Tissue Engineering for Cardiovascular Diseases

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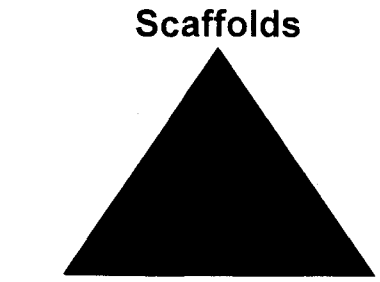
조직재생의 3가지 방법

- Cell transplantation
- Tissue-inducing substance delivery
- Cells on or within matrices

(Langer R and Vacanti JP. Tissue engineering. Science 260: 920-926, 1993)

Three Key Elements in Tissue Engineering



Revascularization Approaches for Myocardial Ischemia

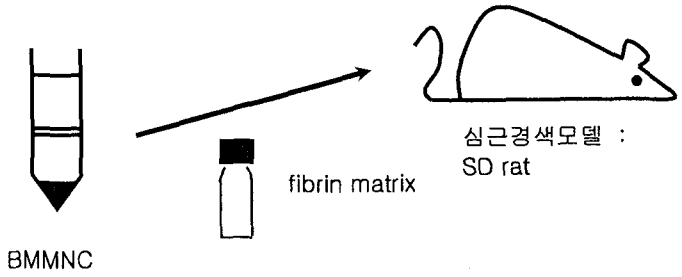
- **Cell transplantation**
  - Allogeneic: Embryonic stem cells, Fetal, neonatal or adult cardiomyocytes
  - Autologous : Bone marrow-derived stem cells, Skeletal myoblasts
  - with or without matrix
- **Angiogenic factor administration**
  - bFGF, VEGF
  - systemic delivery, sustained local delivery
- **Gene therapy**
  - VEGF
  - naked DNA, gene in viral vector

Outline

- 심근 재생
  - 골수단핵세포 이식: 피브린 매트릭스의 효과
  - 배아줄기세포 이식
- 하지허혈 치료
  - 소동물실험 : bFGF delivery, G-CSF, 골수단핵세포 이식
  - 임상실험
- 동맥 재생

Hypothesis

피브린 매트릭스를 이용한 골수단핵세포의 심근경색 동물의 심근에 이식하는 것은 세포치료제의 효능을 향상시킬 것이다.



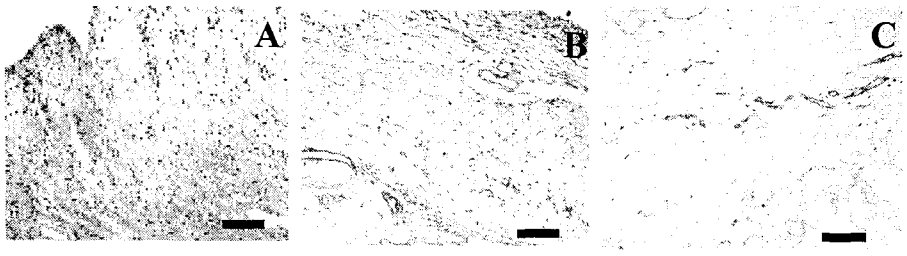
Experimental Groups

실험군: BMMNC & Fibrin matrix injection

대조군 1: BMMNC injection

대조군 2: Medium injection

Histology (H&E, 8 weeks)

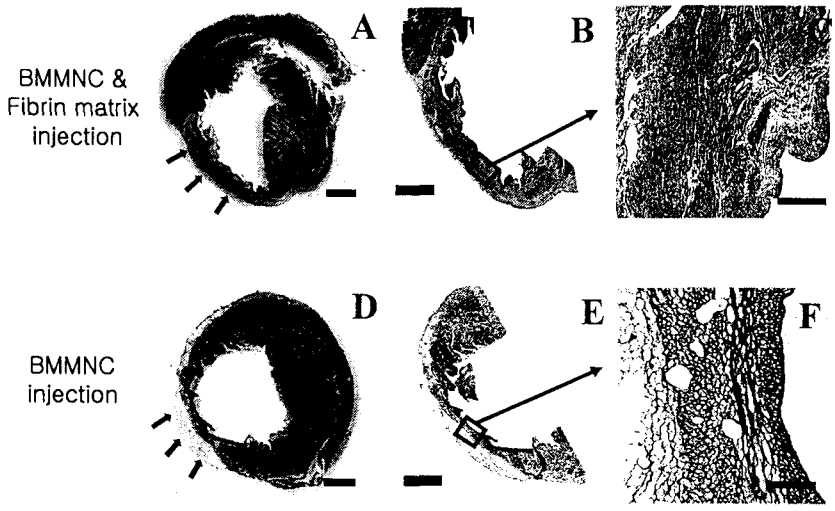


BMMNC & Fibrin matrix injection

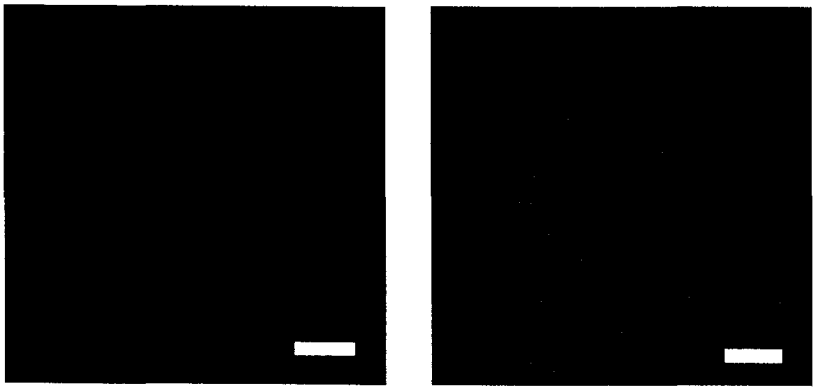
BMMNC injection

Medium injection

Histology (H&E, 8 weeks)



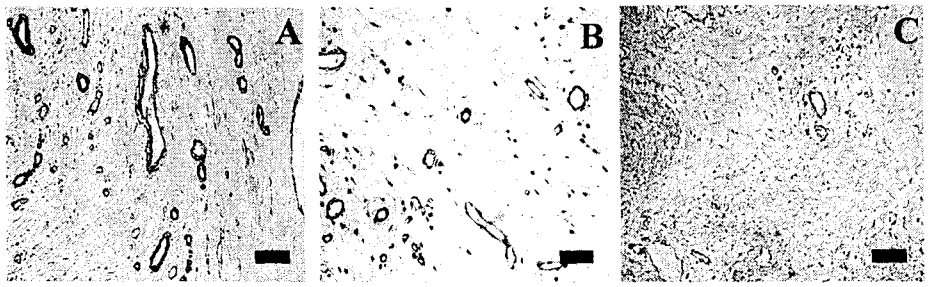
CM-DiI



BMMNC & Fibrin matrix injection

BMMNC injection

Vessels in Infarcted Myocardium (SM α-actin)

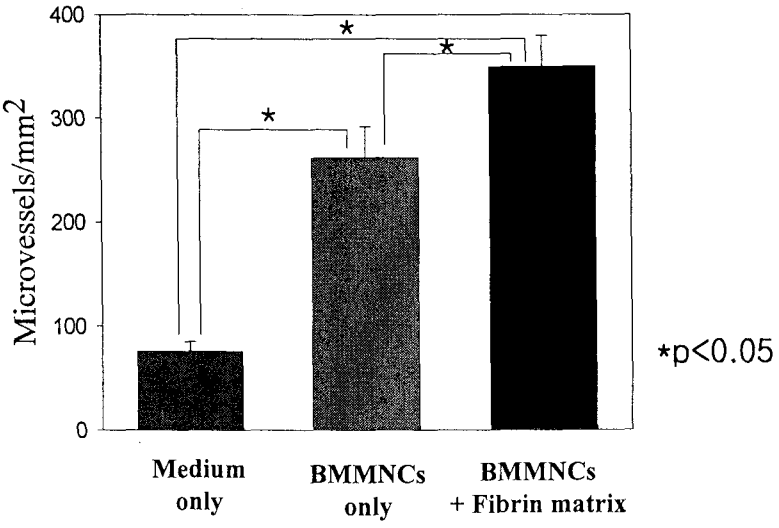


BMMNC & Fibrin matrix injection

BMMNC injection

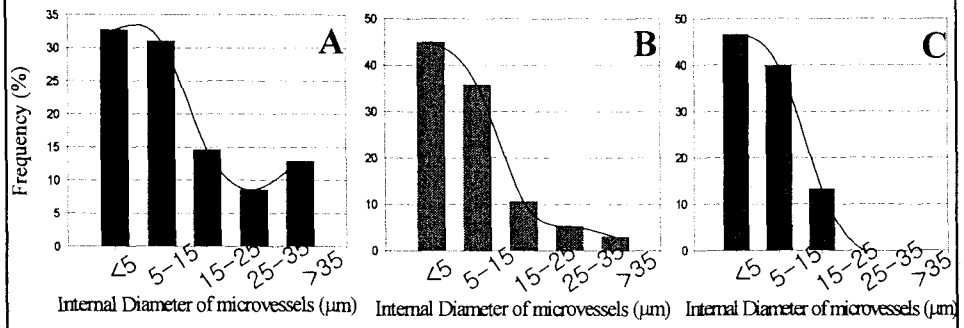
Medium injection

Vessels Density in Infarcted Myocardium



*p<0.05

Vessel Diameter Distribution



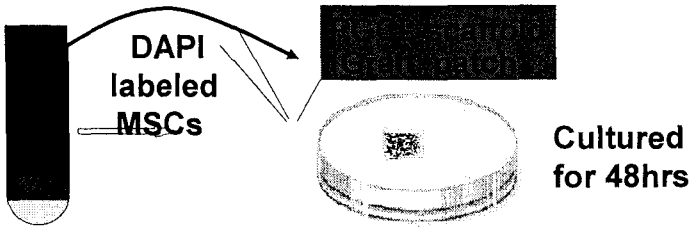
BMMNC & Fibrin matrix injection

BMMNC injection

Medium injection

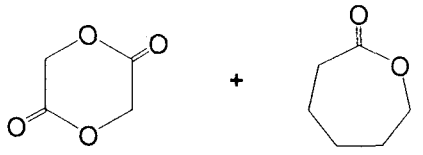
MSC Seeding on the PGCL Scaffold (Graft)

