Supporting information for

Osteopontin-Loaded Nanoarchaeosomes for Enhanced Osteogenesis and Bone Regeneration in Osteoporotic Zebrafish Models

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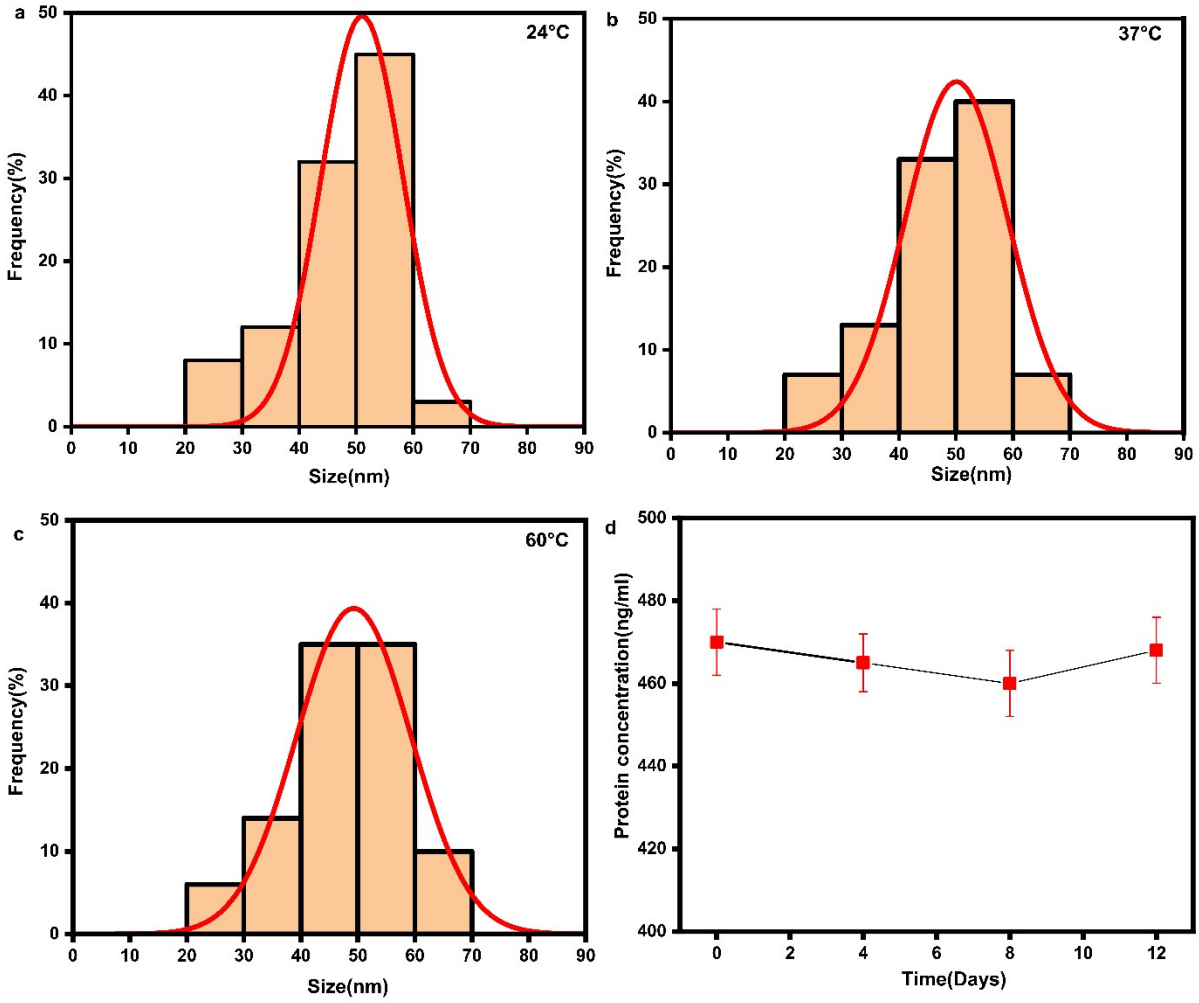
1.1.NAO exhibits stability at different temperatures:

