

# Fabrication of Tannic Acid coated Polyglactin Surgical Suture to Prevent Bacterial Infection

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# 1 Fabrication of Tannic Acid coated Polyglactin Surgical Suture to Prevent Bacterial Infection

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## ABSTRACT

Polyglactin surgical (PLGA) suture is a biodegradable suture frequently used in clinical surgery. Due to its braided structure, polyglactin suture is easier contaminated by bacterial during surgery process. Thus, this study was coated by tannic acid (TA) to prevent Staphylococcus aureus binding. Suture surface modification was carried out by immersing PLGA in tannic acid-ferri chloride (TA-Fe) solution pH 8. The fabrication process was confirmed by FTIR analysis, and the antibacterial preventing activity was determined by bioinformatics analysis. Thus, this engineered suture increased the wettability in DI water, serum, and saline solutions, which could be promising to increase the biocompatibility in wound healing process. To sum up, our method would be applied in surgical suture to prevent bacterial infection and potentially elevate the biocompatibility by increasing wettability in biological solutions.

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## INTRODUCTION

Sutures play a critical role in surgeries and trauma management with the primary function to hold opposing tissue together thus facilitating the wound healing process with no or minimal scar formation (10-nnis *et al.*, 2016). There are various types of sutures, in general, sutures are categorized according to the type of materials natural or synthetic, the lifetime of sutures in the body (absorbable and non-absorbable), and the structure of the suture (monofilament, braided, twisted) (Pillai & Sharma, 2010). The absorbable suture unrequired any revision surgery to remove the suture since the suture is absorbed into the body made this type of suture more likely to use (Baghel, *et al.*, 2015). The use of absorbable sutures gives some advantages such as minimizing clinical time and patient anxiety toward post-operative procedures and also giving a better aesthetic appearance rather than non-absorbable sutures (Dixit *et al.*, 2018).

One of the emerging problems during surgery is surgical site infections (SSI). They are a common type of nosocomial infection and responsible to higher medical costs due to increased risk of intensive care unit admission, hospital readmissions after surgery procedures, and delayed

adjuvant systemic therapy (De Simone *et al.*, 2020). The material and types of sutures used in surgical procedures contribute to the occurrence of SSI (Dixit *et al.*, 2018). The monofilament suture is made of single strand materials therefore it served a small microbial contact area and reduce the possibility of bacterial growth (Xu *et al.*, 2022). Thus, however; braided suture consists of multiple strands braided thus providing a larger and more complex surface for bacterial adherence rather than monofilament sutures resulting higher risk of contamination in surgical sites (Mahesh *et al.*, 2019). Although braided sutures derived a higher risk of SSI, the advantages of braided sutures such as provided better knots than the microfilaments suture which required fewer knots during the stitching process, have good operability, flexibility, and stronger than microfilament sutures made braided sutures is commonly used in surgery (Debbabi & Abdessalem, 2015). Therefore, the SSI risk from the braided suture application should be solved to augment its application in clinical practice.

There are enormous studies to prevent SSI during surgery, such as skin cleansing procedures, administration of pre-operative antimicrobial prophylaxis, instrument sterility, etc. (De Simone *et al.*, 2020). Administration of antibiotics as prophylaxis could lead to prolonged and irrational use of antibiotics, thus antibacterial sutures could be an alternative to prevent SSI (De Simone *et al.*, 2020; Dixit *et al.*, 2018). Sutures coating with antibiotics is one of the techniques which could reduce the possibility of bacterial colonization in braided sutures (Dhom *et al.*, 2017). Instead of antibiotics, Shin and colleagues coated fibronectin on polydioxanone suture to prevent bacterial binding and improve tissue healing (Setiawati *et al.*, 2021). Since protein need to be kept in cold storage, the handling need high cost. Therefore, the simple and low-cost method is urgently needed.

