

Supplementary Data

**Fabrication of electrospun poly (ethylene oxide)-poly (capro lactone) composite
nanofibers for co-delivery of niclosamide and silver nanoparticles exhibits enhanced
anti-cancer effects *in vitro***

*Poornima Dubey^a, P. Gopinath ^{*a,b}*

^aNanobiotechnology Laboratory, Centre for Nanotechnology,

^bDepartment of Biotechnology,

Indian Institute of Technology Roorkee, Roorkee, Uttarakhand-247667, India.

Corresponding author: Tel. +91-1332-285650; Fax. +91-1332-273560;

*E.mail: pgopifnt@iitr.ernet.in; gene gopi@gmail.com

	Mathematical Models for drug release	Kinetic Equations	Factors
1	Zero order	$f_t = K_0 t$	t - time f_t - fraction of drug dissolved in time t , K_0 - apparent dissolution rate constant.
2	First order	$Q_t = Q_0 e^{-K_1 t}$	Q_t - amount of drug released in time t Q_0 - initial amount of drug in the solution K_1 - first order release constant
3	Higuchi	$f_t = K_H t^{1/2}$	K_H - Higuchi dissolution constant,
4	Korsmeyer-Peppas	$M_t/M_\infty = a t^n$	M_t - mass of drug released at time t M_∞ - total mass of drug loaded in the fiber a, n - constant
5	Hixson-Crowell model	$W_0^{1/3} - W_t^{1/3} = \kappa t$	W_0 - the initial amount of drug W_t - the remaining amount of drug in the pharmaceutical dosage form at time t κ (kappa) - a constant incorporating the surface-volume relation.
6	Weibull model	$M = M_0 [1 - e^{-(t-T)^b/a}]$	M - the amount of drug dissolved as a function of time t . M_0 - total amount of drug being released. T accounts for the lag time measured as a result of dissolution process. b describes the shape of the dissolution curve progression. For $b = 1$, the shape of the curve corresponds exactly to the shape of an exponential profile with the constant $k = 1/a$

Table S1 Different kinetic models studied for niclosamide release from alone niclosamide and nic@Ag NPs composite nanofibers.

Genes	Primer sequence
p53	Forward: 5' TGGCCCCTCCTCAGCATCTTAT 3' Reverse : 5' GTTGGGCAGTGCTCGCTTAGTG 3'
cmyc	Forward : 5' CCAGGACTGTATGTGGAGCG 3' Reverse : 5' CTTGAGGACCAGTGGGCTGT 3'
Caspase	Forward:5'TTCAGAGGGGATCGTTGTAGAAGTC 3' Reverse :5'CAAGCTTGTCGGCATACTGTTTCAG 3'
Bax	Forward:5'AAGCTGAGCGAGTGTCTCAAGCGC 3' Reverse : 5' TCCCGCCACAAAGATGGTCACG 3'
Bacl2	Forward : 5' TCCGCATCAGGAAGGCTAGA 3' Reverse : 5' AGGACCAGGCCTCCAAGCT 3'
Bcl xl	Forward : 5' ATGGCAGCAGTAAAGCAAGC 3' Reverse : 5' CGGAAGAGTTCATTCACTACCTGT 3'
β -actin	Forward: 5' CTGTCTGGCGGCACCACCAT 3' Reverse : 5' GCAACTAAGTCATAGTCCGC 3'

Table S.2: List of gene specific primers used for apoptotic gene expression studies

NMR spectral analysis:

The ^1H and ^{13}C NMR were recorded on a Bruker 500 MHz NMR instrument using TMS as internal standard reference and DMSO d_6 as a solvent. Chemical shifts (δ) are given in parts per million (ppm) scales. The chemical shift δ values of ^1H and ^{13}C NMR have been given in Table S.3 a,b.

