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Effect of Natural Antimicrobial Agents on the Characteristics of Surgical Sutures

Doğal Antimikrobiyal Maddelerin Cerrahi İpliklerin Özelliklerine Etkisi

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Abstract

Surgical site infections (SSI) occur after the surgery in body parts where the operation took place. In surgeries, wounds are closed by thread-like materials known as sutures. Some types of sutures may promote bacteria proliferation which is one of the leading causes of the SSI. Sutures undergo coating procedure to prevent infection occurrence. In this study, different types of surgical sutures were coated with natural antimicrobial agents to evaluate their effect on morphological and mechanical properties of the surgical sutures. In this context, due to its antimicrobial ability, chitosan was selected and dissolved in acetic acid solution with other natural antimicrobial agents (aloe vera and olive leaf extract) through ultrasound technology. Multifilament silk, multifilament polyester, and monofilament polyamide sutures were then dipped into those solutions prepared at different concentrations in order to study the synergistic effect of antimicrobial agents. Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) was performed to identify the functional groups on the surface of the coated sutures. Suture surfaces were also analyzed by scanning electron microscope (SEM) to observe the coating on the surface of sutures. Strong adhesion was determined between the suture surface and the coating material after long duration of dipping and drying procedure. It was also found that the coating process increased the mechanical properties of the sutures.

Keywords: *Biopolymer, Chitosan, Aloe Vera, Olive Leaf Extract, Surgical Site Infections, Mechanical Properties*

Öz

Cerrahi girişim uygulanan vücut bölgelerinde, ameliyat sonrası cerrahi alan enfeksiyonları (CAE) ortaya çıkmaktadır. Cerrahide yaralar cerrahi iplik olarak bilinen materyallerle kapatılmaktadır. Bazı cerrahi iplik türleri, CAE'ye sebep olan bakterilerin çoğalmasına neden olmaktadır. Cerrahi iplik kaynaklı enfeksiyonun önüne geçmek için antimikrobiyallerle kaplama yapılmaktadır. Bu çalışmada, CAE'nin oluşmaması için farklı tipteki cerrahi iplikler doğal antimikrobiyal maddelerle kaplanmıştır ve bu ajanların cerrahi ipliklerin morfolojik ve mekanik özelliklerine olan etkisi değerlendirilmiştir. Bu bağlamda, doğal bir polimer olan kitosan antimikrobiyal özelliğe sahip olması sebebiyle seçilmiştir ve kitosan yine doğal antimikrobiyal madde olan aloe vera ve zeytin yaprağı ekstresi ile beraber asetik asit çözeltisi içerisinde ultrason teknolojisi kullanılarak karıştırılıp kaplama solüsyonu hazırlanmıştır. Doğal maddelerin sinerjistik etkisini incelemek için farklı konsantrasyonlarda hazırlanan kaplama solüsyonlarına multifilament ipek, multifilament poliester ve monofilament poliamid cerrahi iplikler daldırılarak kaplama yapılmıştır. Fourier dönüşümlü kızılötesi spektroskopisi kullanılarak kaplanmış cerrahi ipliklerin üzerindeki fonksiyonel gruplar belirlenmiştir. Cerrahi ipliklerin yüzeyleri taramalı elektron mikroskobuyla incelenerek uzun daldırma ve kurutma işlemleri sonrası kaplama malzemesinin yüzeye güçlü bir şekilde tutunduğu gözlemlenmiştir. Ayrıca yapılan mekanik testler sonucunda kaplama işleminin cerrahi ipliklerin mekanik özelliklerini arttırdığı tespit edilmiştir.

Anahtar Kelimeler: *Biyopolimer, Kitosan, Aloe Vera, Zeytin Yaprağı Ekstresi, Cerrahi Alan Enfeksiyonları, Mekanik Özellikler*

