Chemical-modified natural nanogels crosslinked with S-Benzyl-L-cysteine exhibit potent antibacterial activity

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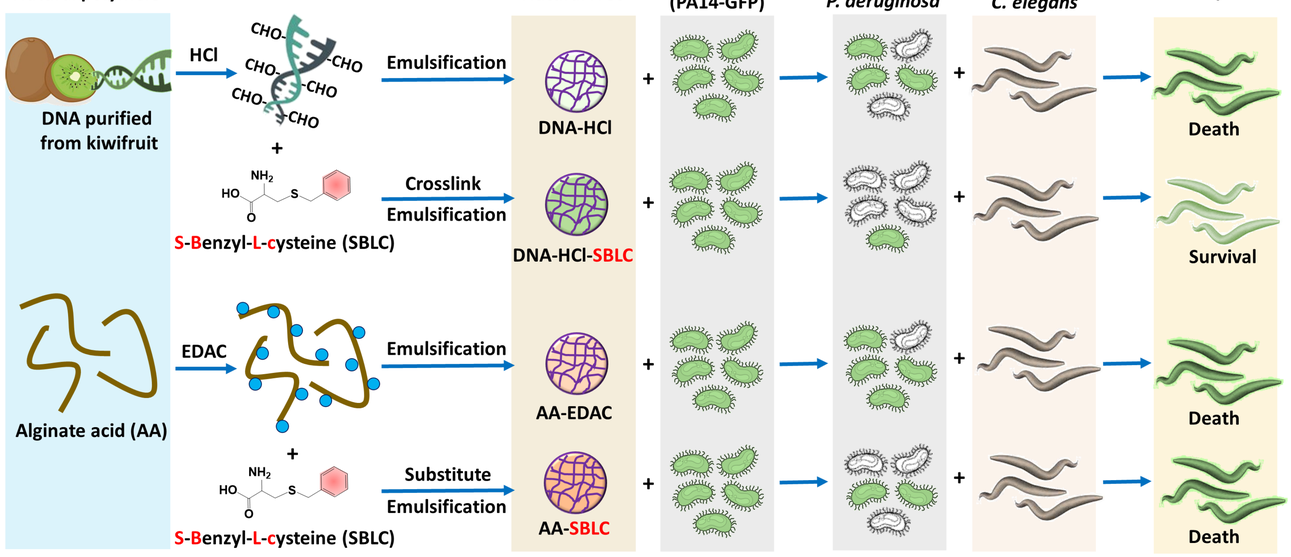
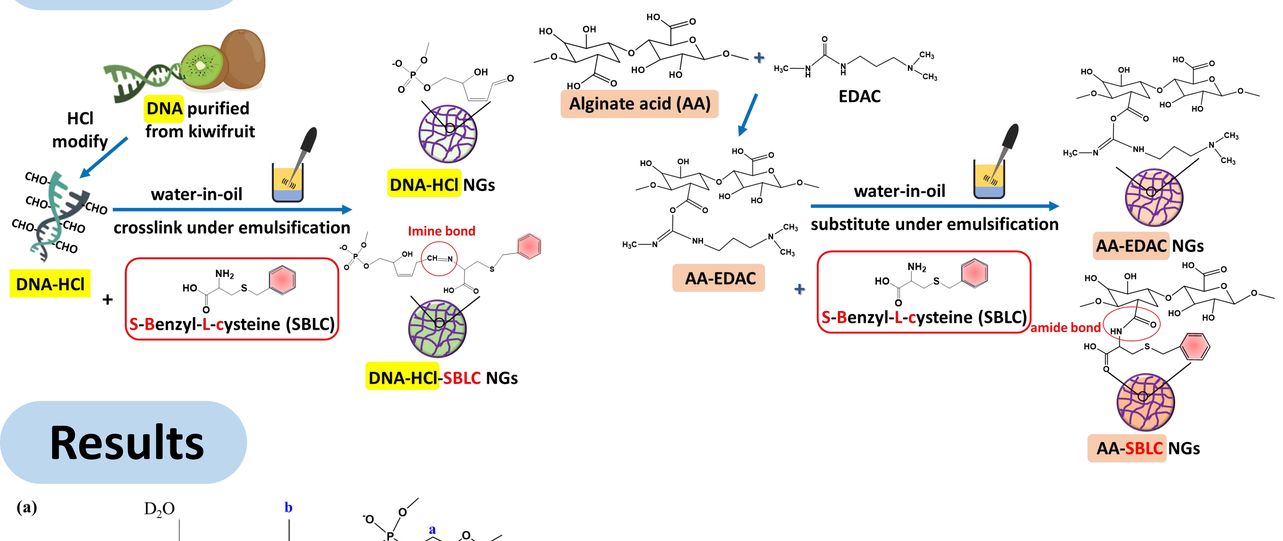
Graphical abstract



