Construction of an Aptamer-SiRNA Chimeras Modified Tissue-Engineered Blood Vessel for Cell Type-Specific Capture and Delivery

Wen Chen, Wen Zeng, Jiansen Sun, Mingcan Yang, Li Li, Jingting Zhou, Yangxiao Wu, Jun Sun, Ge Liu, Rui Tang, Ju Tan, Chuhong Zhu

Supplementary Material

1. Apoptosis detection

After 5 days of selective culturing, CD133-positive cells were divided into three groups—low-glucose (LG), high-glucose (HG), and HG plus CD133-ADK chimera groups. The LG and HG culture medium contained 5.6 mM and 30 mM glucose, respectively. After the cells were stimulated for 48 h, Annexin V protein and corresponding nucleic acid dyes were added, followed by 15 min of reaction in the dark at room temperature. Finally, after the addition of binding buffer, apoptotic cells were counted using a flow cytometer.

2. Detection of adenosine and cytokine concentrations

After 5 days of selective culturing, CD133-positive cells were stimulated with different factors. After 24 hours of culture, the cells were washed with PBS 3 times, and the culture medium was replaced with fresh medium containing 1% serum. After culturing for 48 hours, the supernatants were collected from all groups. Detection of adenosine was based on the generation of the fluorescent derivative 1, N^6-ethenoadenosine. Briefly, 1 ml of the sample in PBS was transferred into a 1.5-ml

Eppendorf tube, and chloroacetaldehyde was added until a final concentration of 220 μM was reached. The mixture was heated in boiling water for 20 min, and its fluorescence value was determined using a fluorescence spectrophotometer (excitation wavelength = 310 nm; emission wavelength = 410 nm). Vascular endothelial growth factor (VEGF), interleukin-8 (IL-8) and stromal cell-derived factor-1 (SDF-1) concentrations in the culture medium were determined using enzyme-linked immunosorbent assay (ELISA) kits (R&D) following the manufacturer's instructions.

3. Scratch assay and Transwell assay

Scratch assays and Transwell assays were used to evaluate the ability of human umbilical vein endothelial cells (ECs) (ATCC) to form capillary-like structures in different conditioned mediums.

Scratch assay: After reaching confluence in six-well plates, ECs were cultured in the culture medium containing 0.1% FBS for 24 h. Next, after a sterile P1000 pipette tip was used to generate scratches, the cells continued to be cultured in a 1:5 dilution of corresponding conditioned medium containing 2 mM hydroxyurea (Sigma) at 37℃ with 5% CO2 for 24 h. Finally, the cell cultures were fixed with 4% paraformaldehyde (Hyclone), and the EC scratches were stained using 0.5% crystal violet (Sigma). The healing rate of scratches was microscopically assessed.

Transwell assay: ECs were cultured in the 0.1% FBS-containing CD133-positive cell-conditioned medium for 24 h. Subsequently, 1×10^4 cells were added into in a

25-mm-diameter Transwell apparatus (8.0-μm pore size) (Corning), and 600 μl of serum-free EC culture medium containing 1:5 dilution of corresponding conditioned medium was added into the lower chamber. After 6 h of incubation at 37°C with 5% CO2, the Transwell membrane was fixed with 4% paraformaldehyde and stained using 0.5% crystal violet. The cells migrating through the membrane were microscopically counted.

4. Detection of the recruitment phenomenon *in vivo*

The diabetic rats were anesthetized with 1% sodium pentobarbital and underwent TEBV grafting into the right common carotid artery, followed by vascular anastomosis. At post-grafting day 1, the grafted TEBVs were removed for frozen sectioning. A portion of slices was stained with HE staining, and another portion of slices underwent immunofluorescence assays for CD133.

Supplementary Results

Figure S1. CD133-ADK chimeras inhibited HG-inducing CD133 positive cell apoptosis. The CD133 positive cells were treated with different concentrations of glucose. After 24 hours culture, the cells were labeled by using Annexin V-FITC Apoptosis Detection Kit and then examined by flow cytometry. *p < 0.05 (n =6) *versus* LG. Values are mean ± SE; # p < 0.05 (n =6) *versus* HG. Values are mean ± SD.

