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 Na₂PO₄, 100 mM NaCl) and adjusted to 25 °C 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, Neuro Probe). To coat the membrane filter of the upper chamber, 50 μL of 20 μg/mL collagen I (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 (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 (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% CO₂ incubator for 12 h. The capillary-like tube structures were stained with 2 μM calcein AM (Thermo Fisher Scientific) at 37°C in a 5% CO₂ 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 % CO₂ 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.