Biological Activity of Lyophilized Chitosan Scaffolds with Inclusion of Chitosan and Zinc Oxide Nanoparticles

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The constant demand for biocompatible and non-invasive materials for regenerative medicine in accidents and various diseases has driven the development of innovative biomaterials that promote biomedical applications. In this context, using sol-gel and ionotropic gelation methods, zinc oxide nanoparticles (NPs-ZnO) and chitosan nanoparticles (NPs-CS) were synthesized with sizes of 20.0 nm and 11.98 nm, respectively. These nanoparticles were incorporated into chitosan scaffolds through the freeze-drying method, generating a porous morphology with small (<100 µm), medium (100–200 µm), and large (200–450 µm) pore sizes. Moreover, the four formulations showed preliminary bioactivity after hydrolytic degradation, facilitating the formation of a hydroxyapatite (HA) layer on the scaffold surface, as evidenced by the presence of Ca (4%) and P (5.1%) during hydrolytic degradation. The scaffolds exhibited average antibacterial activity of F1=92.93%, F2=99.90%, F3=74.10%, and F4=88.72% against four bacterial strains: *K. pneumoniae*, *E. cloacae*, *S. enterica*, and *S. aureus*. In vivo evaluation confirmed the biocompatibility of the functionalized scaffolds, where F2 showed accelerated resorption attributed to the NPs-ZnO. At the same time, F3 exhibited controlled degradation with NPs-CS acting as initiation points for degradation. On the other hand, F4 combined NPs-CS and NPs-ZnO, resulting in progressive degradation, reduced inflammation, and an organized extracellular matrix All the results presented expand the boundaries in tissue engineering and regenerative medicine by highlighting the crucial role of nanoparticles in optimizing scaffold properties.

