Taking together, the assessment of spontaneous transplant expression and/or responsive tissue production of IGF-1 or TGF-β by QQMNCTx for tissue regeneration is counted on revealing the mechanism underlying the preferential efficacy of the cell Tx.

6) Limitation of the present study

In the present study, recipient's T-lymphocyte deficient condition in athymic BALB/c nu/nu nude mice limits the insight into regeneration mechanism by regulatory effects on host immune response through regulatory T cells increased in QQMNCs. Therefore, the relevant animal model studies are required to elucidate the essential effect of QQMNCTx in the future experiments.

Conclusion

The QQ culture system for whole PBMNCs that we described here, may lead to an effective cell-based therapy to alleviate the physical burdens in patients as one feasible strategy for vascular regeneration or tissue repair.

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CONFLICT OF INTEREST DISCLOSURES

None

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FIGURE LEGEND

Figure 1. The characteristics of QQMNCs vs. PBMNCs.

(a) The graph shows total cell counts of PBMNCs isolated from 100 mL PB and the respective QQMNC counts. (b) The left graph indicates linear regression analysis between the cell count ratio of QQMNCs vs. PBMNCs (2×10^6 cells/well) and PBMNC counts isolated from 100 mL PB. The right indicates linear regression analysis of cell counts between PBMNCs and QQMNCs per

100 mL PB. (c) The representative pictures of pEPC-CFU and dEPC-CFU. Scale bar= 500 μm. (d) The left and middle graphs are EPC-CFU counts generated from PBMNCs or QQMNCs per dish (2 x10^5 cells/dish) (left) or in 100 mL PB (middle). The right graph shows the percentage of each EPC-CFU count vs. total EPC-CFU count per dish. Each column in the graph represents a mean ± SE. The white and gray areas in the bar graphs indicate the values of pEPC-CFU and dEPC-CFU. (e) Linear regression graphs on the interrelation of each EPC-CFU count per dish in QQMNCs to that in PBMNCs. $^{*}P < 0.05$, $^{***}P < 0.001$. N = 18 volunteers. (f) The comparison of EPC colony forming activities of post QQ cells among CD34+ cells, CD34−MNCs and their repopulated cells. QQ-34−MNC: QQ cells of CD34−MNCs (2 x10^6 cells/2mL of QQc medium), QQ-34+: QQ cells of CD34+ cells alone (4 x10^3 cells/2mL of QQc medium), QQ-34+/34−MNC: QQ cells of CD34+ cells repopulated CD34−MNCs (4 x10^3 cells for CD34+ cells with 2 x10^6 cells for CD34−MNCs/2mL of QQc medium). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. QQ-34−MNC. $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ vs. QQ-34+. Each column in the graph represents a mean ± SE. N = 3 volunteers. Aliquots of each QQ cell cultured in the equal volume of QQc medium were applied to EPC-CFA; the aliquots were respectively seeded at 2 x10^5 cells/dish (3 dishes each for 3 volunteers) for QQ-34−MNC and QQ-34+/34−MNC, and at the ratio of 2 x10^5 cells to the QQ-34+/34−MNCs for QQ-34+.

Figure 2. Flow cytometry analysis of PBMNCs and QQMNCs.

(a) The representative features of PBMNCs at 3 h after seeding, and QQMNCs after 7 days. Scale bar= 100 μm. (b) Scatter diagrams of PBMNCs and QQMNCs in flow cytometry. The red lines indicate the cellular- sized gates of lymphocyte (A), monocyte (B), or the larger cell (C).-(c) The bar graph shows the ratio of each % cell positivity in QQMNCs to that in PBMNCs. N = 4 to 6 volunteers. The investigated cell surface markers were as follows: hematopoietic stem cell (CD34, CD133), endothelial cell (VEGFR-2, CD31, vWF, CD105, CD146), T cell (CD3, CD4, CD8, CD3/CXCR4/CD31), NK cell (CD16, CD56), B cell (CD19), monocyte (CD14), dendritic cell (CD11c), M1 macrophage (CCR2), M2 macrophage (CD206), erythroid progenitor (CD235a). (d) The bar graph indicates the ratio of each % helper T subset positivity in CD4+ cells of QQMNCs to that of PBMNCs. N = 6 volunteers. $^{*}P < 0.01$, $^{**}P < 0.01$ in (c, d). The gray or white column represents a mean ± SE in each increase or decrease. The number associated with each graph column shows the mean value.