1. Introduction

Sutures are medical devices used to hold the edges of a wound together after an injury or a surgery [1]. They are classified based on their origin (natural or synthetic), absorbability, and configuration (monofilament or multifilament) [1-3]. They act like an artificial and temporary support until the natural fiber of the tissue is generated [4]. The aims of the wound closure contain elimination of the dead space, maintenance of the tensile strength around the wound until the tissue strength is adequate, and even distribution of the tension along deep suture lines [5]. Since the suture material is exposed to different factors such as knot tension, tissue reaction, tensile strength, knot security, static and dynamic fatigue, and friction, it is important to choose the right material for suturing [6]. Ideal suture material should be bioinert, biocompatible, and have easy handling characteristics for the surgeon [7-11]. However, suture material can provide microbial colonization on the wound area, especially multifilament sutures allow bacteria to hide [12-16]. Thus the same suture material in multifilament form causes higher wound infection risk than its monofilament form. Among suture materials, silk has a higher risk of infection due to its protein nature, and causes proliferation of the bacteria, leading to biofilm formation which increases the surgical site infection (SSI) risk [17,18]. Coating the suture surface with antimicrobials that inhibit bacteria formation and uncompromising the mechanical properties is one treatment to prevent suture associated SSI. Majority of the research was focused on metals such as silver and chemicals such as triclosan because of the low toxicity [19-22]. But their excessive usage caused bacteria to develop resistance and it was found that triclosan has safety issues such as affecting immune functions and cell reproducibility negatively [23-25]. For this reason, many researches are focused on new antibacterial products to develop antimicrobial sutures. Recently, naturally occuring materials have become popular due to their biocompatibility and antimicrobial properties. Among these materials, chitosan has gained attention for not only its antimicrobial activity but also wound healing properties [26-36]. Besides, it is hard to develop bacterial resistance, since the antimicrobial activity of chitosan destroys the cell membrane of the bacteria through electrostatic interaction [26]. Along with chitosan, olive leaf extract [37-41] and aloe vera [42-44] possess antibacterial properties as well as antifungal and antiviral activities.

In the current work, the effect of chitosan, olive leaf extract, and aloe vera on the development of antimicrobial sutures was studied. Since the antimicrobial effects of these agents are already proven [45-53], we have focused on improving the mechanical properties of the sutures. Biocompatible, biodegradable, and non-toxic compounds have been used as coating agents on non-absorbable monofilament polyamide, multifilament polyester, and multifilament silk sutures. Functional groups of the coating agents on the surface of the sutures have been investigated. Mechanical and morphological properties of the sutures have been examined.

2. Material and Method

2.1. Materials

Multifilament braided silk sutures with USP (United States Pharmacopeia) 3, monofilament polyamide (nylon 6,6) with USP 2, and multifilament braided polyester with the USP size of 5 were obtained from Doğsan, Turkey. Chitosan (M_w = 300,000 Da) with a deacetylation degree of approximately 90-95% was purchased from Adaga, Turkey. Olive leaf extract was obtained from Zeytinistasyonu, Turkey. Glacial acetic acid (ACS reagent, ≥99.8%) was purchased from Sigma-Aldrich Chemical Company. The Aloe vera gel was extracted from the leaves of the Aloe vera plant and was used as extracted without further treatment.

2.2. Preparation of antimicrobial coating solution

The coating solution was prepared by dissolving 0.5 wt% chitosan powder into 1 wt% acetic acid aqueous solution. The solution was stirred in a magnetic stirrer for 15 minutes by gradually adding chitosan. In order to obtain a homogeneous solution, an ultrasonicator (UP400S, Hielscher Ultrasonics GmbH, Teltow, Germany) at a setting of cycle 0.5 and amplitude 50% was used to disperse the solution for extra 20 min. The other coating formulations including 0.2 wt% aloe vera and 0.2 wt% olive leaf extract were also prepared with the same procedure. The concentrations were determined by considering already published studies which demonstrate the antimicrobial effect of chitosan at low concentrations [54,55,56]. Non-absorbable sutures were coated by dip coating method, being immersed into the solutions for 48 h. Sutures were dried inside an oven at 45 °C for two days, and later kept at room temperature for 7 days before testing. Coating formulations were prepared according to Table 1.

2.3. Suture surface investigation by FTIR-ATR

Fourier transform infrared spectroscopy with Attenuated Total Reflectance (FTIR-ATR) was performed with 16 scans in 4000-400 cm^{-1} on a Thermo Scientific Nicolet iS500 with a diamond crystal UATR. It was used to identify the functional groups on the surface of the coated sutures by examining the peaks of specific kinds of bonds to see if a functional group of coating compound is present or disappear after the treatment.

