Supporting Information

Application of Periostin Peptide-Decorated Self-Assembled Protein Cage Nanoparticles for Therapeutic Angiogenesis

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Supplementary Materials and Methods

Mass spectrometry

The molecular mass of AaLS-PP subunit was analyzed using an ESI-TOF mass spectrometer (Xevo G2 TOF, Waters) interfaced with a water UPLC and autosampler. Samples were loaded onto a MassPREP Micro-desalting column (Waters) and eluted with a gradient of 5 - 95 % (v/v) acetonitrile containing 0.1 % formic acid at a flow rate of 500 μL/min. ESI generally produces a series of variously charged ions, and the charges are distributed as a continuous series with a Gaussian intensity distribution. The molecular mass was determined from the charges and observed mass-to-charge (*m/z*) ratio values. Mass spectra were acquired in the range of *m/z* 500 - 3000 and deconvoluted using MaxEnt1 from MassLynx to obtain the average mass from multiple charge-state distributions. For clarity, only deconvoluted masses are presented.

Characterization of the protein cage nanoparticles

The hydrodynamic diameter of the AaLS-PP was measured using dynamic light scattering (DLS, Malvern Zetasizer) with a disposable rectangular polystyrene cuvette. Each sample solution was prepared in phosphate buffer (pH 7.4, 50 mM Na_2PO_4, 100 mM NaCl) and adjusted to 25 ℃ before introducing the instrument. The system was operated at 25 ˚C, equilibrated for 2 min, and the scattered light was measured at a 90˚ angle with the projected light. The samples were further analyzed by size exclusion chromatography (SEC, Superose® 6 column, GE Healthcare). The system was operated at a flow rate of 0.5 mL/min with FPLC. TEM experiments were conducted on a JEOL-1400 Bio-TEM operated at an acceleration voltage of 120 kV. TEM samples were prepared by placing 10 μL of the samples on carbon-coated copper grids (Electron Microscopy Sciences). The samples were incubated on the grid for 1 min, and the residual solutions were removed with filter paper. The samples were negatively stained by applying 5 μL uranyl acetate (1 % w/v) onto the grid and incubating for 1 min. The excess uranyl acetate solution was removed with filter paper, and the samples were allowed to dry overnight before imaging.

Cell migration assay

ECFCs migration was assayed using a disposable 96-well chemotaxis chamber (ChemoTx, 116-8, Neuro Probe). To coat the membrane filter of the upper chamber, 50 μL of 20 μg/mL collagen I (BC-354236, Corning) was placed on the lower side and dried overnight at RT. ECFCs were harvested with 0.05 % trypsin-EDTA, washed once, and suspended in EBM-2 at a concentration of 1×10^5 cells/mL. EBM-2 with each supplement of experimental groups was then placed in the lower chamber, and suspended cells were loaded onto the upper chamber at a density of 5×10^3 cells/well. After incubation at 37 ˚C for 12 h, the filters in the upper chamber were disassembled, and the upper side of the filter was wiped with a cotton swab to remove non-migrated cells. The cells that migrated to the lower side were stained with 5 μM Hoechst 33342 dye (H3570, Thermo Fisher Scientific) for 30 min in a 37 ˚C incubator, and the number of cells on each filter was determined by counting cells in four locations under a fluorescence microscope at ×100 magnification.

Tube formation assay

GFR-Matrigel (BC356230, BD Biosciences) was added at 50 μL/well to a 96-well plate, maintained at 4 ˚C, and polymerized for 30 min in a 37 ˚C incubator. ECFCs were suspended in an EBM-2 medium containing 1 % FBS, which was the basal medium, and supplements were treated according to the experimental groups. ECFCs were seeded at 1×10^4 cells/well on polymerized Matrigel and incubated at 37 ˚C in a 5 % CO_2 incubator for 12 h. The capillary-like tube structures were stained with 2 μM calcein AM (C1430, Thermo Fisher Scientific) at 37 ˚C in a 5 % CO_2 incubator for 30 min, and then photographed with a fluorescence microscope (Leica, Germany). Tube length was quantified using ImageJ software (version 1.50i).

Cell proliferation assay

To adhere the ECFCs, coverslips were placed in each well of a 24-well plate and coated with 0.1 % gelatin (G9391, Sigma-Aldrich) in a 37 ˚C incubator for 1 h. Subsequently, 5×10^4 EPCs suspended in EBM-2 containing 0.1 % FBS were seeded on the well, followed by treatment with supplementation of experimental groups.

After incubation of the cells at 37 ˚C and 5 % CO_2 for 24 h, the cells were fixed with 4 % paraformaldehyde at RT for 30 min. The fixed cells were permeabilized with PBS containing 0.2 % Tween 20 for 15 min and blocked with 5 % BSA (A6003). The specimens were incubated with anti-Ki67 antibody (NCL-Ki67p, Leica Biosystems) for 2 h, and then with Alexa 488 goat anti-rabbit secondary antibodies for 1 h. Antibodies were diluted in 5 % BSA, and after the incubation was completed, the specimens were washed three times with PBS for 15 min. Finally, the specimens were mounted in Vectashield medium containing 4′,6-diamidino-2-phenylindole (DAPI) (H1200, Vector Laboratories, Burlingame, CA). Images were collected with a confocal microscope (Olympus, Tokyo, Japan) and measured using ImageJ software (version 1.50i).

Immunocytochemistry analysis

For histological analysis of tissue specimens, the animals were sacrificed, and hind limb muscles were excised. The specimens were fixed in 4 % paraformaldehyde (HP2031, Biosesang) and embedded in paraffin. The paraffin-embedded specimens were sectioned into three 6 μm at 150 μm intervals. For analysis of angiogenesis in the hindlimb, sectioned specimens were stained with anti-CD31 and anti-αSMA antibodies. Subsequently, the cells were incubated with Alexa Fluor 568 goat anti-mouse and Alexa Fluor 488 goat anti-mouse antibodies and washed and mounted in a Vectashield medium containing DAPI to stain the nuclei. The stained sections were visualized under a laser confocal microscope (Olympus FluoView FV1000). Twelve randomly chosen microscopic fields from three serial sections in each tissue were examined for the CD31-positive capillary density and number of αSMA-positive arteries in each mouse. The numbers of CD31+ and αSMA + were quantified using ImageJ software.