- Seeding and cultivation of MSCs on the patch
  - MSCs ($2x10^6$ cells) : Pre-labeled with DAPI
  - PGCL scaffold ($5x5\ mm^2$)



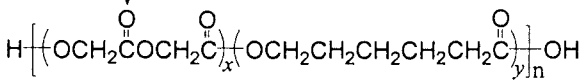
- Charaterization of tissue engineered graft
  - H&E staining
  - Scanning EM

Copolymerization of PGCL



Poly(glycolic acid) (50%) : Poly(ε-Caprolactone) (50%)

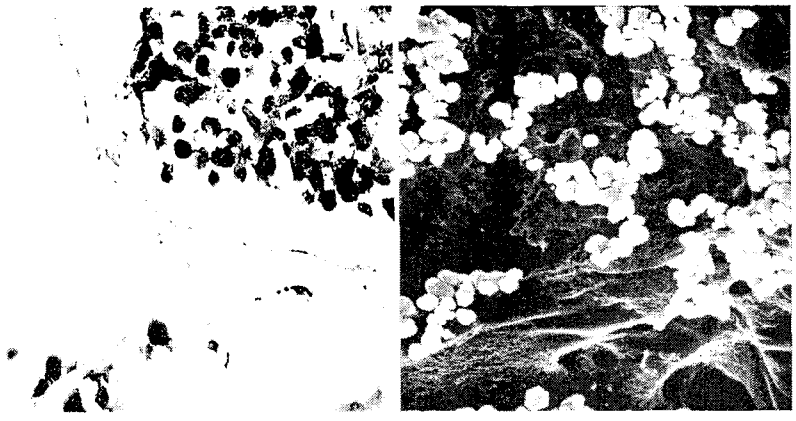
Sn-oct 170 °C, 20 hrs



Poly(glycolic acid)/Poly(ε-Caprolactone) Copolymer
Resorbed within 2 months

MSCs Seeded on the PGCL Scaffold

Culture for 48hrs

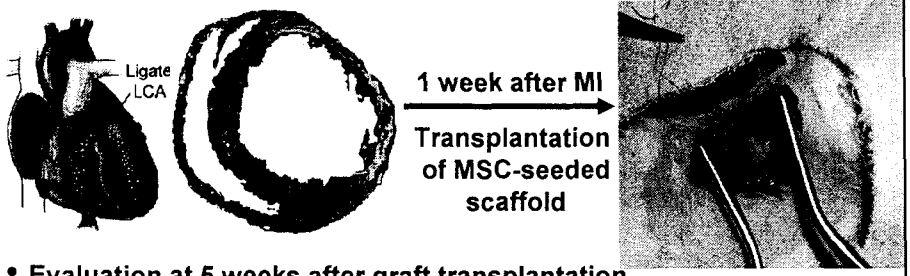


H&E Stain

Scanning EM

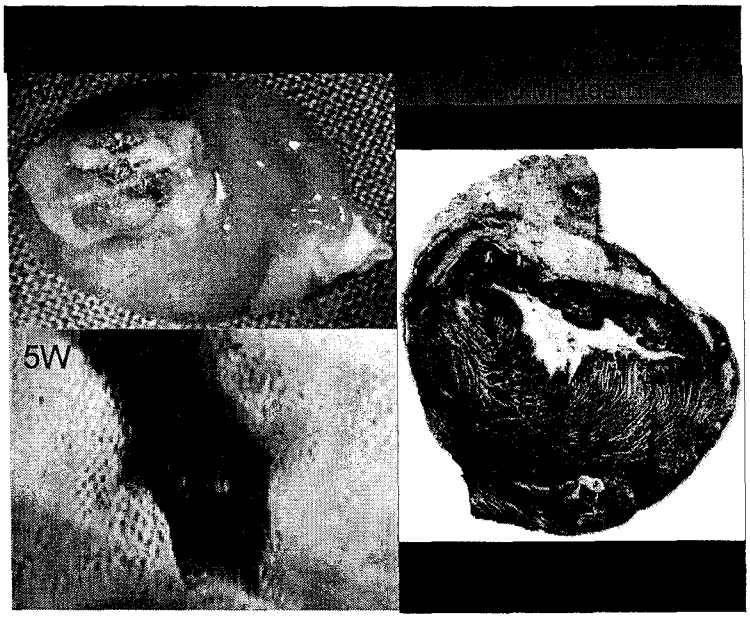
Transplantation of MSC-seeded PGCL Graft

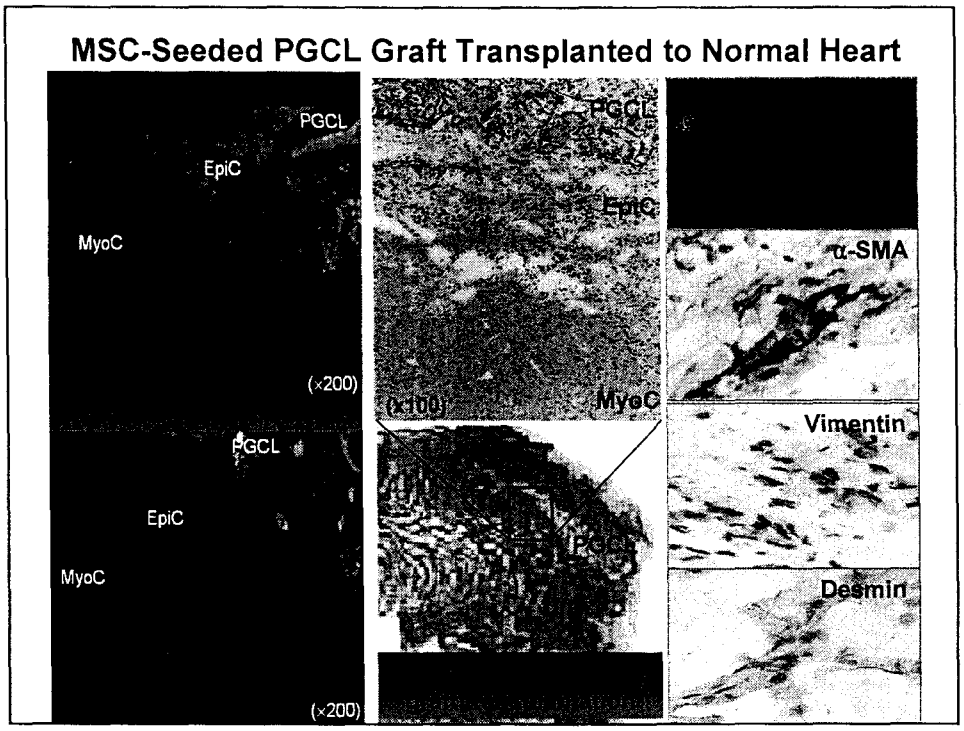
- Sham operation : Normal myocardium
- MI model :LCA ligation with reperfusion after 5hrs

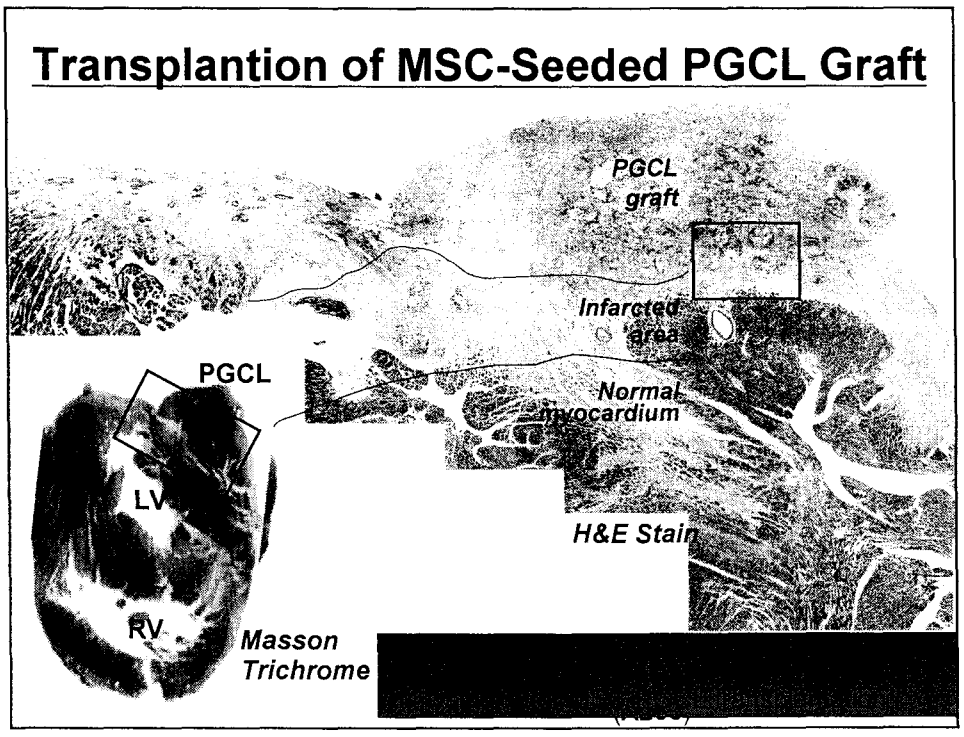


- Evaluation at 5 weeks after graft transplantation
  - Histopathologic examination : H&E, MT staining
  - Confocal microscopy : DAPI
  - Immunostaining : MHC, desmin, vimentin, αSMA
  - Echocardiography : LV chamber, FS

