Electronic Supplementary Information

1. Materials and methods

1.1. Chemicals

$AlCl_3.6H_2O$ (97%), sodium hydroxide (97%), $KMnO_4$ (99%), the anionic surfactant sodium dodecyl benzene sulfonates (SDBS, Analytical reagent) were obtained from Aladdin Industrial co., Shanghai, China. Ethylene glycol (99.8%), xylene and other solvents were supplied from Acros Organics Co., Belgium.

1.2. Synthesis procedures

1.2.1. Preparation of γ–AlOOH NRs

γ-AlOOH NRs were prepared through an easy surfactant-based solvothermal method: 1.2 g of $AlCl_3.6H_2O$ in 23 mL mixture of water and xylene (mixing ration 1:2 by volume) solution was stirred for 15 min. Another solution of 0.5 g of SDBS in 12.5 mL NaOH (1 M) was added to the first solution drop by drop to form a suspension solution. This solution is composed of two phases (aqueous and organic); which were separated through a separating funnel. The upper organic phase was transferred into a Teflon-lined stainless-steel autoclave and heated for 24 h at 200 °C. The product was naturally cooled to RT, centrifuged for 20 min at 6500 rpm, and then washed with water and ethyl alcohol for three times. The washed product was dried for 12 h at 80 °C to finally yield a white powder of γ–AlOOH NRs.

1.2.2. Preparation of γ–MnOOH NRs

A controlled hydrothermal procedure was employed for preparing γ–MnOOH NRs with single crystallinity. An oxidation-reduction reaction between ethylene glycol and $KMnO_4$ was followed in this method, where:
To a 100 mL beaker containing 66 mL of distilled water, $KMnO_4$ (0.18 g, 2.25 mmol) was added and stirred for 15 min. Then, ethylene glycol (0.9 mL) was drop wisely added to the above solution, to reduce the $KMnO_4$ during the hydrothermal procedure, and the mixture was stirred (pH=9). After stirring for 4 h, the mixture was put into a Teflon-lined stainless-steel autoclave, subjected to heating for 10 h at 120 °C, and then cooled naturally to RT. The produced solution was centrifuged for 10 min at 4000 rpm for 10 min, and washed with water and ethyl alcohol for three times. The washed product was dried for 8 h at 65 °C for yielding γ–MnOOH NRs.

1.2.3. Preparation of α-Mn_2O_3 NRs

To produce α-Mn_2O_3 NRs, the prepared γ–MnOOH was thermally annealed at 700 °C for 5 h using a crucible of alumina put in an electric furnace. Through this procedure, γ–MnOOH NRs were dehydrated to produce α-Mn_2O_3 NRs.

1.3. Characterization techniques

A Thermo-Fischer scientific (Nicolet™ iS50R, United States) analyzer tested the FTIR of the NRs at the scan range of 500–4000 cm^{-1}. A PANalytical Aeris diffractometer (Netherlands) was employed to investigate the XRD of the as-synthesized NRs by CuKα X-rays between 2θ angle ranges of 10° to 80°. An XPS Thermo-Fischer scientific analyser (K-Alpha, United States) was used to test the NRs' XPS at 650 μm diameter of spot and 5×10^{-10} mBar by the Al Kα monochromator. The pass energy used to determine the elements' core level was 0.1-20 eV.
A thermo-Fischer scientific field emission TEM (FETEM, FEI Talos F200S, Czech) was conducted to capture the NRs' sizes and morphologies at 200 kV. The FETEM samples were prepared through their ultrasonication in an appropriate liquid, and then two drops were put on the TEM grid. The selected-area-electron-diffraction (SAED) was used to determine the facets and crystallinity of the prepared NRs.
A Hitachi field emission SEM (FESEM, SU8220, Japan) instrument captured the morphology of the prepared NRs at 20 kV. Before imaging, the FESEM sample was sputter-coated by Pt for preventing the electron beam charging. An EDX (X-Max 50, Oxford Inst. United States) was carried out at 30 kV for testing the elemental composition of NRs.

