Biomaterials Science

Supporting Information

Maintenance of ischemic β cell viability through delivery of lipids and ATP by targeted liposomes

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Table S1 Liposome characterization. Data are presented as the mean ± standard deviation from 3 – 8 samples. Samples marked with * contain 1 mol% lissamine rhodamine B labelled DPPE.

Liposome formulation	Encapsulate	Diameter (nm)	Zeta potential (mV)	PR_b (mol%)	ATP Encapsulation % (mole ATP/mole lipid)
PEG CAL	Calcein	117.4 ± 2.0	-2.9 ± 6.2	-	-
PEG CAL*	Calcein	128.4 ± 4.5	-5.4 ± 2.0	-	-
PEG HBSE*	HBSE buffer	134.2 ± 6.8	-2.6 ± 3.1	-	-
PEG HBSE	HBSE buffer	113.0 ± 5.4	-1.1 ± 2.1	-	-
PEG Tris	Tris buffer	122.0 ± 2.8	-2.0 ± 1.8	-	-
PEG ATPL	ATP	126.0 ± 13.8	-4.7 ± 5.5	-	27 ± 12
PR_b-PEG CAL	Calcein	119.9 ± 6.4	0.2 ± 1.7	1.8 ± 0.7	-
PR_b-PEG CAL*	Calcein	127.6 ± 0.7	0.3 ± 4.6	1.4 ± 1.0	-
PR_b-PEG HBSE*	HBSE buffer	118.6 ± 9.0	-2.9 ± 4.3	3.6 ± 1.8	-
PR_b-PEG HBSE	HBSE buffer	122.8 ± 9.3	1.3 ± 0.3	1.6 ± 1.0	-
PR_b-PEG Tris	Tris buffer	119.4 ± 2.5	-1.3 ± 2.0	3.3 ± 0.5	-
PR_b-PEG ATPL	ATP	126.6 ± 8.0	-1.3 ± 3.5	1.5 ± 0.5	33 ± 11