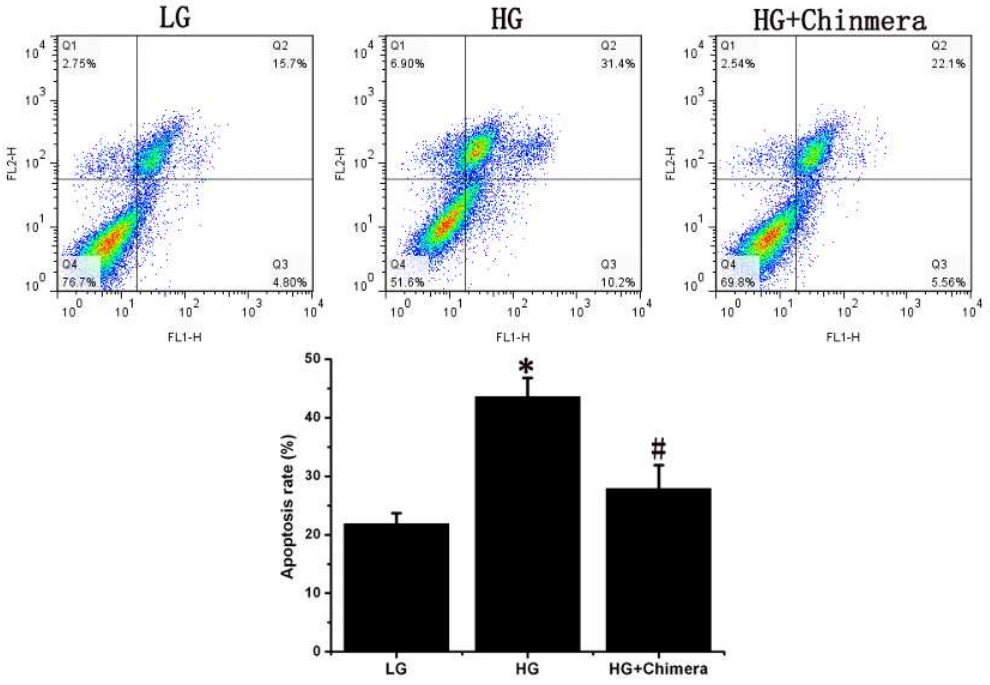

Figure S2. CD133-ADK chimeras promoted cell secretion of adenosine and angiogenic factors. (a) The cells in chimeras group could release a high concentration of adenosine for more than 7 days. (b, c, d) The IL-8, VEGF and SDF-1 concentration in the supernatant was determined by ELISA. *p < 0.05 (n =6) *versus* LG. Values are mean ± SE; # p < 0.05 (n =6) *versus* HG. Values are mean ± SD.