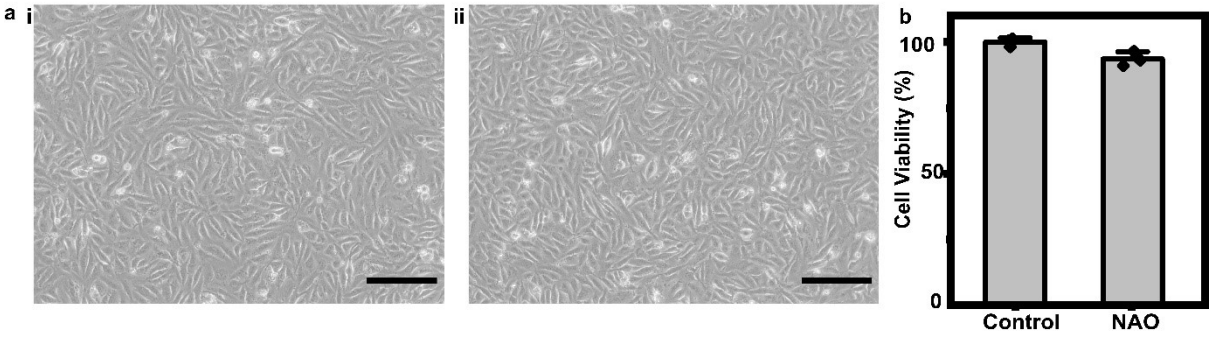
To evaluate the physicochemical stability of NAO under varying storage conditions, the samples were incubated at 24 °C (room temperature), 37 °C, and 60 °C for a period of 14 days after which NAO was analysed for changes in hydrodynamic diameter using Dynamic Light Scattering (DLS).The DLS histograms (FigSi1, a-c) demonstrate that NAO maintained a narrow size distribution with only minimal fluctuations across all tested temperatures. At 24 °C, the particles showed an average size of 51± 1 nm with a symmetric distribution. Similar size profiles were observed at 37 °C, indicating that physiological temperature did not induce aggregation or structural disruption. Even at 60 °C, a condition chosen to impose thermal stress, only a slight broadening of the distribution was detected with average size of 49± 0.8 nm, confirming that NAO remains structurally stable. Consistent with the DLS results, the protein quantification performed using HPLC (Fig Si1d) showed negligible variation over 12 days, with concentrations remaining close to the initial 470± 8 ng/mL. Together, these results reveal that NAO exhibits excellent thermal and storage stability, retaining both its nanoscale dimensions and protein integrity even under elevated temperature conditions.



Supplementary information figure 1: DLS Histogram distribution of NAO at different temperatures (a) 24°C (b) 37°C (c) 60°C . (d) Graph showing concentration of protein in NAO for 14 days.

1.2.NAO shows good biocompatibility in MG-63 cells:

The first and essential step in evaluating any new drug formulation or nanomaterial is to confirm its non-toxic nature towards cells, ensuring its suitability for therapeutic applications.The cytocompatibility of NAO was investigated by the di-methyl thiazolyl diphenyl tetrazolium (MTT) assay using MG63 osteoblast cells, as described in the Experiment Section 2.6 in the main manuscript. The results obtained, as observed in Fig Si2, showed excellent biocompatibility, ensuring its proper effectiveness as a therapeutic agent.As shown in Fig Si2 a(i–ii), both the control and NAO-treated MG63 cells displayed healthy morphology with no visible signs of stress, rounding, or detachment. The cells remained confluent and proliferative, indicating the absence of morphological toxicity.The NAO-treated cells retained 98% viability(fig S1 (b)), comparable to the untreated control group.



Supplementary information figure 2: NAO shows good biocompatibility when treated on MG-63 cells.(a)(i) Control cells (ii) Treated cells with NAO- 100ng/ml. (b) Cell viability graph . Scale=200µm