Scheme



Results

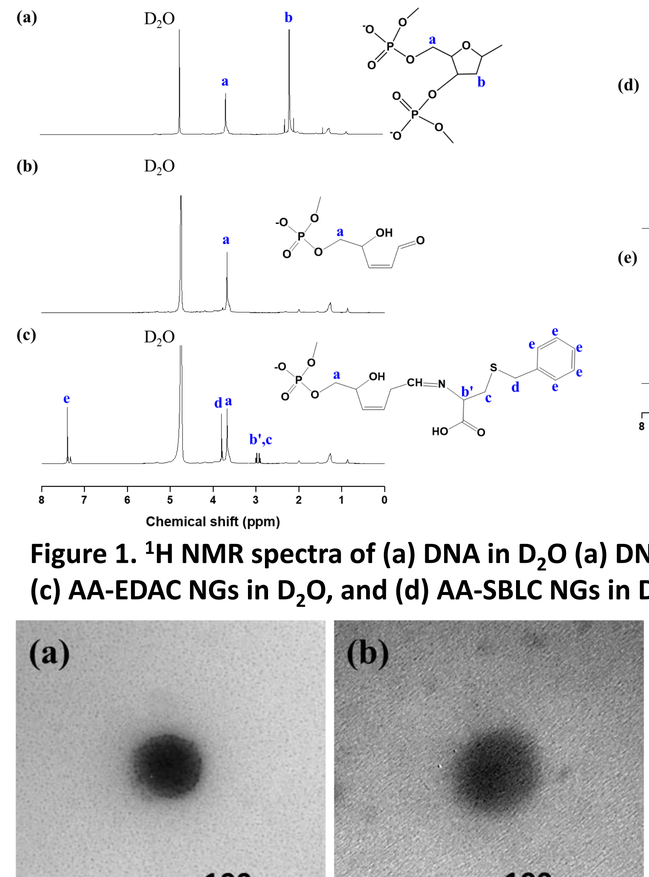


Figure 1. ^{1}H NMR spectra of (a) DNA in D_2O (a) DNA-HCl NGs in D_2O, (b) DNA-HCl-SBLC NGs in D_2O, (c) AA-EDAC NGs in D_2O, and (d) AA-SBLC NGs in D_2O.

Figure 2. TEM images of (a) DNA-HCl, (b) DNA-HCl-SBLC, (c) AA-EDAC and (d) AA-SBLC NGs.

Table 1. Hydrodynamic diameter (size), polydispersity index (PDI), zeta potential, and pH value of natural NGs in MQ-water (n = 3).

Nanogels (NGs)	Size (nm)	PDI	Zeta potential (mV)	pH value
DNA-HCl	42.02 ± 0.44	0.286 ± 0.006	-15.1 ± 0.56	7.34 ± 0.01
DNA-HCl-SBLC	97.01 ± 1.18	0.267 ± 0.01	-21.4 ± 1.06	7.03 ± 0.01
AA-EDAC	40.93 ± 0.12	0.18 ± 0.007	-20.9 ± 1.7	7.25 ± 0.02
AA-SBLC	78.09 ± 0.32	0.381 ± 0.008	-22 ± 0.83	7 ± 0.01

Table 2. Antimicrobial activity of natural NGs by determining MIC values (nM)

Bacteria	DNA-HCl NGs	DNA-HCl-SBLC NGs	AA-EDAC NGs	AA-SBLC NGs
EHEC	196.12	23.32	2010	30.09
P. aeruginosa	515.05	19.79	1250	43.22

