Tissue Engineering

Tissue Engineering

- Recovery of Lost Body Parts or their functions
- Develop Biocompatible Artificial Tissues or Organs
  (Artificial Bone, Artificial Skin, Artificial Vessel, Artificial Hearing)
- Necessary to understand the characteristics of the cell and tissue and to control their growth

Robert Langer and Jay Vacanti, MIT, 1995

Human ear grown on mouse

Objective

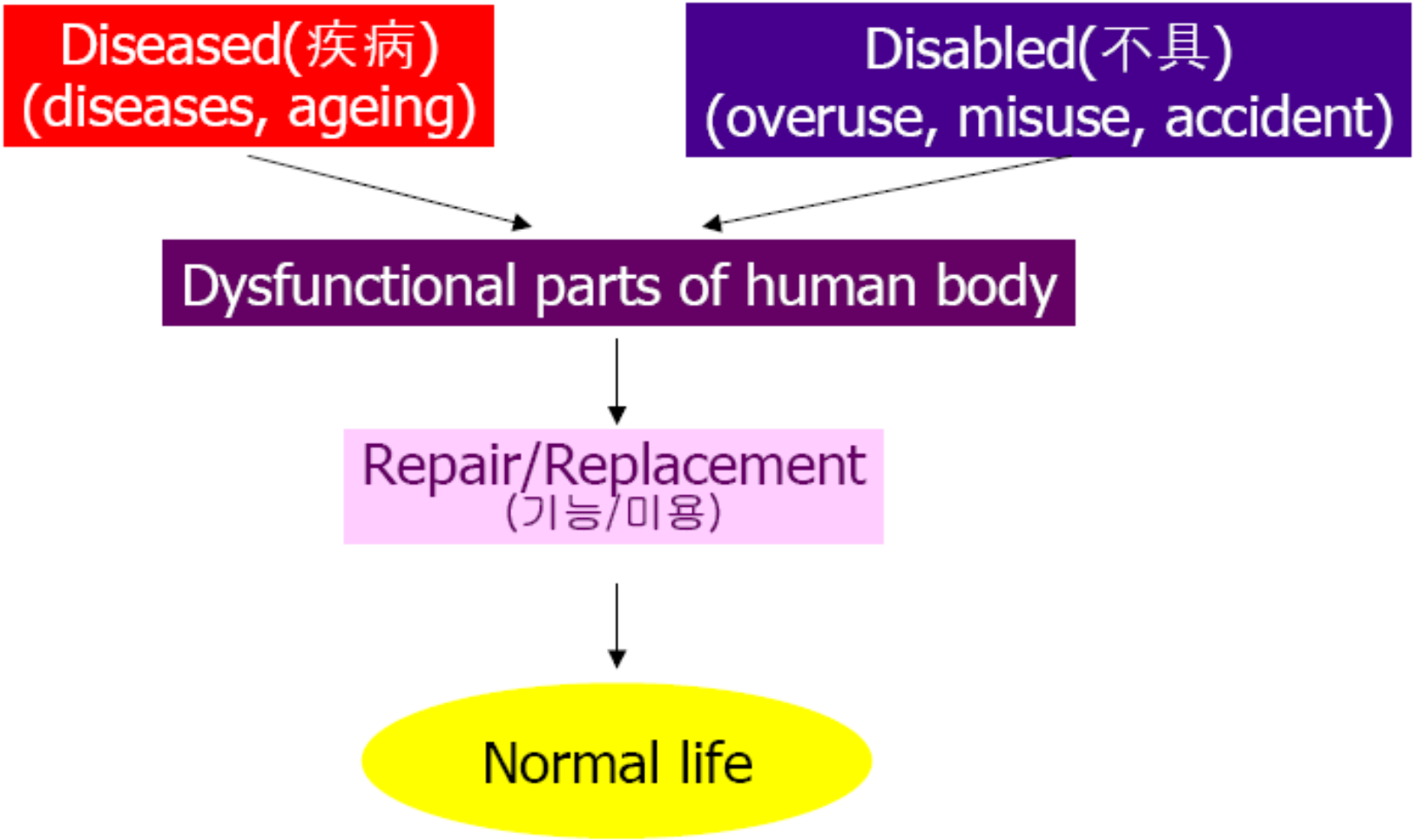
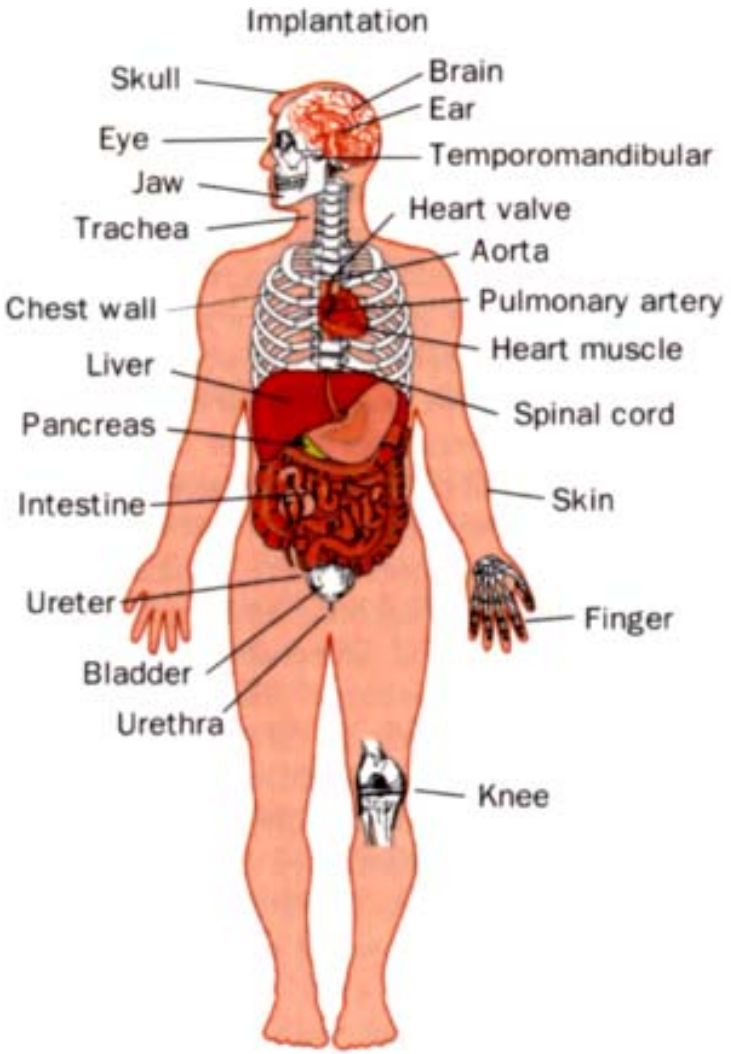

