Dual nanomaterial systems aiming antimicrobial activity and cancer treatment

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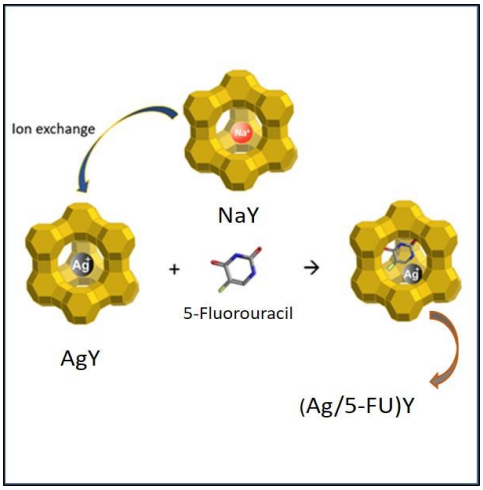

In the 21st century, nanotechnology has been emerging as a very promising field, with numerous applications in distinct areas, such as textiles, food agriculture, environment, electronics and in the biomedical field. Among the different categories of nanomaterials, zeolites are a very important class of inorganic materials that have been used in a wide range of applications. In the current work, we used zeolite NaY as host to incorporate silver ions and the antineoplastic drug 5-Fluorouracil (5-FU) for microbial infections and non-melanoma skin cancer treatment, respectively. Both situations present a major threat to public health: microbial infections due to their increasing resistance to antibiotics and cancer because of the difficulty of an efficient treatment especially when the disease has progressed considerably. So, since zeolites have a range of structural and physical properties that make them suitable for therapeutic delivery systems, they could be interesting candidates for this purpose. Our goal is to create an efficient and biocompatible dual topical delivery system with zeolites and, this way, combine the antimicrobial and the antineoplastic activity in the same formulation.

Introduction

Zeolites are microporous crystalline nanomaterials that contain alkaline metal ions (that can be readily exchanged by other cations in solution) and water molecules within their structure and are composed by silicon, aluminum and oxygen. Their 3D framework of SiO_4 and AlO_4 tetrahedra results in a uniform network of nano-channels and pores. Water molecules can be easily removed upon heating, resulting in a high surface area and an accessible pore volume that allows diffusion of the molecules to the interior of the zeolite particle [1-3]. The number of areas utilizing zeolites has grown tremendously and several studies have reported its use in the industrial sector, as detergent water softeners, as food additives, as adsorbents for wastewater treatment [4-5], as catalysts in industrial processes and in the biomedical field, such as magnetic resonance imaging, wound treatment, as drug delivery systems (DDS) and as antimicrobial agent (by incorporating cations with antimicrobial action) [6-8]. In this work, we explored and combined these last two applications loading zeolite NaY with silver ions by ion exchange followed by loading with 5-Fluorouracil (5-FU), originating a dual biocompatible nanomaterial system, (Ag/5-FU)Y as DDS for topical delivery.

Methods

1. Preparation of DDS. A solution of 0.01 M of silver(I) nitrate (Sigma-Aldrich) was added to the zeolite NaY (Zeolyst International) and the resulting suspension was maintained under constant stirring at 300 rpm for 24 h at room temperature. Suspension was then filtered and washed with deionized water and dried overnight at 60 ºC to obtain the AgY. The resulting sample was calcined at 350 ºC for 4 h. Loading of the drug 5-FU into the zeolitic structure was achieved by adding 200 mg of AgY to a solution of 5-FU (0.023 mM) in acetone (80 % acetone/20 % water; v/v). The suspension was kept under stirring at room temperature for 48 h. The resulting mixture was filtered and washed and the DDS ((Ag/5-FU)Y) was dried at 60 ºC for 12 h to evaporate the solvent. Na(5-FU)Y was prepared as described in [2].

2. Evaluation of antimicrobial activity. To evaluate the antimicrobial activity of the prepared samples, several bacterial strains which are known to be capable of triggering several infections (including skin infections) and therefore compromise human health, were selected as predictive models. 5 µL drops of each inoculum (bacterial cultures at OD ≈ 0.4–0.6) were placed on top of agar plates containing the culture medium supplemented with zeolite nanomaterials. Assays were performed in the presence of 0.2, 0.5, 1 and 2 mg/mL of each zeolite sample. Minimum inhibitory concentration (MIC), defined as the lowest concentrations that prevent bacterial growth, were determined for each pair sample/bacterial strain tested.