a) Proton	H1	H2	H3	H4	H5	H6	H7	H8	H9
Alone Niclosamide	3.32 (singlet)	7.1 (doublet)	7.5 (triplet)	7.9 (doublet)	8.29 (triplet)	8.44 (doublet)	8.8 (doublet)	11.34 (singlet)	12.53 (singlet)
Nic composite nanofiber	3.356	Peak disappears	Peak disappears	Peak disappears	8.33 (singlet)	Peak disappears	8.918 (doublet)	Peak disappears	Peak disappears
Chemical shift ($\Delta\delta$)	0.036	-	-	-	0.04	-	0.12	-	-

b) Carbon	C	C	C	C	C	C	C	C	C	C
Alone Niclosamide	119.18	122.42	123.92	124.81	130.07	134.05	141.15	142.60	155.18	162.59
Nic composite nanofiber	Peak disappears	Peak disappears	Peak disappears	Peak disappears	Peak disappears	Peak disappears	Peak disappears	Peak disappears	Peak disappears	Peak disappears
Chemical shift ($\Delta\delta$)	-	-	-	-	-	-	-	-	-	-

Table S.3 ^1H NMR and ^{13}C -NMR spectral analyses of alone niclosamide and nic composite nanofiber. Chemical shifts are expressed in ppm.

Functional groups	O-H (s)	N-H (s)	C-H (s) aromatic	C-H (s)	H-C=O, aldehyde	C=O (s)	C=C (s)	C-C (s) aromatic	C-C (s) aromatic	N-O (s)	N-O (s)	C-N (s)	C-H (w)	O-H (b)	C-Cl (s)	N-H (w)
Wavenumber (cm-1) of niclosamide alone	3579	3493	3093.38	2913.82	2698.94	1918	1674.57-1648.08	1606.87	1412.10	1351.20	1519.75	1218.59	1113.78	1045.15	585	894.07-665
Functional groups	O-H (s)	N-H (s)		C-H (s)	H-C=O, C-H (s)	C=O (s)	C=C (s)				N-O (as)	C-N (s)	C-H (b)	O-H (b)	C-Cl (s)	
Wavenumber (cm-1) of nic composite nanofiber	3779.23-3800	3425.08		2923.86	2740.15	1721.50	1683.21-1642				1551.18-1464.72	1246.53	1096.13	955.14	842.15-585	

Table S.4 Major vibrational frequencies showed in the IR spectrum of niclosamide alone and nic composite nanofiber (where notation s=symmetric, as= asymmetric and b= bending vibrations).

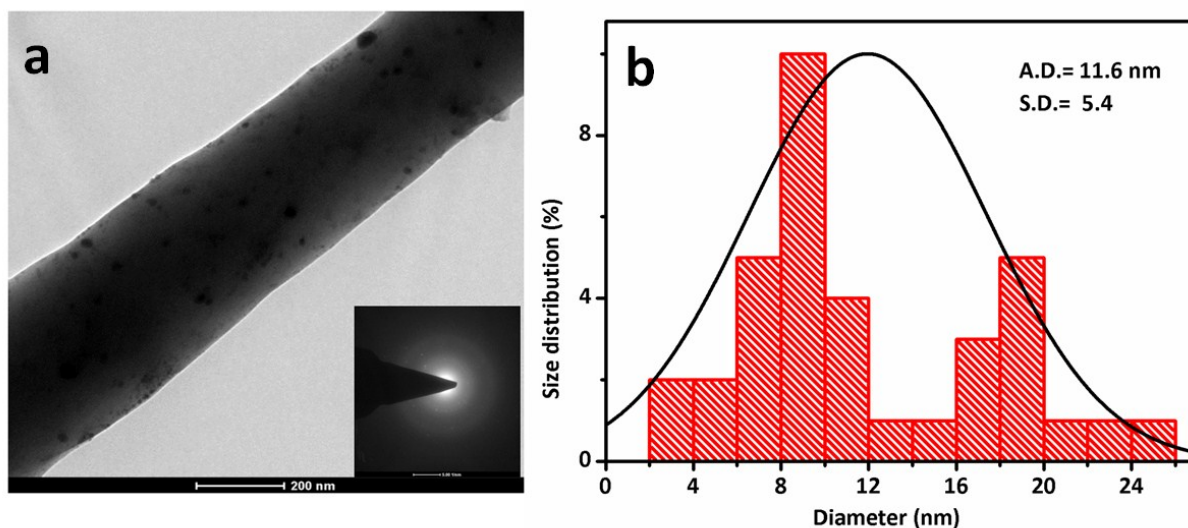


Fig S1. a)TEM micrograph of nic@Ag NPs composite nanofiber, Inset showing SAED pattern of drugs incorporated in the nanofiber. b) The histogram shows the diameter distribution of Ag NPs incorporated into the nic@Ag NPs composite nanofiber. Where A.D.= average diameter, S.D= standard deviation.

