Electronic Supplementary Information (ESI)

Electrospun polycaprolactone (PCL) scaffolds embedded with europium hydroxide nanorods (EHNs) with enhanced vascularization and cell proliferation for tissue engineering applications

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1 Methods

1.1 Transmission electron microscopy (TEM)

The size, shape and morphological characteristics of europium hydroxide nanorods (EHNs) nanorods coated on the carbon-coated copper grids was recorded on an FEI Tecnai G2 S-Twin TEM at an accelerating voltage of 200 kV. The average particle size length and diameter were calculated using the ImageJ software (NIH, Bethesda, MD).

1.2 Energy Dispersive X- ray (EDX) analysis

The presence of EHNs in the PCL scaffolds was confirmed by EDX analysis using an Oxford Swift ED (Oxford-Instruments, UK) attached to JEOL JSM 6390 SEM, based on the energy and intensity distribution of X-ray signals generated by the electron beam striking the surface of the specimen. The EDX chamber was cooled by liquid nitrogen during the analysis.

1.3 Fourier Transform Infrared Spectroscopy

The FTIR spectra of the scaffolds were collected over a range of 500–4000 cm-1 with a FTIR spectrometer (Spectrum 400, Perkin Elmer, USA) with attenuated total reflectance (ATR) attachment (GladiATR, PIKE, USA) with 15 scans at 4 cm-1 resolution using Spectrum 400 software 62 (version 6.3). Since ATR was used, semi-quantitative information regarding the relative amount of various crystalline phases can be obtained.

1.4 Contact angle measurement

Water contact angles on the surface of the PCL and PCL-EHNs scaffolds were measured by a Digital Contact Angle Measurement System equipped with a CCD camera (Phoenix, Surface Electro Optics, Korea). A series of images of the water droplets on the surface of the scaffolds were automatically taken by the machine and contact angles were measured from these images. All the experiments were carried out at 26°C and about 65% relative humidity. The experiment was repeated for three set of samples and the contact angle was expressed as mean ± SD (n = 3).

1.5 Water uptake capacity

Tissue engineering scaffolds should show moderate water uptake capacity to provide adequette diffussion of medium during *in vitro* cell culture and diffussion of body fluids during *in vivo* application. The diffusion of aqueous solution into polymer can be determined by measuring the increase in weight during incubation in phosphate buffered saline (PBS). Water uptake capacity was determined using ASTM D570 standard with minor modifications. The neat PCL as well as PCL-EHNs nanocomposite scaffolds of two inch diameter disks were placed in PBS solution of pH 7.4 (37°C and a relative humidity of 60%). After particular time period, the samples were taken out, gently wiped with a lint free cloth, and weighed. Then, each sample was completely dried and weighed again. Water uptake is expressed as increase in weight percent as shown in equation (2).

Percentage of water uptake = $[(W_w - W_d)/W_d] \times 100$ (2)

where W_w and W_d are the wet weight and dry weight of the membranes respectively. The experiment was repeated for three set of samples and percentage of water uptake was expressed as mean ± SD (n = 3).

2 Results and discussions

2.1 Transmission electron microscopy (TEM).

The size and shape parameters of the as-synthesized nanorods were determined using the TEM. Fig.S1. represents the TEM picture of the EHNs, which clearly demonstrates the rod shape nature with an average length and diameter of about 280 nm and 50 nm, respectively (Calculated from the TEM image using ImageJ software).

