SUPPLEMENTARY INFORMATION

"BLOOD COMPATIBLE CARBON NANOTUBES – NANO-BASED NEOPROTEOGLYCANS"

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Methods and Materials:

Materials:

Sodium heparin from porcine intestinal mucosa was obtained from Celsius Laboratories. Multi walled carbon nanotubes (MWNTs) (average diameter 40 nm, length 10 μm) were obtained from Carbon Nanotechnology Inc. and used as is for the PEI coating. All other chemical used were obtained from Fisher Scientific. Human blood plasma and human whole blood used for the APTT and TEG studies were pooled samples obtained from healthy donors.

Methods:

(a) Poly(ethyleneimine) coating of MWNTs:

MWNT (80 mg) was sonicated in 1% PEI aqueous solution for 3 h, followed by filtration using 0.8 μm polycarbonate filter and washed using double distilled water three times and dried in a dessicator to yield PEI coated MWNTs (PEI-MWNT).

(b) Preparation and Activation of tetrabutylammonium salt of heparin using cyanogen bromide:

Heparin sodium salt from porcine intestinal mucosa (150 mg) was passed through a 30 ml column packed with cationic exchange resin (Dowex® H^+ resin) to afford protonated heparin, which was then neutralized with 50% solution of tetrabutylammonium hydroxide in water to pH 7.0. The solution was then freeze dried to give TBA salt of heparin. The activation was done by using a previous protocol (ref 11 in the manuscript). Briefly, 100 mg of tetrabutylammonium heparin was dissolved in 1 ml of acetonitrile. 1 ml of 100

mg/ml of cyanogen bromide solution in acetonitrile was then added to the above solution in ice bath followed by the addition of 1.2 ml of 100 mg/ml solution of triethylamine in acetonitrile. The reaction contents became cloudy upon adding the base which then became a clear solution in a couple of minutes. Figure 1S represents the schematic representation this step.

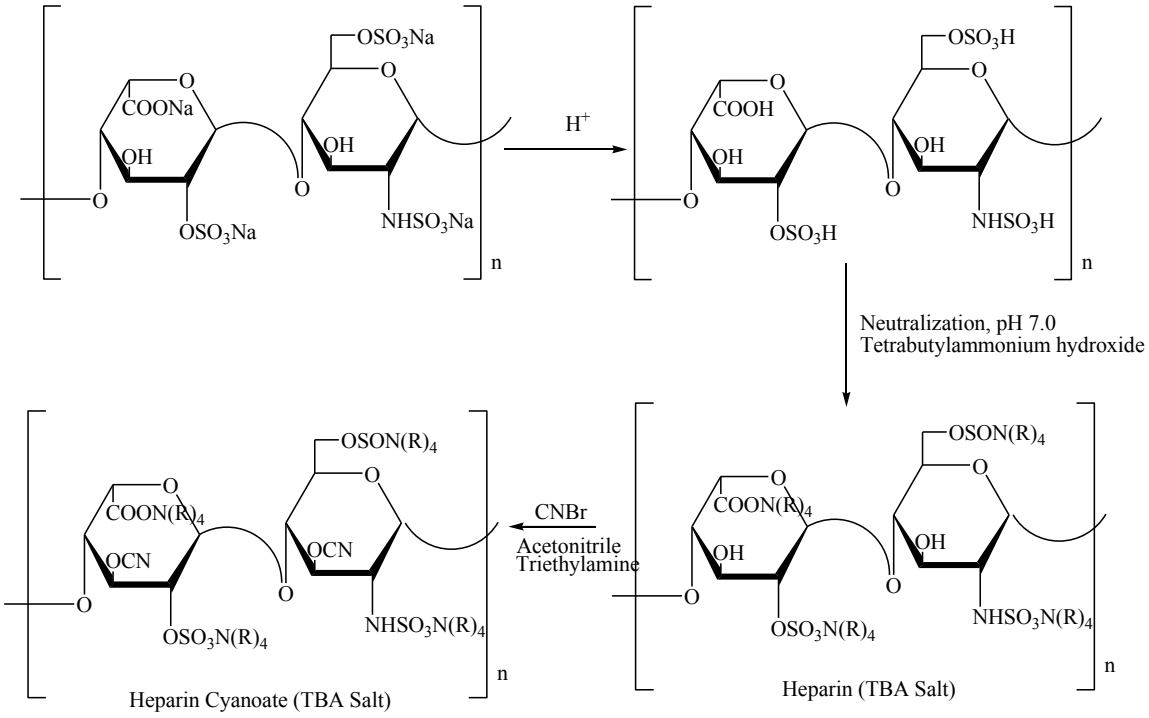

Figure 1S: Schematic representation of the activation of heparin through TBA salt.

