Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Surface functionalization induced enhancement in surface properties and biocompatibility of polyaniline nanofibers

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1. Experimental:

1.1. Isolation, culture, and treatment of lymphocytes

The cytotoxicity effect of Polyaniline nanofibers (PNFs) and surface functionalized polyaniline nanofibers (SF-PNFs) was investigated by using isolated human lymphocytes, collected voluntarily. Anti-coagulated human blood was diluted with a phosphate buffer solution (PBS) (v/v 1:1). 3mL histopaque (1.07 g mL^{-1}) was taken in a centrifuge tubes followed by equal volume of blood was tipped on the histopaque layer. Subsequently, it was centrifuged at 400 g for 30 min and lymphocytes were collected from the buffy layer. The isolated lymphocytes were then washed thrice with 2 mL PBS followed by 2 mL RPMI-1640 media through centrifugation steps separately for 10 min at 250 g. The pelleted lymphocytes were then suspended in RPMI-1640, and viability was tested by the Trypan blue exclusion method using a hemocytometer [1]. Aliquots of 200 mL of isolated cells were seeded in RPMI-1640 supplemented with 10% heat-inactivated FBS. Initially, cells were incubated (at 37 °C in 5% CO_2) for 8 h in RPMI-1640 without FBS. The cells were then treated as per experimental requirement and maintained with the inclusion of FBS for 12 h.

1.2. Erythrocytes separation

Fresh human blood was collected from healthy donors among laboratory personnel. Citarate buffer was used as anticoagulant. Erythrocytes were separated from plasma and buffy coat by centrifugation at 800 ×g, 10 min, and washed three times in isotonic phosphate buffer saline (PBS, pH 7.4) [2]. After three washings with the phosphate-buffered saline solution (centrifugation for 5 min at 700 g), the erythrocytes were suspended in 20 ml PBS diluted the cells $7.068x10^8$ for membrane stability assay.

1.3. MTT assay

The cytotoxicity assay was performed by measuring the viability of cells according to the method described by Denizot and Lang [3] with slight modification. The key component [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT) is yellowish in color and the mitochondrialdehydrogenase of viable cells cleave the tetrazolium ring, yielding purple insoluble formazan crystals, which were dissolved in a suitable solvent [4]. In this report, 0.1% TGA was used as the vehicle in which samples were suspended. The resulting purple solution was evaluated through spectrophotometrical means. An increase or decrease in cell number resulted in a concomitant change in the amount of formazan formed and would indicate the degree of cytotoxicity caused by the test material. Briefly, after treatments, cells were treated with 1% of MTT for 2 h, then the formazan crystals were dissolved in solvent and the absorbance of the solution was measured at 570 nm. The background absorbance was measured at 690 nm and was subtracted from 570 nm. The absorbance of the control cells was set at 100% viability and the values of the treated cells were calculated as a percentage of the control.

The cell viability was assessed quantitatively on the basis of reduction of tetrazolium salt to water soluble colored formazan product by viable cells in culture [5, 6]. Viable cell metabolism produces a reducing equivalent NADH, which passes its electron to an intermediate electron transfer reagent capable of reducing tetrazolium salt to formazan,

whereas non viable cells lose this ability. Therefore, the production of this aqueous colored product is directly proportional to no. of viable cells. Accordingly, the observed absorbance value at 570 nm shown by the colored formazan for each sample is used to calculate the percentage of cell viability using the following formula:

$$\%\ Cell\ Viability = \frac{A_s}{A_c} \times 100$$

Where A_s and A_c represent the absorbance values of sample and control (PBS at p^H = 7.4) respectively.

1.4. Membrane stability test

Membrane stability test was performed as described [7] with slight modification. Direct evidence of damages due to the action of detergents on erythrocyte membranes can be obtained by measuring the extent of haemolysis, i.e. the leakage of haemoglobin from the cytoplasm, upon detergent incubation and the A540 of the supernatant was determined. The 1 ml reaction mixture contains 160 µl of RBC, 0.1% triton-X 100, various concentration of nano particle or standard (ascorbic acid). After 35 min incubation at 37^0 C, agitate the cells for 10min on ice followed by centrifugation at 1300g for 5 min. After that supernatant was taken and absorbance was read at 540 nm against reagent blank and the percentage membrane activity was calculated by comparing the test with positive (triton-x100) and negative (PBS) control. Ascorbic acid was taken as standard.

