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Functionalized nanomaterials: are they effective to perform gene delivery to difficult-to-transfect cells with no cytotoxic?

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Electronic supplementary materials

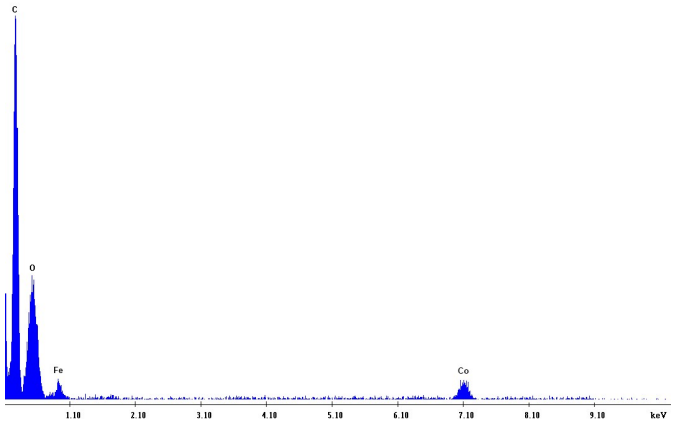

Figure S1: Energy dispersive X-ray (EDX) spectrum of fMWCNTs.

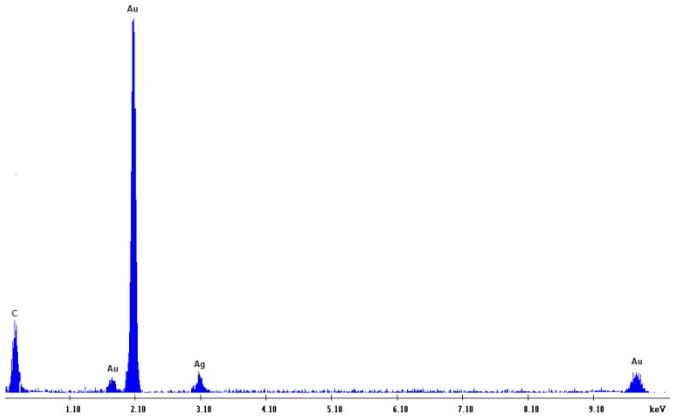

Figure S2: Energy dispersive X-ray (EDX) spectrum of NRs.

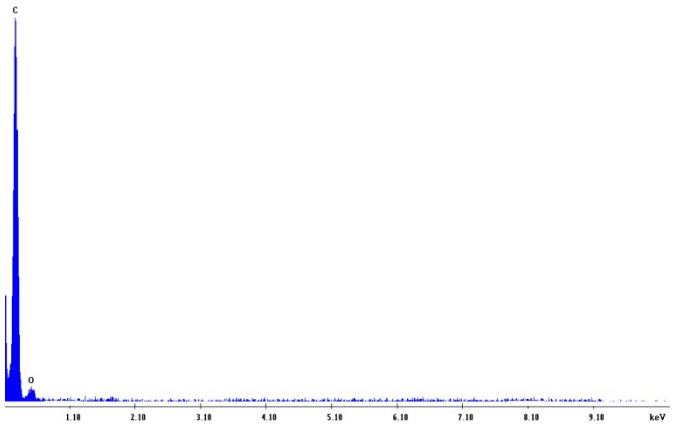

Figure S3: Energy dispersive X-ray (EDX) spectrum of NDs.