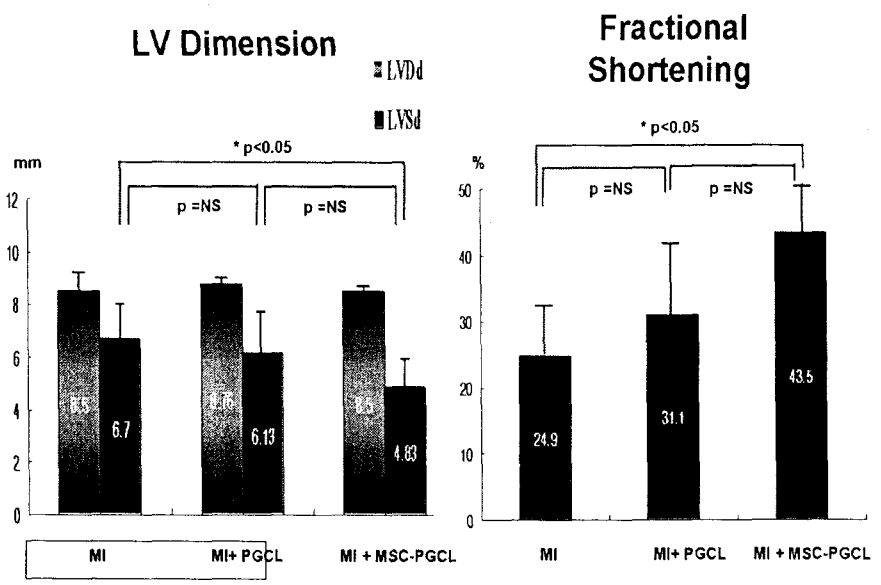
Gross Findings of MSC-Seeded PGCL Graft




MSC-Seeded PGCL Graft Transplanted to Normal Heart
PGCL
EpiC
MyoC
(×200)
PGCL
EpiC
MyoC
(×200)
PGCL
EpiC
(×100)
MyoC
α-SMA
Vimentin
Desmin


Transplantion of MSC-Seeded PGCL Graft
PGCL graft
Infarcted area
Normal myocardium
H&E Stain
PGCL
LV
RV
Masson Trichrome

MSC-Seeded PGCL Graft Improves LV Function



Myocardium regeneration using embryonic stem cell-derived cardiomyocytes and G-CSF

Materials and methods

- Cell source : mouse embryonic stem cell (R1)-derived cardiomyocytes
- Animal model : SD rat heart infarction model (cryoinjury)
- Granulocyte-colony stimulating factor (G-CSF) treatment (50 μg/kg/day)
- 8 weeks after implantation → histology (H&E, Masson's trichrome), immunohistochemistry (cardiac troponin I), heart function measurement

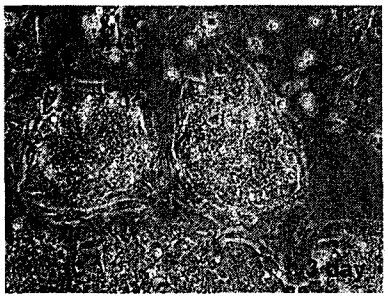
Group 1 : Embryonic stem cells + G-CSF

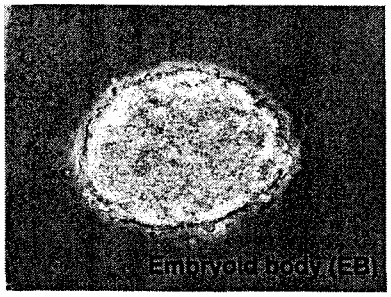
Group 2 : Embryonic stem cells

Group 3 : G-CSF

Group 4 : Medium injection

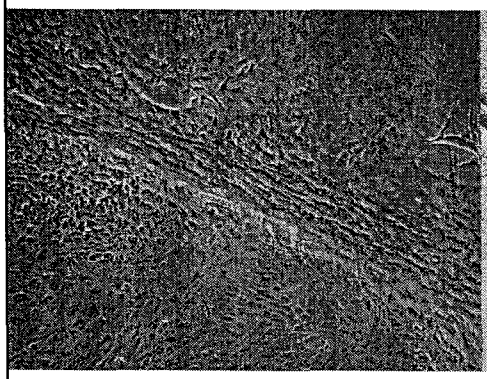
Mouse embryonic stem cells (R1)

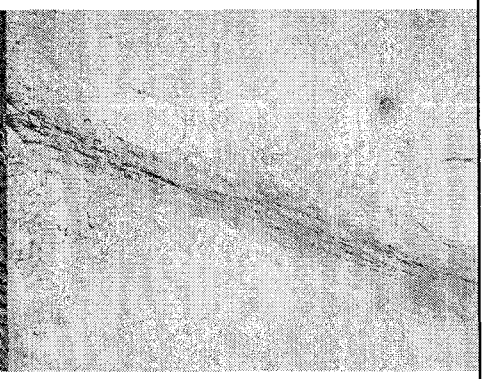




Hanging drop culture (2 day) &
Suspension culture (5 day)

Embryonic stem cell-derived cardiac structure

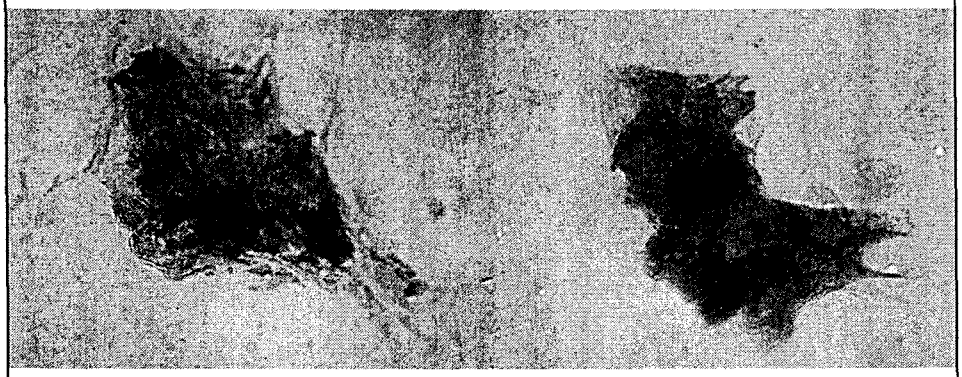




Myotube-like structure

Immunostaining
(cardiac troponin I)

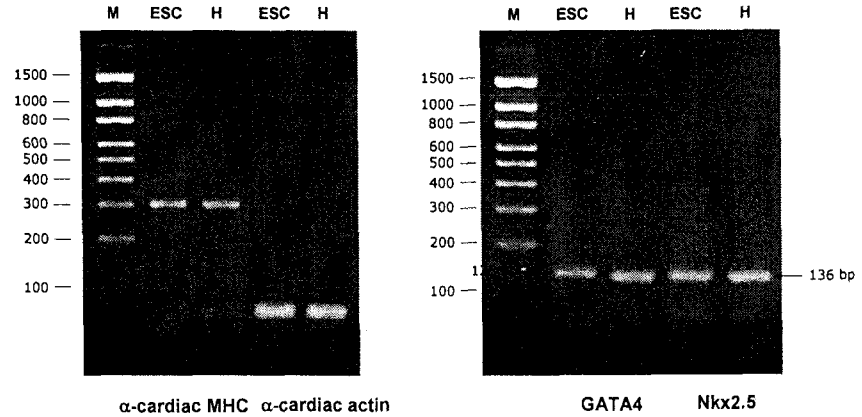
Cell characterization (immunostaining)



Cardiac myosin heavy chain

Cardiac troponin I

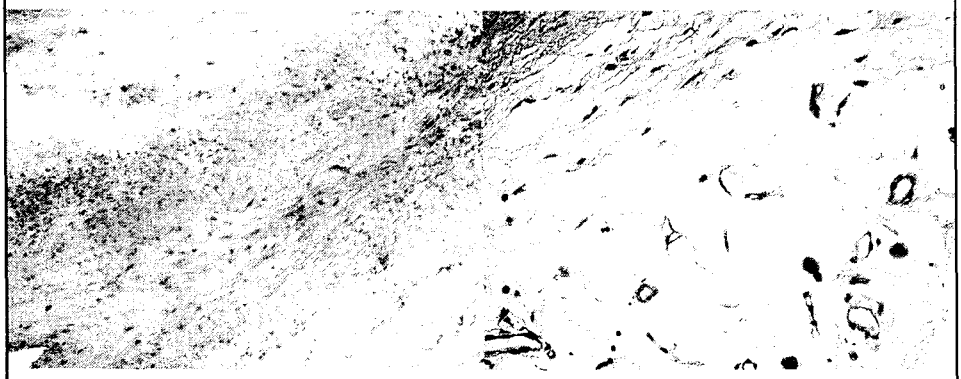
Cell characterization (RT-PCR)



α-cardiac MHC α-cardiac actin

GATA4 Nkx2.5

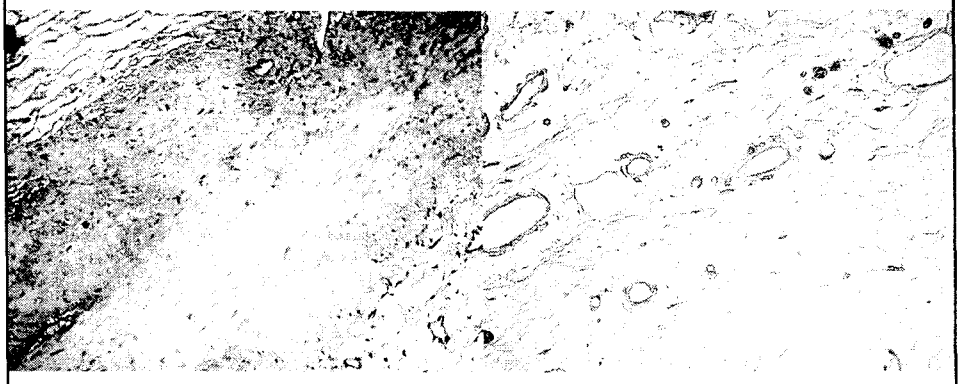
Histological analysis (H&E staining)



ES cell injection

Medium injection

Immunohistochemistry (cardiac troponin I)



ES cell injection

Medium injection

Left ventricular function (n=3)

	ES cells	Medium
LVSP (mmHg)	114.0 ± 25.1	105.0 ± 11.6
LVEDP (mmHg)	7.0 ± 2.6	7.3 ± 3.1

Echocardiographic measurement of left ventricular function (n=3, *: p<0.05)

	ES cells	Medium
LVDd (mm)	9.03 ± 0.15	9.70 ± 0.70
LVDs (mm)*	5.53 ± 0.46	6.90 ± 0.76
EF (%)*	73.7 ± 7.2	60.3 ± 4.7
FS (%)*	39.0 ± 6.1	29.0 ± 3.6
LVPWT (mm)	2.03 ± 0.23	1.90 ± 0.20

