GelMA-based nanocomposite film loaded with Neomycin sulphate and Carvacrol in Halloysite nanotubes against *Staphylococcus aureus* skin infections.

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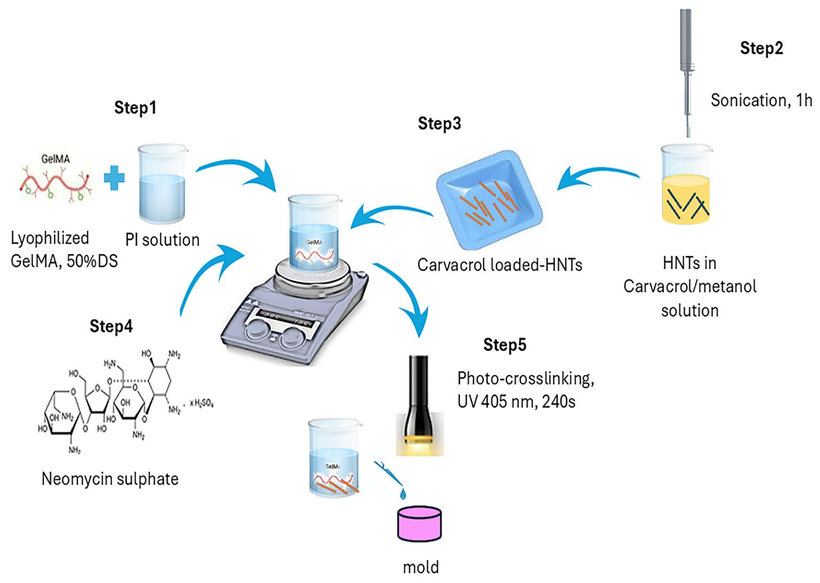
Introduction

The identification of effective treatment and alternative agents for the management of wound infections represents a challenge for the healthcare system due the economic and public health implications. Recently the development of dual-drug delivery systems to release in a controlled manner two therapeutics, antimicrobial and anti-inflammatory agents, to prevent and control infections, avoid antibiotic resistance appearance, and promote tissue regeneration has garnered increasing attention. Nanocomposite hydrogels based on gelatin, modified with photocrosslinkable methacrylamide groups (GelMA), represents an effective system to develop a differentiated delivery platforms for antimicrobial and anti-inflammatory agents, while maintaining a moist wound environment and promoting wound healing.

AIM

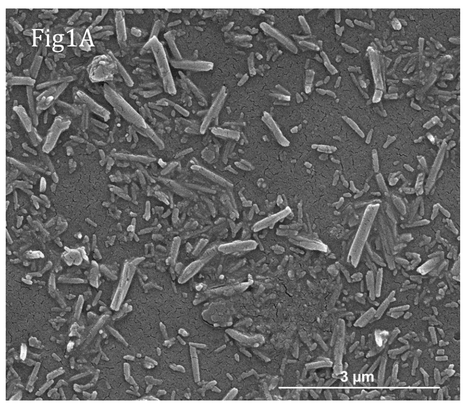
The aim of this work was to develop a dual delivery system using GelMA enriched with Halloysite nanotubes (HNTs) to improve mechanical properties of GelMA and obtain a controlled delivery of two distinct therapeutic agents, Neomycin sulphate loaded directly into the hydrogel, and Carvacrol entrapped into HNTs. To achieve this goal, we determined the better preparation of GelMA film enriched both with HNTs and Carvacrol loaded-HNTs in terms of physico-chemical, mechanical and swelling properties. Moreover, we evaluated the antibacterial effect of GelMA enriched with Carvacrol loaded-HNTs against *S. aureus* to identify the optimal content to add to GelMA, as well as cytotoxicity and antimicrobial properties of the optimized formulation of nanocomposite GelMA enriched with Neomycin sulphate and Carvacrol-loaded HNTs.

Schematic representation of GelMA nanocomposite preparation

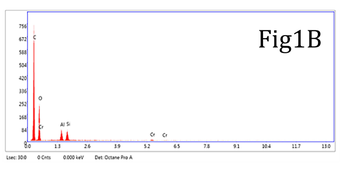


Step1: Reconstitued GelMA solution constituted by lyophilized GelMA in 5ml PI solution, containing Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) in DPBS, was placed on rotating plate at 60 °C to obtain GelMA at 10% concentration. **Step2**: HNTs were transferred in Carvacrol solution in methanol and subjected to ultrasonication, then transferred in oven to remove Carvacrol not adsorbed onto HNTs by evaporation. **Step3**: Dry loaded HNTs were added in the reconstituted GelMA solution and maintained under stirring overnight at 50°C. **Step4**: Neomycin sulphate was added in GelMA solution enriched with Carvacrol loaded-HNTs. **Step 5**: 2ml of preparation were transferred into a mold and photocrosslinked with a UV lamp

