

Safety and Effect of Adipose Tissue derived Mesenchymal Stem Cell Implantation in Patients with Critical Limb Ischemia

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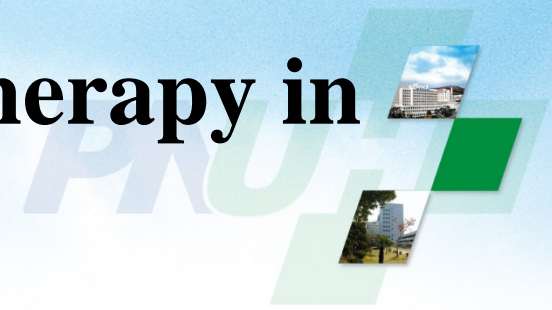
Critical Limb Ischemia



- Revascularisation : 60%-70% in CLI
- 30-40% cannot be successfully revascularised
- PTA and bypass op. are difficult in Buerger' disease

- Severe pain at rest, tissue loss, infection, amputation
- 1 yr major amputation risk for CLI patients with failed revascularisation: 80-90%

Cell Therapy: BM-MNC therapy in PAD/TAO

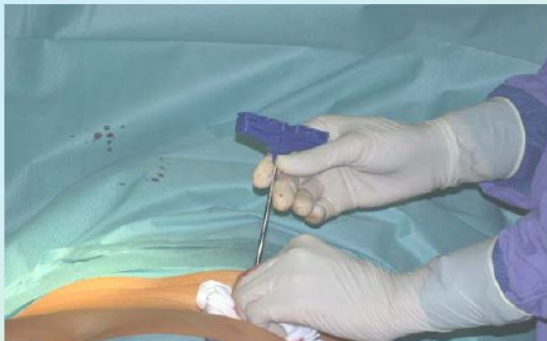


Author/yr	Study Level	Subjects,	ABI	TcP02	Pain	Amp.	+/-
Tateishi (Lancet 2002)	1b	45, PAD Diabetes	↑	↑	↓	↓	+
Esato (CellTransplant2002)	4	8, PAD, TAO	↑	--	↓	↓	+
Saigawa (2004)	4	8 PAD, Diabetes	↑	↑	↓	↓	+
Higashi (Circulation 2004)	4	8, PAD	↑	↑	↓	↓	+
Durdu (J Vasc Surg2006)	1b	28, TAO	↑	↑	↓	↓	+
Huang (Diabetes Care 2005) peripheral stim.stem c.+MNC	3	25, PAD, Diabetes	↑	↑	↓	↓	+

BM EPC : Critical Limb Ischemia

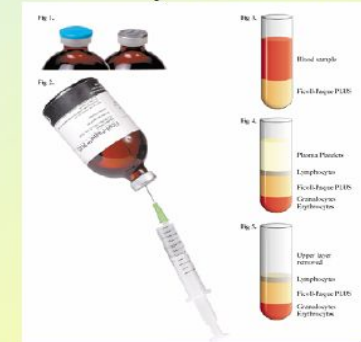
BONMOT 1: Harvesting + Separation

Extraction of bone marrow



BM-Purification/separation of the monocytic cell fraction

Pts #1-12 with Ficoll®



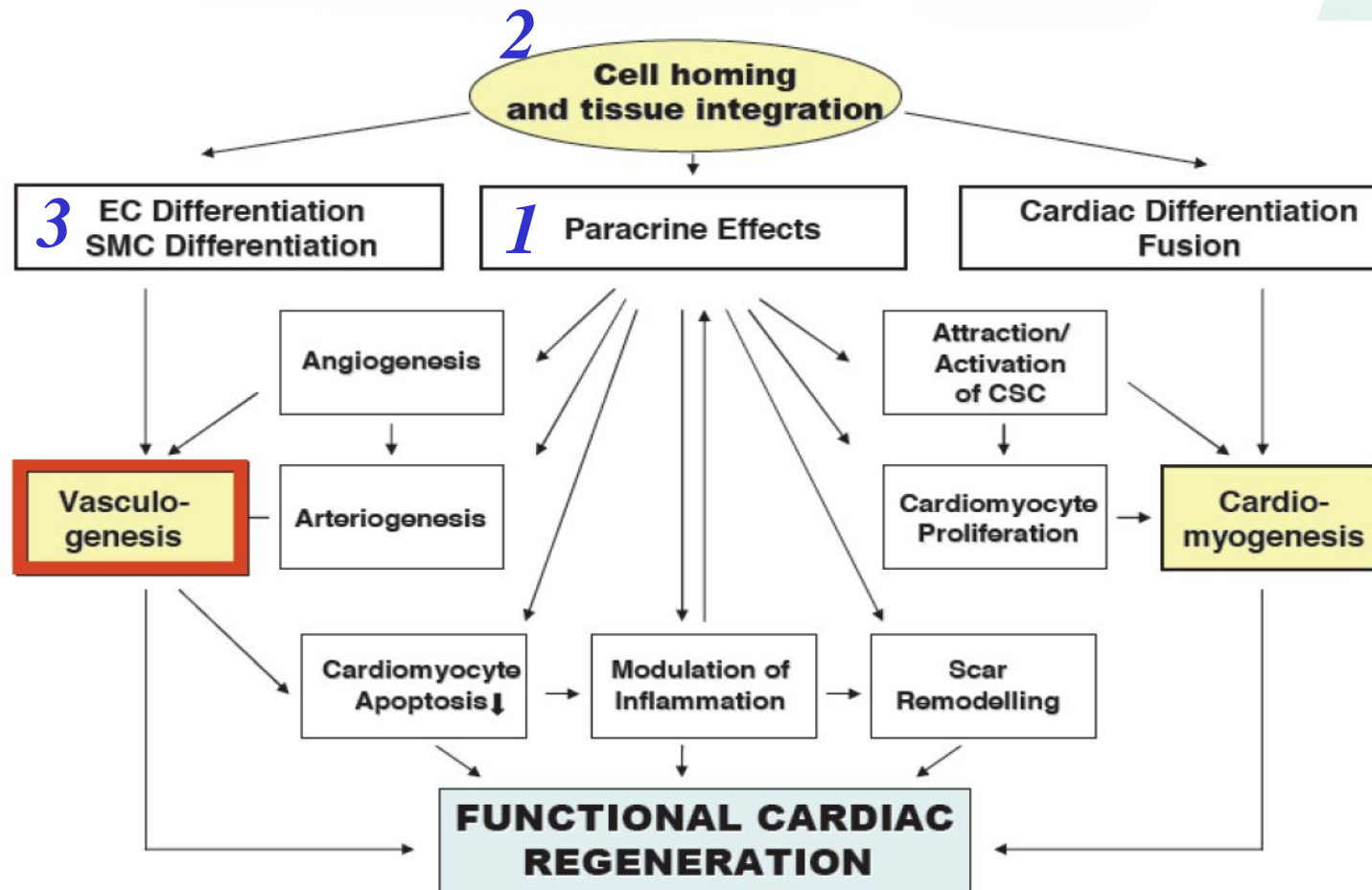
Pts #13- 71 with
Harvesttech SmartPrep®
centrifugation (bed-side)



Adipose Tissue Derived Mesenchymal Stem Cell in CLI

- Convenient and easy to get the adipose tissue
- Larger abundance of MSC and stromal cells
- Basically same potency compared to BMC
- Effects : paracrine effect, homming effect and differentiation into endothelial cell, smooth m. cell

Adipose Tissue Derived Mesenchymal Stem Cell in CLI

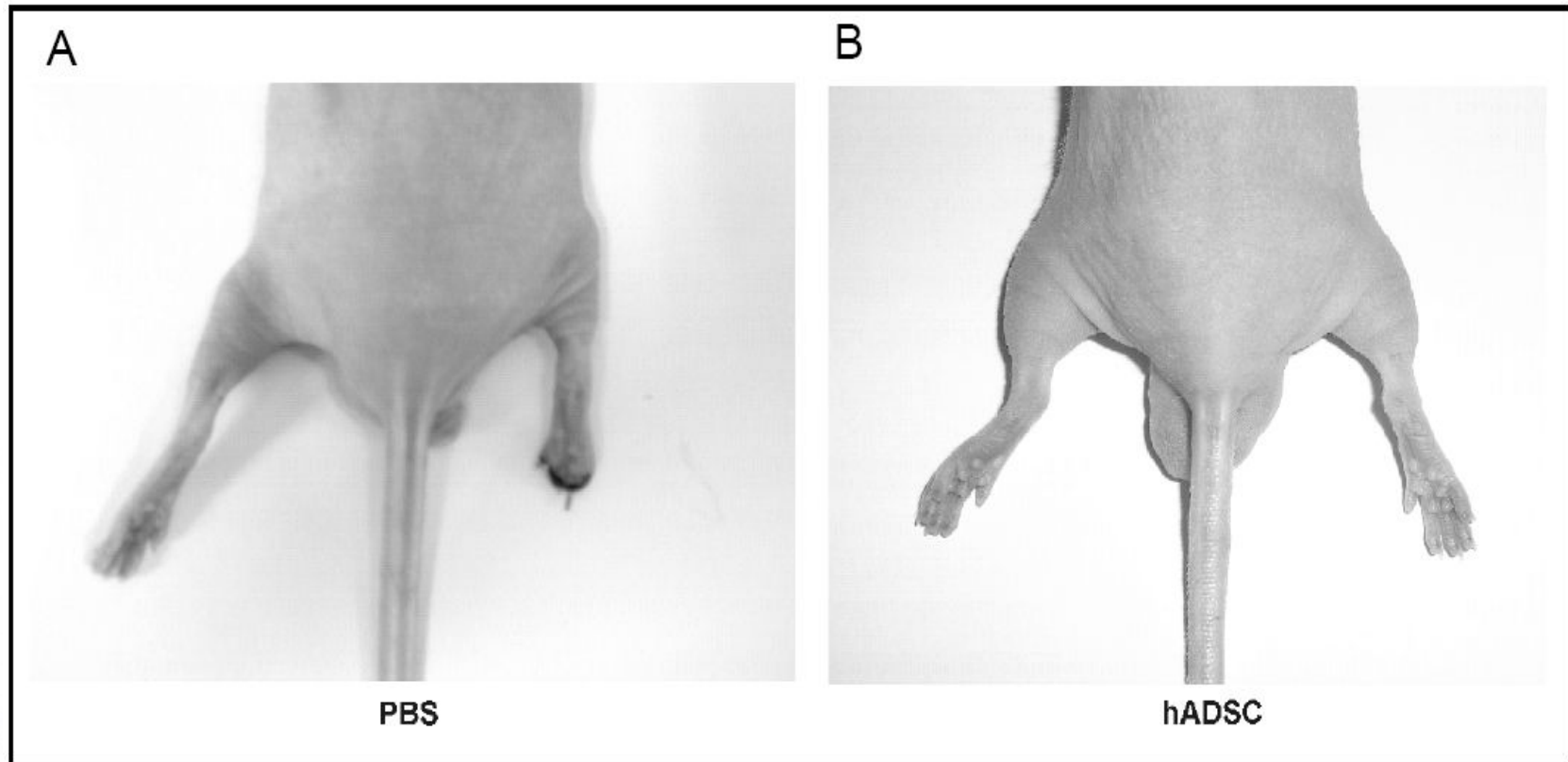


Zuk PA et al. Mol Biol Cell 2002;13(12);4279-95

Background : Animal Model

- Mice hindlimb ischemia model :
ADSC 1×10^5 , 5×10^5 , 1×10^6 intramuscular injection
- 60% autoamputation in the control group
0% autoamputation in the ADSC group