Membrane stability test of both PNFs before and after functionalization has been carried out by evaluating the percentage of haemolysis using the following formula:

$$\%\ Haemolysis = \frac{A_S - A_N}{A_P - A_N} \times 100$$

Where A_S, A_P and A_N are the absorbance of the sample, positive control and negative control, respectively. The results are shown in Fig.11. According to the ASTM F 756-00 standard [8, 9], all materials can be categorized on the basis of their haemolysis activity as follows:

% Haemolysis < 2: non-haemolytic

2 < % Haemolysis (%) < 5: slightly haemolytic

% Haemolysis > 5: haemolytic

References:

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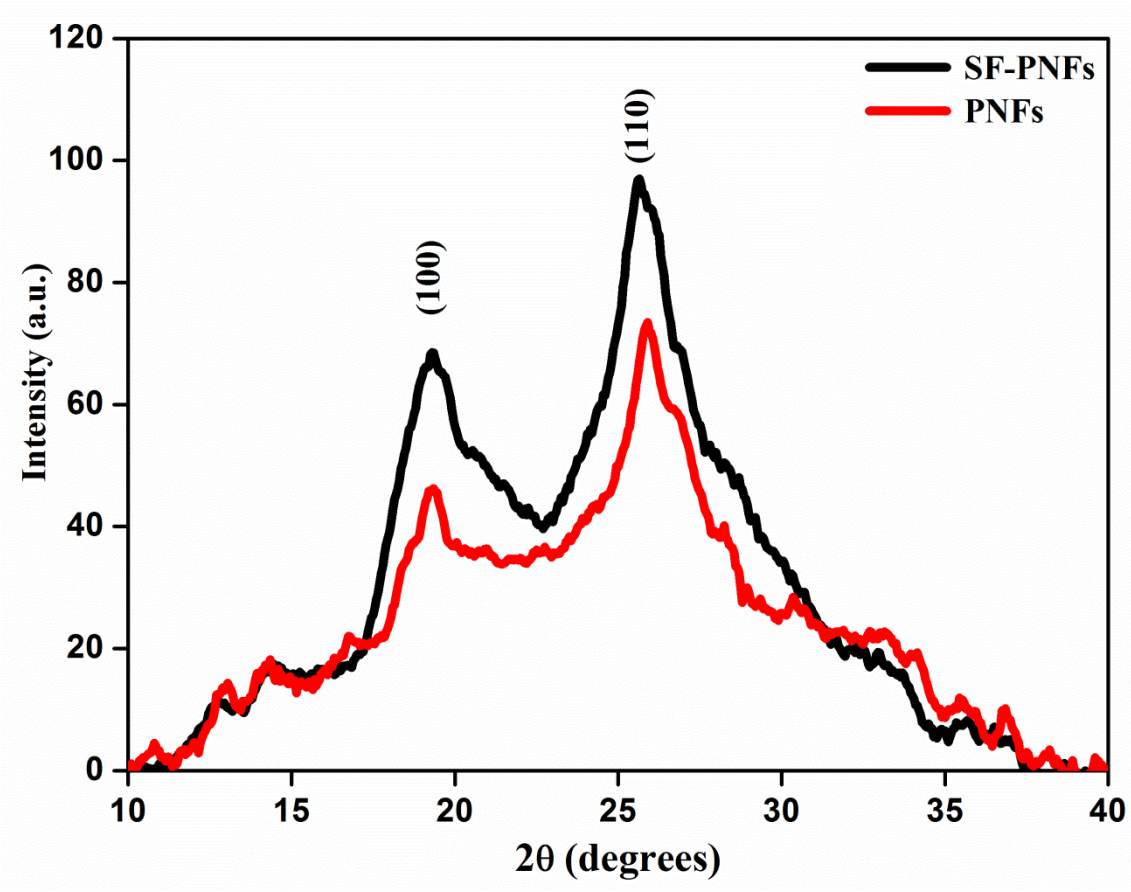

Figure S1: XRD pattern of PNFs (black) and SF-PNFs (red). XRD suggests that PNFs are semicrystalline in nature and after functionalization crystallinity of PNFs has been observed to be reduced.