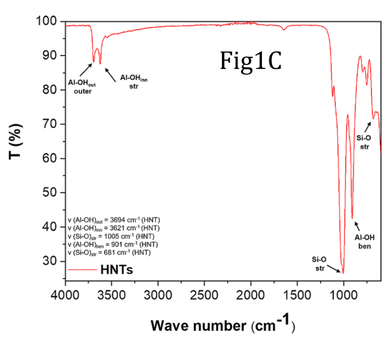
HNT characterization

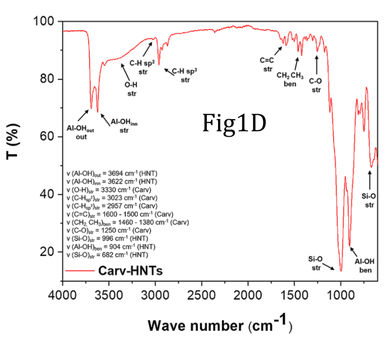


Leight (µm)	Diameter (nm)	ζ potential (mV)
1,09 ± 0,47	167 ± 51	-25,2 ± 0,7



EDX spectrum acquired by HNTs

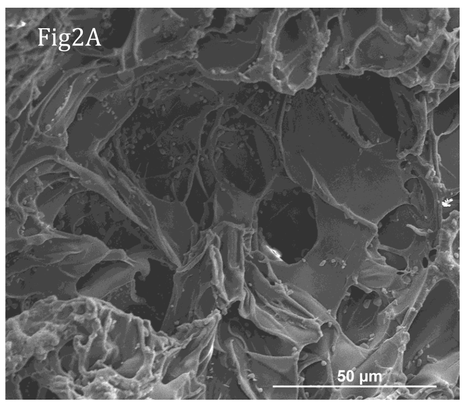


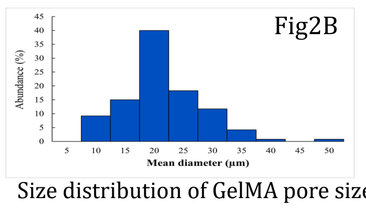


FTIR spectra acquired by HNTs

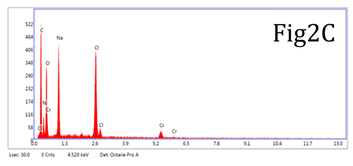
SEM analysis of HNTs showed that the length of the nanotubes was between 0,3 and 3 µm, while the width between 50 and 400 nm (Fig1A). HNTs showed a negative surface charge, due to the presence of the Si-O-Si groups on the outer surface. X-ray microanalysis showed that the nanomaterial is composed of Si and Al (Fig1B). FTIR spectra of Carv-HNTs showed the interaction between HNTs and Carvacrol (FIg1D). HNT showed bands consistent with Al-OH stretching and bending and Si-O stretching; carvacrol showed O-H, aromatic and aliphatic C-H stretching, and a C-O stretching band

GelMA –HNT nanocomposite film characterization

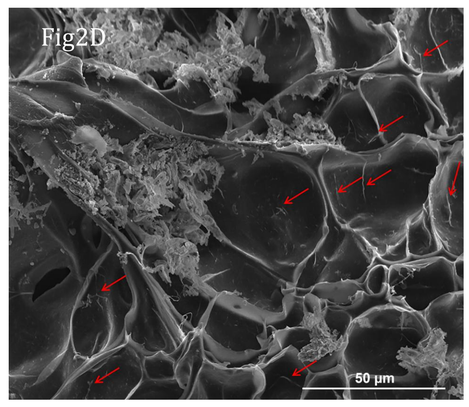


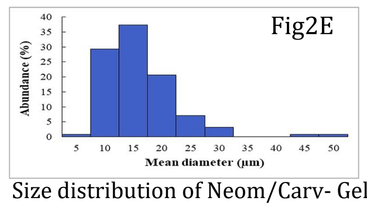


Size distribution of GelMA pore sizes

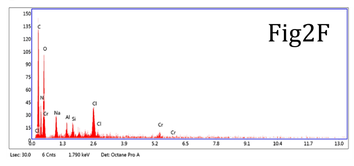


EDX spectrum acquired by GelMA film



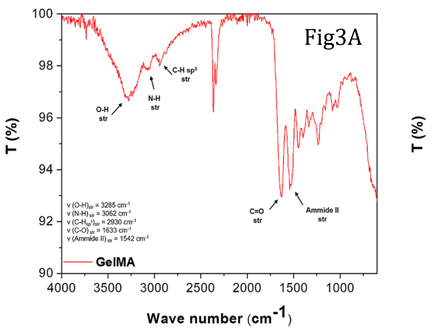


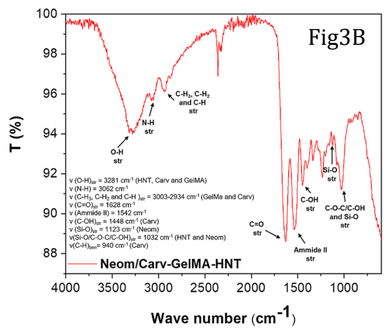
Size distribution of Neom/Carv- GelMA-HNT10% pore sizes