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Supplementary information

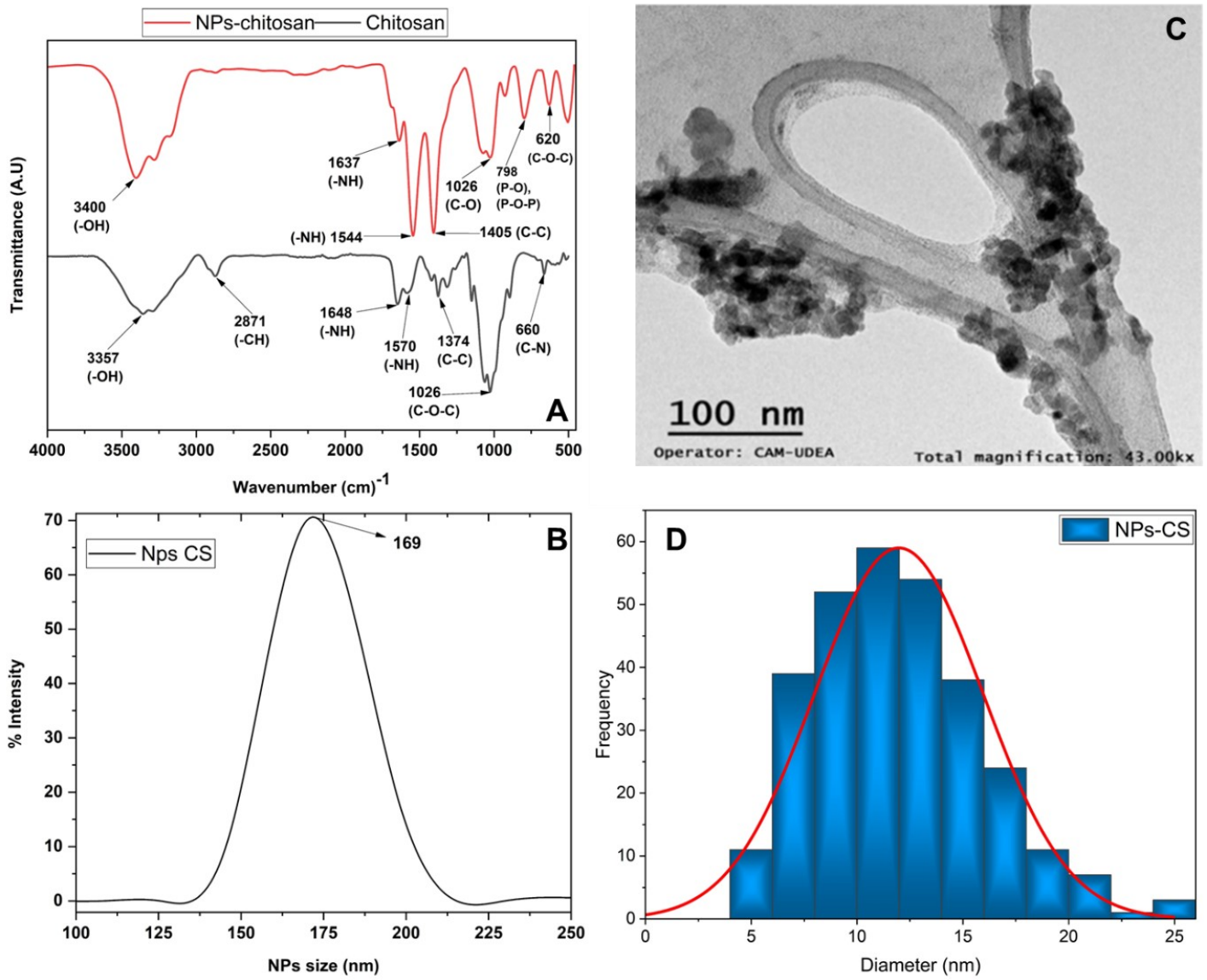

Figure S1. A) FTIR Spectrum of chitosan and NPs-CS. B) Size distribution profile of NPs-CS. C) TEM images of NPs-CS. D) Size histogram of NPs-CS.

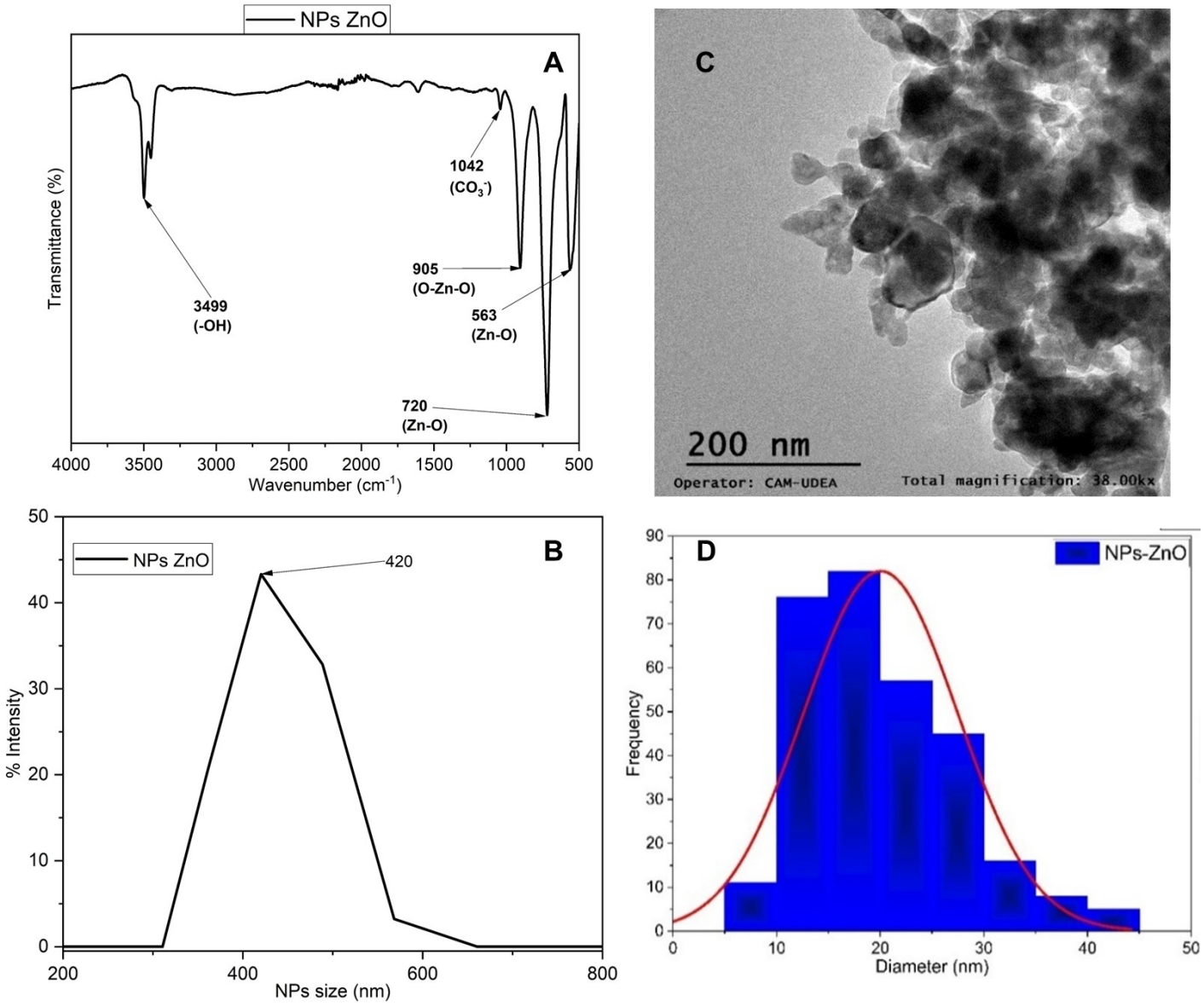

Figure S2. A) FTIR Spectrum of NPs-ZnO. B) Size distribution profile of NPs-ZnO. C) TEM images of NPs-ZnO. D) Size histogram of NPs-ZnO.