In this study, we coated polyglactin suture, one of high demand absorbable braided suture, with tannic acid molecule under basic condition. Tannic acid (TA), a natural polyphenolic compound used in biomaterials, has been reported as antibacterial agent as well as antiviral molecule (Kaczmarek, 2020; Akiyama *et al.*, 2001; Sathiskumar *et al.*, 2022). TA is commonly extracted from grape, tea extract, and many different plants (Sathiskumar *et al.*, 2022). Our study offered simple, low cost, and versatile method to bind tannic acid to the polyglactic suture by immersing it in Ferri ion solution. Additionally, based on bioinformatics analysis tannic acid revealed strong interaction to the proteins and genes of *Staphylococcus aureus* (*S.aureus*), a major bacterium causing SSI in hospital (Pal *et al.*, 2019). Since those gene related to *S.aureus* survival and pathogenicity during the infection stage in human, we proposed that our engineered suture prevented the bacterial infection. Additionally, our engineered suture increased the surface wettability in biological solution proposing its potential to emphasize of the biological activity.

## RESEARCH METHOD

### Preparation of TA coated polyglactin suture

The suture was cut in 2-cm length then kept in sealed plastic at -20 °C. TA-Fe<sup>3+</sup> solutions were prepared by mixing TA and FeCl<sub>3</sub>.6H<sub>2</sub>O (0.1 mg/ml) in deionized water to make solutions with TA concentration of 10 mg/ml and 30 mg/ml. Then, pH value of solutions was adjusted to 8 with NaOH solution (1.0 M). Polyglactin sutures were submerged in 1.5 mL TA-Fe<sup>3+</sup> solution and incubated in room temperature for 24 hours. TA coated polyglactin sutures were rinsed in deionized water once and dried in room temperature. (Lv *et al.*, 2020; Wang *et al.*, 2017)

### FTIR confirmation of coated suture

Dried fabricated suture, after which measured total- reflectance. Fourier-transform infrared (ATR-FTIR), 64 scans accumulation in the wavenumber range 600-4000 cm<sup>-1</sup>. The successfully fabricated suture was shown by OH peak in the wavenumber range 3300-3600 cm<sup>-1</sup>.

### Bioinformatics Analysis of Tannic Acid to *Staphylococcus aureus* protein

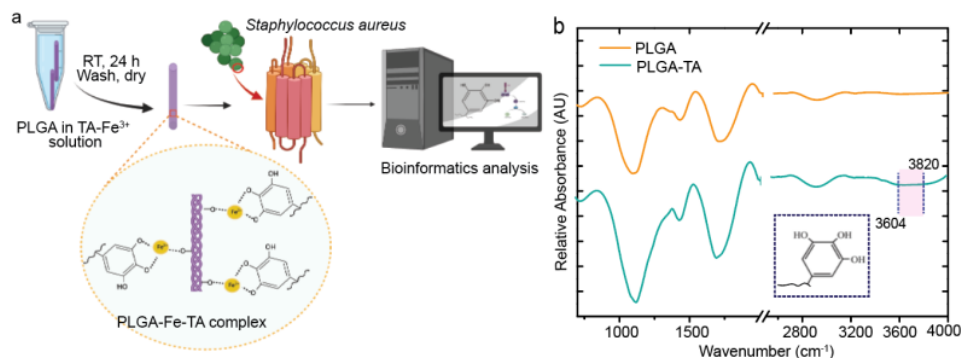
Potential target proteins (PTP) were obtained from NCBI (<https://www.ncbi.nlm.nih.gov/>). Proteins that interacted with tannic acid (PTA) were obtained from STITCH (<https://stitch.embl.de>). PTP and PTA were analyzed using Venn diagram to get intercept as potential tannic acid target proteins. PPI network was constructed by using STRING-DB v11.0 with medium confidence score ( $>0.4$ ) and Cytoscape. Top 4 proteins were determined by using Maximal Clique Centrality (MCC) method from CytoHubba plugin with default setting.

### Surface Wettability

The contact angle measurement was evaluated by imaging the droplets which is recorded in the flat surface of the suture using camera. The contact angle was analyzed using Drop analysis-LB-ADSA plugin at ImageJ software.