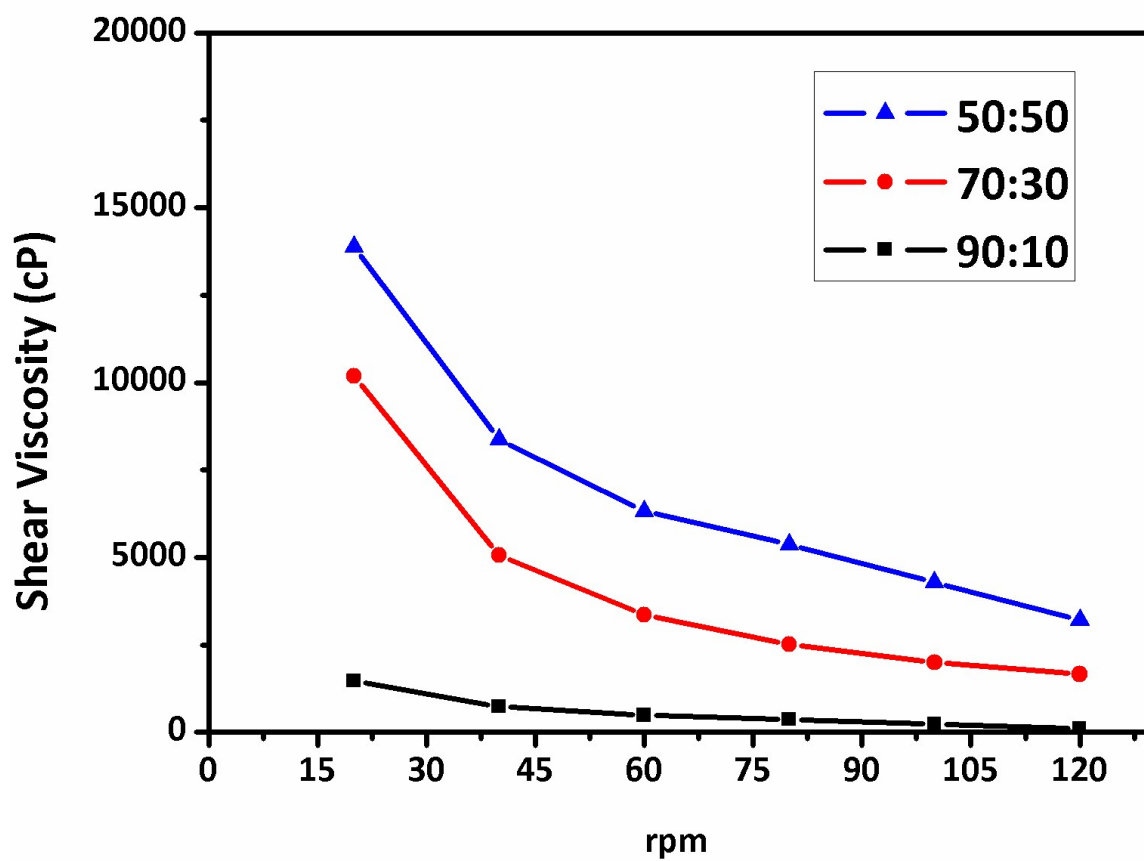


Fig S2. Shear viscosities of niclosamide PEO-PCL blend solutions at different proportions of PEO and PCL as a function of shear stress.