Safety and Effect of Adipose Tissue derived Stem Cell Implantation in Patients with Critical Limb Ischemia : Animal Model



Human Study : Inclusion Criteria

- PNUH IRB and KFDA Approved this human study
(생물의약품 정책과-1273호, 2008. 12. 23.)
- Candidates : 15 patients
- At least 6 months since the onset CLI(Chronic ASO, DM foot or Buerger disease)
- 20 yrs < Age < 80 yrs
- Rutherford's class is II-4, III-5 or III-6,
Rest pain or ischemic ulcer/necrosis
- Patients with CLI were not suitable for percutaneous vascular intervention and bypass operation
- Patients who wrote informed consent

Method

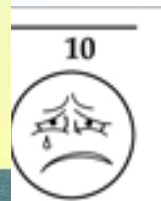


- Liposuction 10 cc
- Culture during 2 weeks, 3 passage
- Increase dose : ADSC $1 \times 10^8 \rightarrow 3 \times 10^8$
- Intramuscular injection, lower leg
0.5 cc * 20-60 points
- Mouse model dose: $1 \times 10^5 - 1 \times 10^6$
- Human dose : $1 \times 10^8 - 3 \times 10^8$
reduction of dose

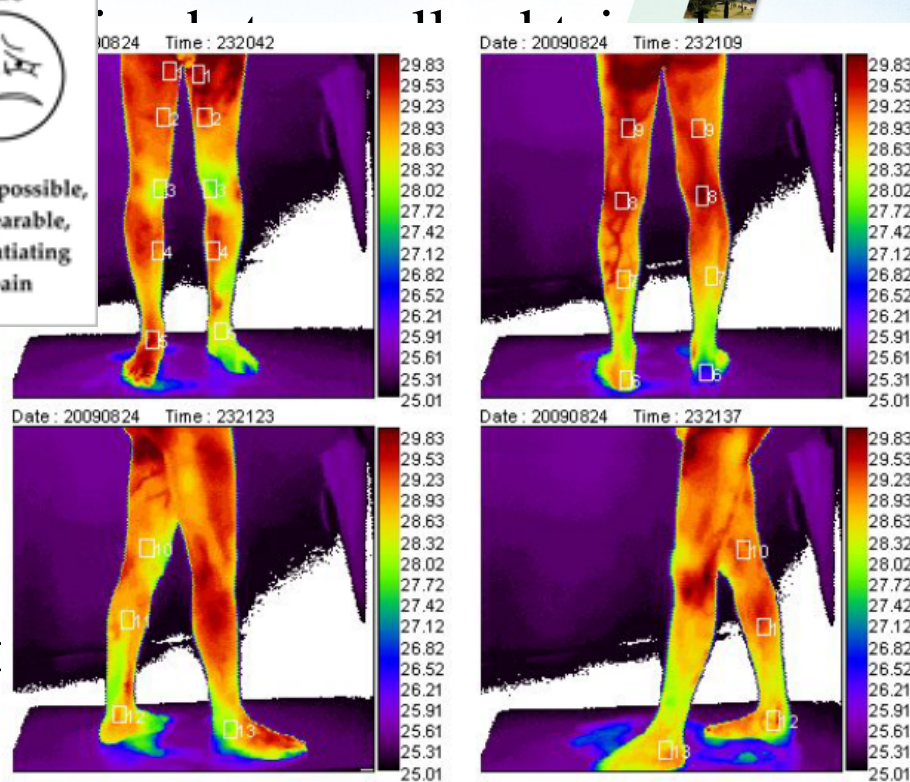




Method



Worst possible, unbearable, excruciating pain



of
0.5
mc
star
Ev

Region of Interests

No	Rt	Lt	Difference
1	29.39	29.49	-0.10
2	29.07	29.16	-0.09
3	28.15	27.95	0.20
4	28.89	29.04	-0.15
5	29.29	28.30	0.99
6	26.47	28.58	-2.11
7	28.56	28.80	-0.24
8	29.63	29.46	0.17
9	29.16	29.41	-0.25

No	Rt	Lt	Difference
10	28.62	29.03	-0.41
11	28.61	29.13	-0.52
12	28.65	28.72	-0.07
13	28.02	28.67	-0.65

Method



At 1, 3 months :

3-month adverse events Analysis(MAE)

At 6 months :

Check ABI, DSA, Thermography, Pain scale rate,
Treadmill test

6-month adverse events Analysis(MAE)

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➤ Surface Antigen :

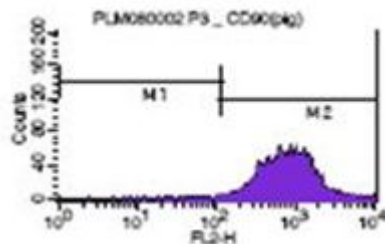
CD73(+), CD90(+)(mesenchymal stem cell marker)

CD31(-)(endothelial cell marker),

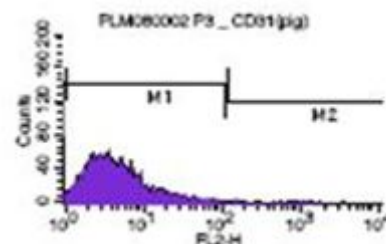
CD34(-)(hematopoietic or hemangioblast marker)

CD45(-)(blood - derived cell marker)

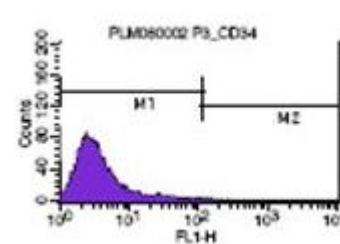
CD 90(+)



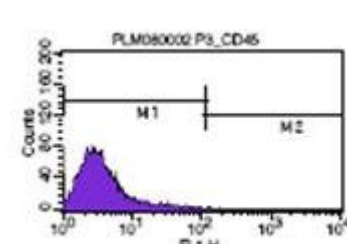
CD 31(-)



CD34(-)



CD45(-)



Method : ADSC proliferation and differentiation



➤ **Proliferation assay** : CFU(colony forming unit) assay

SVF (a seeding density of 10^5 cells/10cm²)

ADSC (a seeding density of 100 cells/10cm²)

➤ **Differentiation**

AM (adipogenic differentiation)

: adipogenic medium (incubation period 7-10 days)

Oil Red O stain (an indicator of intracellular lipid accumulation)

OM (osteogenic differentiation)

: osteogenic medium (incubation period 14-16 days)

alizarin red S stain(extracellular matrix calcification)

Results



- Enrolled 15 patients : 12 Buerger's disease
3 DM foot
- Mean follow up : 6 months
- We followed up 15 patients during 6 months

Results : Patient characteristics

Case No.	Age /Gender	Diagnosis	Ischemic Site/ Status	Past History	Previous Tx. For CLI
1	33/M	Buerger's ds	Left toe/ Resting pain(II-4)	Smoking	Minor amputation, sympathectomy
2	52/M	Buerger's ds	Left toe/ non-healing ulcer(II-5)	Smoking , quit hyperlipidemia	F-F bypass op Major amputation
3	24/M	Buerger's ds	Left toe/ non-healing ulcer(II-5)	Smoking , quit	Minor amputation
4	46/M	Buerger's ds	Right toe/ Necrosis(III-6)	Smoking , quit	Minor amputation
5	36/M	Buerger's ds	Right toe / Resting pain(II-4)	Smoking , quit	Rt F-P bypass op
6	42/M	Buerger's ds	Left foot/ Necrosis(III-6)	Smoking , quit	SFA-PTA bypass op
7	64/M	DM foot	Left foot/ non-healing ulcer(II-5)	DM, HT	
8	55/M	Buerger's ds	Right toe/ non-healing ulcer(II-5)	Smoking	

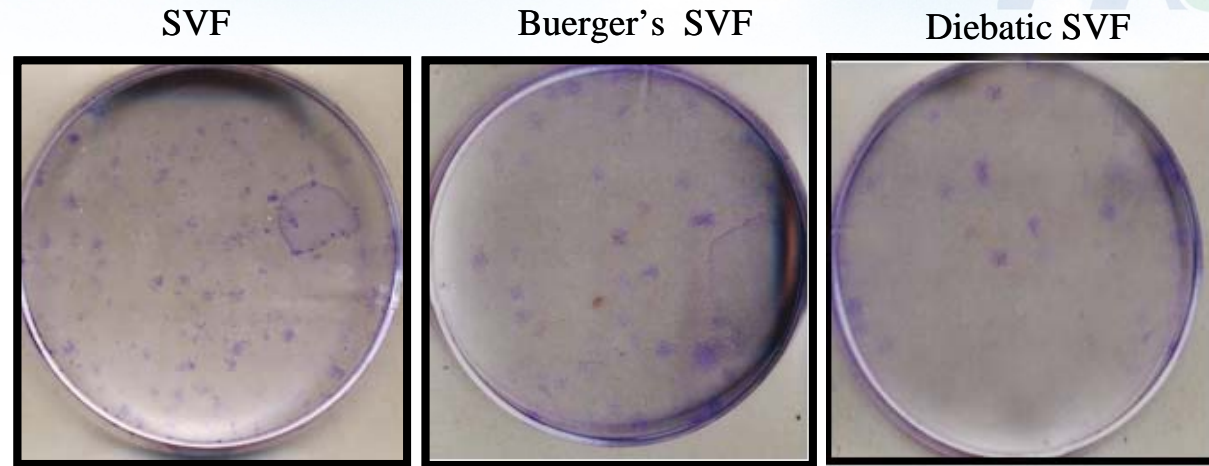
Results : Patient characteristics

Case No.	Age /Gender	Diagnosis	Ischemic Site/ Status	Past History	Previous Tx. For CLI
9	55/M	Buerger's ds	Right toe/ non-healing ulcer(II-5)	Smoking	Rt F-P bypass op
10	69/M	DM foot	Right foot/ Necrosis(III-6)	DM, HT	
11	60/M	Buerger's ds	Left foot/ non-healing ulcer(II-5)	Smoking, HT	Rt Axillo-F-F bypass
12	46/M	Buerger's ds	Left toe/ non-healing ulcer(II-5)	Smoking	Minor amputation
13	73/M	Buerger's ds	Left foot/ Resting pain(II-4)		
14	39/M	Buerger's ds	Left toe/ non-healing ulcer(II-5)	Smoking, DM, HT, Hyperlipdemia	Minor amputation
15	73/M	DM foot	Left toe/ non-healing ulcer(II-5)	HT, DM	PTA

