# Efficient and safe gene transfection in fish spermatogonial stem cells using nanomaterials‡

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## Supplementary Material

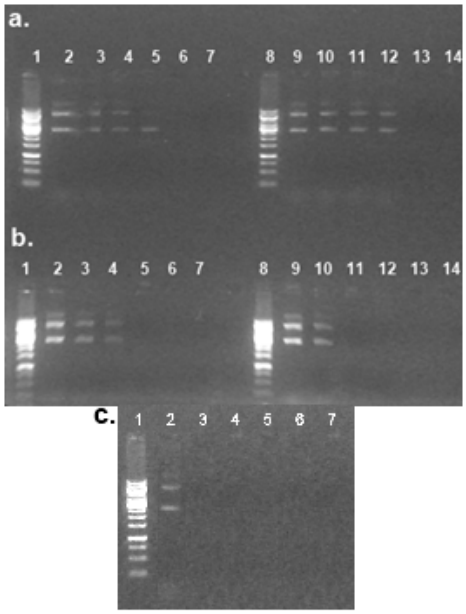


**Figure S1:** Binding capacity. **a.** Nanographene oxide (NGO) from $10^{-7}$ (lane 2) to $10^{-2}$ (lane 7) and nanodiamonds (NDs) from $10^{-7}$ (lane 9) to $10^{-2}$ (lane 14). **b.** gold nanorods (NRs)from $10^{-7}$ (lane 2) to $10^{-2}$ (lane 7) and multiwalled carbon nanotubes(fMWCNT) from $10^{-7}$ (lane 9) to $10^{-2}$ (lane 14). **c.** Phosphate based nanocomposites (NPC) from $10^{-7}$ (lane 2) to $10^{-2}$ (lane 7). Lanes 1 and 8 – ladder 1kb. Nanomaterials where able to bind to plasmid DNA from concentrations equal or superior to: 10 ng/mL for NGO; 100 ng/mL for ND; 1.3 x $10^{9}$ particles/mL for NRs; 2.5 ng/mL for MWCNTs and 1 ng/mL for NPCs