2.4. Morphological analysis

Scanning Electron Microscope (SEM) images were taken for having the knowledge about surface topography of the sutures. The SEM used is a Carl Zeiss 300VP model and works with Gemini Optic Technology. Before SEM analysis, the samples were sputter coated with gold by using QUORUM Q150 RES coating device.

2.5. Tensile tests

A computer controlled Shimadzu Autograph AG-IC mechanical testing device with 10 kN load cell was used to conduct tensile testing of the specimens for all the formulations including control groups. For sutures, straight-pull and knot-pull strength tests were performed. During the test, each suture material (USP sizes; Polyamide 2, Silk 3, Polyester 5) was placed in the grips of the testing machine, carefully ensuring that the grips were tightened evenly and firmly to prevent the slippage of the specimen while testing. During straight-pull measurements, extension rate of the machine was set at the rate of 100 mm/min and gauge length was 50 mm for each suture type. And for knot-pull tests, gauge length was 180 mm while the extension rate was 300 mm/min. For each coating formulations, including uncoated sutures, a total of twenty sutures (N = 20) were used, ten sutures for straight-pull tests (N = 10) and ten sutures for knot-pull tests (N = 10) were tested for each formulation to obtain mean values. These parameters were determined according to United States Pharmacopeia standards for non-absorbable sutures [57]. The specimens were pulled at the constant rate of extension. In knot-pull strength, we formed the surgeon's knot illustrated in Figure 1, around a flexible rubber tubing (ID = 6.5 mm, Thickness = 1.6 mm) by using surgeon's knot method. Later, knot efficiency was calculated using the equation

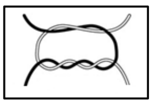

Figure 1. Illustration of the surgeon's knot

which is the ratio of the tensile strength of knotted suture to unknotted suture [58].

Table 1. Coating formulations of PA, PES, and Silk sutures.

Suture Type	Suture Material	Suture Code	Coating Formulation*		
			Chitosan (wt%)	*Aloe Vera (wt%)*	*Olive Leaf Extract (wt%)*
Monofilament & Non-absorbable	Polyamide (PA)	PA-C1	0.5	_	_
		PA-C2	0.5	0.2	_
		PA-C3	0.5	_	0.2
		PA-C4	0.5	0.1	0.1
		UC-PA		*uncoated*	
Multifilament & Non-absorbable	Polyester (PES)	PES-C1	0.5	_	_
		PES-C2	0.5	0.2	_
		PES-C3	0.5	_	0.2
		PES-C4	0.5	0.1	0.1
		UC-PES		*uncoated*	
Multifilament & Non-absorbable	Silk (S)	S-C1	0.5	_	_
		S-C2	0.5	0.2	_
		S-C3	0.5	_	0.2
		S-C4	0.5	0.1	0.1
		UC-S		*uncoated*	

*Chemicals were dissolved in 1 wt% acetic acid solution.

3. Results

3.1. FTIR-ATR analysis

The FTIR spectrum of chitosan in Figure 2(a) confirms the presence of the functional groups in the structure of the chitosan polymer. The peak in the range 3400-3500 cm^{-1} is attributed to N-H stretching vibrations of the amine group in the chitosan where the bending vibrations can be seen at 1647 cm^{-1}. The C-N stretching vibrations are assigned to two bands at 1027 cm^{-1} and 1150 cm^{-1}. The absorption at 1423 cm^{-1} can be attributed to bending vibrations of the -OH groups. In the polymer, C=O stretching vibration is observed at 1647 cm^{-1} [59]. The broad absorption peak in the range 3300-3600 cm^{-1} is attributed to O-H stretching in the aloe vera plant. The significant band occurring at 1644 cm^{-1} is attributed to the C=O stretching vibrations. The O-H stretching vibration is observed at 3282

cm^{-1} and the peak at 1649 cm^{-1} is assigned to C=O stretching vibrations, those are groups possibly of oleuropein, elenoic acid, and/or ligrostride found in it.

The strong band at 1037 cm^{-1} could be attributed to the C-OH vibrations of the protein in the olive leaf.

The uncoated silk has a broad amide band which is observed at 3278 cm^{-1} and 2963 cm^{-1}. Also amide I (C=O stretching vibrations) and amide II (N-H bending vibrations) peaks can be seen at 1620 cm^{-1} and 1515 cm^{-1}, respectively in Figure 2(b).

