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Sutures modified by incorporation of chlorhexidine and cinnamaldehyde: anti-*Candida* effect, bioavailability and mechanical properties

Suturas modificadas pela incorporação de clorexidina e cinamaldeído: efeito anti-*Candida*, biodisponibilidade e propriedades mecânicas

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Resumo

Introdução: Fios de suturas com antimicrobianos são uma alternativa terapêutica para o controle de infecções orais. **Objetivo:** Incorporar Clorexidina (CHX) e Cinamaldeído (CN) em fios de sutura e avaliar o efeito anti-*Candida*, liberação de antimicrobianos e as propriedades mecânicas. **Material e método:** Fios de Seda (S) e Poliglactina 910 (P) foram seccionadas assepticamente (20 mm) e imersos para incorporação em CHX a 0,12%, CN a 0,4% e solução fisiológica a 0,9% sob agitação por 60 minutos (n = 10 / grupo). Suspensões de 500 µL de *Candida albicans* (ATCC 90028/1 × 106 UFC / mL) foram utilizadas para avaliar a aderência fúngica após o período de 48 horas a 37 ° C. A liberação de CLX e CN foi avaliada em 0, 24 e 48 horas (n = 3 / grupo) por espectrofotômetro UV-VIS (275 nm). A resistência à tração e o deslocamento (n = 5 / grupo) foram avaliados após a incorporação (30 mm / min, 50N). Os dados foram analisados por Anova e Tukey (α = 5%). **Resultado:** Não foi observado efeito anti-*Candida* nas suturas S e P incorporadas com CLX e CN (p> 0,05). No entanto, a liberação progressiva foi verificada até 48 após o tratamento com CLX (S = 0,075 / P = 0,073 µg / mL) e CN (S = 35,33 / P= 5,72 µg / mL). Houve uma diminuição na resistência à tração em S (CLX = 9,9 / CN = 9,9 N) e P (CLX = 14,4 / CN = 15,5 N) (p <0,05). Não foram observadas diferenças para o deslocamento para S (CLX = 19,3 / CN=20,7 mm) e P (CLX = 16,2 /CN= 15,8 mm) (p> 0,05). **Conclusão:** A incorporação de CLX e CN não teve efeito positivo sobre as propriedades biológicas e mecânicas das suturas avaliadas.

Descritores: Suturas; *Candida albicans*; libertação de drogas; resistência à tração.

Abstract

Introduction: Antimicrobial sutures are a therapeutic alternative for the control of oral infections. **Objective:** Incorporate Chlorhexidine (CHX) and Cinnamaldehyde (CN) in sutures and evaluate the anti-*Candida* effect, release of antimicrobials and mechanical properties. **Material and method:** Silk (S) and Polyglactin 910 (P) sutures were aseptically sectioned (20 mm) and immersed for incorporation in 0.12% CHX, 0.4% CN and 0.9% saline solutions under stirring for 60 minutes (n = 10 / group). Suspensions of 500 µL of *Candida albicans* (ATCC 90028/ 1 × 10^6 CFU/mL) were used to evaluate fungal adhesion after the 48 h period at 37°C. The release of CLX and CN were evaluated at 0, 24 and 48 hours (n=3/group) by UV-VIS spectrophotometer (275 nm). The tensile strength and displacement (n=5/group) were evaluated after incorporation (30 mm/min, 50N). Data were analyzed by Anova and Tukey (α = 5%). **Result:** No anti-*Candida* effect was observed on S and P sutures incorporated with CLX and CN (p>0.05). However, progressive release was verified up to 48 after treatment with CLX (S = 0.075 / P = 0.073 µg/mL) and CN (S = 35.33 /P= 5.72 µg/mL). There was a decrease in tensile strength in S (CLX = 9.9 / CN = 9.9 N) and P (CLX

= 14.4 / CN = 15.5 N) ($p<0.05$). No differences were observed for the displacement for S (CLX = 19.3 / CN=20.7 mm) and P (CLX = 16.2 / CN=15.8 mm) ($p>0.05$). **Conclusion:** The incorporation of CLX and CN did not have a positive effect on the biological and mechanical properties of the sutures evaluated.

Descriptors: Sutures; *Candida albicans*; drug liberation; tensile strength.

INTRODUCTION

Surgical wound repair is a complex physiological process that includes inflammation, cell proliferation, matrix deposition and tissue remodeling[1]. Sutures are materials of first choice for the synthesis of these surgical sites because they exert less influence in the healing stages and promote tissue repair by first intention[2]. The main problem with surgical sutures is the deposition of biofilm on the surface and direct contact with the wound, increasing the risk of surgical site infections and altering the quality of the scar tissue[3].