## RESULTS AND DISCUSSIONS

Polyglactic acid (PLGA) is one the most common absorbable sutures used in clinics. This polymer formed of lactide and glycolide which is covered by lubricant and has braided structure. PLGA, such as Vicryl, retains tensile strength of 65% at 2 weeks and 40% at 3 weeks while its complete absorption reaches in 90 days (Tajirian and Goldberg, 2010). Since its braided structure, it has higher risk to be contaminated with bacteria. Therefore, many study engineered PLGA suture by antibacterial coating such as triclosan, a broad spectrum antibacterial (Marzo *et al.* 2008). Yet, however, there is no data it effectively against *Staphylococcus aureus*, the gram-positive bacteria in surgical site infection (SSI).



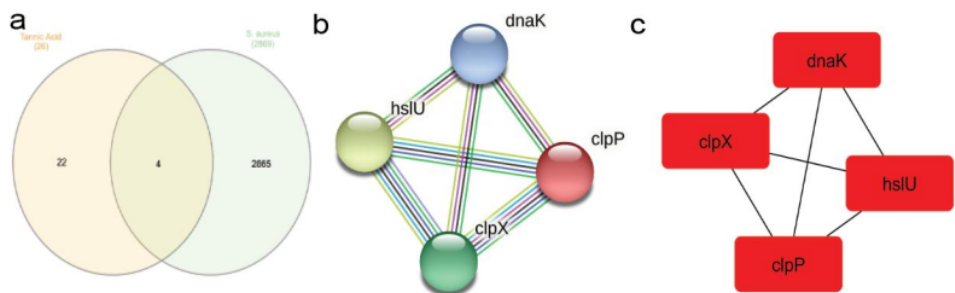
**Figure 1.** Fabrication of TA coated PLGA Surgical Suture. a. Scheme of TA-PLGA Fabrication Process, b. FTIR Spectra of coated PLGA suture

This study offered a simple, low-cost, and effective antibacterial coating method to PLGA suture by immersing tannic acid in ferri solution (TA-Fe). Tannic acid, polyphenolic compound, had revealed antibacterial activity in several previous study (Kaczmarek, 2020; Akiyama *et al.*, 2001; Sathiskumar *et al.*, 2022). The scheme of this study was presented in Figure 1a, after immersing in TA-Fe solution, suture then washed and be investigated its antibacterial property against *S. aureus*. Our method successfully cross-linked tannic acid to PLGA suture confirmed by FTIR spectra of hydroxyl (OH) group of TA at broad peak 3604-3820  $\text{cm}^{-1}$ . The OH phenolic could be appeared 3440 to 3320  $\text{cm}^{-1}$ , thus it was the peak of O-H stretching vibration (Tinti *et al.*, 2015). Owing many hydroxyl moieties as well as catechol and pyrogallol groups, TA had been known an antibacterial

natural compound (Wang *et al.*, 2017). Due to its excessive phenolic compound, it might be engineered for biomaterial surface modification.

| Tabel 1. Tannic acid target genes and their activity |      |   |   |  |
|--|------|---|---|--|
| No   | Code | Protein Name                                    | Description   | Reference  |
| 1  | clpP | Caseinolytic Protease (Clp) Proteolytic Subunit | Protein degradation misfolded and defective proteins  | Moreno-cinos <i>et al.</i> 2019                              |
| 2  | hslU | AAA+ATPase component of heat shock protein      | Unfolds protein target of the heat shock protein complex  | Jeong <i>et al.</i> 2020<br>Bochtler <i>et al.</i> , 2000    |
| 3  | dnaK | Chaperone protein DnaK                          | Increase bacterial tolerance to heat, oxidative and antibiotic stresses, carotenoid production, and increase its survival in a murine host. | Singh <i>et al.</i> , 2012                                   |
| 4  | clpK | Caseinolytic Protease (Clp) ATPase              | Heat tolerance/ thermal survival of the bacteria  | Bojer <i>et al.</i> , 2010<br>Motiwala <i>et al.</i> , 2021. |