Diseased(疾病)
(diseases, ageing)
Disabled(不具)
(overuse, misuse, accident)
Dysfunctional parts of human body
Repair/Replacement
(기능/미용)
Normal life

TABLE 12.1 Incidence of Organ and Tissue Deficiencies, or the Number of Surgical Procedures Related to These Deficiencies in the United States[a]

Indicator	Procedure or Patients per Year
Skin	
Burns[b]	2,150,000
Pressure sores	150,000
Venous stasis ulcers	500,000
Diabetic ulcers	600,000
Neuromuscular disorders	200,000
Spinal cord and nerves	40,000
Bone	
Joint replacement	558,200
Bone graft	275,000
Internal fixation	480,000
Facial reconstruction	30,000
Cartilage	
Patella resurfacing	216,000
Chondromalacia patellae	103,400
Meniscal repair	250,000
Arthritis (knee)	149,900
Arthritis (hip)	219,300
Fingers and small joints	179,000
Osteochondritis dissecans	14,500
Tendon repair	33,000
Ligament repair	90,000
Blood Vessels	
Heart	754,000
Large and small vessels	606,000
Liver	
Metabolic disorders	5,000
Liver cirrhosis	175,000
Liver cancer	25,000
Pancreas (diabetes)	728,000
Intestine	100,000
Kidney	600,000
Bladder	57,200
Ureter	30,000
Urethra	51,900
Hernia	290,000
Breast	261,000
Blood Transfusions	18,000,000
Dental	10,000,000

[a] From Langer and Vacanti (1993).
[b] Approximately 150,000 of these individuals are hospitalized and 10,000 die annually.



Tow Methods for Artificial Organ Development

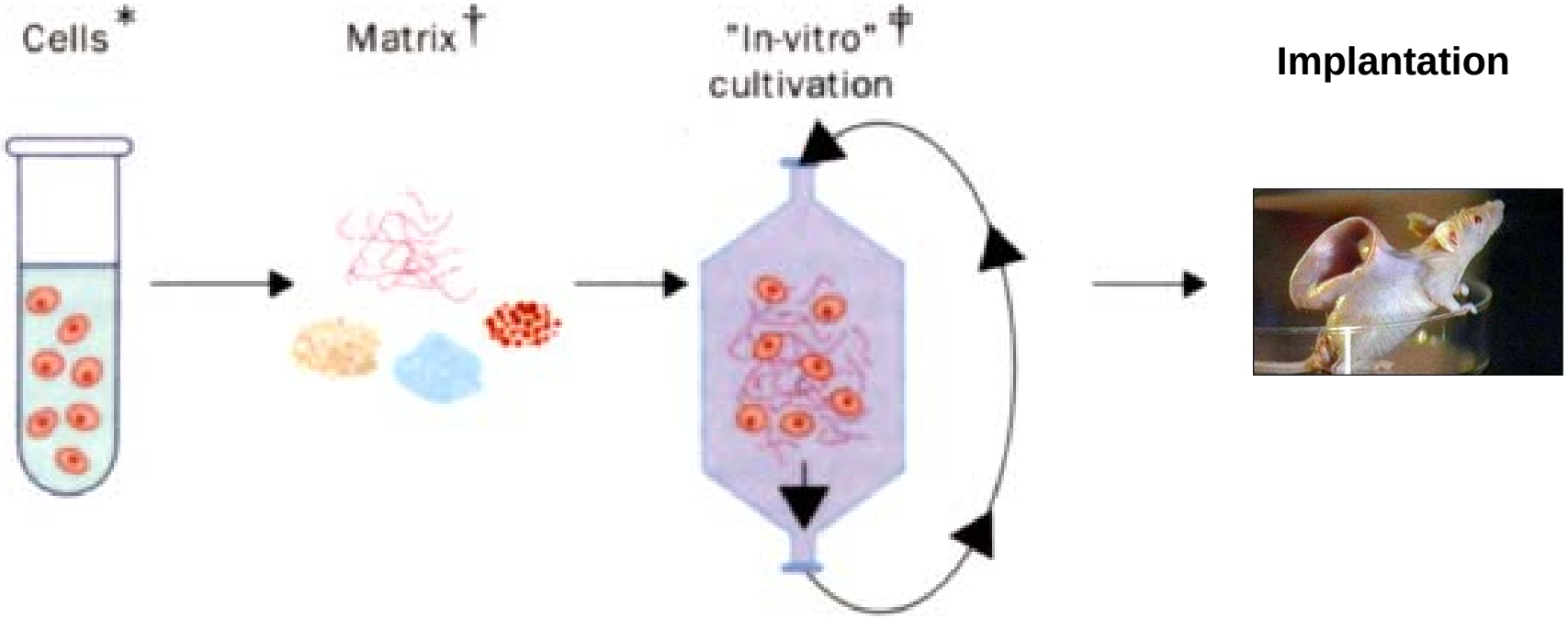
Organ Transplantation (장기이식)