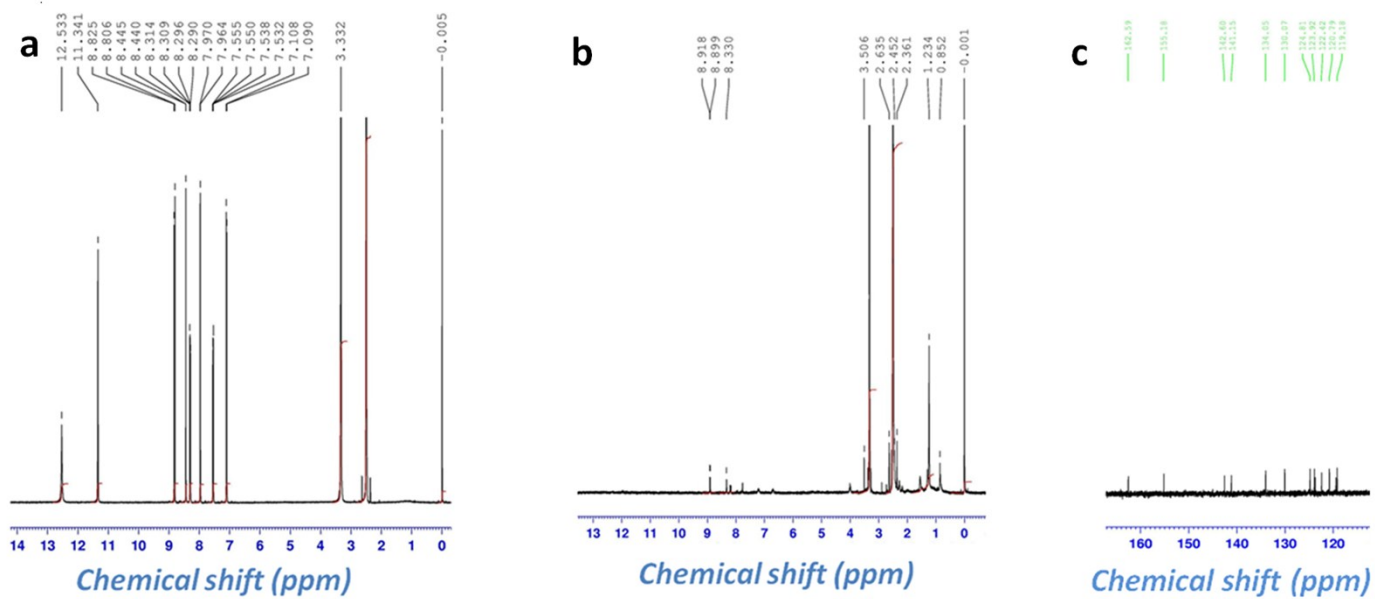


Fig S3 ^1H and ^{13}C NMR analysis of alone niclosamide nanofiber (a, c) and nic composite nanofiber (b).

