Electronic Supplementary Information

Biological activity of dendrimer-methylglyoxal complexes for improved therapeutic efficacy against malignant cells

Srabanti Ghosh[a]*, Prabal Chakraborty[b], Adrita Chakrabarti[c], Manosij Ghosh[d], Amit Mandal[e], Partha Saha[b], Anita Mukherjee[d], Somobrata Acharya[a], Manju Ray[c,f]

[a]Centre for Advanced Materials, Indian Association for the Cultivation of Science, Kolkata-700032, India.

[b]Crystallography and Molecular Biology Division, Saha Institute of Nuclear Physics, Kolkata-700064, India.

[c]Biological Chemistry, Indian Association for the Cultivation of Science, Kolkata-700032, India.

[d]Centre of Advanced Study, Department of Botany, University of Calcutta, Kolkata, India

[e]Molecular Medicine, Bose Institute, Kolkata 700 054, India.

*Corresponding Author, E-mail: *ghosh.srabanti@gmail.com*

Hemolytic Activity

Heparin-stabilized human blood was freshly collected and used within 3 hours of being drawn. A 4 mL sample of whole blood was added to 8 mL of Dulbecco's phosphate-buffered saline (PBS), and the RBCs were isolated from serum by centrifugation at 1000 rpm for 5 min. The RBCs were further washed five times with sterile phosphate-buffered saline solution. Following the wash, the RBCs were diluted to 40 mL of PBS (10% v/v RBC). The resulting stock (10% v/v RBC) was diluted 3-fold in buffer to give the assay stock (3.3% v/v RBC). The assay stock, RBC was then mixed with the PAMAM/MG formulations to give a final solution of 3% v/v RBC, which corresponds to approximately 10^8 red blood cells per mL based on counting in a haemacytometer. Buffer or Triton X-100 (1% v/v) was added as negative and positive hemolysis controls, respectively. The eppendrof were secured in a shaker at 37°C for 60 min and then centrifuged at 1000 rpm for 10 min.

Development of tumour

The carcinogen was dissolved in olive oil by placing it in warm water-bath and 0.1 mL of olive oil containing 0.2 mg of the carcinogen was injected into each mouse thrice with one-week interval. After 12-14 weeks full-grown tumour was developed. The malignancy was confirmed by histological examination where differentiated muscle cells are conspicuously visible in normal mouse muscle and highly differentiated sarcoma tissue is being observed, where tumour was developed by 3-methyl-cholanthrene.

Cytotoxicity assays (MTT assay)

Cell toxicity was determined using MTT assay based on the reduction of a soluble tetrazolium salt by mitochondrial dehydrogenase of the viable cells to water insoluble colored product (i.e. formazan). The amount of the formazan produced proportional to the number of live cells. Reduction of the absorbance value can be attributed to the killing of the cells or inhibition of cell proliferation by the PAMAM/MG. Briefly, the confluent C2C12, WI38 and Hela cells were seeded in triplicate at a density 2×10^5 cells per well in a 96-well microtiter plate for 12-24 h before the assay. The cells were then incubated for 24 h at 37°C under 5% CO_2 in presence of varying concentration of PAMAM/MG. The untreated cells and media alone were used as positive and negative controls, respectively. Then, 50 µl of a 2 mg mL^{-1} MTT (Sigma; St Louis, MO) was added into each well, followed by 4 h of incubation at 37°C. The formazan crystals were then solubilized in 200 µl DMSO. The optical density (OD) at 570 nm was measured using an automated BioTek® Elisa Reader.

Isolation of peritoneal macrophages

After isolation, cells were enumerated with a haemocytometer and then re-suspended in tissue culture plates (at 10^6 cells/mL) and allowed to adhere for four hours in a humidified atmosphere with 5% CO_2 at 37^0C. Then cells were suspended in complete medium and cell viability was checked by the trypan blue dye exclusion test. The untreated macrophages were 95% viable as identified by their morphology. The macrophages were then treated with PAMAM/MG complexes for the entire corresponding time period. After incubation, PAMAM-MG complexes was removed from the medium and the cells were washed with PBS for further analysis.