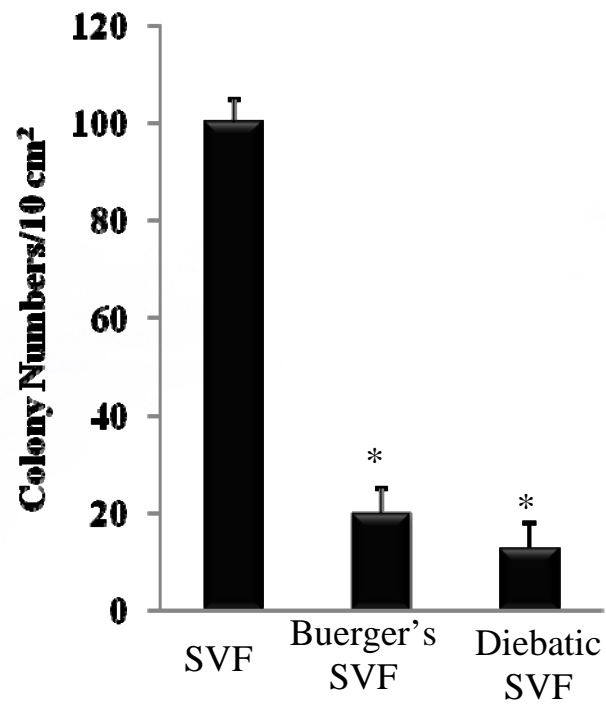
Results

: CFU assay of Stromal vascular fraction(SVF)

