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Biocompatibility and antimicrobial activity essays of surgical sutures loaded with oil of *Lippia sidoides* Cham. with and without natural polymer

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Ensaios de biocompatibilidade e atividade antimicrobiana de fios de sutura carreados com óleo de *Lippia sidoides* Cham. com e sem polímero natural

Lília van der Linden

2021

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Dissertação apresentada ao Programa de Pós-Graduação em Odontologia, da Universidade Federal da Paraíba, como parte dos requisitos para obtenção do título de Mestre em Ciências Odontológicas.

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RESUMO

O triclosan tem sido usado para revestir fios de sutura cirúrgico e reduzir a ocorrência de infecções do sítio cirúrgico. No entanto, devido às questões ambientais e de segurança biológica, há um interesse crescente em encontrar antimicrobianos mais seguros e biocompatíveis, dos quais os óleos essenciais são candidatos promissores. O objetivo do presente estudo foi investigar a atividade antimicrobiana e a biocompatibilidade de um fio sutura contendo o óleo essencial de *Lippia sidoides* Cham (LSEO) com e sem polímero natural (LSEOP) como material de revestimento. Foi realizada Cromatografia líquida de alta eficiência (HPLC) para determinar a concentração de timol na sutura revestida. *Staphylococcus aureus* (ATCC 15656) e *Escherichia coli* (ATCC 25922) foram cultivados em meio de infusão de cérebro e coração. O método de microdiluição em caldo para determinar a concentração inibitória mínima (CIM), a difusão em ágar e o experimento de biofilme monoespécie para teste de aderência bacteriana foram realizados em triplicata. Além disso, para determinar a biocompatibilidade do ensaio de suturas, foi usada a linhagem celular epitelial de rim de macaco verde africano (VERO). Para o ágar de difusão, biofilme multiespécies e ensaio de biocompatibilidade, LSEO e LESEOP foram incorporados em um fio de sutura teste (poliglactina 910) usando procedimentos químicos. Valores de CIM do óleo essencial das folhas de *Lippia sidoides* Cham. foram <15 μg / mL para ambas as cepas. Para comprovar a adesão, a HPLC quantificou o componente majoritário da *Lippia sidoides* Cham. óleo essencial presente nas suturas revestidas (LSEO e LSEOP). A microscopia eletrônica de varredura (SEM) demonstrou LSEO e LSEOP nas suturas revestidas. Os halos de inibição (comprimento x largura) das suturas LSEO, LSEOP e triclosan contra *S. aureus* foram 25 x 10 mm, 22 x 4 mm e 31 x 13 mm, respectivamente. Os halos de inibição das suturas LSEO, LSEOP e triclosan contra *E.coli* foram 25 mm x 0,5 mm, 16 mm x 4 mm e 23 mm x 2 mm, respectivamente. Todos os ensaios antimicrobianos mostraram que o uso de suturas revestidas pode reduzir a aderência bacteriana. O LSEO e LSEOP depositados nas suturas exibiram efeito citotolerável. Os resultados demonstram que o fio de sutura contendo o óleo essencial de *Lippia sidoides* Cham. com e sem revestimento de polímero natural é um material antimicrobiano promissor que pode ser usado para revestir suturas e reduzir a ocorrência de infecções do sítio cirúrgico.

Palavras-chave: Infecção da ferida cirúrgica; Suturas; Biofilme; *Lippia*; Testes de Sensibilidade Microbiana; Biocompatibilidade.

ABSTRACT

Triclosan has been used to coat sutures and reduce the occurrence of surgical site infections. However, due to environmental issues and biological safety, there is growing interest in finding safer and biocompatible antimicrobials, among which essential oils are promising candidates. The aim of the present study was to investigate the antimicrobial activity and biocompatibility of a suture containing the essential oil from the leaves of *Lippia sidoides Cham* (LSEO) with and without a natural polymer (LSEOP) as a coating material. High Performance Liquid Chromatography (HPLC) was performed to determine the concetration of timol in the coated suture. *Staphylococcus aureus* (ATCC 15656) and *Escherichia coli* (ATCC 25922) were cultivated in a brain heart infusion medium. The broth microdilution method to determine the minimum inhibitory concentration (MIC), the agar diffusion and the monospecies biofilm experiment for testing bacterial adherence were performed in triplicate. In addition, to determine the biocompatibility of the sutures assay was used African Green Monkey Kidney Epithelial (VERO) Cell Line. For the diffusion agar, multispecies biofilm and biocompatibility assay, LSEO and LESEOP was incorporated in a testing suture (polyglactin 910) using chemical procedures. MIC values of the essencial oil from the leaves of *Lippia sidoides* Cham. were < 15 μg/mL for both strains. To approve the adherence, HPLC quantified the majority componente of *Lippia sidoides* Cham. essential oil present in the coated sutures (LSEO and LSEOP). Scanning electron microscopy (SEM) demonstrated LSEO and LSEOP in the coated sutures. The antimicrobial halos (length x width) of the LSEO, LSEOP and triclosan sutures against S. aureus were 25 x 10 mm, 22x 4 mm and 31 x 13 mm respectively. The halos of the LSEO, LSEOP and triclosan sutures against *E.coli* were 25 mm x 0,5 mm, 16 mm x 4 mm and 23mm x 2 mm, respectively. All the antimicrobial assays showed that the use of coated sutures could reduce bacterial colonization. The LSEO and LSEOP deposited on the sutures exhibited cytotolerable effect. The findings demonstrate that essential oil from Lippia sidoides Cham with and without a natural polymer coating is a promising antimicrobial material that can be used to coat sutures and reduce the occurrence of surgical site infections.

Keywords: Surgical wound infection; Sutures; Biofilm; *Lippia*; Microbial Sensitivity Tests; biocompatibility.

LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

ATCC	*American Type Culture Collection*
BHI	*Brain Heart Infusion*
CFU	*Colony Forming Unit*
CIM	Concentração Inibitória Mínima
CLAE	Cromatografia Líquida de Alta Eficiência
CLX	Clorexidina
E.coli	*Escherichia coli*
HPLC	*High Performance Liquid Chromatography*
ISC	Infecção do Sítio Cirúrgico
kV	Quilovolts
mg, mL, mm	Miligrama, mililitro, milímetro
mg/kg	Miligramas/Quilogramas
LSEO	*Suture containing the essential oil from the leaves of Lippia sidoides Cham .*
LSEOP	*Suture containing the essential oil from the leaves of Lippia sidoides Cham.*
MIC	Minimum inhibitory concentration
MRSA	*Methicillin-resistant Staphylococcus aureus*
NC	*Negative Control*
PC	*Positive Control*
RENISUS	Relação Nacional de Plantas Medicinais de Interesse ao Sistema Único de Saúde
SEM	*Scanning electron microscopy*
SSI	*Surgical site infection*
UFC	Unidade Formadora de Colônias
VERO	*African Green Monkey Kidney Epithelial*
µg, µL	Micrograma e microlitro
µg/cm	Micrograma/centímetro
µg/mL	Micrograma/mililitro
g	Grama
°C	Graus Celsius

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1. INTRODUÇÃO

Infecções do Sítio Cirúrgico (ISC) são as complicações mais comuns em cirurgias convencionais, estima-se que ocorram entre 160.000 a 300.000 por ano (1,2). São caracterizadas por infecções que se apresenta nas incisões cirúrgicas ou próximo a elas, durante o processo de cicatrização, gerando internações prolongadas, afetando a qualidade de vida e aumentando a mortalidade (1,3).

Nesse processo infeccioso, os microrganismos comumente isolados são *Staphylococcus aureus* (4,5)seguido da *Escherichia coli*, *Klebsiella*, *Staphylococcus coagulase negativa*, *Pseudomonas aeruginosa*, *Proteus*, *Enterococcus*, *Citrobacter*, *Acinetobacter spp.* (5).

Com a finalidade de reduzir a possibilidade de contaminação e infecção por bactérias e acelerar o processo de cicatrização com qualidade, as suturas cirúrgicas são utilizadas como suporte principal no pós-operatório. Porém, essas suturas constituem fatores retentivos para adesão bacteriana, assim, as pesquisas mais promissoras concentraram-se no desenvolvimento de revestimentos antibacterianos para fios cirúrgicos no pós-operatório.

Os fios de sutura podem ser classificados em absorvíveis e não absorvíveis (6,7). Os fios de sutura absorvíveis são de colágeno, categute e polímeros sintéticos, e são preferidos na maioria das cirurgias periodontais por evitar possíveis danos ao tecido inerentes a remoção do nó cirúrgico (8).

Os fios de sutura sintéticos absorvíveis mais utilizados são: poliglecaptona 25 (Monocryl), ácido poliglicólico (Dexon) e Poliglactina 910 (Vicryl®). O fio de poliglactina 910 é multifilamentar, trançado, composto por copolímero obtido a partir de ácido glicólico e L-lactida na proporção 9:1, vendido comercialmente como vicryl®. É indicado para ligações e/ou aproximações dos tecidos lisos em geral, incluindo procedimentos oftálmicos, mas não indicado para uso em tecidos cardiovasculares e neurológicos. A absorção acontece por hidrólise entre 2 a 3 semanas (7).

A Poliglactina 910 constitui, atualmente, um típico fio de sutura biodegradável, considerado um best-seller (9). Ele é indicado em fixação na reconstrução de mandíbula após ressecção segmentar na região do dente com resultados satisfatórios do ponto de vista cosmético e funcional (10) e na fixação transósseas de fraturas mandibulares em crianças (11)

Nas últimas décadas, o uso indiscriminado de antibióticos tem impulsionado o aumento de bactérias resistentes aos medicamentos. Para reduzir a ligação bacteriana às suturas cirúrgicas, foram desenvolvidas suturas de poliglactina 910 revestidas com Triclosan (Vicryl Plus ®) com potencial antibacteriano, único fio de sutura disponível no mercado com ação antimicrobiana (12,13).

Atualmente, a preocupação quanto à segurança biológica do triclosan (tricloro-2,4,4' hidroxi-2'difenileter), o seu potencial de toxicidade e efeitos colaterais aumentaram (14–16).

Aproximadamente cerca de 93% do Triclosan descartado é biodegradado ou adsorvido em Estação de Tratamento de Esgoto (ETEs), enquanto que os outros 7 % podem derivar produtos mais tóxicos e se transformar por fotólise direta em produtos secundários, com um grau mais elevado de persistência ambiental e associados a atividade cancerígena (17,18)

Por isso, visando o tratamento de água para remoção deste composto, se faz necessário a utilização de processos de tratamento mais apropriados, como, por exemplo, processos oxidativos avançados (POAs). Estes processos transformam contaminantes em dióxido de carbono, considerado grave para o meio ambiente (19). Além disso, o triclosan é uma substância fisiologicamente prejudicial, além de promover resistência a múltiplos fármacos (14–16).

No Brasil, a recente publicação da Anvisa, RDC Nº 528, de 4 de agosto de 2021, autorizou o uso de baixas concentrações de triclosan em dois grupos de produtos: a) 0,3% em dentifrícios, sabonetes para as mãos, sabonetes para o corpo, géis corporais para banho, desodorantes artificiais para axilas e pés, pós e corretivos faciais e produtos para higiene das unhas antes da aplicação de unhas artificiais; b) 0,2% em enxaguatórios bucais. Entretanto, já se proíbe o triclosan em sistemas pulverizáveis (como aerossóis e sprays) (20).

Estudos comparativos com o Vicryl Plus ® e Vicryl ®, demonstraram que não houve diferenças na diminuição de incidência de infecção do local cirúrgico em implantes dentários e até mesmo houve equivalência na aderência bacteriana ao fio (21–23).