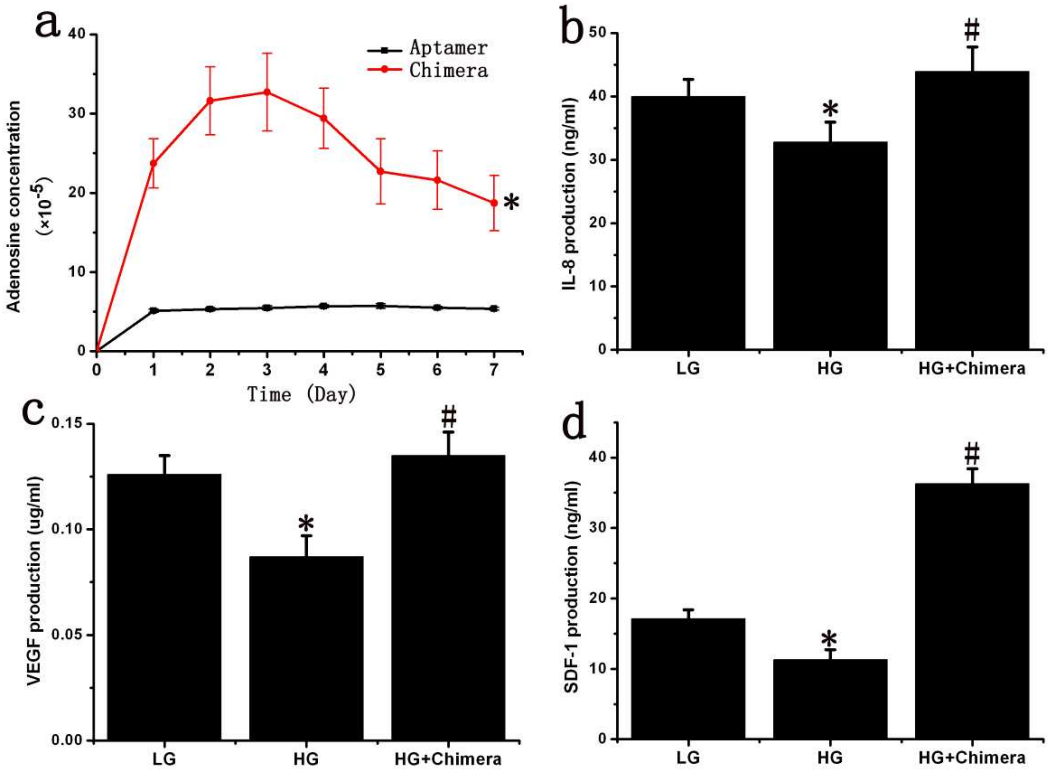

Figure S3. Cell scratch experiment showed the supernatant of CD133 positive cells in CD133-ADK chimeras group promoted healing of the endothelial cell scratches.

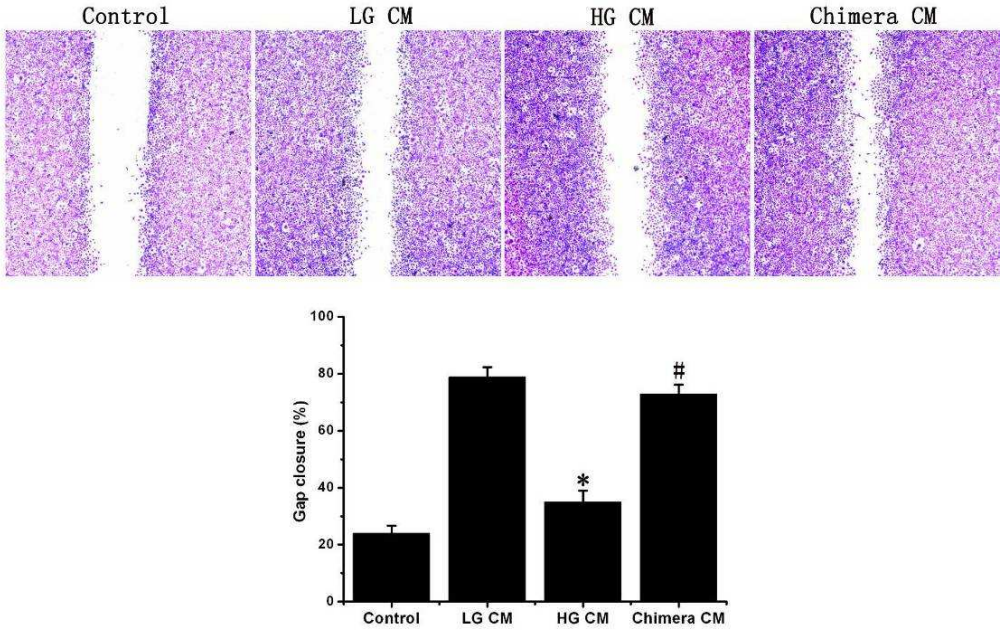

Figure S4. The Transwell migration assay showed that the supernatant of CD133 positive cells in CD133-ADK chimeras group promoted endothelial cell migration.

