Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Realizing the label free sensitive detection of carcinoembryogenic antigen (CEA) in blood serum *via* MNC decorated flexible immunosensor

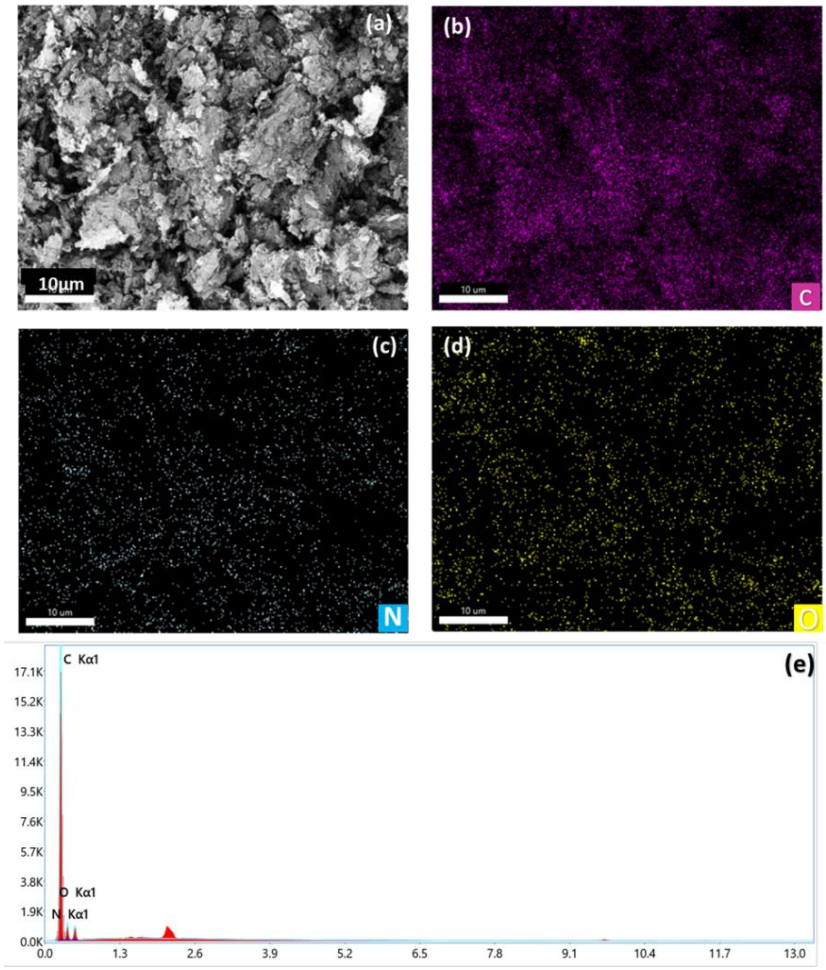

Fig. S1 SEM image for (a) MNC-600, (b-d) Elemental dot mapping of C, N and O respectively, (e) graph showing the EDS spectra of different elements present in MNC-600.