Os óleos essenciais podem representar uma alternativa de substituição aos agentes antimicrobianos sintéticos convencionais, pois, além de evitar o desenvolvimento de resistência bacteriana, devido à variedade de mecanismos de atuação dos metabólicos, eles apresentam menor incidência de efeitos colaterais, baixo custo e geralmente possuem toxicidade baixa (24).

A Relação Nacional de Plantas Medicinais de Interesse do Sistema Único de Saúde (RENISUS) incluiu espécies vegetais com potencial de gerar produtos de interesse do Ministério da Saúde do Brasil. A espécie *Lippia siddoides* Cham., popularmente conhecida como alecrim-pimenta, está inclusa nesta relação, sendo uma planta nativa brasileira que possui em sua composição, flavonoides, quinonas, triterpenos, lignanas, esteroides livres e glicosilados e ácidos orgânicos. O óleo essencial de *L. sidoides* Cham. apresenta alto valor comercial, sendo o timol e o cravacol seus principais constituintes, os quais possuem propriedades antisséptica, antimicrobiana, antifúngica, antioxidante , anti-inflamatória e até larvicida(COSTA et al., 2002) . O óleo essencial de *Lippia sidoides* Cham. destacou-se em um estudo *in vivo* no qual foi avaliado o grau de toxicidade aguda e subcrônica, administrando doses entre 100 e 300 mg/kg do óleo essencial via oral, sendo concluído que não houve nenhuma alteração de comportamento em nenhuma das doses administradas. A dose da *Lippia siddoides* Cham. de administração oral subcrônica foi calculada em 117,95 mg/kg/dia, desprovido de toxicidade aparente calculada entre 110.61–125.29 mg/kg (25,26).

Através de estudos que resultaram na patente (BR102018010699-6), comprovou-se a eficácia do fio de sutura absorvível com óleo de *Lippia sidoides* Cham. frente a microrganismos comuns presentes na ferida cirúrgica. Porém este óleo essencial tende a ser muito volátil (27), propriedade a qual pode ser diminuída pelo acréscimo de revestimentos com polímeros naturais, que encontra-se em processo de depósito de patente.

Devido à resistência bacteriana e os problemas de segurança biológica com a substância antimicrobiana atualmente utilizada nos fios de sutura, é necessária a criação de novos agentes antibacterianos e sua implementação nos fios de sutura.

2. CAPÍTULO 1

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Antimicrobial and biocompatibility evaluation of sutures containing essential oil from *Lippia sidoides* Cham.

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Triclosan has been used to coat sutures and reduce the occurrence of surgical site infections. However, due to environmental issues, there is increasing interest in finding safer antimicrobials, among which essential oils are promising candidates. The present study aimed to investigate the antimicrobial activity and biocompatibility of sutures containing essential oil from the leaves of *Lippia sidoides* Cham. (LSEO). High-performance liquid chromatography (HPLC) was performed to determine the concentration of thymol in the coated sutures. *Staphylococcus aureus* (ATCC 15656) and *Escherichia coli* (ATCC 25922*)* were cultivated in a brain heart infusion medium. The broth microdilution method was used to determine the minimum inhibitory concentration (MIC), and agar diffusion and monospecies biofilm experiments were used to test bacterial adherence, which were performed in triplicate. Additionally, to determine the biocompatibility/cytotoxicity of the LSEO-coated sutures, an assay using the African green monkey kidney epithelial (Vero) cell line was conducted. For the diffusion agar, multispecies biofilm and cytotoxicity experiments, LSEO was incorporated into a test suture (polyglactin 910) using chemical procedures. A suture containing triclosan (Vycril® Plus) was used as a positive control. The MIC values of the LSEO were < 15 µg/mL for both strains. The amount of LSEO absorbed into the coated sutures was estimated by the HPLC technique using thymol as an analyte. Scanning electron microscopy (SEM) showed LSEO in the coated sutures. The halos of the LSEO and triclosan sutures against *S. aureus* were 25x10 mm and 31x13 mm, respectively. The halos of the LSEO and triclosan sutures against *E. coli* were 25 x 0.5 mm and 23 x 2 mm, respectively. The antimicrobial assays showed that the use of coated sutures reduced bacterial colonization. The cytocompatibility test using the Vero cell line exhibited greater than 70% cell viability. The findings demonstrate that essential oil from *Lippia sidoides* Cham. is a promising antimicrobial material to coat sutures for reducing the occurrence of surgical site infections.

Keywords: Surgical wound infection, sutures, biofilm, *Lippia*, biocompatibility, microbial sensitivity tests.

Introduction

Surgical site infections (SSI) are the most common complications that occur in conventional surgeries, and approximately 160,000 to 300,000 SSI are estimated to occur each year[1,2]. They are characterized by infections that present in or close to surgical incisions during the healing process and cause prolonged hospitalization, affect the quality of life and increase mortality[1,3–5].

Sutures are used as the main support in the postoperative period to reconnect tissues and promote rapid healing. However, sutures can facilitate infection, as they tend to attract bacteria that can adhere to the threads[6,7]. In a hospital setting, the most common bacteria are *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli, Klebsiella,* coagulase-negative *Staphylococcus, Proteus, Enterococcus, Citrobacter* and *Acinetobacter spp* [6,8–10]. Due to the risk of bacterial adhesion and consequent SSI, the most promising potential solutions have focused on the development of antibacterial coatings for surgical threads[11].

Surgical threads are classified as nonabsorbable and absorbable sutures[12]. Absorbable sutures are preferred in most periodontal surgeries to avoid damage to the tissue during the removal of the

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surgical knot. These sutures are also used at the point of fixation in mandible reconstruction after segmental resection in the tooth region and provide satisfactory results from a cosmetic and functional point of view, and in the fixation of mandibular fractures in children[13–15]. Polyglactin 910 is the best-selling absorbable suture currently in use and is available in two versions: with antimicrobial activity due to the incorporation of triclosan (Vycril® Plus by Ethicon Inc., Somerville, New Jersey, USA) or without an antimicrobial agent (Vycril® by Ethicon Inc., Livingston, West Lothian, Scotland)[16]. The polyglactin 910 monofilament suture is synthesized through the copolymerization of lactic acid and glycolic acid, and it is composed of 90% glycolide and 10% lactide[17].

Both *in vitro* and *in vivo* studies have demonstrated the antimicrobial efficacy of Vycril® Plus[18–21], and other studies have reported the effectiveness of surgical threads containing triclosan in abdominal surgeries[19,20]. However, several studies have highlighted the disadvantages of triclosan in antimicrobial sutures[18,19]. For example, in oral cavity surgeries, a decrease in pathogenic bacteria was not observed when sutures coated with this material were used[5]. Fowler and colleagues tested Vicryl® and Vicryl® Plus sutures and observed qualitatively equivalent bacterial adherence. Moreover, the use of triclosan (5-chloro-2-[2,4-dichlorophenoxy)phenol]) results in a toxic secondary product (dioxide), which is considered to be a serious threat to the environment and promotes multidrug resistance[19].

Thus, there is a requirement for new antimicrobial substances for sutures as a substitute for triclosan. Among the potential candidates, essential oils (aromatic oily liquids) are considered to be promising antimicrobials due to their availability and generally low toxicity and antimicrobial activity, which are associated with the individual susceptibility of bacteria[22]. Many essential oils have a minimum inhibitory concentration (MIC) lower than 100 μg/mL against many pathogens, and these low MIC values have piqued the interest of researchers[23].

Lippia sidoides Cham. (Family Lamiaceae) is a plant, commonly known as rosemary pepper, that is native to northeastern Brazil. The essential oil from the leaves of this plant contains flavonoids, quinones, triterpenes, lignans, free and glycosylated steroids. The oil also contains organic acids that have a high commercial value, such as thymol and carvacrol, which have antiseptic, antimicrobial, antifungal, antioxidant, anti-inflammatory and larvicidal properties[24,25]. Furthermore, studies have demonstrated the low cytotoxicity of *Lippia sidoides* Cham. in mammalian cells[26], and the oral administration of the essential oil at doses ranging from 1 to 3 g/kg did not induce any alterations, as demonstrated during a 30-day oral administration of *L. sidoides* oil (117.95 mg/kg/day) to rodents[27].

The present study aimed to investigate the antimicrobial activity and biocompatibility of a suture containing essential oil from *Lippia sidoides* Cham.

Materials and methods

Lippia sidoides Cham. essential oil

Lippia sidoides Cham. essential oil (LSEO) was purchased from Laszlo® (Belo Horizonte, Minas Gerais, Brazil). The major phenolic components of this oil are thymol (2-isopropyl-5-methylphenol) (71%) and carvacrol (also known as cymophenol) (2-methyl5-[1-methylethyl]phenol) (1%). LSEO has a density of 0.934 mg/mL at 20°C[28].

Microorganisms

The microorganisms tested were *S. aureus* (ATCC 15656) and *E. coli (*ATCC 25922*)*. The bacterial strains were cultured in brain heart infusion (BHI) broth (Kasvi, Curitiba, Paraná, Brazil) and incubated under aerobic conditions at 37°C for 24 hours. The microbial inocula were then standardized by absorbance (Fluorstar Optima-BMG Labtech, Ortenberg, Baden-Württemberg, Germany) in 0.5 McFarland suspensions for the experiments.

Minimum inhibitory concentration of _Lippia sidoides_ Cham. against _S. aureus_ and _E.coli._

The microdilution technique described by Andrews and colleagues (2001) was used in triplicate, with several modifications[29]**.** Two different MICs were determined: one for the essential oil from *Lippia sidoides* Cham. and one for triclosan (Alchemistry, São Paulo, São Paulo, Brazil). Both substances were dissolved (1 mg/mL) in 5% Tween 80 (Sigma, St. Louis, Missouri, USA) and diluted with BHI to achieve concentrations ranging from 15 to 400 μg/mL. Saline solution and 5% Tween 80 were used as negative controls, whereas a 0.12% chlorhexidine solution (Sigma, São Paulo, São Paulo, Brazil) was used as the positive control. The plates were incubated in a microbiological oven for 24 hours at 37°C. After this period, 35 µL of the oxidation-reduction indicator resazurin (0.1%) (Sigma-Aldrich, São Paulo, Brazil) was added to each well. The lowest concentration of oil at which there was no visible growth was recorded as the MIC[29–31].

Development of surgical suture coatings

The coatings were prepared using the fatty acid technique involving methanol (Sigma, St. Louis, Missouri, USA) and LSEO, with several modifications[19]. Sutures (Polyglactin 910, Vycril® by Ethicon Inc., Livingston, West Lothian, Scotland) of a standard size (length: 2 cm; diameter: 2-0) were immersed in the coating solution. As this material is undergoing the patent process (BR 10 2018 010699 6) at the Brazilian National Institute of Industrial Property (INPI), no further details are presented.

Scanning electron microscopy

Pieces of the resorbable sutures, with and without a coating, were placed on stell stubs and sputter-coated with a 15 nm layer of gold using a Leica™ ACE 200 gold metallizer. The samples were then observed and photographed along the entire length of the suture under a scanning electron microscope (SEM) (JSM-5600LV, Jeol, Peabody, MA, USA). The images were taken at 50, 100 and 2000× magnification at a voltage of 15 kV[32].

High-performance liquid chromatography

The identification and quantification of most of the compounds in the surgical sutures were performed by dipping 27.5 cm of the thread in 5 mL of P.A. ethanol, followed by incubation in an ultrasound bath for ten minutes. Subsequently, 5 mL of deionized water was added to the solution, and the samples were placed in an ultrasound bath for an additional 10 minutes. The theoretical final concentration of thymol in this solution was 40 µg/ml. After extraction of the thymol present on the threads, the solution was filtered and injected into a Shimadzu HPLC system with the following set-up: a diode array detector (DAD) (SPDM20A), an automatic injector (SIL-20ª), a multisolvent pump (LC-20AT), an NST® C8 column (250 mm x 3.9 mm x 5 µm), an oven operating temperature of 40 °C, a 6:4 methanol: NaH2PO4 0.03M + 0.4% triethylamine mobile phase and a pH of 2.7 that was compatible with phosphoric acid. The flow was 0.8 mL/min and the sample injection volume was 20 µL. An evaluation of the thymol peak was conducted at a wavelength of 275 nm[33].