1.4. Effect of NRs on the mature biofilm

Similarly, the used protocol was the same as described earlier with a slight modification.[1] Briefly, Luria-Bertani (LB) medium (with 2.5 % w/v glucose) was separately inoculated by each bacterial strain until getting the bacterial concentration of 3 x 10^7 CFU/mL. To get a thick mature biofilm, the plates were sealed incubated under suitable shaking conditions for 48 h directly. The well's solution was subjected to discarding, washing gently three times using PBS, and re-filling with PBS containing higher-MIC values tested substances. The higher-MIC values were chosen because removing the bacterial strains requires a long time and more concentrations of the antibacterial agent as compared with the free-floating planktonic cells. As mentioned before, the positive and negative controls were used. Following the contacting time, every well's content was subjected to discarding and washing using PBS. A CV solution (0.1 %) was dispensed into every well and underwent incubation for 20 min at RT; and finally, the percent of eradication was determined as mentioned earlier.[1] Each sample was repeated three times to measure the mean value.

1.5. Bacteriostatic assay

To measure the bacteriostatic property of the γ–AlOOH, γ–MnOOH, and α-Mn_2O_3 NRs, Luria-Bertani (LB) medium was inoculated by overnight bacterial cultures *E. coli* (gram-negative) and *S. aureus* (gram-positive) and dispensed into sterilized conical centrifuge tubes 50 mL (10 mL/tube). Then sub MICs values of every dispersed solution of the NRs were added to each tube. The tubes with the inoculated medium were used as control only without adding the nano-suspension. The inoculated tubes were kept for incubation at 37 °C for 12 h under constant shaking. Aliquots of samples were withdrawn intermittently from the tubes to measure the time dependent bacteriostatic rate of the nanomaterial using the UV-visible spectrophotometry then compared with the control group.[2]

2. Results

The suspensions of the prepared NRs were tested against the pre-formed and mature biofilm and the percentages of residual biofilm after treatment for all tested strains (Figure S1). There was a massive eradication of α-Mn2O3 NRs against mature biofilm formed by not only S. aureus, B. pertussis, and B. subtilis, but also E. coli, and P. aeruginosa with double-MIC values of 79.3±2.1%, 78.7±3.5%, 78.3±3.5%, 74.3±4%, and 74.3±3.1%, respectively. This showed a broad-spectrum activity. While γ-ALOOH exhibited antibiofilm activity with double-MIC values of 70.7±2.1%, 65±2.6%, 63.3±4.2%, 50±5%, and 57±3%, for B. pertussis, S. aureus, B. subtilis, E. coli, and P. aeruginosa, respectively. While, γ–MnOOH NRs showed the lowest eradication effect through the double-MIC evaluation. This test illustrated that the antimicrobial activity of NRs is decreasing in the order of α-Mn_2O_3 NRs > γ-AlOOH > γ-MnOOH. This assessment confirmed that the antimicrobial efficiency is decreasing in the order of α-Mn_2O_3 > γ-ALOOH > γ–MnOOH NRs.

The bacteriostatic rate of the bacterial suspension treated with the prepared NRs in conical centrifuge tubes for 12 h was detected quantitatively. [3] It is a vastly used technique to monitor the growth of bacteria to estimate the antibacterial abilities.[4] After cultivation of the bacterial suspension for a definite time, the lower optical density at (OD_{600} nm) demonstrated the higher antibacterial ability of the tested NRs. As illustrated in Figure S2 (A and B), the OD_{600} values for different prepared antibacterial γ–MnOOH, γ–AlOOH, and α-Mn_2O_3 NRs, which are almost congruent with the agar well diffusion test and MIC assay. α-Mn_2O_3 showed the lowest OD_{600} after incubation period among the three NRs. These results emphasized that α-Mn_2O_3 possesses the highest capability in hindering the bacteria growth agreeing with the previous results. The three NRs exhibited a higher inhibitory effect towards *S. aureus* than *E. coli* as compared with the non-treated samples which proved that the NRs are more effective towards gram-positive bacteria than gram-negative bacteria. It is anticipated that the prepared NRs are effective antibacterial agents for various biological applications. The results emphasized the preferable antimicrobial activity of the prepared α-Mn_2O_3 NRs because of their large surface area, small diameter size, uniform NR architectures, single crystallinity, and highly exposed [110] Mn-polar surfaces outwards; which are promising structural features for α-Mn_2O_3 NRs.