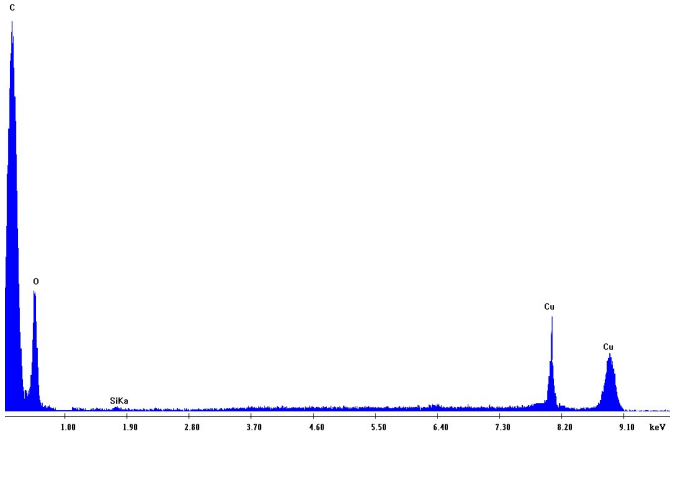

Figure S4: Energy dispersive X-ray (EDX) spectrum of NGOs.

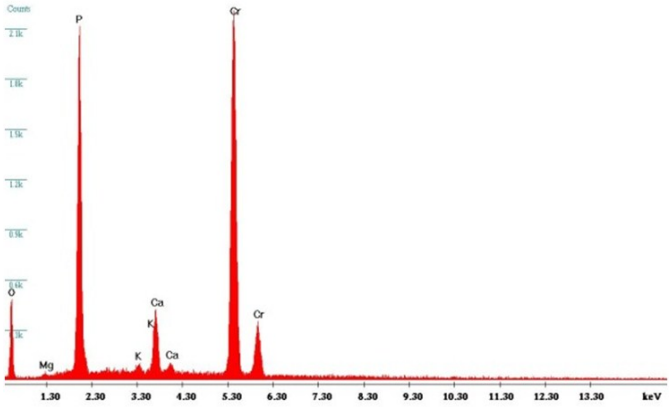

Figure S5: Energy dispersive X-ray (EDX) spectrum of NPCs.

Cell culture

Cardiomyocytes were obtained from rats and cultivated as previously described[1]. Briefly, in the primary culture cardiac cells were plated in dishes using M199 medium supplemented with: 100 units/ml penicillin, 100µg/ml streptomycin, 10% Fetal Bovine Serum, 2 mmol/L L-glutamine, and 20 µg/mL cytosine-D-arabinofuranoside (ARA-c) (to prevent growth of fibroblasts). After 48 hours, neonatal cardiomyocytes were exposed to the nanomaterials, with and without DNA[1].

DRG neurons were also obtained and cultivated as previously described[2]. Briefly, cells were obtained from animals lumbar segments using enzymatic procedure. Then they were washed in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma) supplemented with 10% Fetal Bovine Serum and 1% penicillin (Cultilab), and platted in dishes pre-treated with poly-D-lysine 0,1% (Sigma). After 24 hours, cells were exposed to the samples containing nanomaterials or Lipofectamine (with or without DNA)[2].

Lineage C6 cells(ATTC CCL-107) were cultivated in T-75 bottles until 90% of confluence was achieved, and then the cells wereplated in dishes using DMEM high glucose (Gibco) supplemented with 10% Fetal Bovine Serum,(Gibco), 2,5UI/mL of penicillin, 2,5ug/mL de streptomycinand 5ug/mL of gentamicin.

Lineage U373 cells were cultivated as same as C6 cells. In T-75 bottles until 90% of confluence was achieved, and then wereplated in dishes using DMEM high glucose (Gibco) supplemented with 10% Fetal Bovine Serum,(Gibco), 2,5UI/mL of penicillin, 2,5ug/mL de streptomycin and 5ug/mL of gentamicin.

Reference List

1. C. Rocha-Resende, A. Roy, R. R. Resende, M. S. Ladeira, A. Lara, E. R. M. Gomes, V. F. Prado, R. Gros, C. Guatimosim, M. A. M. Prado, S. Guatimosim *J Mol Cell Cardiol*, 2012,**53**, 206-16.

2. H. C. Joca, D. C. Vieira, A. P. Vasconcelos, D. A. Araújo, and J. S. Cruz, *Eur J Pharmacol.*, 2015**, 756**, 22-27.