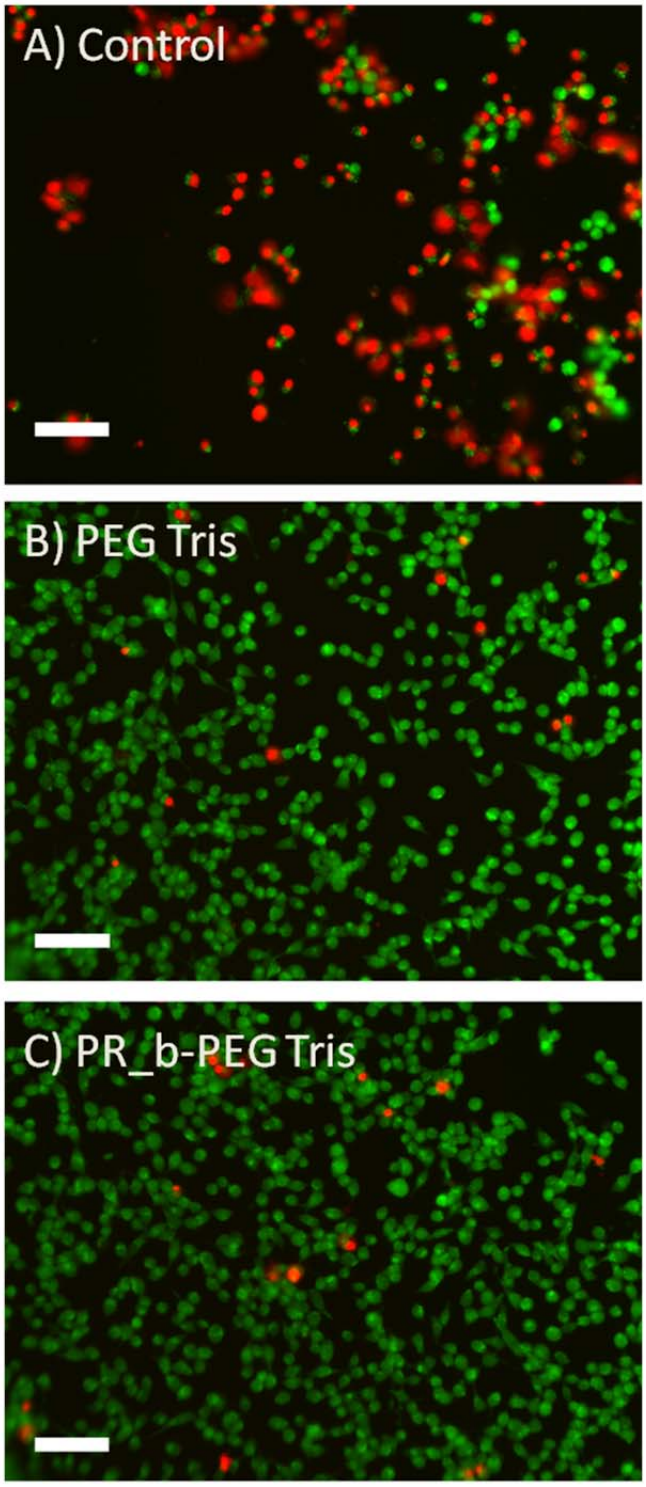

Figure S1 Live/dead staining of INS-1 cells exposed to ischemic conditions (glucose depleted minimal medium, 37 °C, 95% N_2, 5% CO_2) for 9 hours. Cells were treated with 250 μM PEG Tris or PR_b-PEG Tris liposomes, stained with calcein AM (green-live) and propidium iodide (red-dead), and imaged with EVOS®*fl* microscope. Scale bar is 50 μm.

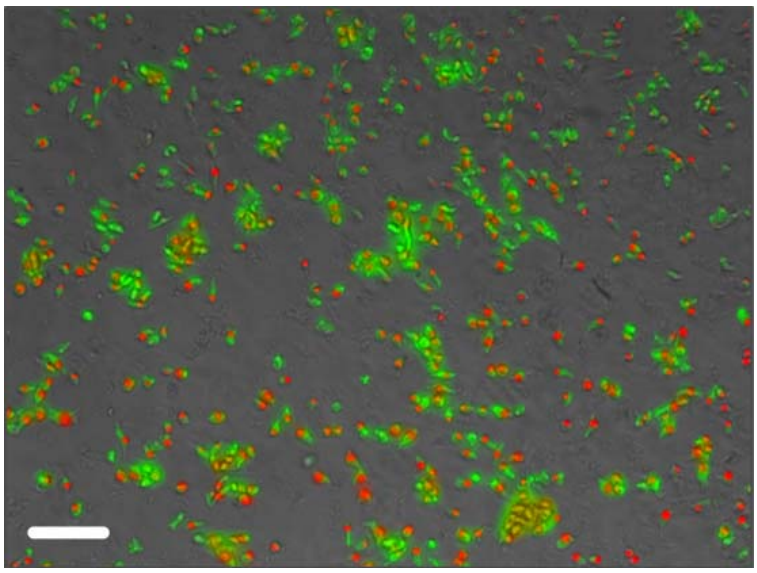

Figure S2 Live/dead staining of INS-1 cells exposed to ischemic conditions (glucose depleted minimal medium, 37 °C, 95% N_2, 5% CO_2) for 6 hours. Cells were treated with 250 μM DPPC, stained with calcein AM (green-live) and propidium iodide (red-dead), and imaged with Nikon Eclipse. Scale bar is 20 μm.