Figure 3. qRT-PCR analysis of PBMNCs and QQMNCs.

(a) Angiogenic growth factors. (b) Angiogenic cytokines. (c) Pro- or anti-inflammatory cytokines. (d) MMPs. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Each graph column represents a mean ± SE. N = 8 volunteers.

Figure 4. *In vitro* angiogenic assay of HUVEC co-cultured with QQMNC.

(a) The features of tubes formed by HUVECs. (b) The bar graphs represent the number of tubes counted under x 2 HPF. $^{*}P < 0.05$ vs. HUVEC. $^{\#\#}P < 0.01$ vs. HUVEC+PBMNC. (c) The incorporation of PBMNCs or QQMNCs uptaking acLDL-DiI into tubes of HUVECs. Arrow heads indicate PBMNCs or QQMNCs that are labeled with acLDL-DiI and incorporated into tubes formed by HUVECs. (A): HUVEC alone, (B): HUVEC + PBMNC, (C): HUVEC + QQMNC in (a, c). (d) The bar graphs show numbers of incorporated PBMNCs or QQMNCs, counted under x 4 HPF. $^{***}P < 0.001$. Each graph column represents a mean ± SE. N = 10 wells/group.

Figure 5. Blood flow and distribution of limb salvage patterns in ischemic hindlimbs.

(a, b) Laser Doppler Imaging was used to analyze blood flow 21 days after ischemia. Cells were transplanted at 1 $x10^4$/mouse (5 $x10^3$ cells/each of ATM or GCM). The top panels show the representative features in each group. ROI for blood flow measurement is shown by an yellow square. The bottom line graph presents % blood flow ratio of ischemic to contralateral hindlimb during the observation period for 21 days. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. IMDM control. $^{\#}P < 0.05$ vs. PBMNCTx in (a) or eEPCTx in (b). Each line graph represents a mean ± SE. N = 12 mice/group. (c, d) Limb salvage features of QQMNCTx vs. PBMNCTx day 21 after ischemia. The top pictures in (c) show the representative features of ischemic leg patterns; the severity of the phenotypes are graded from the left to the right. The column graphs in (c, d) show the respective % distributions of severity for each group. N= 19 mice for IMDM control, 21 for PBMNCTx, 23 for QQMNCTx in (c). N = 10 mice per each group in (d). LS; limb salvage, TN; toe necrosis, FN; foot necrosis AA; autoamputation in (c, d).

Figure 6. Assessment of angiogenesis and arteriogenesis in ischemic hindlimbs.

(a, d) The representative pictures of angiogenesis and arteriogenesis in ATM in each group. (A, B, C) in (a) and (A-D) in (d): mouse microvessels stained with Isolectin B4-FITC. (D, E, F) in (a) and (E-H) in (d): pericyte recruited microvessels stained with Cy3-conjugated anti- SMα actin antibodỹThe graphs present the counts of microvessels in (b, e) and pericyte recruited

microvessels in (c, f). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. IMDM control in (b, c, e, f). $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ vs. PBMNCTx in (b, c). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. eEPCTx in (e, f). x 40 HPF. Each column on the graph represents a mean ± SE. N = 6 mice per group.

Figure 7. Evaluation of vasculogenesis by transplanted cells in ischemic hindlimbs.

(a) The representative 2D and 3D images by 3D spectrum analysis using a confocal microscope to assess vasculogenesis and angiogenesis in cell (GmCD34) transplanted ATM. (Green): mouse microvessels stained with Isolectin B4-FITC. (red); vasculogenic microvessels by transplanted human cells, stained with human specific anti-CD31 antibody-Alexa-594. (blue): nuclei stained with TOTO-3. The unit of numbers in their images is μm. (b, e) The panels show the representative similar images in each treatment group. Cells were transplanted at 2 $x10^5$ cells per ATM. Scale bar = 20 μm. (c, f) Microvessel counts/mm^2 in each group. (d, g) %microvessel volume per 3D image cube (142.58 μm x 142.58 μm x 8 μm) in each group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. IMDM control in (c, d, f, g). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. PBMNCTx in (c, d) or eEPCTx in (f, g). Each column on the graph represents a mean ± SE. N = 3 to 4 mice per group.

