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PLLA/Gelatin Composite Fiber Membrane Doped with Cerium Oxide Nanoparticles as Bioactive Scaffolds for Future Angiogenesis

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Table S1. Crosslinking process parameters

Crosslinker		Solvents		Reaction conditions	
EDC	NHS	Ethanol	Deionized water	Temperature	Time
50 mM	20 mM	90% (V/V)	10% (V/V)	RT	12 h

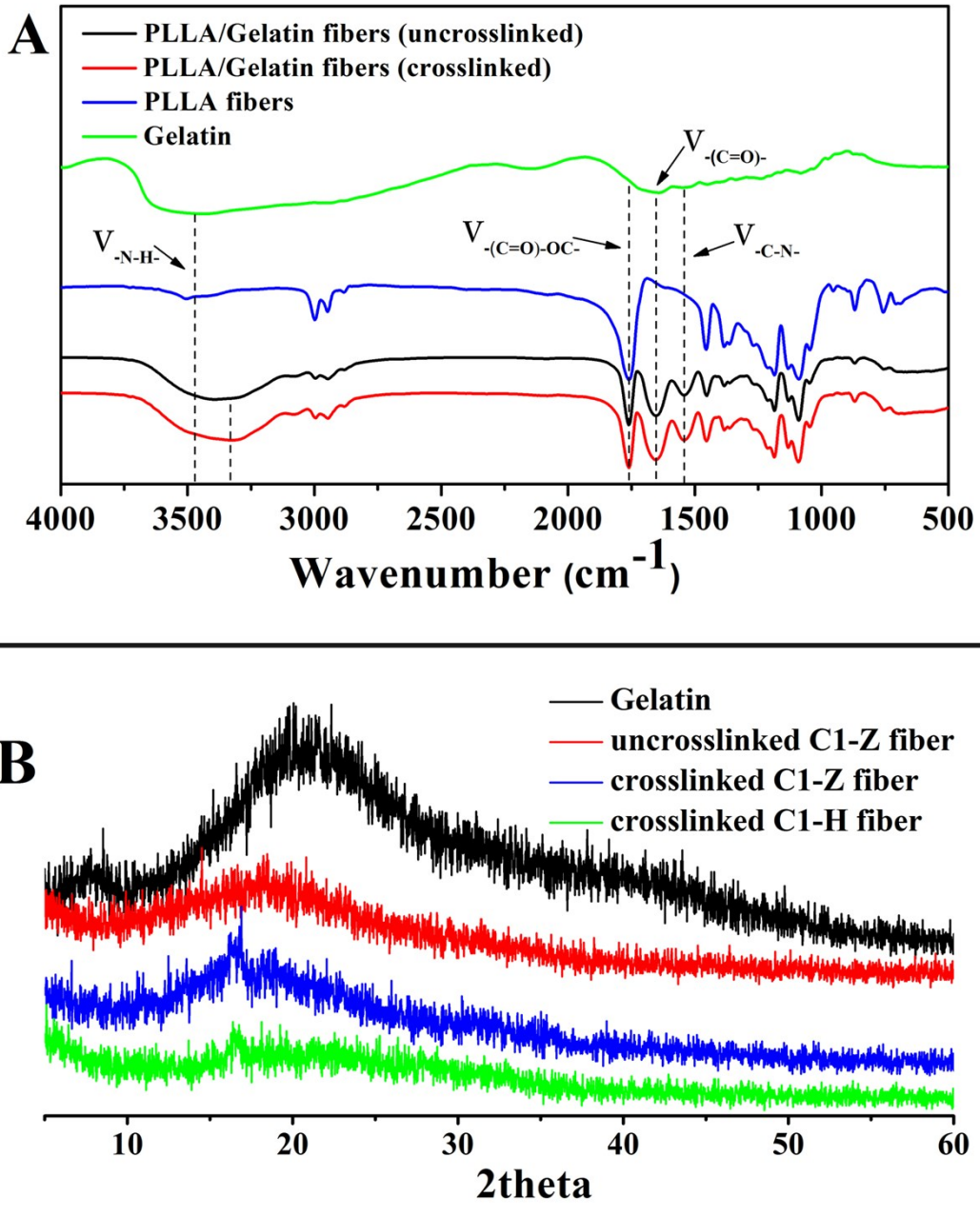

Fig. S1 A: FTIR spectra of Gelatin powder (line of green), PLLA fibers (blue), uncrosslinked C1-H fibers (black) and crosslinked C1-H fibers (red). B: XRD pattern of Gelatin and PLLA/Gelatin composite fibers with different CeNPs content.

