SUPPORTING INFORMATION

Mucolytic and antibiotic combination therapy through silica-based nanocarriers to eradicate *Escherichia coli* biofilms

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Characterization techniques

Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) were performed in a Perkin Elmer Pyris Diamond TG/DTA analyser (Perkin Elmer, California, USA) by placing approximately 5 mg of sample in an aluminium crucible and applying 5 °C/min heating ramps from room temperature to 600 °C under a flow rate of 100 mL/min of air. TGA-DTA analyses were used to determine the total organic content (in percentage) present in each sample. Chemical microanalyses, which was used to determine the drugs loading efficiency, were performed with a Perkin Elmer 2400 CHN and a LECO CHNS-932 thermoanalyzers (Leco Corporation, Michigan, USA). The amount of levofloxacin (LVX) loaded in the nanoparticles was determined from the carbon and the nitrogen content, quantified by elemental CHN chemical microanalysis, and averaged. In the case of gelatin-coated nanosystems, the amount of LVX loaded was determined upon subtracting the amount of carbon and nitrogen present in the drug-free MSN@Gel nanoparticles, prepared as the reference sample. On the other hand, the amount of N-acetylcysteine (AC) in samples was determined from the carbon, nitrogen and sulfur content, quantified by elemental CHNS chemical analysis, and averaged. In this case, MSN-L@Gel and MSN-L@Gel_L samples were used as references of MSN-L@Gel_{AC} and MSN-L@Gel_L-$_{AC}$ nanosystems, respectively. Finally, the amount of fluorescein loaded into the MSN@Gel_F nanosystem was calculated from the carbon content of samples quantified by elemental CHN chemical analysis, upon subtraction of the carbon content present in MSN@Gel as the reference.

Transmission Electron Microscopy (TEM) was carried out with a JEOL JEM 2100 instrument operated at 200 kV (JEOL Ltd., Tokyo, Japan). Sample preparation was performed by dispersing *ca.* 1 mg of sample in 1 mL of ethanol followed by sonication in a low power bath sonicator (Selecta, Spain) for 5 min and then depositing one drop of the suspension onto carbon-coated copper grids. Negative staining of organic matter was made by treating the deposited sample with a 1% solution of phosphotungstic acid (PTA) during 20 seconds.

The surface characterisation of materials was carried out by N_2 adsorption/desorption analysis at −196 °C on a Micromeritics ASAP2020 analyser (Micromeritics Co, Norcross, USA). In all cases, 30-50 mg of the functionalized materials were outgassed under a vacuum lower than 10^{-5} Torr during 24 h at 60 °C prior to the measurements. The Brunauer-Emmett-Teller (BET) equation was used to calculate the surface area S_{BET}. Single-point total pore volume (V_T, cm^3/g) was measured at $P/P_0 \approx 0.97$. The average mesopore size (D_P) between 0.5 and 40 nm was obtained from the maximum of the pore size distribution curve calculated from the adsorption branch of the isotherm using the BJH (Barrett-Joyner-Halenda) method.

Electrophoretic mobility measurements for the materials suspended in water were used to calculate the zeta-potential (ζ) values of the nanosystems. Measurements were performed in a Zetasizer Nano ZS (Malvem Instruments Ltd., United Kingdom) equipped with a 633 nm "red" laser. For this purpose, 1 mg of nanoparticles was added to 10 mL of water followed by vortex and ultrasound to get a homogeneous suspension. Measurements were recorded by placing *ca.* 1 mL of the suspension in a DTS1070 disposable folded capillary cells (Malvern Instruments). Dilutions of the initial suspension were performed if needed. Values presented are mean ± SD from triplicate measurements. The hydrodynamic size of the nanoparticles was measured by

dynamic light scattering (DLS) with the same Malvern instrument. Values presented are mean ± SD from quintuplicate measurements.

Fourier transformed infrared (FTIR) spectra were collected in a Thermo Nicolet Nexus spectrometer equipped with a Goldengate attenuated total reflectance (ATR) device.