Surgical incisions in the oral cavity are even more vulnerable to infections, occurring in 2 to 12% of surgeries[2,4]. Some factors, such as the diversity of microorganisms, the presence of saliva, rich vascularization, contamination by food residues and trauma resulting from speech and hygiene increase the inflammatory process, the healing time and the discomfort of the patient[5]. In addition, removal of the suture in infectious areas may lead to bacteremia which is a risk factor for the development of bacterial endocarditis in high-risk patients[6].

The oral mucosa when covered by acrylic surfaces of dental prostheses as in pre-prosthetic, peri-implant or immediate rehabilitation surgeries also becomes susceptible to fungal infections[7]. *Candida albicans* is the main pathogen involved in oral fungal infections due to the ability to colonize biotic and abiotic surfaces, promoting epithelial invasion, and may lead to systemic complications, mainly in immunosuppressed patients[8]. The interaction of bacteria and *Candida* inside biofilms is increasingly proven[7,9], however, the role of fungi in the progression of inflammation and in the prognosis of oral infections remains uncertain.

In the literature, *in vitro* studies[2,3,10], *in vivo*[11,12] and clinical trials[13,14] analyzed the adherence of bacteria present in the oral cavity in sutures. Moreover, there are not still studies that considered the adhesion of C. albicans and the antifungal effect of coatings on surgical sutures under conditions that simulate the oral environment.

In addition, many studies have proposed various methods to develop antimicrobial sutures by using antimicrobial agents[15,16]. Cinnamaldehyde (C_9H_8O), a phytoconstituent extracted from cinnamon essential oil, has demonstrated antibacterial and antifungal effects in previous studies with cell viability assays[17,18] and reduced cytotoxicity in epithelial models of oral mucosa[9]. The use of cinnamaldehyde is considered a promising alternative for the control of the *C. albicans* biofilm, and for this reason the incorporation of this antimicrobial into sutures could be a viable alternative for use in oral surgeries in areas prone to infections and this association not been yet investigated.

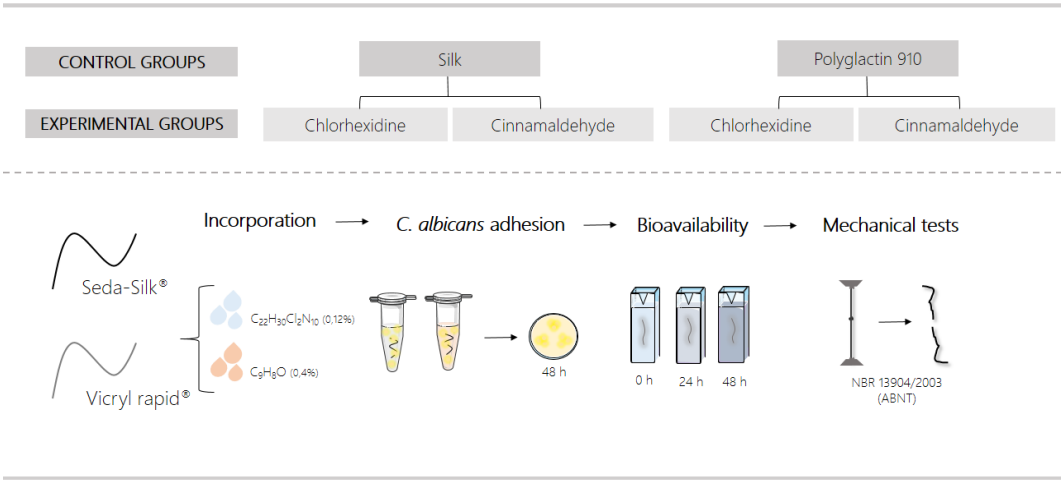
Therefore, the aim of this study was to perform the incorporation of chlorhexidine and cinnamaldehyde into sutures and to evaluate the anti-*Candida* effect, the release of these antimicrobials and the influence of this incorporation on the mechanical properties of the sutures.

MATERIAL AND METHOD

Preparation and Incorporation of the Samples

The schematic diagram shows of the experimental design this study (Scheme 1). Two groups of sutures were used synthetic biodegradable - Polyglactin 910 (Vicryl-rapid®, Ethicon, USA) and

other nonbiodegradable - Silk (Seda-Silk®, Ethicon, USA) with same thickness and quantity of filaments (3.0). These materials were selected because they are the most used in oral surgeries. The antimicrobial substances used for incorporation into the sutures were Chlorhexidine Gluconate 0.12% (PerioGard®, Colgate-Palmolive, New York, USA) and Cinnamaldehyde 0.4% (Sigma-Aldrich®, São Paulo, Brazil) as specified in Table 1.