DNA damage analysis using Comet assay

Slides were prepared in triplicates per concentration. A total of 100 μl of cell suspension was mixed with 100 μl of 1% low melting agarose onto microscope slides. The suspension was pipetted onto the pre-coated slides. Slides were immersed in cold lysis solution at pH 10 (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris pH 10, 1% Triton X-100 and 10% DMSO) and kept at 4°C for 60 min. To allow denaturation of DNA, the slides were placed in alkaline electrophoresis buffer at pH > 13 (1 mM Na_2EDTA/300 mM NaOH), left for 20 min and subsequently were transferred to an electrophoresis tank with fresh alkaline electrophoresis buffer. Electrophoresis was performed at field strength of 0.7 V/cm for 30 min at 4°C (20 V–300 mA). Slides were neutralized and washed in 0.4 M Tris pH 7.5 for 5 min, thrice and finally rinsed with water. Slides were stained with ethidium bromide and images of 150 (50 cells per replica x 3 replicates) nuclei per concentration were obtained.

Isolation of human lymphocytes

Isolation of lymphocytes was performed from human peripheral blood (HPB) that was obtained by venipuncture from healthy volunteers (20–25 year old male donors, non-smokers, non-alcohol consuming and not undergoing any medication) into heparinised vacutainers. The cells were washed with PBS and re-suspended in RPMI-1640 media at a concentration of 10^6 cell/mL and were immediately processed for further experiment. All experiments were conducted in accordance with the institutional guidelines. Freshly isolated lymphocytes were incubated for 3 h at 37°C in RPMI-1640 media with different concentration of PAMAM-NH_2/MG.

Table S1 Size distribution by dynamic light scattering and average zeta potential data of PAMAM dendrimer, MG, PAMAM-NH_2/MG, PAMAM-COOH/MG and PAMAM-OH/MG complexes at pH 6.5.

Material	**Average Diameter (nm)**	**Zeta Potential (mV)**
PAMAM-NH_2 /MG	55	+27.5
PAMAM-COOH/MG	78	-14.3
PAMAM-OH/MG	85	+12.5
MG	-	-11.2
PAMAM/NH_2	-	+32
PAMAM/COOH	-	-13.3
PAMAM/OH	-	+10.5

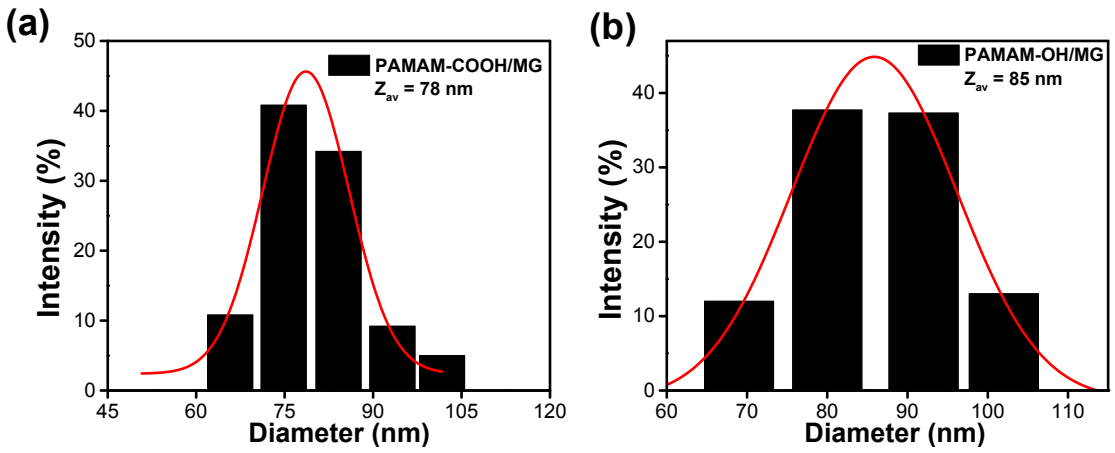

Fig. S1 Size distribution histogram of (a) PAMAM-COOH/MG and (b) PAMAM-OH/MG.