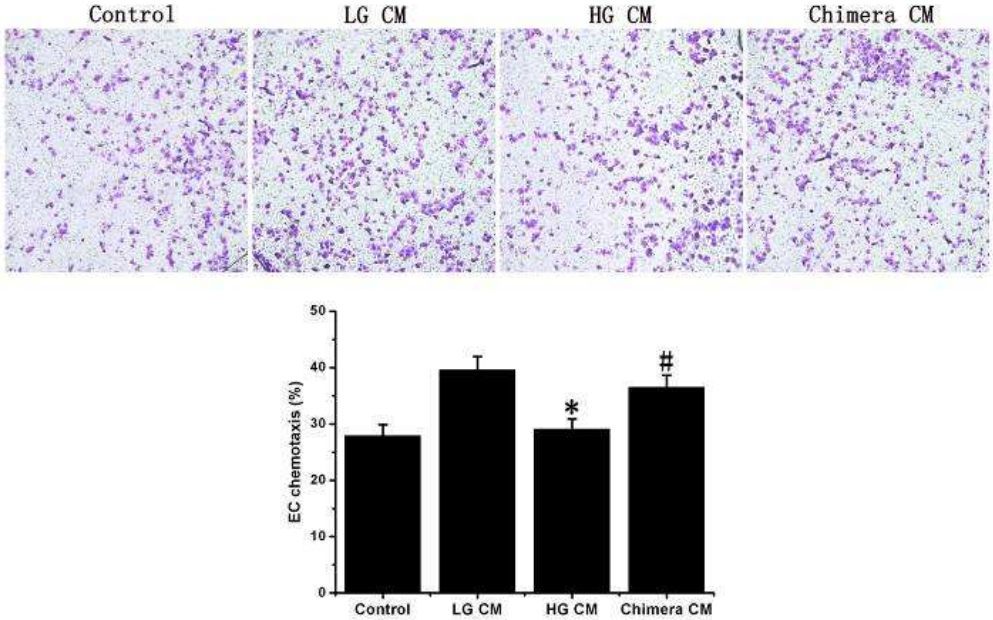

Figure S5. CD133-ADK chimeras captured circulating CD133 positive cells *in vivo*. At post-grafting day 1, CD133 positive cells in cryosections were immunostained with CD133 antibody. The results showed that CD133 aptamer and CD133-ADK chimeras induced more CD133 positive cells to TEBVs and promoted rapid endothelialization.

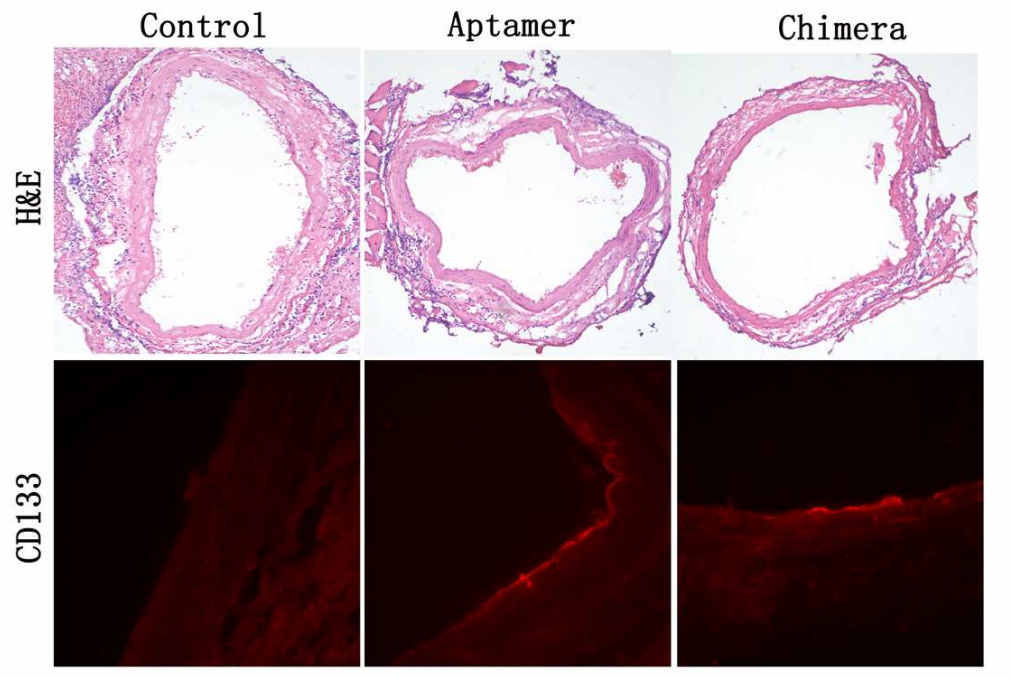

Figure S6. Characterization of prepared TEBV. (a) HE staining of acellular vascular matrix. (b, c, d) The SEM images of acellular vascular matrix, collagen incubated over the TEBV, the high-density PEI/PEG-AuNPs AuNP crosslinked over the collagen.