In Figure 2(c), the stretching vibration frequency of N-H groups in polyamide is observed at 3296 cm^{-1} whereas the bending vibrations of the N-H groups can be seen at 1537 cm^{-1}. The stretching vibrations at 1639 cm^{-1} is attributed to C=O functional groups. The peaks of C-H in polyamide are observed at 2932 cm^{-1} and 2859 cm^{-1}.

The strong absorption peak at 1713cm^{-1} corresponds to ester groups shown in Figure 2(d). The peaks at 1241 cm^{-1} and 1338 cm^{-1} is attributed to C-O stretching vibrations in esters. While the stretching vibrations of C-C aromatic ring are determined in the region of 1400 cm^{-1} and 1500 cm^{-1}, the C-H stretching vibrations are detected at 2963 cm^{-1}.

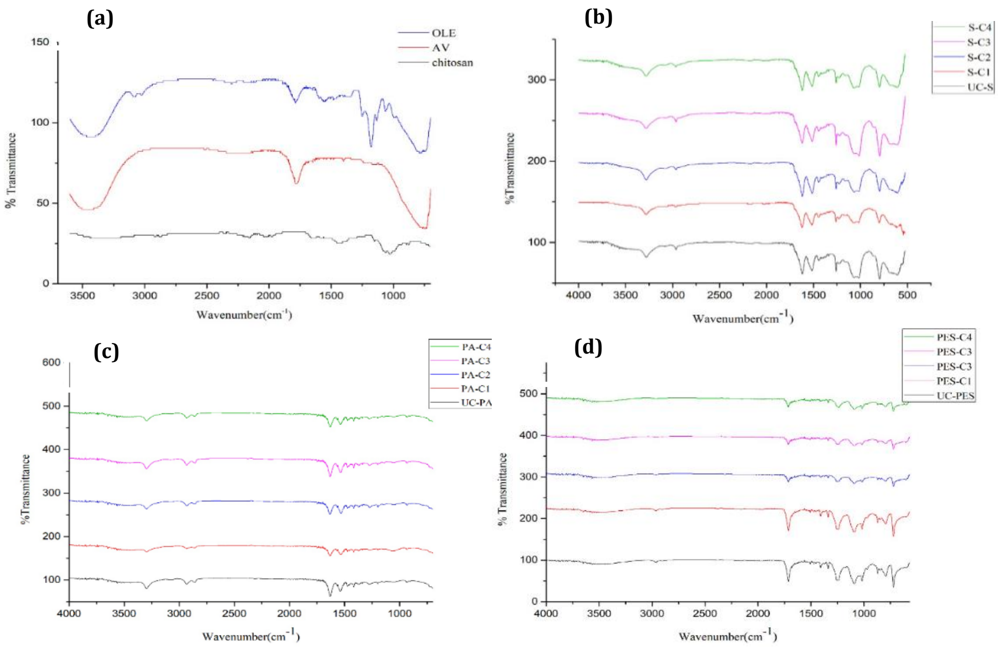

Figure 2. (a) FTIR spectra of antimicrobial agents (b) FTIR spectra of the silk suture coating formulations (c) FTIR spectra of the polyamide suture coating formulations (d) FTIR spectra of the polyester suture coating formulations

3.2. Morphological analysis

Scanning electron microscopy (SEM) was employed to image the surface of both coated and uncoated sutures. SEM images of the uncoated silk (UC-S), and C4 coated silk suture (S-C4) are presented with different magnifications of 100x, 250x, 500x, and 1000x in Figure 3.

3.3. Mechanical properties of coated sutures

Tensile properties of surgical sutures are important for the right selection of the sutures because, if the knotting force of the material is greater than its

tensile strength, suture may easily break apart while performing the knot [60]. Therefore, it is crucial to know the mechanical properties of the coated sutures. Figures 5, 6, and 7 show the straight-pull (tensile) strength values of the coated silk, polyester, and polyamide sutures, respectively, comparing with their uncoated (UC) counterparts.