Agar diffusion: Testing the antimicrobial activity of *Lippia sidoides* Cham. coated sutures

The antimicrobial activity of the suture threads containing LSEO was evaluated by diffusion in agar culture (performed in triplicate)[34] and the bacterial adhesion test (biofilm model). The modified agar diffusion assay was conducted using strains of *S aureus* (ATCC. 15656) and *E. coli* (ATCC 25922) that were standardized at 0.5 on the McFarland scale. One ml of the strains was inoculated in a Petri dish containing BHI agar medium. After drying, the coated suture threads were placed in the dishes, which were left for 30 minutes at room temperature for pre-diffusion prior to incubation at 37°C for 24 hours. After this period, the inhibition halos were measured in mm. The positive control consisted of commercial polyglactin 910-Vycril® containing triclosan and the negative control consisted of polyglactin 910.

Monospecies biofilm model with LSEO-coated sutures

Suture segments (2 cm) were immersed in 24-well cell culture plates containing 1.6 mL of BHI broth and 0.4 mL of bacterial inocula containing *S. aureus* (ATCC 15656) and *E. coli* (ATCC 25922) that were standardized at 0.5 on the McFarland scale, as described above. The plates were incubated in a microbiological oven at 37°C for 24 hours. The surgical threads were first placed into a well containing saline solution to remove plankton cells, then transferred to a test tube containing 2 mL of 0.9% saline solution and sonicated. Next, serial dilutions were performed and 20 µL of the diluted solutions were placed in Petri dishes containing BHI agar medium. The dishes were incubated at 37°C for 24 hours, this was followed by counting the colony-forming units (CFUs). The positive and negative controls consisted of commercial Vycril® polyglactin 910 containing triclosan and polyglactin 910 without triclosan, respectively[35]. All analyses were performed in triplicate.

Cell cultures

African green monkey kidney epithelial (Vero) cells were grown and maintained in Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich™) that was supplemented with 1% penicillin/streptomycin (Sigma-Aldrich™), 0.25µg/mL and 10% fetal bovine serum (FBS) (Gibco™). The cell culture was kept in a greenhouse at 37°C, in a humid atmosphere at 5% CO_2.

Biocompatibility assay

The Vero cells were seeded in 24-well plates (3 x 10^5 cells/well) in DMEM medium supplemented with 2% FBS and incubated for 24 hours. The media from the plates was aspirated and placed with the LSEO-coated sutures and Vicryl and Vicryl Plus (2 cm and 20 cm per well, respectively). The untreated cells (negative control) and the cells in DMEM medium supplemented with 10% dimethylsulfoxide (DMSO) (Sigma, São Paulo, Brazil) (positive control) were also included in this trial. The plates were incubated in an oven at 37°C and 5% CO_2 for 24 hours. After incubation, the culture medium was aspirated with the treatments and the cells were trypsinized. After trypsinization, the cells were suspended in culture medium and diluted 10 times, and the cell viability was evaluated in a hemocytometer using the trypan blue colorimetric method[36]. All analyses were conducted in triplicate and the results are shown as the mean value ± standard error. The Kruskal-Wallis and Dunn's test for multiple comparisons were performed.

Results

The MIC of the LSEO was < 15 μg/mL for *S. aureus* (ATCC 15656) and *E. coli* (ATCC 25922). The MIC of the triclosan was > 400.0 μg/mL. As expected, no cellular activity (< 0.0053 μg/mL) was observed in the treatment with the positive control (chlorhexidine).

The SEM assay showed that the surfaces of all the sutures (coated and uncoated) were uniform and presented surface integrity without debris (Fig. 1).

The HPLC assay quantified the primary substance, thymol. The LSEO suture that was created one day before the experiment contained 142 μg/cm of thymol and the suture that was created two months before the experiment contained 1.09 μg/cm of thymol.

The agar diffusion method was performed to observe the inhibition halos. The inhibition zones were measured perpendicular to the middle of the suture threads. The plates cultivated with *S. aureus* that were exposed to the LSEO sutures had inhibition halos measuring 25 x 10 mm. A halo of 31 x 13 mm was observed when this bacterium was exposed to the commercial Vycril® polyglactin 910 containing triclosan (positive control) (Fig. 2). In the experiments conducted using *E. coli* (ATCC 25922), the halo sizes were 25 x 0.5 mm with the LSEO sutures and 23 x 2 mm with the triclosan sutures (Fig. 3). Neither of the negative controls exhibited inhibition halos.

The biofilm technique was performed to reproduce a more clinical setting of bacterial attachment, similar to an oral cavity. These results are presented in Fig. 4. The mean cell survival rates in the LSEO treatments were 18% for *S. aureus* and 30% for *E. coli*.

The biocompatibility test using trypan blue allowed for live and dead cells to be distinguished. Trypan blue is a vital stain that is not absorbed by healthy viable cells, but stains cells with a damaged membrane. The mean values of the Vero cell viability in all the treatments remained above 70%, and no statistical differences were observed between the various treatments or between the treatments and the positive and negative controls. The cell viability values were 81% and 96.29% for the LSEO at 2 cm and 20 cm, respectively. Additionally, the 2cm and 20 cm commercial sutures demonstrated a cytocompatible effect, at 83.03% and 81.27% for Vycril® and 77% and 70% for Vycril® Plus, respectively (Figs. 5 and 6).

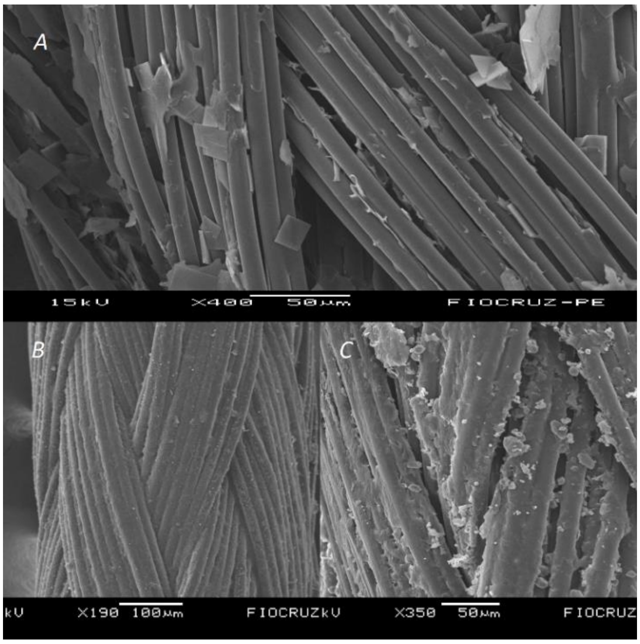

Figure 1. Photomicrographs of the threads studied. A: Scanning electron micrograph of an LSEO suture showing the coating (x400); B: Scanning electron micrograph of the polyglactin 910 showing a multi-filament structure (x190); C: Scanning electron micrograph of the polyglactin 910 with triclosan suture showing the coating (x350).

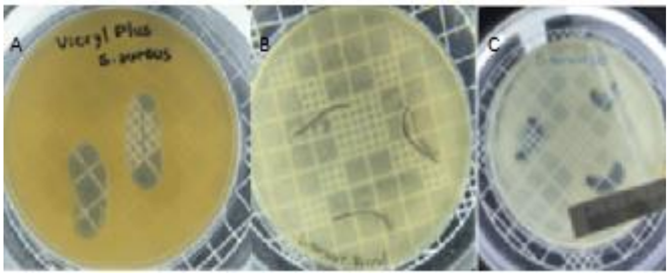

Figure 2. Measuring the antibacterial efficacy of coated sutures via zones of inhibition against *S. aureus*. A: Vicryl Plus with zones of inhibition measuring 31 x 13 mm; B: Vicryl without zones of inhibition and C: LSEO with zones of inhibition measuring 25 x 10 mm.

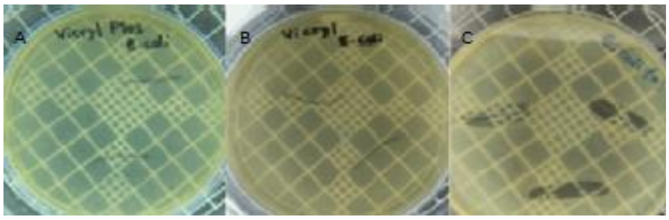

Figure 3. The antimicrobial efficacy on agar plates containing *E.coli A:* Vicryl Plus with zones of inhibition measuring 23 x 2 mm; B: Vicryl without zones of inhibition and C: LSEO with zones of inhibition measuring 25 x 0.5 mm

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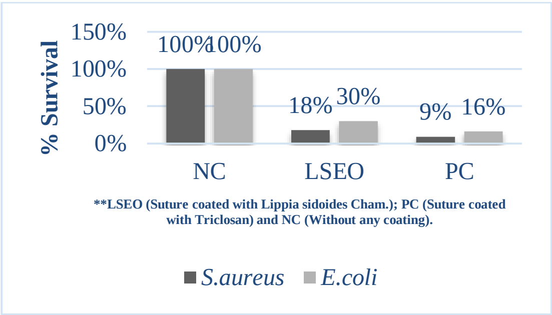

Figure 4. Mean cell survival rates (monospecies biofilm method) based on colony-forming units per mL with a dilution of 10^{-4}.

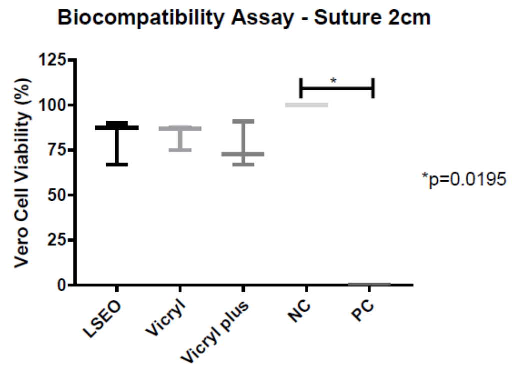

Figure 5. The viability averages of the Vero cells exposed to the treatment with the 2 cm suture. The Kruskal-Wallis test was conducted, followed by Dunn's test for multiple comparisons. LSEO = suture coated with essential oil from *Lippia sidoides* Cham.; Vicryl = polyglactin 910 without coating; Vicryl plus = polyglactin 910 coated with triclosan; NC = negative control- cells only; PC = positive control with 10% DMSO.

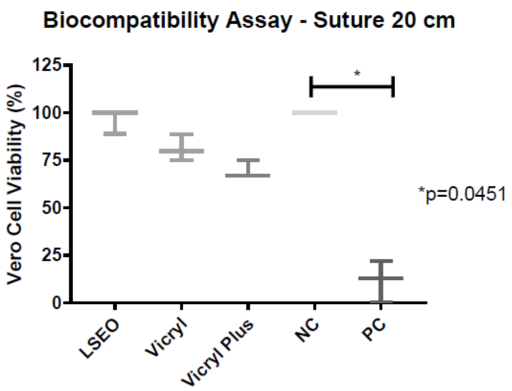

Figure 6. The viability averages of Vero cells exposed to the treatment with the 20 cm suture. The Kruskal-Wallis test was conducted, followed by Dunn's test for multiple comparisons. LSEO = suture coated with essential oil from *Lippia sidoides* Cham.; Vicryl = polyglactin 910 without coating; Vicryl plus = polyglactin 910 coated with triclosan; NC = negative control- cells only; PC = positive control with 10% DMSO.