Figure 8. Histological evaluation of myogenesis in ischemic hindlimbs.

(a, c) The representative images of muscle tissues in ATM by HE staining. (A): normofused tissue with normal morphology of skeletal muscle fibers with the sub-sarcolemmal nuclei in contralateral hindlimb of IMDM control, (B): ischemic hindlimbs from IMDM control, (C): PBMNCTx, or (D): QQMNCTx in (a). (A): IMDM control, (B): eEPCTx (C): QQMNCTx, (D): GmCD34Tx in (c). Note the smaller size of the fibers in combination with centrally located nuclei indicated that a muscle fiber had been actively regenerating. (b, d) The graphs present the counts of regenerating muscle fibers in each group. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. IMDM control in (b, d). $^{\#}P < 0.05$ vs. PBMNCTx in (b) or eEPCTx in (d). Each column on the graph represents a mean ± SE. x 20 HPF. N= 6 mice per group.

Figure 9. Histological evaluation of fibrosis in ischemic hindlimbs.

(a, c) The representative pictures of fibrosis in ATM assessed by Azan staining (blue) in each group. x 40 HPF. (A): contralateral hindlimb of IMDM control, (B, C, D): ischemic hindlimbs of IMDM control, PBMNCTx, and QQMNCTx in (a). (A, B, C, D): ischemic hindlimbs of IMDM control, eEPCTx, QQMNCTx, and GmCD34Tx in (c). (b, d) The graphs show %fibrotic area in

each group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. IMDM control in (b, d). $^{\#\#}P < 0.01$ vs. PBMNCTx in (b). $^{\#}P < 0.05$ vs. eEPCTx in (d). Each column on the graph represents a mean ± SE. N = 6 mice per group.

Figure 10. Histological evaluation of inflammation in ischemic hindlimbs.

(a, c) The representative pictures of inflammation in ATM by immunohistochemistry using anti-iNOS antibody (brown colour) in each group. x 20 HPF. (A): control of rabbit polyclonal IgG, (B): IMDM control, (C): PBMNCTx, (D): QQMNCTx in (a). (A): control of rabbit polyclonal IgG, (B): IMDM control, (C): eEPCTx, (D): QQMNCTx, (E): GmCD34Tx in (c). (b, d) The graphs show % iNOS expressing area in each group. $^{*}P < 0.05$, $^{***}P < 0.001$ vs. IMDM control in (b, d). $^{\#}P < 0.05$ vs. PBMNCTx in (b) or eEPCTx in (d). The each graph column is shown by a mean ± SE. N = 6 mice per group.

Figure 11. qRT-PCR assay of murine gene expression in ischemic hindlimbs after cell transplantation.

The graphs show the relative gene expression levels of myogenic (MyoD1, myogenin, IGF-1), anti-inflammation (TGF-β) and angiogenic (IL-1β) factors. The comparison of the levels in QQMNCTx to those in IMDM control or PBMNCTx in (a), and in IMDM control, eEPCTx, GmCD34Tx in (b). H; healthy (contralateral) hindlimb of IMDM control mice. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. IMDM control in (a, b). $^{\$}P < 0.0.5$, $^{\$\$}P < 0.01$ vs. H in (a, b). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. eEPCTx. The each graph column is shown by a mean ± SE. N= 4 to 6 mice per group.

TABLE 1. Materials for QQc and EPC-CFA.

	Company, Cat No.	Application
6-well Primaria tissue culture plate	BD Falcon, #353846	QQc
35-mm Primaria tissue culture dish	BD Falcon, #353801	EPC-CFA
Blunt-end needle	Stem Cell Tec., #28110	Applying semi-solid medium
Gridded scoring dish	Stem Cell Tec., #27500	Guide when counting EPC-CFU

TABLE 2. Contents of QQc medium.

	Company, Cat No.	Final concentration
Stemline II ™ Hematopoietic Stem Cell Expansion Medium	Sigma-Aldrich, #S0192	
rh SCF	Peprotec, #300-07	100 ng/mL
rh Flt-3 ligand	Peprotec, #300-19	100 ng/mL
rh TPO	Peprotec, #300-18	20 ng/mL
rh VEGF	Peprotec, #100-20	50 ng/mL
rh IL-6	Peprotec, #200-06	20 ng/mL

rh: recombinant human.