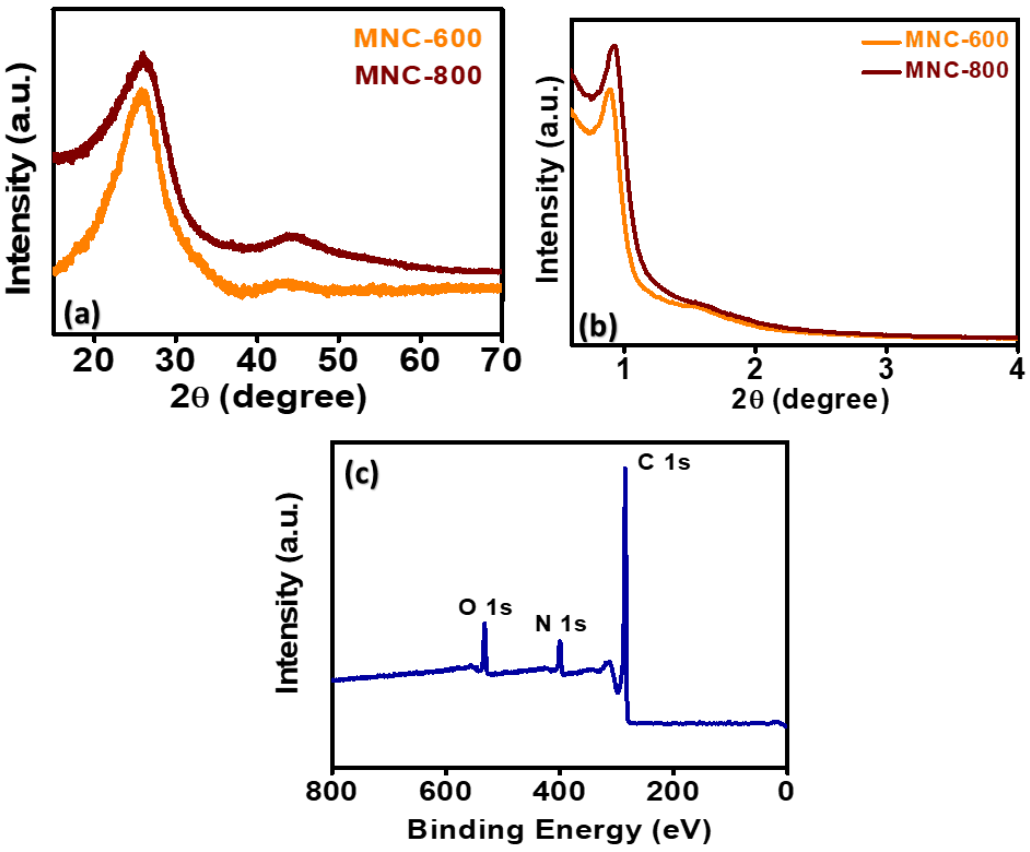

Fig. S2 (a-b) P-XRD patterns for MNC-600 and MNC-800, (c) XPS survey spectrum of showing the presence of different elements.

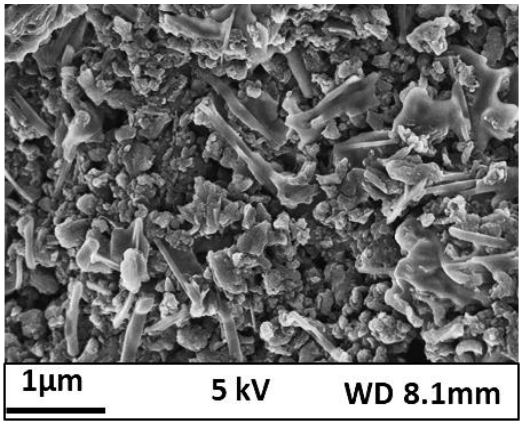

Fig. S3 FE-SEM image of MNC-600 after the immoblization of AbCEA molecules.