3. Cell viability assays. Cell viability studies were performed using a human skin cancer cell line, A375. The cell line was routinely cultured in DMEM, supplemented with 10 % (v/v) Fetal Bovine Serum and 1 % (v/v) penicillin–streptomycin and maintained in a 5 % CO_2 humidified atmosphere. Cells were subcultured every 2 to 3 days (approximately when they reached 80% confluence) to ensure their proper growth and health. To evaluate the effect of zeolite-mediated cytotoxicity and cell proliferation *in vitro*, Sulforhodamine B (SRB) colorimetric assay was performed.

Results

We observed that bacterial growth was unaffected by the presence of parent zeolite, NaY, regardless of the concentration tested, meaning that the zeolite itself revealed no antibacterial effects. However, AgY exhibited antibacterial activity against all the bacterial strains tested and no viable bacterial cells were detected in the presence of 1 mg/mL of AgY, proving that the introduction of silver ions in the zeolitic structure gives the antibacterial properties. The DDS (Ag/5-FU)Y also exhibited inhibitory effects on bacterial growth, showing that the zeolite maintains its antibacterial capacity in the presence of both

agents, so the presence of 5-FU does not interfere with the release and action of silver ions. Interestingly, the MIC values obtained with (Ag/5-FU)Y were lower than the values for AgY, meaning that the hybrid system exhibited enhanced antibacterial properties which may be caused by the combined action of 5-FU and silver ions (Table 1).

Table 1. MIC values for NaY, AgY and (Ag/5-FU)Y against each of the tested indicator bacterial strains

Microorganism	MIC (mg/mL)		
	NaY	AgY	(Ag/5-FU)Y
P. acnes	>2	0.5	0.5
MRSA	>2	1	0.5
S. aureus	>2	1	0.2
E. coli	>2	1	0.5
P. aeruginosa	>2	1	0.5

P. acnes - Propionibacterium acnes, MRSA - Methicillin-resistant Staphylococcus aureus, S. aureus - Staphylococcus aureus, E. coli - Escherichia coli and P. aeruginosa - Pseudomonas aeruginosa

Regarding the cancer cell viability tests (Figure 1), NaY did not interfere with the cell viability in the tested range of sample concentrations and throughout the entire period of exposure of the cells to the samples (up to 72 h), indicating its suitability as a drug delivery system for this type of cancer cells. (Ag/5-FU)Y caused a very accentuated decrease in cell viability. Similarly, AgY, which does not contain the antineoplastic drug 5-FU, also showed the same behavior, indicating that silver ions are highly cytotoxic to A375 cells. This way, both agents incorporated in the zeolite structure, silver ions and 5-FU, have an active role on the decrease of cell viability.

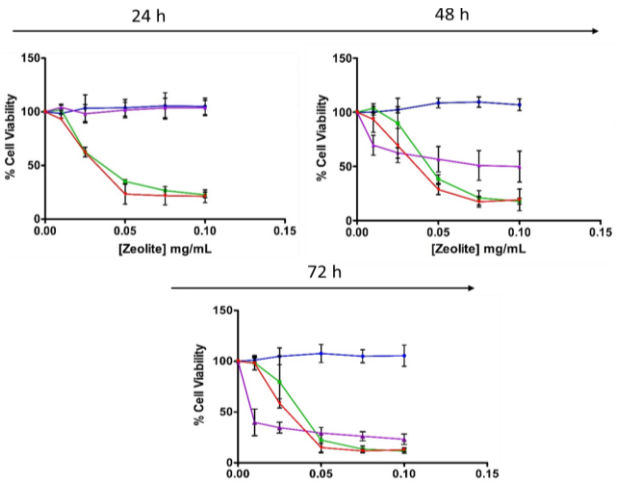

Figure 1. Effect of NaY (blue), AgY (green), Na(5-FU)Y (purple) and (Ag/5-FU)Y (red) on A375 human cells viability.

Conclusions

(Ag/5-FU)Y showed good antimicrobial properties, and in some cases with lower MIC values than the sample with only silver ions, suggesting that 5-FU itself has some antibacterial effect. Moreover, the DDS proved to be highly cytotoxic to A375 cells, an effect that is not only the result of 5-FU action but is also due to the presence of Ag^+ ions. These results highlight the great potential of the dual nanomaterial system and, with further work, it could be eventually incorporated into a biocompatible cream to be used for topical delivery, and this way combine the advantageous effects of the antimicrobial and antineoplastic activity in the same formulation.

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