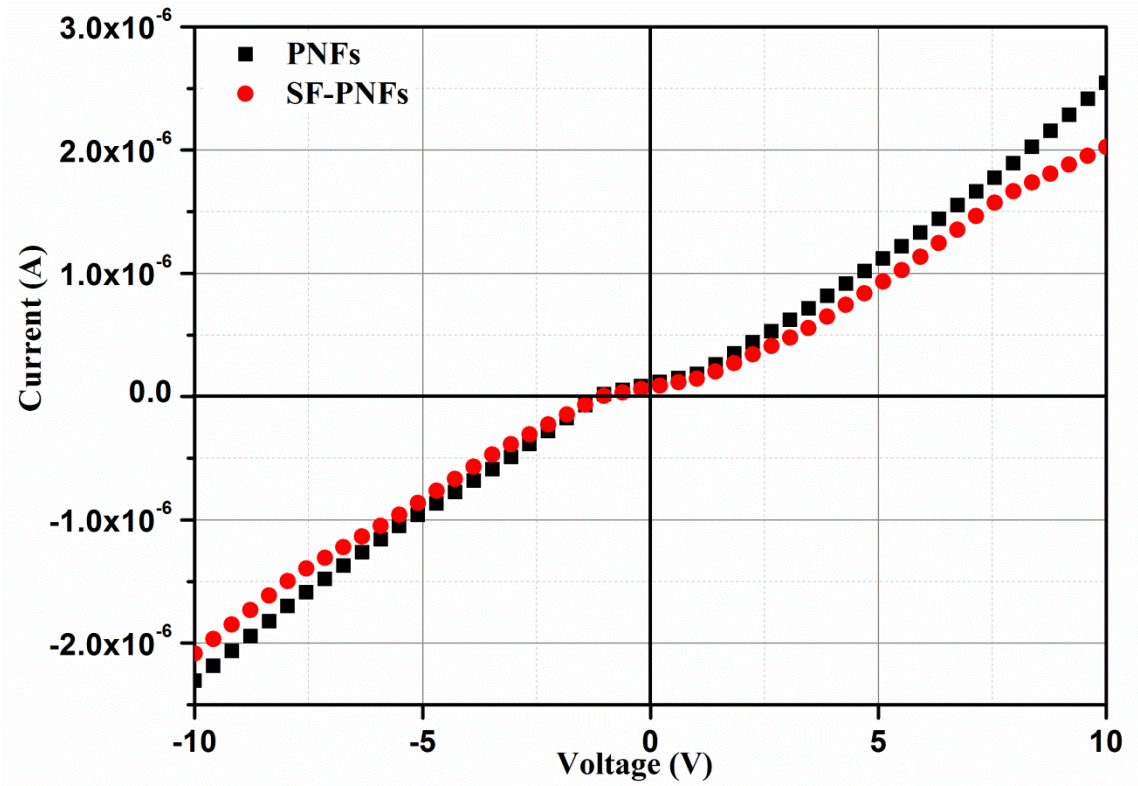

Figure S2: I-V characteristics of polyaniline films before (PNFs: black) and after functionalization (SF-PNFs: red). The characteristic is almost nonlinear and can be attributed due to the intrinsic charge carriers of the polymer. After functionalization, the current is observed to be slightly decreased.