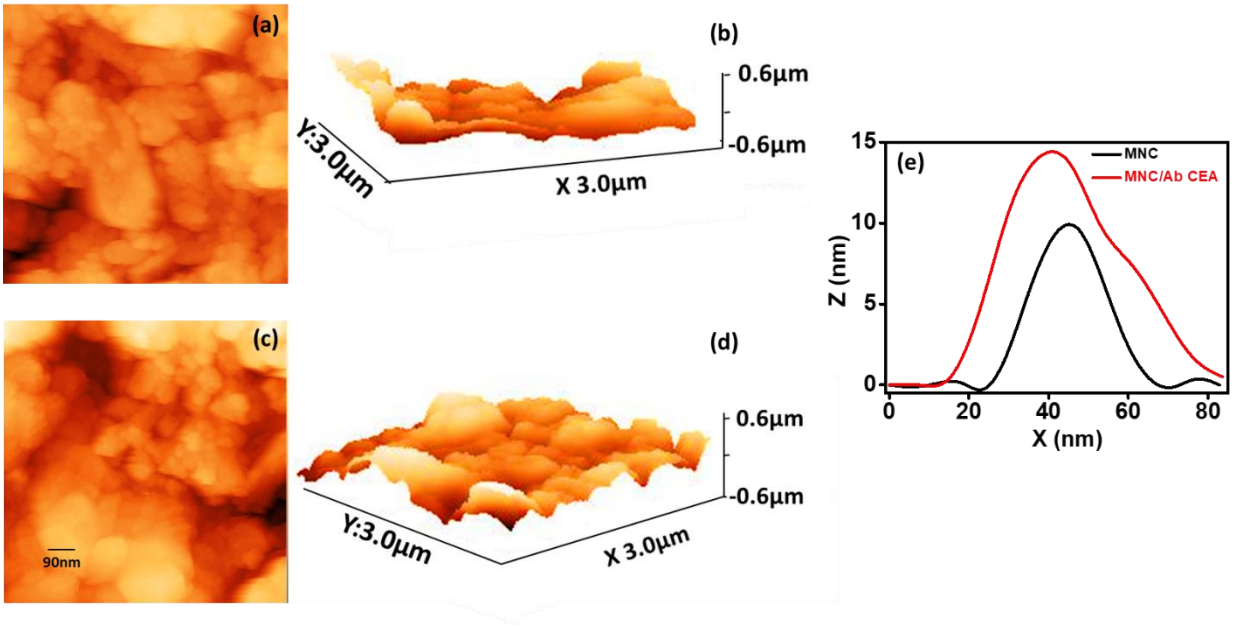

Figure S4. AFM images (a) Before immoblization of AbCEA (b) corresponding 3D image (c) After immobilization step (d)Corresponding 3D-image.

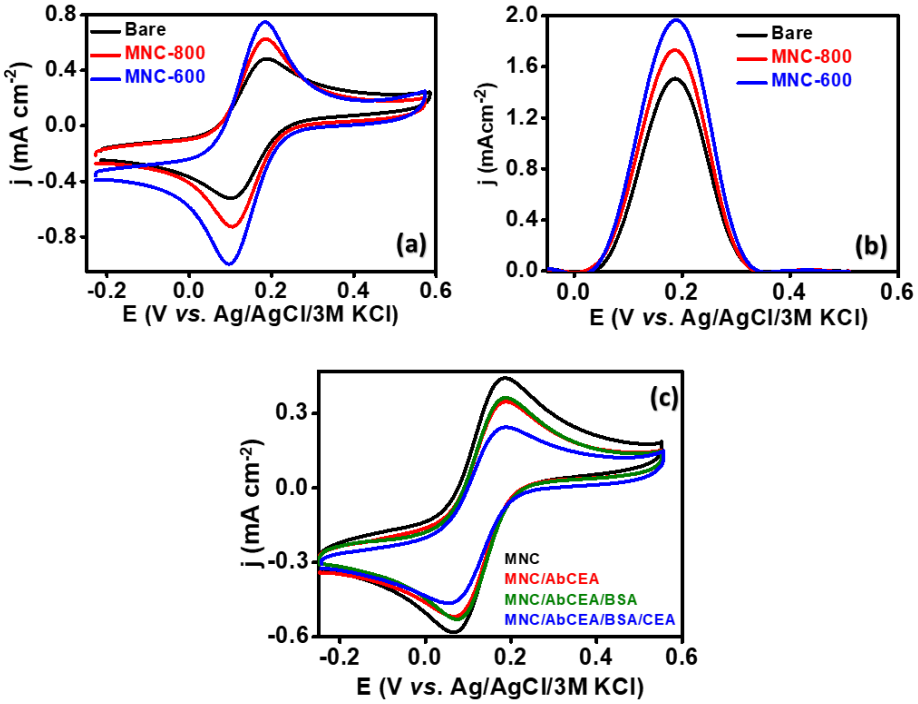

Fig. S5 (a) CV, (b) SWV curves showing the comparison electrochemical activities of different variants of MNC in 0.1 M PBS solution containing 5 mM of Fe^{3+}/Fe^{2+} solution, (d) CV plot showing the effect of BSA on modified electrode.

Table S1. Calculation of charge transfer resistance (R_{ct})			
Material	**R_{ct} (Ω)**	**Rate constant (K_s) (s^{-1})**	**Surface Coverage (θ)**
MNC	136	12.6×10^{-6}	18.6×10^{-3}
MNC/Anti-CEA	162.5	10.5×10^{-6}	14.9×10^{-3}
MNC/Anti-CEA/CEA	202.2	8.5×10^{-6}	10.6×10^{-3}

Electrochemical surface area (ECSA) analysis:

The electrochemical surface area (ECSA) was ascertained by calculating the "double-layer pseudo-capacitance" (C_{dl}) in 0.1 M PBS (pH 7.4) containing 5 mM of Fe^{3+}/ Fe^{2+}.Cyclic voltammetry tests were performed in the non-faradic region at various scan rates from 10 to 300 mV s^{-1} over a potential range from 0.0 to 0.2 V. Slope obtained from the plot of averaged current $(I_a+I_c)/2$; ('I_a' denotes anodic current and 'I_c' is for cathodic current) density at a potential 0.11 V *vs.* the scan rate gave $C_{dl.}$ The obtained C_{dl} was divided with the specific capacitance of the flat standard surface (20-60 μF cm^{-2}) which in the current study is considered to be 40 μF cm^{-2}, gives electrochemical surface area (ECSA).[1] The roughness of the surface was calculated by dividing the obtained ECSA with the geometrical surface area to result in the roughness factor (R_f).

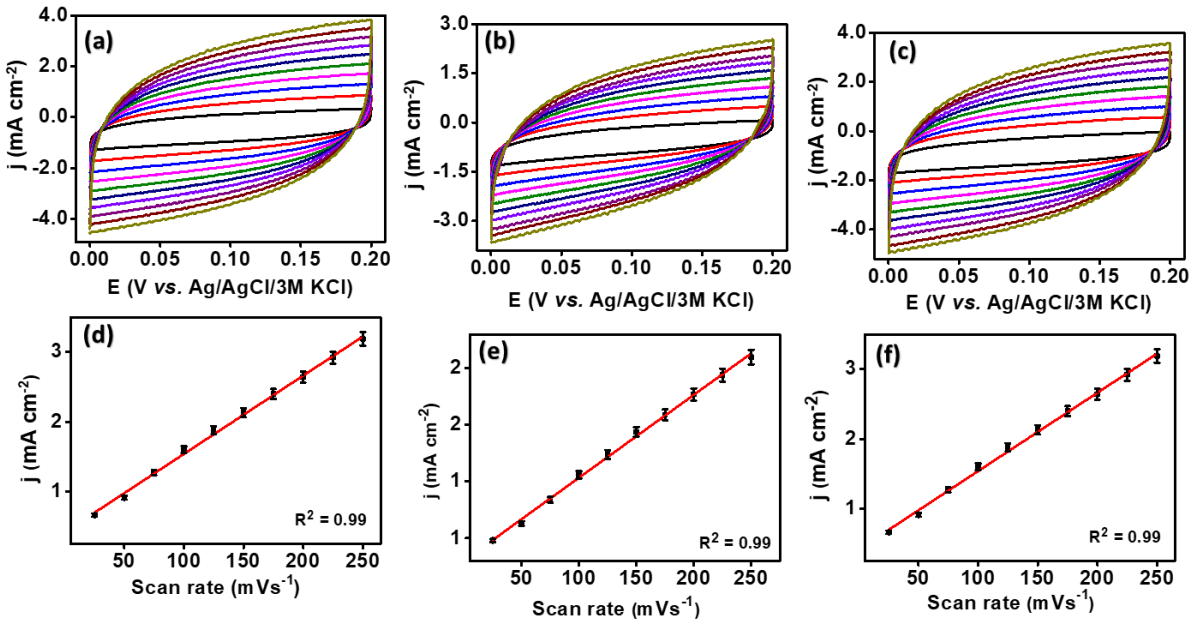

Fig. S6 Cyclic voltammogram of (a) MNC (b) MNC/Ab CEA and (c) MNC/Ab CEA/CEA respectively in the non-faradic region at various scan rates ranging from 10 to 300 mV s^{-1} and (d-f) corresponding plot of average current densities versus scan rate.