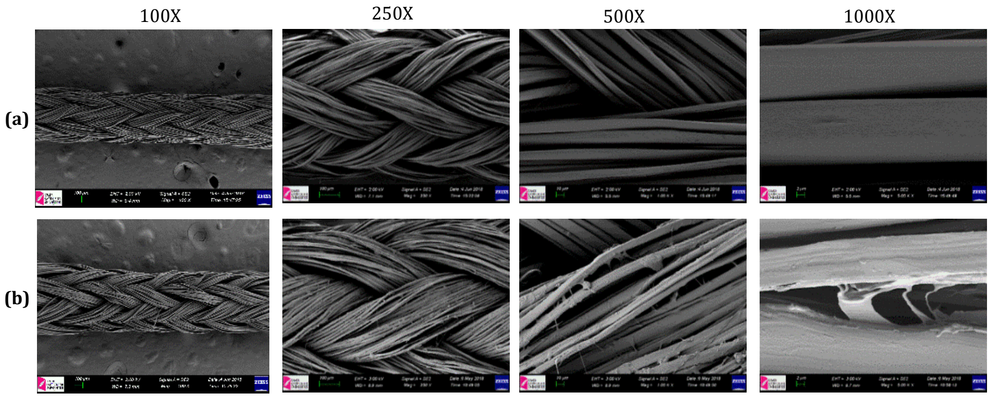

Figure 3. SEM Micrographs of (a) uncoated silk, (b) coated (S-C4) silk at 100x, 250x, 500x, and 1000x magnifications

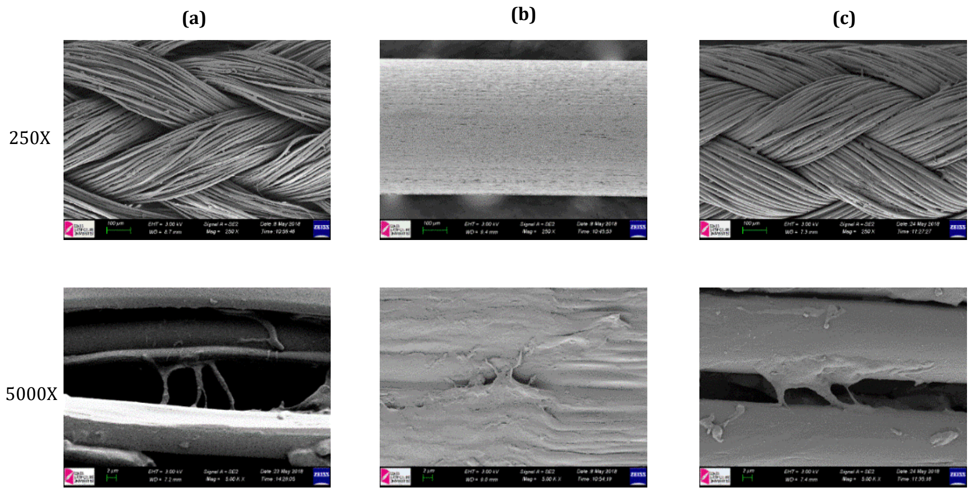

Figure 4. SEM Micrographs of chitosan coated (a) silk, (b) polyamide, (c) polyester sutures at 250x and 5000x magnifications

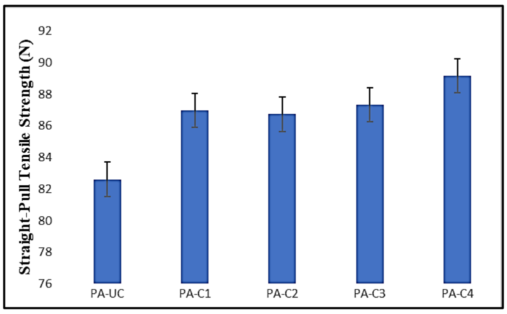

Figure 5. Straight-pull tensile strength values for non-absorbable polyamide sutures with respect to different coating formulations (C1, C2, C3, and C4; UC: Uncoated)

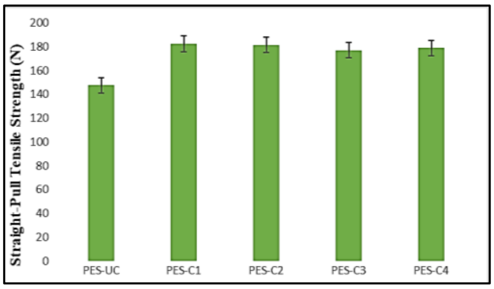

Figure 6. Straight-pull tensile strength values for non-absorbable polyester sutures with respect to different coating formulations (C1, C2, C3, and C4; UC: Uncoated)