Fluorescence spectroscopy was performed by using a Microplate fluorescence reader Synergy4 (Biotech) by using λ_{ex} = 292 nm and λ_{em} = 494 nm for levofloxacin and λ_{ex} = 485 nm and λ_{em} = 528 nm for fluorescein. Calibration curves were first established in a concentration range from 0.01 to 12 μg/mL.

In vitro stability assays in physiological media

In vitro stability studies in PBS 1×

In vitro stability assays were carried out by suspending the different nanosystems in PBS 1× (pH = 7.4) at 37 °C under orbital shaking. A 6 Transwell® plate with two different compartments (sample and analysis) was employed for the experiment. Both compartments are separated by a membrane (12 kDa molecular weight) that only allows the diffusion of released ions and medium. A sample of 5.4 mg of each nanosystem was suspended in 1.5 mL of medium and was placed in the sample compartment, and 2.6 mL of PBS 1× was placed in the analysis compartment. After 96 h, the medium located in the analysis compartment was extracted and the concentration of silicon released from nanosystems in aqueous media was assayed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) through the Si emission line at 250.690 nm in a Varian Vista AX Pro spectrometer. Three measurements were made on each of the two replicates per composition examined. On the other hand, the nanoparticles were recovered and submitted to a TEM study, to evaluate the changes produced in the nanosystems after this incubation period, and colloidal stability tests by DLS and ζ-potential measurements after suspending the nanoparticles in PBS 0.1×.

In vitro colloidal stability tests in DMEM + 10% FCS

The in vitro colloidal stability of the nanosystems was also investigated in a standard complete cell culture medium consisting of Dulbecco's Modified Eagle Medium (DMEM) with 10 % Fetal Bovine Serum (FBS) (DMEM + 10 % FBS) (Gibco, Thermo Fisher Scientific, Wilmington, DE, USA). To this aim, 1 mg of the nanoparticles was suspended in the culture medium and sonicated for 5 min and the hydrodynamic size distribution was measured by DLS (t = 0 h). Then, the suspensions were incubated at 37 °C under orbital shaking during 96 h. After such period of time, the suspensions were sonicated for 5 min and the DLS measurements were recorded.

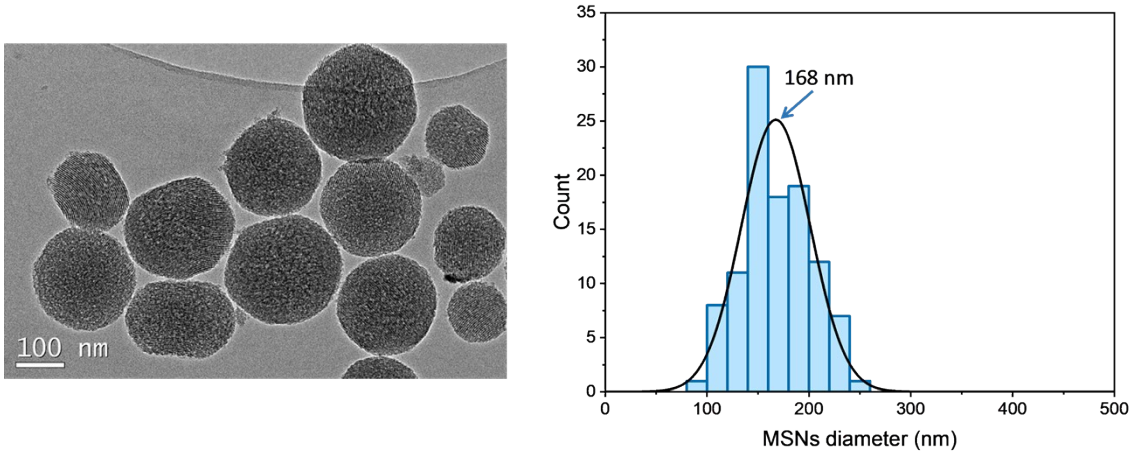

Fig. S1. A) TEM micrograph of pristine MSNs. B) Histogram of measured nanoparticle diameters (n = 107) in the TEM images with the best-fit lognormal distribution superimposed, indicating 168 nm as the maximum of the distribution. The normality test using the Kolmogorov-Smirnov approximation gives a p-value of 0.51475, which indicates that at the 0.05 level the data are significantly drawn from a normally distributed population.