- **Homotransplantation(동종이식)**
  - **better performance**
  - **limited supply**
- **Xenotransplantation(이종이식)**
  - **unlimited supply**
  - **unknown infection**
  - **ethical problem**
- **Autotransplantation(자가이식)**
  - **ideal solution**
  - **ethical problem**

Artificial Organs (인공장기)

- **Mechanoelectric(전자기계식)**
  - **mechanical organ**
  - **sensory organ**
  - **size, biocompatibility**
  - **power requirement**
- **Biomaterial(생체재료)**
  - **tissue**
  - **biocompatibility**
  - **longevity**
- **Biological(생물학적)**
  - **secretory organ**
  - **biochemical organ**
  - **longevity**

Xenotransplantation (이종이식)



* Cells may be tissue specific, stem cells, or embryonic stem cells. They may be autologous or allogenic

† The matrix may be natural or synthetic. It may be fibrous, a foam, a hydrogel, or capsules

‡ In-vitro culture may be in static, stirred, or dynamic flow conditions

The Lancet, 354 (1001):S32-S34 , 1999

Autotransplantation (자가이식)

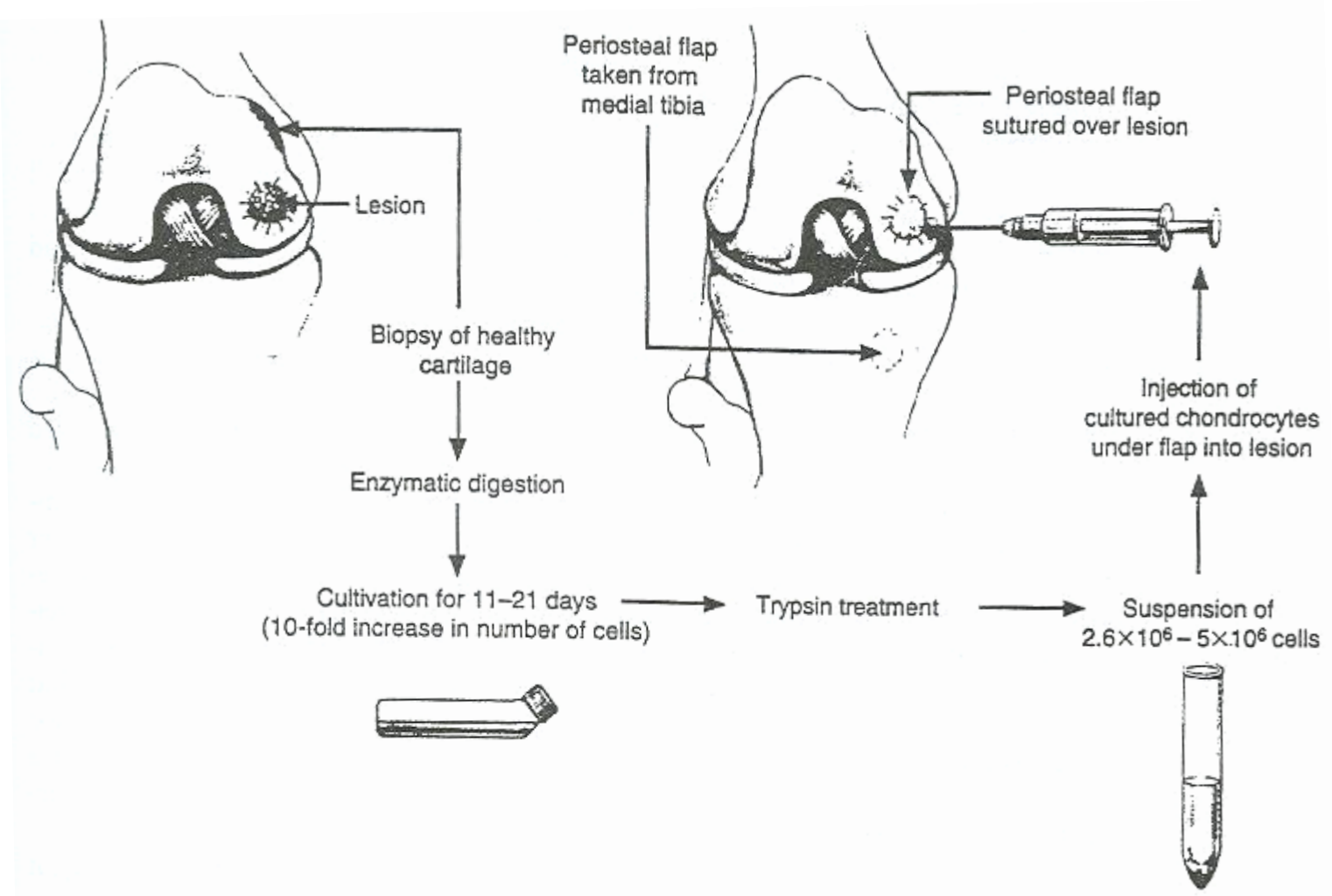


Fig. 12.3 Diagram of chondrocyte transplantation in the right femoral condyle (from Brittberg, 1994).

