Employing topical film forming spray for the antibiotic delivery in the management of chronic wounds

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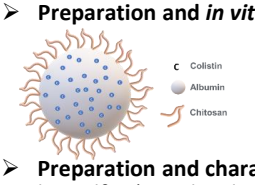
INTRODUCTION

Microbial infections represent a recurrent severe complication in the management of chronic non-healing wounds. In addition, the increasing appearance of Multidrug-resistant (MDR) bacterial strains makes the treatment of wound infections a growing challenge. Interestingly, the combination of nanocarriers with film forming spray formulations can represent a promising strategy for antibiotic delivery to counteract resistant infections, increasing drug efficacy and penetration at the infection sites.

AIM OF THE WORK → Development of a topical film forming spray formulation containing Colistin-loaded albumin nanoparticles (Col/haNPs) for the treatment of wound infections.

EXPERIMENTAL METHODS

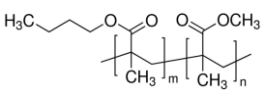
➢ **Preparation and *in vitro* characterization of Col/haNPs**



- Tuning of albumin NPs containing aqueous nanoreservoir preparation method
- Col encapsulation in the aqueous nanoreservoirs within the albumin matrix
- Chitosan coating of albumin NPs

➢ **Preparation and characterization of film forming spray formulation**

Plastoid® B (Evonik Industries,supplied by Rofarma Italia S.r.l)



butyl methacrylate-methyl methacrylate copolymer

Composition	Formulation A	Formulation B	Formulation C
Plastoid® B	1%	1%	1%
water	28.5%	28%	27.5%
glycerol	1.5%	2%	2.5%
ethylacetate	70%	60%	50%

➢ **Addition of ha/NPs and Col/haNPs to the film forming formulation**

➢ ***In vitro* characterization of Col/haNPs composite film forming formulation**

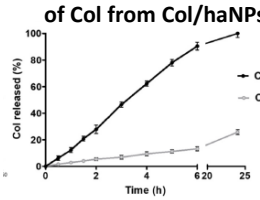
RESULTS

IN VITRO CHARACTERIZATION OF Col/haNPS

Table 1. Physico-chemical parameters of blank and Col loaded haNPs.

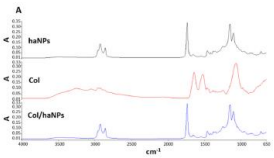
Sample	Average diameter ± SD (nm)	PDI	Zeta potential ± SD (mV)	pH
blank haNPs	174.7 ± 2.1	0.20	29.76 ± 1.5	7.52
Col/haNPs	176.1 ± 0.9	0.12	28.05 ± 2.1	7.46

➢ ***In vitro* release kinetics of Col from Col/haNPs**

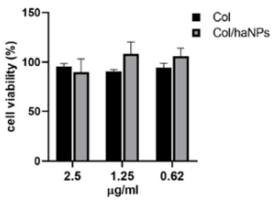


Encapsulation efficiency: 98.6%

➢ **FTIR spectra**



➢ **Biocompatibility of Col/haNPs**



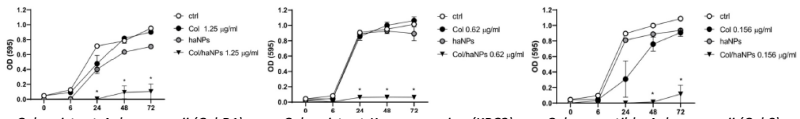
In vitro cytotoxic effect on HFF fibroblasts

➢ **Mucoadhesive properties**

Sample	Mucoadhesion (%) ± SD
Blank haNPs	96.4 ± 0.2
Col/haNPs	95.5 ± 0.4

ANTIMICROBIAL CAPACITY and ANTIBIOFILM EFFECTS OF Col/haNPS

➢ **Effect of Col/haNPs on Bacterial Growth**



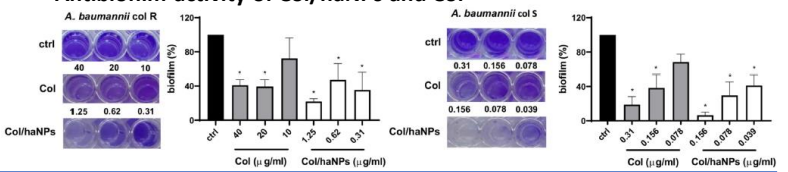
Col-resistant A. baumannii (Col R1) *Col-resistant K. pneumoniae (KPC2)* *Col-susceptible A. baumannii (Col S)*