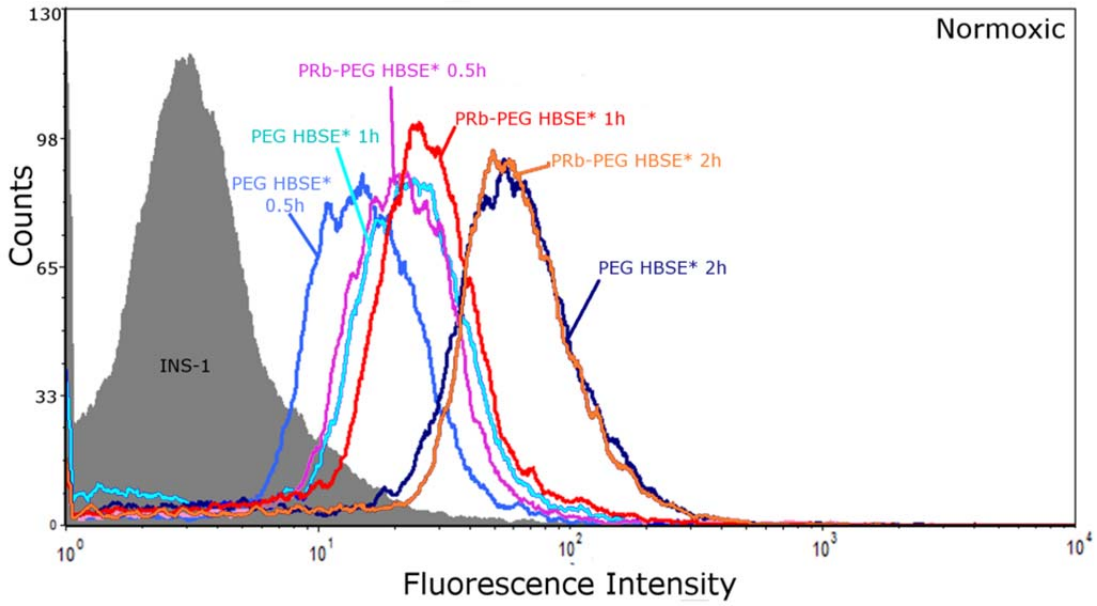

Figure S3 Representative flow cytometry histogram for INS-1 cells incubated with PEG HBSE* and PR_b-PEG HBSE* liposomes. Both liposome formulations contain 1 mol% lissamine rhodamine B DPPE. Liposomes were incubated for indicated periods at 37 °C and the fluorescence monitored after washing via flow cytometry.

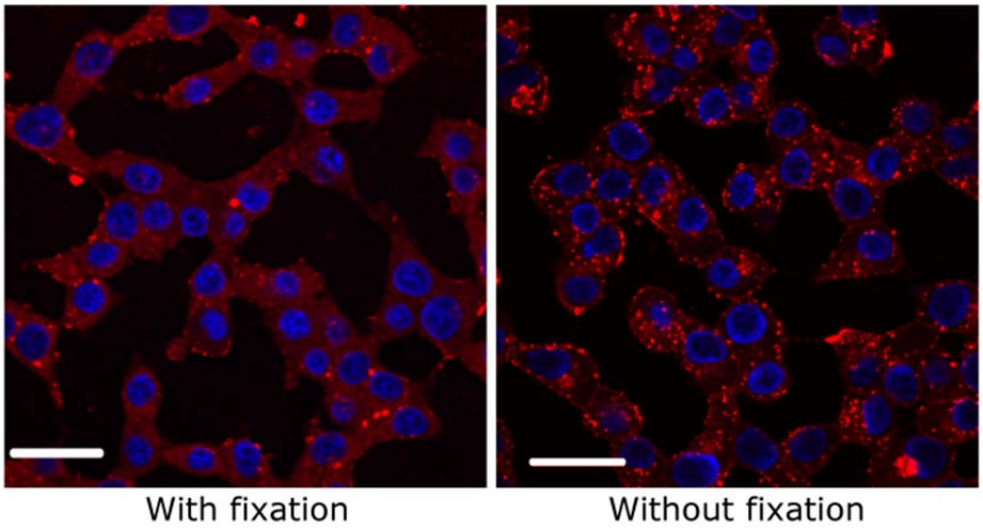

Figure S4 Cell fixation induced artifacts in lissamine rhodamine B DPPE labelling. Cells were incubated with PEG HBSE* liposomes for 1 hour at 37 °C and imaged with or without fixation in 4% paraformaldehyde. Nuclei are shown in blue and rhodamine fluorescence in red. In samples that were fixed the rhodamine appeared diffuse within the cell while samples imaged without fixation showed rhodamine fluorescence primarily on the periphery of the cells and in a punctate pattern within the cells. Scale bar is 20 μm.