A



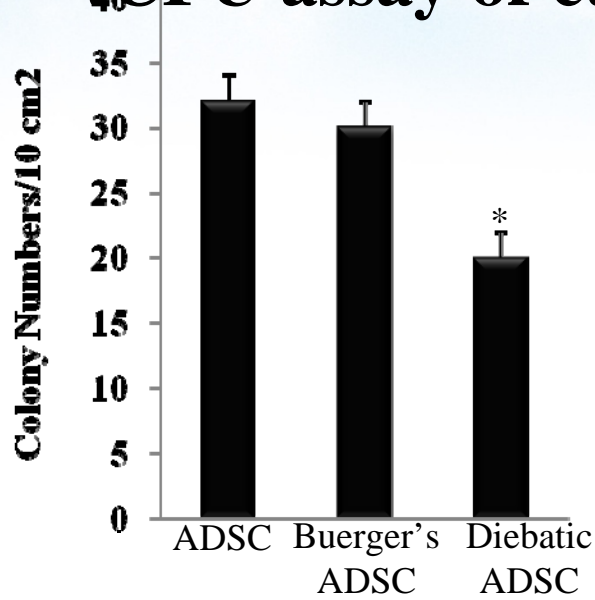
B



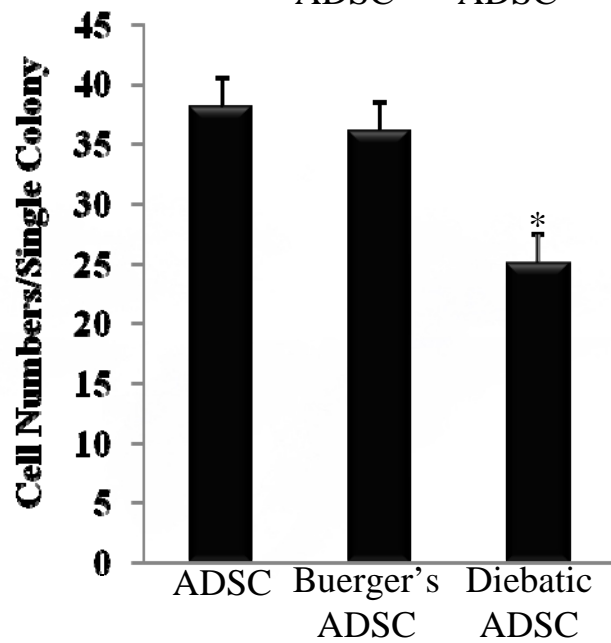
Results

CFU assay of culture-expanded cells

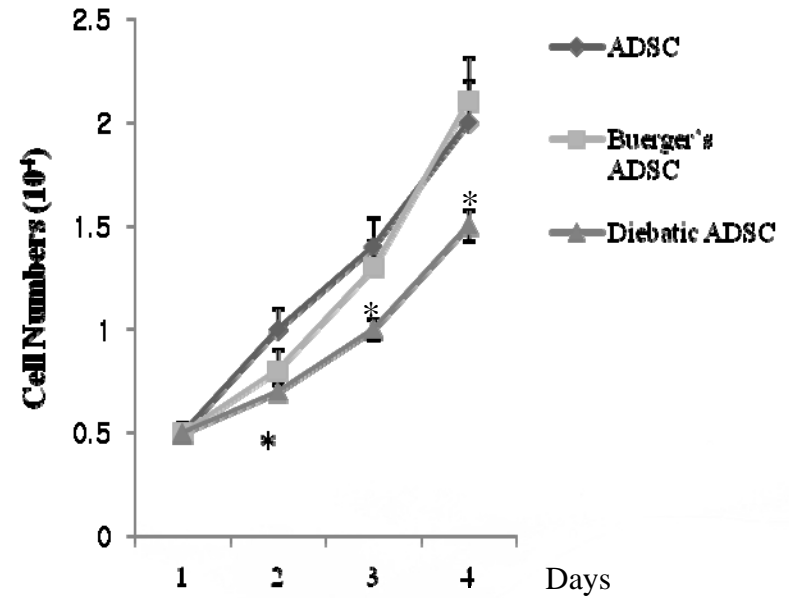
A



B



C



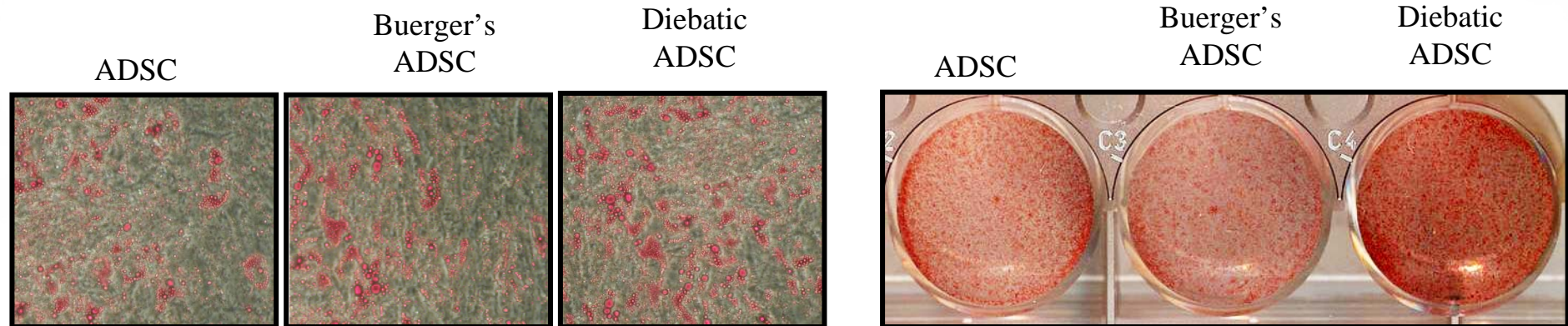
Results

:CFU assay of culture-expanded cells

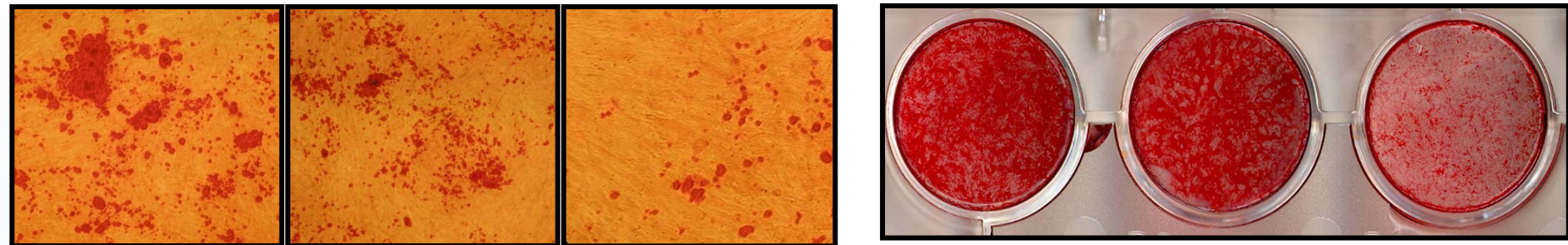


Figure A

AM



OM

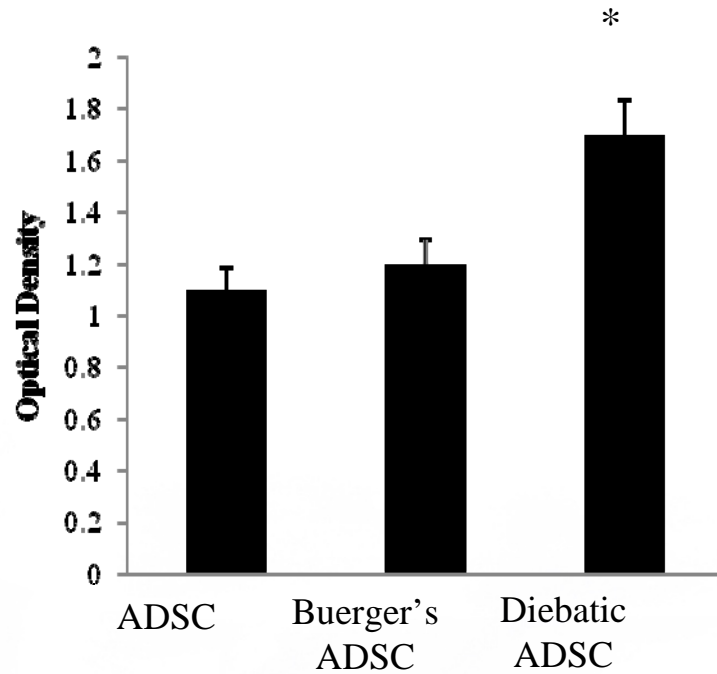


Results

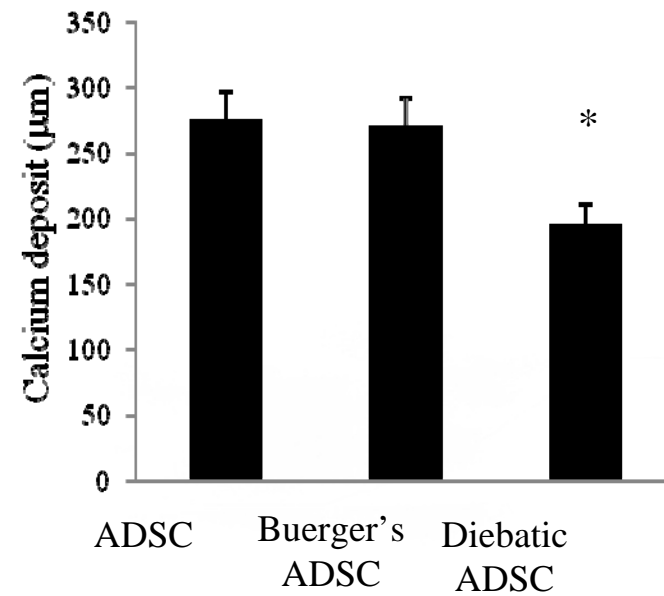
:CFU assay of culture-expanded cells



B

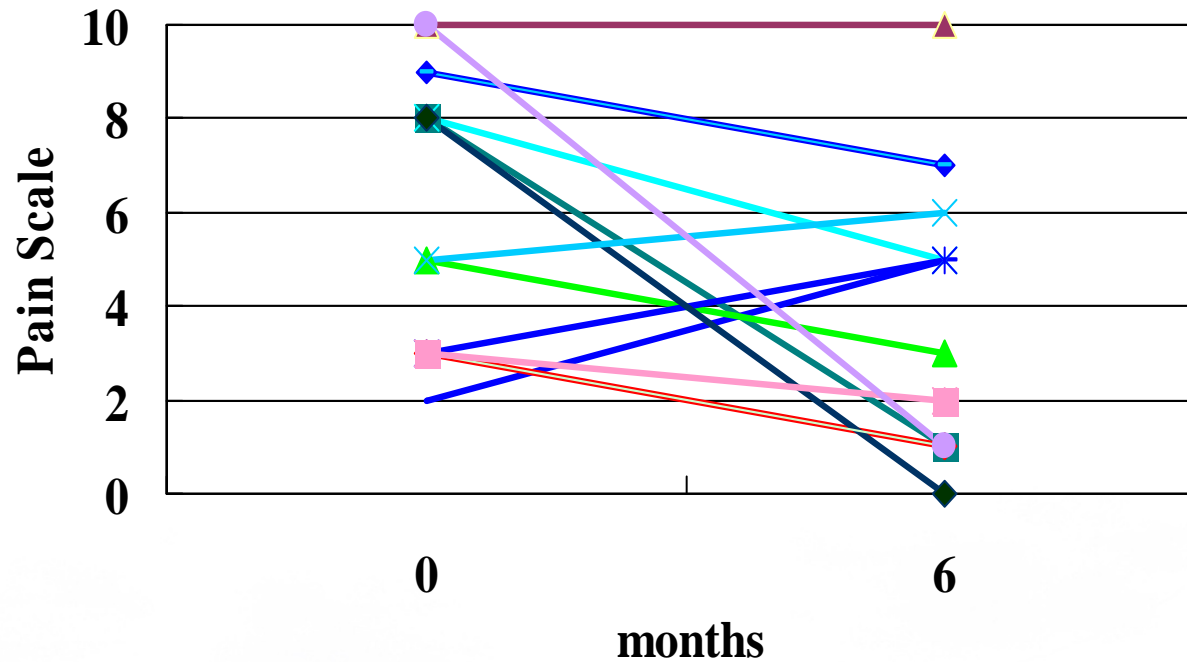
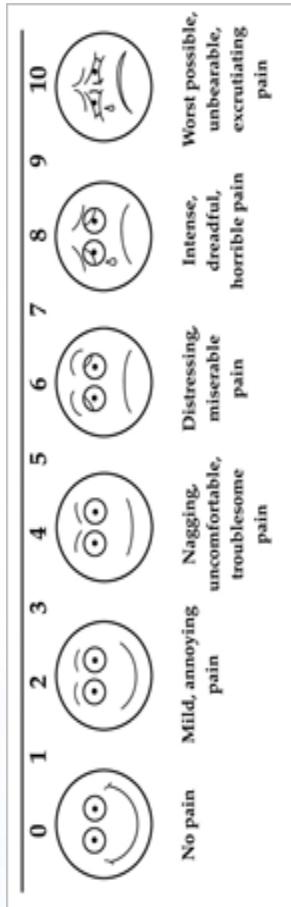