Table S2. Electrochemical surface area (ECSA) analysis

Sr. No	Catalyst	Electrolyte	C_{dl} (μF) at 0.11 V	ECSA (cm^2)	Roughness factor (a.u.)
1	MNC	0.1 M PBS + 5 mM $K_4[Fe(CN)_6]$	12.3	0.31	10
2	MNC/AntiCEA	0.1 M PBS + 5 mM $K_4[Fe(CN)_6]$	11.2	0.28	9.03
3	MNC/Anti-CEA/CEA	0.1 M PBS + 5mM $K_4[Fe(CN)_6]$	7.3	0.18	5.80

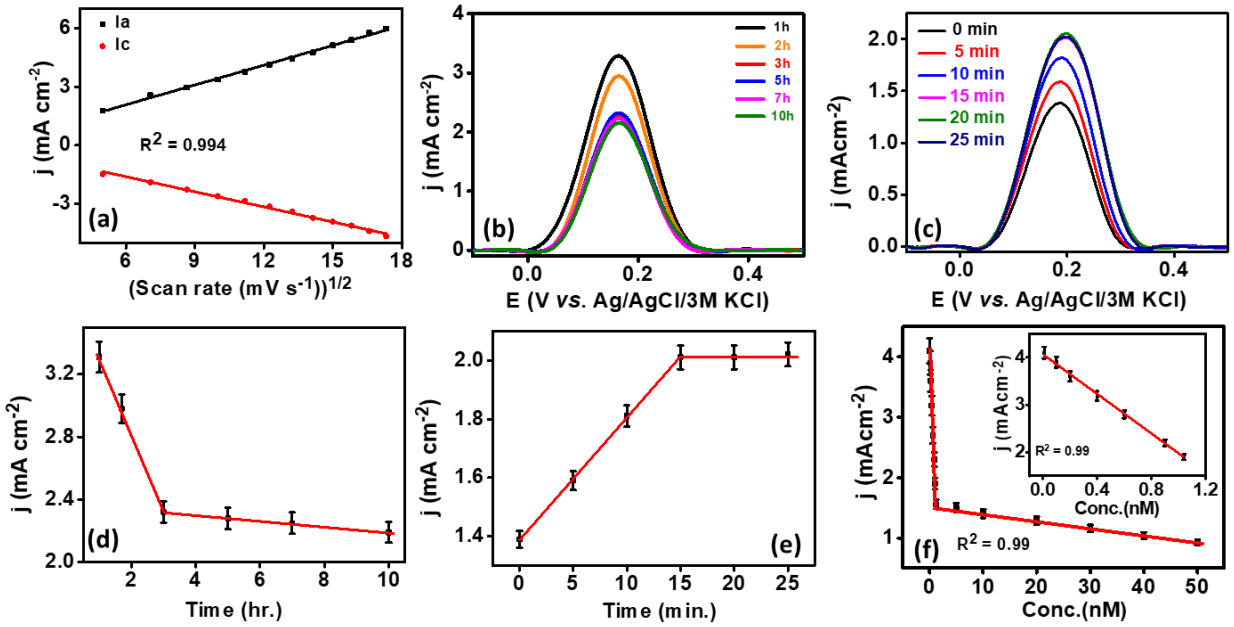

Fig. S7 (a) The linear curves between average current density versus scan rate extracted from fig. 2d.SWV showing optimization of incubation time for (b) AbCEA (c) CEA in 0.1 M PBS (pH 7.4) containing 5 mM of Fe^{3+}/Fe^{2+} solution and(d-e) corresponding calibration graphs. (f) Linear range curves extracted from Fig. 2e, (inset: graph showing the linearity from 10 pM to 1 nM)**.**

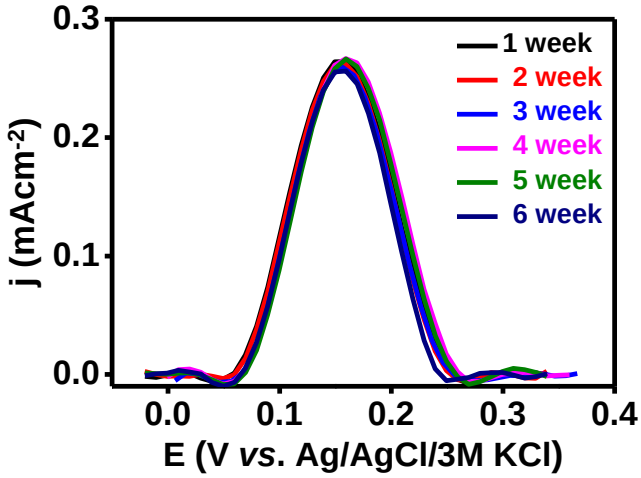

Fig. S8 SWV representing the long term storage stability of immunosensor.

Table S3. Comparison of MNC based immunosensor with previously reported labelled and label free immunosensors.