Purposes of Cellular Engineering

- To understand cellular dynamics
- To control cellular behavior
- To make cellular networks
- To develop artificial organs, tissue
- To enhance biocompatibility of implant material

Tissue Dynamics

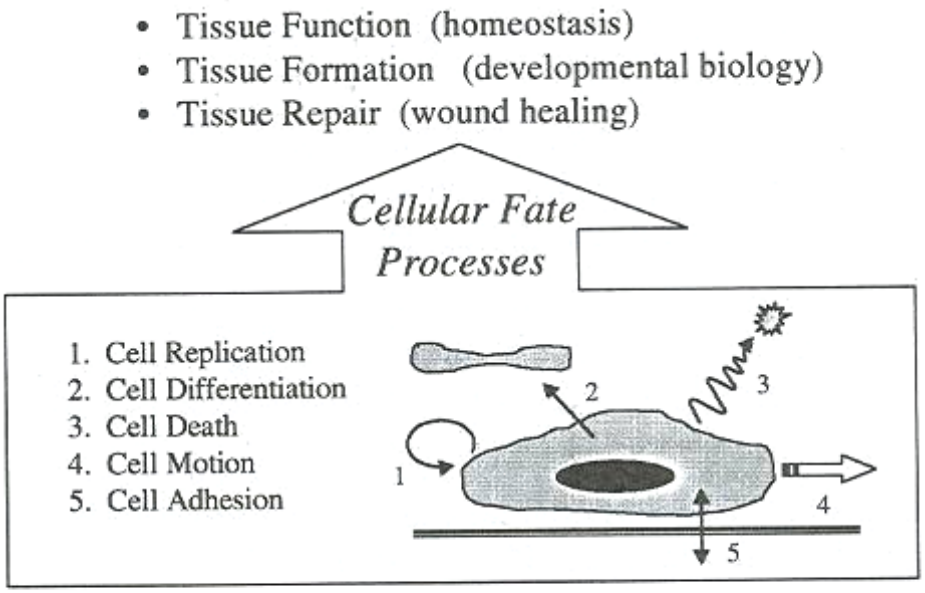


Fig. 12.5 Tissue dynamics. The three dynamic states of tissues and the underlying cellular fate processes.

1. **Cell replication**-an increase in cell number
2. **Cell differentiation**-changes in gene expression and the acquisition of a particular function
3. **Cell motility**-the motion of a cell into a particular niche or location
4. **Cell apoptosis (programmed death)-** the controlled death of a cell
5. **Cell adhesion**-the physical binding of a cell to its immediate environment, which may be a neighboring cell, extracellular matrix, or an artificial surface.

세포 공학의 현황과 전망: 세포 치료에 있어서 줄기 세포의 역할과 활용

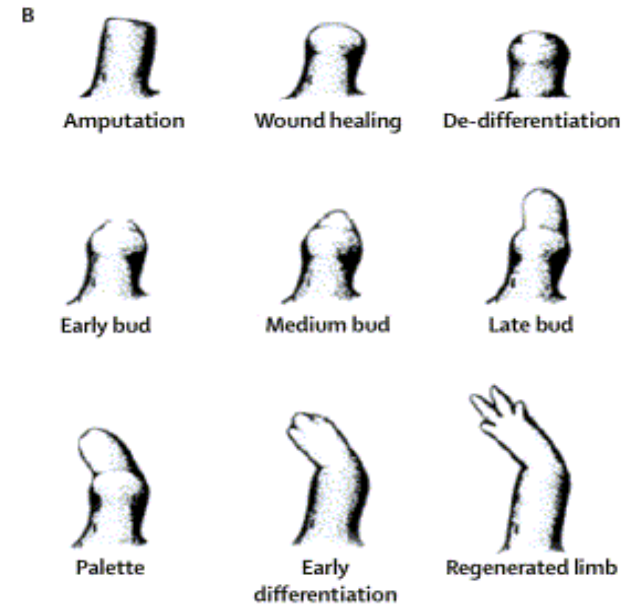
목적

- 난치병치료-현재는?
- 줄기 세포 치료기술의 현재와 미래
- 줄기세포의 경제성
- 무엇이 중요한가?

세포 치료

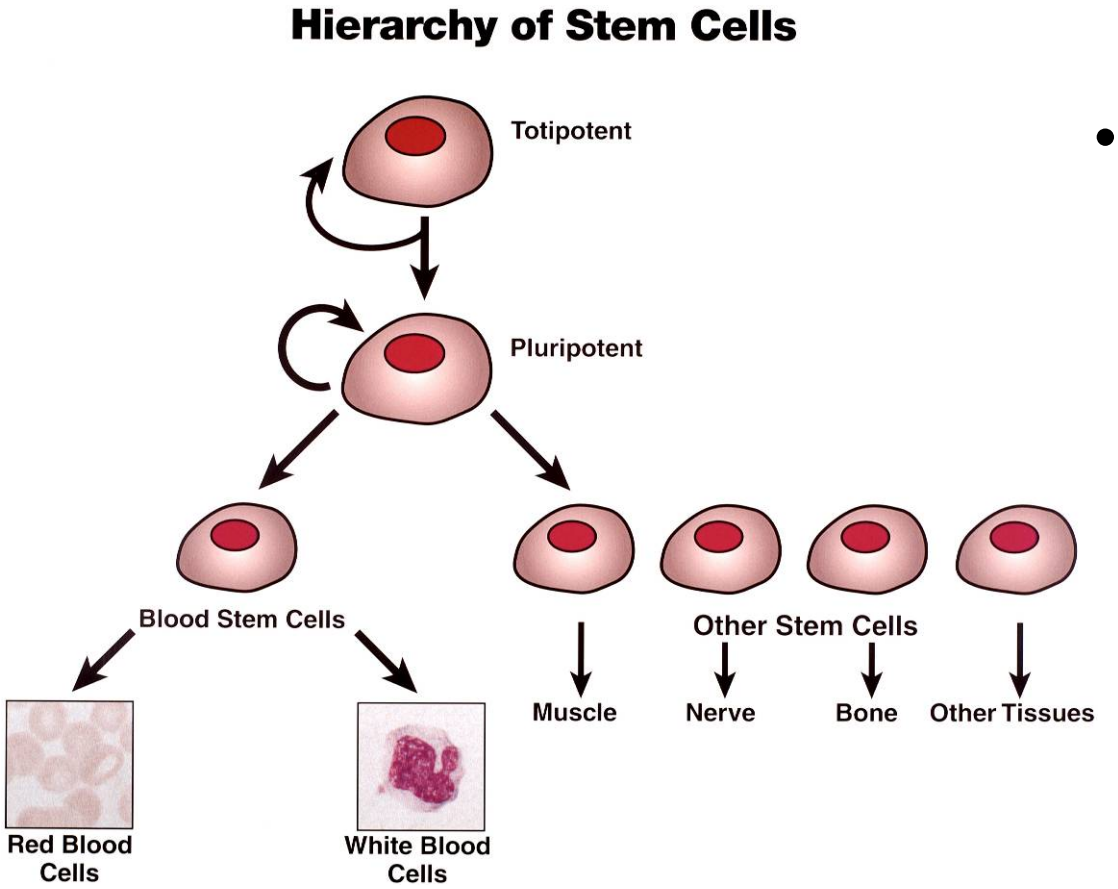
- 세포치료
  - 세포 치료란 환자의 손상된 장기나 조직을 치료하기 위하여 외부에서 세포를 주입하거나 세포의 성장 및 분화를 촉진하여 치료 대상이 되는 부위의 재활 및 재생을 유도하는 치료 방법.
- 최초의 세포 치료
  - 1912년에 갑상선 항진 환자에 갑상선 세포를 이식한 것
- 1971년 노벨상을 수상한 골수 이식 수술도 훌륭한 세포 치료의 한 예

