Supporting Information

Investigation of the role of nitric oxide driven angiogenesis by zinc oxide nanoflowers

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Fig. S1: Characterization of as synthesized ZONF using different analytical methods. XRD analysis shows the crystalline nature of ZONF with wurtzite phase. The inset TEM image reveals the flower like morphology of the nanomaterials.

Fig. S2: Determination of formation of intracellular $O_2^{-}$ in ECV-304 cells using DHE reagent. Row 1 (a1-a4): control untreated cells; Row 2 (b1-b4): cells treated with VEGF (40 ng/mL, 24 h); Row 3 (c1-c4): cells treated with 10 µg/mL ZONF; Row 4 (d1-d4): cells treated with 20 µg/mL ZONF; Column 1 (a1-d1): bright field images; Column 2 (a2-d2): red fluorescence images; Column 3 (a3-d3): merging of bright field and red fluorescence images; Column 4 (a4-d4): Hoechst 33258 stained blue fluorescence images. The untreated control cells exhibit weak red fluorescence (autofluorescence) while cells treated with ZONF show red fluorescence with higher intensity in a dose dependent manner (10-20 µg/mL, 24 h), suggesting the production of intracellular $O_2^{-}$ in response to nanoflowers. Scale bar: 50 µm.
**Fig. S3** Determination of generation of intracellular NO in ECV-304 cells using DAR reagent. Row 1 (a1-a3): control untreated cells; Row 2 (b1-b3): cells treated with VEGF (40 ng/mL, 24 h); Row 3 (c1-c3): cells treated with ZONF (20 µg/mL, 24 h); Column 1 (a1-c1): Hoechst 33258 stained blue fluorescence images; Column 2 (a2-c2): red fluorescence images; Column 3 (a3-c3): merging of blue and red fluorescence images. The result demonstrates that cells treated with ZONF exhibit red fluorescence with higher intensity compared to untreated control cells, indicating the enhanced level of intracellular NO in response to nanoflowers.
Fig. S4: Scratch wound healing assay in ECV-304 cells. (a) The result exhibits that L-NAME (200 µM, 8 h) attenuates the ZONF (20 µg/mL, 8 h) induced EC migration significantly, suggesting the role of eNOS signaling pathway during nanoflowers induced angiogenesis. Scale bar: 300 µm. (b) The migration capability of cells in response to different treatments is presented as % wound healing. Values are mean ± SD of three independent experiments. **p<0.01 compared to control; ##p <0.01 compared to ZONF treatment.