Supplementary Material

Dendrimer-PLGA based multifunctional immuno-nanocomposite mediated synchronous and tumor selective delivery of siRNA and cisplatin: Potential in treatment of hepatocellular carcinoma

Mohd. Asif Sherwani[1], Saba Tufail[1], Aijaz Ahmed Khan[2], Mohammad Owais*[1]

[1]Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh, 202002, India

[2]Department of Anatomy, Jawaharlal Nehru Medical College, Faculty of Medicine, Aligarh Muslim University, Aligarh, 202002, India

***Name and address of corresponding authors:**

Prof. Mohammad Owais

Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh-202002, INDIA.

Phone: +91-571-2720388, Fax: +91-571-2721776.

E-mail address: owais_lakhnawi@rediffmail.com

Materials and Methods

Peripheral blood mononuclear cell (PBMC) isolation

Fresh blood (20–15 ml) was procured from Blood Bank, Department of Pathology (R. No.-BBL-04/SC/P/1996), Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, India. PBMCs from fresh blood were isolated following the published procedure.[1,2] Briefly, the blood sample (50% hematocrit) was carefully layered on Ficoll-Histopaque and the mixture was then centrifuged at 400 g for 30 min at 20–22˚C. The undisturbed lymphocyte layer was carefully taken out and the lymphocytes obtained were washed and pelleted down with three volumes of PBS two times and re-suspended in RPMI-1640 media with antibiotic and antimycotic solution 10%, v/v fetal calf serum (FCS). Cell counting was performed to determine the PBMC cell number with equal volume of trypan blue and further MTT assay was performed following the protocol as follows.

MTT assay for Ab@PAMAM-siRNA@Cis-PLGA NC toxicity on PBMCs

Cytotoxicity of Ab@PAMAM-siRNA@Cis-PLGA NC formulation on PBMCs was examined by performing MTT assay following the procedure described elsewhere.[2] Briefly, PBMCs maintained in RPMI 1640 culture medium supplemented with 10% heat-inactivated fetal calf serum and antibiotic antimycotic solution were plated at a density of 5×10^3 cells per well in a

96-well plate, and cultured for 24 hours at 37 °C. The cells were treated with various PLGA-dendrimer NC formulations for 24 and 48 hours separately. After stipulated incubation periods, cell proliferation was measured by adding 20 µl of MTT dye (5 mg/ml in phosphate-buffered saline) per well. The plates were incubated further for 4 hours at 37 °C in a humidified chamber containing 5% CO_2. Formazan crystals formed due to reduction of dye by viable cells in each well were dissolved in 150 µl dimethyl sulfoxide, and absorbance was read at 570 nm. The absorption values were expressed as the cell viability (%), with reference to the control group taken as 100 %.

Biodistribution of nanocomposites upon intravenous administration of Ab@PAMAM-siRNA@Cis-PLGA NC formulation

The biodistribution of nanocomposites was analyzed by examining the *in vivo* distribution of cisplatin entrapped in them. Previous studies have also reported tracking the nanoparticles by examining the biodistribution of the payload entrapped within the nanoparticles[3] because it is accepted that location of drug release is the site of nanoparticle homing. Cisplatin loaded formulations viz. PAMAM-siRNA@Cis-PLGA NC and Ab@PAMAM-siRNA@Cis-PLGA NC were injected intravenously to the animals and the biodistribution study was performed at 0.25 hr (15 min), 1 hr, 2 hr, 6 hr and 24 hr time points post injection. At these time points, blood was collected by retro-orbital puncture and animals were sacrificed to isolate various vital organs like liver, kidney and spleen for studying the biodistribution of nanoparticles. The organs were removed aseptically and then washed, dried and weighed. Further, each organ was homogenized and the tissue lysate obtained was centrifuged at 10, 000 g. The supernatant was collected and analysed for cisplatin content using HPLC as described elsewhere.[4] For determination of cisplatin in systemic circulation, blood was centrifuged at 1, 000 ×g for 10 minutes, and serum was collected. Serum proteins were precipitated, and further centrifuged at 10, 000 ×g for 10 minutes. Supernatant was then collected and examined for cisplatin content by HPLC.