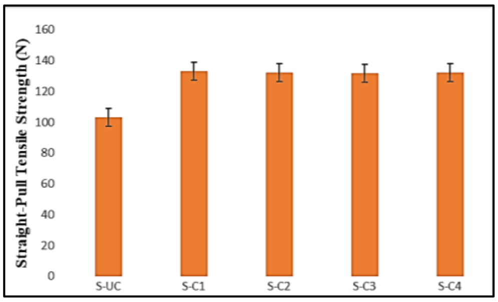

Figure 7. Straight-pull tensile strength values for non-absorbable silk sutures with respect to different coating formulations (C1, C2, C3, and C4; UC: Uncoated)

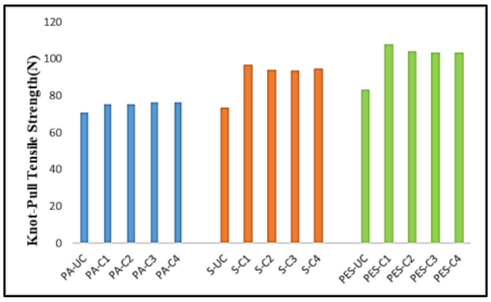

Figure 8. Knot-pull tensile strength values for polyamide, silk, and polyester sutures with respect to different coating formulations (C1, C2, C3, and C4; UC:Uncoated)

Figure 8 indicates that knotting does not have the same effect among all suture types. In surgeon's knot strength, there was a significant increase in especially PES and Silk sutures compared with their uncoated formulations.

4. Discussion and Conclusion

In this paper, the naturally occurring agents chitosan, olive leaf extract, and aloe vera were demonstrated to be able to increase the mechanical properties of the sutures.

The results indicate that there was a slight increase in the tensile strength of the coated sutures. While the highest straight-pull tensile strength was revealed at polyester sutures (182.4 N) coated with C1 formulation, the lowest value obtained (103.63 N) was for uncoated silk sutures. Chitosan increased the tensile strength, while the addition of aloe vera and olive leaf extract did not have a significant effect on straight-pull tensile strength. As for knot-pull tensile test, data show an increase in the suture knotting strength. The increase might be due to coating formulation which could cause lowering the surface friction of the coated sutures. Moreover, we can conclude that the weakest part of the suture is knot which is also proven by other studies [61, 62], as the knot-pull strength of all types of sutures are lower than the straight-pull strength for both uncoated and coated sutures, which means the knot itself provides high stress area that causes reduction in the tensile strength of the sutures.

The slight increase in the suture tensile strength was also reported by Masood et al. [58] they coated the polyethylene terephthalate and polyamide sutures with chitosan, turmeric, and clove oil. Another study performed the mechanical tests for the polyamide sutures [59] coated with chitosan-citric acid biopolymer; however we could not compare our results with the previous findings, due to the unknown USP sizes of the sutures in the previous studies.

As for morphological analysis, a strong adhesion was observed between coating and suture surface along the length of the sutures. SEM images of C1 and C4 formulations prove that the surface of the sutures coated well and strong adhesion between suture surface and coating solution can be observed which is an indication of the efficacy of the coating formulation and coating technique. Figure 4 indicates the adhesion between coating and all types of sutures provided by the long duration of the dipping and drying procedure to minimize the occurrence of detachment of the coating layers.

Our results are in good agreement with previous findings by Yang et al. [54] who coated the absorbable sutures by dip coating method with hydroxypropyltrimethyl ammonium chloride chitosan (HACC) for duration of 24 h. The same type of surface morphology of sutures was also observed by Li et al. [63] in their study of which Vicryl Plus sutures were coated with an amphiphilic polymer which is poly[(aminoethyl methacrylate)-co-(butyl methacrylate)]. The roughness of the surface of sutures was observed, validating an effective coating process.

As a conclusion, based on our findings, the research conducted into development of antimicrobial surgical suture with improving mechanical properties has been successful. Since the antimicrobial activity has not yet been tested, further work will be carried out to validate the antimicrobial properties of the sutures.

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