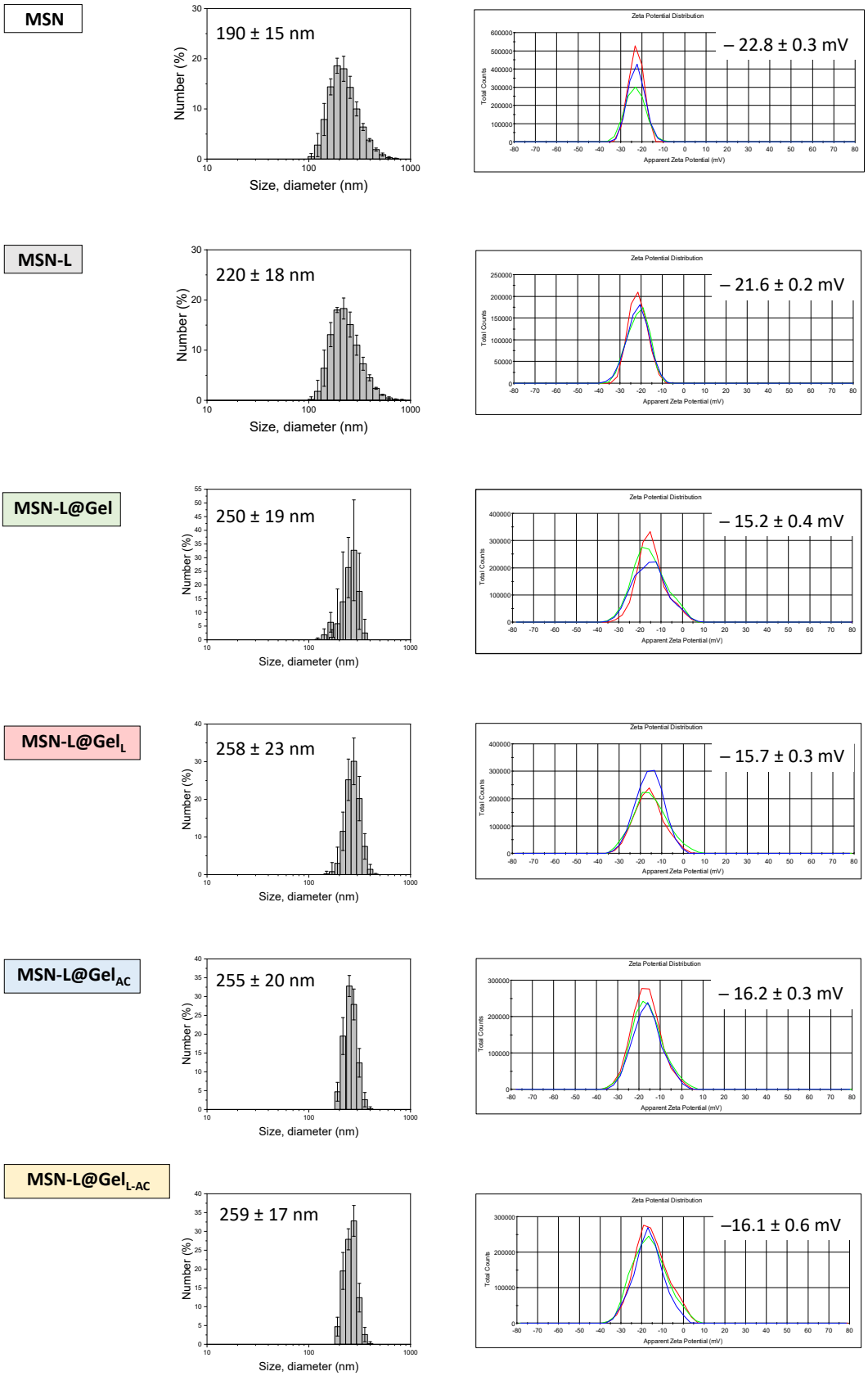

Fig. S2. Hydrodynamic size distribution (in number) obtained by dynamic light scattering (average ± standard deviation of five measurements) and ζ-potential distribution (average ± standard deviation of three measurements) in Milli-Q water for the different nanomaterials prepared in this work.