➢ **Minimum Inhibitory Concentration (MIC) of Col/haNPs and Col**

Pathogenic Bacteria	Col MIC (µg/ml)	Col/haNPs MIC (µg/ml)
Acinetobacter baumannii ATCC19606	0.31	0.156
Acinetobacter baumannii Col S	0.31	0.156
Acinetobacter baumannii Col R 1	>40	1.25
Acinetobacter baumannii Col R 2	>40	1.25
Acinetobacter baumannii Col R 3	>40	2.5
Klebsiella pneumoniae KPC 1	20	2.5
Klebsiella pneumoniae KPC 2	40	0.62

Higher antimicrobial and antibiofilm efficacy compared to free Col

➢ **Antibiofilm activity of Col/haNPs and Col**

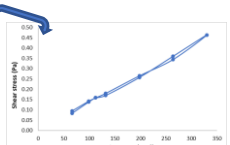


IN VITRO CHARACTERIZATION OF Col/haNPs COMPOSITE FILM FORMING FORMULATIONS

	pH ± SD	Osmolarity ± SD (mOsm)	Viscosity ± SD (mPas-sec)
Formulation B	5.50 ± 0.05	280 ± 1.5	0.95 ± 0.02
Formulation C	6.00 ± 0.05	300 ± 0.7	1.10 ± 0.03
Formulation B + Col/haNPs	5.75 ± 0.05	290 ± 1.2	1.30 ± 0.02
Formulation C + Col/haNPs	6.50 ± 0.05	310 ± 0.5	1.60 ± 0.04

	Average diameters (nm) ± SD	Polydispersity index ± SD	Zeta potential (mV) ± SD
Col/haNPs in Formulation B	180.3 ± 3.3	0.18 ± 0.03	26.2 ± 2.6
Col/haNPs in Formulation B	180.4 ± 4.9	0.20 ± 0.05	27.5 ± 1.4

Physico-chemical parameters of Col/haNPs incorporated in film forming solutions

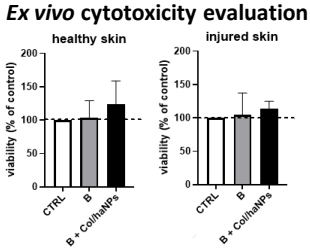


Rheogram of the composite film forming formulation

➢ **Minimum Inhibitory Concentration (MIC) of Col/haNPs composite film forming formulations**

			Formulation B	Formulation C
Pathogenic bacteria	Col MIC (µg/ml)	Col/haNPs MIC (µg/ml)	Col/haNPs MIC (µg/ml)	Col/haNPs MIC (µg/ml)
Acinetobacter baumannii Col R 1	>40	1.25	1.25	1.25
Klebsiella pneumoniae KPC 3	>40	0.62	5	2.5

➢ ***Ex vivo* cytotoxicity evaluation**



CONCLUSIONS

The developed preparation method allowed the production of stable chitosan-coated albumin NPs containing aqueous nanoreservoirs and the Col incorporation within the albumin matrix. The ha/NPs showed biocompatibility and high antimicrobial and antibiofilm efficacy against MDR GNB strains. The feasibility of Col/haNPs incorporation in film forming spray formulations, able to form stable bio-adhesive polymer films, was demonstrated. Based on the results, the topical film forming spray could be a valid approach to obtain a Colistin sustained release in the infection site for the management of chronic wounds.

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REFERENCES: *1) Ahmed M, et al. Emerg. Microbes Infect. 2020, 9, 868; 2) Lee NY, et al. Front. Pharmacol. 2019, 10, 1153; 3) Scutera S, et al. Antibiotics 2021, 10, 57*