Supplementary Information

Functionalized MWCNTs in improving the performance and biocompatibility of potential hemodialysis membranes

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S1 Physico-chemical characterizations of the flat-sheet membranes

Surface functional groups on the MMMs were identified by using an ATR-FTIR (Nicolet Avatar 370, USA) and scanned for the entire spectrum from 400 to 4000 cm^{-1} at a resolution of 2 cm^{-1}.

Top surface membrane morphologies were evaluated using an AFM analysis (Agilent 5500, USA) and the cross sectional images of the MMMs were observed using a SEM (FEI Quanta-400 FEG, USA) with an accelerating voltage of 10 kV. The AFM analysis was carried out for dry membranes affixed on the metal substrate and surface roughness reported is the average of a 5 X 5 micron scan in three different locations carried out in a non-contact mode.

The membranes were dried using a series of alcohol solvent (25, 50, 75 and 100%), mounted on the stub and then sputter coated with gold for 1 min before the SEM analysis.

A contact angle goniometer (OCA15, DataPhysics, Germany) equipped with a video capture device was used to determine the surface hydrophilicity of the prepared membranes. A fixed volume (5 µl) of DI water was dropped at eight different sites and the average values were reported.

Viscosity of the dope solution is instrumental in explaining the membrane morphology. The viscosity was determined by a parallel plate module of the rheometer (MCR 302, Anton Paar, Austria) operated at a shear rate of 0.1 s^{-1} and a temperature of 25 °C. Here n=5 samples.

Thermogravimetric analysis (TGA) was carried out using a TGA instrument (TG, DT-40 Shimadzu, Japan) for all samples weighing 0.3 g. The temperature was increased from RT to 800 °C at a heating rate of 10 °C/ min while maintaining an inert atmosphere.

Membrane porosity (Ɛ %) was measured by the gravimetric method. The membrane porosity is obtained by:

$$\varepsilon\,(\%) = \frac{(W_w - W_d)/D_w}{\frac{W_w - W_d}{D_w} - W_d/D_p} \qquad \text{(S1)}$$

Where; W_w and W_d are weights (g) of wet and dry membrane, respectively, D_w and D_p are densities (g/cm^3) of the water and polymer membrane, respectively. These tests were replicated three times and their average value has been reported.

S2 Blood compatibility of flat-sheet membranes

S2.1 Plasma Collection

Fresh blood was collected from a healthy human (male, 26 years old) and was collected in a EDTA lined sterile vacutainer (EDTA: blood is in 1:9 ratio). The blood was centrifuged at 4000 rpm or 1000 rpm for 20 min to obtain the platelet poor plasma (PPP) or platelet rich plasma (PRP). All of the blood related experiments were performed in accordance with the institutional ethical guidelines.

S2.2 Protein Adsorption

Membrane coupons of 1 cm^2 were incubated in phosphate buffered solution (PBS) for 24 h prior to protein adsorption studies. These coupons were then introduced into 1 ml protein solution (HSA solution-1 mg/ml; IgG-0.5 mg/ml and FN-0.5 mg/ml) at 37°C for 1 h and subsequently washed thrice with PBS followed by rinsing with DI. The membrane coupons were transferred into 2 ml 2 wt% sodium dodecyl sulphate (SDS) solution and shaken for 1 h to elute the adsorbed proteins. The eluted protein concentration was determined using the Micro BCA™ Protein Assay Kit (Thermo Scientific) at a wavelength of 562 nm. The values reported are an average ±SD of at least three measurements performed until we obtained a reliable result.

S2.3 Platelet Adhesion

The PRP was utilized to carry out the platelet adhesion experiments in order to minimize the interference of the other blood components such as leucocytes and erythrocytes. Membrane coupons of 1 cm^2 were equilibrated in PBS at 37 °C for 12 h prior to the experiment. The PBS was removed and the membrane coupons were incubated with 1 ml fresh PRP for 2 h on a shaker. The PRP was then removed and the membrane coupons were washed repeatedly with PBS. The number of platelets attached was determined by the well-established lactate dehydrogenase (LDH) assay (here n=3). Another set of membranes were treated using 2.5 wt% glutaraldehyde for 6 h in order to affix the bound platelets. The coupons were then washed with DI and then treated with osmic acid followed by drying in a series of alcohol solutions. The samples were then sputtered with gold and immediately observed using a SEM (FEI Quanta-400 FEG).

S2.4 Antithrombogenity tests

The antithrombogenity of a surface is an indication of its blood compatibility which is evaluated by activated partial thromboplastin times (aPTTs) and prothrombin times (PTs) using a semi-automatic blood coagulation analyzer (CA- 50, Sysmex Corp., Kobe, Japan). All membrane coupons (1 cm^2) were incubated in PBS for 24 h before carrying out the experiments. For aPTT analysis, 100 µl of fresh PPP was added and the membranes were incubated for 30 min; after which 50 µl of this PPP was drawn, mixed with 50 µl of aPTT reagent and further incubated for 3 min. This was followed by the addition of 50 µl of $CaCl_2$ solution (25 mM) and measurement. The PT tests were carried out by adding 100 µl of the PT reagent to 50 µl of incubated PPP and then analyzed by the coagulation analyzer. n=5 samples.