(c) Immobilization of activated heparin onto nanotubes:

PEI coated nanotubes (20 mg) were suspended in 28.8 ml of 0.1 M sodium phosphate solution (pH 3.5) using sonication for 5 min. This solution was then added to the reaction solution prepared in step (b) (1:10 dilution of the solution made in step (c)). The resulting reaction mixture was then stirred for 2 h at room temperature followed by filtration using 0.2 μm polycarbonate filters. The heparinized nanotubes thus obtained were then washed with 25 % saline solution for 15 min to remove the ionically and physically adsorbed heparin from the covalently formed heparinized nanotubes.

(d) Characterization by atomic force microscopy:

Heparinized MWNTs were characterized using Multimode IIIa atomic force microscopy (Digital Instruments/Veeco Metrology Group). Heparinized MWNTs were first suspended in DMF solution by sonication for 40 min and then spin-cast on silicon substrates. Tapping Mode (TM) AFM topography and phase images were recorded simultaneously in air. The driving frequency was adjusted to the resonant frequency (~160 kHz) of a sharp probe (tip radius ~ 2 nm, Mikromasch) scanned at a rate of 0.5027 Hz with 512 sample lines at a scale of 2 μm.

(e) Carbazole assay:

This assay looks for the presence of uronic acid (either as iduronic acid as in heparin or as glucuronic acid as in heparan sulfate) in a particular sample (ref 12 in the manuscript). Briefly, 1 mg of pristine MWNT was added to five test tubes (1-5), heparin in the amounts of 0 μg, 1 μg, 10 μg, 100 μg, 250 μg and 500 μg was added to test tubes 1-5 respectively. 1 mg of PEI-NT was added to test tube 6 and 1 mg of heparinized CNT was added to test tube 7. Test tube 8 was left without any MWNT as one of the controls. All the samples were then subjected to carbazole assay. The presence of heparin (precisely uronic acid) gives a pink color to the solution, the absorbance of which was then taken at 525 nm. The concentration of heparin loaded on to the MWNTs was found by using the standard obtained through the samples 1-5.

(f) Activated partial thromboplastin time (APTT):

This assay measures the prolonged clotting time as a function of heparin concentration. APTT is the time needed for plasma to form a clot after the addition of calcium and a phospholipid reagent such as activated cephaloplastin reagent. This assay is one of the available ways to determine the blood compatibility of a particular material. The protocol involves the addition of pristine MWNT (1 mg) or hep-MWNT (0.25 mg, 0.35 mg, 1.0 mg) into test tubes. 100 μl of citrated human plasma (platelet poor plasma) and 100 μl of automated APTT reagent were added to all the test tubes followed by incubation at 37 °C for 5 min. 100 μl of 0.025 M $CaCl_2$ was then added to recalcify the citrated blood plasma. The clotting time was measured by using automated Fibrometer which stops the timer as soon as the clot is formed.

(g) Clotting kinetics:

The clotting kinetics of the human whole blood was also assessed in the presence of the heparinized nanotubes by using thromboelastography (TEG). TEG has been a widely useful technique in hospitals to study the abnormalities in the coagulation pathway of the patients. TEG works by measuring the physical viscoelastic characteristics of blood. Typically, MWNTs (0.5 mg) was placed in a TEG cup, followed by the addition of 350 μl human whole blood and incubated for 5 min. 10 μl of 0.01 M $CaCl_2$ was added to recalcify the citrated blood. A coaxially suspended stationary piston was then placed on the cup with a clearance of 1 mm. This pin is suspended by a torsion wire which transduces the torque. The cup is oscillated at an angle of 4° 45' in either direction every 4.5 s. During the clot formation, fibrin fibrils link the cup to the pin which influences the rotation of the pin, and the disturbance is measured and displayed by a computer. The display called thromboelastogram plots the torque experienced by the pin as a function of time. TEG studies the coagulation by measuring various factors including the latent time for clot initiation (R), the time to initiate a fixed clot firmness of around 20 mm amplitude (k), the kinetics of clot development (angle α) and the maximum amplitude (MA) of the clot. This is another way of measuring the blood compatibility of the heparinized nanotubes.

(h) Lyase digestion of heparin immobilized on MWNTs:

Heparinized nanotubes (1 mg) were treated with heparin lyase I (5 U (Sigma units)) in sodium phosphate buffer (1 ml, pH 7.1) at 37 °C for 24 h. After treatment, the heparinized nanotubes were washed with the sam