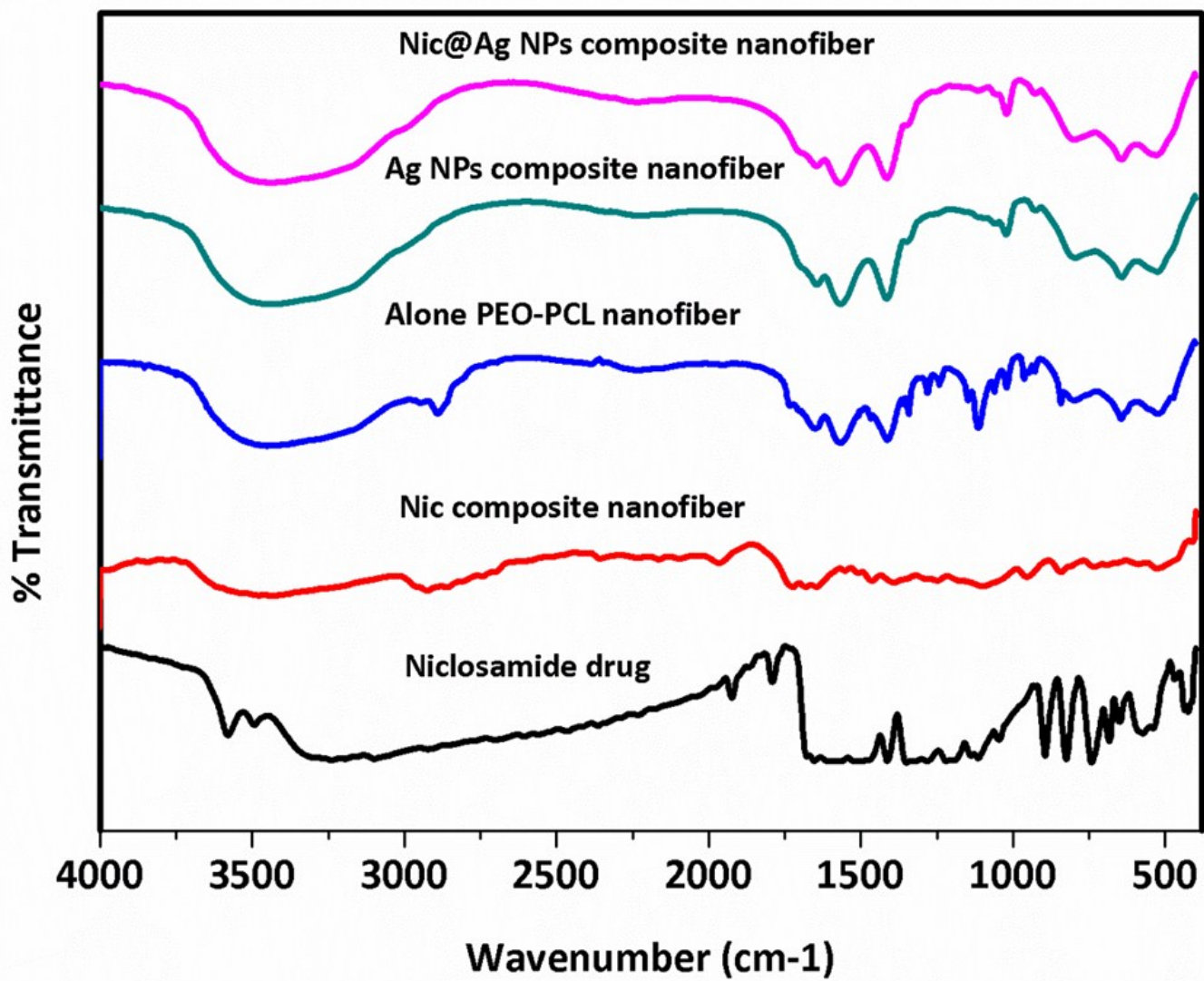


Fig S4. FTIR spectral analysis of alone drug and various composite nanofibers.