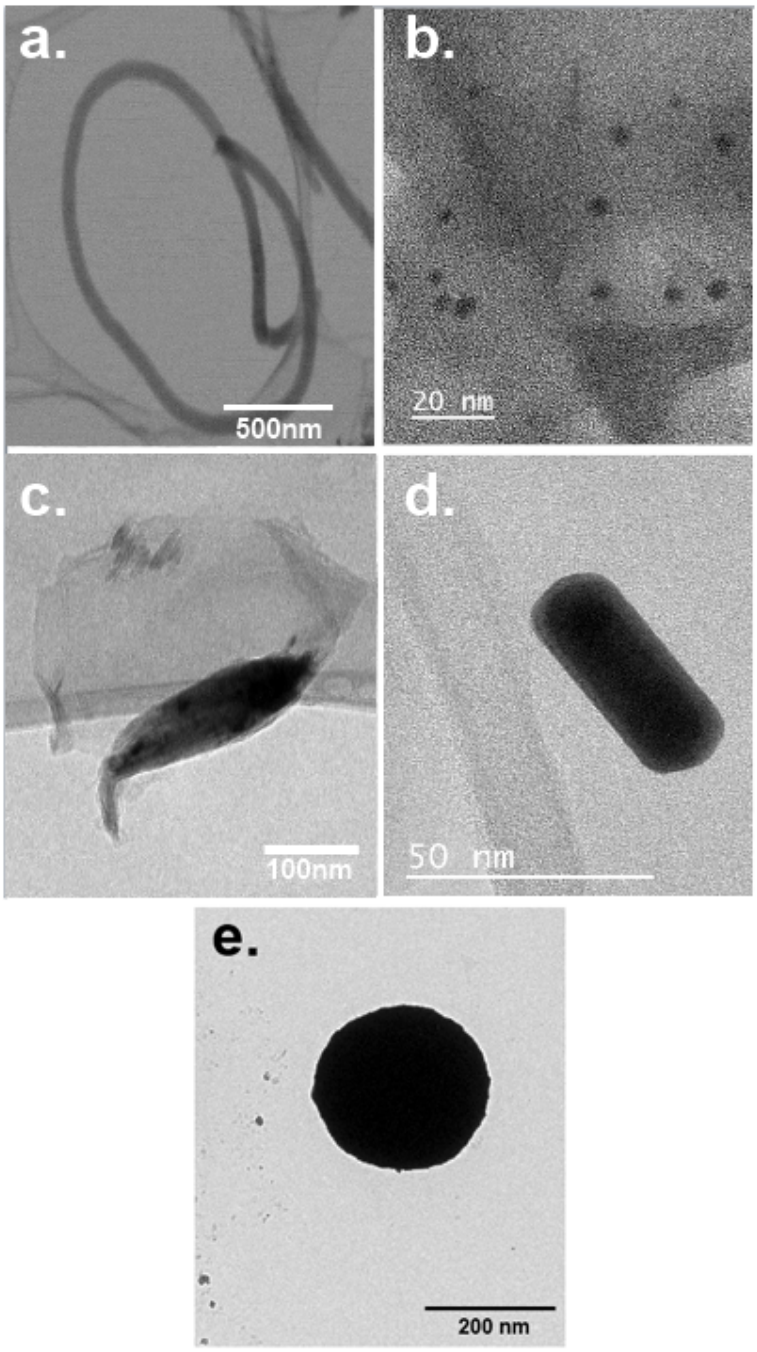


**Figure S2.**Transmission electron microscopy (TEM) images of nanomaterials. **a.**Multiwalled carbon nanotubes (MWCNTs). **b.**Nanodiamonds (ND). **c.**Nanographene oxide (NGO).**d.**Gold nanorods (NRs). **e.**Phosphate based nanocomposites (NPC).