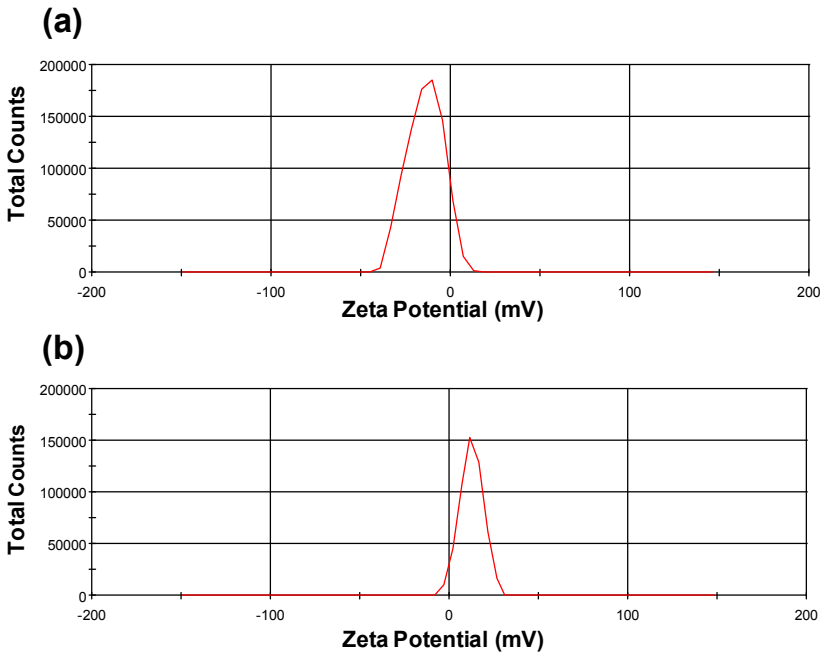

Fig. S2 Zeta potential distribution of (a) PAMAM-COOH/MG and (b) PAMAM-OH/MG.

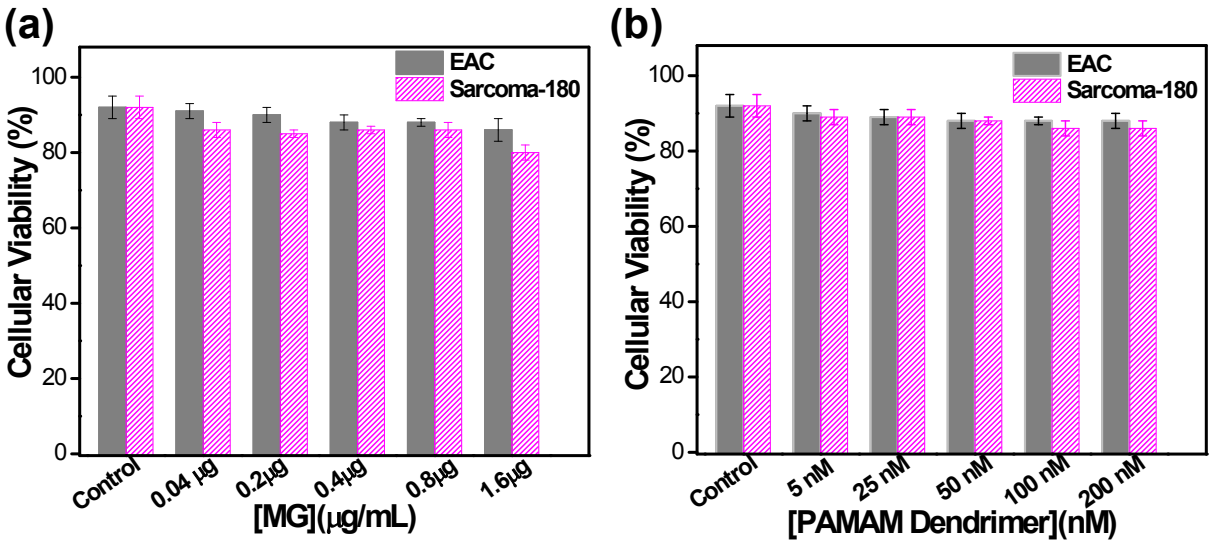

Fig.S3 (a) Cellular viability of EAC and Sarcoma-180 cells after treatment with different concentration of MG and amine terminated PAMAM dendrimer by Trypan Blue Exclusion assay.

