silk is one of the most cost-effective suture materials currently used2,3. A dental surgeon needs to understand the physical and mechanical properties of commonly used suture materials. At present, no suture fulfills all the requisites of an ideal suture material. Most of the published data on the tensile behavior of sutures mainly focus on the tensile strength. Comprehensive reports on other vital properties of suture material such as percentage elongation, modulus and full stress-strain curves are quite limited in the field of periodontal and oral surgery4,5–21.

Lycopene, commonly delivered as a gel, shows acceptable kinetics in the mucosal surfaces of the human oral cavity4,22. This drug reaches a peak concentration within 15-36 hours and returns to baseline levels between approximately 80 and

104 hours. When compared to the placebo and the sham sites, lycopene-treated sites showed a significant reduction in PDs and attachment loss levels, but only during the 12-24 week period. Lycopene is known to exhibit an inhibitory effect on bacterial colonization and the results seem to suggest that lycopene can be used as an adjunct to SRP in the control of gingivitis23. The ability of gum tissue to withstand oxidative stress, retain normal tissue, and limit bacterial damage appears to be harmed when antioxidants are low. Krol's 24 investigation of total antioxidant status in peripheral and gingival serum associated with periodontal clinical status, revealing that total antioxidant status in venous blood serum was considerably lower in each category compared to controls. He came to the conclusion that oxidative stress in periodontitis, as evidenced by elevated levels of reactive oxygen species (ROS) and decreased antioxidant activity in gingival blood, may hasten the establishment of periodontal lesions. Waddington et al 25. discussed more evidence for a function for ROS in tissue damage in inflammatory periodontal disease. Hence, lycopene can help in wound healing as well as reduce the bacterial load during surgical procedures. It is of utmost importance to make sure the wound closure is perfect and it does not allow any bacterial contamination. However, the wicking effect of the braided sutures, commonly used silk suture is unavoidable. Hence, the purpose of this study was to compare the effects of lycopene, on the physical and mechanical properties of the suture material, which is commonly used in periodontal, oral and maxillofacial surgeries.

## MATERIALS AND METHODS

The present in-vitro experimental study used non-resorbable (silk). The suture materials used in the study were of uniform gauge (4-0). The type of suture material and their gauge were selected because of their frequent use in periodontal and oral surgery. Lycopene gel was used to evaluate the physical and mechanical properties of these suture materials. The experimental media used were as follows: Control group (G 1) = Plain Silk suture, Test group (G 2) = Silk suture Immersed in lycopene

gel. A total of 5 suture specimens were collected from sterile, unexpired and commercially available individual packs. All the suture specimens were cut to a uniform length of 14 cm. Each specimen in the control group was tested in the dry state for tensile strength (TS) before immersing it into the test media and was labeled as the control group. The rest of the specimens were kept in a lycopene gel. All the suture specimens were exposed to the test media for 24 hrs and returned for evaluating properties.

### ***Mechanical Testing Method***

Each sample was prepared with a acrylic knob holding the material in the form of a knot and this is placed around two metal poles installed in the Universal Testing Machine (INSTRON E 3000 UTM) at a crosshead speed of 10mm/unit - Instron Industrial Products, 900 Liberty Street, Grove City, PA 16127, USA) with a fixed distance of 15.0 mm between the two poles. The study was carried out in a heavy duty testing lab in saveetha dental college that is specialized in evaluating tensile strengths. Tensile strength (TS) was measured on a tensile meter in a unit of Newton (N) in the universal testing machine. TS is the force applied per unit original cross-sectional area, to a test specimen at any given time. Each sample was stretched until the material failed, and the maximum load was recorded in Newtons (N).

### ***Wound Healing Assay***

The 3T3 cell line was used for wound healing assay. The cells were seeded into the plate and incubated for 24 hrs. After incubation, the cells were observed for growth and assay proceeded. A sterile tip was used to create the wound. The sample to be tested was placed inside the well aseptically. Control was maintained with medium.

### ***Anti-Bacterial Property***

A loop of bacteria, E.coli and S. aureus, were inoculated in a freshly prepared nutrient broth and incubated for 24 h. The samples were sterilized under UV radiation for 30 min. The

bacterial culture was swapped using sterile cotton swabs on the surface of the freshly prepared agar plates. Then the sample was placed over the surface and incubated for 24 h.

## **RESULTS**

### ***Structural and chemical characterization of silver-treated sutures***

The distribution of lycopene deposited on the sutures was evaluated by Scanning Electron Microscopy (SEM). SEM analysis revealed the characteristic braided nature of the multifilament non-absorbable suture (Fig. 1) and the effective deposition of lycopene on the surface of the treated samples (Fig.2).