2.2 Fourier transformed infrared spectroscopy (FTIR)

FTIR spectra of EHNs as exhibited in Fig.S3 demonstrates characteristic peak at 705.17 cm^{-1} can be attributed to the bending deformation of Eu-O bond.[1] The second peak in EHNs spectra at around 1383 cm^{-1} might be due to the stretching bond of Eu-O.[2] The characteristic peak at 3608.92 cm^{-1} is attributed to the stretching frequency of O-H bond.[3]

2.3 Wettability and swelling of the scaffolds

Wettability of the PCL-EHNs scaffold surface was characterized by water contact angle measurement (Table S1). Compared with the bare PCL scaffolds (108 ± 6°), the water contact angle value of modified scaffolds were much less, which indicated that a relatively hydrophilic surface was formed after incorporating EHNs in the PCL scaffolds. The water contact angle value of PCL-EHNs-0.25 scaffolds decreased to 97 ± 4°. The value of PCL-EHNs-0.5 and PCL-EHNs-1 scaffolds decreased to 88 ± 6° and 78 ± 4° respectively. PCL-EHNS-2 scaffolds showed high hydrophilicity with very low contact angle with water (76 ± 6°). These results indicated that after the incorporation of EHNs, the hydrophilicity of the modified scaffolds was improved significantly.

The effect of the EHNs on the water absorption of PCL-EHNs scaffolds is presented in Figure S5 as percentage of swelling of the scaffold. Swelling due to the water uptake of the PCL-EHNs scaffolds were faster than the bare PCL scaffolds, because EHNs were relatively hydrophilic and the presence of hydrophilic nanofillers could improve the absorption of water. During the entire period of study, PCL-EHNs-2 scaffolds exhibited better water uptake than other scaffolds which were manifested about 125% of water uptake after 5 h of incubation in PBS which was much higher than the bare PCL scaffolds ($P < 0.005$). Scaffolds containing 0.25, 0.5 and 1% w/w of EHNs were also showed considerable improvement in swelling in a concentration dependent manner.

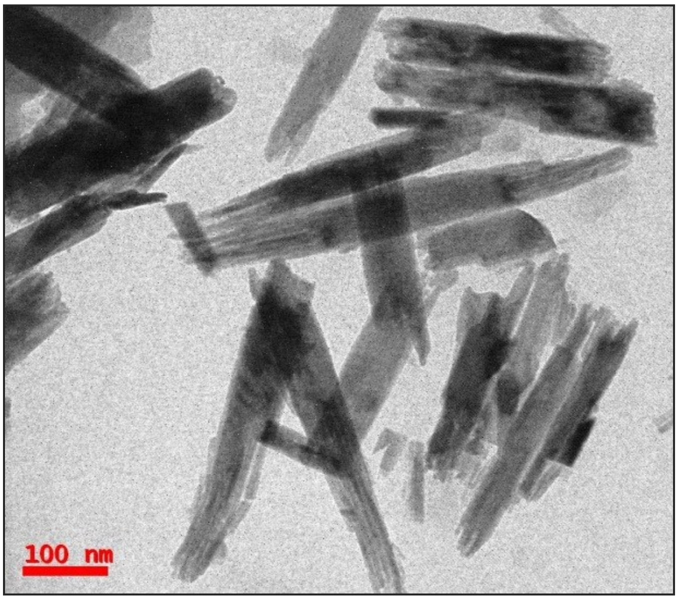

Fig. S1. Transmission electron microscopy (TEM) image of the EHNs clearly indicating the rod shape nature.