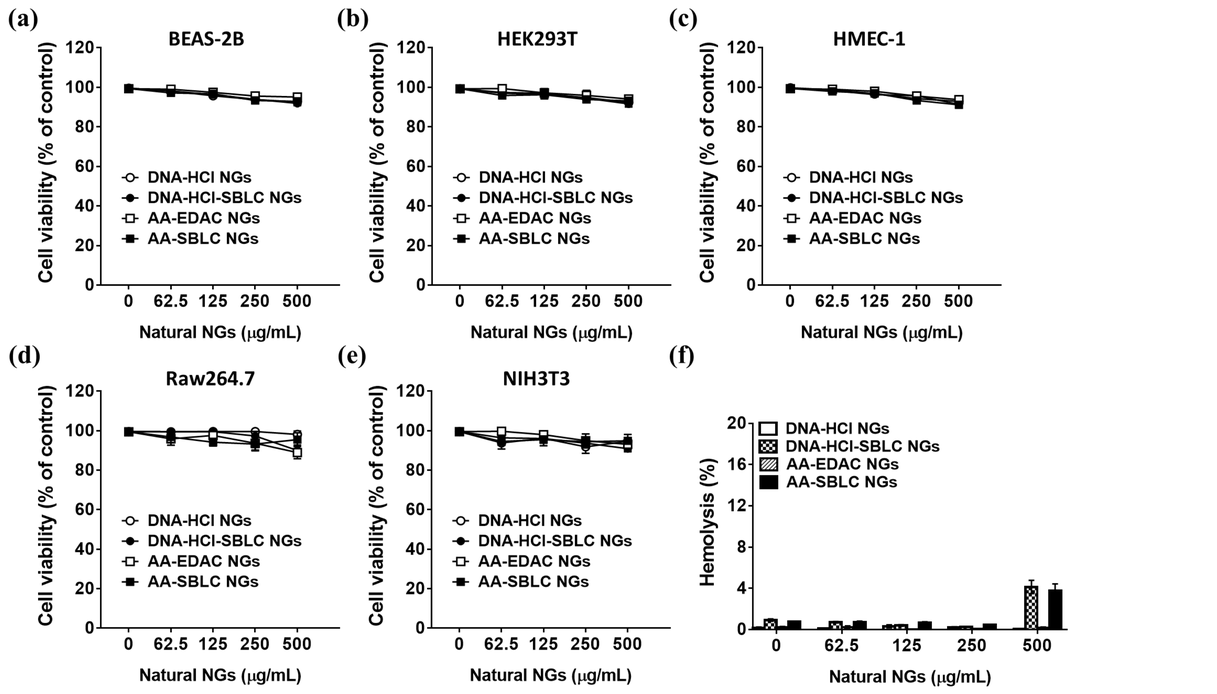


Figure 3. *In vitro* cytotoxicity of DNA-HCl, DNA-HCl-SBLC, AA-EDAC, and AA-SBLC NGs in normal cells. (a-e) Cells were treated with various concentrations of natural NGs for 24 h and cell proliferation was determined by CCK-8 assay (n = 3). (f) human RBCs were treated with various concentrations of natural NGs for 1 h and hemoglobin in supernatant was measured at 405 nm (n = 3).

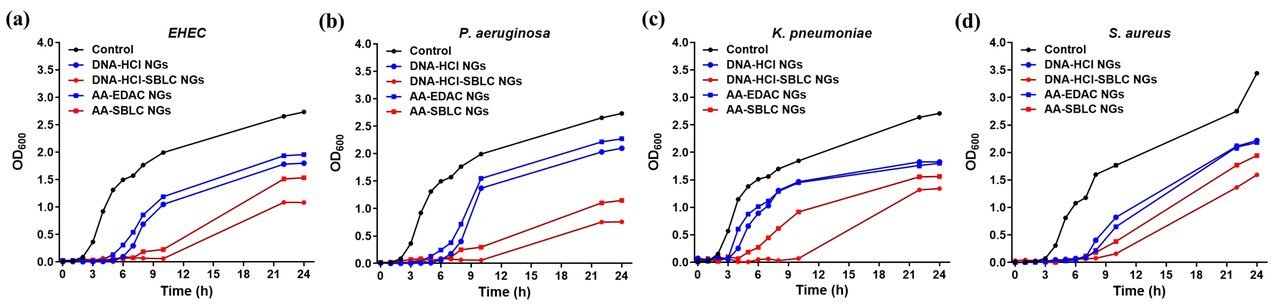


Figure 4. Growth curves of bacteria *EHEC* (a), *P. aeruginosa* (b), *K. pneumoniae* (C), and S. *aureus* (d) incubated with natural NGs under the concentration of respective MIC for 24 h.