### ***Antibacterial properties***

Lycopene alone did not show any specific antibacterial activity<sup>26</sup>.

### ***Wound healing properties***

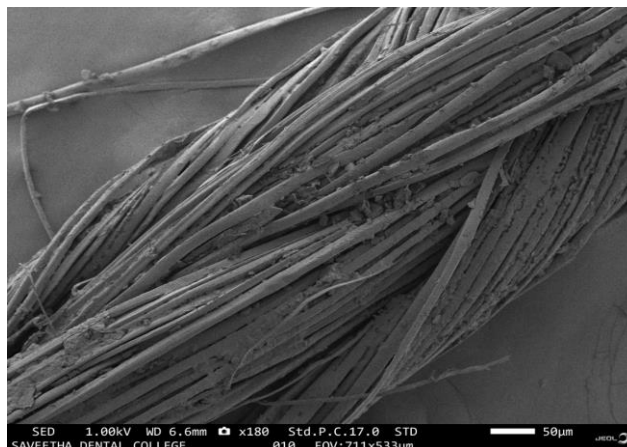
Suture degradation eluates have been tested for their ability to alter the rate of migration of 3T3 fibroblasts<sup>26</sup>. The effect of the lycopene coating on wound healing was assessed in vitro using the scratch assay at time  $t = 0$  as well as following one day of incubation. Light microscopy images of the scratched cell monolayer appear in Fig. 3, and varied diameters of the scratch were noticed when comparing the untreated and lycopene-treated samples. The percent of migration was additionally measured, where the stronger the ability to stimulate cell migration in samples that were treated at each point in time can be shown. Indeed, although lower than 0.5 mg/L, the presence of silver in the extracts produced an increased number of fibroblasts in the denuded surface compared to fibroblasts cultured in untreated eluates (Fig. 3). Comparing the results with the negative control (culture medium only), a significant difference was observed in the distance between the scratches.

### ***Tensile Strength***

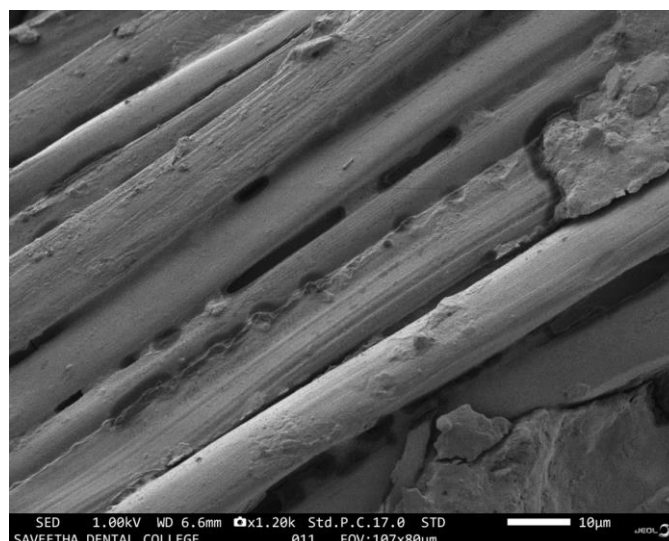
When analyzing the results (Table-1), it was found that in the lycopene group, the average

tensile strength was stable for the preoperative and postoperative times (baseline and 24 hrs). The highest force (7.13 N) was found on the next day. Therefore, there were no significant differences in tensile strength of the lycopene

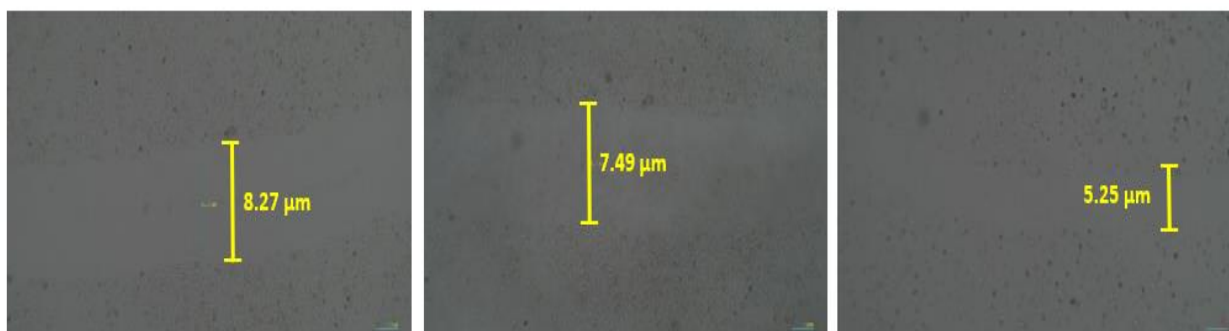
suture with respect to the different sutures. Therefore, it is shown that lycopene coated suture remains stable over time even after 24 hrs postoperative.



