SUPPORTING INFORMATION

Investigation of molecular mechanisms and regulatory pathways of pro-angiogenic nanorods

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Modified microwave oven – MAS II (SINEO)

Figure S1. Modified microwave oven (MAS II- SINEO) used for the synthesis of EHNs.



Figure S2. Particle size distribution of nanorods (a) length and (b) width using ImageJ software. (c) Analysis of EHNs particle size using DLS. (d) Zeta potential distribution (surface charge) of EHNs using DLS.



Figure S3. FTIR analysis of as-synthesized EHNs showing the characteristic peaks at 3610 cm⁻¹ and 705.58 cm⁻¹.



Figure S4. Fluorescence imaging of nitric oxide production in EA.hy 926 cells upon treatment with (g-g1) EHNs + catalase and (h-h1) EHNs + MnTBAP + catalase respectively using DAF-2DA dye.



Figure S5. Nitric oxide imaging in HUVEC in response to (a) Control, (b) TE, (c) VEGF, (d) L-NAME, (e) EHNs (20 µg/mL) and (f) EHNs+ L-NAME treatments respectively using DAF-2DA.





Figure S7. Immunofluorescence of eNOS- phosphorylation at ser-1177 residue in ECV-304 cells in response to (a) Control, (b) EHNs (5 μ g/mL), (c) EHNs + MnTBAP, (d) VEGF, (e) EHNs + catalase and (f) EHNs + MnTBAP + catalase respectively.



Figure S8. Densitometric analysis of Phospho-Akt (Tyr 450) expression in HUVECs incubated with EHNs: 5-20 μ g/mL. VEGF 40 ng is used as a positive control treatment.



Figure S9(a-b). Densitometric analysis of (a) Phospho-Akt (Tyr 450) & (b) Phoshpho-eNOS (Ser 1177) expression in HUVECs incubated with EHNs in a time dependent manner. VEGF 40 ng is used as a positive control treatment.



Figure S10(a-b). Densitometric analysis of (a) Phospho-Akt (Tyr 450) & (b) Phospho-eNOS (Ser 1177) expression in EA.hy 926 cells incubated with EHNs in a time dependent manner. VEGF 40 ng is used as a positive control treatment.





Table. S1

Figure S11. Tube formation assay performed in ECV-304 cells incubated with (a) Control, (b) Sodium nitro prusside (SNP), (c) L-NAME, (d) EHNs and (e) EHNs + L-NAME respectively. (f) Corresponding representation of number of tubes.

Gene	Forward primer	Reverse primer	Size (Kb)	Annealing
				Temp
GAPDH	AGTAGAGGCAGGGATGATGTT	CTTTGGTATCGTGGAAGGACTC	133	57
eNOS	ACCCTCACCGCTACAACAT	GCTCATTCTCCAGGTGCTTC	198	58
iNOS	ACAAGCCTACCCCTCCAG	TCCCGTCAGTTGGTAGGTTC	158	57
VEGF	ACACATTGTTGGAAGAAGCAGCCC	AGGAAGGTCAACCACTCACACACA	179	60
VEGFR	ACGGACAGTGGTATGGTTCT	TGTCTGTGTCATCGGAGTGA	175	58

Table S1. Detailed sequence of the primers used for the Real Time-PCR analysis.