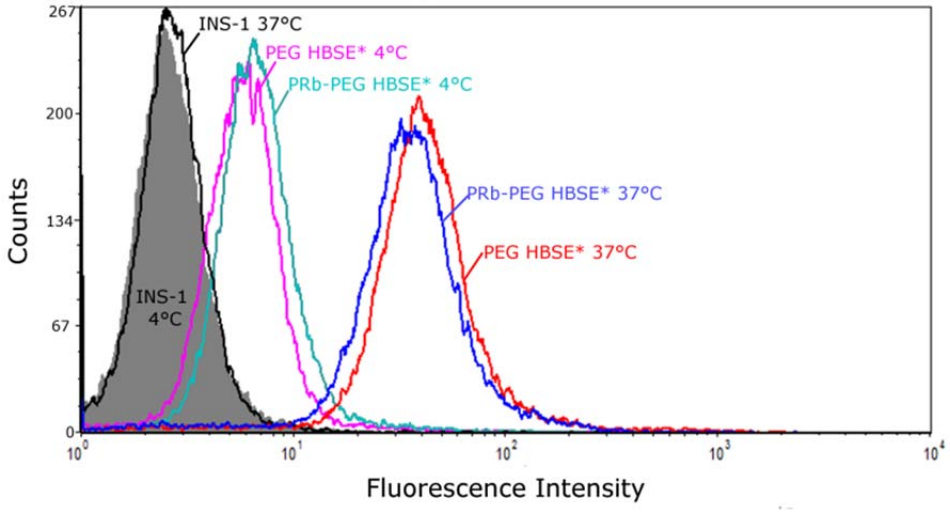

Figure S5 Flow cytometry histogram from a representative experiment monitoring the transfer of fluorescence from PEG HBSE* and PR_b-PEG HBSE* liposomes to INS-1 cells at 4 °C and 37 °C. Cells were incubated with liposomes for 1 hour. Both liposome formulations contain 1 mol% lissamine rhodamine B DPPE.