TABLE 3. Contents in semi-solid culture for EPC-CFA.

	Company, Cat No.	Final concentration
MethoCult™ SF^{BIT} H4236	Stem Cell Tec, #04236	
rh SCF	Peprotec, #300-07	66.7 ng/mL
rh VEGF	Peprotec, #100-20	33.3 ng/mL
rh basic FGF	Peprotec, #100-18B	33.3 ng/mL
rh EGF	Peprotec, #100-15	33.3 ng/mL
rh IGF-1	Peprotec, #100-11	33.3 ng/mL

rh IL-3	Peprotec, #200-03	13.3 ng/mL
Heparin	Shimizu Pharmaceutical Co.	1.33 IU/mL
FBS	SAFC Biosciences, #12303	30% (vol/vol)

rh: recombinant human.

TABLE 4. Antibodies recognizing hematopoietic cell populations for flow cytometry.

Antibody	Clone	Isotype	Company, Cat No.
CD34-FITC	581	Mouse IgG1 κ	BD Pharmingen, #555821
CD133-APC	AC133	Mouse IgG1	Miltenyi Biotece, #130-090-826
VEGFR-2-PE	89106	Mouse IgG1	R&D, #FAB357P
CD31-FITC	WM59	Mouse IgG1 κ	BD Pharmingen, #555445
vWF	4F9	Mouse IgG1 κ	Abcam, #ab20435
CD105-APC	SN6	Mouse IgG1 κ	eBioscience, #17-1057-42
CD146-PE	P1H12	Mouse IgG1 κ	BD Pharmingen, #550315
CD3-Alexa700	HIT3a	MouseIgG2a κ	BioLegend, #300324
CD4-APC/Cy7	RPA-T4	Mouse IgG1 κ	BioLegend, #300518
CD8-Pacific Blue	SK1	Mouse IgG1 κ	BioLegend, #344718
CD11c-PE	S-HCL-3	MouseIgG2b κ	BD Biosciences, #347637
CD14-Pacific Blue	M5E2	Mouse IgG2a κ	BioLegend, #301828
CD16-APC/Cy7	3G8	Mouse IgG1 κ	BioLegend, #302018
CD19-PE/Cy7	HIB19	Mouse IgG1 κ	BioLegend, #302215
CD56-APC	HCD56	Mouse IgG1 κ	BioLegend, #318309
CD192(CCR2)-PerCP/Cy5.5	TG5/CCR2	Mouse IgG2b κ	BioLegend, #335303
CD206-APC/Cy7	15-2	Mouse IgG1 κ	BioLegend, #321119
CD235a-FITC	GA-R2(HIR2)	Mouse IgG2b κ	BD Pharmingen, #559943
CD184(CXCR4)-PE/Cy7	12G5	Mouse IgG2a κ	BioLegend, #306514

TABLE 5. Antibodies recognizing helper T cell subsets for flow cytometry.

Antibody	Clone	Isotype	Company, Cat No.
CD4-PerCP/Cy5.5	OKT4	Mouse IgG2b κ	BioLegend, #317428
CD25-PE	BC96	Mouse IgG1 κ	BioLegend, #302606,
INF-γ-Pacific Blue	4S.B3	Mouse IgG1 κ	BioLegend, #502522
IL-4-APC	8D4-8	Mouse IgG1 κ	BioLegend, #500713
Foxp3-FITC	206D	Mouse IgG1 κ	BioLegend , #320105

TABLE 6. Isotype antibodies and reagents for flow cytometry.

