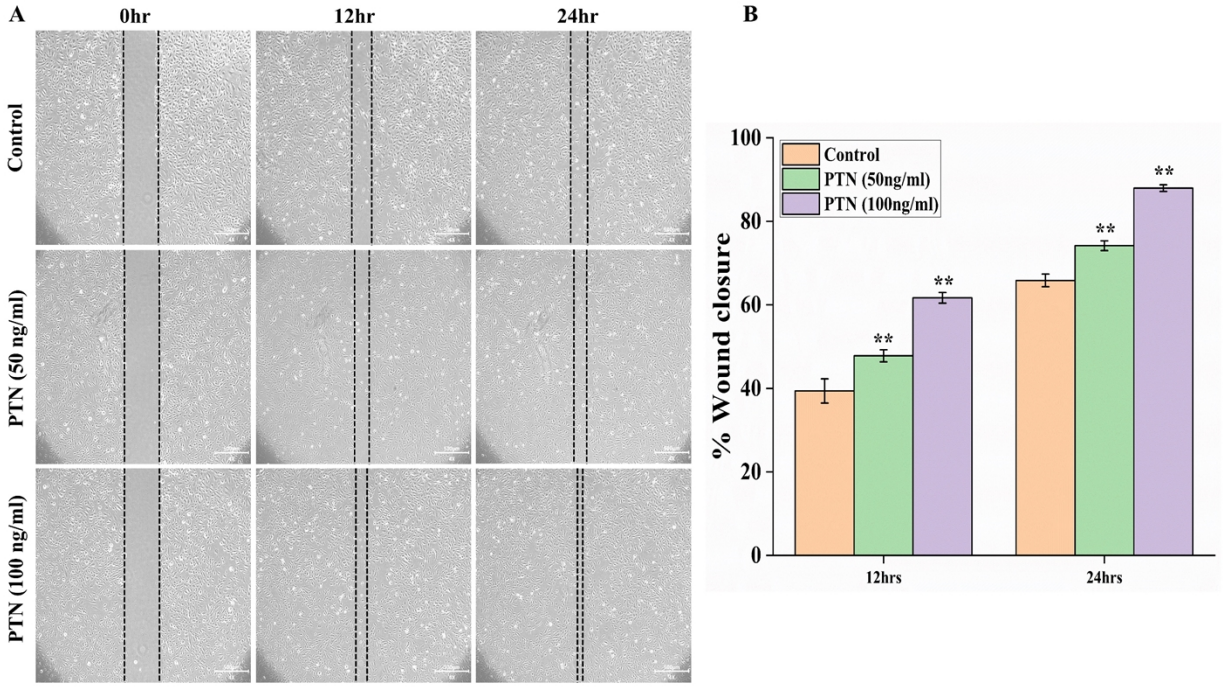
Heparin-Functionalized Scaffold Loaded with Pleiotrophin Enhances Endothelialization and Angiogenic Potential in Liver Tissue Engineering

Chandra Jit Yadav, Usha Yadav, Sadia Afrin, Jun-Yeong Lee, Jihad Kamel, Kyung-Mee Park, *