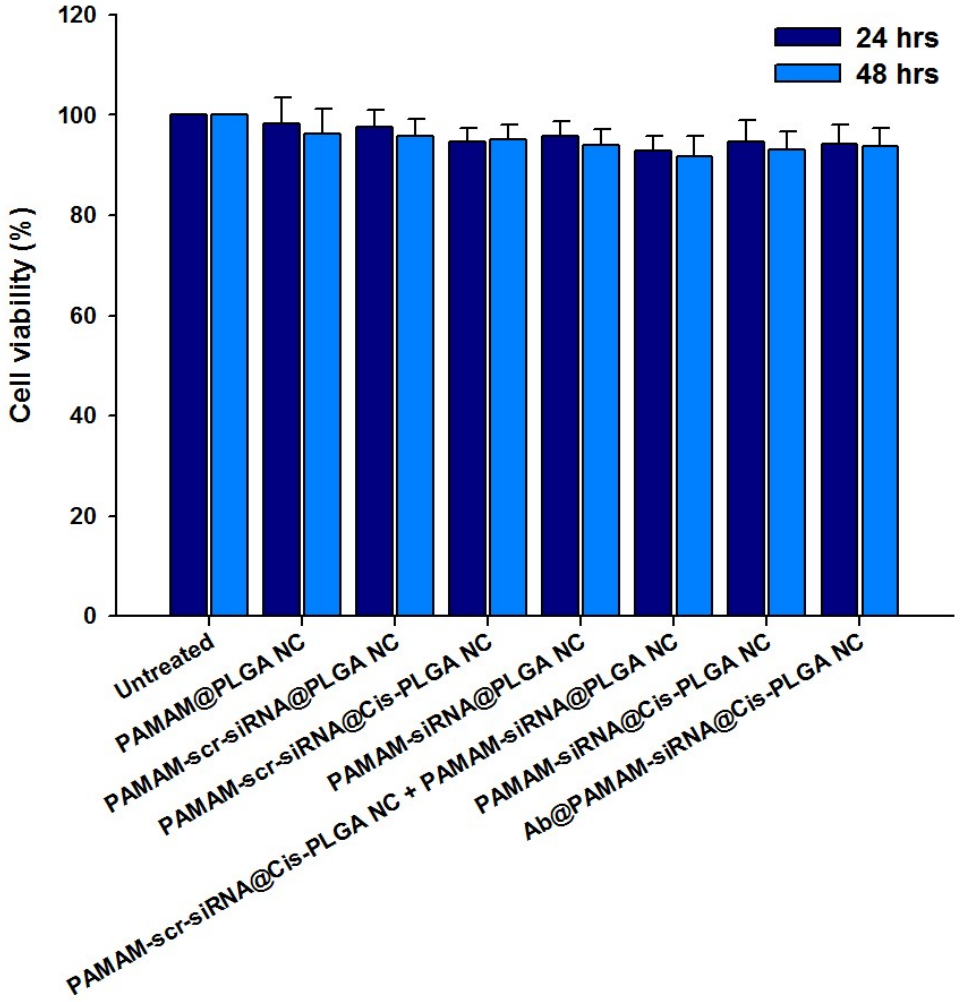

Figure S1. MTT assay. MTT assay for analyzing toxicity of various dendrimer-PLGA nanocomposites to PBMCs after 24 hrs and 48 hrs of treatment.

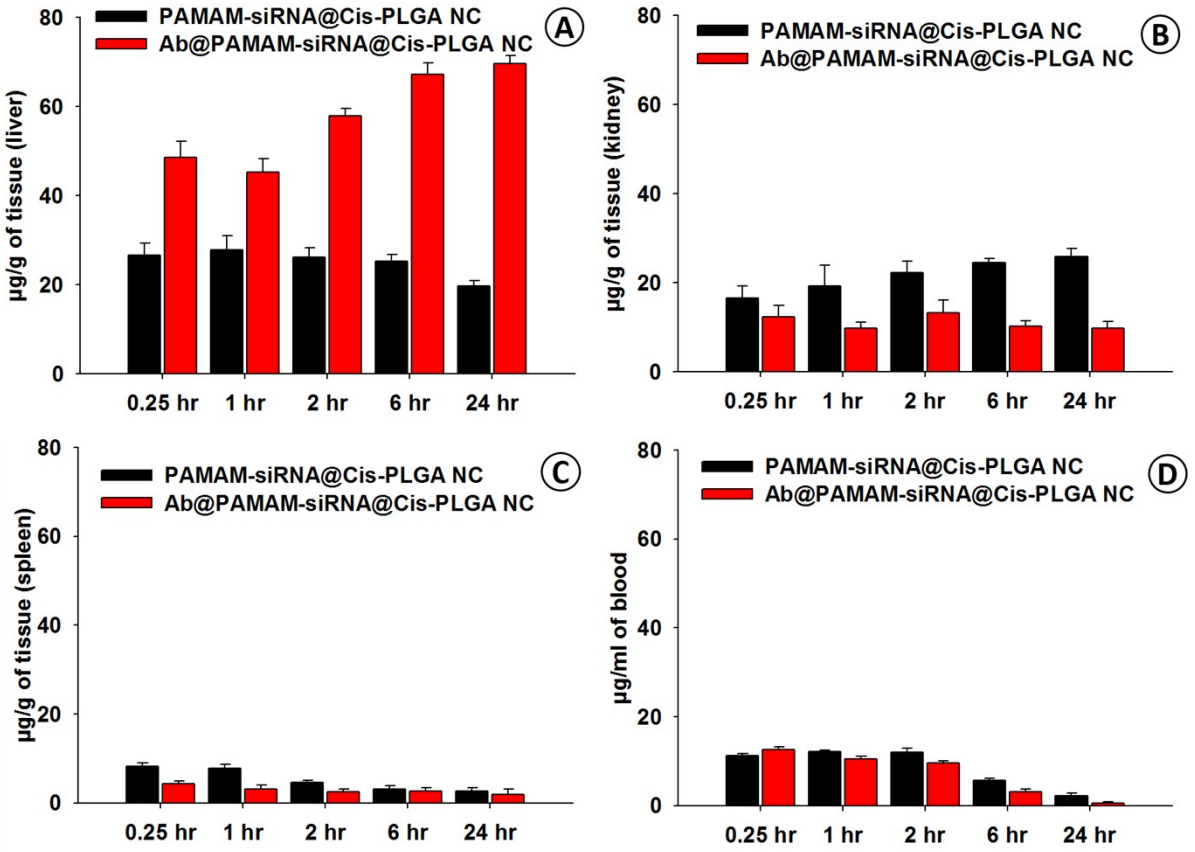

Figure S2. Figure 6. Biodistribution of immunonanocomposites as analysed by *in vivo* dissemination of cisplatin entrapped therein. Concentration of cisplatin in Liver **(A)** Kidney **(B)** Spleen **(C)** and Blood **(D)** upon intravenous injection of cisplatin carrying nanocomposites at 0.25 hr, 1 hr, 2 hr, 6 hr and 24 hr time points. Data are mean values ± standard deviation from three different experiments (For Ab@PAMAM-siRNA@Cis-PLGA NC: Liver vs other organs and blood, $P < 0.001$).

References

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