Scheme 1. Schematic diagram of the experimental design[19].

Table 1. Morphological and commercial characterization of the sutures and substances evaluated

Commercial name	Thickness	Material	Configuration	Lot number
Seda-Silk[a]	3-0	Black Silk	Multifilamentado	AE0068
Vicryl rapid[a]	3-0	Polyglactin 910	Multifilamentado	524605
Commercial name	**Composition**	**Substance**	**Concentration**	**Lot number**
PerioGard[b]	Liquid	Chlorhexidine Gluconate	0.12%	5030BR121A
Cinnamaldeido[c]	Liquid	Cinnamaldehyde	0.4%	MFCD00007000

[a]Ethicon US LLC®, Cincinnati, USA[20]; [b]Colgate-Palmolive Company®, New York, USA[21]; [c]Sigma-Aldrich®, São Paulo, Brazil[22]

The silk and polyglactin 910 sutures were sectioned in 20 mm length samples under aseptic conditions and with tweezers, scissors and sterile millimeter ruler. Subsequently, the samples were immersed in 500 µl of the test-solutions[15]. These samples were separately deposited in 0.12% chlorhexidine, 0.4% cinnamaldehyde and 0.9% saline solution (control), remaining under stirring on a vibratory table for 60 minutes for homogeneous incorporation of the substances in all parts.

Anti-*Candida albicans* Effect

After incorporation, the samples (n = 30/group) were inserted into 500 µL of brain and heart infusion broth (BHI Broth, Difco®, Rio de Janeiro, RJ, Brazil) with a suspension of *C. albicans* (ATCC 90028) standardized at the optical density of 0.1 to 600 nm, equivalent to $1x10^6$ colony forming units per milliliter (CFU/mL). Then the samples were incubated in aerobiose at 37 °C for a period of 48 hours. After incubation, the samples were removed immersed in saline solution and shaken for 30 seconds in vortex (Phoenix®, São Paulo, SP, Brazil) for the detachment of cells adhered to the sutures. Serial dilutions (10^{-1} to 10^{-5}) were performed for further evaluation of cell viability by the drop technique. Subculture of the serial dilutions was performed on Saburaund agar plates (Difco®, Rio de Janeiro, RJ, Brazil) and the number of viable microorganisms was determined after

24 h of incubation. All microbiological assays were performed in triplicate in three independent experiments.

Antimicrobial Release

The samples (n=9/group) were distributed in a 24-well plate for cell culture (Zellkultur Testplatte 24®; Trasadingen, Switzerland) containing 1 ml of 0.9% NaCl. The release was measured by the analysis of the change in optical density obtained by divided beam UV/Vis spectrometry (BioDrop®, São Paulo, Brazil), using a wavelength of 275 nm, with volume of 2 µL, and readings were taken immediately after the incorporation (t_0) and after 24 hours (t_1) and 48 hours (t_2). Based on previously established linear calibration, the amount of antimicrobial (µg) released in each 1 mL was measured (µg/mL). The assays were performed in triplicate to verify the highest release peaks of the incorporated agents.

Mechanical Tests

The samples (n=5/group) were submitted to mechanical tests of tensile strength on node pull and displacement after incorporation with antimicrobials. The parameters described in standard NBR 13904:2003[19] of the Brazilian Association of Technical Standards (ABNT) were followed for mechanical tests and control of traction results. The analyzes were performed in a universal machine of mechanical tests (Shimadzu®, Kyoto, Japan) with a speed of 30 mm/min and a load cell of 50 N (Recommended parameters for 3.0 diameter sutures). A previously trained researcher, according to standardization, reproduced a surgical node and, later, the wire was positioned in the machine. Initially, the knot was positioned equidistant from the claws, with the ends making 3 turns around the fixing cylinder, establishing a distance of 50 cm between them. In this condition, the device was activated generating force (N) and displacement (mm) values until the wire broke. These parameters were evaluated to verify the influence of the treatment on surgical node rupture (tensile strength) and manipulation and dehiscence of the sutures (displacement).

Data Analysis

The data were analyzed by statistical software SPSS (IBM SPSS Statistics v. 21.0; IBM-Corp). The variables were submitted to the normality and homoscedasticity test (Shapiro-Wilk; α=5%). The tested sutures present specific clinical indications and for this reason the analysis of variance (ANOVA 1-way; α=5%) was used only for intra-group comparison of the effect of the incorporated substances (chlorhexidine and cinnamaldehyde) in the UFC (*C. albicans*) data, the tensile strength and the displacement of each group. Tukey test (HSD) was used as a post hoc for multiple paired comparison (α = 5%). The antimicrobial release was analyzed descriptively.