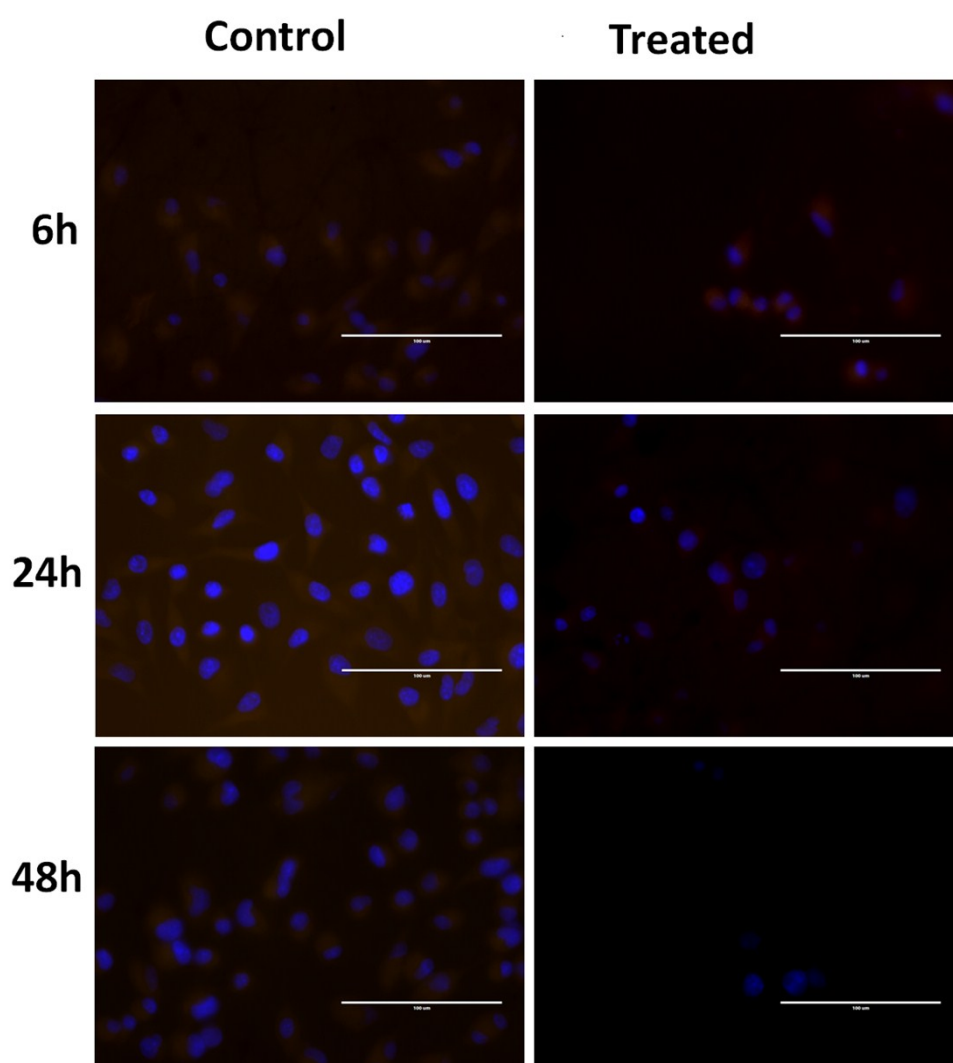


Fig S5. Hoechst 33342 and rhodamine B staining of MCF-7 cells seeded over nanofibers at different time point (12 h, 24 h and 48 h) to measure the changes in cellular morphology.

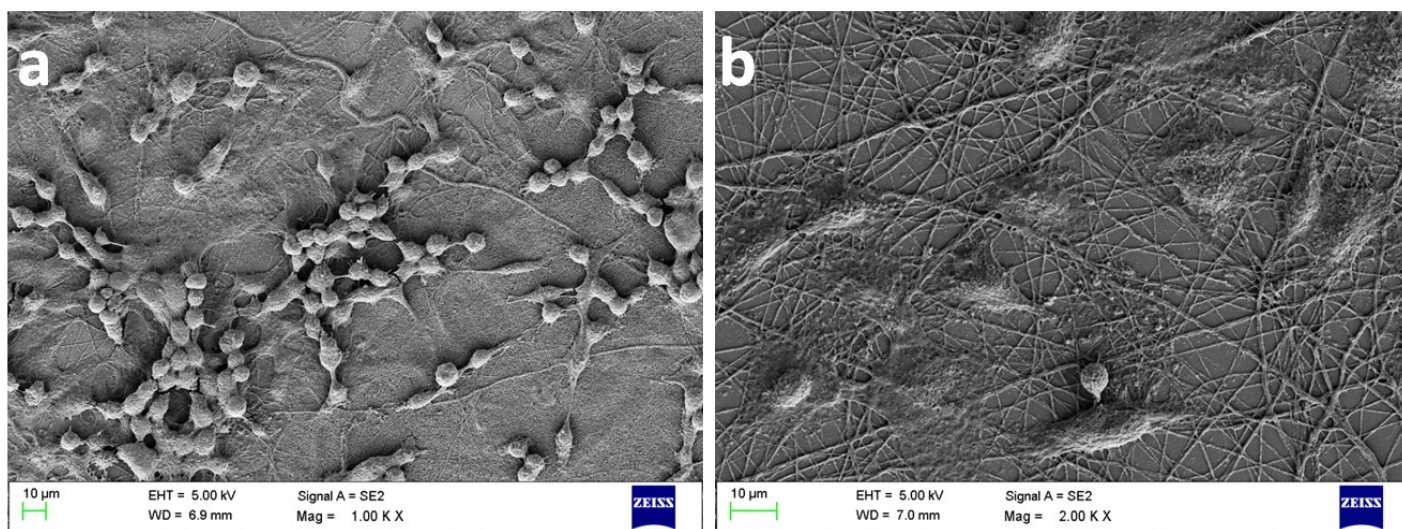


Fig S6. FE-SEM images of cancer cells seeded over alone PEO-PCL blended nanofiber for period of 24 h to observe the cell proliferation and cell attachment over seeded nanofibers.