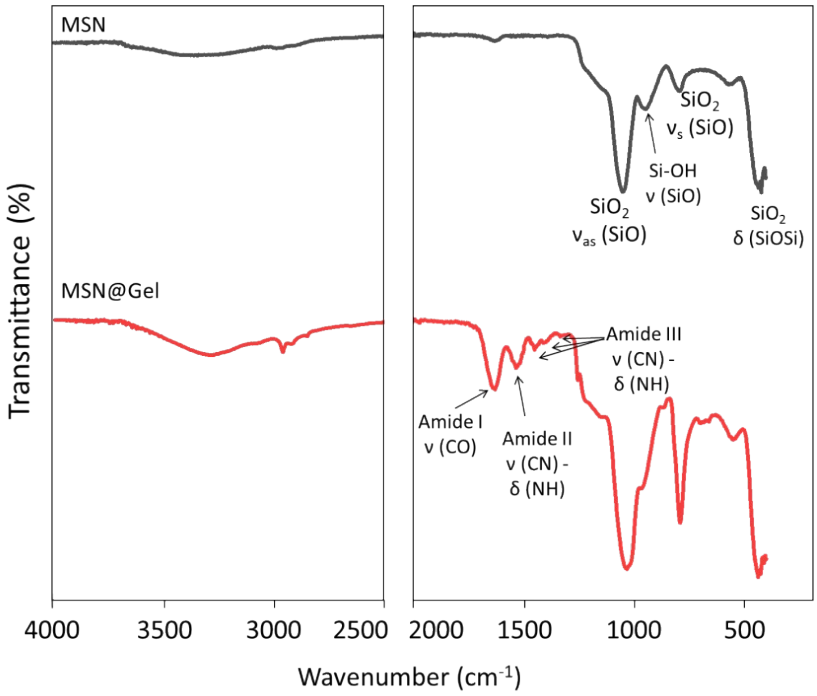

Fig. S3. FTIR spectra (4000-2500 cm^{-1} and 2000-400 cm^{-1} regions) of MSN and MSN@Gel nanosystems.

