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Microbiological analysis of chitosan membranes with n-acylydrazone associations: evaluation of efficiency

Análise microbiológica de membranas de quitosana com associações de n-acilidrazona: avaliação da eficiência

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Abstract

Objective: To verify the potential and bactericidal activity of chitosan (CS) films combined with N-acylhydrazone (JR19) in different concentrations for wound treatment. **Methodology**: Films were developed from chitosan (CS) + polyvinyl acetate (PVA) solutions. The F1 film consisted of CS and the F2 film consisted of CS + PVA in a 1:1 ratio. To incorporate JR19 in the polymeric solutions, 1 mg of sulfadiazine (AgSD) + 8.6 mg of JR19 were added in 10 mL of CS: PVA. Films formed from this solution were named F3, F4, and F5. The antimicrobial activity of films with JR19 derivatives was assessed using the disc diffusion technique in an Agar Müeller Hinton solid culture medium. **Results:** The F1 film was discarded due to strong electrostatic repulsion with solid agar culture medium, deforming the film and hampering the culture. The F2, F3, and F5 films showed bacterial colony formations in front and verse, indicating no microbial inhibitory activity on *Staphylococcus aureus*. **Conclusion:** In the F4 film, the synergy between JR19 and AgSD showed complementary properties that facilitate wound healing. However, the safety and efficacy of this alternative therapy need further investigation in clinical trials.

Keywords: Chitosan; Healing; Membranes; Wounds.

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Resumo

Objetivo: Verificar a potencialidade e a ação bactericida de membranas de quitosana (QTS) com associações de N-acilidrazona (JR19) em diferentes concentrações para o tratamento de feridas. **Metodologia:** Os filmes foram desenvolvidos a partir das soluções de QTS e acetato de polivinila (PVA). Foram analisados: FI, filme de QTS; F2, filme de QTS+PVA na proporção de 1:1; F3, filme com 8,6 mg de JR19 adicionados à solução de QTS usada em F1; F4, filme com 8,6 mg de JR19 e 1 mg de Sulfadiazina de prata (AgSD) adicionados a 10 ml da solução de F2; F5, filme com 1 mg de JR19 e 1 mg de AgSD adicionados a 10 ml da solução de F2. A constatação da atividade antimicrobiana dos filmes de polímero de QTS com derivados JR19 foi realizada pela técnica de difusão de discos em meio de cultura sólido Ágar Müeller Hinton. **Resultados:** O F1 foi desprezado devido à forte repulsão eletrostática com meio de cultura sólido ágar, que causou deformação e inviabilizou a cultura. A ausência de atividade inibitória microbiana dos filmes F2, F3 e F5 sobre os S. aureus foi evidenciada pela formação de colônias bacterianas frente e verso sobre os filmes de polímero de QTS com derivados de JR19. **Conclusão:** A sinergia entre JR19 e AgSD na membrana F4 é vantajosa para a cicatrização de feridas devido às suas propriedades complementares. No entanto, a segurança e a eficácia em ensaios clínicos precisam ser mais exploradas para uma terapia alternativa segura e eficaz.

Palavras-chave: Cicatrização; Feridas; Membranas; Quitosana.

INTRODUCTION

By 2050, almost 25% of Western populations may experience chronic wounds This situation worsens when associated with physical impairment, poor quality of life, risk of infection, hospitalization, amputation, and death[1].

Recently, the incidence of skin wounds in health facilities has increased, affecting 42.6% of patients. This increase, independent of age or sex, causes prolonged hospitalizations[2]. Since skin wounds are a major concern in medicine, therapies for wound healing have been developed focusing on effectiveness and positive outcomes[4, 5, 6, 7, 8].

Research has been conducted to develop promising, cost-effective, and highly efficient tools such as polymer membranes. These membranes are easy to handle, not requiring daily maintenance (e.g., dressing changes and wound cleaning), and can replace damaged skin, prevent infections, and absorb exudates[9,10,11].

Several products have been developed to facilitate wound healing. Evidence indicates over 2,000 types of synthetic or biological dressings are available on the international market. These products are biodegradable and biocompatible, and some techniques are used to prevent reabsorption and material failure[12,13,14,15].