Discussion

Surgical site infections (SSI) are the most common surgical complications that occur in conventional surgeries and are mainly caused by infected sutures. As the most common bacteria involved in SSI are *S. aureus* and *E. coli*, these bacteria were analyzed in the present study[9,10]. To minimize the occurrence of SSI, we developed a novel antimicrobial suture coated with essential oil. Our findings obtained through SEM observation reinforce the success of coated suture technique. Moreover, HPLC analysis of the samples confirmed the impregnation of the essential oil into the sutures. The results observed after 2 months that showed the amount of essential oil (thymol) absorbed by the threads decreased by 99.24% may have been due to the oil being extremely volatile and/or an inadequate form of storage[37].

Minimum inhibitory concentrations are more reliable than agar diffusion test results due to a greater possibility of errors associated with the latter method[31]. Thus, MICs are considered the “gold standard” for determining the sensitivity of organisms to antimicrobials[29]. Previous studies have reported that the MIC of triclosan ranges from 0.5 µg/mL to 0.025 and 1 mg/L against *S. aureus MRSA*[38]. In the present study, the MIC of triclosan was measured at > 400 µg/mL for *S. aureus* and *E. coli*. It is, therefore, important to evaluate effective alternatives that have suitable antimicrobial activity as a substitute for triclosan. Antimicrobial agents with an MIC below 100 µg/mL are considered to be the most promising since a lower MIC minimizes the chances of adverse effects during clinical use[23]. The findings of the present study demonstrate that sutures containing LSEO are promising materials against *S. aureus* and *E. coli*.

The results of the agar diffusion assay that compared sutures containing LSEO to the commercially available suture Vicryl Plus that contains triclosan revealed that the sutures containing LSEO may decrease the occurrence of SSI.

A biofilm is a persistent form of microbial contamination that enables bacteria to adhere to the surface of a material[26]. In the biofilm tests conducted in the present study, the sutures coated with LSEO presented similar results to those observed with the positive control, whereas the negative control exhibited 5.07-fold more CFUs/mL for *S. aureus* (ATCC 15656) and 3.3-fold more CFUs/mL for *E. coli* (ATCC 25922). These results demonstrate the efficacy of the essential oil regarding the non-adherence of the bacteria to the coated threads.

However, the mature biofilm used in this study is not of the multispecies type that is generally observed in surgical wounds.

Greater than 70% cell viability was observed in the cytocompatibility test using the VERO cell Line with the LSEO. Previous studies have demonstrated the cytotoxic potential of materials using Vero cells, following ISO 10993-5:2009. For example, one study showed the low toxicity of *Lippia sidoides* Cham. when applied to mammalian cells[26]. Moreover, in a separate study conducted on rats, 30 days of oral administration of LSEO at doses ranging from 1 to 3 g/kg did not induce significant changes[27].

The results of the present study suggest that antimicrobial essential oil incorporated into sutures is a promising strategy for reducing SSI. However, this work has limitations that should be addressed. Firstly, the 24-hour evaluation time is a short period of evaluation; hence, it is unknown whether bacterial adhesion to the suture would occur after this timeframe. Secondly, we did not use MRSA strains in our antimicrobial coated suture studies, only ATCC strains of *E. coli* and *S. aureus*. Thirdly, the biofilm test was not conducted using multiple bacterial species, which hinders a comparison with results obtained in previous *in situ* and *in vivo* studies. Therefore, future *in vivo* studies are required that involve analysis of the mechanisms of action of the oil on the sutures for greater than 48 hours ('the time at which the essential oil is fully absorbed by the threads') and determine whether the oil interferes with the absorption process.

Conclusions

The findings of the present study demonstrate that the essential oil from *Lippia sidoides* Cham. has promising antimicrobial efficacy when incorporated into suture threads. Moreover, LSEO-coated sutures represent an alternative substitute for triclosan-coated sutures.

Acknowledgements

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Conflicts of interest

The authors declare no conflict of interest.

Notes and references

1 CDC, Oid, Ncezid and DHQP, 2020, 1–36.

2 B. H. Garner and D. J. Anderson, *Infect. Dis. Clin. North Am.*, 2016, **30**, 909–929.

3 J. Ruiz Tovar and J. M. Badia, *Cirugía Española (English Ed.*, 2014, **92**, 223–231.

4 H. Kim, B. H. Kim, B. K. Huh, Y. C. Yoo, C. Y. Heo, Y. Bin Choy and J. H. Park, *Biomater. Sci.*, 2017, **5**, 1670–1677.

5 L. Gao, J. Chen, W. Feng, Q. Song, J. Huo, L. Yu, N. Liu, T. Wang, P. Li and W. Huang, *Biomater. Sci.*, 2020, **8**, 6930–6945.

6 B. D. Masini, D. J. Stinner, S. M. Waterman and J. C. Wenke, *J. Surg. Educ.*, 2011, **68**, 101–104.

7 R. Urie, M. McBride, D. Ghosh, A. Fattahi, R. Nitiyanandan, J. Popovich, J. J. Heys, J. Kilbourne, S. E. Haydel and K. Rege, *Biomater. Sci.*, 2021, **9**, 3791–3803.

8 M. Belusic-Gobic, M. Car, M. Juretic, R. Cerovic, D. Gobic and V. Golubovic, *Oral Oncol.*, 2007, **43**, 77–81.

9 D. M. Sievert, P. Ricks, J. R. Edwards, A. Schneider, J. Patel, A. Srinivasan, A. Kallen, B. Limbago and S. Fridkin, *Infect. Control Hosp. Epidemiol.*, 2013, **34**, 1–14.

10 M. SenGupta, D. Banerjee, M. Sengupta, S. Sarkar, S. Nag and R. M. Singh, *Int. J. Infect. Control*, 2014, **10**, 2–7.

11 K. Pelz, N. Tödtmann and J. E. Otten, *Ann. Agric. Environ. Med.*, 2015, **22**, 551–555.

12 R. F. Edlich, K. Gubler, A. G. Wallis, J. J. Clark, J. J. Dahlstrom and W. B. 3rd Long, *J. Environ. Pathol. Toxicol. Oncol. Off. organ Int. Soc. Environ. Toxicol. Cancer*, 2010, **29**, 339–361.

13 M. Maksoud, S. Koo, K. Barouch and N. Karimbux, *J. Investig. Clin. Dent.*, 2014, **5**, 45–50.

14 B. Aluko-Olokun, A. A. Olaitan and O. A. Aluko-Olokun, *Oral Maxillofac. Surg.*, 2017, **21**, 13–20.

15 S. Chandan, R. Halli, S. Joshi, G. Chhabaria and S. Setiya, *J. Craniofac. Surg.*, 2013, **24**, 2050–2052.

16 B. Azimi, P. Nourpanah, M. Rabiee and S. Arbab, *J. Eng. Fiber. Fabr.*, 2014, **9**, 47–66.

17 S. J. Aston and T. D. Rees, *Aesthetic Plast. Surg.*, 1976, **1**, 289–293.

18 J. R. Fowler, T. A. Perkins, B. A. Buttaro and A. L. Truant, *Clin. Orthop. Relat. Res.*, 2013, **471**, 665–671.

19 A. Obermeier, J. Schneider, P. Föhr, S. Wehner, K. D. Kühn, A. Stemberger, M. Schieker and R. Burgkart, *BMC Microbiol.*, 2015, **15**, 186.

20 A. Obermeier, J. Schneider, S. Wehner, F. D. Matl, M. Schieker, R. Von Eisenhart-Rothe, A. Stemberger and R. Burgkart, *PLoS One*, , DOI:10.1371/journal.pone.0101426.

21 J. Guo, L. H. Pan, Y. X. Li, X. Di Yang, L. Q. Li, C. Y. Zhang and J. H. Zhong, *J. Surg. Res.*, 2016, **201**, 105–117.

22 S. Chouhan, K. Sharma and S. Guleria, *Medicines*, 2017, **4**, 58.

23 J. L. Ríos and M. C. Recio, *J. Ethnopharmacol.*, 2005, **100**, 80–84.

24 S. M. O. Costa, T. L. G. Lemos, D. L. Pessoa, J. A. C. Carlos and R. Braz-Filho, *Rev. Bras. Farmacogn.*, 2002, **12**, 66–67.

25 F. P. de Oliveira, E. de O. Lima, J. P. de Siqueira Júnior, E. L.

de Souza, B. H. C. Santos and H. M. Barreto, *Rev. Bras. Farmacogn.*, 2006, **16**, 510–516.
26 M. das G. F. de Medeiros, A. C. da Silva, A. M. das G. L. Citó, A. R. Borges, S. G. de Lima, J. A. D. Lopes and R. C. B. Q. Figueiredo, *Parasitol. Int.*, 2011, **60**, 237–241.
27 R. O. S. Fontenelle, S. M. Morais, E. H. S. Brito, M. R. Kerntopf, R. S. N. Brilhante, R. A. Cordeiro, A. R. Tomé, M. G. R. Queiroz, N. R. F. Nascimento, J. J. C. Sidrim and M. F. G. Rocha, *J. Antimicrob. Chemother.*, 2007, **59**, 934–940.
28 L. P. Fernandes, W. P. Oliveira, J. Sztatisz and C. Novák, *J. Therm. Anal. Calorim.*, 2008, **94**, 461–467.
29 J. M. Andrews and J. M. Andrews, *J. Antimicrob. Chemother.*, 2001, **48 Suppl 1**, 5–16.
30 H. A. Montejano, M. Gervaldo and S. G. Bertolotti, *Dye. Pigment.*, 2005, **64**, 117–124.
31 E. G. Alves, A. H. C. Vinholis, L. A. Casemiro, N. A. J. C. Furtado, M. L. A. Silva, W. R. Cunha and C. H. G. Martins, *Quim. Nova*, 2008, **31**, 1224–1229.
32 L. Palhares Neto, L. M. de Souza, M. B. de Morais, E. Arruda, R. C. B. Q. de Figueiredo, C. C. de Albuquerque and C. Ulisses, *J. Plant Growth Regul.*, 2020, **39**, 26–40.
33 V. M. Muniz, 2018.
34 CLSI, *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement - document M100S22*, 2012, vol. 32.
35 M. Iñiguez-Moreno, M. Gutiérrez-Lomelí, P. J. Guerrero-Medina and M. G. Avila-Novoa, *Brazilian J. Microbiol.*, 2018, **49**, 310–319.
36 W. Strober, *Curr. Protoc. Immunol.*, 2015, **111**, A3.B.1-A3.B.3.
37 A. R. Bilia, C. Guccione, B. Isacchi, C. Righeschi, F. Firenzuoli and M. C. Bergonzi, *Evidence-based Complement. Altern. Med.*, , DOI:10.1155/2014/651593.
38 O. Assadian, K. Wehse, N.-O. Hübner, T. Koburger, S. Bagel, F. Jethon and A. Kramer, *GMS Krankenhhyg. Interdiszip.*, 2011, **6**, Doc06.
39 N. Devi and J. Dutta, *Int. J. Biol. Macromol.*, 2017, **104**, 1897–1904.

3. CAPÍTULO 2

Este artigo será submetido após o aceite do artigo contido no capítulo 1, mencionando-o como referência, por isso estará em * já que este ainda não foi publicado.