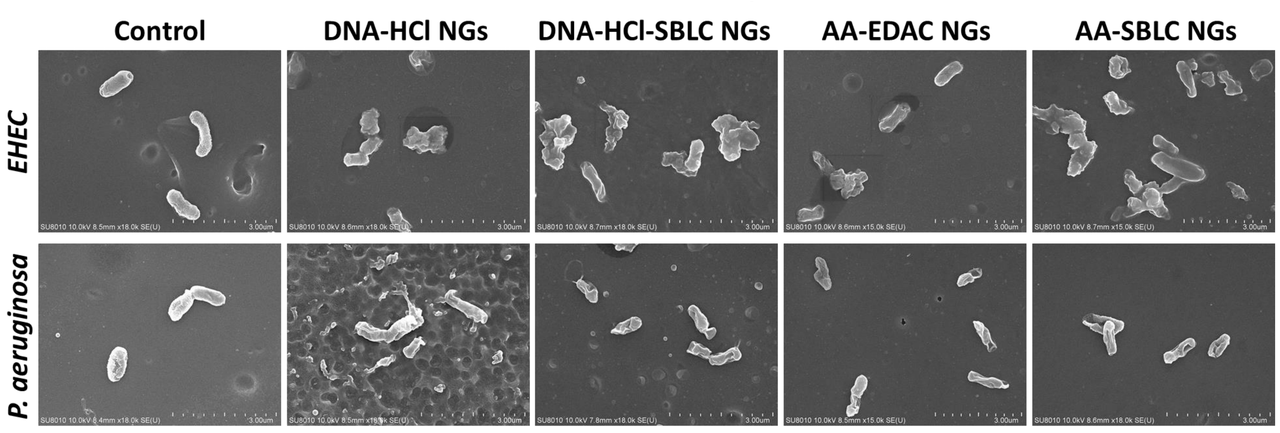


Figure 5. FE-SEM images of *EHEC* and *P. aeruginosa* bacteria treated with natural NGs for 1 h.

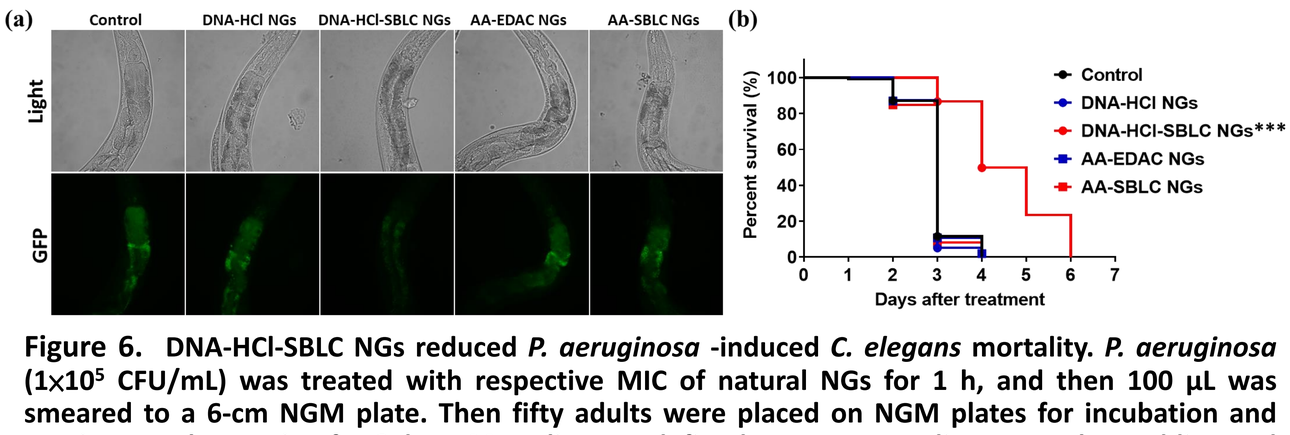


Figure 6. DNA-HCl-SBLC NGs reduced *P. aeruginosa* -induced *C. elegans* mortality. *P. aeruginosa* (1×10^5 CFU/mL) was treated with respective MIC of natural NGs for 1 h, and then 100 μL was smeared to a 6-cm NGM plate. Then fifty adults were placed on NGM plates for incubation and continuous observation for 6 days. Mortality was defined as not responding to gentle prodding and displaying no pharyngeal pumping under a dissection microscope.

Conclusion

This experiment applied HCl to open the deoxyribose rings in DNAs, and used the final product (DNA-HCl) with exposed aldehyde groups as the backbone. The control biomaterial used the polysaccharide conjugates, alginate acid modified with EDAC, as the backbone. We then applied hydrophobic S-benzyl-L-cysteine as a cross-linker in conducting the Schiff reaction, and ran a water-in-oil emulsification process to synthesize NGs ranging from 40 to 100 nm. The cross-link between each natural polymeric backbone and S-benzyl-L-cysteine is verified through FTIR and NMR, while the amorphous characteristic of the natural NGs are further confirmed with X-ray diffraction patterns. We also found that the natural polymers in the form of NGs increased considerably in antibacterial activity from SEM and bacterial growth curves. We therefore presume that both the increased surface area resulting from being nanosized and the hydrophobicity of S-benzyl-L-cysteine have encouraged the interaction between NGs and bacteria, which explains why the NGs inhibited the four biofilm bacteria effectively and prolonged the lifespan of *C. elegans* in *P. aeruginosa*-induced sepsis model. From this study, we conclude that NGs modified by S-benzyl-L-cysteine can serve as a solution to the antibiotic resistance in biofilm bacteria, and therefore a potential antibiotic of the next generation.