Dressings are composed of synthetic and biological polymers, such as hyaluronic acid, al-

ginate, and chitosan (CS). These polymers present adequate safety, compatibility with the human body, natural degradation, and antibacterial properties. Polymers composition includes nanofibers that improve wound healing due to a high-volume ratio, surface area, proliferation, and cell differentiation. These nanofibers can mimic tissue compositions, facilitating cell organization, adhesion, migration, and transport. Also, multiple porous fibers which are randomly[3] dispersed and interconnected facilitate tissue, cartilage, bones, and blood vessel regeneration[12,13,14].

Therefore, this study aimed to evaluate the efficacy and bactericidal action of CS membranes associated with N-acylhydrazone (JR19) at different concentrations for wound healing.

MATERIALS AND METHODS

Films development

Films were developed using two polymeric solutions. The first was a CS solution prepared in a 1% (v/v) acetic acid solution, with a final concentration of 1% (m/v). The second was a polyvinyl acetate (PVA) solution prepared in heated water, with a final concentration of 1% (m/v). From these polymeric solutions, two films (F1 and F2) were produced (Table 1). F1 was a CS-only film, and F2 was a CS + PVA film, in a 1:1 ratio. JR19 was incorporated in the polymeric solutions with 1 mg of silver sulfadiazine (AgSD) + 8.6 mg of JR19 in 10 ml of CS: PVA (1:1) under magnetic agitation for 24 hours. This solution produced films named F3, F4, and F5 (Table 1). JR19 quantity was optimized using 1 mg of AgSD + 1 mg of JR19 in 10 ml of a CS: PVA (1:1) solution under the same conditions described for F2. Ten milliliters of the resulting solutions were poured in plastic Petri dishes (5.5 cm diameter) and kept in an oven with air circulation at 50°C for 24 hours to solvent evaporation and film formation. The formulations were developed in the Laboratory of Development and Characterization of Pharmaceutical Products at the Universidade Estadual da Paraíba.

Table 1. Samples used in the study

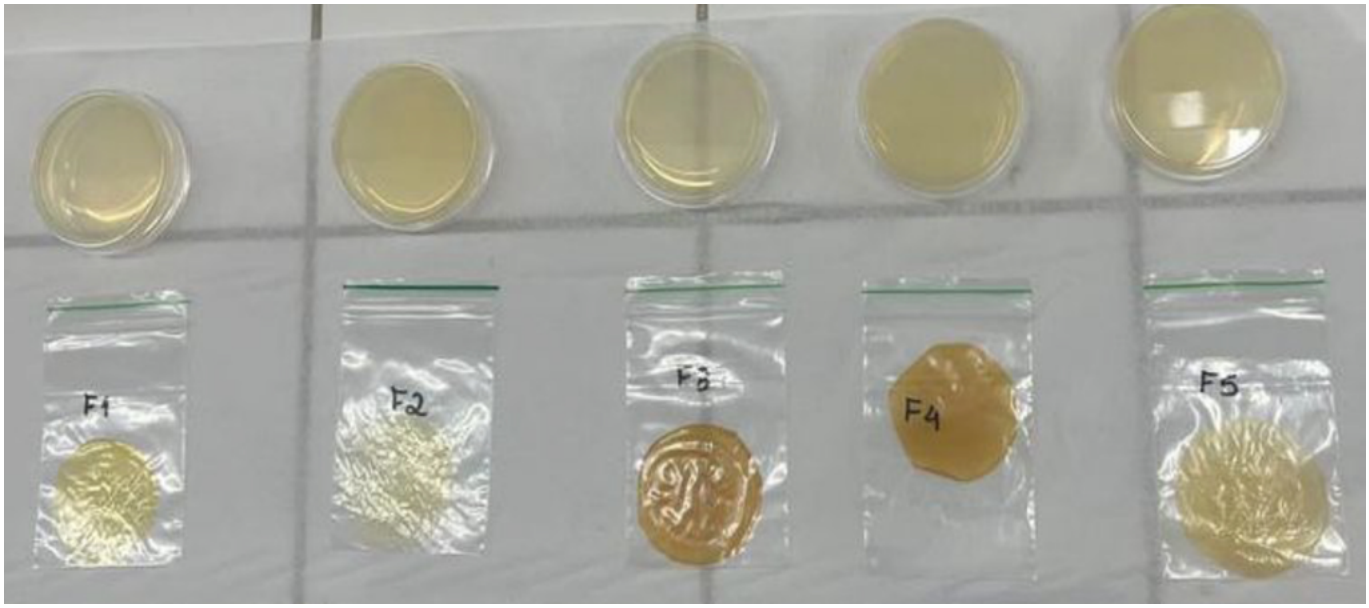
Sample	CS:PVA ratio (m/m)	JR19 (mg)	AgSD (mg)
F1	1:0	-	-
F2	1:1	-	-
F3	1:0	8.6	-
F4	1:1	8.6	1.0
F5	1:1	1.0	1.0