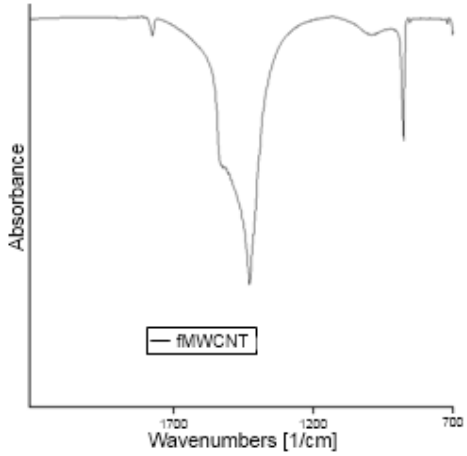


**Figure S3.** FTIR spectra of multiwalled carbon nanotubes(fMWCNTs).

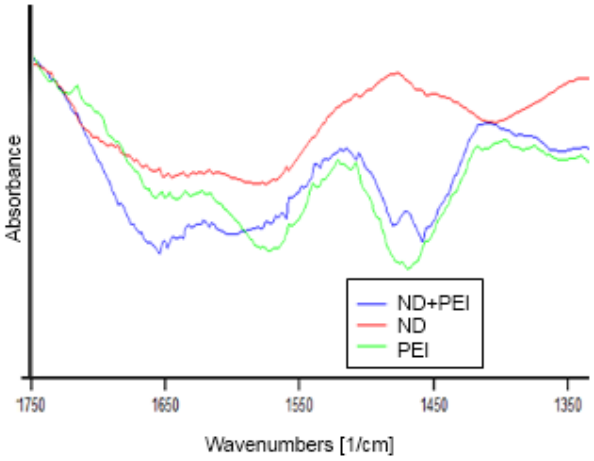


**Figure S4.** FTIR spectra of nanodiamonds (NDs).

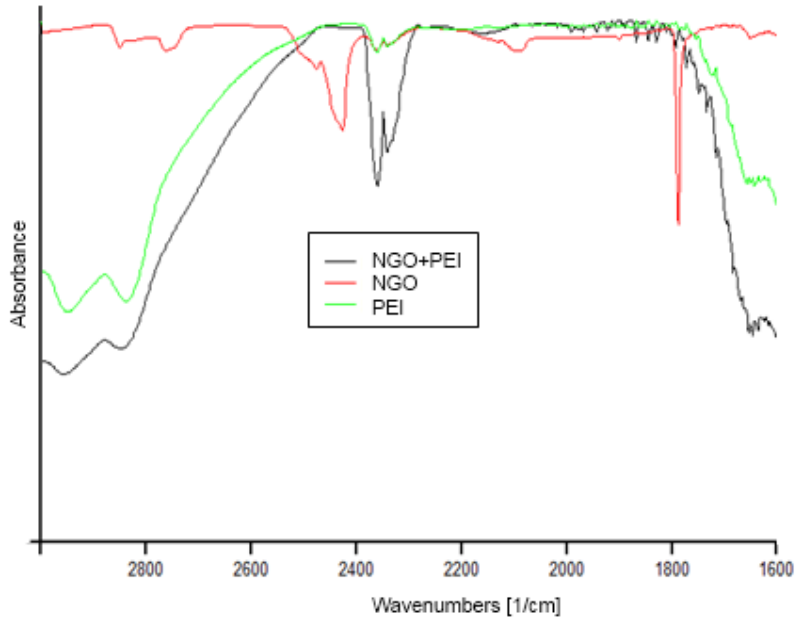


**Figure S5.** FTIR spectra of Nanographene oxide(NGOs).

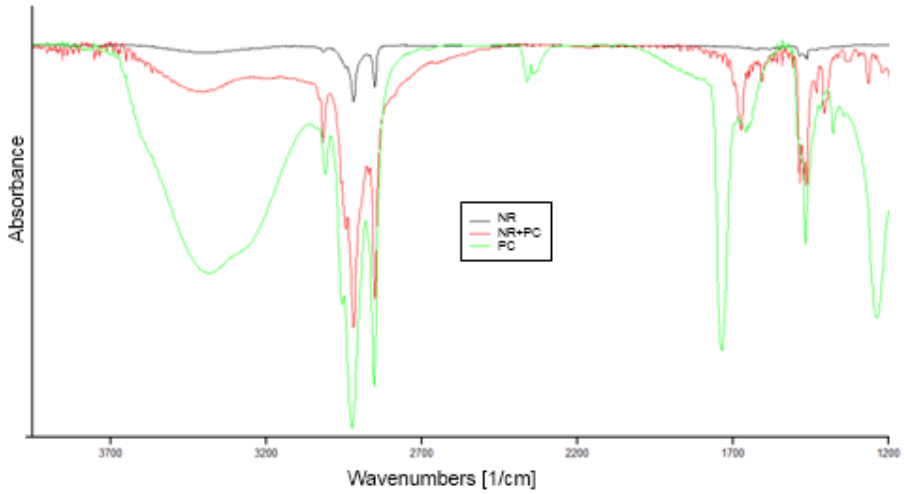


**Figure S6.** FTIR spectra of gold nanorods(NRs).

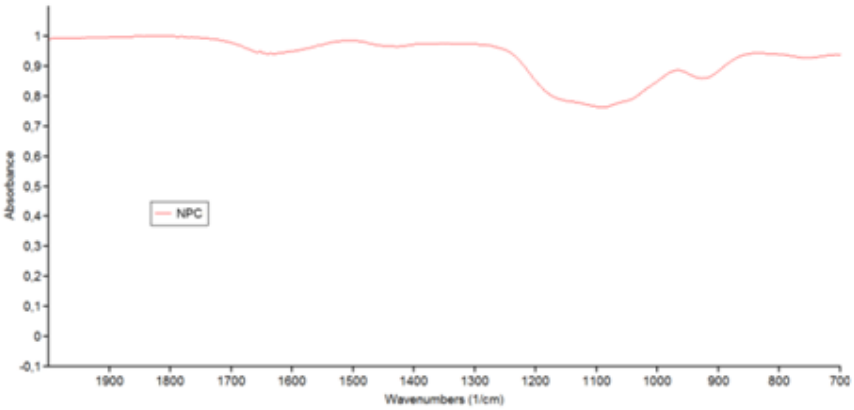


**Figure S7.** FTIR spectra of Phosphate based nanocomposites (NPCs).

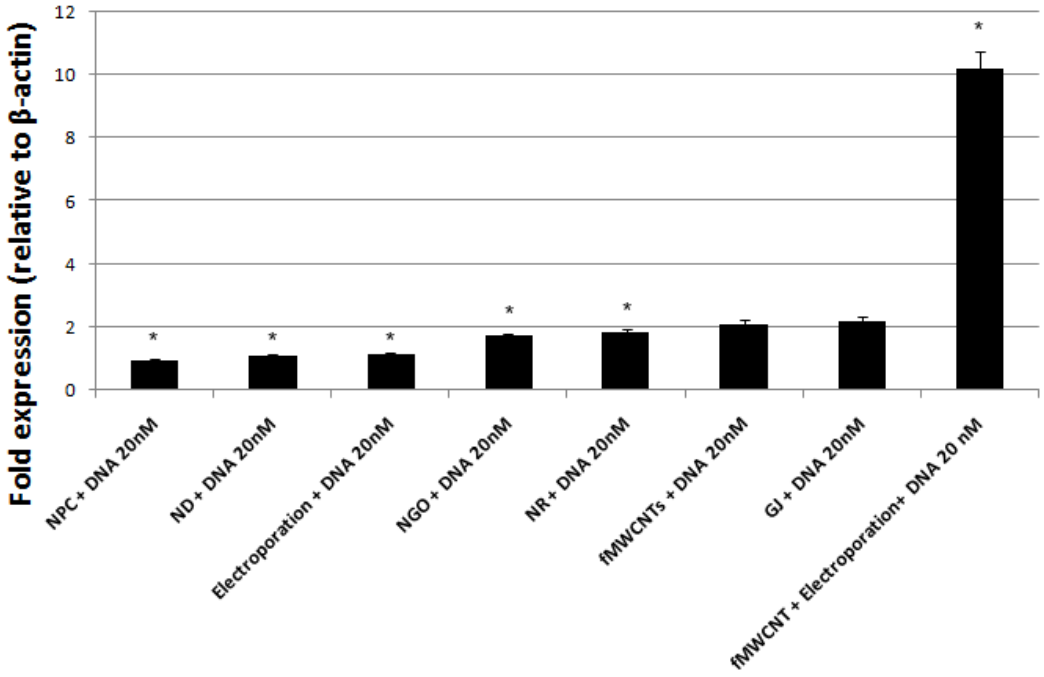


**Figure S8.** Dosage of transgene expression by qPCR. AmCyan1 mRNA synthesis in relation to β-actin using different strategies to promote transfection of SSCs. The results were compared to the ones from the