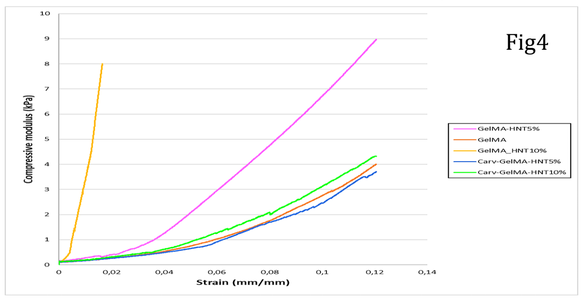
EDX spectrum acquired by Neom/Carv- GelMA-HNT10%

SEM observations of GelMA film cross sections (Fig2A) showed an irregular and honeycomb structure with well-separated pores and thin edges. Cross sections of nanocomposite GelMA enriched with Carvacrol-loaded HNTs and Neomycin sulphate (Fig2D) showed a more compact structure with reduced pore sizes and increased wall thickness. EDX spectra (Fig2F) highlighted that the HNTs were completely incorporated into the polymer matrix within the pores

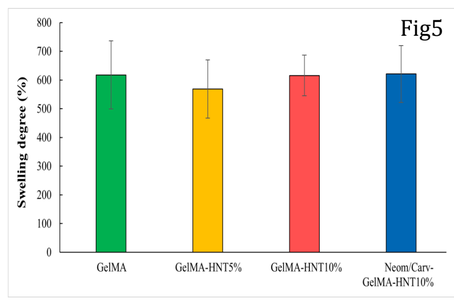




FTIR spectra confirmed the successful incorporation of HNT, Carvacrol and Neomycin sulphate into the GelMA network (Fig3A,3B), as shown by the persistence of the characteristic amide I/II bands alongside new Si–O and C–OH signals from HNT and carvacrol, and an overlapping Si–O–Si/C–O–C band attributable to HNT and Neomycin sulphate.

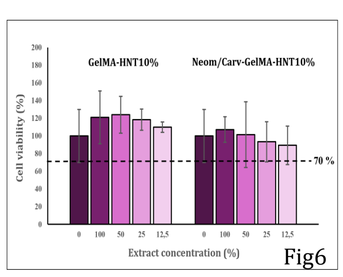


The addition of HNTs to GelMA enhanced the elastic modulus and compressive strength. Conversely, the entrapment of carvacrol within the HNTs reduced the compressive strength compared to GelMA-HNT films; however, Carv-GelMA-HNT10% exhibits a better response than GelMA (Fig4).



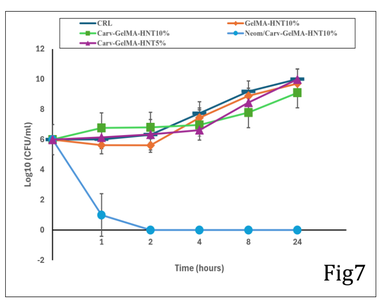
The enrichment of HNTs in GelMA as well as the loading of drug and Carvacrol did not influence the swelling properties of GelMA (Fig5), indicating the ability of GelMA-HNT films and Neomycin sulphate/carvacrol GelMA-HNT film to absorb and retain water

Cytotoxicity and Antibacterial activity



Viability of HaCat cells treated with extracts from both GelMA-HNT10% and Neom/Carv-GelMA-HNT10%.

Cytotoxicity of extracts from optimised nanocomposite GelMA, unloaded or loaded both with Neomycin sulphate and carvacrol, was assessed using HaCat cells. Results showed that both GelMA-based nanocomposite films did not show cytotoxicity (Fig6).



Antibacterial activity of different GelMA formulations against *S. aureus* 6538p strain.

Antibacterial activity against *S. aureus* 6538p strain (Fig7) showed that only HNT-GelMA containing both Neomycin and Carvacrol was able to kill bacteria, starting 1 h incubation, whereas the other GelMA formulations did not exert some antimicrobial effect, compared to untreated control culture.

Conclusions

The enrichment of GelMA with 10% HNTs allowed obtaining:

- a compact and honeycomb structure with smaller pore sizes, able to facilitate the efficient transport of encapsulated drugs
- good mechanical performance compared to GelMA10% hydrogel
- good swelling capacity indicating the ability of GelMA-HNT films to absorb exudates
- an effective dual drug delivery system of Neomycin sulphate and Carvacrol exhibiting enhanced antibacterial activity.

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