C

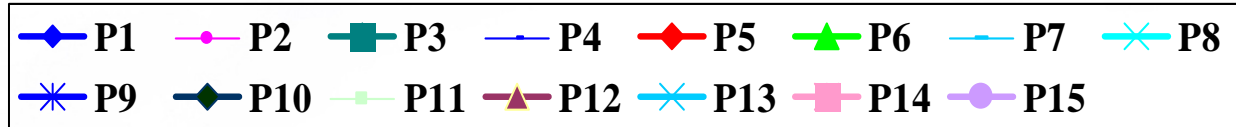


Results

: Wong-Baker FACES Pain Rating Scale

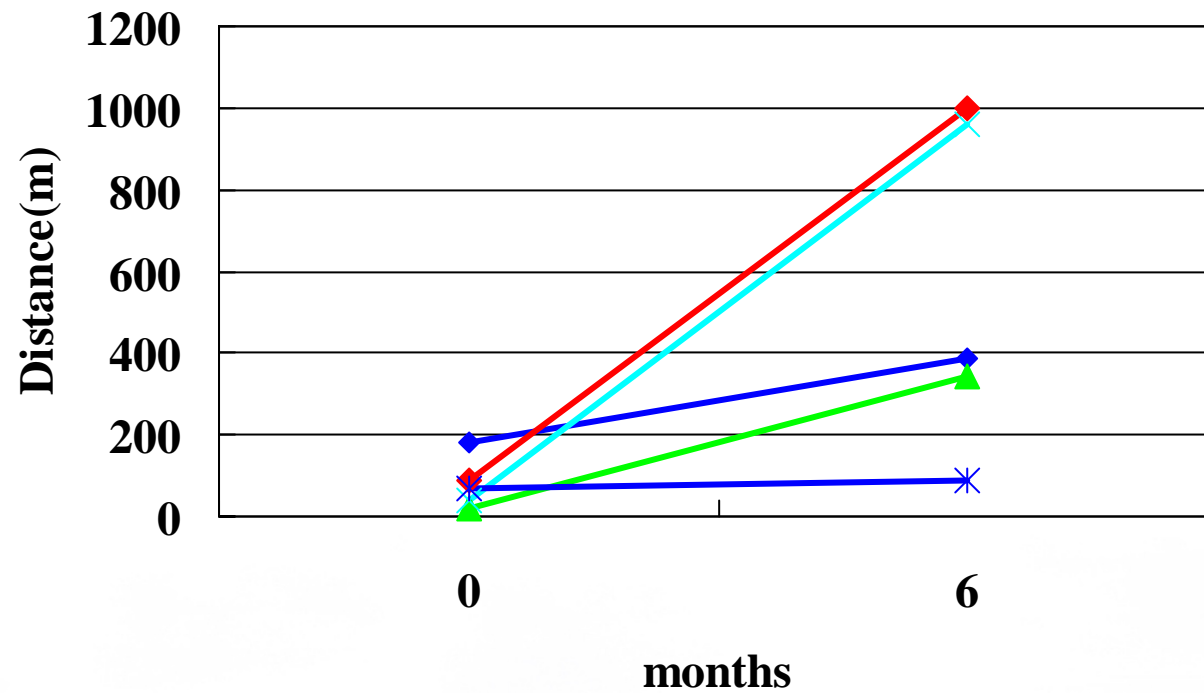


P=0.032 by paired Wilcoxon test



Results

: Claudication Distance

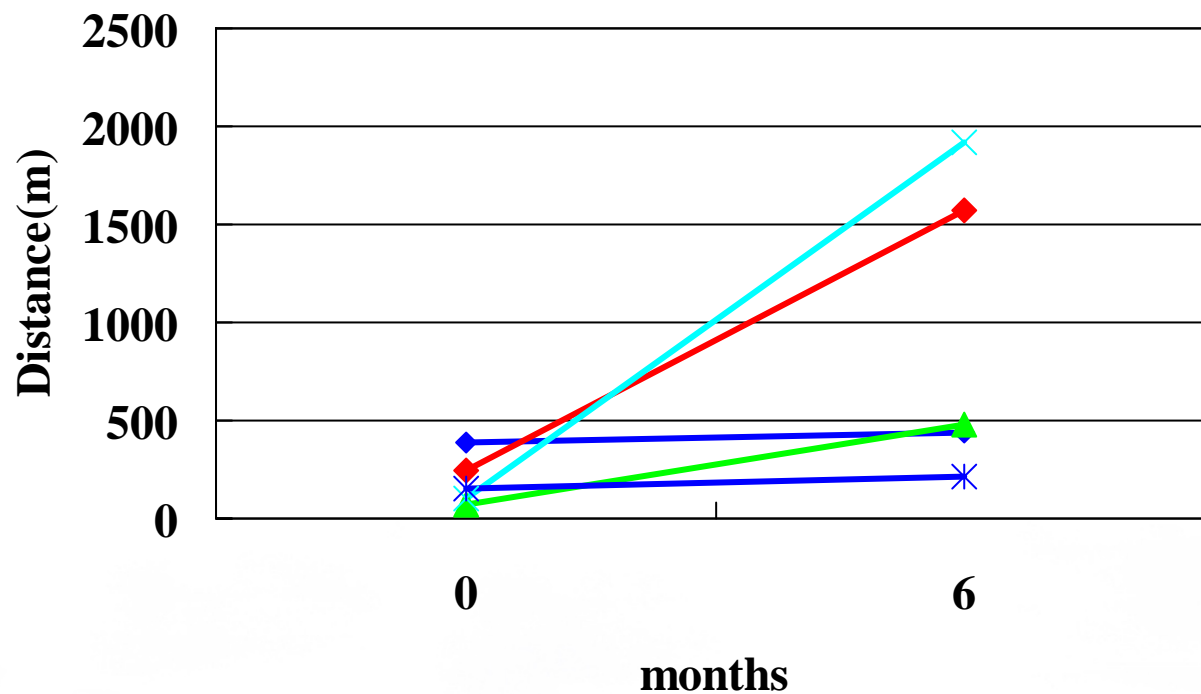


P=0.046 by paired
Wilcoxon test



Results

: Maximal Walking Distance

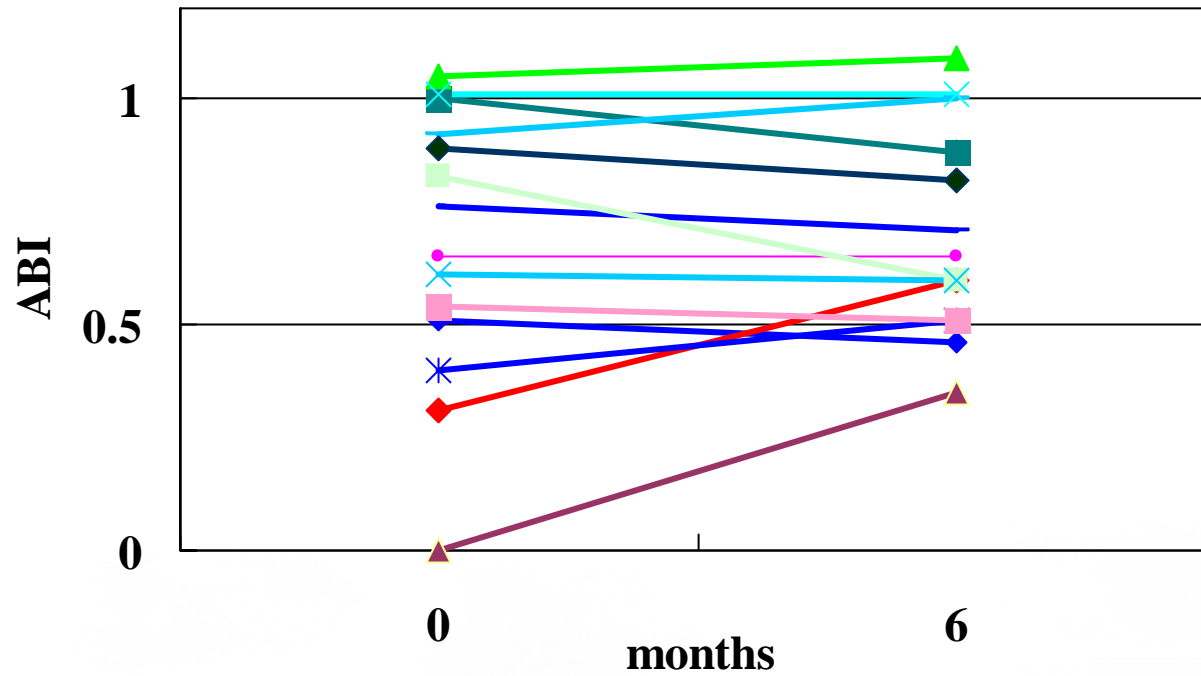


P=0.075 by paired
Wilcoxon test

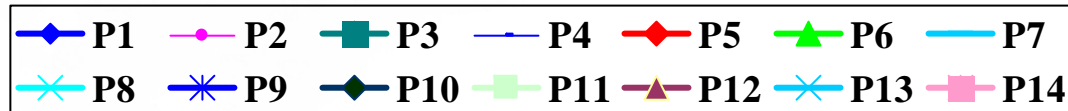
—◆— P1 —◆— P5 —▲— P6 —×— P8 —*— P9

Results

: Ankle Brachial Index

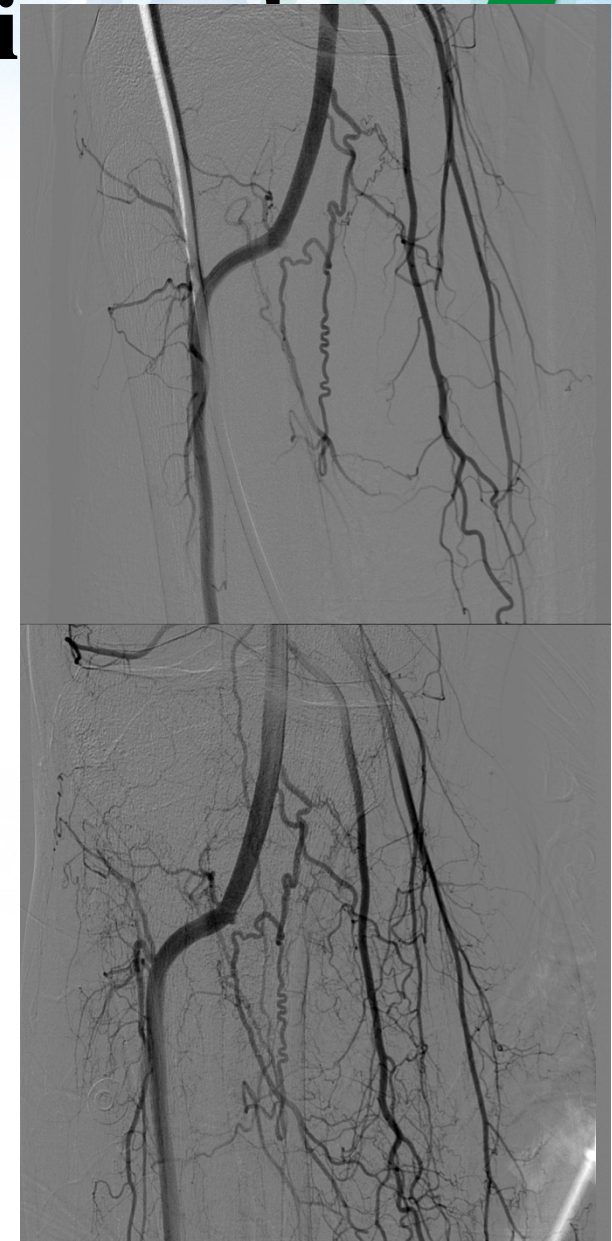
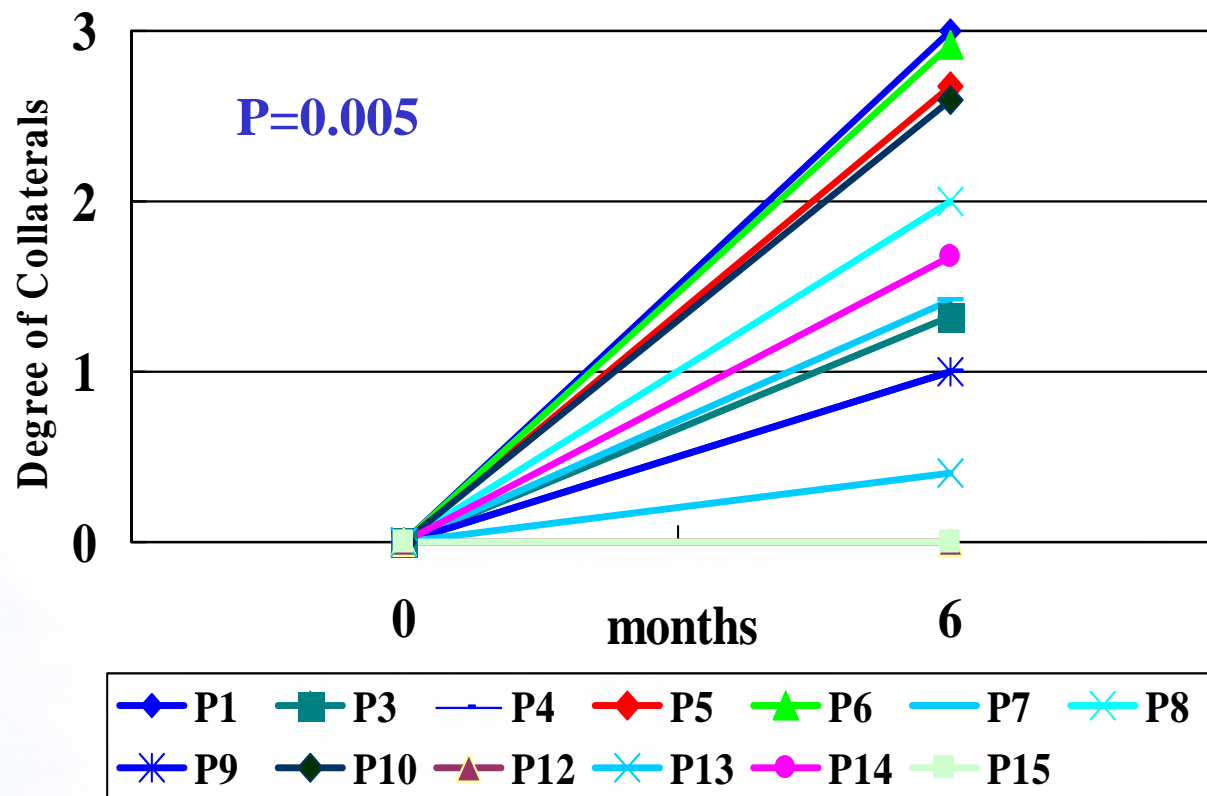


