Figures and figure supplements

Capillary pericytes mediate coronary no-reflow after myocardial ischaemia

Fergus M O’Farrell et al
Figure 1. Cardiac pericyte morphology is appropriate for regulating capillary diameter. (A) Coronary capillaries in rat left ventricle (dashed arrows in left panel show longitudinal capillaries, solid arrows show connector capillaries) labelled for basement membrane (FITC-isoelectin B4) and for pericytes (example somata labelled with arrows in right panel) with antibody to the proteoglycan NG2. (B) A larger percentage of parallel capillaries (in rat.)
receive pericyte contacts (<1 μm away) than do connector capillaries running between the parallel capillaries. (C) Mouse pericytes labelled with antibody to PDGFRβ (example somata labelled with arrows) and with NG2-DsRed. (D) Percentage of 2874 NG2-expressing pericytes (left) that also express PDGFRβ and of 3047 PDGFRβ—expressing pericytes (right) that also expressed NG2 (averaged over 36 confocal stacks each, from 4 mice, 2–9 months old) in the left ventricle of NG2-dsRed mice. (E) Pericyte in NG2-DsRed mouse showing soma (white arrow) and circumferential and longitudinal processes (yellow arrows). (F) The mean rat pericyte inter-soma distance is similar in the lateral wall of the left ventricle (LLV), the septal wall of the left ventricle (SLV), and anterior and posterior walls of the left ventricle (APLV). (G) Mouse pericyte circumferential processes can extend over much of the capillary surface between pericyte somata. (H) Labelling of tyrosine hydroxylase (blue) shows a close association of sympathetic axons (white arrows) with rat pericytes (cyan arrows). (I) At higher magnification, tyrosine hydroxylase labelled axon varicosities (putative transmitter release sites, cyan) can be seen apposed to pericyte soma and processes. (J) Both capillaries and pericytes (including soma and processes) frequently have sympathetic axon varicosities within 1 μm (in rat). (K–M) Examples of pericytes labelled with Alexa647-isolectin B4 (red) that also labelled (green) for α-SMA (K), β-actin (L) or γ-actin (M). White arrows indicate somata; yellow arrows indicate actin-labelled processes. (N) Percentage of pericytes expressing the 3 actin isoforms in three hearts. Data are mean ± s.e.m. Numbers on bars are of capillaries (panels B, J), images (D), intersoma distances (F) or pericytes (J, N). DOI: https://doi.org/10.7554/eLife.29280.003
Figure 2. Ischaemia and reperfusion lead to no-reflow mediated by capillary block. (A, B) Low power view of sham-operated heart (A) and a heart after LAD coronary artery occlusion and reperfusion (B), with perfusion volume assessed as intensity of FITC-albumin (green). In (B) vessels are also labelled with isolectin B4 - Alexa Fluor 647 (purple) to define location of unperfused tissue. Regions of interest (ROIs) for analysing the intensity of FITC-albumin fluorescence are shown in yellow. (C) Perfusion volume (assessed from mean FITC-albumin intensity), in ROIs indexed with numbers starting at the interventricular septum and proceeding clockwise around the left ventricle (as seen from above), for five sham-operated hearts (control), six hearts made ischaemic and reperfused (ischaemia), and eight hearts made ischaemic and exposed to adenosine starting 5 min before reperfusion (isch + ado). (D) Percentage of capillaries blocked in the anterior wall of the left ventricle for the three experimental conditions (numbers on bars are of ‘capillaries examined, image stacks examined’). Data are mean ± s.e.m. P values are corrected for multiple comparisons.

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Figure 3. No-reflow reflects blockage by pericyte constriction. (A) Image of perfused and non-perfused capillaries in post-ischaemic left ventricle. Isolectin B4 labelling (white) defines positions of all vessels, while FITC-albumin labelling (green) shows vessels that are perfused. Bottom left capillary is blocked vessel. (B) Site of block is near pericyte soma. (C) Pericyte process is squeezed by pericyte soma. (D) Site of block is near pericyte soma. (E) Pericyte processes are blocked near pericyte soma. (F) Block near pericyte soma. (G) Graph showing FITC-albumin intensity vs. distance from block (µm). Line indicates expected for random position of block. (H) Cumulative probability vs. distance of block from pericyte (µm). (I) Diameter at soma/diameter far from soma vs. distance from block (µm). Error bars indicate standard deviation. (J) Diameter at soma vs. distance of block from pericyte (µm). Error bars indicate standard deviation. (K) FITC NG2 IB4 isolectin B4 pericyte blocked vessel. (L) Isolectin B4 glycophorin A RBCs pericyte. (M) Isolectin B4 FITC-albumin pericyte. α-SMA FITC-albumin.
Figure 3 continued

completely non-perfused; top green capillary is fully perfused; lower green capillary is blocked halfway across the image. (B, C) NG2-labelling of pericytes (B) and merge (C) of the images (A) and (B) show pericyte processes constricting vessel at block site. (D–F) Another example set of images as in (A–C), showing two capillaries blocked near pericyte somata. (G) Normalised intensity of (background-subtracted) FITC-albumin (green) labelling along the centre of the capillary lumen across 20 block sites. (H) Cumulative probability distribution for the distance from capillary blockage sites to the nearest pericyte soma (black) and for the position expected (see Figure 3—figure supplement 1) if blocks occurred at positions independent of pericyte locations (significantly different, \(p=3.9 \times 10^{-5}\)). Comparing the experimental distribution with a theoretical distribution increasing linearly to one at a distance of 30 \(\mu\)m (see Image Analysis in Materials and methods) also showed a significant difference \(p=7.6 \times 10^{-5}\). (I) Ratio of capillary diameter at pericyte somata to the diameter at positions \(-10 \mu\)m upstream after ischaemia, after ischaemia with adenosine (ado), and for sham-operated hearts (Con). (J) Diameter at pericyte somata after ischaemia, after ischaemia with adenosine, and for sham-operated hearts (Con) (all pericyte locations were measured, not just those associated with capillary blockages, for which the mean diameter after ischaemia was smaller: 3.19 ± 0.24 \(\mu\)m, \(n=30\)). (K) Capillary blockage in an area of the heart with a neutrophil (labelled for neutrophil elastase, NE, bottom left) present outside the capillaries. (L) Pericyte (labelled with isolectin B4) near a blockage constricting a vessel with two red blood cells (RBCs, labelled for glycophorin A) trapped in the constriction. (M) Blockage-associated pericyte (labelled for NG2) that also labels for \(\alpha\)-SMA. Numbers on bars are of pericytes. Data are mean ± s.e.m. P values in I–J are from Mann-Whitney tests and are corrected for multiple comparisons.

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For a pericyte soma at a distance A from the frame edge, potential blockage positions are represented twice for x<A, but only once for A<x<L-A.

For a randomly positioned block, the probability of the block being between x and x+dx is:

2.dx/L for x<A

and
dx/L for A<x<L-A

Figure 3—figure supplement 1. Calculation of probability distribution for distance from a randomly placed block to a chosen pericyte soma. (A) Diagram showing the calculation of the probabilities on the right of the panel. (B) Cumulative probability distribution for the position of a randomly placed block on this capillary. Figure 3H was calculated by averaging over 42 such distributions for capillaries where block was observed.

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