To investigate the antibacterial activity of *S.aureus*, bacterial-caused SSI, we analyzed the *S.aureus* NCT 8325 protein interacted with tannic acid. There are four main proteins as the targets of tannic acid in *S. aureus* (Figure 2a), protein-protein interaction networks revealed that all the proteins (clpP, hskU, dnaK, and clpX) interact each other's (Figure 2b), thus all their genes have strong interactions to the tannic acid based on MCC analysis (Figure 2c). Based on Table I, overall proteins play important roles for cell survival especially thermal heat tolerance which crucial during bacterial infection stage. Thus, this study showed the potential to minimize SSI by applying our engineered suture to uphold the wounded tissue.



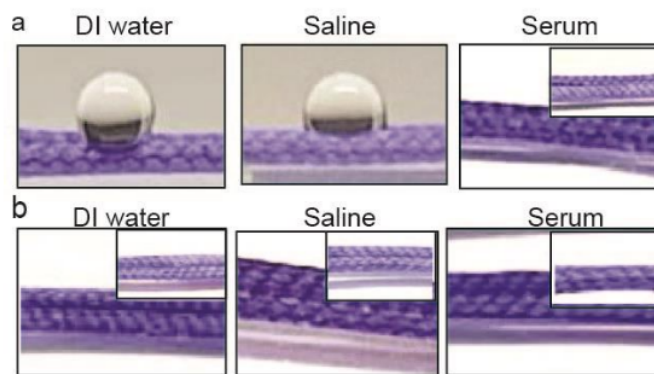
**Figure 2.** Bioinformatics Analysis of *Staphylococcus aureus* protein interacting with tannic acid a. Venn Diagram showing interacting protein of *S.aureus* to tannic acid. b. Protein-protein interaction network of tannic acid and the interacting proteins. Top 4 rank of *S.aureus* gen interacting to tannic acid based on MCC (Maximal Clique Centrality).

This study also investigated another important parameter of biomaterial, surface wetting. Surface wetting is the process of biomaterial surface to be interacting with water which is mostly water and biological fluid (Menzies and Jones, 2010, Huhtamäki *et al.*, 2018). The wettability of biomaterials *in vitro* is measured by calculating the contact angle (CA) at the liquid-PLGA interface. We employed water, serum, and 18% NaCl solution for representing human biological fluid (Setiawati *et al.*, 2021). The lower contact angle values represented the tendency of the water adherence to the surface. In some previous studies, the lower CA values indicated the more biocompatible biomaterials thus also better wound healing activity (Zhou *et al.*, 2020; Setiawati *et al.*, 2021). Our finding revealed that overall, our engineered suture dropped the contact angle value indicating by solution spreading on the PLGA surface (Figure 3a). The contact angle of water, and 0.9% NaCl solution in pristine suture were  $89.76 \pm 2.37$ , and  $92.47 \pm 2.49$ ; however, we could not be



determined the contact angle in serum due to it was quickly absorbed into the suture (Figure 3a). After coated with TA, the suture surface was absorbed all the solution. Then, the wettability degree of our modified sutures was better than control, especially in term of water and 0.9% NaCl solution. (Figure 3b).

To sum up, our study proposed simple, fast, and low-cost method to fabricate antibacterial surgical suture by cross-linking to the polyphenolic compound. Since, it increased the wettability against biological solution, we strongly believed our engineered suture is potential to be further investigated for *in vitro* and *in vivo* wound healing study. To support this hypothesis, the following study to investigate biocompatibility of the engineered suture is remarkably needed.



**Figure 3.** Surface wettability of Engineered Suture in Biological Solution. a. Images of pristine suture dropped with DI water, saline and serum b. Images of the engineered suture in water, saline and serum solution. The small boxes on the top of each figure were suture before contact angle experiment.

## CONCLUSION

This study successfully applied simple, fast, and versatile surface modifications of polyglactin surgical suture to have antibacterial activity and increase the surface wettability of biological solution.

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