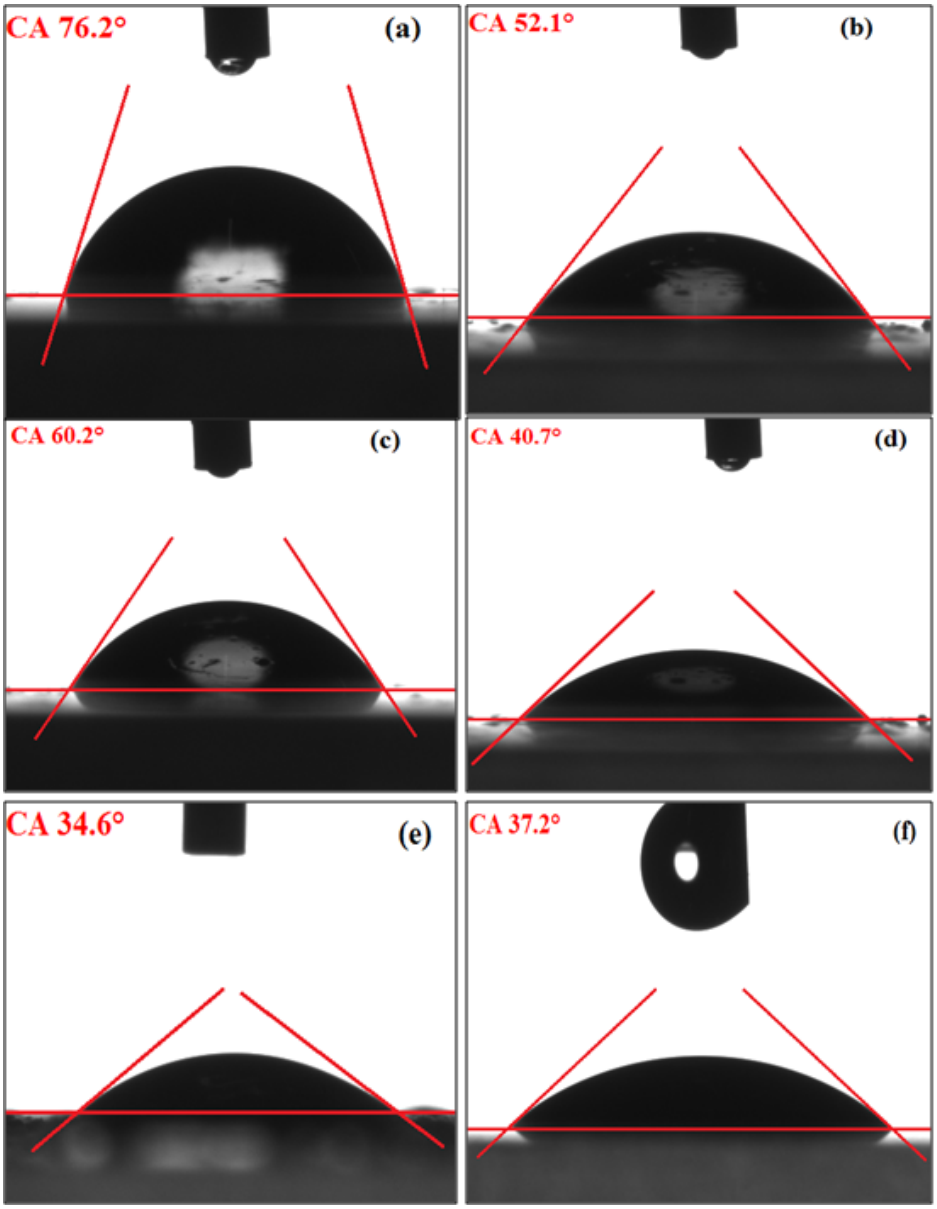

Figure S3: Photographic images of sessile liquid drops on pristine polyaniline nanofibers (PNFs) and surface functionalized polyaniline nanofibers (SF-PNFs). Figures (a), (c) and (e) show contact angle images on pristine film for water, ethylene glycol and diiodomethane respectively. Figures (b), (d) and (f) show contact angle images on functionalized film for water, ethylene glycol and diiodomethane respectively.