RESULT

In the analysis of fungal cell viability (CFU/mL), there was no difference in the adhesion of C. albicans on silk and polyglactin 910 sutures incorporated with chlorhexidine and cinnamaldehyde in relation to their respective controls (p>0.05) (Figure 1).

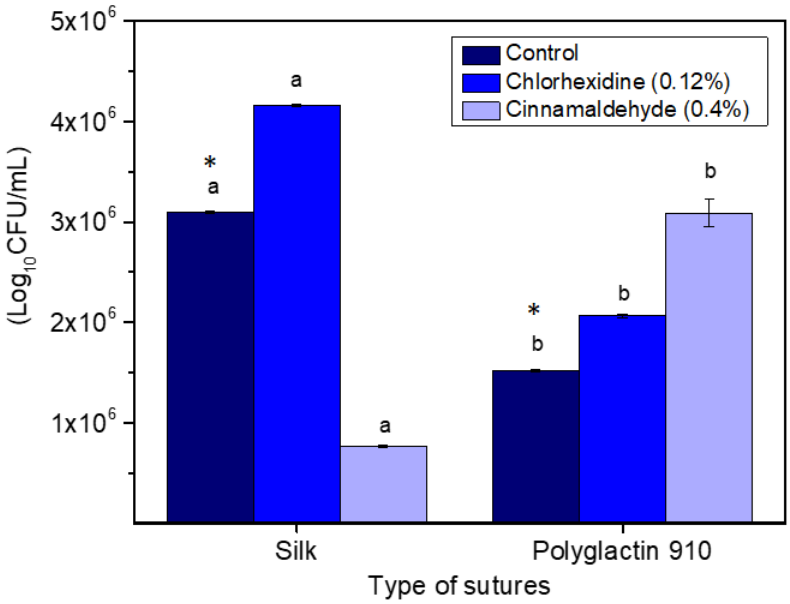


Figure 1. Colony forming units (Log_{10}CFU/mL) of sutures incorporated with antimicrobials. *Non-significant differences (p>0.05).

With regard to the release of antimicrobials, these were incorporated and presented constant and increasing release in the periods evaluated, with higher release peaks after 48 hours of incorporation. For both materials (Silk and Polyglactin 910), the released cinnamaldehyde concentrations were higher when compared to the chlorhexidine concentration in the respective groups and evaluation periods (Figure 2).

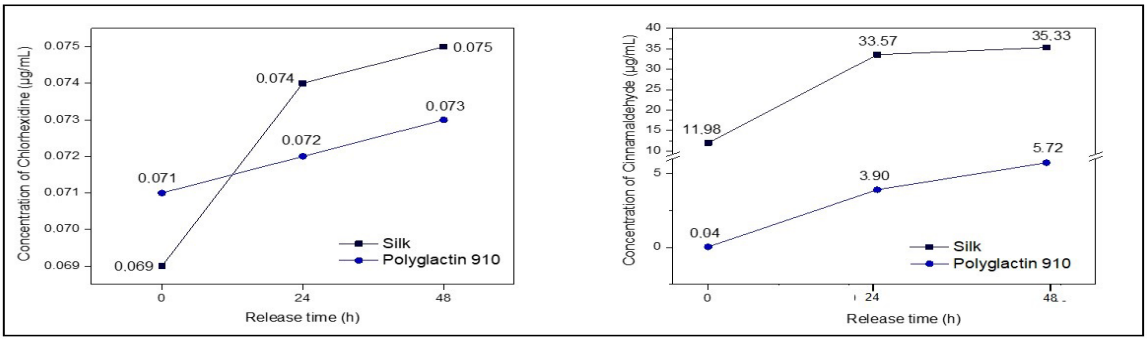


Figure 2. Graphical distribution of the release of chlorhexidine 0.12% and cinnamaldehyde 0.4% in 0, 24 and 48 hours after incorporation into silk (S) and polyglactin 910 (P) sutures.

The mechanical tests demonstrated a reduction of the rupture force after incorporation of the antimicrobials to the sutures when compared with the respective control groups (p<0.05). However, displacement values were not influenced by incorporation, with no difference between chlorhexidine and cinnamaldehyde for Silk and Polyglactin 910 (p>0.05) (Table 2).