**FIGURE 1:** Scanning electron microscope image showing remains of lycopene on the suture.



**FIGURE 2:** Scanning electron microscope image showing remains of lycopene on the suture.



**FIGURE 3:** Wound healing appreciated by attracting cells towards the suture line at different time intervals- baseline and 24 hrs.



**TABLE 1:** Tensile strength of the AgNP coated suture material samples at 24 hrs

S.No	Maximum Force	Tensile Strength (%)
1 (BASELINE)	5.78	39.84
2	6.57	10.23
3	7.13	10.78
4	5.96	9.57
5	6.25	9.21
6	6.87	9.34

## DISCUSSION

Research on the synthesis of antioxidant materials has increased recently in order to provide a variety of uses, including catalysis, wound healing, and antibacterial action. A different technique for producing particles is known as "green synthesis," which uses elements from plants or natural chemicals. These methods prevent the toxicity of chemicals and are therefore environmentally friendly. Algae, bacteria, fungi, and plants were used to make NPs naturally, without the need of extra reducing agents or stabilisers. The bioactive compounds found in the plant extracts include ascorbic acid, carotenes, retinoic acid, and ascorbic acid among others that may be crucial to the process.

In addition to its many health advantages, lycopene is helpful for the management of a number of oral disorders, including as periodontitis, oral cancer, and precancerous lesions<sup>27</sup>. These attributes can be used to explain humans: 1. Antioxidant action; 2. Reduction in cancer cell development; 3. Reduction in growth factor stimulation. Enzyme induction during phase II, transcriptional control, and gap junction restoration round out the list. Lycopene is the most important carotenoid for quenching single oxygen and exerts antioxidant effects through physically and chemically quenching free radicals<sup>27,28</sup>. Lycopene's distinctive metabolic characteristics may make it feasible to shield certain cellular components from harm brought on by extremely reactive oxygen species<sup>29</sup>. As a result, the creation of lycopene gel may have significant medical significance in addition to being employed in a number of dental procedures. Due to their incredibly small size and large surface area, this gel can permeate tissues, making it potentially useful in drug-resistant

bacteria <sup>29,30</sup>. Because they are simple to implement, control, and interpret, cytotoxicity studies are typically carried out in vitro. These are the preliminary studies that mimic in vivo circumstances. Numerous qualitative as well as quantitative assays can be used to study cytotoxicity in vitro, but since they count the total number of viable cells<sup>31</sup>, quantitative assays are the most useful.

Despite the widespread use of antioxidant gels in the medical field, relatively few in vitro studies have been performed on healthy cells, most of which have been performed on tumor cells<sup>32</sup>. Different cell lines were used to evaluate cytotoxicity because diploid/primary cells, mainly oral fibroblasts, were considered to be more suitable biological tools for experiments. Compared with permanent cells<sup>32</sup>. However, the literature is sparse on cytotoxicity studies of lycopene gel in cells such as human fibroblast cells, which are considered common in vitro models for these studies for biocompatibility studies <sup>33</sup> and were therefore used in this study.

Numerous physiological processes, including tissue regeneration as well as wound healing, depend on cell migration<sup>34</sup>. Whenever a cell monolayer gets damaged, it reacts by boosting the amount of growth factors as well as cytokines at the wound edge, which starts the proliferation and migration of several cell types, like keratinocytes and fibroblasts<sup>35</sup>. The scratch assay mimics the second stage of wound healing, which is marked by keratinocyte or fibroblast migration and proliferation <sup>36,37</sup>. In this study, the indirect method of the scratch assay was used to examine any potential interactions between the silver coating and the wound-healing process. For this, a scratch was made on the fibroblast monolayer, and light microscopy was used to

monitor and quantify cell migration towards the wound site (Fig. 3). Interestingly, the findings showed that the extracts increased the total amount of the fibroblasts in the region that was scratched by improving cell migration and proliferation, which improved their ability to seal the wound 38.

### CONCLUSION

The use of different modified sutures is an essential method for wound infection treatment. Lycopene-coated sutures were developed in this study. The silver coating developed was distinguished by a uniform cluster distribution and a robust adherence to the substrate. The scratch assay results demonstrated how the presence of lycopene increased proliferation and migration of cells in the wound area, implying that lycopene plays an active role in the process of wound healing. This study found that antioxidant coatings produced on silk sutures may offer significant benefits in terms of surgical infection prevention and wound healing, and it proposed a unique method to antioxidant biomaterials for usage in dentistry.

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