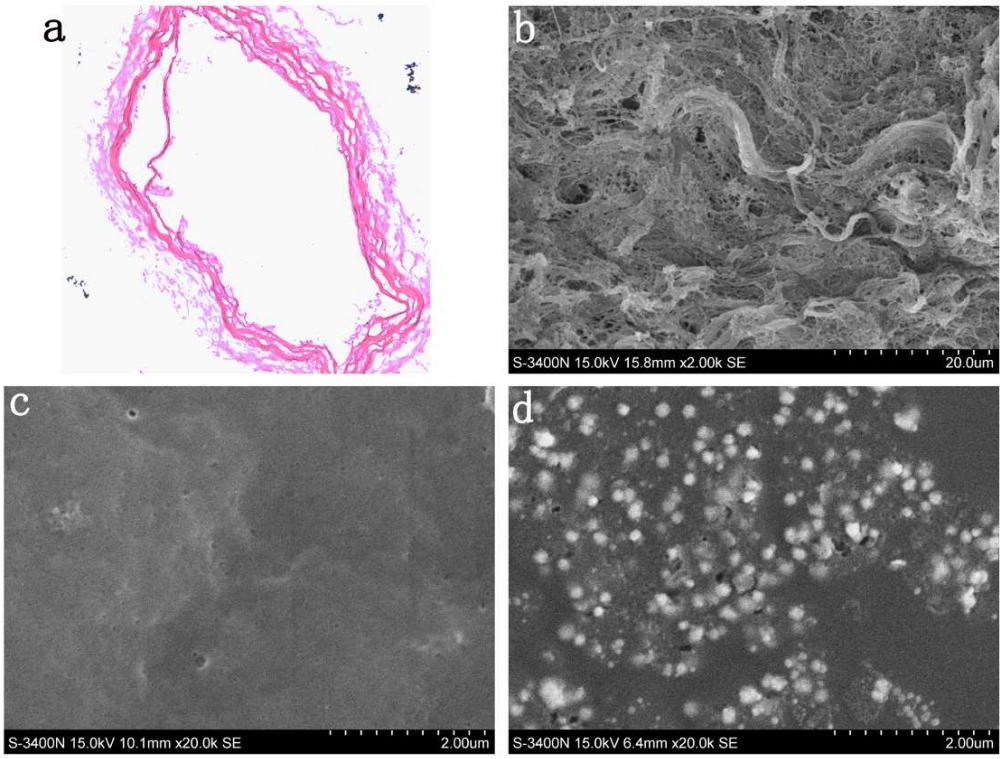

Figure S7. CD133 aptamer increased TEBV patency rate in the rats not induced to be hyperglycemic. At post-grafting day 30, the patency rate of control siRNA modified TEBV is only 10%. TEBVs in CD133 aptamer group and CD133-ADK chimera group had a patency rate of 70% and 90%, and had no significant intimal hyperplasia, respectively.

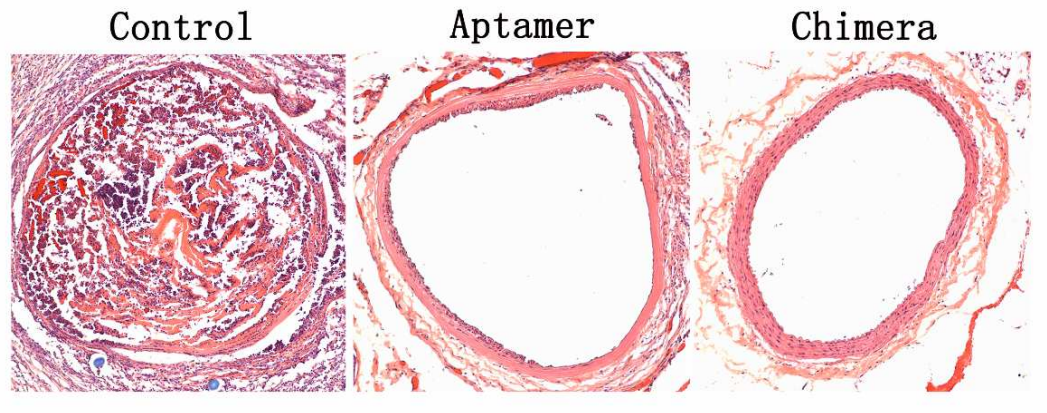

Figure S8. CD133-ADK chimeras maintained TEBV endothelialization. (a) CD31 staining of CD133-ADK chimeras modified TEBV for 30 days. (b) SEM showed the endothelialization of CD133-ADK chimeras modified TEBV at 30 days after implantation.