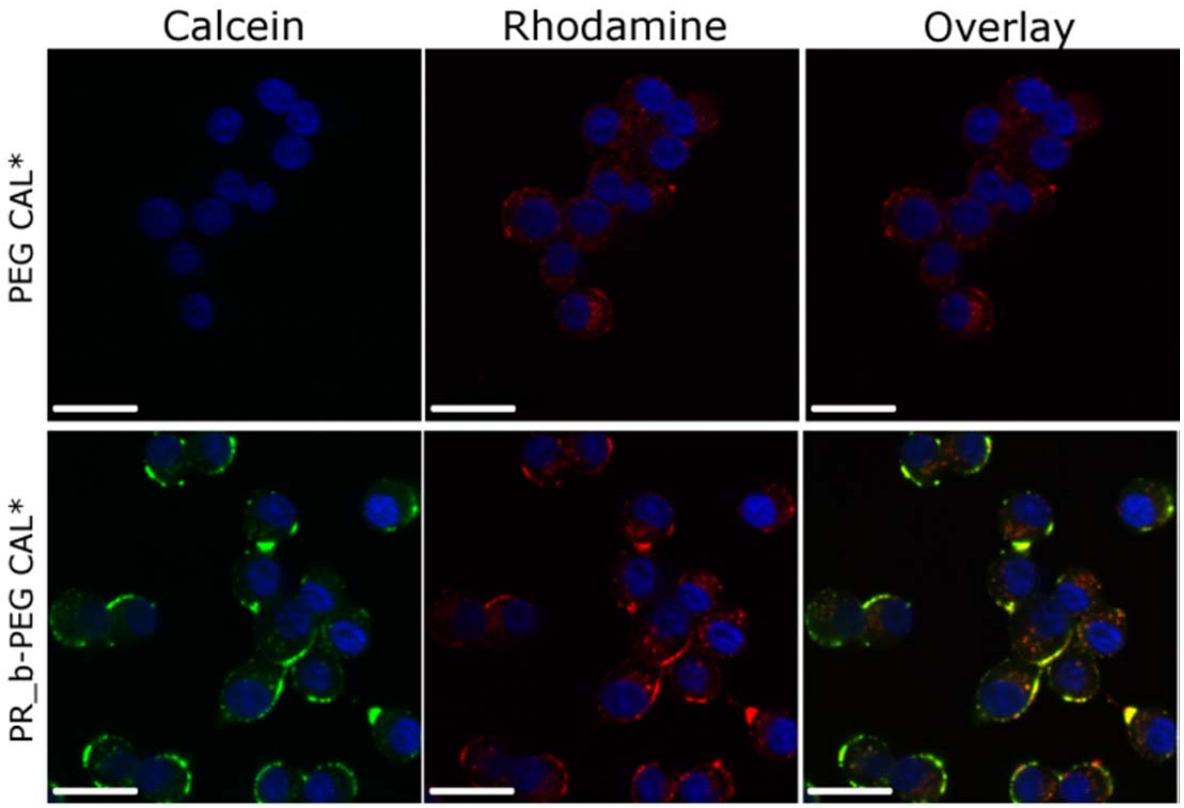

Figure S6 INS-1 cells were treated with liposomes containing 1 mol% rhodamine labelled DPPE and encapsulating 2 mM calcein for 1 hour in glucose depleted minimal medium at 37 °C, 5% CO_2. Confocal micrographs show the nucleus in blue, calcein in green, and rhodamine in red. Scale bars are 20 μm.

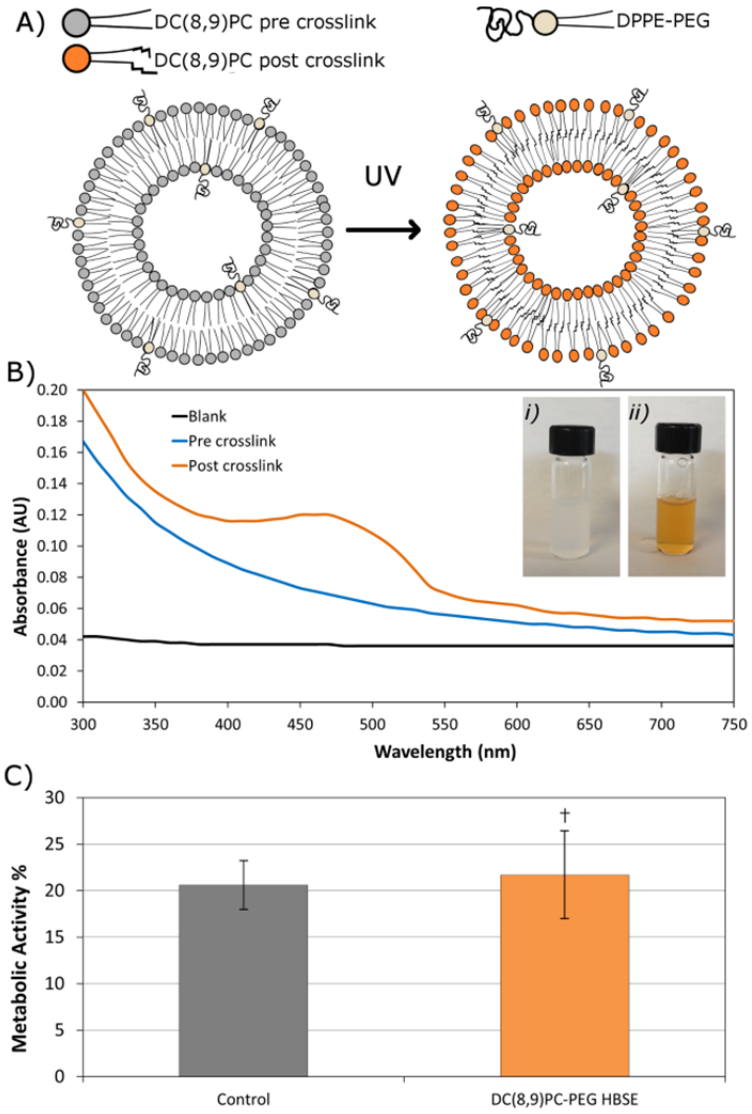

Figure S7 Polymerizable liposomes are formed by crosslinking lipids under UV light. A) Schematic of liposome formation. B) UV-vis absorbance spectrum of liposomes before and after polymerization. Blank refers to buffer only. Liposomes that are polymerized have an increase in absorbance and an indicative colour change. Inset pictures represent liposomes *i)* before and *ii)* after polymerization. C) Polymerized liposomes do not significantly increase the metabolic activity of INS-1 cells exposed to 6 hours of ischemia at 37 °C compared to the control. Data are presented as mean ± SE of n=7 experiments as a percentage of cells cultured in normal conditions (3 repetitions per experiment). †, no significance compared to control.