Title:

Efficacy of *Lippia sidoides* Cham. with natural polymer coated suture against Surgical Site Infection

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ABSTRACT

Surgical sutures it’s a potential factor that causing Surgical Site Infection (SSI). Antimicrobial sutures are development to reduce sutures colonized by bacteria, consequently decrease SSI and mortality. Triclosan coated sutures engender concerns about the biological safety of triclosan and its potential toxicity and side effects and therefore alternative substances with essencial oils and natural polymer are promising candidates. The aim of this study is an optimization of a suture with essential oil of *Lippia sidoides* Cham., coated with a natural polymer (LSEOP) in order to reduce the oil volatility in the surgical suture and to reach antimicrobial efficacy. To determine the concentration of the majority compound in the coated suture was performed by High-performance liquid chromatography (HPLC). *Staphylococcus aureus* (ATCC 15656) and *Escherichia coli* (ATCC 25922*)* were used at the antimicrobial assays. Biocompatibility assay was use the African green monkey kidney epithelial (Vero) cell line. Scanning electron microscopy (SEM) showed LSEOP in the coated suture. HPLC technique was estimated the amout LSEOP into the coated suture in 2 moths. The halos of the LSEOP and triclosan sutures against *S. aureus* were 22x4mm and 31x13 mm, respectively. The halos of the LSEO and triclosan sutures against *E. coli* were 16 x 4 mm and 23 x 2 mm, respectively. The *Biofilm test* showed that the use of coated sutures LSEOP reduced bacterial colonization better than with triclosan coated. The cytocompatibility test using the Vero cell line exhibited greater than 70% cell viability. LSEOP it's biocompatible and a promising antimicrobial material to reduce the occurrence of SSI.

INTRODUCTION

Surgical site infection (SSI) is characterized by the appearance of a purulent secretion and/or fistulas in surgical wounds(1). According to the US Centers for Disease Control and Prevention, SSI is the third most common type of healthcare-associated infection (23.1%) after central line-associated bloodstream infection (40%) and catheter-associated urinary tract infection(2). *Staphylococcus aureus* and *Escherichia coli* are present in the SSIs among the hospitals that report to the CDC (3).

The presence of suture material in a surgical wound is known as a risk to infections due to increased bacterial adherence (4,5). Studies demonstrate that the physical properties and composition of suture material play an important role in bacterial adhesion and suture, causing it to be a major contributor to the induction, severity and persistence of a wound infection(5)

In recent decades, the overuse of antibiotics increased the existence of drug-resistant bacteria. To reduce bacterial binding to surgical sutures, Triclosan (Vicryl Plus®) coated polyglactin 910 sutures with antibacterial potential has been developed(6–9).

Recently, concerns about the biological safety of triclosan and its potential toxicity and side effects have grown. Some studies indicate that this substance is physiologically harmful to reproductive health in humans and animals(10,11)

Industries has focused on developing coated surgical sutures to decrease SSI, and then, mortality. Recent patent (BR102018010699-6) showed that essential oil of Lippia *sidoides* Cham. coated sutures (LSEO) demonstrated high efficacy against *S. aureus* and *E.coli* and related low cytotoxicity. However, essential oils are volatile and in order to minimize this property in coated sutures(LSEO), chitosan is a promising option (ARTICLE 1*;(12).

Chitosan is a natural polymer that is a potential useful pharmaceutical material - mainly as a carrier for drugs delivery - because of their excellent biocompatibility, biodegradability, mucoadhesive properties and non-toxic properties for humans and animals with an LD_{50} in rates of 16 g/kg (13–17).

The aim of this study is an optimization of a suture with essential oil of *Lippia sidoides* Cham., coated with a natural polymer (LSEOP) in order to reduce the oil volatility.

MATERIALS AND METHODS

Coated Surgical Suture with a Natural Polymer

Chitosan was solubilized and coated onto the coated suture (LSEO) using a strong acid. The technique is in the patent process at the Brazilian National Institute of Industrial Property (INPI), no further details are presented.

Scanning Electron Microscopy (SEM)

Individual coated sutures with chitosan (LSEOP) were prepared for SEM by sputtering using a Leica™ ACE 200 gold metallizer and examined with a high vacuum SEM (Scanning Electron Microscope, mod. JSM-5600LV, Jeol, Pea- body, MA, USA) (18).

High Performance Liquid Chromatography (HPLC)

The identification and quantification of the majority compound (2-Isopropyl-5-methylphenol, known as thymol) in the surgical suture was performed by dipping the thread in the P.A. ethanol and, in another moment, a bath of deionized water, followed by an ultrasound bath. The theoretical final concentration of thymol in this solution was 40 µg ml^{-1}. After extracting the thymol present in the wire, the solution was filtered and injected in a Shimadzu High Performance Liquid Chromatography(HPLC) equipment, diode array detector (DAD) (SPDM20A), with automatic injector (SIL-20ª), multisolvent pump (LC-20AT), NST® C8 column (250 mm x 3.9 mm x 5 µm), oven operating temperature 40 °C, using as mobile phase 6: 4 methanol: NaH2PO4 0.03M + 0.4% triethylamine, pH 2.7 compatible with phosphoric acid. Flow was 0.8 mL min-1 and sample injection volume 20 µL. The evaluation of the thymol peak was carried out at a wavelength of 275 nm (19).

Antimicrobial efficacy of coated sutures via agar diffusion test

According to Clinical and Laboratory Standard Institute (CLSI), suspensions of *Staphylococcus aureus* (ATCC 15656) and *Escherichia coli* (ATCC 25922) were prepared to an optical density of 0.5 McFarland standard. Then, 1 ml of this suspension was plated uniformly on BHI Agar plates. After removal of the supernatant and drying the petri dishes, surgical sutures samples were placed on the inoculated Agar plates and incubated at 37 °C overnight. After 24 h, zones of inhibitions were measured in millimeter (mm). The positive control was the commercial polyglactin 910-Vycril® containing triclosan (Vycril® plus by

Ethicon Inc., Somerville, New Jersey, USA) and the negative control was polyglactin 910 (Vycril® by Ethicon Inc., Livingston, West Lothian, Scotland)(20).

Biofilm Test

Suture segments (2 cm) were immersed in 24-well cell culture plates containing 1 BHI broth (Kasvi, Curitiba, Paraná, Brazil) and inoculum of *Staphylococcus aureus* (ATCC 15656) and *Escherichia coli* (ATCC 25922) standardized at 0.5 of the McFarland scale, as described above. The plates were incubated at 37 °C overnight. After 24 hours, serial dilutions were performed and 20 µL of the diluted solutions were plated in Petri dishes containing BHI agar medium (Kasvi, Curitiba, Paraná, Brazil). The dishes were incubated at 37°C for 24 hours, followed by counts of the colony-forming units (CFUs). The positive and negative control were the commercial Vycril® polyglactin 910 containing triclosan (Vycril® plus by Ethicon Inc., Somerville, New Jersey, USA) and polyglactin 910 without triclosan (Vycril® by Ethicon Inc., Livingston, West Lothian, Scotland), respectively (21).

Biocompatibility study

Analysis of *in vitro* cytotoxicity of coated sutures was performed by using VERO cells in 24-well plates ($3x10^5$ cells/well) in DMEM medium supplemented with 2%FBS and incubated for 24h. Media from the plates were aspirated and placed with LSEOP coated sutures, Vicryl and Vicryl Plus of 2 cm and 20 cm per well. Untreated cells (negative control) and cells on DMEM medium supplemented with 10% DMSO – Dimethylsulfoxide, Sigma, São Paulo - (positive control) were also included for this trial. The plates were incubated in an oven at 37°C and 5% CO_2 per 24h. After incubation, the culture medium was aspirated with treatments and the cells were trypsinized. After trypsinization, the cells were suspended in culture medium and diluted 10 times to evaluate cell viability using the Trypan blue colorimetric method in a hemocytometer (22). All analyses were carried out in triplicate and the results are shown as the mean values ± standard errors. Kruskal Wallis followed by Dunn´s test for multiple comparisons were performed.

RESULTS

Scanning electron microscopy (SEM)

Analysis of polyglactin 910 pieces with essencial oil of *Lippia sidoides* Cham., with and without chitosan coating, revealed the multifilament characteristic of the suture thread and showed that the surfaces of all sutures were uniform, with and without coated, showing no debris (Figure 1).

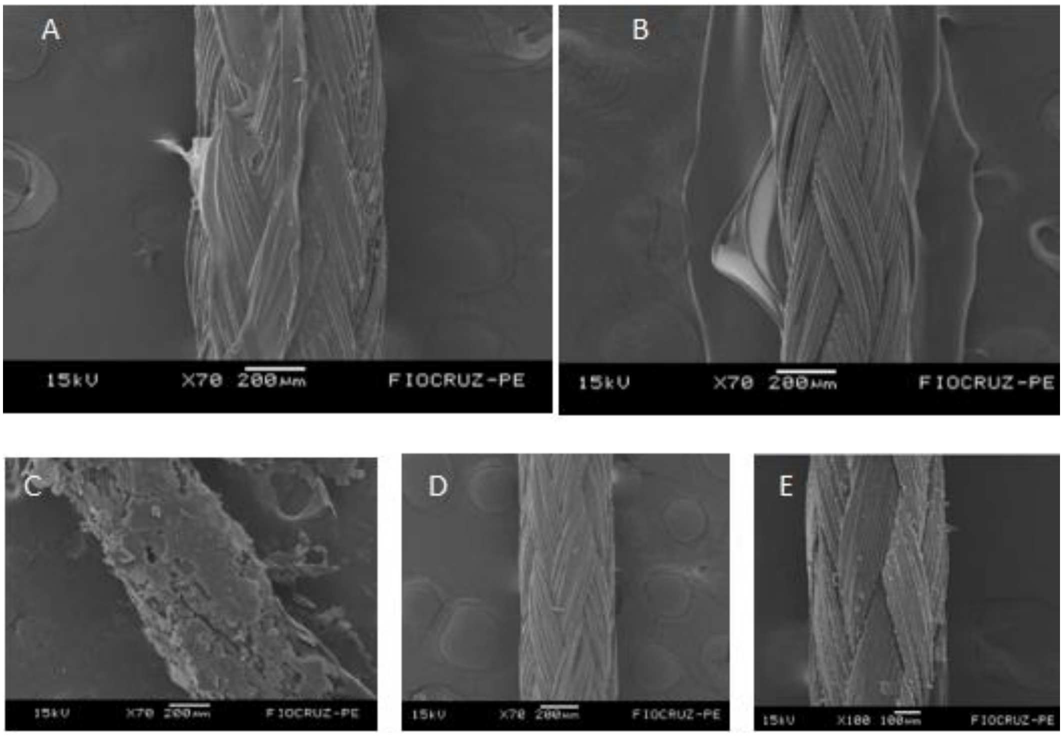

Figura 1 - Photomicrographs of the threads studies. A- Scanning electron micrograph of the LSEOP suture showing the coated (x70);B- Polyglactin 910 with chitosan; C- Lippia sidoides Cham. coated suture (Polyglactin 910) D- Scanning electron micrograph of the Polyglactin 910 showing multi-filamental structure (x70); E- Scanning electron micrograph of the Polyglactin 910 with Triclosan suture showing the coated (x350).

HPLC

LSEOP was analysed by high performance liquid chromatography (HPLC) to quantified the marjority substance of *Lippia sidoides* Cham (thymol). The suture LSEOP that made one day before the experiment has 153,75 μg/cm and the two sutures that made in approximately 60 days before the experiment, in which one of the suture threads was stored in the bag with air and the other one was stored in the bag with less air, simulating a vacuum bag has 1,47 μg/cm (4,06 μg/mL) and 45,49 μg/cm (125,11 μg/mL) respectively.

Antimicrobial Assays

Antimicrobial efficacy of coated sutures was daily assessed by using an Agar diffusion and a single species biofilm. Sutures coated LSEOP revealed a large growth inhibition zones against *S. aureus* and *E. coli* with (length x width) 22 x 4 mm and 16 x 4 mm respectively (Figure 2 and 3). The 31x13 mm halo was found when the *S. aureus* was exposed to the commercial Vycril® Plus polyglactin 910 containing triclosan (positive control) and 23 x 2 mm against *E.coli*. Chitosan suture coated without *Lippia sidoides* Cham. and the commercial suture without antimicrobial agent (Vycril®) did not show inhibition halos.