P=0.21

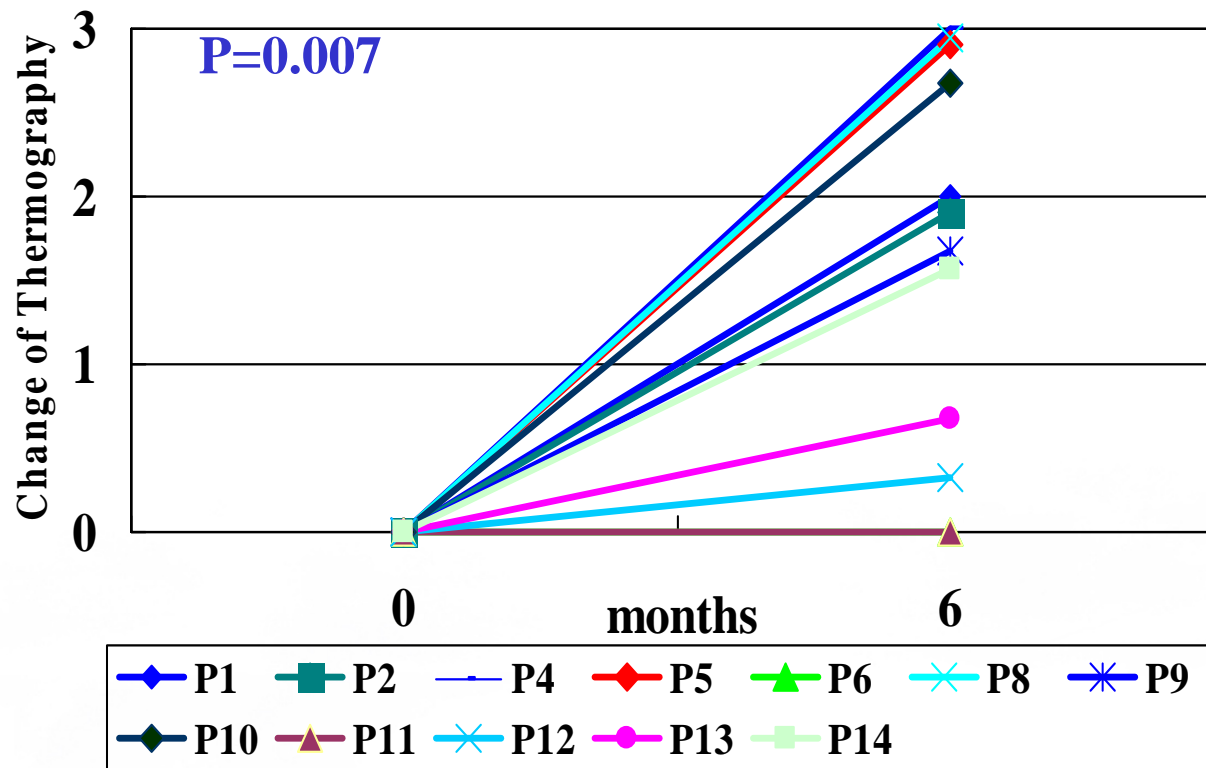


Results

: Digital Subtraction Angi



Results : Thermography

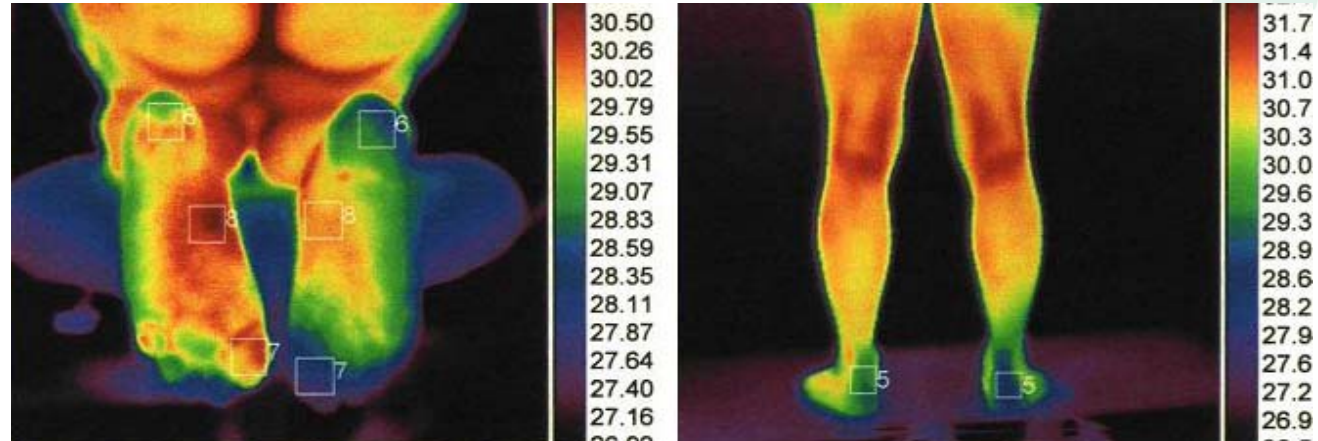


Results : Thermography

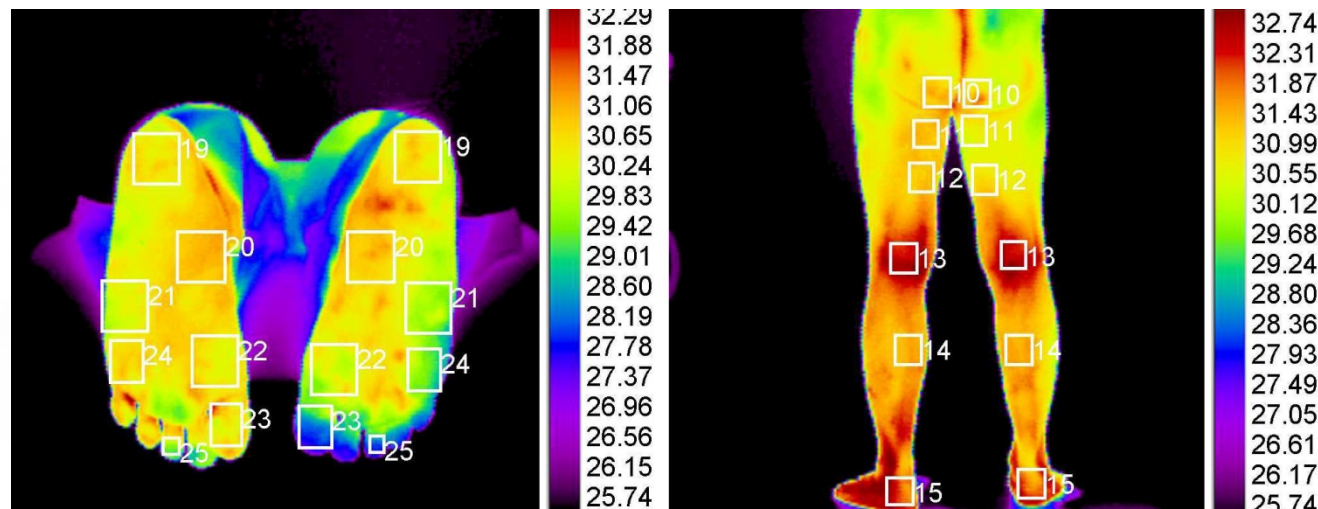


Patient 8

Baseline



6 month



Results : Clinical Outcomes

Case No.	Age /Gender	Diagnosis	Ischemic Site/ Status	Past History	Clinical symptom Wound Healing
1	33/M	Buerger's ds	Left toe/ Resting pain(II-4)	Smoking	Well improved
2	52/M	Buerger's ds	Left toe/ non-healing ulcer(II-5)	Smoking , quit hyperlipidemia	Ulcer healing Well improved
3	24/M	Buerger's ds	Left toe/ non-healing ulcer(II-5)	Smoking , quit	Minor amputation Well improved
4	46/M	Buerger's ds	Right toe/ Necrosis(III-6)	Smoking , quit	Minor amputation No change
5	36/M	Buerger's ds	Right toe / Resting pain(II-4)	Smoking , quit	Well improved
6	42/M	Buerger's ds	Left foot/ Necrosis(III-6)	Smoking , quit	Minor amputation Mild improved
7	64/M	DM foot	Left foot/ non-healing ulcer(II-5)	DM, HT	Ulcer healing Well improved
8	55/M	Buerger's ds	Right toe/ non-healing ulcer(II-5)	Smoking	Ulcer healing Well improved

Results : Clinical Outcomes

Case No.	Age /Gender	Diagnosis	Ischemic Site/ Status	Past History	Previous Tx. For CLI
9	55/M	Buerger's ds	Right toe/ non-healing ulcer(II-5)	Smoking	Mild improved Ulcer healing
10	69/M	DM foot	Right foot/ Necrosis(III-6)	DM, HT	Minor amuptaion Well improved
11	60/M	Buerger's ds	Left foot/ non-healing ulcer(II-5)	Smoking, HT	Well improved Ulcer healing
12	46/M	Buerger's ds	Left toe/ non-healing ulcer(II-5)	Smoking	No change
13	73/M	Buerger's ds	Left foot/ Resting pain(II-4)		No change
14	39/M	Buerger's ds	Left toe/ non-healing ulcer(II-5)	Smoking, DM, HT, Hyperlipdemia	Minor amputaion Well improved
15	73/M	DM foot	Left toe/ non-healing ulcer(II-5)	HT, DM	Well improved Ulcer healing

well \geq 2step, mild \geq 1step in Wong-baker Score

Results : Clinical Outcomes



Results : Adverse Events



NCI CTCAE (Version 4) Grade	Adverse Events
Grade 5 (death)	None
Grade 4 (life-threatening)	None
Grade 3 (Severe)	None
Grade 2 (Moderate)	None
Grade 1 (Mild)	Clinical symptoms Fever (n=1) Flu-like symptom (n=1) Injection site pain (n=2) Headache (n=1)

Results : Clinical Outcomes

	Responders(n=10)	Non-Responders(n=4)
Lesion suprapopliteal	4 (40%)	2 (50%)
infrapopliteal	6 (60%)	2 (50%)
DM	4 (40%)	0
Age	48.7 yr	51.8 yrs
Smoking	8 (80%)	3 (75%)
Current Somker	4 (50%)	2 (67%)
Initial Claudication Distance (m)	137	244
Maximum Walking Distance (m)	263	413
ABI	0.75	0.60
DSA	1.96	1.08
Thermography		

P value : insignificant

Conclusion



- **Intramuscular ADSC therapy for CLI is effective and safe.**
- Response rate is about 71% in our study
- Factors that influence on therapeutic response were not definite
- **Proliferation and differentiation ADSC in Buerger's disease are normal compare to normal control group.**
But, SVFs are fewer than normal control group.

Next Step



- Large scaled study as Phase II, III

- Strategy to increase activity of ADSC
 1. Selective collection and culture of high activity ADSC
 2. Heterogenous ADSC implantation

Our Team

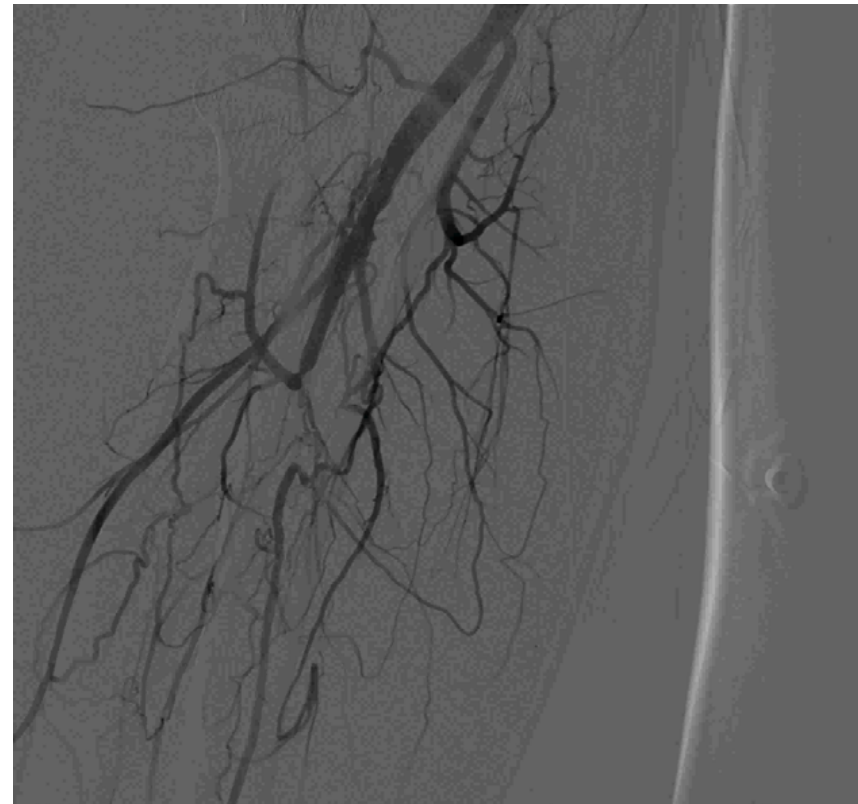


- ❑ Han Cheol Lee, MD PhD, Cardiology
- ❑ Yong Chan Bae, MD PhD, Plastic Surgery
- ❑ Sung Wun Chung, MD PhD, Vascular Surgery
- ❑ Jin Sup Jung, PhD, Physiology