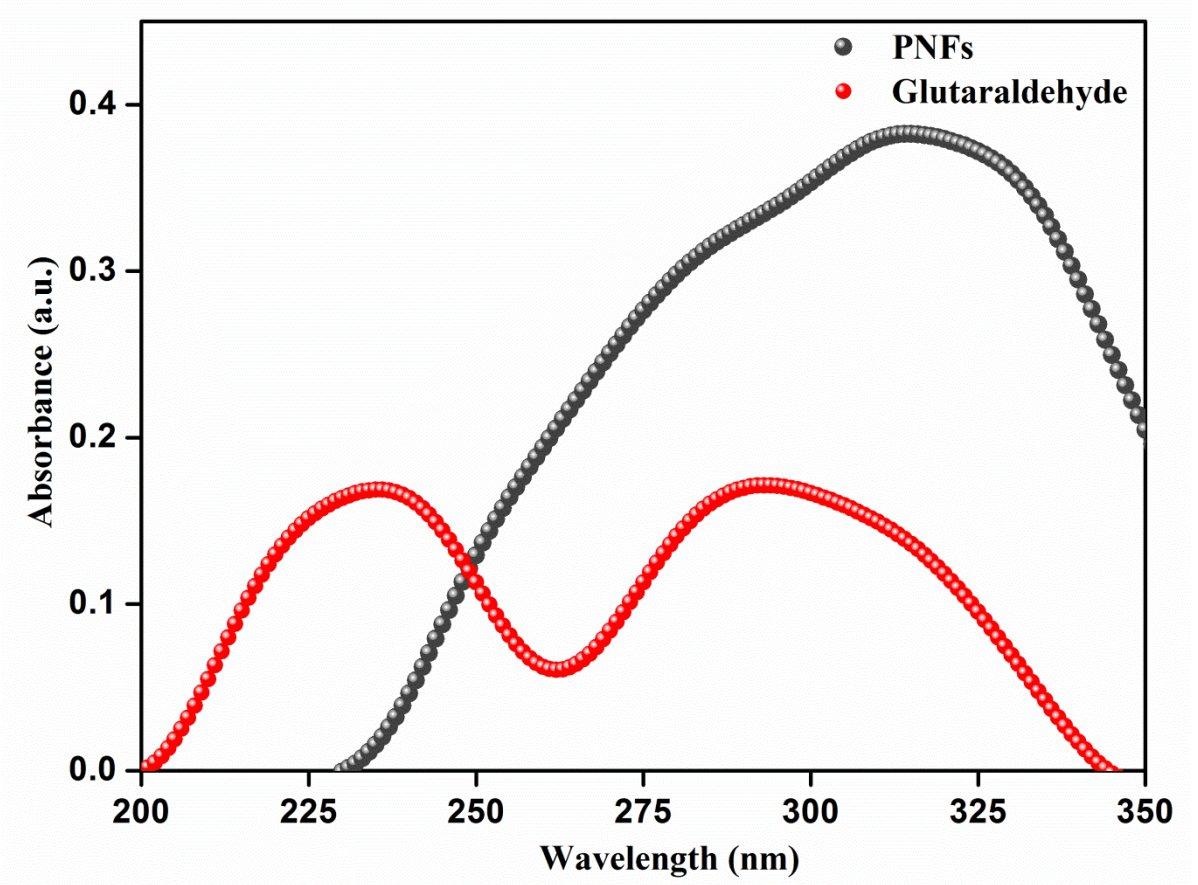

Figure S4: Absorption spectrum of (a) 1% glutaraldehyde solution (b) PNFs in 0.1M PBS at $p^H = 7.4$. Absorption spectra are shown only to show the overlapping between the absorption spectrum of PNFs and glutaraldehyde. Glutaraldehyde shows two absorption peaks at 237 nm and 284 nm, while EB of PNFs shows absorption peaks in the region 300 nm (shown here) and 600 nm (not shown here) .