줄기세포



- 양서류의 경우 다리가 절단 된 후에 줄기 세포의 역할에 의해서 다리가 재생되는 사실이 이미 보고되어 있음.

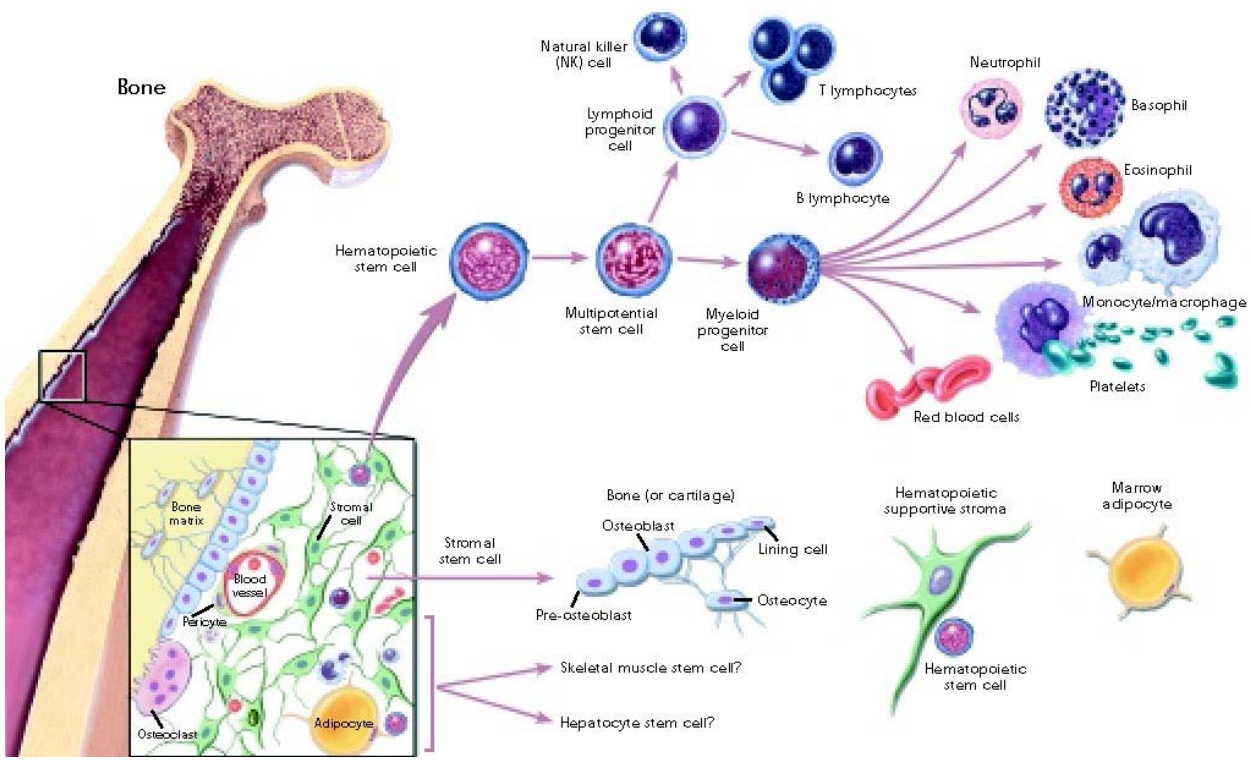
줄기 세포



- 줄기 세포의 종류
  - 배아 줄기 세포: 전능(Pluripotent) 세포, 거의 모든 세포로 분화 가능
  - 성체 줄기 세포: 다능(Multipotent) 세포 , 분화능에 있어서 일정 한계