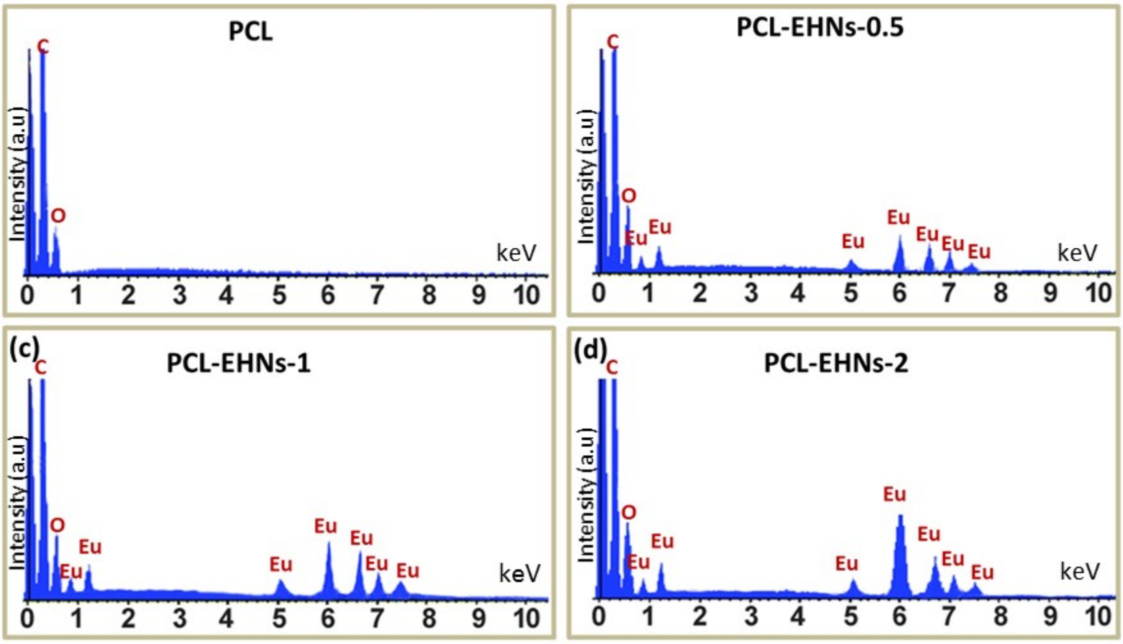

Fig. S2. EDX analysis of the scaffolds clearly indicating the presence of EHNs in PCL matrix.

