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WILMER RAMÍREZ CARMONA

Nanocarreador dual de clorexidina e miconazol baseado em nanopartículas de óxido de ferro revestidas com quitosana: caracterização físico-química e efeito antibiofilme

Araçatuba - SP
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Tese apresentada à Faculdade de Odontologia de Araçatuba da Universidade Estadual Paulista (Unesp), como parte dos requisitos para a obtenção do título de Doutor em Ciências, área de concentração Saúde Bucal da Criança.

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À minha querida filha Valentina, você é o maior presente que a vida me deu, e à minha amada esposa, obrigado pelo amor, compreensão, carinho e apoio incansável nesta longa jornada de trabalho em equipe onde, mesmo muitas vezes separados, tivemos a coragem para seguir em frente, acreditar e ficar ainda mais unidos.

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"Educar é dar ao homem as chaves do mundo, que são a independência e o amor, e prepará-lo com forças para percorrê-lo sozinho, com o passo alegre do homem natural e livre"

José Martí

RESUMO

CARMONA, W. R. **Nanocarreador dual de clorexidina e miconazol baseado em nanopartículas de óxido de ferro revestidas com quitosana: caracterização físico-química e efeito antibiofilme**. 2024. Tese (Doutorado) - Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba, 2024.

Este estudo preparou e caracterizou um novo nanocarreador dual de clorexidina (CLX) e miconazol (MCZ), usando nanopartículas de óxido de ferro (NPsFeO) revestidas com quitosana (QTS), bem como avaliou seu efeito sobre biofilme microcosmo de candidíase bucal. CLX e MCZ foram imobilizados sobre NPsFeO previamente funcionalizadas com QTS. Microscopia eletrônica de transmissão, análise termogravimétrica e espectroscopia de infravermelho com transformada de Fourier foram usadas para caracterizar o novo nanocarreador dual. A concentração inibitória mínima (CIM) do nanocarreador dual sobre cinco isolados clínicos de *Candida albicans* foi avaliada pelo método da microdiluição em caldo, seguida pela determinação do índice de concentração inibitória fracionada. A mistura das salivas de cinco usuários de próteses removíveis com estomatite protética foi o inóculo usado na formação de biofilmes microcosmos. Os biofilmes foram formados por 72 horas sobre espécimes de resina acrílica posicionados no modelo de adesão ativa de Amsterdam e tratados por 24 horas com NPsFeO-QTS carreando, isoladamente, 4 diferentes concentrações de CLX-MCZ (7,8, 19,5, 39 ou 78 μg/mL). NPsFeO, QTS, CLX e MCZ sozinhos foram testados como controles, enquanto o biofilme não tratado foi o controle negativo (CN). Os ensaios quantitativos de biomassa total, atividade metabólica, contagem de unidades formadoras de colônias (UFCs) e produção de ácido láctico foram conduzidos para se avaliar o efeito antibiofilme. A viabilidade celular e a espessura do biofilme também foram analisadas por microscopia confocal de varredura a laser. Todos os resultados foram analisados por ANOVA ou teste de Kruskal-Wallis, seguidos pelo teste *post hoc* de Student-Newman-Keuls (α=0,05). O nanocarreador mostrou forma prevalentemente esférica, com diâmetro médio de 6,6 nm. Para todos os isolados clínicos de *C. albicans*, os valores de CIM do nanocarreador dual foram menores do que aqueles encontrados para CLX e MCZ na forma isolada, e o efeito sobre células planctônicas foi classificado como sinergístico. O nanocarreador dual na maior concentração testada (78 μg/mL) aumentou significativamente a biomassa total do biofilme em comparação ao CN. Por outro lado, esse composto reduziu significativamente a atividade metabólica e a produção de ácido láctico, superando o efeito promovido por CLX-MCZ. Para as cargas microbiana total e de *Streptococcus mutans*, o nanocarreador contendo a maior concentração dos antimicrobianos

comportou-se semelhantemente ao composto CLX-MCZ, e ambos levaram a reduções significativas em relação ao CN. Por microscopia confocal, o nanocarreador dual e CLX-MCZ reduziram e aumentaram significativamente a viabilidade celular e a espessura do biofilme polimicrobiano, respectivamente, comparados ao CN. Conclui-se que o nanocarredor dual foi efetivo na inibição de células planctônicas de *C. albicans*. Esse composto também mostrou efeito antimicrobiano sobre biofilme microcosmo de candidíase bucal igual ou superior à combinação de CLX com MCZ sem nanocarreador, dependendo do método analisado. A nanoterapia testada neste estudo tem potencial para ser usada em diversas aplicações clínicas em Odontologia.