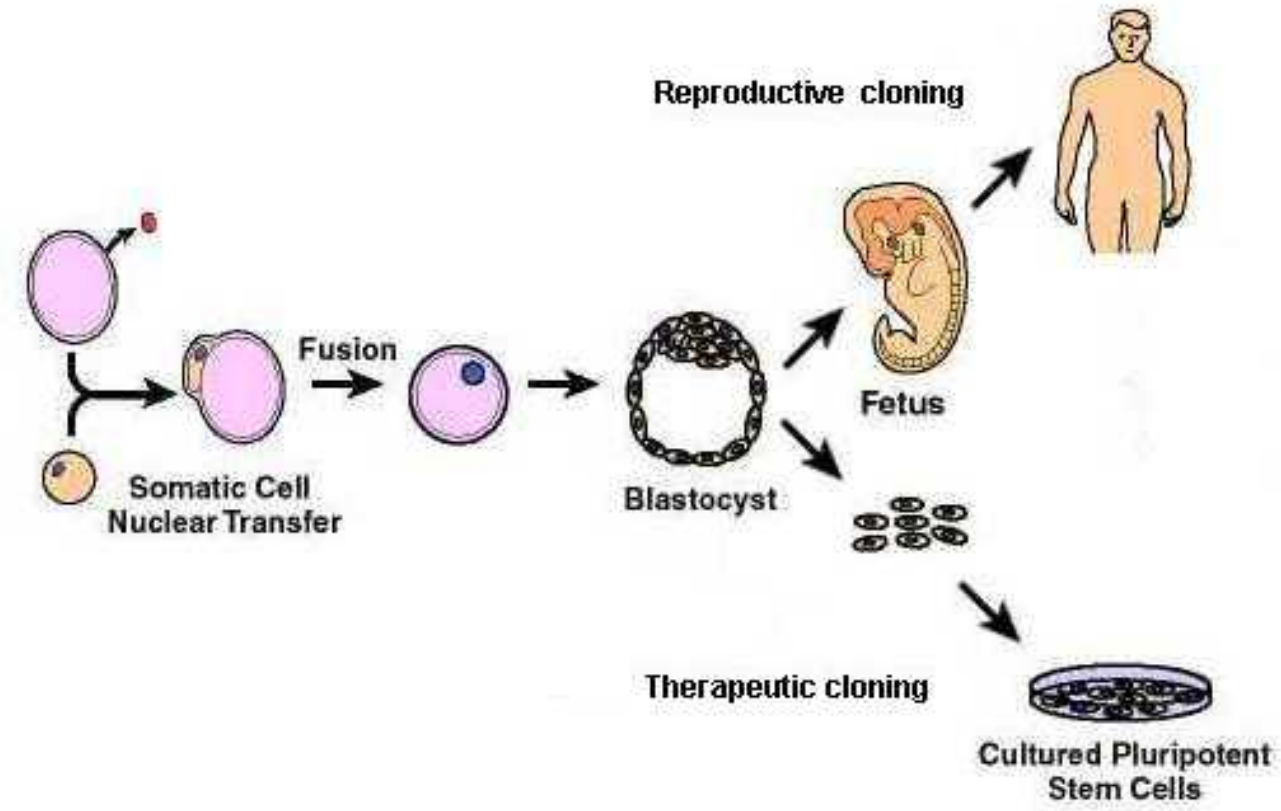
- 줄기 세포 획득 방법
  - 태아 줄기 세포: 유산된 태아
  - 성체 줄기 세포: 골수, 제대혈, 조직
  - 배아 줄기 세포: 수정란, 체세포 핵치환

성체 줄기 세포



- 신체의 거의 모든 부위에서 발견
  - 골수, 제대혈, 뇌, 간, 지방, 장, 표피, 망막, 췌장 등
- 자신의 세포이므로 면역 거부 반응 문제 최소화
- 분화능에 있어서는 일정 한계

배아 줄기 세포

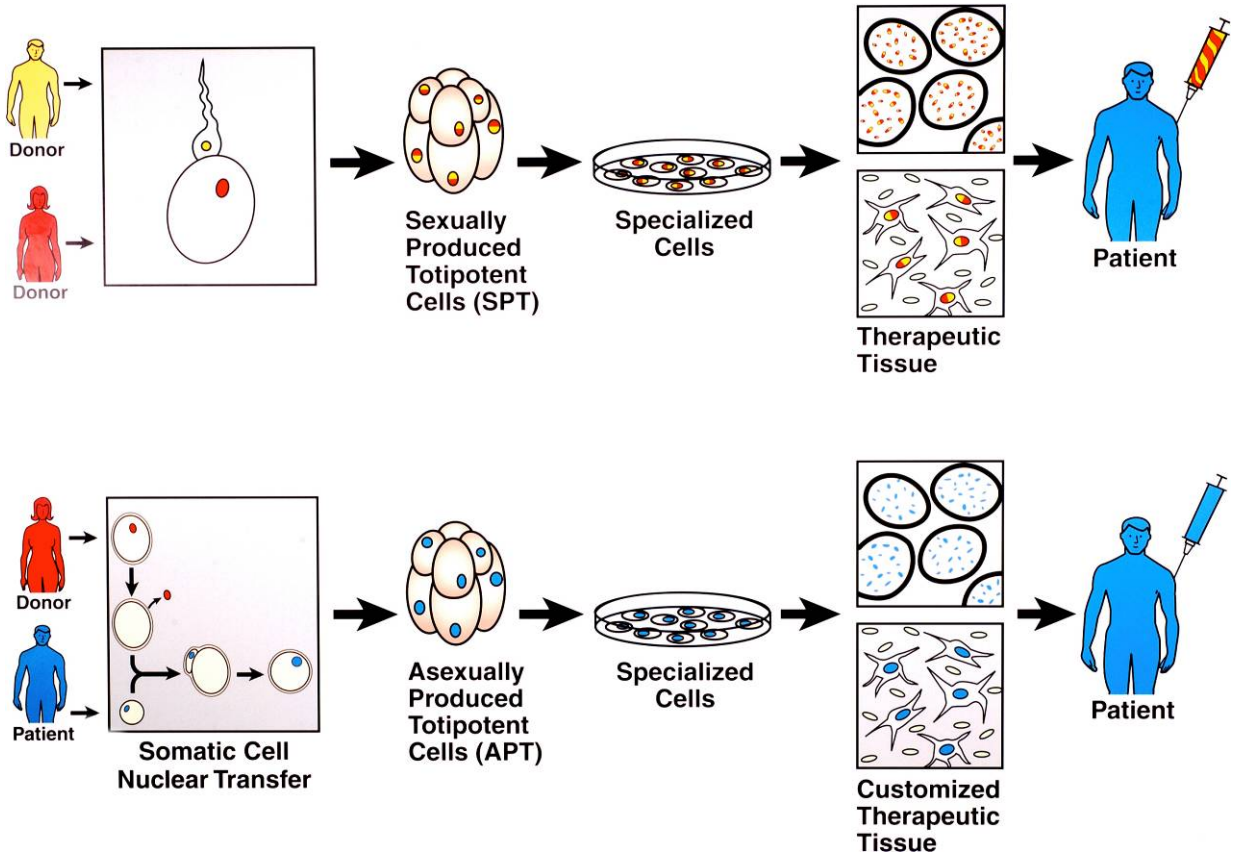


- 1998년 톰슨에 의해 인간 포배(blastocyte)에서 최초로 배아 줄기 세포를 분리 배양
- 수정란 사용으로 인간 생명 파괴의 윤리적 문제 야기
- 거의 모든 세포로 분화 가능한 전능 세포