CS: chitosan; PVA: polyvinyl acetate; JR19: N-acylhydrazone; AgSD: silver sulfadiazine
Source: research data.

Antimicrobial activity

The antimicrobial activity of CS polymer films with JR19 derivatives was assessed using an adapted diffusion-disk technique in a solid Agar Müeller Hinton culture medium (Figure 1) on 60 x 15 mm Petri dishes. The bacterial inoculum was seeded with a sterile swab[14,18.]

Figure 1. CS films with and without JR19 derivatives.



Source: authors

Antimicrobial sensitivity tests were conducted using the *Staphylococcus aureus* (*S. aureus*) ATCC® 25923 strain. Strains were activated in test tubes containing nutrient agar and kept in an oven at 37 °C for 18 hours. Next, a 0.9% saline solution was prepared, and a bacterial inoculum was adjusted to the MacFarland scale of 0.5, corresponding to approximately 10^8 CFU/ml [18,19].

From a culture of the selected strain, a single colony was inoculated in BHI broth and incubated at 37 °C for approximately 18 hours. Subsequently, after medium turbidity, with a sterile swab previously dipped in bacterial suspension, the culture was inoculated in streaks onto the Müeller Hinton agar plates in two directions across the entire medium surface. Moreover, the swab was passed around the whole edge of the plate, and the inoculum dried from 5 to 10 minutes[15,17,18]. Last, the CS polymer films with different concentrations of JR19 derivatives were placed in the inoculated plates using sterile forceps.

The F1 film showed strong repulsion towards the agar medium and was discarded, while the F2 and F4 films showed less repulsion and were hydrated with a saline solution to improve adherence[5, 6, 7]. The plates were incubated at 37 °C for 24 hours in an oven. The results were interpreted following the recommendations of the National Committee for Clinical Laboratory ob-

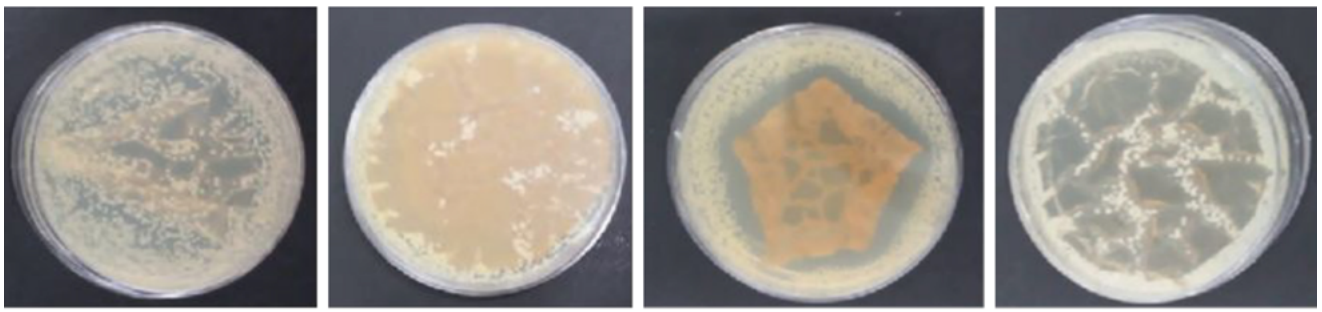
serving isolated colonies around and over the films[2, 3].

The antimicrobial susceptibility profile was conducted in the Laboratory of Microbiology of the Universidade Federal de Pernambuco according to the ISO 17025 and following specific standards for laboratories performing microbiological studies. Strict biosafety protocols were adopted to prevent human and environmental related risks[3, 4].

