Figure S1 Mannitol exposure showed no effects on morphology changes and cell viability of dermal microvascular endothelial cell. (A) Both cells treated in mannitol transient on day 4 shows spindle shape and appear in oval shape. The magnification bar equals 50 μm. (B) Effects of mannitol on proliferation profile in dermal microvascular endothelial cell culture over time. (C) Effect of mannitol on apoptosis of dermal microvascular endothelial cell over time.
Figure S2. There are no significant difference between control and Mannitol group of dermal microvascular endothelial cells in a wound “scratch” assay. (A) Dermal microvascular endothelial cells were treated in M131 with or without elevated glucose and fatty acid for 2 days, and then scratched. The scratched cells were culture in conditional medium as follows: control Group, continuous M131 without elevated glucose; Mannitol Group, continuous M131 with elevated 30 mM mannitol. Images were captured immediately post scratch 0, 24, 48, 72, 96 and 120 h. (B) Data displayed indicate mean ± SD of a single representative experiment with N=6 for individual condition. And the value of magnification bar is 200 μm.
Figure S3 Transient elevated Glucose exposure induces a persistent change on Timp3 gene expression by dermal microvascular endothelial cell. Timp3 mRNA measured by RTPCR shows down-regulated in both transient Glucose group and Chronic group (*P<0.05 compared with control group on day 4). There is no significant difference between control group on day 0 and day 4. Mannitol as a Control treatment to control for osmolarity did not affect TIMP3 gene expression significantly. The Data displayed indicated mean ± SD of a single representative experiment with N=6 for individual condition.