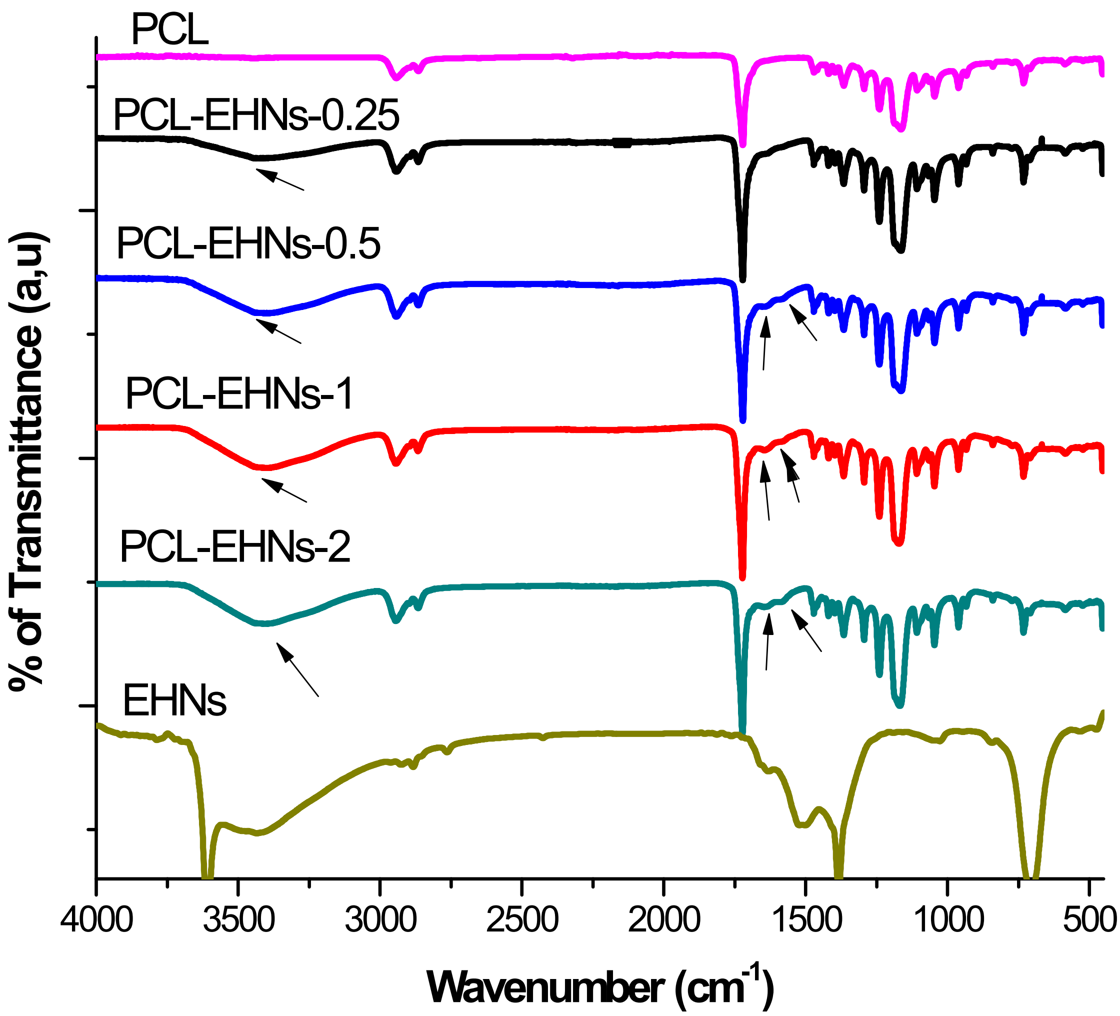

Fig. S3. FTIR spectra of EHNs, PCL and PCL-EHNs showing the characteristic peaks of EHNs in PCL-EHNs nanocomposite.