RESULTS

After incubation at 37°C for 24 hours, F4 film presented antibacterial activity with no bacterial colonies identified and formed an *S. aureus* inhibition halo (Figure 2)[3, 6, 7]. The F1 film was discarded due to strong electrostatic repulsion against the agar medium, which deformed the film and hampered the culture[10, 11]. The F2, F3, and F5 films showed no antibacterial activity over *S. aureus* since bacterial colonies were observed front and verse (Figure 2).

Figure 2. Antibacterial activity of CS polymer films with JR19 derivatives against S. aureus.



F2 **F3** **F4** **F5**

Source: authors

DISCUSSION

RF1 film was discarded due to strong electrostatic repulsion towards the agar medium, which deformed the film and hampered the analysis. The repulsion might be explained by a reaction between the culture medium and monomeric units of β-(1→4)-2-amino-2-deoxy-D-glucose and β-(1→4)-2-acetamido2-deoxy-D-glucose rt[10, 11, 12].

F2 showed no antimicrobial activity towards *S. aureus*, allowing greater bacterial growth than the other films. In this sense, JR19 and AgSD, included in the final composition of the film, might be the main agents of antimicrobial activity P[13, 14, 15]. Similarly, the F3 composition (CS associated with 8.6 mg of JR19) also lacked antimicrobial activity towards S. aureus, which may suggest that AgSD is the main agent of bacterial sensitivity[4, 6, 7].

In contrast, F4 presented high antimicrobial activity with no bacterial colony formation and showed an *S. aureus* inhibition halo. Therefore, F4 (1mg of AgSD) was the most promising

film for antibacterial activity. F4 antibacterial activity might be explained by the interaction with the peptidoglycan cell wall, which disrupted the bacterial wall. Also, the F4 bacteriostatic effect, represented by the *S. aureus* inhibition halo, might have contributed to the antibacterial activity.

Although AgSD and JR19 were added to the F5 film, no antibacterial activity was observed. F4 significant antimicrobial activity suggests specifically 8.6 mg of JR19 as an efficient concentration for this outcome. Combining AgSD and JR19 with CS as adjuvant therapies for wound healing is important for *in vitro* experiments[6, 7, 8].

JR19 has shown promising results for wound healing and is considered a privileged structure in medicinal chemistry due to its pharmaceutical relevance. JR19 shows antimicrobial activity towards various pathogens, which is crucial to prevent infections during wound healing[17, 18].

The combined activity of JR19 with AgSD in wound healing may be beneficial, considering their complementary properties. While JR19 is antimicrobial and anti-inflammatory, AgSD shows antimicrobial activity towards various bacteria frequently associated with cutaneous infections[10, 11].

Wanderley *et al*. (2020) developed CS films containing JR19 to create a therapeutic formulation. The authors analyzed the physicochemical properties, mechanics, and biocompatibility of the films. Results indicated JR19 in the films, with characteristics identified by differential exploratory calorimetry, thermogravimetry, and X-ray diffraction[17].

Besides the high antimicrobial activity, AgSD is safe and efficient in preventing secondary infections and promoting wound healing. By adding AgSD in films for wound healing, tissue regeneration is facilitated, inducing damaged tissue repair. Nevertheless, additional clinical studies are necessary to evaluate the safety and efficiency of AgSD as an alternate therapy for wound healing[19].

Thus, F4 film may inhibit bacterial growth due to structural and chemical differences from the other films tested. This potential inhibition might be related to the structural and chemical differences of the CS polymer with JR19 derivatives, which may interfere with bacterial cell wall adherence. Also, the antimicrobial activity of F4 compounds through diffusion might explain the *S. aureus* inhibition halo[18, 19].

CONCLUSION

The synergy between JR19 and AgSD in the F4 film was beneficial. Combining these agents in cicatrization membranes can regenerate damaged tissue, stimulating wound healing. However, the safety and efficiency of this combination must be further tested in clinical trials to ensure its potential for wound healing.

Moreover, these results are specific for *in vitro* tests and may partially reflect the perfor-

mance of the film *in vivo*. Also, the efficiency of a membrane as a healing agent depends on factors, such as wound severity and individual response of the patient.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

BBTO, MEAN e CABL: conceptualization, investigation, methodology, and writing. **GGG:** methodology. **LRT e BPGLD e TKBO:** conceptualization, writing – original draft and writing – revision and editing. All authors approved the final version.

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