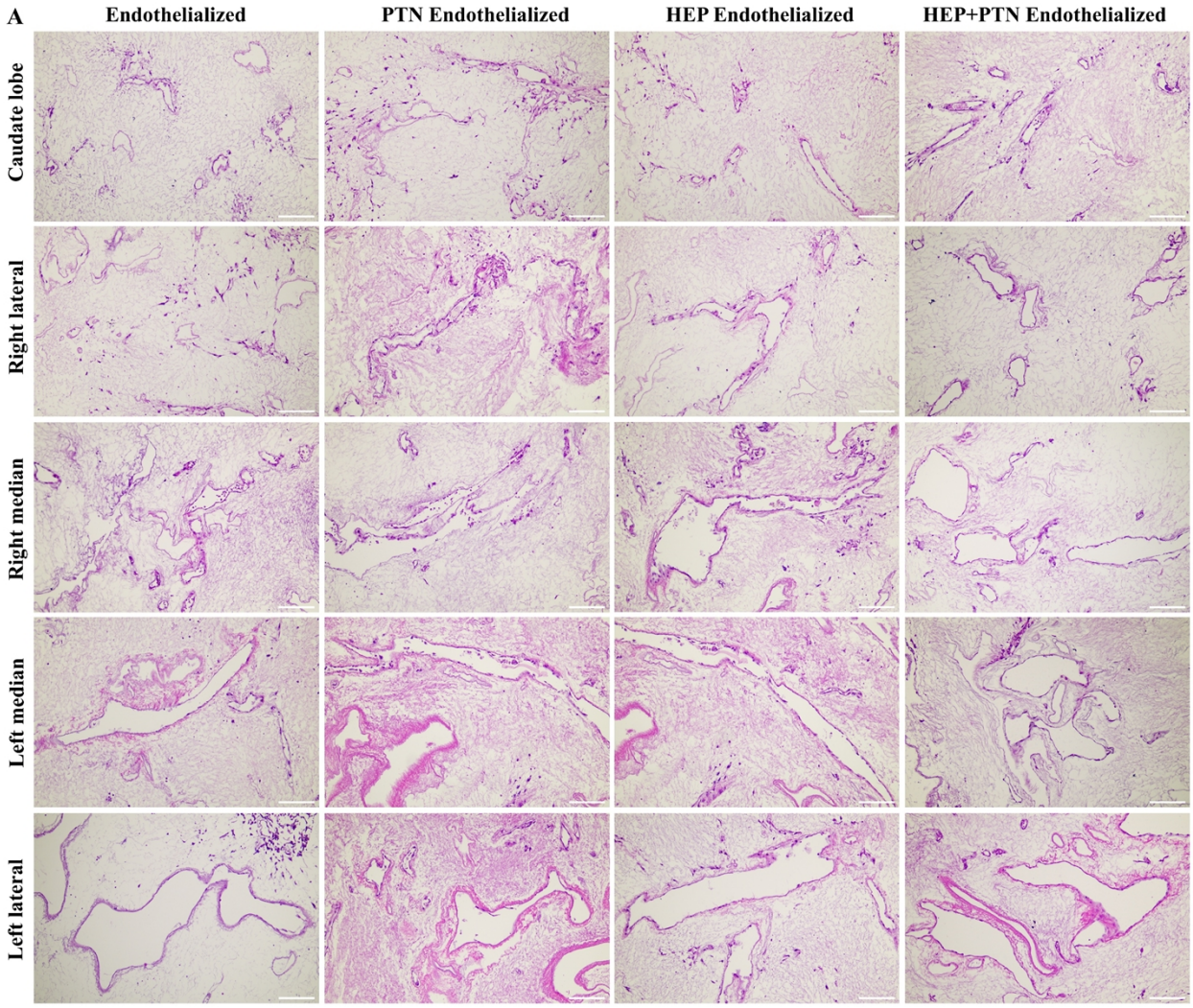
College of Veterinary Medicine, Chungbuk National University, Cheongju 28644, Republic of Korea.
* Correspondence: parkkm@cbnu.ac.kr

Supplementary Figure 1



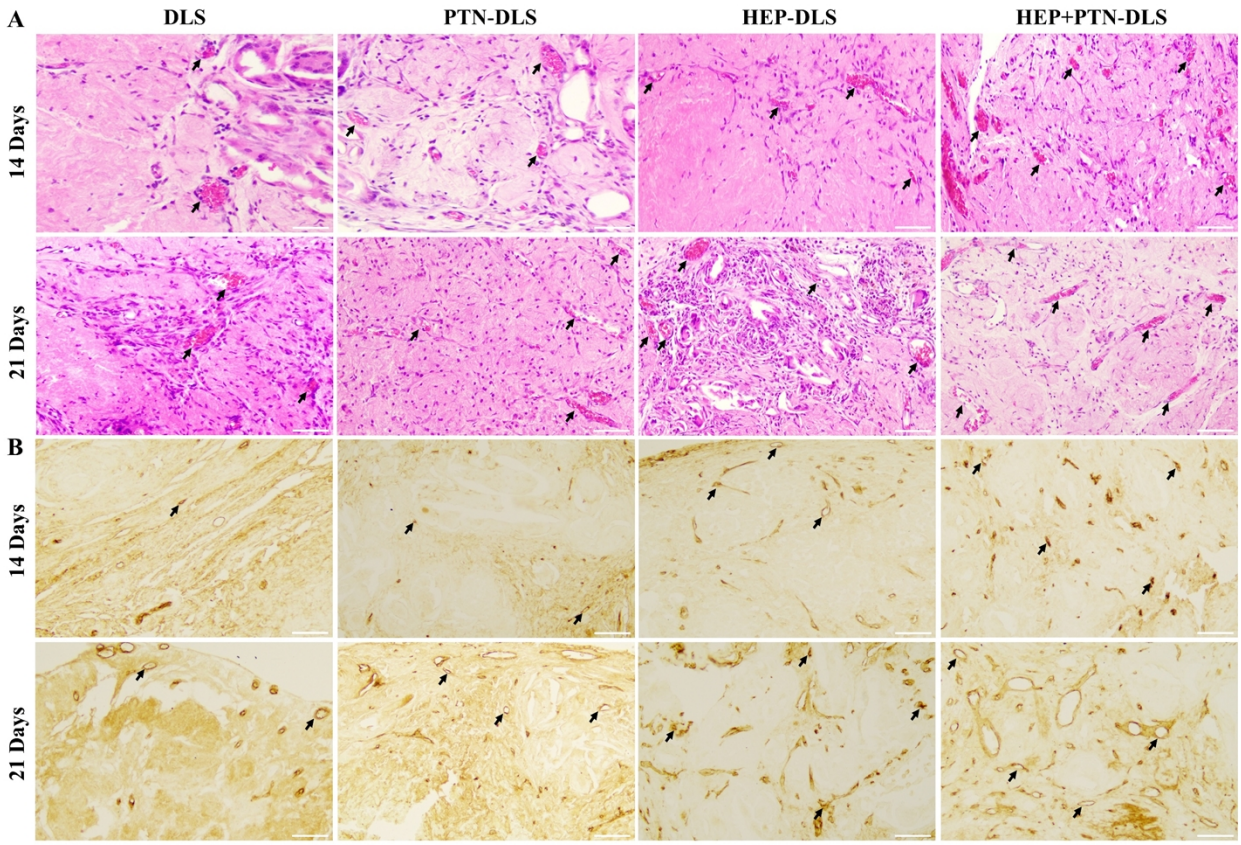
Supplementary Fig. S1 Effects of PTN on HUVECs migration. (A) Representative images of scratch assay treated with different concentration of PTN for the indicated time. The initial wounded area was defined by black dotted lines. (B) Quantification of wound closure rates showed a significantly higher percentage in the 100 ng/mL treated group at 12- and 24-hours intervals.