Left ventricular function

	ES cells + G-CSF	G-CSF
LVSP (mmHg)	108.5 ± 44.5	113.0 ± 8.1
LVEDP (mmHg)	6.0 ± 8.5	7.5 ± 6.6

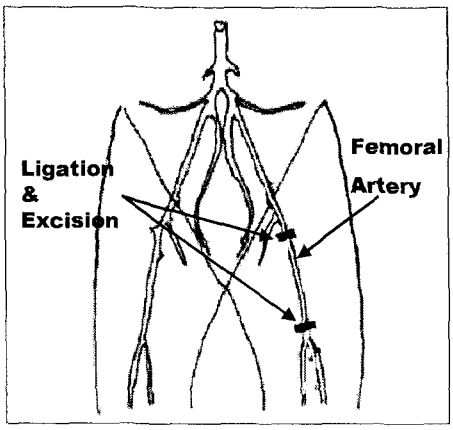
Echocardiographic measurement of left ventricular function

	ES cells + G-CSF	G-CSF
LVDd (mm)	8.45 ± 0.05	8.63 ± 0.54
LVDs (mm)	5.65 ± 1.34	5.70 ± 0.27
FS (%)	33.5 ± 12.0	33.8 ± 6.3
LVPWT (mm)	2.20 ± 0.28	2.13 ± 0.01

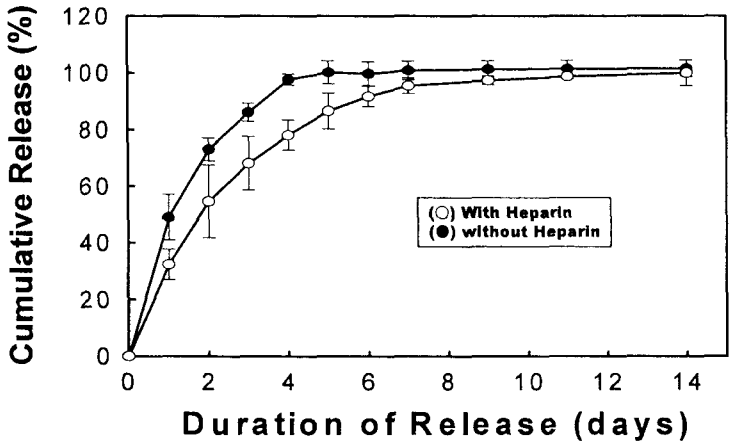
Summary 1

- 피브린 매트릭스를 이용한 골수단핵세포의 이식은 혈관생성을 향상시킨다.
- 골수중간엽줄기세포가 부착된 생분해성 고분자 패치의 이식은 허혈성 심장에서 혈관을 재생시킨다.
- 배아줄기세포로부터 분화된 심근세포의 이식은 허혈성 심장에서 심근을 재생시킨다.

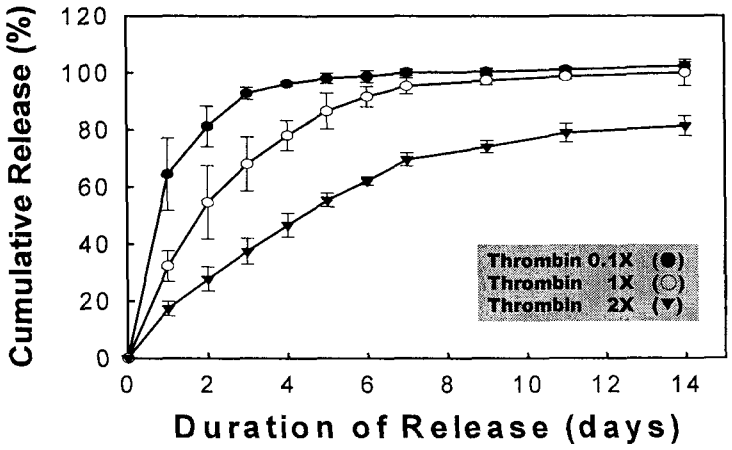
Mouse Model of Hind Limb Ischemia



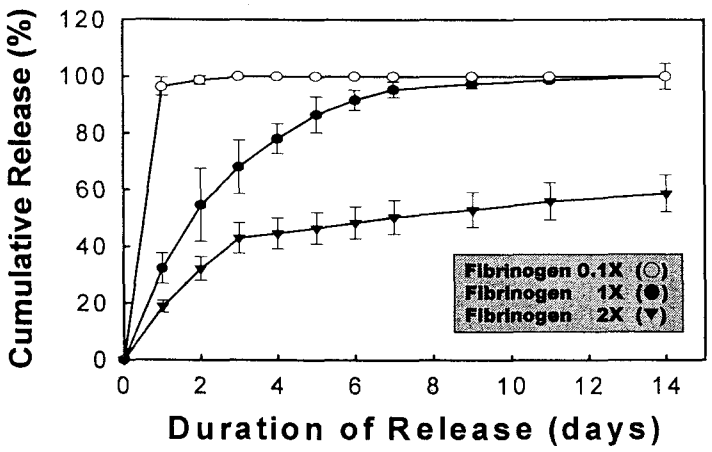
bFGF Release from Fibrin Matrix



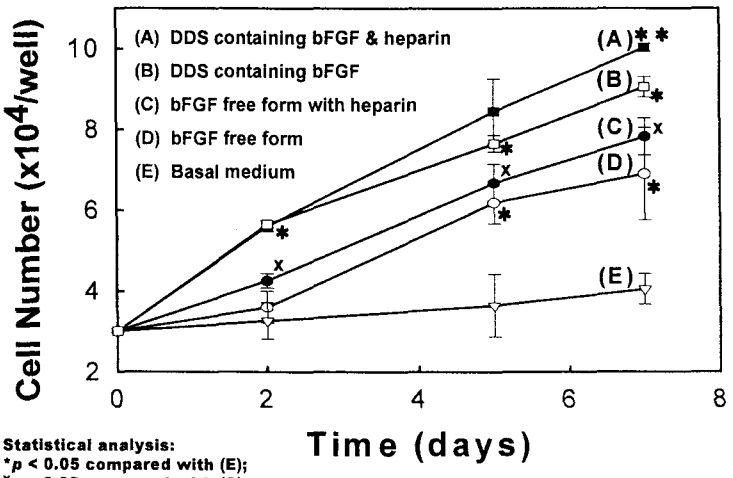
Release Profile of bFGF (Various Thrombin Concentration)



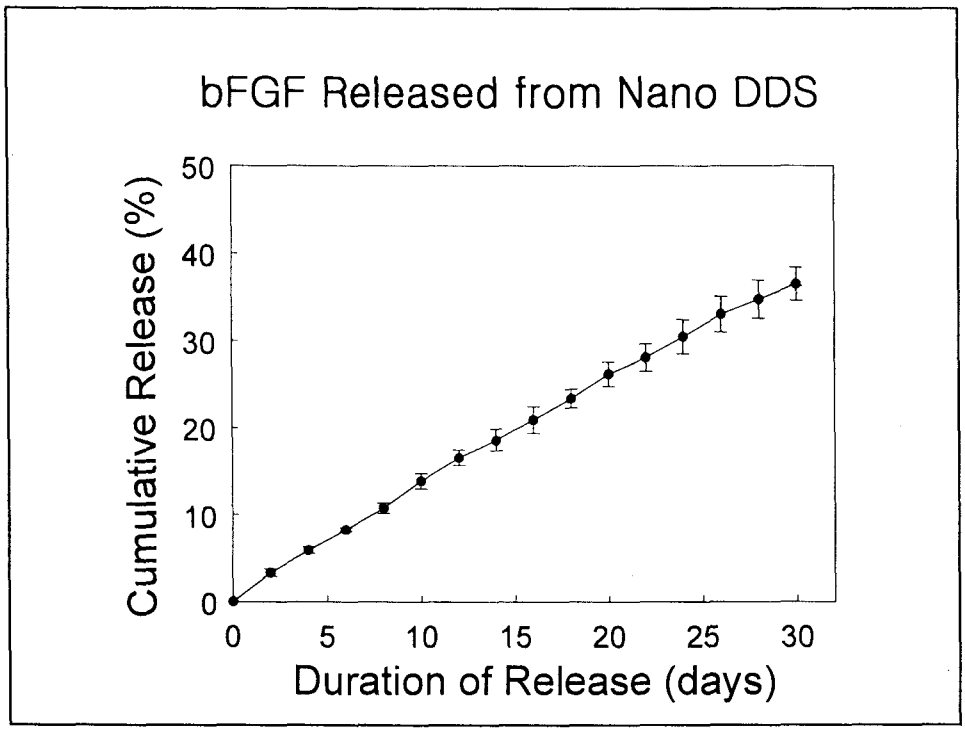
Release Profile of bFGF (Various Fibrinogen Concentration)