Material	Technique	Detection Range ng ml^{-1}	Incubation Time of immunocomplex (min.)	Storage stability (Weeks)	LOD (ng ml^{-1})	Ref
Rh@PdNDs/MWCNTs -SO_3H	Label free	25x 10^{-6} - 100	25	2.1	8.3x 10^{-6}	2
polyCBMA/PANI	Label free	0.01 x 10^{-3} - 0.1	45	01	3.05 x 10^{-6}	3
Ag/MoS_2@Fe_3O_4	Labelled	0.0001– 20	90	3.6	0.03 x 10^{-3}	4
Au-Ag/rGO@PDA	Label free	0.001 - 80	40	04	0.286 x 10^{-3}	5
Graphene-zirconia nanocomposite (GZ)	Label free	0.01 - 10	75	2.8	4.25 x 10^{-3}	6
PPI and CNDTs	Label free	0.005 - 300	50	02	0.00145	7
Au@Bi nanospheres (NSs)	Labelled	50 – 100	30	2.1	9.83 x 10^{-6}	8
Ni/C@SiO_2	Labelled	0.006 - 12	20	04	1.56 x 10^{-6}	9
GO/Fe_3O_4-Pd-Ag	Label free	1 x 10^{-3} - 80	30	04	0.2 x 10^{-3}	10
CSAuNPs-PEDOT-PB cry/SPCE	Label free	1.0 × 10^{-4} - 1.0 × 10^{3}	15	05	5.05 X 10^{-5}	11
MoS_2-AuNPs	Labelled	10× 10^{-4} –1.0	50	2.2	1.2 X 10^{-5}	12
Au-β-CD/MXene @PANI/FTO	Label free	0.5 – 350	40	1.4	0.0429 X 10^{-5}	13
Au@Bi NSs	Label free	50 x 10^{-6}–100	30	2.1	9.83 X 10^{-5}	14
PtNPs@rGO@PS NS	Label free	0.05 - 70	60	0.7	0.01	15
Cu-THQ	Labelled	1 x 10^{-6} - 40	35	3.5	0.477X10^{-6}	16
Ag@CNCs	Label free	0.0001 - 100	30	1.4	5.12 X10^{-3}	17
CuCo/CNC	Labelled	0.0001–80	25	05	0.031 X 10^{-5}	18
Ag@SiO_2	Label free	0.5 - 10	17	04	0.01	19
tCHI/dPNMA/SPCE	Label free	0.01 – 30	40	3.8	0.01	20
Mesoporous nitrogen carbon (MNC)	**Label free**	**0.009 – 1000**	**15**	**06**	**9.04 X** 10^{-3}	**This Work**

Table S4. Human serum sample analysis for CEA.					
	Actual conc. (pM)	**Conc. Added (pM)**	**Conc. Found (pM)**	**Recovery (%)**	**RSD (%)**
1.	23.2	200	223	99.3	1.8
2	23.2	400	438	103.7	0.64
3	23.2	600	642	101.4	0.04
4	23.2	800	861	103.3	0.05

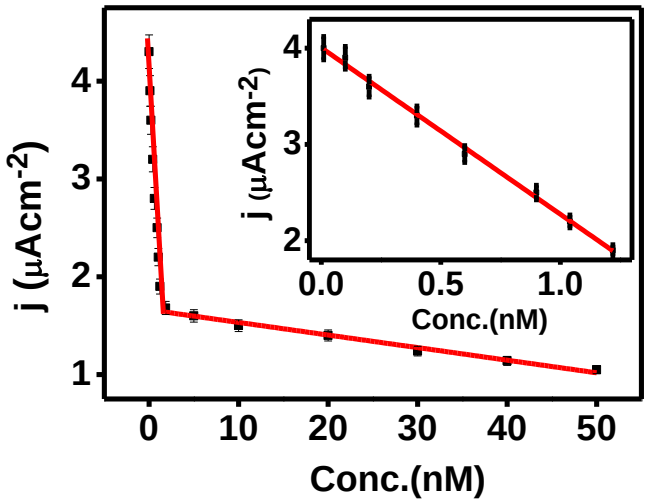

Fig. S9 The linear curves between average current density versus concentration extracted from figure 4d.

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