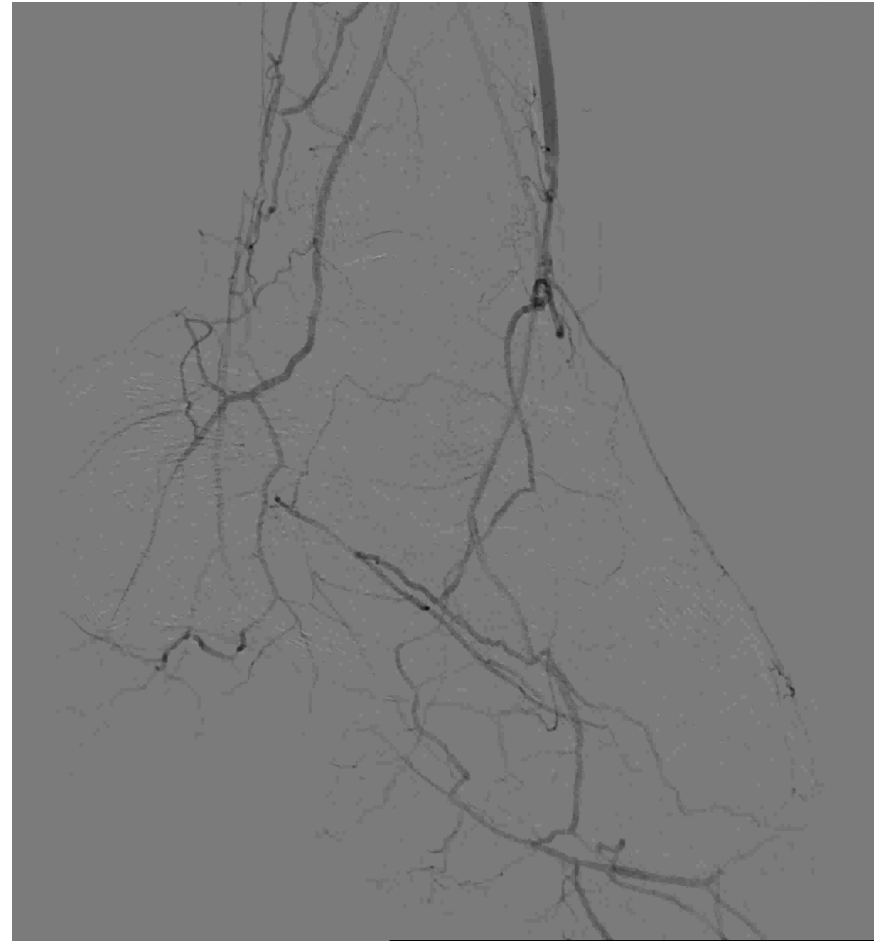


Thank you for your attention

DSA : Patient 1



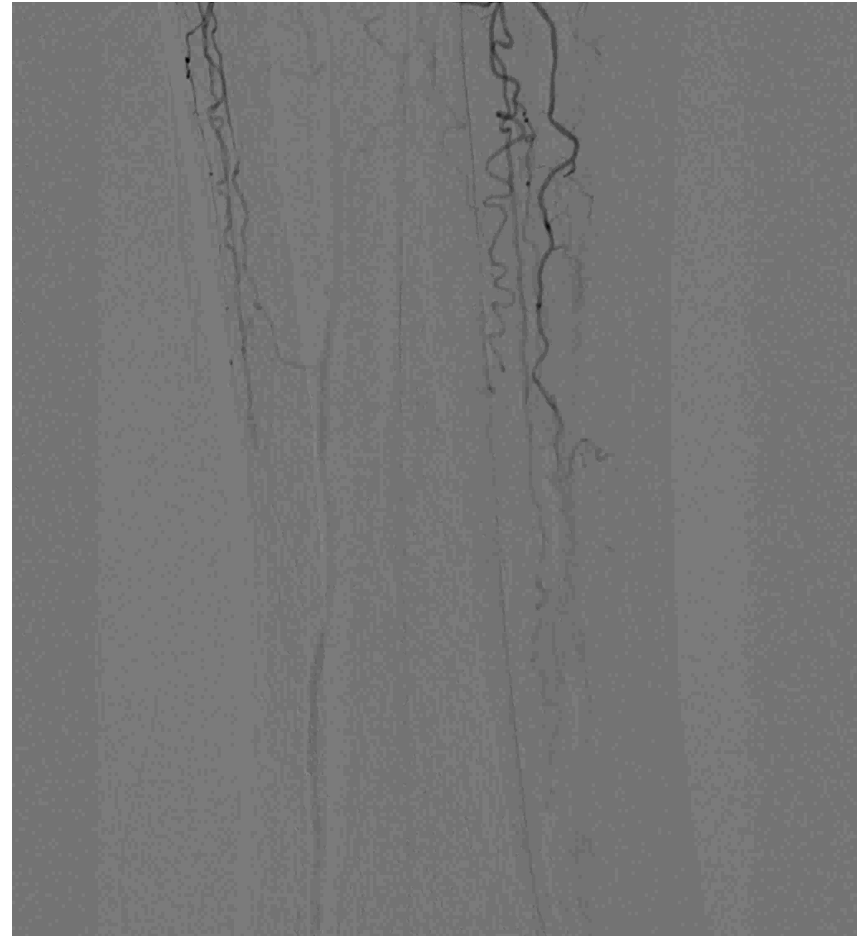
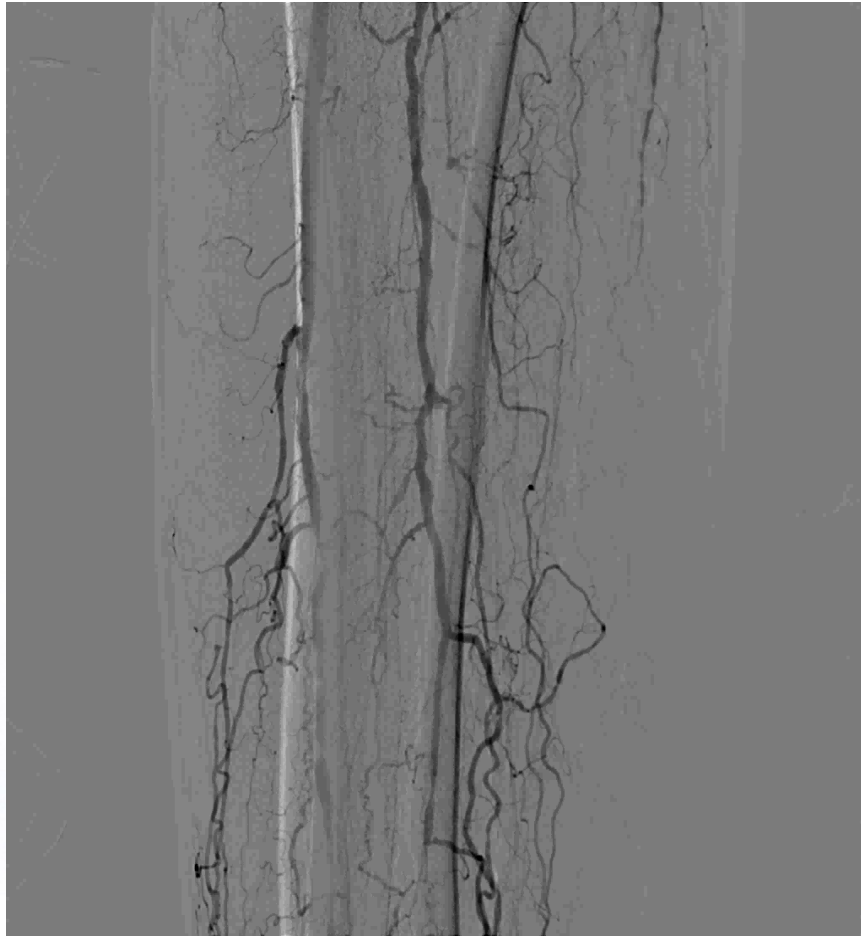
DSA : Patient 6



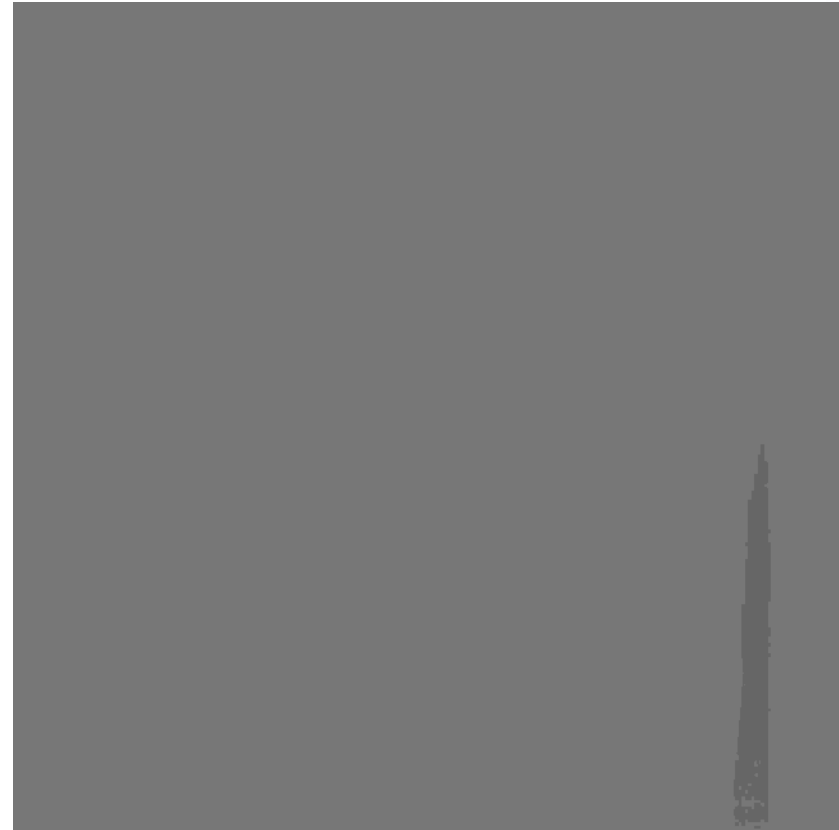
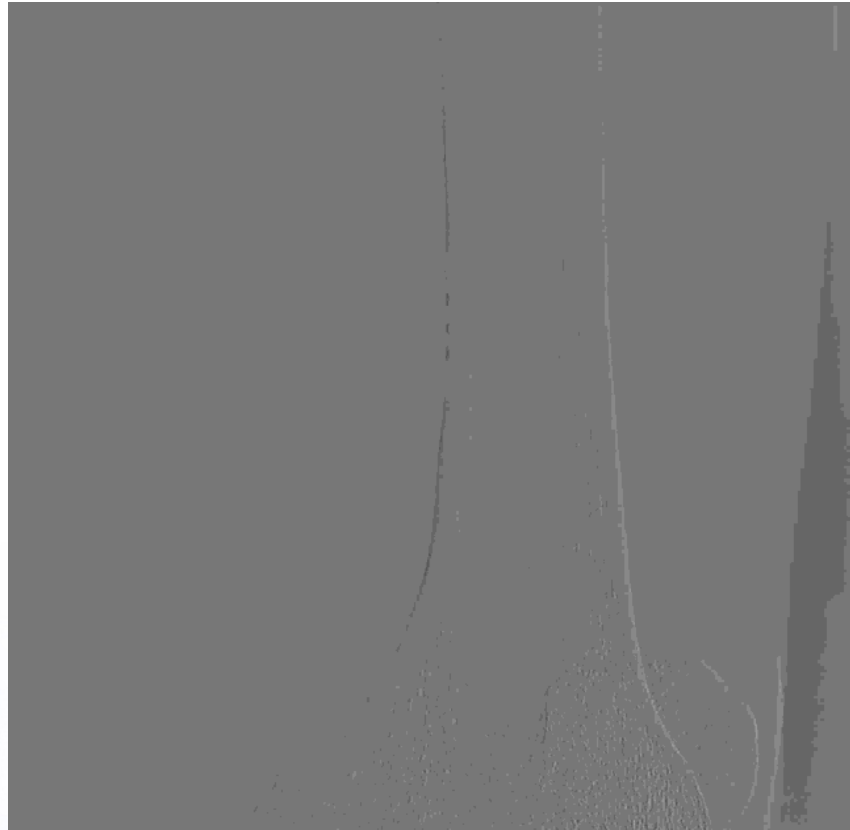
DSA : Patient 9