Palavras-chave: biofilmes; candidíase bucal; clorexidina; miconazol; nanopartículas magnéticas de óxido de ferro; quitosana.

ABSTRACT

CARMONA, W. R. **Dual nanocarrier of chlorhexidine and miconazole based on chitosan-coated iron oxide nanoparticles: physicochemical characterization and antibiofilm effect**. 2024. Thesis (PhD) - Faculty of Dentistry, São Paulo State University, Araçatuba, 2024.

This study prepared and characterized a new dual nanocarrier of chlorhexidine (CHX) and miconazole (MCZ) using chitosan (CS)-coated iron oxide nanoparticles (IONPs), as well as evaluated its effect on an oral candidiasis microcosm biofilm. CHX and MCZ were immobilized on IONPs previously functionalized with CS. Transmission electron microscopy, thermogravimetric analysis, and Fourier transform infrared spectroscopy were used to characterize the novel dual nanocarrier. The minimum inhibitory concentration (MIC) of the dual nanocarrier on five clinical isolates of *Candida albicans* was evaluated by the broth microdilution method, followed by the determination of the fractional inhibitory concentration index. The mixture of saliva from five removable denture wearers with denture stomatitis was the inoculum used in the formation of microcosm biofilms. The biofilms were formed for 72 hours on acrylic resin specimens positioned in the Amsterdam active attachment model and treated for 24 hours with IONPs-CS carrying, alone, 4 different concentrations of CHX-MCZ (7.8, 19.5, 39, or 78 μg/mL). IONPs, CS, CHX, and MCZ alone were tested as controls, while untreated biofilm was the negative control (NC). Quantitative assays of total biomass, metabolic activity, colony-forming unit (CFU) count, and lactic acid production were conducted to evaluate the antibiofilm effect. Cell viability and biofilm thickness were also analyzed by confocal laser scanning microscopy. All results were analyzed by ANOVA or Kruskal-Wallis test, followed by the Student-Newman-Keuls *post hoc* test (α=0.05). The nanocarrier showed a predominantly spherical shape, with an average diameter of 6.6 nm. For all clinical isolates of *C. albicans*, the MIC values of the dual nanocarrier were lower than those found for CHX and MCZ in isolated form, and the effect on planktonic cells was classified as synergistic. The dual nanocarrier at the highest concentration tested (78 μg/mL) significantly increased the total biofilm biomass compared to NC. On the other hand, this compound significantly reduced metabolic activity and lactic acid production, surpassing the effect promoted by CHX-MCZ. For the total microorganism and *Streptococcus mutans* loads, the nanocarrier containing the highest concentration of antimicrobials behaved similarly to the CHX-MCZ compound, and both led to significant reductions in CFUs compared to NC. By confocal microscopy, the dual nanocarrier and CHX-MCZ significantly reduced and increased cell viability and polymicrobial biofilm thickness, respectively, compared to NC. It was possible to conclude that the dual

nanocarrier was effective in inhibiting *C. albicans* planktonic cells. This compound also showed an antimicrobial effect on oral candidiasis microcosm biofilm equal to or greater than the combination CHX-MCZ without nanocarrier, depending on the method analyzed. The nanotherapy tested in this study has the potential to be used in several clinical applications in Dentistry.

Keywords: biofilms; chitosan; chlorhexidine; magnetic iron oxide nanoparticles; miconazole; oral candidiasis.

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J and K, respectively. Different lowercase letters in images J and K show significant differences among groups. Data were analyzed by one-way analysis of variance (ANOVA) and Student-Newman-Keuls *post hoc* test (for biofilm thickness (n = 6; α = 0.05); K) or Kruskal-Wallis and Student-Newman-Keuls *post hoc* test (for percentage of dead cells (n = 6; α = 0.05); J)

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LISTA DE SIGLAS

CFU	colony-forming units
CHX	chlorhexidine
CIM	concentração inibitória mínima
CLSM	confocal laser scanning microscopy
CLX	clorexidina
CN	controle negativo
CS	chitosan
CV	crystal violet
FICI	fractional inhibitory concentration index
FTIR	fourier transform infrared spectroscopy
IONPs	iron oxide nanoparticles
LAc	lactic acid
MCZ	Miconazol/miconazole
MIC	minimum inhibitory concentration
NC	negative control
NPsFeO	nanopartículas de óxido de ferro
QTS	quitosana
TEM	transmission electron microscopy
TG	termogravimetric analysis
UFCs	unidades formadoras de colônias
XTT	sodium 3-[1-(phenylaminocarbonyl)- 3,4- tetrazolium]-bis (4-methoxy6-nitro) benzene sulfonic acid hydrate

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