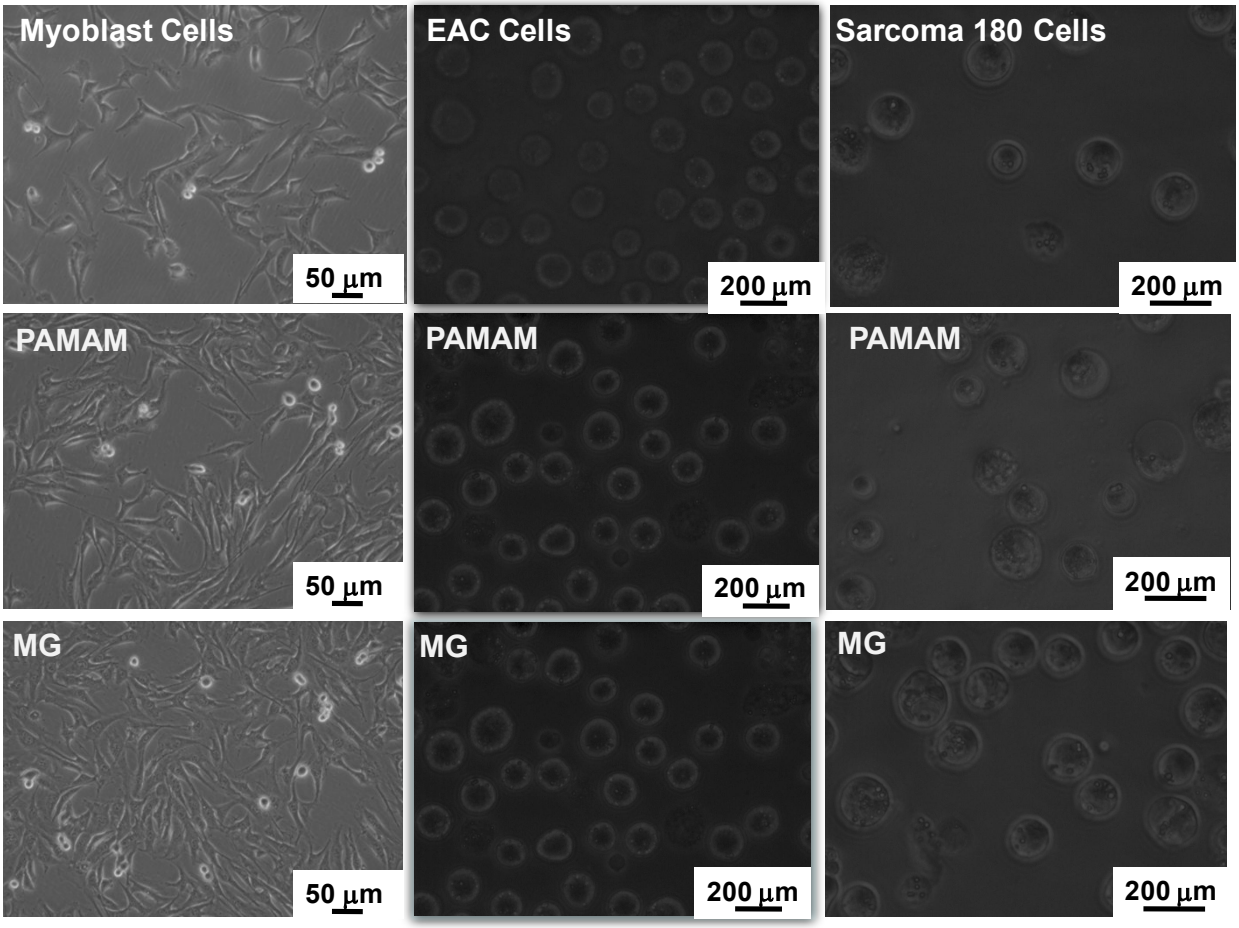

Fig.S4 Effect of amine terminated PAMAM dendrimer and MG on the morphology of mouse myoblast cells, EAC and Sarcoma-180 cells by phase contrast microscopy.