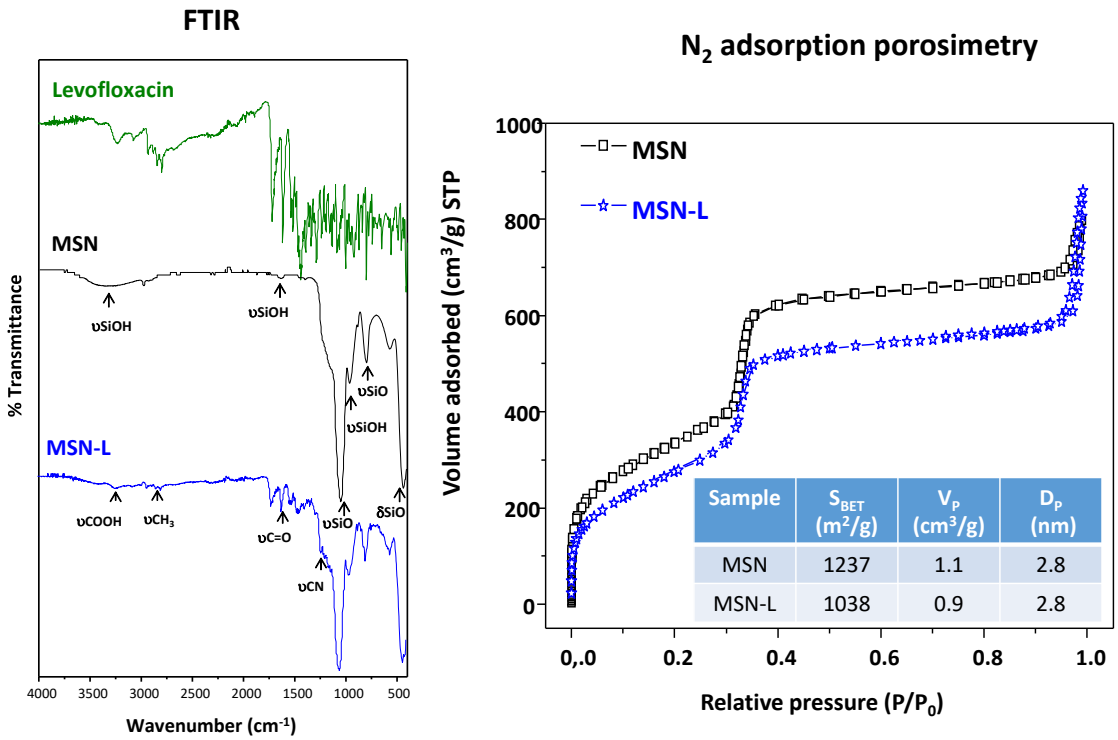

Fig. S4. Left: FTIR spectra (4000-400 cm^{-1} region) of MSN and MSN-L nanosystems. For comparative purposes, the FTIR of free LVX is also displayed. The results evidence the presence of LVX in the MSN-L nanosystems by the appearance of new vibration bands of the organic functional groups of LVX molecule [1]. Right: N_2 adsorption/desorption isotherms of MSN and MSN-L nanomaterials. The table displayed as an inset shows the main textural data for these samples, namely, specific surface area (S_{BET}), pore volume (V_P) and pore diameter (D_P). The decrease in the S_{BET} and V_P values confirm the loading of LVX into the mesoporous cavities, as reported elsewhere [2].