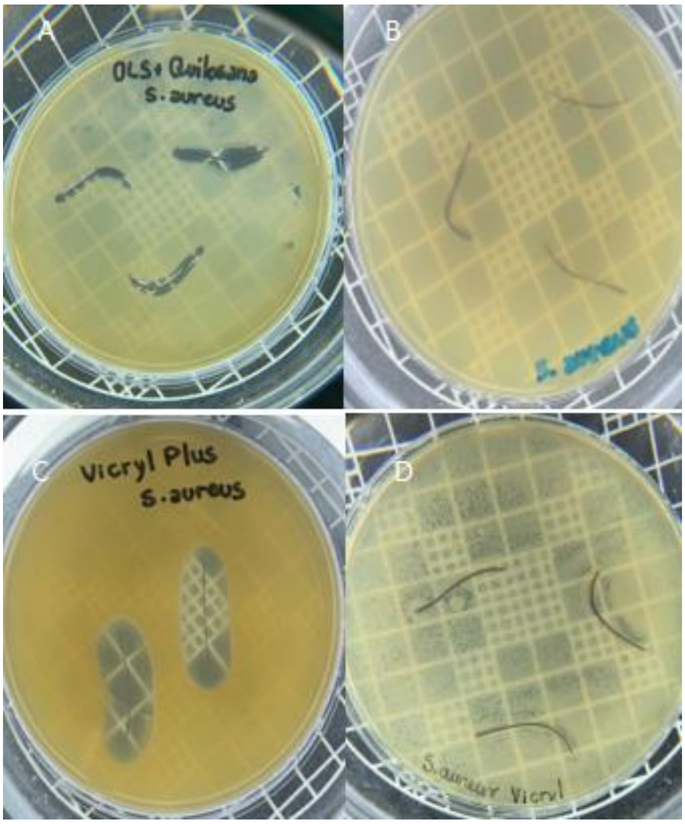

Figura 2 Measuring anti-bacterial efficacy of coated sutures via zones of inhibition against *S.aureus*. A LSEOP 22x 4 mm; B Vicry only with chitosanl without zones of inhibition; C Polyglactin 910 with triclosan coated 31 x 13 mm and D Vicryl without halos of inhibition.

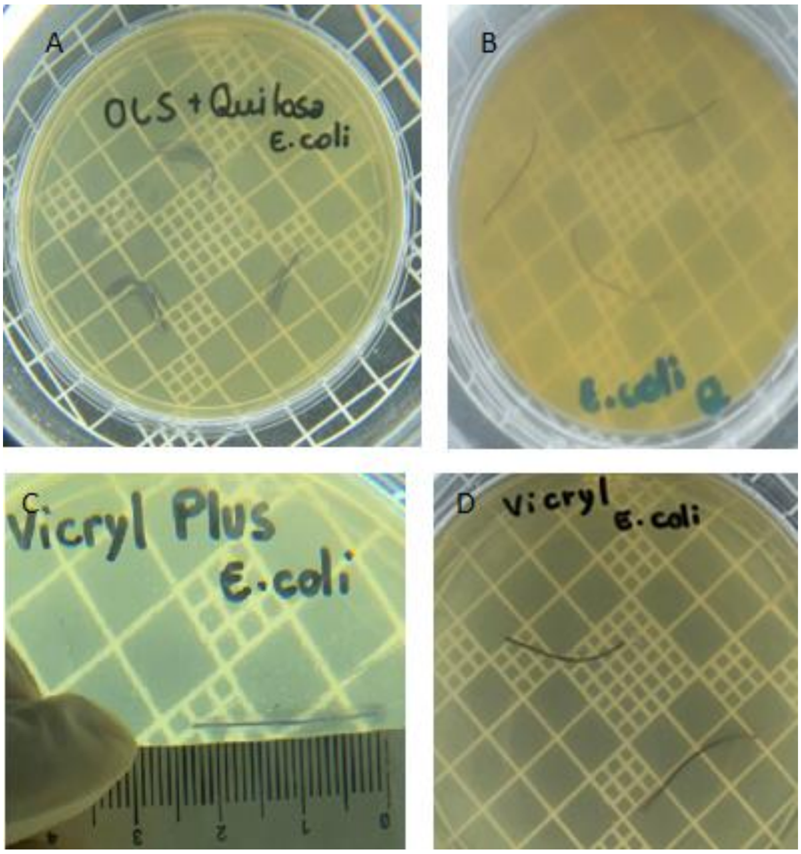

Figura 3 Measuring anti-bacterial efficacy of coated sutures via zones of inhibition against E.coli..A LSEOP 16 x 4 mm; B Vicryl only with chitosanl without zones of inhibition; C Polyglactin 910 with triclosan coated 23 x 2 mm and D Vicryl without halos of inhibition.

In order to simulate an oral cavity, the monospecies biofilm was performed. These results are presented in Fig. 4.

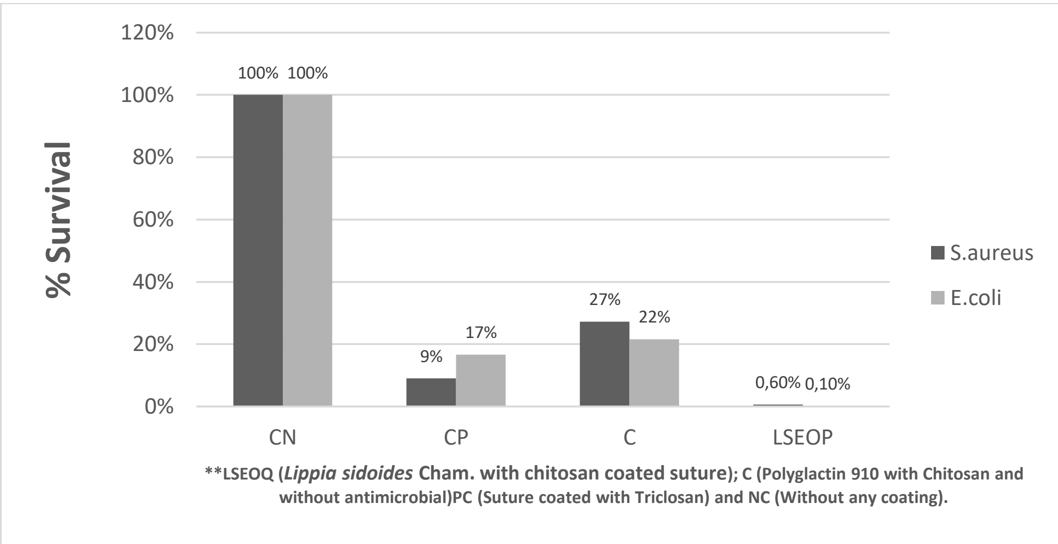

Figure 4 Mean cell survival rates (monospecies biofilm method) based on colony-forming units per mL with dilution of 10^{-4}.

Biocompatibility Study

This study was performed to evaluate the cytotoxicity of the LSEOP in VERO cells, according to ISO 10993-5:2009 (International Organization for Standardization, 2009). The percentage of cell viability resulted 87,36% and 84,23% for LSEOP 2 cm and 20 cm respectively. In addition, the commercial sutures Vycril® Plus with 2cm and 20 cm demonstrated cytocompatible effect with 77% and 86,31%, for Vycril® 83,03% and 81,27%, for Chitosan, 90,77% and 70% respectively (Figs. 5 and 6).

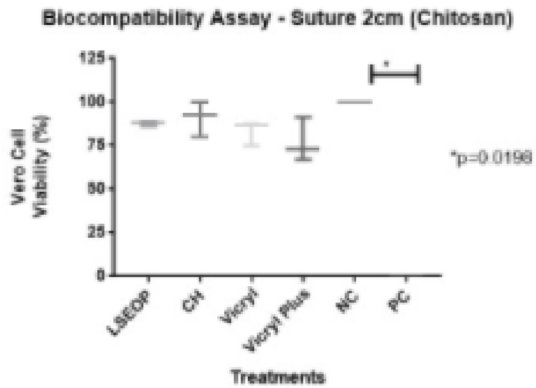

Figure 5. The viability averages of the Vero cells exposed to the treatment with the 2 cm suture. The Kruskal-Wallis test was conducted, followed by Dunn's test for multiple comparisons. LSEOP = suture coated with essential oil from *Lippia sidoides* Cham. and chitosan; CH= suture coated with chitosan; Vicryl = polyglactin 910 without coating; Vicryl plus = polyglactin 910 coated with triclosan; NC = negative control- cells only; PC = positive control with 10% DMSO.

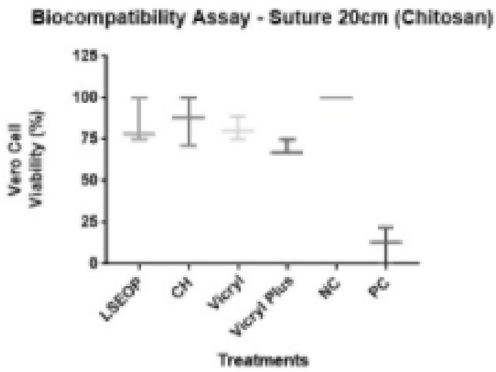

Figure 6. The viability averages of the Vero cells exposed to the treatment with the 20 cm suture. The Kruskal-Wallis test was conducted, followed by Dunn's test for multiple comparisons. LSEOP = suture coated with essential oil from *Lippia sidoides* Cham. and chitosan; CH= suture coated with chitosan; Vicryl =

polyglactin 910 without coating; Vicryl plus = polyglactin 910 coated with triclosan; NC = negative control- cells only; PC = positive control with 10% DMSO.

DISCUSSION

Sutures with antimicrobial coating should reduce SSI because they can inhibit bacterial growth(23). However, there is only one commercial antimicrobial suture, which is the polyglactin 910 with triclosan (Vycril® Plus).

Triclosan is a substance that is physiologically harmful, may result photolysis in carcinogenic products, as well as promoting resistance to multiple drugs (10,11,23–27). Hence, to minimize the ISC and substitute triclosan, coated sutures with another antimicrobial agents should be studied.

The search to replace the commercial suture thread containing triclosan has been growing in the pharmaceutical industries. Recently, a study demonstrated the effectiveness of suture threads containing *Lippia sidoides* Cham. essential oil against *S.aureus* and *E.coli* and their biocompatibility, but it showed a weakness: the volatility of the oil in the suture thread. Therefore, in this study we developed a chitosan wrapped in the LSEO sutures thread in order to minimize the volatility. The SEM test confirmed the success of the coated surgical suture with a natural polymer technique, because the chitosan evenly wrapped the suture thread with the essential oil.

HPLC confirmed the incorporation of essential oil to the suture thread, as it quantified a high level of thymol in all threads containing the essential oil of *Lippia sidoides* Cham, except for one that did not obtain a favorable storage form. However, the form of storage with little air changed the thymol concentration after approximately 60 days. Despite having decreased the final concentration of thymol after 60 days, the storage form with little air had a concentration of 125.11 µg/mL, a concentration which is considered to be very good since the MIC of *Lippia sidoides* essential oil against *S. aures* and *E.coli* is <15 µg/mL according to (ARTICLE 1*).

Chitosan is a natural polymer that is an excellent biocompatible and biodegradable mucoadhesive, with practically non-toxic properties to humans and animals (16,17). According to ARTICLE 1 * in the agar diffusion test against *S.aureus* and *E.coli* for sutures coated with essential oil of *Lippia sidoides* Cham.. It was 25 x 10 mm and 25 x 5 mm respectively. In this assay, we obtained an inhibition halo against *S.aureus* and *E.coli* for LSEOP of 22 x 4 mm and 16 x 4 mm, respectively. However, we did not obtain a halo of inhibition for both strains for the thread containing only the chitosan coating. Hence, that technique is not as effective to demonstrate the antimicrobial effect compared to other sutures thread coated with an antimicrobial agent without chitosan.