commercial reagent Gene Juice® (GJ). Phosphate based nanocomposites (NPC), nanodiamonds (NDs), Nanographene oxide (NGO), gold nanorods (NRs),multiwalled carbon nanotubes (fMWCNTs) as vehicles. (asterisk, $p < 0.05$, *t* test).

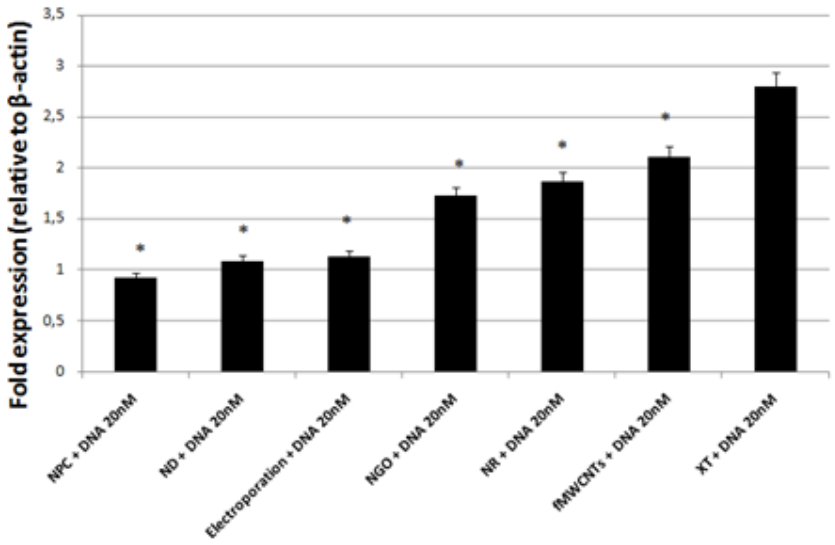


**Figure S9.** Dosage of transgene expression by qPCR. AmCyan1 mRNA synthesis in relation to β-actin using different strategies to promote transfection of SSCs. The results were compared to the ones from the commercial reagent X-treme. Phosphate based nanocomposites (NPC), nanodiamonds (NDs), Nanographene oxide (NGO), gold nanorods (NRs),multiwalled carbon nanotubes (fMWCNTs) as vehicles. Transfection was also assessed using the commercial reagent X-tremeGENE™ (XT) (asterisk, $p < 0.05$, *t* test).