Table 2. Tensile strength (mean and standard deviation) and displacement of sutures after incorporation with antimicrobials

	Experimental groups					
	Silk (S)			Polyglactin 910 (P)		
	Control	Chlorhexidine 0.12%	Cinnamaldehyde 0.4%	Control	Chlorhexidine 0.12%	Cinnamaldehyde 0.4%
Tensile strength (N)	13.3^{A*}	9.9 ±0.4^{B}	9.9 ±0.4^{B}	17.7^{C}	14.4 ±0.7^{D}	15.5±0.4^{D}
Displacement (mm)	-**	19.3±1.7	20.7±3.3	-	16.2±1.3	15.8±1.2

*Similar capital letters on the same line indicate statistically significant values ($p < 0.05$); ** Reference values for displacement tests are missing from ABNT standard (NBR 13904:2003)[19].

DISCUSSION

The present study demonstrated that it is possible to incorporate antimicrobials into sutures by the immersion method with constant release of the substances for up to 48 hours. Different from other studies it was considered the indication of the material and the time required for incorporation, availability and adhesion of the *C. albicans* biofilm, in order to simulate an oral clinical condition. In addition, it is the first study that to use cinnamaldehyde as a possible clinical alternative in preoperative antisepsis of sutures to be used at surgical sites susceptible to fungal infections.

In view of the peculiarities of the oral cavity and the absence of an ideal suture the use of antimicrobial solutions for mouthwash is among the main postoperative recommendations[2]. Chlorhexidine gluconate (0.12%) is the most indicated antimicrobial because it has a broad spectrum of action[6,15]. However, the dose-response effect through indiscriminate use may affect the viability of epithelial cells and fibroblasts, interfering in the regulation of proinflammatory cytokines and growth factors, contradictorily, hindering tissue repair and increasing the resistance of microorganisms[23]. For this reason, it is believed that the previous incorporation of antimicrobials into surgical sutures is a promising alternative in the control of adhesion and biofilm formation because it allows controlled release for a longer period of time and with less toxic effect.

In this study, chlorhexidine and cinnamaldehyde incorporated into sutures were not able to exert anti-*Candida* effect. One possible explanation for this result is the chemical interaction that may have occurred between the antimicrobials tested and the synthetic components used in prefabricated coatings of the sutures inactivating their effect as demonstrated in other studies[4,24]. The incorporation of substances into sutures by the immersion method is a technique used for ease of execution, rapid incorporation and low cost when compared to other coating techniques but that may also have influenced these results. In addition, biofilms of *C. albicans* present more organized and complex structures than bacterial biofilms, which hinders the penetration of substances and their possible antimicrobial effect in small doses[7].

Regarding bioavailability, chlorhexidine and cinnamaldehyde were absorbed by the sutures and released gradually at low concentrations over 48 hours. Similar results on the kinetics of progressive release are demonstrated in the literature only for chlorhexidine for 48 hours[15,16]. This time is strategic for antimicrobial sutures that impede the infectious process without selecting resistant microorganisms[3]. The low concentrations of the released substances may be, according to cell viability studies[15,16,25], considered to be biocompatible with oral tissues for chlorhexidine and cinnamaldehy because they are below 11 µg/cm and 65 µg/mL, respectively. This is a relevant finding in this study because it demonstrates the possibility of incorporation of

controlled release antimicrobials with low concentration for long times through this coating technique.

After the incorporation, a reduction in tensile strength of the sutures was observed. However, the values evidenced in the present study are still higher than others reported in the literature that tested silk sutures without incorporation[26] and polyglactin 910[27]. This fact reaffirms that external aspects such as storage conditions, manufacturing time and types of materials can influence this parameter[26,28]. Another point is that the incorporation of the antimicrobial agents did not influence the displacement of the sutures, which clinically may not have a negative effect on the elasticity and plasticity of the sutures. In addition, it is necessary to also analyze the impact of saliva on these properties.

This study presents some limitations because it is an *in vitro* assay, however, we emphasize that these results may guide future research for the synthesis of new antimicrobial coatings with a focus on fungal infections. Other studies should be carried out by increasing the concentration of antimicrobials and reducing the time of exposure, seeking an effective antifungal effect that exerts less influence on the physical structure of the sutures. In summary, we believe that the treatment proposed in this study presents a promising alternative for surgical sutures to allow the incorporation and controlled release of antimicrobials. Nevertheless, this treatment requires preliminary alterations before use in clinical practice.

CONCLUSION

The incorporation of chlorhexidine and cinnamaldehyde into sutures showed no anti-*Candida* effect, even with the progressive release of antimicrobials with greater bioavailability after 48 hours of incorporation. In addition, the incorporation adversely interfered in the tensile strength without influencing the displacement of the sutures. Thus, there is no increase in the effectiveness of surgical sutures incorporated with antimicrobials the biological and mechanical properties evaluated.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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