성체 줄기 세포와 배아 줄기 세포 비교

	배아 줄기 세포	성체 줄기 세포
분화 능력	거의 모든 세포	일정한 세포
기형종	발생 가능성 큼	발생 가능성 작음
수명	제한 없음, 많은 수의 증식 가능	일정 시간 배양 후 증식 능력 상실
윤리 문제	윤리적 부담	비교적 크지 않음
극복 과제	면역학적 거부 반응 특정 세포로 분화	줄기 세포의 획득 및 증식, 성질 유지

체세포 핵치환 줄기 세포주

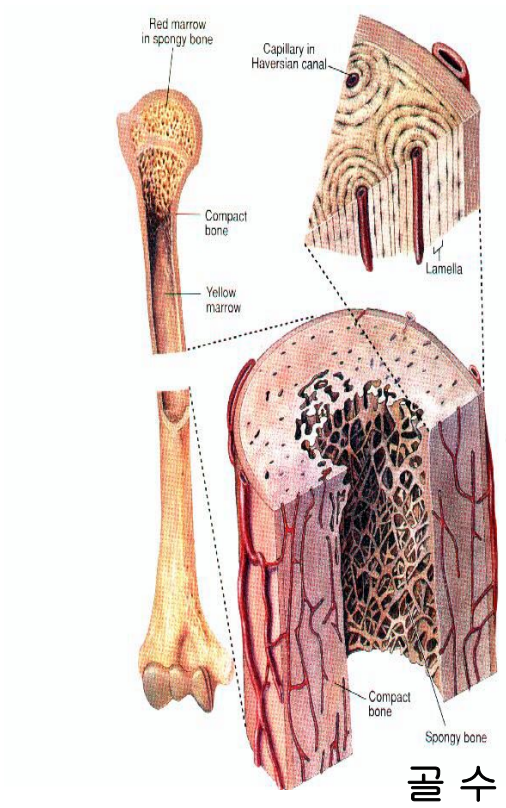


- 인간 난자에 환자의 체세포의 핵을 이식하여 환자 자신의 유전 정보를 가진 세포주 확립
  - 면역 거부 반응이 없고, 환자 자신의 맞춤 세포주