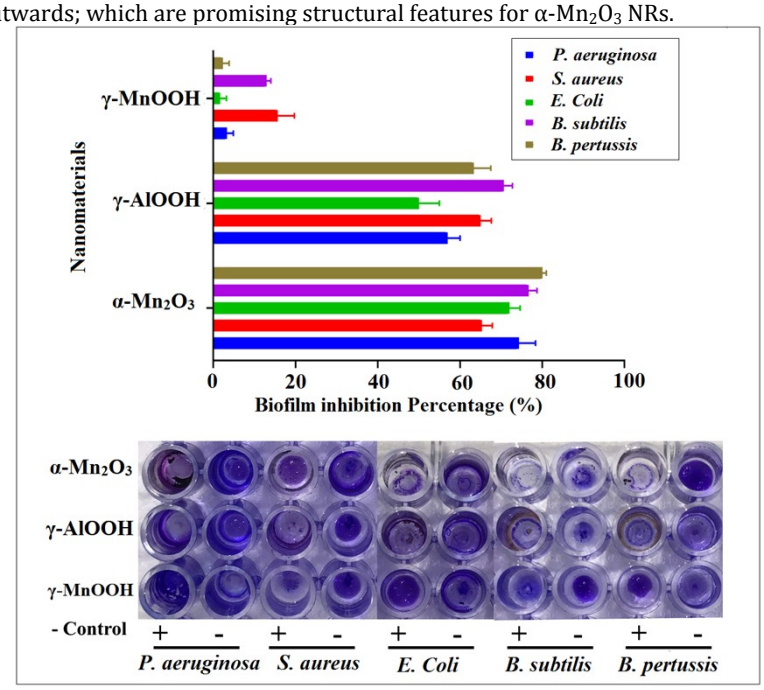

Figure S1. The effect of different NRs on 48 h old biofilms of gram-positive and gram-negative bacterial strains was tested through the CV test. Their wells were carefully stained through a CV solution and captured using a digital camera. The bar represents the average ±SD of each result which was determined for three times.

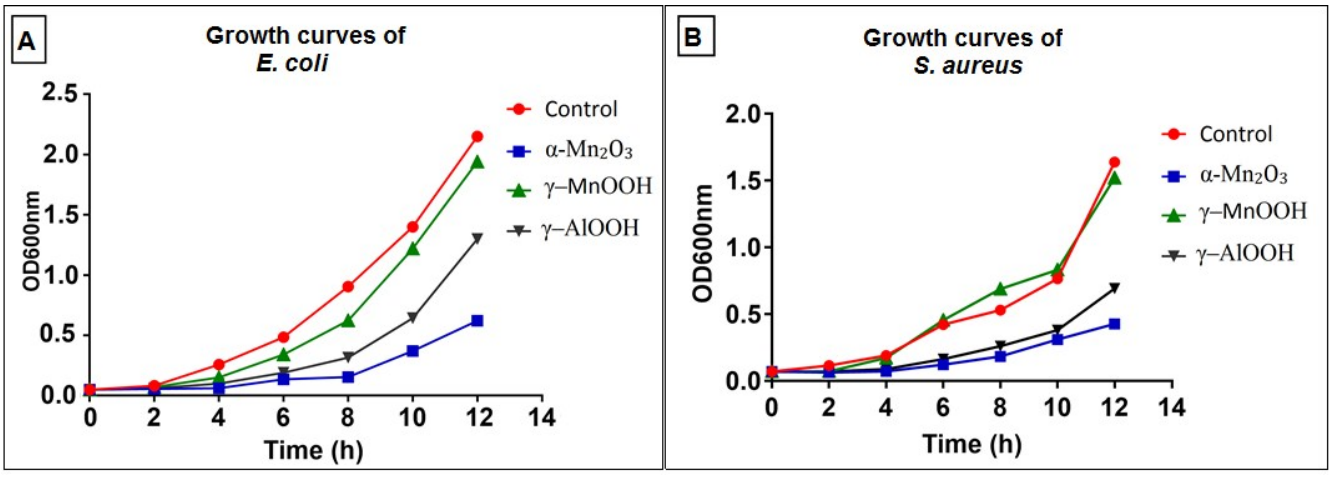

Figure S2. Time dependent optical density of bacterial cultures control and treated with different NRs for 12 h; where (A and B) represent the growth curves for *E. Coli* and *S. aureus*, respectively.

References:

[1] M. S. Selim, N. A. Samak, Z. Hao, J. Xing, *Mater. Chem. Phys.,* 2020, **239**, 122300.
[2] L. Yu, Y. Zhang, B. Zhang, J. Liu, *Sci. Rep.,* 2015, **4,** 4551.
[2] T. J. Erb, P. Kiefer, B. Hattendorf, D. Günther, J. A. Vorholt, Science, 2012, **337**, 467–470.
[3] L. Yu, Y. Zhang, B. Zhang, J. Liu, H. Zhang, C. Song, J. Membrane Sci., 2013, **447**, 452-462.