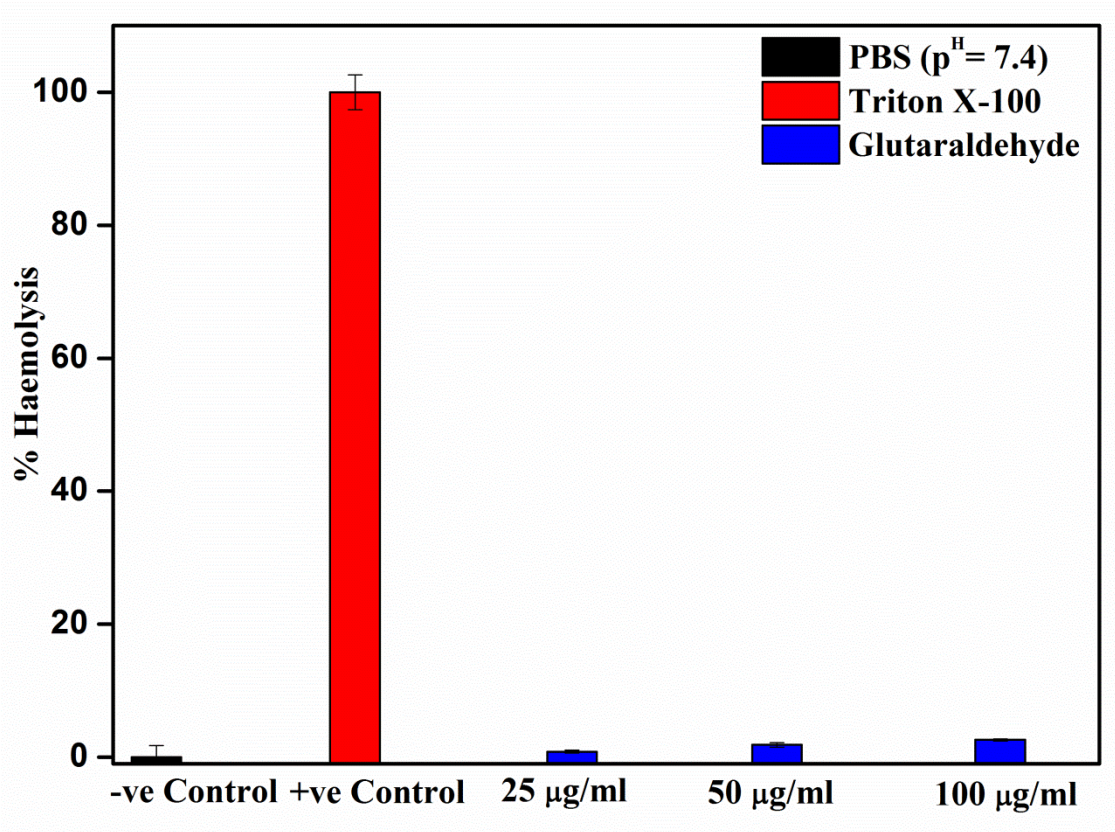

Figure S5: Haemolysis activity of 1% glutaraldehyde solution at three different doses showing its blood compatibility nature.

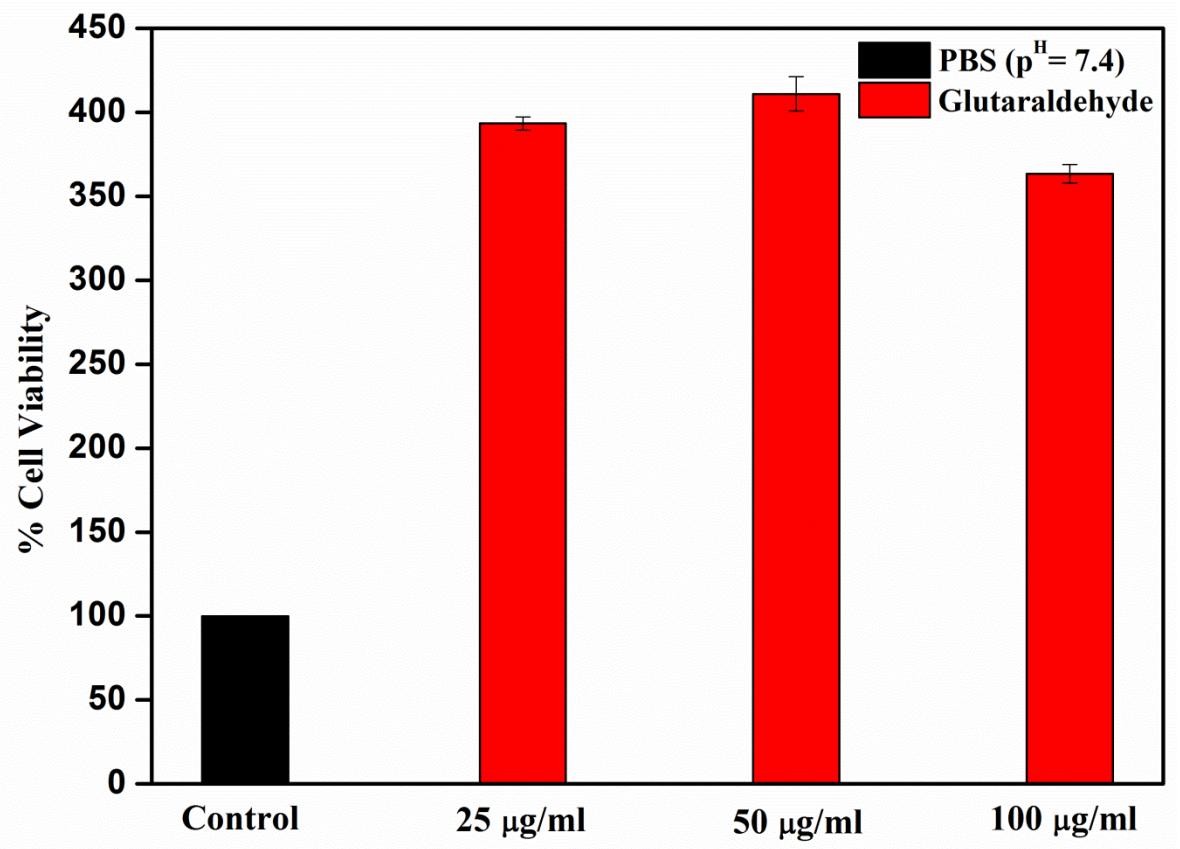

Figure S6: Percentage of cell viability of PBMC for 1% glutaraldehyde solution at three different doses. As the concentration of the doses increases, percentage of cell viability observed to be decreased. It indicates the biocompatibility nature of glutaraldehyde at lower dose level.