Antibody	Clone	Company, Cat No.
Mouse IgG1 κ -FITC	MOPC-21	BD Pharmingen, #555748
Mouse IgG1 κ -APC	679.1Mc7	Beckman Coulter, #IM2475
Mouse IgG1 κ -PE	MOPC-21	BD Pharmingen, #555749
Mouse IgG1 κ	MOPC-21	BD Pharmingen, #555746
Mouse IgG1 κ -Pacific Blue	MOPC-21	BioLegend, #400131
Mouse IgG1 κ -PE/Cy7	MOPC-21	BioLegend, #400125,
Mouse IgG1 κ -APC/Cy7	MOPC-21	BioLegend, #400127
Mouse IgG2a κ -Pacific Blue	MOPC-173	BioLegend, #400235,
Mouse IgG2a κ -Alexa700	MOPC-173	BioLegend, #400247
Mouse IgG2b κ -FITC	27-35	BD Pharmingen, #555742
Mouse IgG2b κ -PE	27-35	BD Pharmingen, #555743
Mouse IgG2 κ -PerCP/Cy5.5	MPC-11	BioLegend, #400337
Mouse IgG2a κ -PE/Cy7	MOP-173	BioLegend, #400232
Biotin-rat anti mouse IgG1	A85-1	BD Pharmingen, #553441
Streptavidin-PE/Cy7		BioLegend, #405206
Fc Blocking reagent, human		Militenyi Biotec, #130-059-901

TABLE 7. Human PCR primers and probes for qRT-PCR in PBMNC and QQMNC.

Gene	Forward primer	Reverse primer	TaqMan probe (5'-FAM, 3'-BHQ)
VEGF-A	5'-CCCAGGAGACCT 5'-GGTTGTGT-3'	5'-TGGATCCTGCCCTGT CTCTCT-3'	5'-AGTGGTTGACCTTCC TCCATCCCC-3'
VEGF-B	5'-AGGTGACACATG GCTTTTCAG A-3'	5'-GTTCCCCCACTGGGA TATAGC-3'	5'-TCAGCAGGGTGACTT GCCTCAGA-3'
Ang-1	5'-AAGCTACTGGGC CTCCTCTCA-3'	5'-CCATTAAGGCAT AGTGGATCAAGTC-3'	5'-AAAAGAGACAGTTGT TGGCAAGGTAGCAA-3'
Ang-2	5'-GCAGGGAGTGG TGAGACAGTT-3'	5'-TGCAGGTGCTATGGT CTTTAGAAT-3'	5'-ACGGCTCCTCAGAAA TCCAGTGACC-3'
IGF-1	5'-GCCCAAAATGCA CTGATGTAAA-3'	5'-AGTGACTTTGCTATGAG TTGGTGAGT-3'	5'-CTCTAAAATCCCTTT CAAGCCACCCAT-3'
Leptin	5'-TCACTAGATGGCGAGC ATCCT-3'	5'-CACGCTCAGCTAACTTT TGTGTTT-3'	5'-CCAACATGGTGAAACCCCG TCTCTAC-3'
IL-8	5'-TTTGATACTCCCAGTC TTGTCATTG-3'	5'-CAAGTTTCAACCAGCAA GAAATTACT-3'	5'-TTAGAACTATTAAAACAGC CAAAACTCCACA-3'
IL-10	5'-GCCTGACCACGCTTTC TAGCT-3'	5'-CCAAGCCCAGAGACAA GATAAATT-3'	5'-TTGAGCTGTTTTCCCTGAC CTCCC-3'
IL-1 β	5'-CGGCCACATTTGGTTC TAAGA-3'	5'-AGGGAAGCGGTTGCTCA TC-3'	5'-ACCCTCTGTCATTCGCTCC CACA-3'
TGF-β	5'-CCCTGCCCCTACATTT GGA-3'	5'-CCGGGTTATGCTGGTTG TACA-3'	5'-TGGACACGCAGTACAGCA AGGTCCT-3'
TNF-α	5'-GAGACCAGGGAGCCT TTGGT-3'	5'-TGTGTCAATTTCTAGGT GAGGTCTTC	5'-CTGGCCAGAATGCTGCAGG ACTT-3'
MMP-2	5'-GGTTCCCCTGTTCACT CTACTTAGC-3'	5'-CGGCTTGGTTTTCCTCCA T-3'	5'-TGTCCCTACCGAGTCTCTTC TCCACTG-3'
MMP-9	5'-CCCGGAGTGAGTTGA ACCA-3'	5'-AGGGCACTGCAGGATGT CA-3'	5'-TGGACCAAGTGGGCTACGT GACCT-3'
GAPDH	5'-GGTGGTCTCCTCTGAC TTCAACA-3	5'-GTGGTCGTTGAGGGCAA TG-3	5'-ACACCCACTCCTCCACCTT TGACG-3

TABLE 8. Primary and isotype antibodies to detect iNOS for immunohistochemistry.