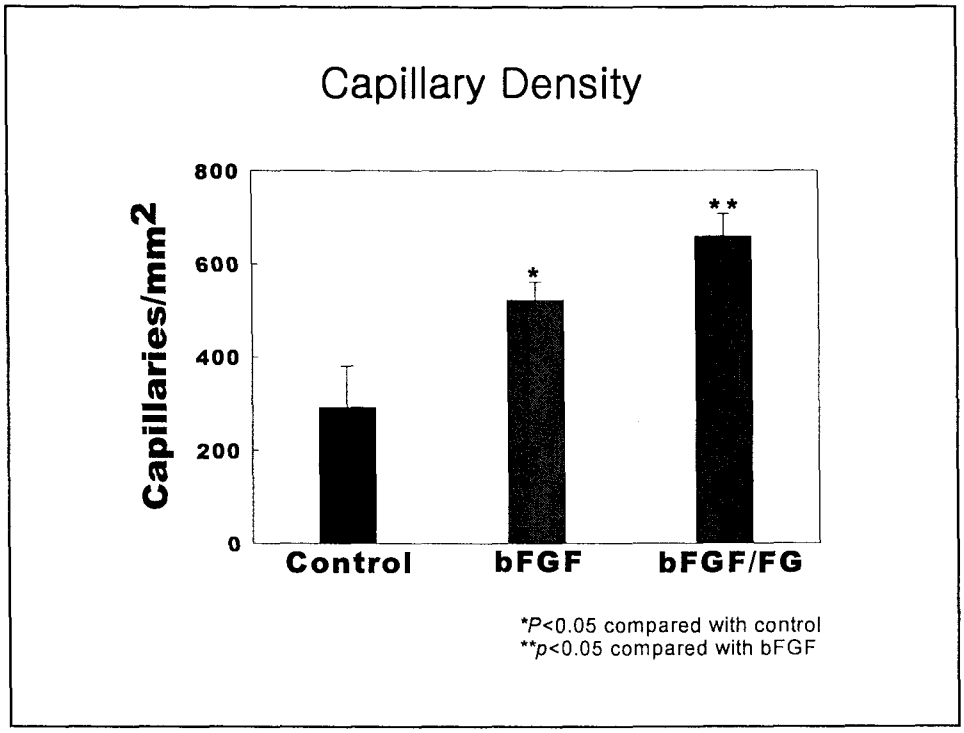


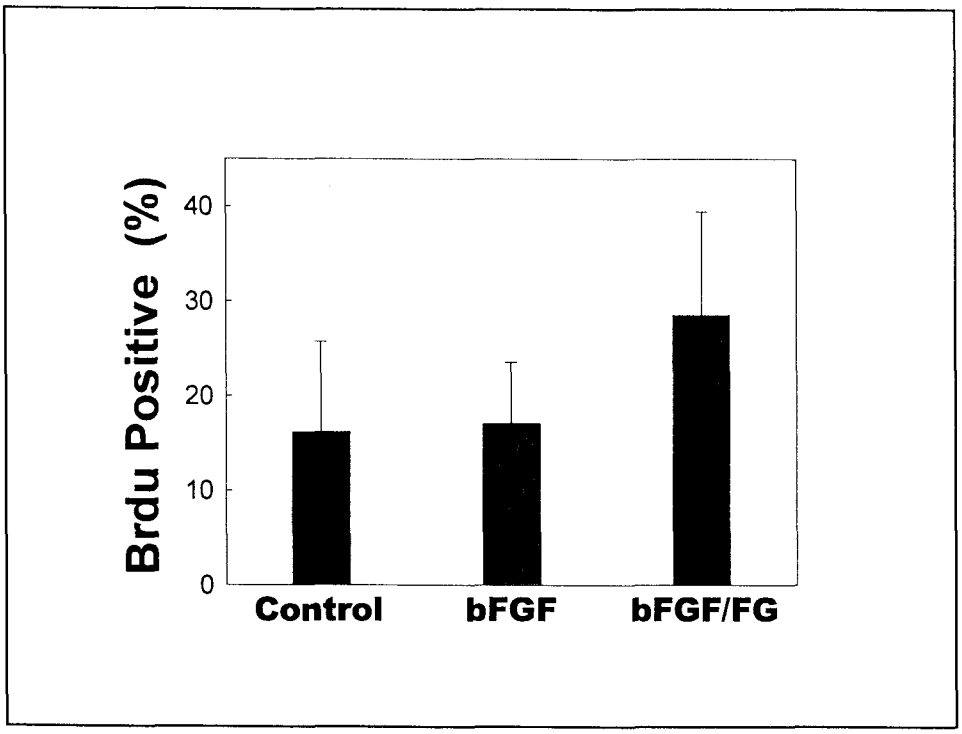
Bioactivity of bFGF

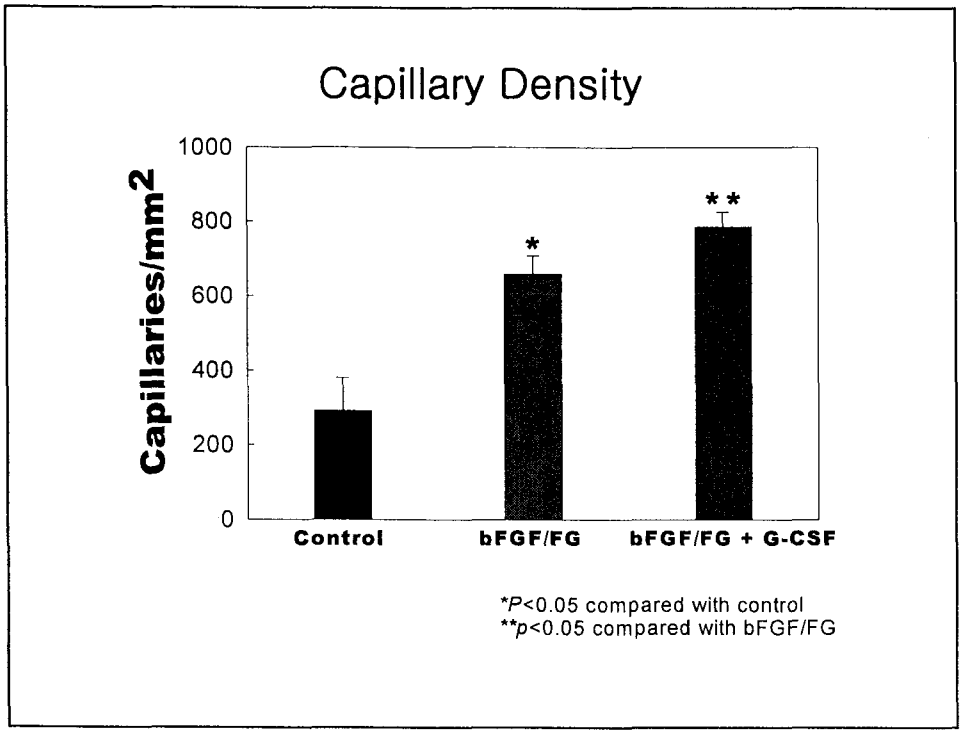


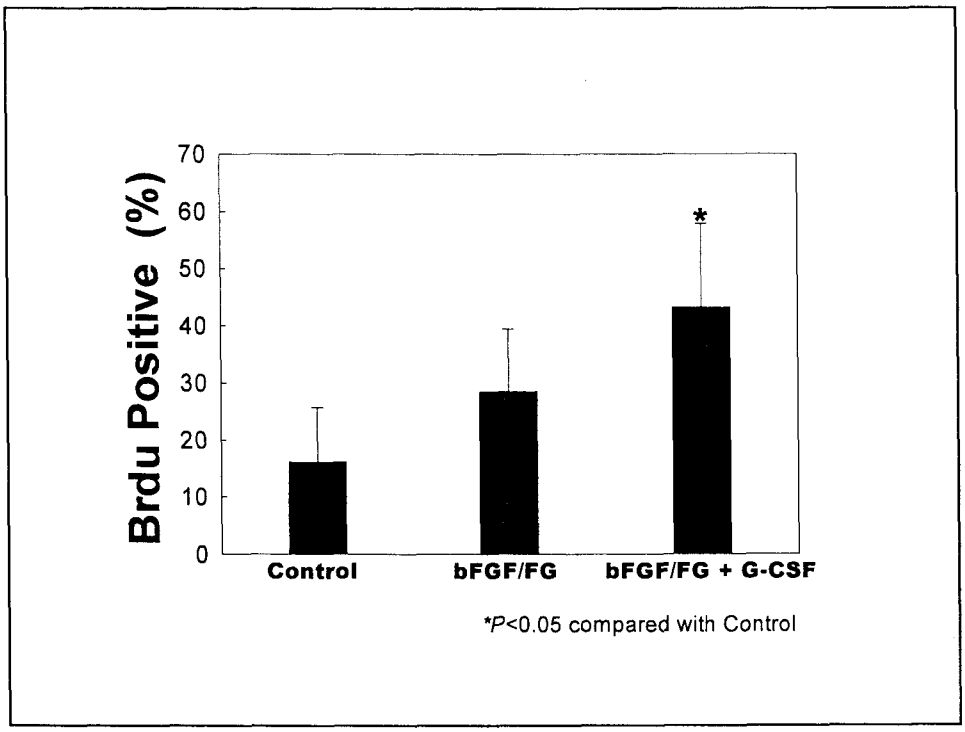
Statistical analysis:
*$p < 0.05$ compared with (E);
x$p < 0.05$ compared with (A);
**$p < 0.05$ compared with (B).

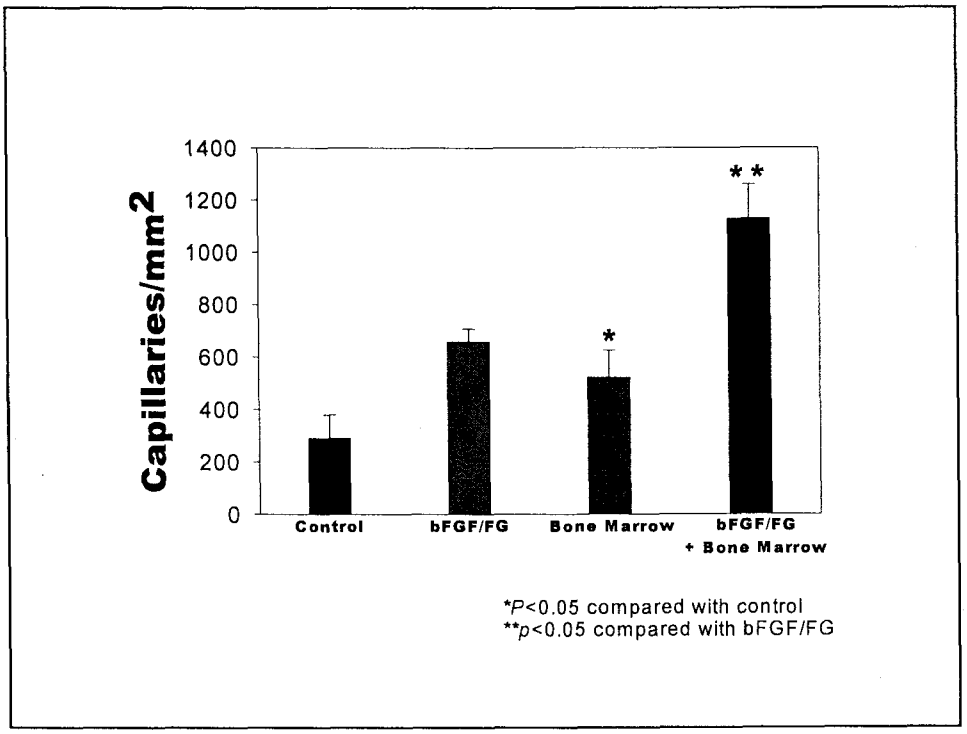

bFGF Released from Nano DDS
Cumulative Release (%)
50
40
30
20
10
0
0
5
10
15
20
25
30
Duration of Release (days)