S2.6 Hemolytic Ratio

The membrane coupons were placed in a 24-well plate and incubated in PBS solution for 12 h before carrying out the test. The erythrocytes obtained from whole blood were washed repeatedly with normal saline (0.90% w/v of NaCl) and then re-suspended in isotonic PBS solution to achieve a concentration of around 10^7cells /ml as measured with a flow cytometer. 500 µl of this re-suspended solution was added into the wells containing the membrane coupons (0.5 cm^2) and were incubated for 1 h at 37°C. The solution was then aspirated from the wells and centrifuged at 2000 rpm for 10 min to separate the lysed RBCs from the pristine ones. The extent of hemolysis corresponds to the presence of hemoglobin present in the supernatant, which was spectroscopically determined at 541 nm. The ratio of hemolysis was determined by the following equation:

$$R_{hemolysis}\,(\%) = \frac{A_{supernatent} - A_{Negative\ Control}}{A_{positive\ control} - A_{negative\ control}} \times 100 \qquad \text{(S2)}$$

In this instance, suspensions of the erythrocytes in DI and PBS were taken to be positive and negative control respectively.

S3 Cytocompatibility Studies

S3.1 Cell culture

Chang liver cell line procured from National Centre for Cell Science (NCCS), Pune, India with the passage number 9 were used. Cells were maintained in Dulbecco's Minimum Essential Media (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and antibiotics -penicillin (100 units/mL), streptomycin (100 µg/mL). The cells were cultured at 37±0.2 °C in a strictly maintained humidified atmosphere with 5% CO_2. The cells were grown in 75 cm^2 culture flasks and after a few passages, they were seeded for experiments. The experiments were done at 70 to 80% confluence. The cells, on reaching the desired confluence were trypsinized using a 0.25% Trypsin-EDTA solution.

S3.2 Cell Proliferation assay

Proliferation of these cultured Chang liver cells was assessed by the standardized MTT assay. The principle of cytotoxicity estimation is based on the ability of the mitochondria of live cells to metabolize MTT to purple colored formazan. Cells were plated over the membranes in 96-well plate at a concentration of 5×10^4 cells/well. Untreated cells served as controls. After the desired incubation period (24, 48 and 72 h), 50 µl of MTT was added to each well. Cells along with the reagents were then incubated for 4 h at 37°C in a CO_2 incubator. MTT was then discarded and the colored crystals of the formazan produced were dissolved in 200 µl of DMSO. This was mixed effectively by pipetting the solution up and down. Spectrophotometric absorbance of the purple-blue formazan dye was measured using an ELISA reader (BIORAD) at 570 nm. Optical density of each sample was compared with control optical density and graphs were plotted. Here n=5.

S3.3 Cell Viability

Acridine orange/ Ethidium bromide (AO/EB) dual staining was carried out to detect apoptosis of the seeded cells. Chang liver cells were plated over the membranes at a density of 5×10^4 in 6-well plates and were allowed to grow at 37°C in a humidified CO_2 incubator until they were 70–80% confluent. After 24 h of incubation, the culture medium was aspirated from each well and cells were gently rinsed twice with PBS at room temperature. Then equal volumes of cells from the control and the membranes were mixed with 100 µl of a 1:1 mixture of AO/EB and viewed immediately under Nikon inverted fluorescence microscope (Ti series) at 10x magnification.

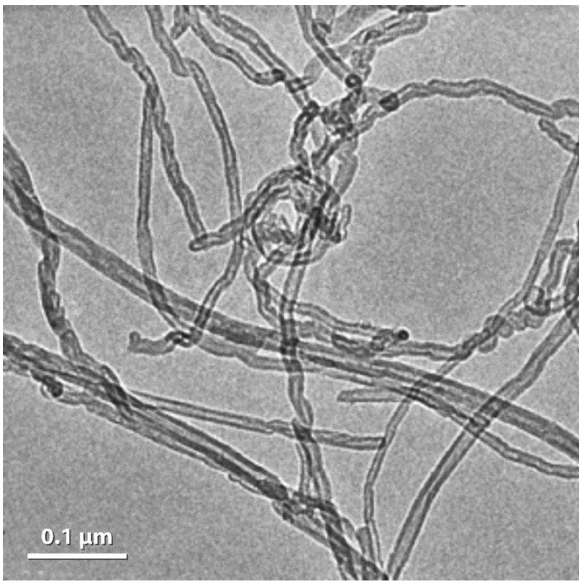

Figure S1: HR-TEM image of the pristine MWCNTs.

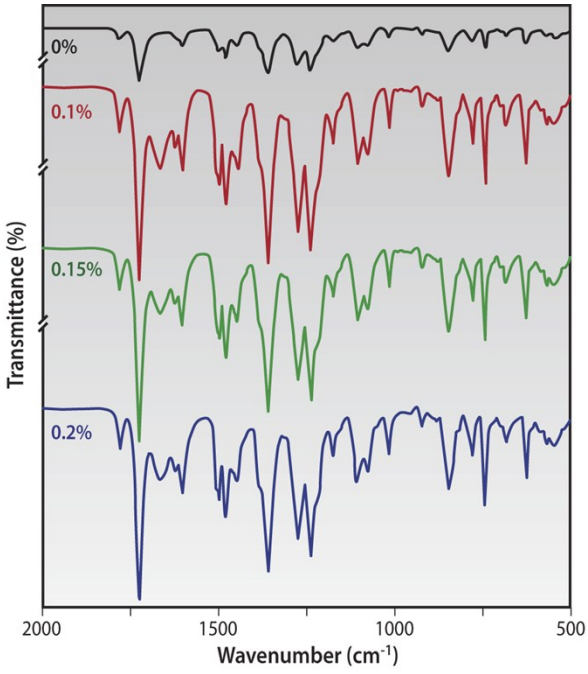

Figure S2: FTIR Spectra (enlarged) of the MMMs.