Inhibition agar diffusion study and the biofilm assay proved to be an excellent test to assess the antimicrobial effect when chitosan is incorporated. In the (ARTICLE 1*) study, also made with the same bacterial strains, it showed that polyglactin 910 coated with the essential oil of *Lippia sidoides* Cham. had a 18% and 30% of survival rate of bacteria for *S.aures* and *E.coli* respectively, while polyglactin 910 with triclosan (Vycril® Plus) obtained 9% and 17% of survival bacteria rate for the same strains. In our study, LSEOP had an excellent antimicrobial response, better when compared without the chitosan coating and the commercial suture that contain triclosan. LSEOP obtained 0.60% and 0.10% of survival rate of bacteria in a single species biofilm of S.aureus and E.coli respectively. The present findings demonstrate that sutures with LSEOP are promising materials against *S. aureus* and *E. coli*.

Biocompatibility tested with LSEOP and the polyglactin 910 with chitosan coated without antimicrobial agents were tested on VERO Cell Line. LSEOP and the others coated suture exhibited more that 70% cell viability. *Lippia sidoides* Cham showed low toxicity to mammalian cells and other studies with rats showed low toxicity ranging from 1 to 3 g/kg, did not induce any significant alterations (28,29). A study with rodent animal showed that chitosan also have low toxicity with an LD_{50} in rats of 16 g/kg(13).

CONCLUSIONS

This present study revealed that the LSEOP absorbable sutures may be an option to prevent SSI, by the reason of a high antibacterial potential and good biocompatibility.

ACKNOWLEDGEMENTS

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Belusic-Gobic M, Car M, Juretic M, Cerovic R, Gobic D, Golubovic V. Risk factors for wound infection after oral cancer surgery. Oral Oncol. 2007;43(1):77–81.

2. Sievert DM, Ricks P, Edwards JR, Schneider A, Patel J, Srinivasan A, et al. Antimicrobial-Resistant Pathogens Associated with Healthcare-Associated Infections Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009–2010. Infect Control Hosp Epidemiol [Internet]. 2013;34(01):1–14. Available from: https://www.cambridge.org/core/product/identifier/S0195941700031921/type/journal_article

3. Garner BH, Anderson DJ. Surgical Site Infections: An Update. Infect Dis Clin North Am [Internet]. 2016;30(4):909–29. Available from: http://dx.doi.org/10.1016/j.idc.2016.07.010

4. Venema S, Abbas F, Van De Belt-Gritter B, Van Der Mei HC, Busscher HJ, Van Hoogmoed CG. In vitro oral biofilm formation on triclosan-coated sutures in the absence and presence of additional antiplaque treatment. J Oral Maxillofac Surg [Internet]. 2011;69(4):980–5. Available from: http://dx.doi.org/10.1016/j.joms.2010.02.030

5. Katz S, Moderdechai I, David M. Katz1981.Pdf. J.B. Lippincott. 1981. p. 35–41.

6. Justinger C, Slotta JE, Ningel S, Gräber S, Kollmar O, Schilling MK. Surgical-site infection after abdominal wall closure with triclosan-impregnated polydioxanone sutures: Results of a randomized clinical pathway facilitated trial (NCT00998907). Surg (United States). 2013;154(3):589–95.

7. Justinger C, Moussavian MR, Schlueter C, Kopp B, Kollmar O, Schilling MK. Antibiotic coating of abdominal closure sutures and wound infection. Surgery [Internet]. 2009;145(3):330–4. Available from: http://dx.doi.org/10.1016/j.surg.2008.11.007

8. SenGupta M, Banerjee D, Sengupta M, Sarkar S, Nag S, Singh RM. In vitro efficacy of triclosan coated polyglactin 910 suture against common bacterial pathogen causing surgical site infection. Int J Infect Control [Internet]. 2014;10(2):2–7. Available from: http://www.ijic.info/article/view/11840

9. Yang Y, Yang SB, Wang YG, Zhang SH, Yu ZF, Tang TT. Bacterial inhibition potential of quaternised chitosan-coated VICRYL absorbable suture: An in vitro and in vivo study. J Orthop Transl [Internet]. 2017;8:49–61. Available from: http://dx.doi.org/10.1016/j.jot.2016.10.001

10. Han J, Won EJ, Hwang UK, Kim IC, Yim JH, Lee JS. Triclosan (TCS) and Triclocarban (TCC) cause lifespan reduction and reproductive impairment through oxidative stress-mediated expression of the defensome in the monogonont rotifer (Brachionus koreanus). Comp Biochem Physiol Part - C Toxicol Pharmacol [Internet]. 2016;185–186:131–7. Available from: http://dx.doi.org/10.1016/j.cbpc.2016.04.002

11. Wang X, Chen X, Feng X, Chang F, Chen M, Xia Y, et al. Triclosan causes spontaneous abortion accompanied by decline of estrogen sulfotransferase activity in humans and mice. Sci Rep. 2015;5(November):1–11.

12. Bilia AR, Guccione C, Isacchi B, Righeschi C, Firenzuoli F, Bergonzi MC. Essential oils loaded in nanosystems: A developing strategy for a successful therapeutic approach. Evidence-based Complement Altern Med. 2014;2014.

13. Candy T, Sharma CP. CHITOSAN - AS A BIOMATERIAL Thomas. Biohat, Art Cells, Art Org. 1990;18(1):1–24.

14. Aspden TJ, Mason JDT, Jones NS, Lowe J, Skaugrud Ø, Illum L. Chitosan as a nasal delivery system: The effect of chitosan solutions on in vitro and in vivo mucociliary transport rates in human turbinates and volunteers. J Pharm Sci. 1997;86(4):509–13.

15. Rao SB, Sharma CP. Use of chitosan as a biomaterial: Studies on its safety and hemostatic potential. J Biomed Mater Res. 1997;34(1):21–8.

16. Giacomello E, Sava G, Vita F, Delhom N, Mahl P, Bergamo A. Chitosan-coated alginate micro-particles delivery of active principles through conventional pelleted food - A study in Tilapia (Oreochromis niloticus). Int J Biol Macromol [Internet]. 2020;165:82–92. Available from: https://doi.org/10.1016/j.ijbiomac.2020.09.162

17. Mao S, Sun W, Kissel T. Chitosan-based formulations for delivery of DNA and siRNA. Adv Drug Deliv Rev [Internet]. 2010;62(1):12–27. Available from: http://dx.doi.org/10.1016/j.addr.2009.08.004

18. Palhares Neto L, de Souza LM, de Morais MB, Arruda E, de Figueiredo RCBQ, de Albuquerque CC, et al. Morphophysiological and Biochemical Responses of Lippia grata Schauer (Verbenaceae) to Water Deficit. J Plant Growth Regul [Internet]. 2020;39(1):26–40. Available from:

https://doi.org/10.1007/s00344-019-09961-6

19. Muniz VM. Desenvolvimento de Micropartícula contendo Clorexidina e Timol [Internet]. Vol. 10, UNIVERSIDADE FEDERAL DE PERNAMBUCO. 2018. Available from: http://dx.doi.org/10.1053/j.gastro.2014.05.023%0Ahttps://doi.org/10.1016/j.gie.2018.04.013%0Ahttp://www.ncbi.nlm.nih.gov/pubmed/29451164%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5838726%250Ahttp://dx.doi.org/10.1016/j.gie.2013.07.022

20. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement - document M100S22 [Internet]. Vol. 32, Clinical and Laboratory Standard Institute (CLSI). 2012. 188 p. Available from: http://www.facm.ucl.ac.be/intranet/CLSI/CLSI-M100S22-susceptibility testing-2012-original.pdf

21. Iñiguez-Moreno M, Gutiérrez-Lomelí M, Guerrero-Medina PJ, Avila-Novoa MG. Biofilm formation by Staphylococcus aureus and Salmonella spp. under mono and dual-species conditions and their sensitivity to cetrimonium bromide, peracetic acid and sodium hypochlorite. Brazilian J Microbiol. 2018;49(2):310–9.

22. Strober W. Trypan Blue Exclusion Test of Cell Viability. Curr Protoc Immunol. 2015;111(1):A3.B.1-A3.B.3.

23. Obermeier A, Schneider J, Föhr P, Wehner S, Kühn KD, Stemberger A, et al. In vitro evaluation of novel antimicrobial coatings for surgical sutures using octenidine. BMC Microbiol [Internet]. 2015;15:186. Available from: http://dx.doi.org/10.1186/s12866-015-0523-4

24. Fidelis MZ. DEGRADAÇÃO DE TRICLOSAN E 2 , 8-DICLORODIBENZENO-p- DIOXINA VIA SISTEMA Fe / Nb 2 O 5 / UV. 2019;1–92.

25. Niu J, Dai Y, Yin L, Shang J, Crittenden JC. Photocatalytic reduction of triclosan on Au-Cu2O nanowire arrays as plasmonic photocatalysts under visible light irradiation. Phys Chem Chem Phys [Internet]. 2015;17(26):17421–8. Available from: http://dx.doi.org/10.1039/C5CP02244D

26. Kanetoshi A, Katsura E, Ogawa H, Ohyama T, Kaneshima H, Miura T. Acute toxicity, percutaneous absorption and effects on hepatic mixed function oxidase activities of 2,4,4′-trichloro-2′-hydroxydiphenyl ether (Irgasan® DP300) and its chlorinated derivatives. Arch Environ Contam Toxicol. 1992;23(1):91–8.

27. 山口悠, 口田圭吾. No Title消費者型官能評. 日本畜産学会報 [Internet]. 2013;84(december):487–92. Available from: http://ir.obihiro.ac.jp/dspace/handle/10322/3933

28. Fontenelle ROS, Morais SM, Brito EHS, Kerntopf MR, Brilhante RSN, Cordeiro RA, et al. Chemical composition, toxicological aspects and antifungal activity of essential oil from Lippia sidoides Cham. J Antimicrob

Chemother. 2007;59(5):934–40.

29. de Medeiros M das GF, da Silva AC, Citó AM das GL, Borges AR, de Lima SG, Lopes JAD, et al. In vitro antileishmanial activity and cytotoxicity of essential oil from Lippia sidoides Cham. Parasitol Int [Internet]. 2011;60(3):237–41. Available from: http://dx.doi.org/10.1016/j.parint.2011.03.004

4. CONSIDERAÇÕES GERAIS

A presente pesquisa visou apresentar a avaliação dos efeitos antimicrobianos do óleo essencial de *Lippia sidoides* Cham. em fio de sutura cirúrgica contra duas espécies de microrganismos, bem como avaliar seu possível efeito citotóxico *in vitro* utilizando-se células VERO. Durante o desenvolvimento da pesquisa e da etapa de revestimento, analisamos através do HPLC a presença do composto majoritário, o timol, confirmando a impregnação do óleo no fio de sutura (LSEO e LSEOP).

Devido ás questões de volatilidade do óleo e potencial perda durante o armazenamento, foi adaptado novo revestimento com um polímero natural que, no qual melhorou sua ação antimicrobiana, bem como sua biocompatibilidade em células de mamíferos. Dessa forma, considerando o nível de maturidade tecnológica do produto, podemos inferir que nesse estudo o produto atinge o nível 3 de TRL/MRL (estudos analíticos e/ou laboratoriais que verificam se a tecnologia é viável e pronta para prosseguir para o processo de desenvolvimento). No entanto, ensaios pré-clínicos são necessários para confirmar a segurança do uso destes revestimentos *in vivo*, bem como utilizando novo espectro de ação antimicrobiana.