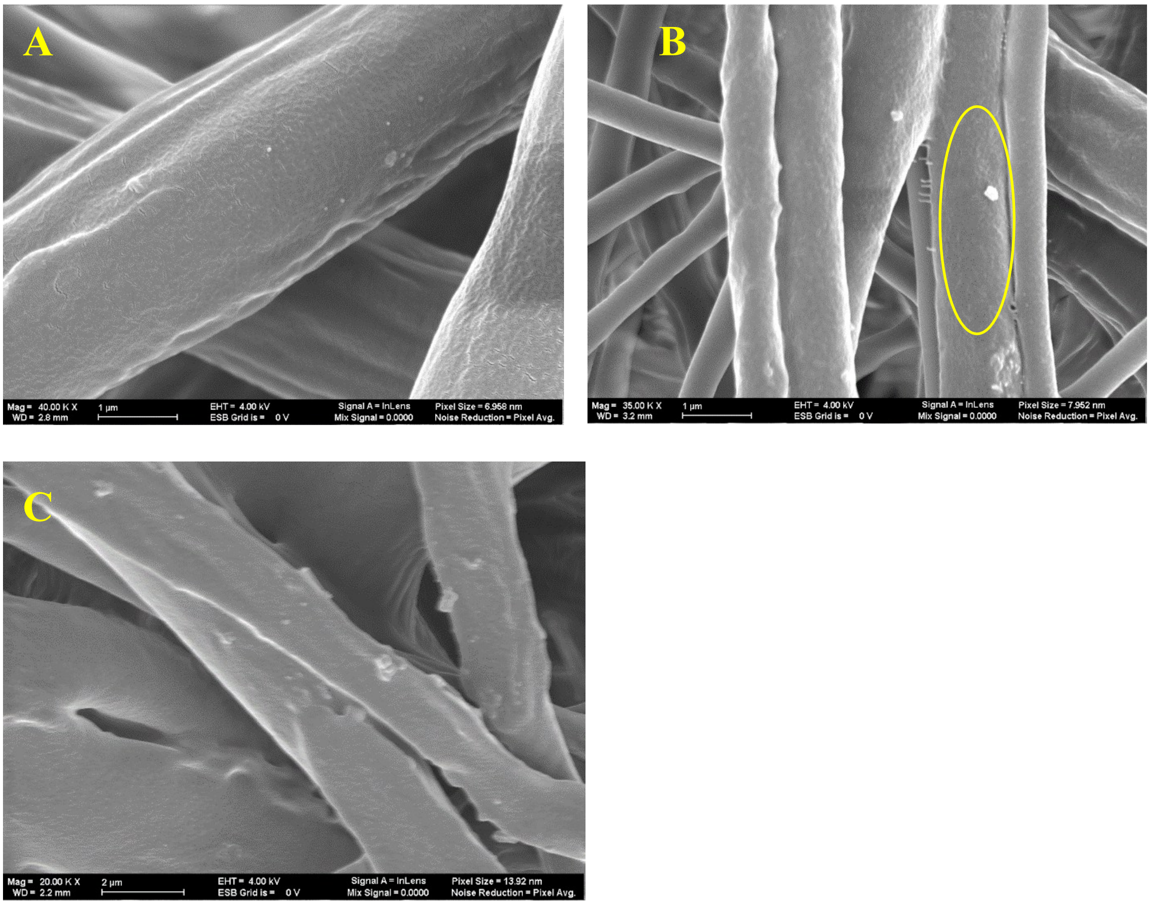

Fig. S4. High resolution scanning electron microscopy (HR-SEM) images of the PCl-EHNs-0.5 (A), PCl-EHNs-1 (B) and PCl-EHNs-2 (C) clearly indicates the presence of nanoparticles on the surface of the scaffolds. On certain regions of the PCL fibers nanostructures of EHNs are visible (denoted by yellow circle in B).

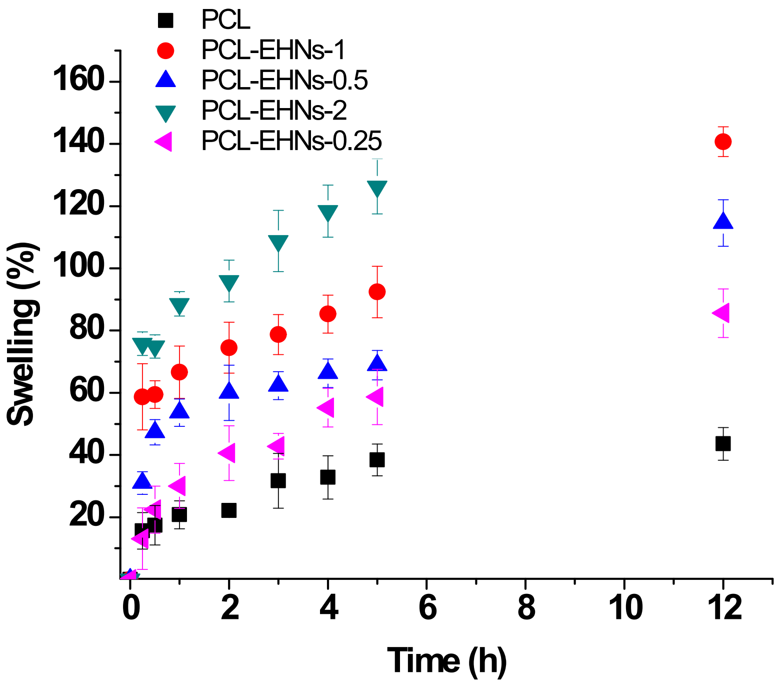

Fig. S5. Swelling of neat PCL membrane and PCL membranes containing various concentrations of EHNs.

Table S1. Water contact angle measurement of neat PCL membrane and PCL membranes containing various concentrations of EHNs.

Scaffolds	Water contact angle (°)
PCL	108 ± 6
PCL-EHNs-0.25	97 ± 4
PCL-EHNs-0.5	88 ± 6
PCL-EHNs-1	78 ± 4
PCL-EHNs-2	76 ± 6

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