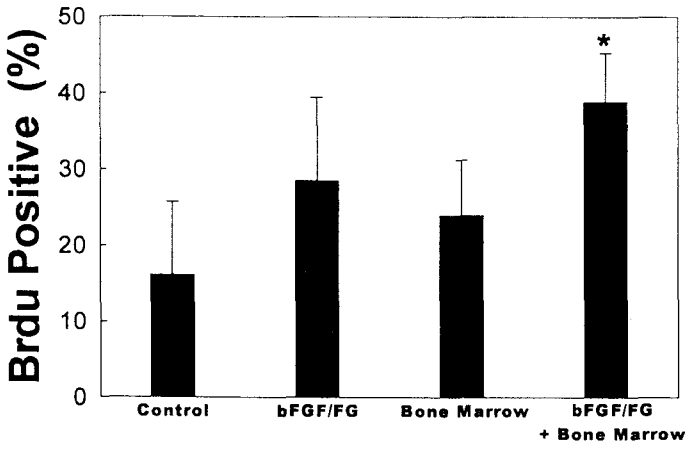

Capillary Density
Capillaries/mm2
800
600
400
200
0
*
**
Control
bFGF
bFGF/FG
*P<0.05 compared with control
**p<0.05 compared with bFGF


Brdu Positive (%)
40
30
20
10
0
Control
bFGF
bFGF/FG

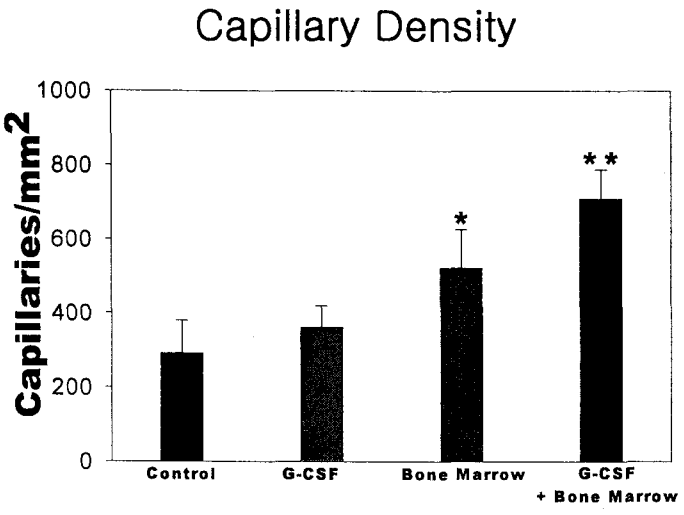

Capillary Density
Capillaries/mm2
1000
800
600
400
200
0
*
**
Control
bFGF/FG
bFGF/FG + G-CSF
*P<0.05 compared with control
**p<0.05 compared with bFGF/FG


Brdu Positive (%)
70
60
50
40
30
20
10
0
*
Control
bFGF/FG
bFGF/FG + G-CSF
*P<0.05 compared with Control


Capillaries/mm2
1400
1200
1000
800
600
400
200
0
*
**
Control
bFGF/FG
Bone Marrow
bFGF/FG
+ Bone Marrow
*P<0.05 compared with control
**p<0.05 compared with bFGF/FG



*P<0.05 compared with Bone Marrow



*P<0.05 compared with G-CSF
**p<0.05 compared with Bone Marrow

Clinical Trial
(BMSCs and G-CSF for Limb Ischemia)

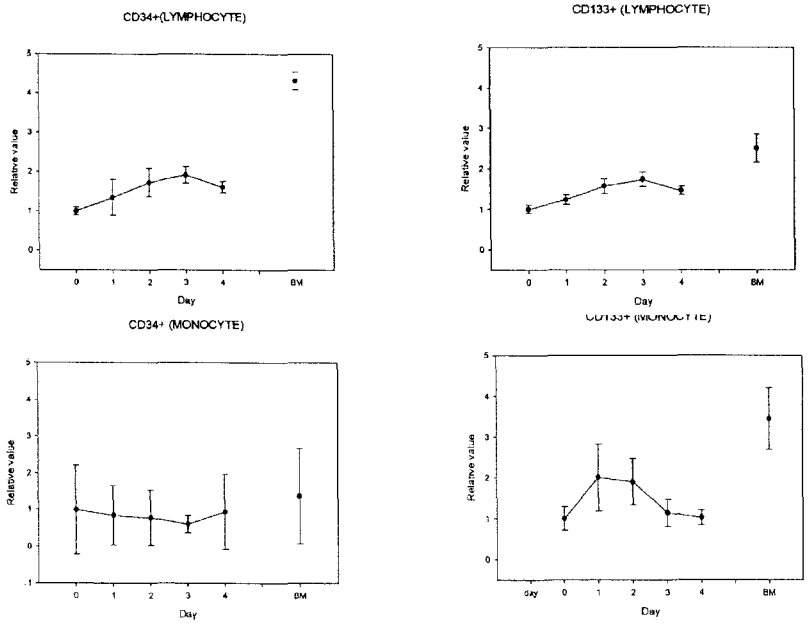
1) 임상 실험 : 임상시험 심의위원회(IRB) 승인을 얻어 시행.

2) 대상 환자 : 수술적 치료 및 방사선 중재시술을 시행 할 수 없었던 환자 중 하지 허혈증상이 악화되어 통증으로 정상 생활이 불가능하거나 하지 절단이 고려되었던 20명의 환자.

3) 효과 검증
- 적외선체열측정기(thermogram) : 하지 온도 변화를 측정
- 동맥조영술 : 신생혈관 유발 정도 검증
- 자각 증상 개선 정도 판단 기준 : 국제표준 임상표에 근거 (J Vasc Surg 1997;26:517-538)

4) 결과; 전체 20명 중 18명(90%)에서 하지허혈증 개선

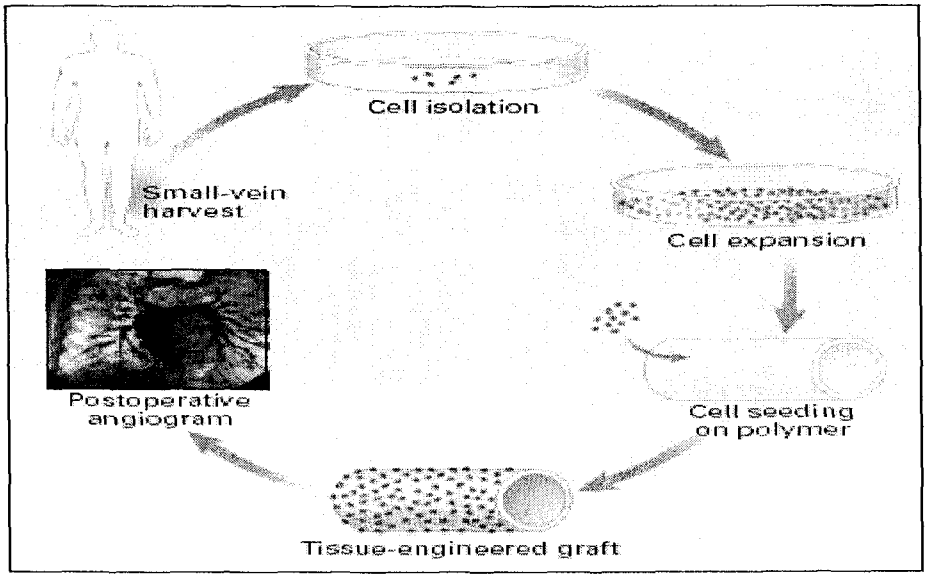
Flowcytometric Analyses of Pheripheral Bloods