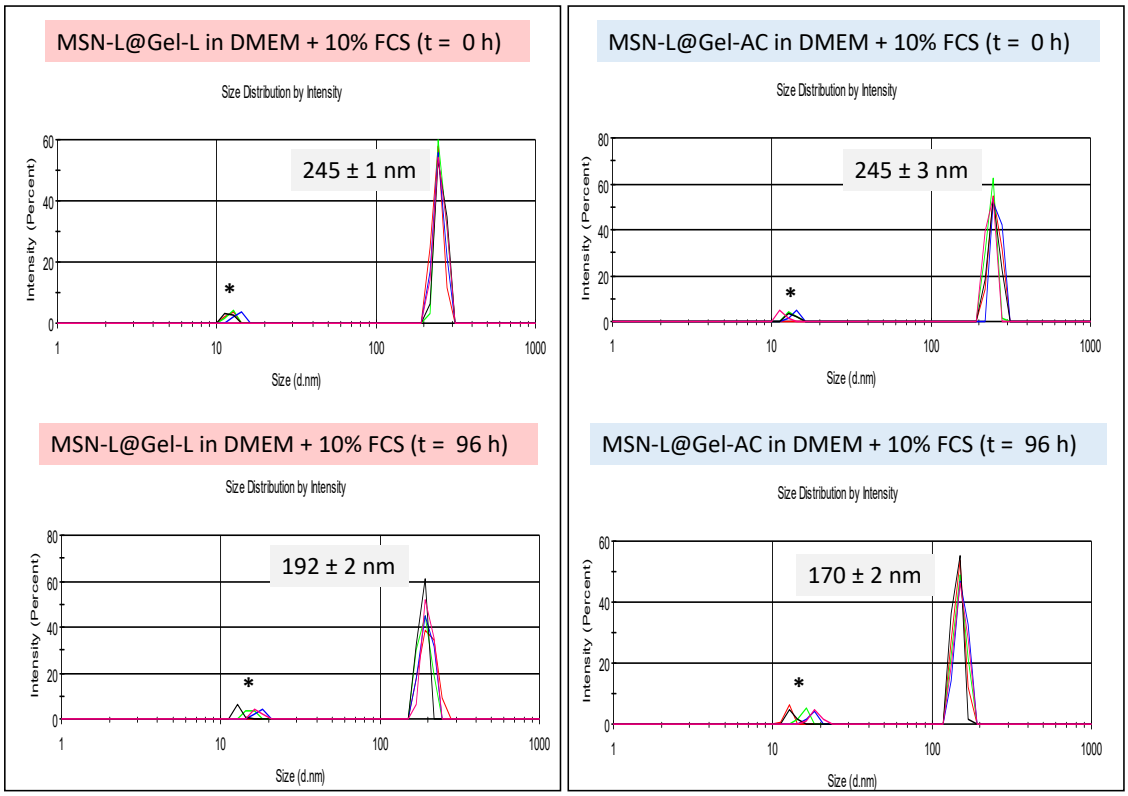

Fig. S5. Hydrodynamic size distributions by intensity measured by DLS of the MSN-L@Gel_L and MSN-L@Gel_{AC} at different time periods in DMEM + 10% FCS medium. The maximum of each nanoparticle size distributions was the resulted from the average ± standard deviation of 5 measurements. The peaks centred at 12-14 nm (indicated by asterisks) correspond to the bovine serum albumin (BSA) protein plus many other proteins present in the cell culture medium.

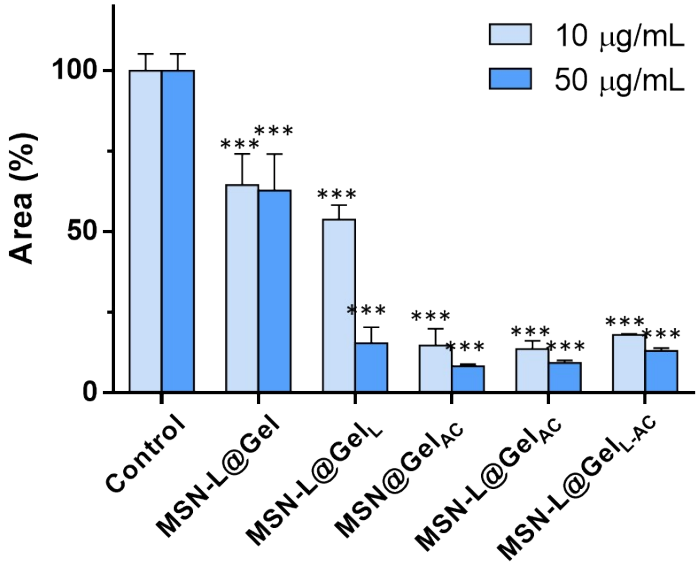

Fig. S6. The percentage of area occupied by the mucopolysaccharide matrix (blue) of *E. coli* biofilms treated with the different MSN@Gel nanosystems at different concentrations 10 and 50 μg/mL, respectively, obtained by confocal microscopy. The occupied area was calculated from eight confocal images of different areas of the biofilm and treated with the Image J program to calculate the percentage of occupied area. Data are mean ± σ (*P < 0.05, compared to control, **P < 0.01, compared to control, ***P < 0.001, compared to control). The experiment was performed in triplicate.

References

[1] J. Tavares Jacon Freitas, L. de Sousa Martins, V. Francioli Magalhães Calisto, H. Beraldo, Renata Diniz, New hydrated salts of levofloxacin: structural analysis, characterization and physicochemical properties of pharmaceutical interest, Journal of Molecular Structure, 132, **2025**, 141698.

[2] B. González, M. Colilla, J. Díez, D. Pedraza, M. Guembe, I. Izquierdo-Barba, M. Vallet-Regí Mesoporous silica nanoparticles decorated with polycationic dendrimers for infection treatment Acta Biomater., 68 (2018), pp. 261-271.