Supplementary Figure 2



Supplementary Fig. S2 Structural characterization of re-endothelialized scaffold. H&E staining of 5 different lobes of re-endothelialized scaffolds indicated as above. HEP and HEP+PTN endothelialized liver scaffolds show robust attachment of HUVECs to the vasculature, forming a well-defined endothelial cell lining within the blood vessels compared to endothelialized and PTN endothelialized scaffolds (scale bar=100μm).

Supplementary Figure 3



Supplementary Fig. S3 Angiogenic assessment of functionalized scaffolds after omentum implantation in mice. (A, B) H&E and CD31 staining shows newly formed blood vessels (black arrows), with increased vascularization in HEP-DLS and HEP+PTN-DLS compared with DLS or PTN-DLS at 14 and 21 days. (scale bar = 100 μm), enlarged inset micrographs are provided to highlight newly formed blood vessels.

Supplementary method

Supplementary method S1. In vitro wound healing assay

To evaluate the effect of PTN on HUVECs migration, a scratch motility assay was performed. HUVECs were seeded in 6-well plates and allowed to reach 90% confluency. A scratch was created in the cell monolayer using a 200-microliter pipette tip, followed by a gentle wash with PBS to remove detached cells. The scratched monolayers were cultured in the presence of different concentrations of PTN (0, 50, 100ng/mL) for 24 h. Images of the scratched areas were captured at 0, 12, and 24 h using a phase-contrast microscope. Wound areas were quantified using ImageJ software, at each time point, the wound area was manually outlined and measured. To ensure consistency, all images were analyzed using the same thresholding parameters, and each measurement was performed on three fields per well across triplicate wells. The migration rate was calculated using the formula: migration area (%) = [(A0 - At) / A0] × 100%, where A0 represents the initial wound area and At represents the wound area at each measurement time point.

Supplementary method S2.

The cell proliferation was presented as the % Reduction of Resazurin sodium salt following the equation below and using the Molar Extinction Coefficient.

% Reduction of Resazurin = (O2 × A1) - (O1 × A2) 100/ (R1 × N2) - (R2 × N1)

O1=Molar Extinction Coefficient of Oxidized Resazurin at 570 nm is 80586

O2=Molar Extinction Coefficient of Oxidized Resazurin at 600 nm is 177216

R1 =Molar Extinction Coefficient of Reduced Resazurin at 570 nm is 155677

R2 =Molar Extinction Coefficient of Reduced Resazurin at 600 nm is14652

A1=Absorbance value of test wells at 570 nm

A2=Absorbance value of test wells at 600 nm

N1=Absorbance value of Negative Control well at 570 nm

N2=Absorbance value of Negative Control well at 600 nm

Supplementary method S3. RNA isolation and Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA was extracted from the liver tissue using the RNeasy® Mini kit (Qiagen, Hilden, Germany). RNA purity and concentration were determined using spectrophotometry. Subsequently, 1μg of total RNA was reverse transcriptase into cDNA using the TOPscriptTM RT DryMix (dN6Plus, Enzynomics, Daejeon, Republic of Korea). The relative expression levels of target genes were analyzed using the ΔΔCt method, with GAPDH serving as the internal normalization control.