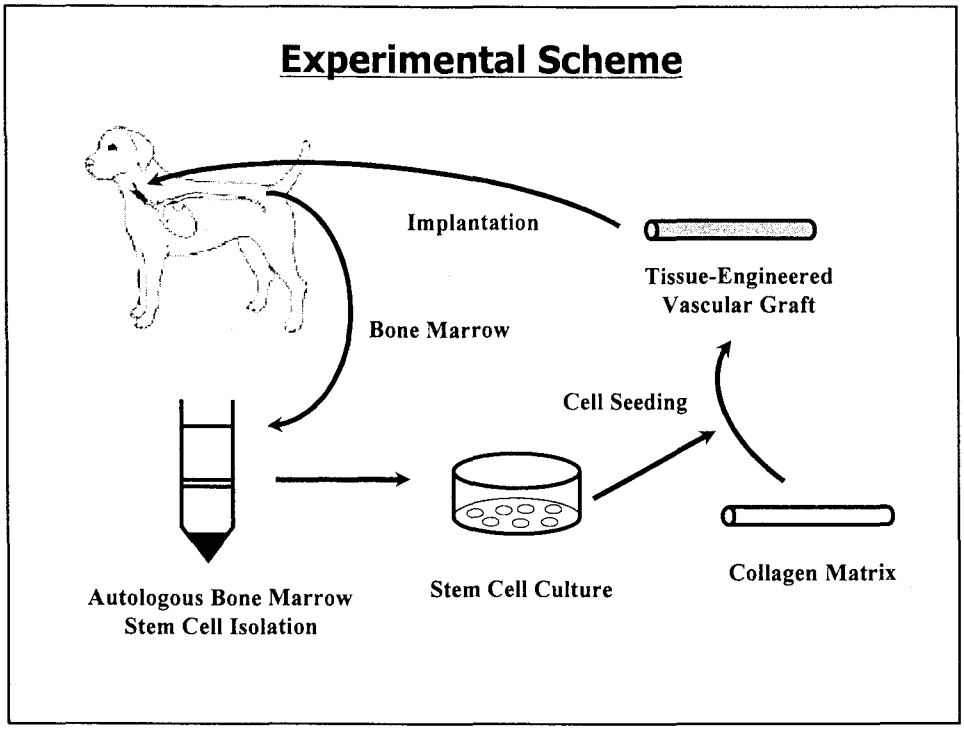


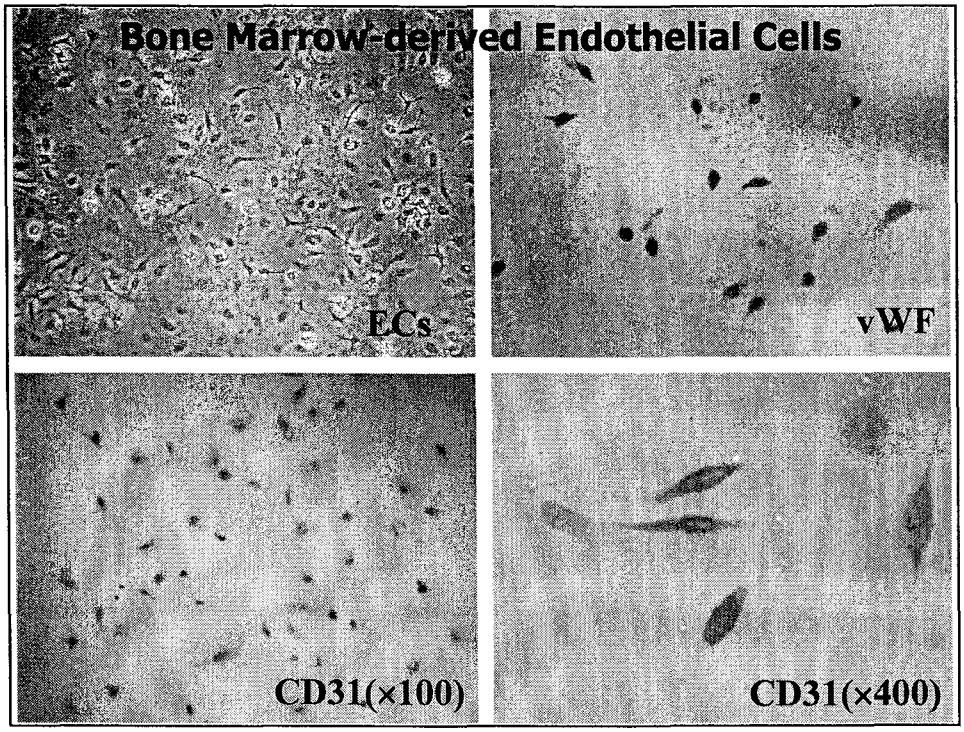
Summary 2

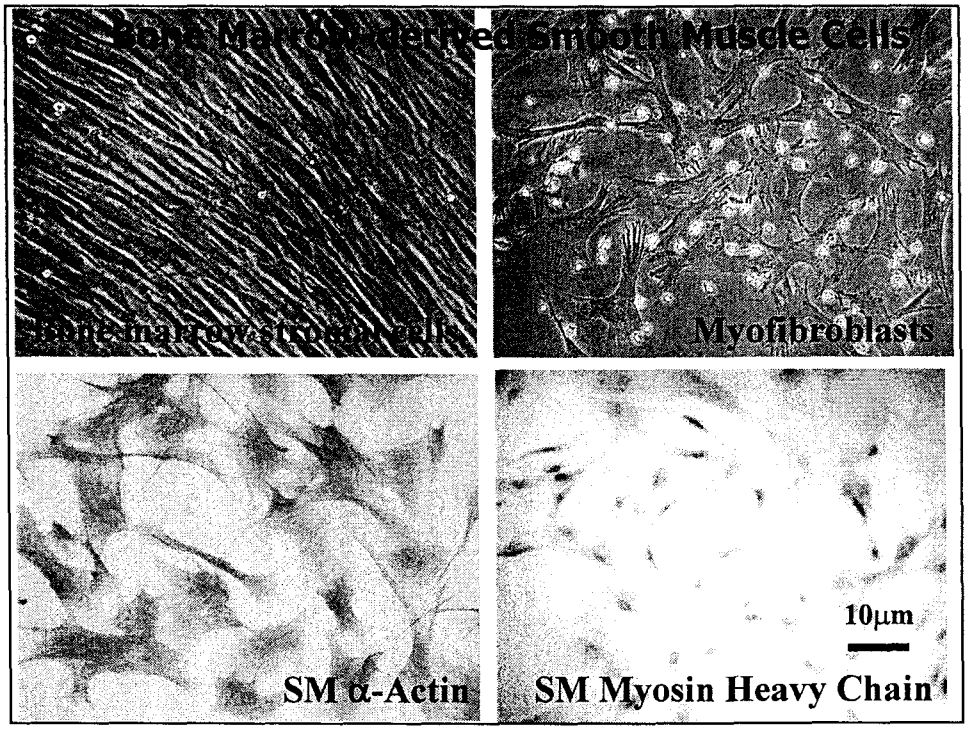
- bFGF를 각각 1주일, 4주일간 전달할 수 있는 약물 전달시스템을 개발하였다.
- bFGF 약물전달시스템 투여는 매일 bFGF bolus injection하는 것보다 신생혈관생성을 향상시킨다.
- 골수단핵세포 이식, bFGF 약물전달, G-CSF 투여의 복합치료는 각각의 단독치료보다 신생혈관생성을 향상시킨다.
- 임상시험에서 골수단핵세포 이식과 G-CSF의 복합치료술은 하지허혈환자를 성공적으로 치료하였다.

Transplantation of a Tissue-Engineered Pulmonary Artery (Shin'oka T, et al., N Engl J Med 344, 532 (2001))

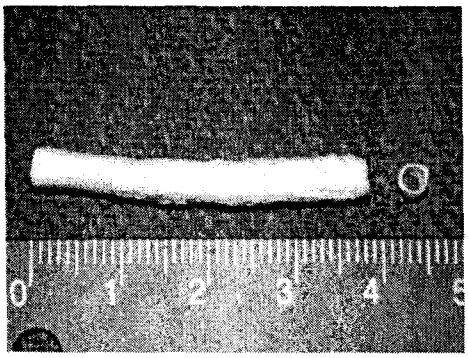


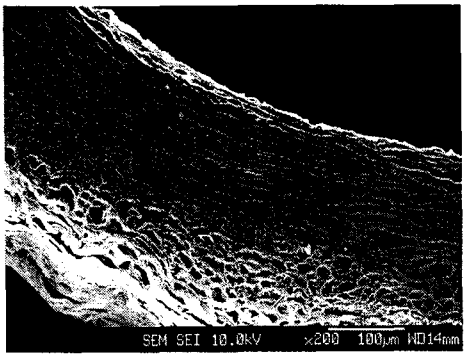

Experimental Scheme
Implantation
Tissue-Engineered
Vascular Graft
Bone Marrow
Cell Seeding
Autologous Bone Marrow
Stem Cell Isolation
Stem Cell Culture
Collagen Matrix


Bone Marrow-derived Endothelial Cells
ECs
vWF
CD31(×100)
CD31(×400)

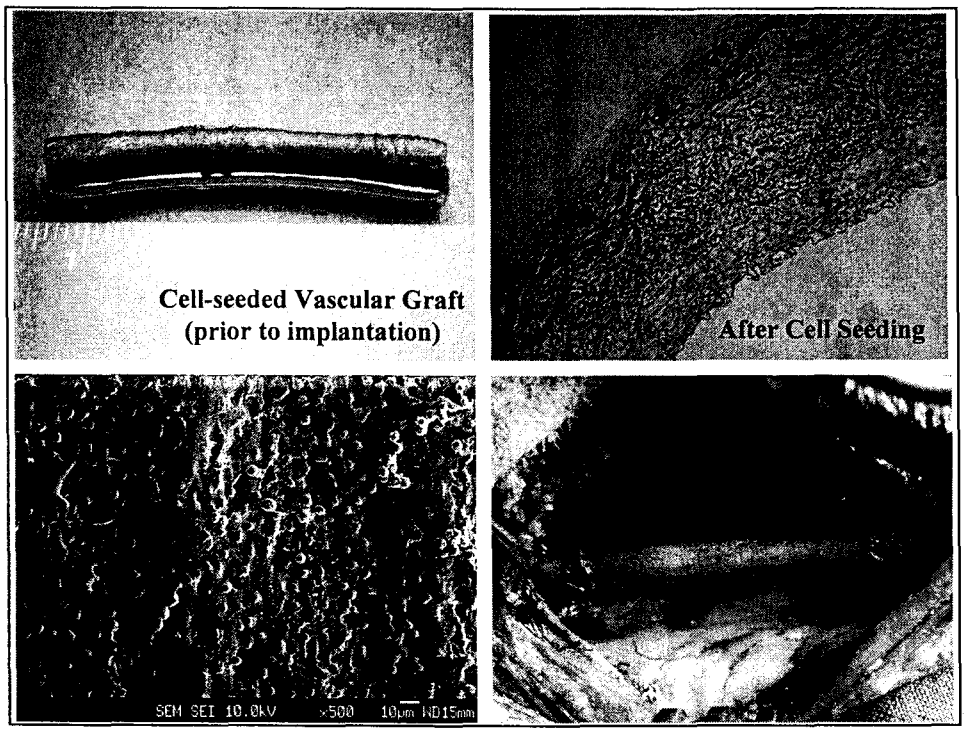


Decellularized Vascular Graft

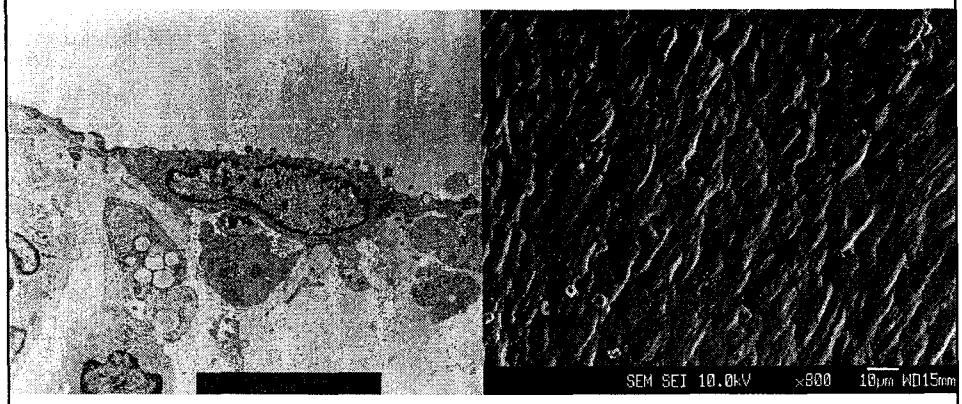




Gross View **SEM: Cross-section**



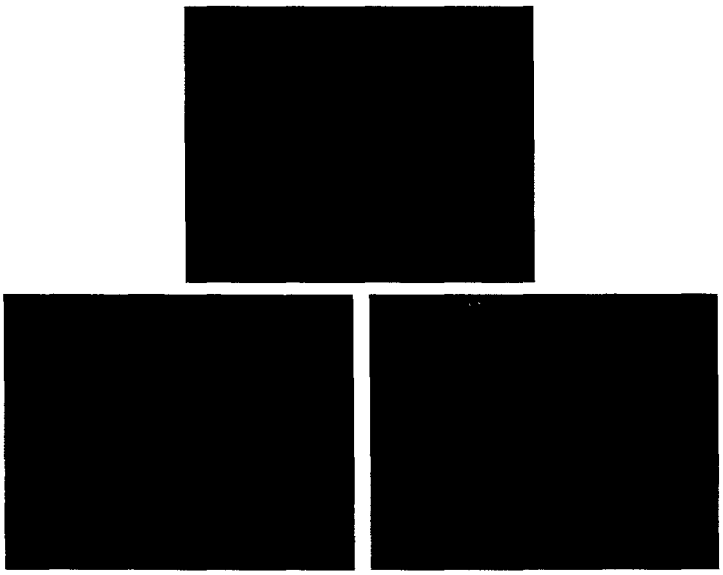
Electron microscopic analyses
(8 week-explanted graft)