Antibodies	Company, Cat No.	Final dilution ratio or concentration
Anti-iNOS antibody	Abcam, #ab15323	1 : 100 in 1% BSA/ PBS
Rabbit Immunoglobulin Fraction (Solid-Phase Absorbed)	DAKO, #X0936	2 μg/mL in 1% BSA/ PBS

TABLE 9. Primary and secondary antibodies for immunohistochemistry to detect vascular structures formed by transplanted human cells.

Antibodies	Company, Cat No.	Final dilution ratio
Mouse anti-human CD31	DAKO, #M0823	1 : 24 in 1% BSA/PBS
goat anti-mouse IgG (H+L) (biotin)	Fitzgerald, # 43C-CB1533	1 : 144 in 1% BSA/PBS
Purified mouse IgG1, isotype control	DAKO Cytomation, #X0931	1 : 6 in 1% BSA/PBS
streptavidin, Alexa Fluor 594 conjugate	Molecular Probes, # S-11227	1 : 90 in 1% BSA/PBS

TABLE 10. Murine PCR primers and TaqMan probes for qRT-PCR.

Gene	Cat No.	Company
MyoD1	Mm01203489_g1	Applied Biosystems
Myogenin	Mm00446195_g1	Applied Biosystems
IGF-1	Mm00439560_m1	Applied Biosystems
IL-1β	Mm00434228_m1	Applied Biosystems
TGF-β	Mm01227699_m1	Applied Biosystems
18S rRNA	Mm03928990_g1	Applied Biosystems

TABLE 11. The cell and EPC colony counts in QQMNC vs. PBMNC.

	PB-MNC	QQ-MNC	P value
Cell counts $x10^5$/well	20.00 ± 0	10.88 ± 1.19***	0.0003
Cell counts $x10^5$/100 mL PB	831.3 ± 75.3	399.2 ± 43.1***	0.0004
pEPC-CFU counts /$2x10^5$cells/dish	1.58 ± 0.34	2.89 ± 0.60*	0.0393
dEPC-CFU counts /$2x10^5$cells/dish	0.71 ± 0.22	29.41 ± 2.34***	0.0002
Total EPC-CFU counts /$2x10^5$cells/ dish	2.31 ± 0.53	31.63 ± 2.66***	0.0002
pEPC-CFU counts $x10^5$/100 mL PB	644.7 ± 125.2	500.4 ± 106.2	0.4331
dEPC-CFU counts $x10^5$/100 mL PB	285.1 ± 78.4	5,407 ± 790.0***	0.0002
Total EPC-CFU counts $x10^5$/100 mL PB	934.3 ± 190.8	5,839 ± 855.2***	0.0002

Each value indicates a mean ± SE. * $P< 0.05$, *** $P< 0.001$ vs. PBMNC. N = 18 volunteers.

TABLE 12. The % positivities of hematopoietic cell populations in QQMNC vs. PBMNC.

	% in PBMNC	% in QQMNC	P value	Ratio	P value
CD34+	0.21± 0.03	1.25 ± 0.26**	0.0078	5.97 ± 0.88**	0.0078
CD133+	0.23 ± 0.08	0.48 ± 0.08	0.0977	3.59 ± 0.71*	0.0195
VEGFR-2+	1.07 ± 0.15	0.67 ± 0.28	0.2500	0.61 ± 0.21	0.2500
CD31+	39.60 ±1.93	40.66 ± 1.89	0.2969	1.03 ± 0.02	0.2969
vWF+	10.52 ± 3.63	10.33 ± 4.07	0.7422	0.85 ± 0.06*	0.0391
CD105+	22.65 ± 1.37	32.05 ± 2.84*	0.0313	1.40 ± 0.05*	0.0313
CD146+	1.57 ± 0.20	2.09 ± 0.16	0.1094	1.48 ± 0.17	0.1094
CD3+	55.16 ± 4.12	71.30 ± 2.61**	0.0078	1.33 ± 0.08**	0.0078
CD4+	34.38 ± 4.27	46.38 ± 3.28*	0.0350	1.41 ± 0.12*	0.0313
CD8+	19.54 ± 2.70	22.03 ± 1.80*	0.0345	1.18 ± 0.08*	0.0313
CD16+	35.66 ± 1.73	31.76 ± 0.88	0.2500	0.90 ± 0.06	0.2500
CD19+	22.00 ± 2.27	6.98 ± 0.16*	0.0350	0.33 ± 0.03*	0.0313
CD56+	24.50 ± 1.17	7.63 ± 1.11*	0.0350	0.30 ± 0.03*	0.0313
CD14+	19.80 ± 1.73	3.47 ± 0.57**	0.0078	0.19 ± 0.04**	0.0078
CD11c+	35.81 ± 2.19	39.57 ± 2.39*	0.0391	1.11 ± 0.03*	0.0391
CCR2+	15.70 ± 1.48	0.22 ± 0.06*	0.0350	0.01 ± 0.004*	0.0355
CD206+	5.53 ± 0.33	26.28 ± 2.69*	0.0350	4.95 ± 0.70*	0.0355
CD235a+	0.88 ± 0.09	0.71 ± 0.03*	0.0211	0.83 ± 0.05*	0.0223
CD3+/CXCR4+ /CD31+	30.28 ± 1.75	41.01 ± 2.07*	0.0350	1.37 ± 0.07*	0.0350