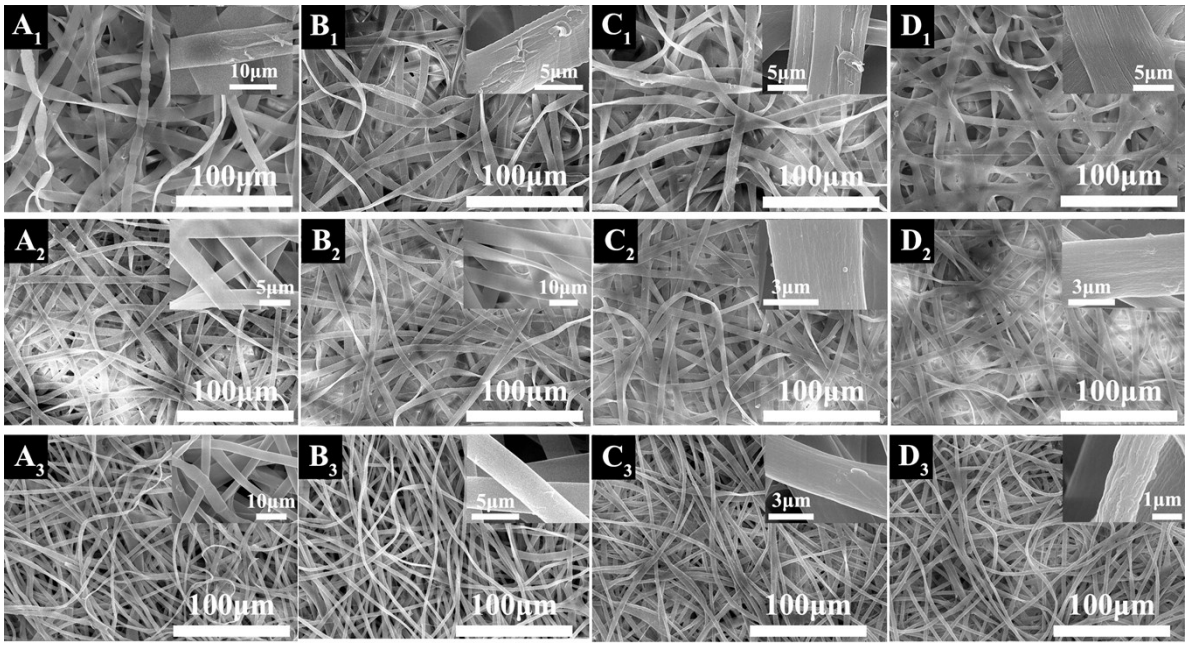

Fig. S2. SEM images of crosslinked C1-L (1), C1-M (2), C1-H (3) fibers immersed in PBS for different times. A: 0 day, B: 1 day, C: 3 days, D: 7 days.

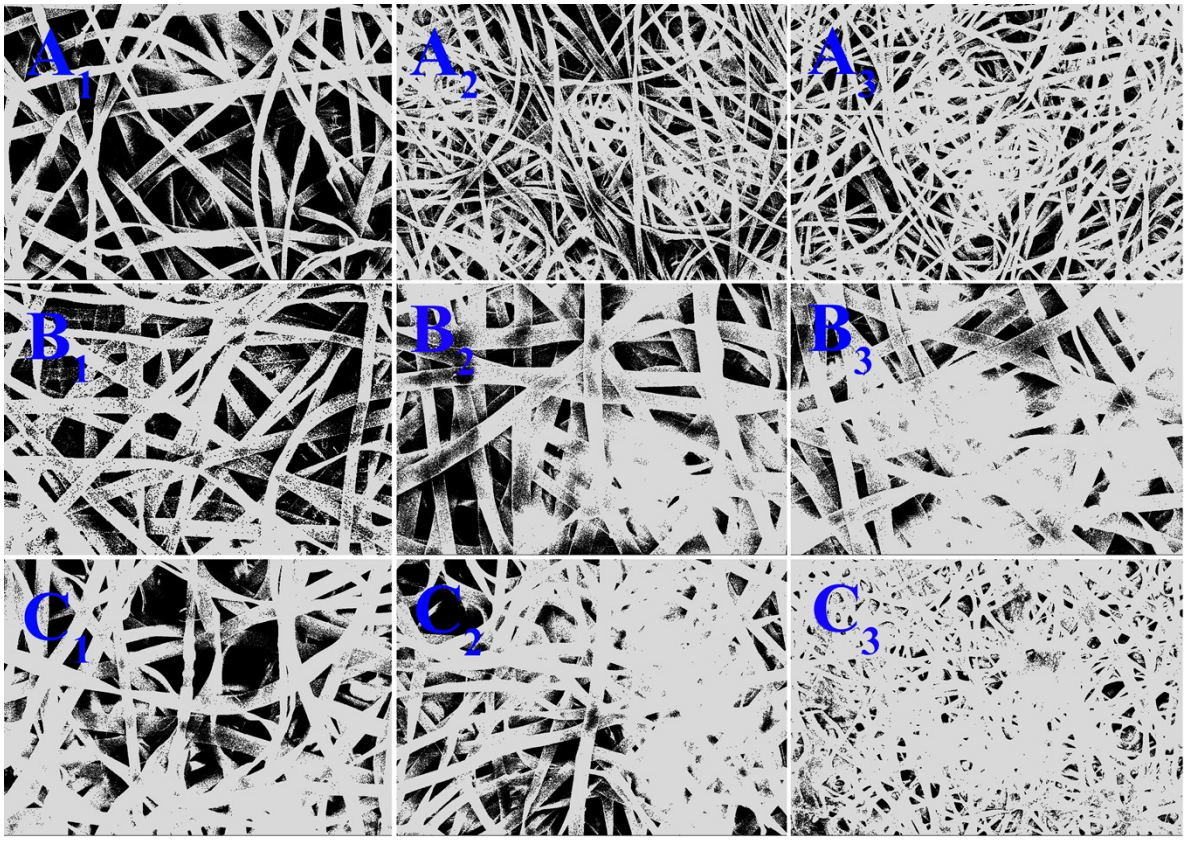

Fig. S3. SEM image processed with MATLAB of crosslinked C1-H (A), C1-M (B), C1-L (C) fibers after degradation and porosity change trend, and 1: 0 day, 2: 3day, 3: 7day.