5. CONCLUSÕES

- Todos os fios de sutura desenvolvidos com o agente antimicrobiano (óleo de *Lippia sidoides* Cham.) com e sem revestimento de polímero natural demonstraram ter uma ação antimicrobiana eficaz contra microrganismos encontrados frequentemente nas feridas cirúrgicas.
- De acordo com ISO 10993- 5:2009 os fios de sutura desenvolvidos possuem biocompatibilidade frente às células VERO. Portanto, essas suturas revestidas representam uma alternativa eficaz para substituição dos fios de sutura com triclosan, principalmente para casos de resistência e/ou efeitos adversos com esta substância.

REFERÊNCIAS*

1. CDC, Oid, Ncezid, DHQP. 9 Surgical Site Infection (SSI) Event. 2020;(January):1–36. Available from: https://www.cdc.gov/nhsn/pdfs/ps-analysis-

2. Garner BH, Anderson DJ. Surgical Site Infections: An Update. Infect Dis Clin North Am [Internet]. 2016;30(4):909–29. Available from: http://dx.doi.org/10.1016/j.idc.2016.07.010

3. Ruiz Tovar J, Badia JM. Prevention of Surgical Site Infection in Abdominal Surgery. A Critical Review of the Evidence. Cirugía Española (English Ed [Internet]. 2014;92(4):223–31. Available from: http://dx.doi.org/10.1016/j.cireng.2013.08.003

4. Sievert DM, Ricks P, Edwards JR, Schneider A, Patel J, Srinivasan A, et al. Antimicrobial-Resistant Pathogens Associated with Healthcare-Associated Infections Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009–2010. Infect Control Hosp Epidemiol [Internet]. 2013;34(01):1–14. Available from: https://www.cambridge.org/core/product/identifier/S0195941700031921/type/journal_article

5. SenGupta M, Banerjee D, Sengupta M, Sarkar S, Nag S, Singh RM. In vitro efficacy of triclosan coated polyglactin 910 suture against common bacterial pathogen causing surgical site infection. Int J Infect Control [Internet]. 2014;10(2):2–7. Available from: http://www.ijic.info/article/view/11840

6. Edlich RF, Gubler K, Wallis AG, Clark JJ, Dahlstrom JJ, Long WB 3rd. Wound closure sutures and needles: a new perspective. J Environ Pathol Toxicol Oncol Off organ Int Soc Environ Toxicol Cancer [Internet]. 2010;29(4):339–61. Available from: http://www.begellhouse.com/download/articles/35e05d6477309b76/JEP2904%28339-361%29.pdf%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/21284597

7. Goel A. Surgical Sutures - A Review. Delhi J Ophthalmol. 2016;26(3):159–62.

8. Maksoud M, Koo S, Barouch K, Karimbux N. Popularity of suture materials among residents and faculty members of a postdoctoral periodontology program. J Investig Clin Dent. 2014;5(1):45–50.

9. Azimi B, Nourpanah P, Rabiee M, Arbab S. Poly(lactide-co-glycolide) Fiber: An Overview. J Eng Fiber Fabr. 2014;9(1):47–66.

10. Aluko-Olokun B, Olaitan AA, Aluko-Olokun OA. Reconstruction of tooth-bearing portion of mandible using polyglactin 910 sutures for internal fixation in the third-world: functional and cosmetic outcome. Oral Maxillofac Surg. 2017;21(1):13–20.

11. Chandan S, Halli R, Joshi S, Chhabaria G, Setiya S. Transosseous fixation of pediatric displaced mandibular fractures with polyglactin resorbable suture - A simplified technique. J Craniofac Surg. 2013;24(6):2050–2.

12. Guo J, Pan LH, Li YX, Yang X Di, Li LQ, Zhang CY, et al. Efficacy of triclosan-coated sutures for reducing risk of surgical site infection in adults: A meta-analysis of randomized clinical trials. J Surg Res [Internet]. 2016;201(1):105–17. Available from: http://dx.doi.org/10.1016/j.jss.2015.10.015

13. Justinger C, Slotta JE, Ningel S, Gräber S, Kollmar O, Schilling MK. Surgical-site infection after abdominal wall closure with triclosan-impregnated polydioxanone sutures: Results of a randomized clinical pathway facilitated trial (NCT00998907). Surg (United States). 2013;154(3):589–95.

14. Han J, Won EJ, Hwang UK, Kim IC, Yim JH, Lee JS. Triclosan (TCS) and Triclocarban (TCC) cause lifespan reduction and reproductive impairment through oxidative stress-mediated expression of the defensome in the monogonont rotifer (Brachionus koreanus). Comp Biochem Physiol Part - C Toxicol Pharmacol [Internet]. 2016;185–186:131–7. Available from: http://dx.doi.org/10.1016/j.cbpc.2016.04.002

15. Obermeier A, Schneider J, Föhr P, Wehner S, Kühn KD, Stemberger A, et al. In vitro evaluation of novel antimicrobial coatings for surgical sutures using octenidine. BMC Microbiol [Internet]. 2015;15:186. Available from: http://dx.doi.org/10.1186/s12866-015-0523-4

16. Wang X, Chen X, Feng X, Chang F, Chen M, Xia Y, et al. Triclosan causes spontaneous abortion accompanied by decline of estrogen sulfotransferase activity in humans and mice. Sci Rep. 2015;5(November):1–11.

17. Niu J, Dai Y, Yin L, Shang J, Crittenden JC. Photocatalytic reduction of triclosan on Au-Cu2O nanowire arrays as plasmonic photocatalysts under visible light irradiation. Phys Chem Chem Phys [Internet]. 2015;17(26):17421–8. Available from: http://dx.doi.org/10.1039/C5CP02244D

18. Kanetoshi A, Katsura E, Ogawa H, Ohyama T, Kaneshima H, Miura T. Acute toxicity, percutaneous absorption and effects on hepatic mixed function oxidase activities of 2,4,4′-trichloro-2′-hydroxydiphenyl ether (Irgasan® DP300) and its chlorinated derivatives. Arch Environ Contam Toxicol. 1992;23(1):91–8.

19. Fidelis MZ. DEGRADAÇÃO DE TRICLOSAN E 2 , 8-DICLORODIBENZENO-p-DIOXINA VIA SISTEMA Fe / Nb 2 O 5 / UV. 2019;1–92.

20. Agência Nacional de Vigilância Sanitária – ANVISA. Resolução De Diretoria Colegiada - Rdc Nº 530, De 4 De Agosto De 2021. 2021;2021:1–46. Available from: http://antigo.anvisa.gov.br/documents/10181/5284308/RDC_530_2021_.pdf/9af17f17-eb62-425d-b04f-bb6acf429400

21. Fowler JR, Perkins TA, Buttaro BA, Truant AL. Bacteria adhere less to barbed monofilament than braided sutures in a contaminated wound model infection. Clin Orthop Relat Res. 2013;471(2):665–71.

22. Pelz K, Tödtmann N, Otten JE. Comparison of antibacterial-coated and non-coated suture material in intraoral surgery by isolation of adherent bacteria. Ann Agric Environ Med. 2015;22(3):551–5.

23. Tabrizi R, Mohajerani H, Bozorgmehr F. Polyglactin 910 suture compared with polyglactin 910 coated with triclosan in dental implant surgery: randomized clinical trial. Int J Oral Maxillofac Surg [Internet]. 2019;48(10):1367–71. Available from: https://doi.org/10.1016/j.ijom.2019.01.011

24. Oliveira FP de, Lima E de O, Siqueira Júnior JP de, Souza EL de, Santos BHC, Barreto HM. Effectiveness of Lippia sidoides Cham. (Verbenaceae) essential oil in inhibiting the growth of Staphylococcus aureus strains isolated from clinical material. Rev Bras Farmacogn [Internet]. 2006;16(4):510–6. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-695X2006000400013&lng=en&nrm=iso&tlng=en

25. Saúde A. MS elabora Relação de Plantas Medicinais de Interesse ao SUS 06/03/2009. MS Elabor Relação Plantas Med Interess ao SUS [Internet]. 2009;3–5. Available from: http://bvsms.saude.gov.br/bvs/sus/pdf/marco/ms_relacao_plantas_medicinais_sus_0603.pdf

26. Fontenelle ROS, Morais SM, Brito EHS, Kerntopf MR, Brilhante RSN, Cordeiro RA, et al. Chemical composition, toxicological aspects and antifungal activity of essential oil from Lippia sidoides Cham. J Antimicrob Chemother. 2007;59(5):934–40.

27. Bilia AR, Guccione C, Isacchi B, Righeschi C, Firenzuoli F, Bergonzi MC. Essential oils loaded in nanosystems: A developing strategy for a successful therapeutic approach. Evidence-based Complement Altern Med. 2014;2014.

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* De acordo com as normas do PPGO/UFPB, baseadas na norma do International Committee of Medical Journal Editors - Grupo de Vancouver. Abreviatura dos periódicos em conformidade com o Medline.

ANEXO I

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso
Cadastro nº ABDE352

A atividade de acesso ao Patrimônio Genético/CTA, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**ABDE352**
Usuário:	**FABIO SAMPAIO**
CPF/CNPJ:	**526.970.964-34**
Objeto do Acesso:	**Patrimônio Genético/CTA**
Finalidade do Acesso:	**Pesquisa**

Espécie

Lippia sidoides

lippia para higiene bucal

Fonte do CTA

CTA de origem não identificável

Título da Atividade:	**Pesquisas com oleos de Lippia sidoides**

Equipe

FABIO SAMPAIO	**Universidade Federal da Paraiba**

Resultados Obtidos

Requerimento de propriedade intelectual

Órgão no qual foi requerido:	**INPI**
Código do requerimento:	**10**
Número da patente:	**BR 10 2018 010699 6**
Órgão no qual foi requerido:	**INPI**
Código do requerimento:	**10**
Número da patente:	**BR 10 2017 018692 0**
Órgão no qual foi requerido:	**INPI**
Código do requerimento:	**10**
Número da patente:	**BR 10 2018 012597 4**
Órgão no qual foi requerido:	**INPI**
Código do requerimento:	**10**
Número da patente:	**BR 10 2018 0689240**
Órgão no qual foi requerido:	**INPI**
Código do requerimento:	**10**
Número da patente:	**BR 10 2018 012597 4**

Divulgação de resultados em meios científicos ou de comunicação

Identificação do meio onde foi divulgado:	**Teses, dissertações e revistas científicas**

Data do Cadastro:	**05/11/2018 21:37:09**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **21:39** de **05/11/2018**.

SISTEMA NACIONAL DE GESTÃO
DO PATRIMÔNIO GENÉTICO
E DO CONHECIMENTO TRADICIONAL
ASSOCIADO - SISGEN

ANEXO II

Biomaterials Science

Antimicrobial and biocompatibility evaluation of sutures containing essential oil from Lippia sidoides Cham.

Journal:	*Biomaterials Science*
Manuscript ID	BM-ART-12-2021-001882
Article Type:	Paper
Date Submitted by the Author:	07-Dec-2021
Complete List of Authors:	de Albuquerque, Lília; Universidade Federal da Paraiba, Postgraduate Program in Dentistry DE SOUZA, JOELMA ; Universidade Federal da Paraíba, Department of Physiology and Pathology FARIAS, ISABELA ; Universidade Federal da Paraíba, Department of Restorative Dentistry DA SILVA, ALAN ; Universidade Federal da Paraiba, Undergraduate student in Biomedicine DANTAS, VANESSA; Instituto Aggeu Magalhães, Postgraduate Program in Biosciences and Biotechnology in Health MUNIZ, VANESSA ; Universidade Federal da Paraíba, Postgraduate student in Drug Development and Technological Innovation DOS SANTOS, ALESSANDRA; Ebserh, Hospital Universitário Lauro Wanderley Sampaio, F. C. ; Universidade Federal da Paraiba Centro de Ciencias da Saude, Department of Clinical and Social Dentistry

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