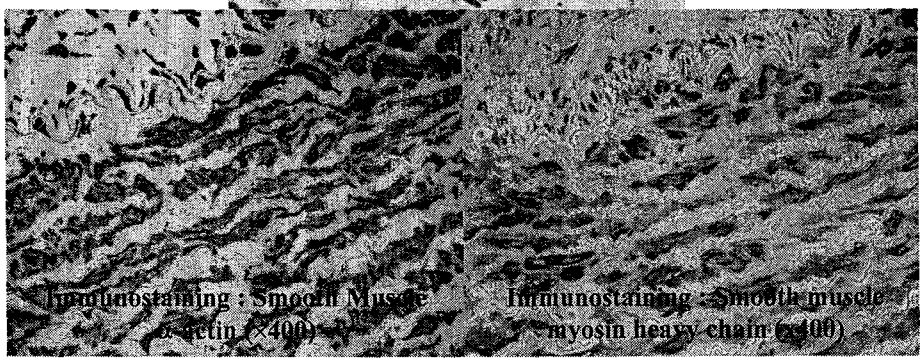
TEM: endothelium

SEM: endothelium

Fluorescence microscopic analysis (CM-DiI)



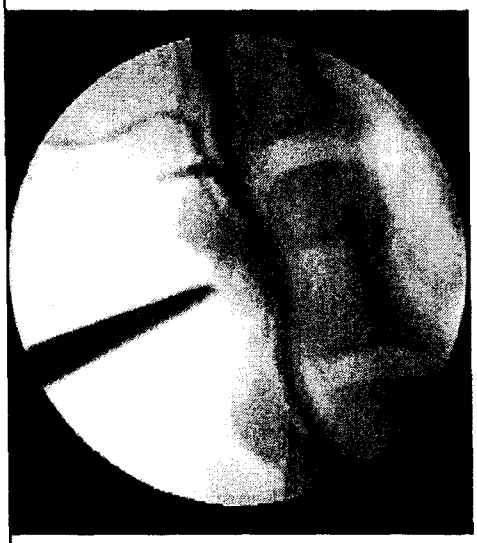
Immunostaining : von Willebrand Factor (×400)



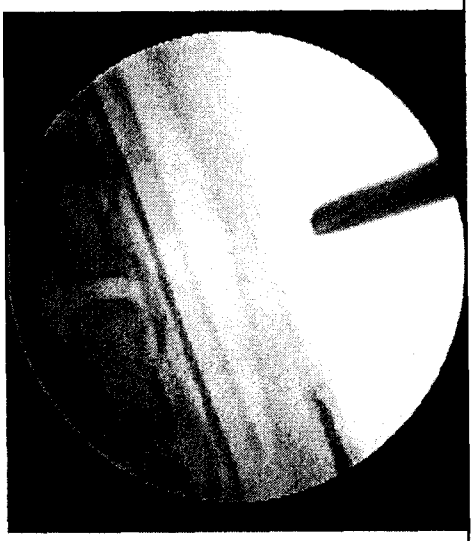
Immunostaining : Smooth Muscle α-actin (×400)

Immunostaining : Smooth muscle myosin heavy chain (×400)

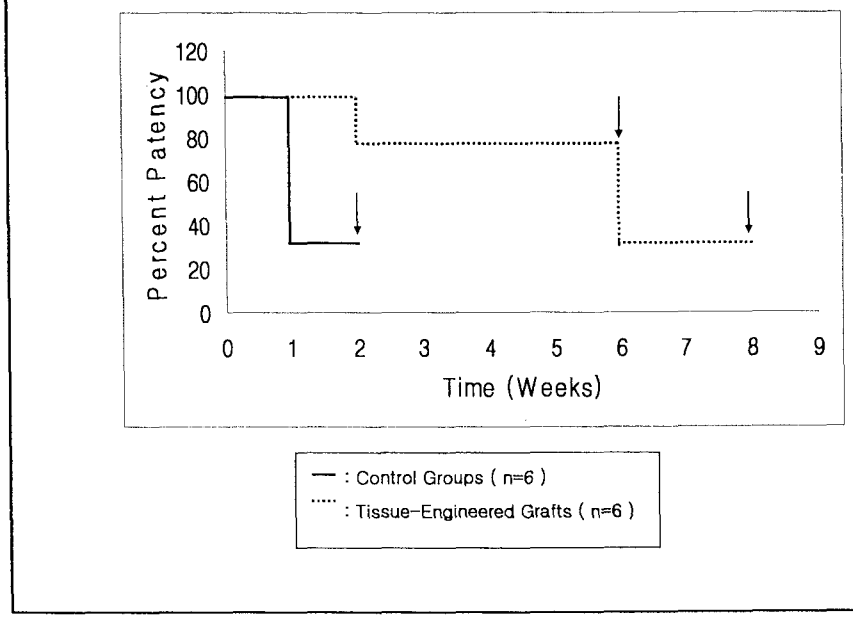
Patency (angiogram)



Stem cells + Matrix
(8 weeks)



Collagen matrix
Matrix only
(2 weeks)



Enhanced endothelial regeneration in tissue-engineered vascular grafts using G-CSF

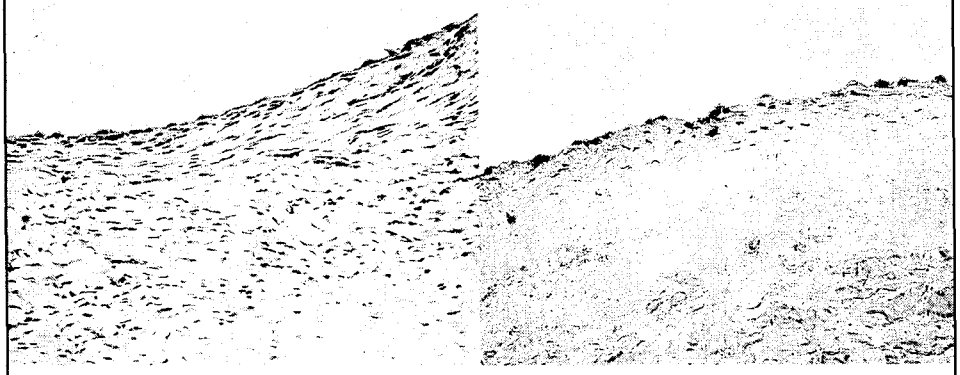
Materials and methods

- Scaffold : decellularized abdominal aorta (ID = 7 mm)
- Cell source : autologous bone marrow-derived cells → endothelial-like cells and smooth muscle-like cells
- Animal model : canine abdominal aorta (8 weeks)
- Granulocyte-colony stimulating factor (G-CSF) treatment (5 μg/kg/day) → for 2 weeks
- Analyses : Histology, Immunohistochemistry, SEM, TEM, RT-PCR (vWF)

Group 1 : Bone marrow-derived cell seeding + G-CSF treatment

Group 2 : Bone marrow-derived cell seeding

Immunohistochemistry (vWF)

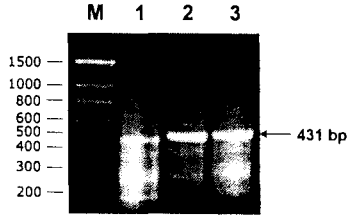


BMC seeding + G-CSF

BMC seeding

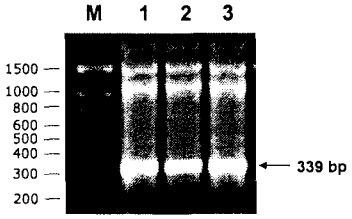
RT-PCR analysis

vWF



Lane 1: BMC seeding
Lane 2: BMC seeding + G-CSF
Lane 3: Native abdominal aorta

Ribosome s17

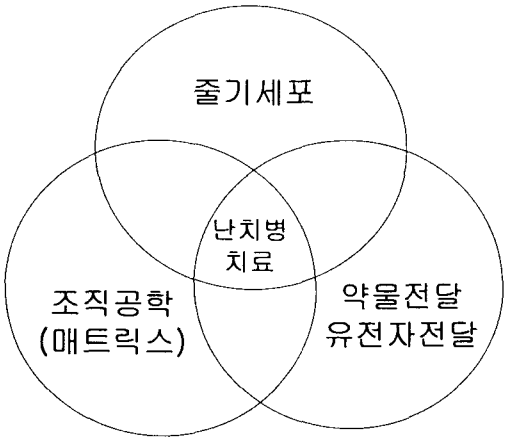


Lane 1: BMC seeding
Lane 2: BMC seeding + G-CSF
Lane 3: Native abdominal aorta

Summary 3

- 골수중간엽줄기세포와 생체재료를 이용하여 소구경 동맥을 조직공학적으로 재생할 수 있다.
- G-CSF의 투여는 조직공학적 소구경 인공동맥의 혈관내피 생성을 촉진시킬 수 있다.

Regenerative Medicine



Acknowledgements

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