DSA : Patient 10



DSA : Patient 14

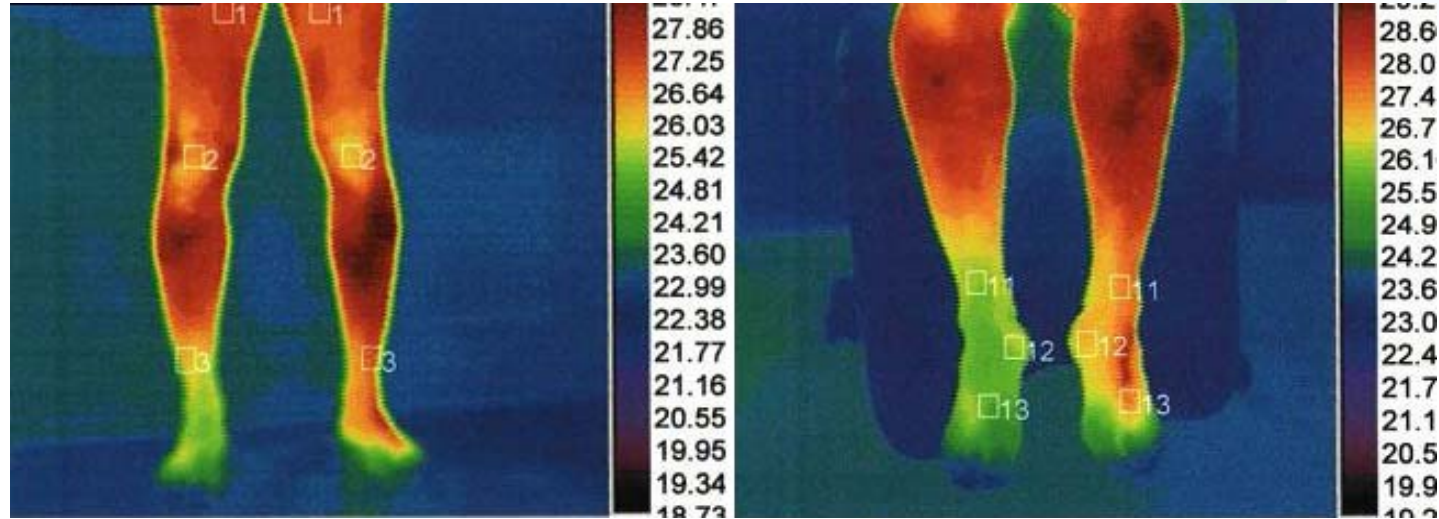


Results : Thermography

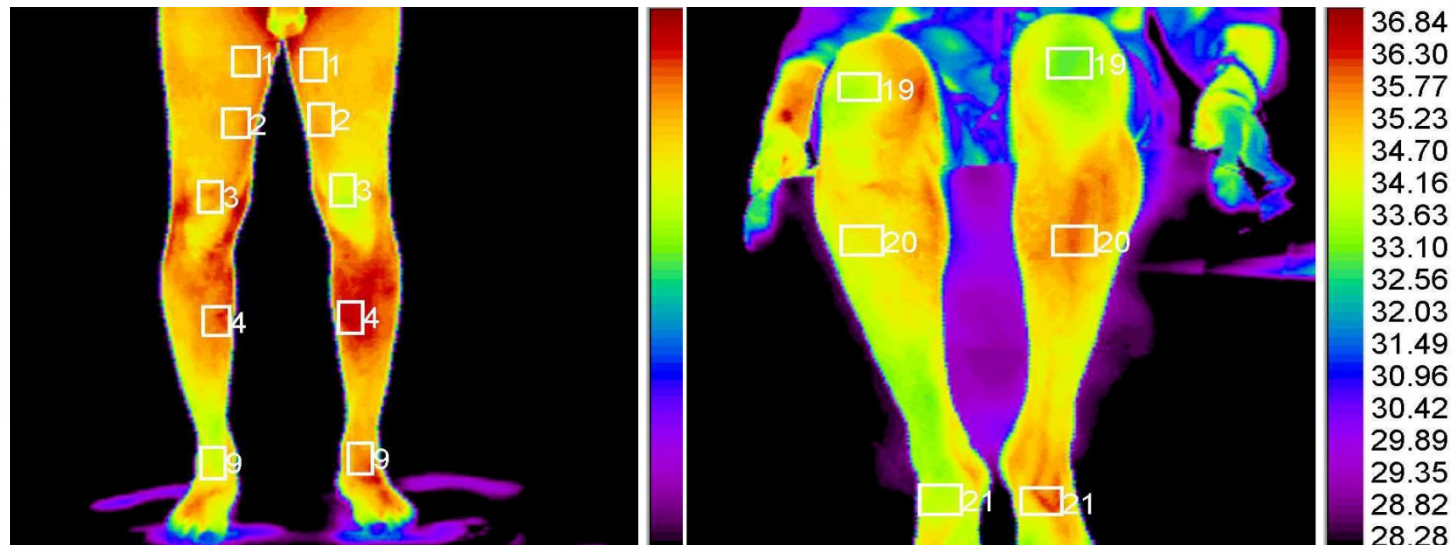


Patient 5

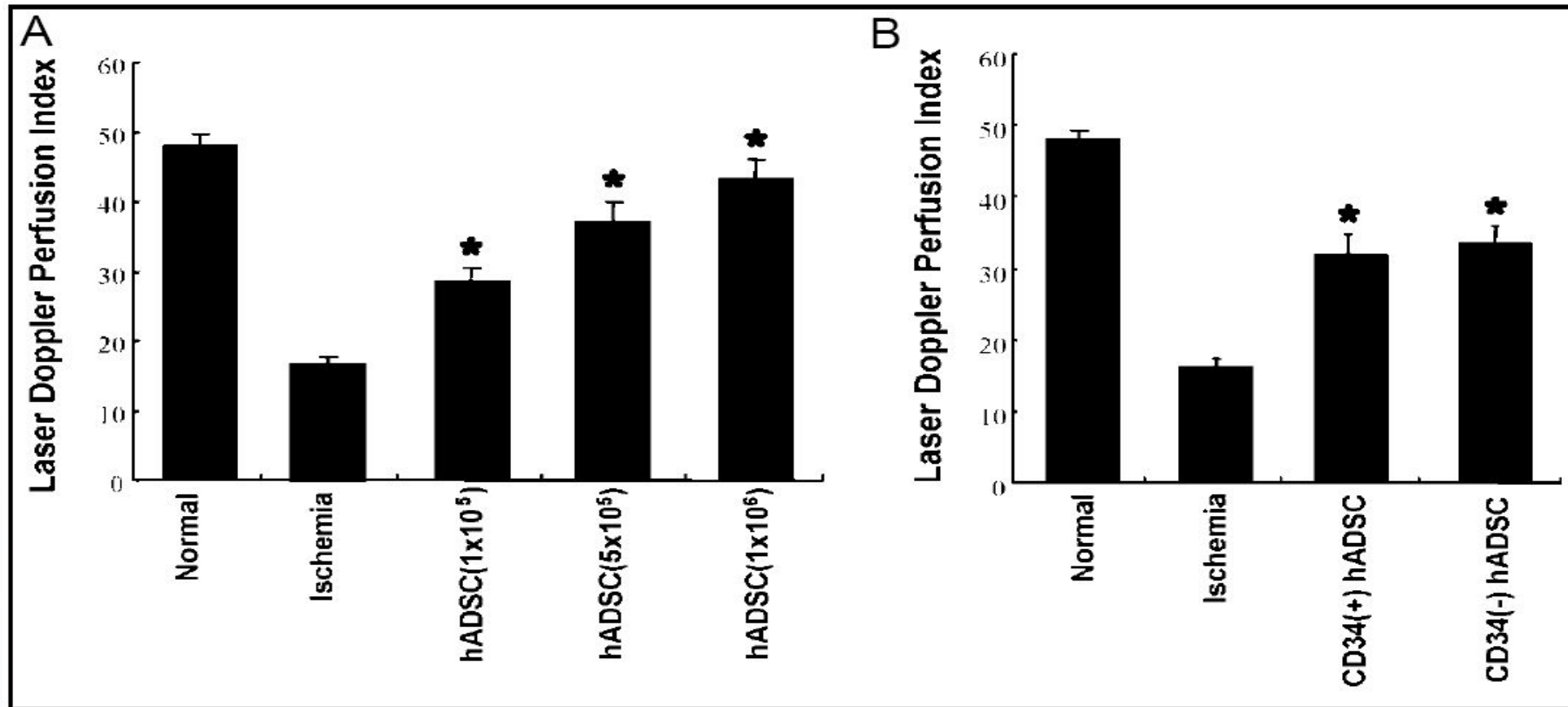
Baseline



6 month



Safety and Effect of Adipose Tissue derived Stem Cell Implantation in Patients with Critical Limb Ischemia : Animal Model



Safety and Effect of Adipose Tissue derived Stem Cell Implantation in Patients with Critical Limb Ischemia : Animal Model