Table S2. Mechanical properties analysis

Category		Cross. C1-Z	Cross. C1-L	Cross. C1-M	Cross. C1-H
Tensile strength (MPa)	Dry	3.49	2.60	3.39	4.29
	Wet	1.28	0.85	0.95	1.45
Elongation at break (%)	Dry	9.28	12.44	13.60	6.94
	Wet	32.85	26.96	33.07	28.35
Young's modulus (MPa)	Dry	81.86	69.56	85.04	104.47
	Wet	10.33	5.98	7.40	19.04

Cell Viability Assay

Method

In a similar experiment, cell viability assay with EA.hy926 was repeated using 96 well plate according to our published protocols.[1] Endothelial cells (EA.hy926 cells) were seeded in 96-well plates, to a density of 6×10^3 cells per well and cultured overnight. The cells were then incubated with different fiber membranes cut equally into square shaped pieces (0.5cm×0.5cm) for 24 hours. Later, the media in each well was replaced by media containing MTT reagent (0.5mg·mL^{-1}) and incubated for 3-4 hours in dark condition. After the incubation period, the media containing MTT was removed and DMSO: Methanol (1:1 V/V) was added to each well. Finally, absorbance was recorded at 570 nm using BioTek Synergy H1 multiplate reader.

Results and Discussions

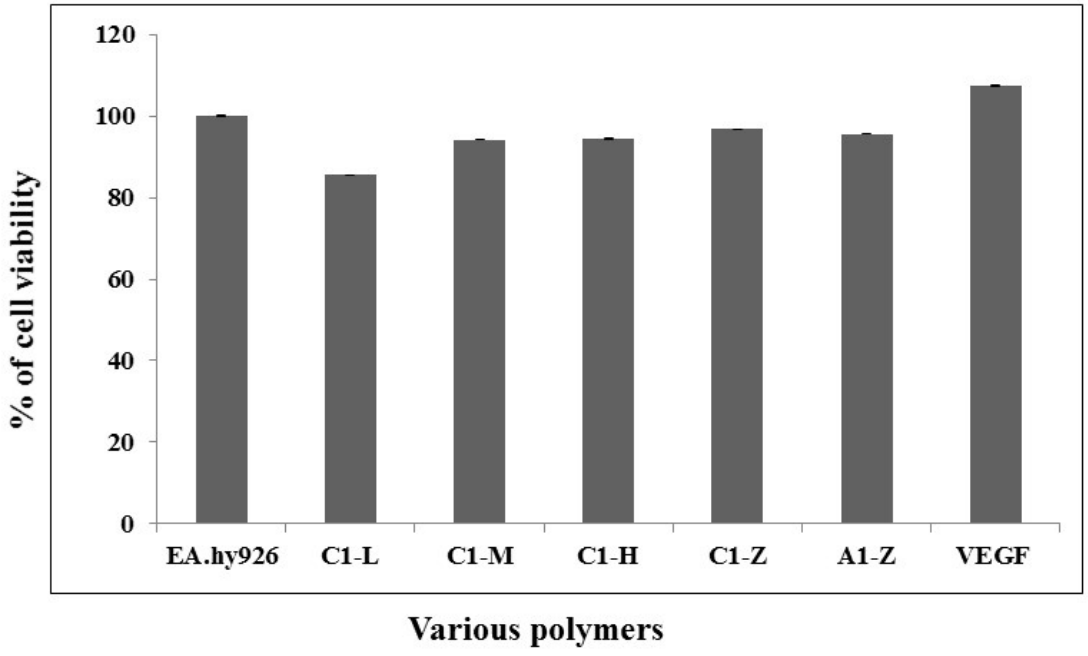

Fig. S4 Cell viability assay (MTT assay) in Endothelial cell line (EA.hy926), incubated with membrane scaffolds loaded with different dose of the Series C1 fiber membranes (C1-L, C1-M, C1-H, C1-Z, A1-Z) for 24 hours. VEGF was used as a positive control.

The results of cell viability assay indicated that the treatments did not inhibit the growth of endothelial cells **(Fig S4).** Additionally, the Series C1 fiber membranes did not induce any cytotoxic effect or proliferation of the endothelial cells. The results further indicated that the Series C1 fiber membranes are biocompatible in nature.

References

1. Nethi, S. K.; Nanda, H.S.; Steele, T.W.J.; Patra. C.R. Functionalized nanoceria exhibit improved angiogenic properties. *J. Mater. Chem. B* **2017**, *5*, 9371-9383.