'% in PBMNC' and '% in QQMNC' indicate the % positivity of each cell population in the whole cell of QQMNC and PBMNC. 'Ratio' means the ratio of the % positivity in the whole cell of QQMNC to that of PBMNC, corresponding to **Figure 2c**. The left P values indicate the comparison of % cell positivities between QQMNC and PBMNC; the right ones indicate that of the ratios. *P < 0.05, ** P < 0.01 vs. PBMNC. N = 4 to 6 volunteers.

TABLE 13. The % positivities of helper T cell subsets in CD4+ T cells of QQMNC vs. PBMNC.

	% in CD4+ cell				
	PBMNC	QQMNC	P value	Ratio	P value
CD4+/INF-γ+/IL-4□	10.34 ± 3.71	5.90 ± 2.28*	0.0355	0.55 ± 0.02*	0.0355
CD4+/INF-γ□/IL-4+	2.94 ± 0.53	14.91 ± 3.10*	0.0355	6.04 ± 1.90*	0.0350
CD4+/CD25+/Foxp3+	3.40 ± 0.82	15.00 ± 1.96*	0.0355	5.82 ± 1.51*	0.0355

'% in CD4+ cell' indicates the % positivity of each helper T subset in CD4+ T cell of QQMNC and PBMNC. 'Ratio' means the ratio of the % positivity in CD4+ T cell of QQMNC to that of PBMNC, corresponding to **Figure 2d**. The left P values indicate the comparison of % cell positivities between QQMNC and PBMNC; the right ones indicate that of the ratios. *P < 0.05, ** P < 0.01 vs. PBMNC. N = 6 volunteers.

TABLE 14. The angiogenic and vasculogenic microvessels in ischemic ATMs 21 days after surgery.

	Microvessel counts/mm^2 in 2D			Microvessel volume % in 3D		
Tx cells	Angio	Vasculo	Total	Angio	Vasculo	Total
IMDM	229.6 ± 37.4	0 ± 0	229.6 ± 37.4	0.15 ± 0.06	0 ± 0	0.15 ± 0.06
PBMNC	602.6 ± 165.4	202.9 ± 97.3	805.5 ± 220.6	0.36 ± 0.16	0.05 ± 0.04	0.41 ± 0.19
eEPC	449.7 ± 90.7	203.8 ± 50.8	653.5 ± 115.5	0.39 ± 0.07	0.06 ± 0.02	0.46 ± 0.07
QQMNC	1008.0 ± 162.1	811.6 ± 178.6	1820.0 ± 191.0	1.17 ± 0.29*	0.76 ± 0.17	1.92 ± 0.37
GmCD34	930.8 ± 118.2	662.2 ± 98.6	1593.0 ± 168.7	1.05 ± 0.35	0.53 ± 0.15	1.58 ± 0.46

Each value indicates a mean ± SE. Angio = angiogenic microvessel, Vasculo = vasculogenic microvessel. Tx cells = 2x10^5 cells/mouse transplanted into ischemic ATM. N = 3 to 4 mice per group.