임상적 적용의 예

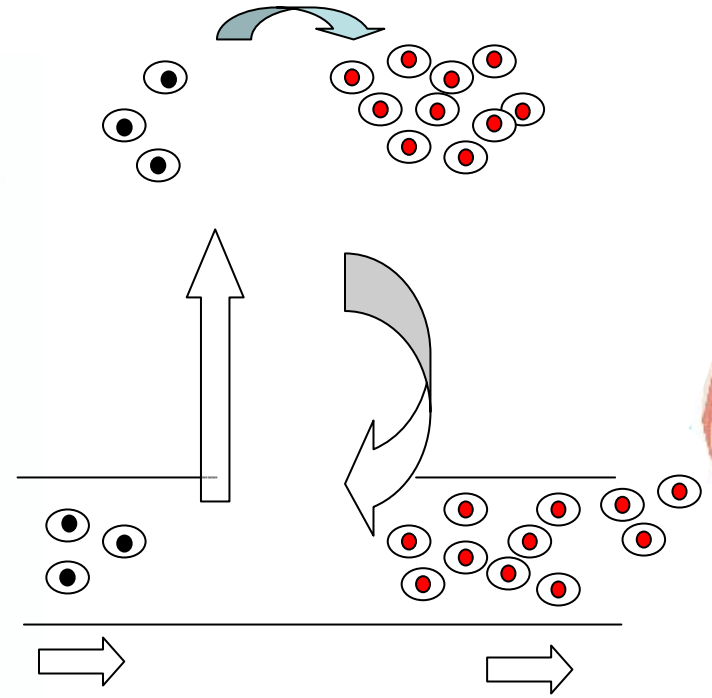
골수 줄기 세포를 이용한 심혈관 재생

Three aspects of modification in stem cell therapy



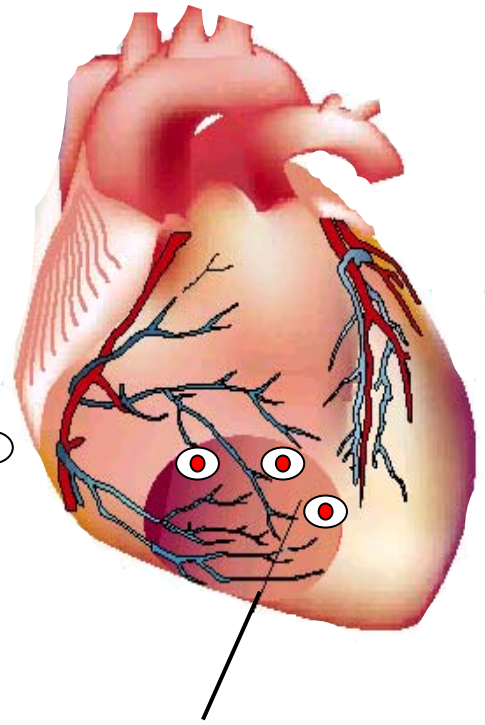
골 수

Ex vivo expansion



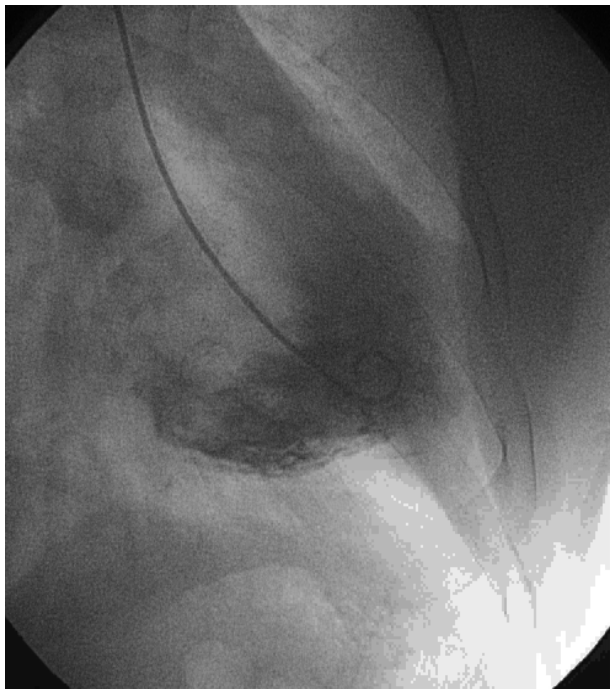
Mobilization

Homing

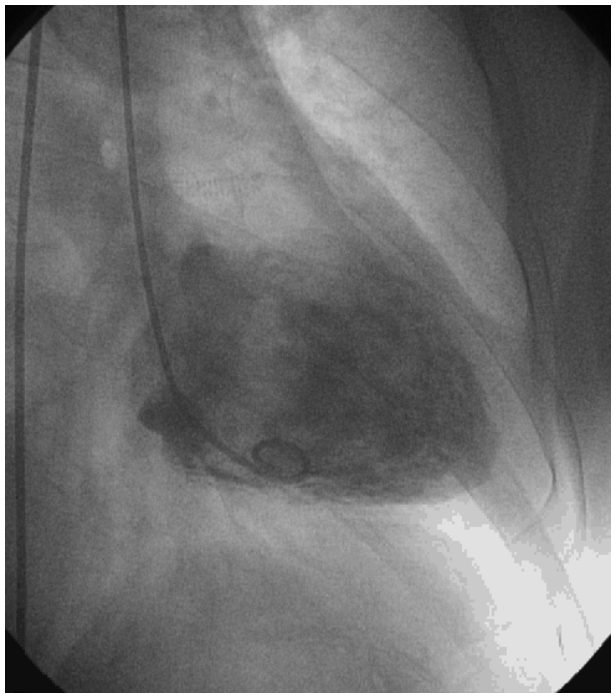


허혈 심근

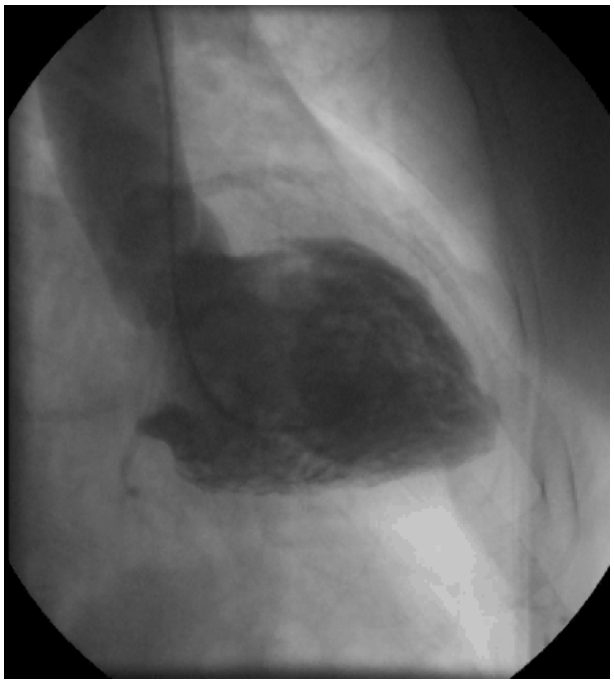
골수 줄기 세포 심혈관 내에 직접 주입



Baseline



6months follow up



1 year follow up

- 심근 경색 환자의 심장 동작 회복을 확인.

척수 손상

- 척수 손상으로 19년간 하반신 마비 환자의 척수에 제대혈 줄기 세포를 주입
- 일부 감각이 회복되는 것이 보고됨
- 안정성과 효과에 대한 추가 연구가 필요

청각 손상

Generation of hair cells by stepwise differentiation of embryonic stem cells

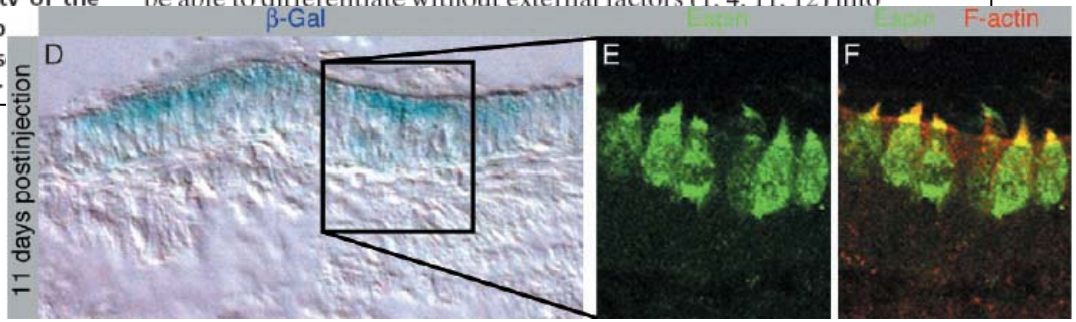
Huawei Li, Graham Roblin*, Hong Liu, and Stefan Heller†

Department of Otolaryngology and Program in Neuroscience, Harvard Medical School and Eaton–Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Boston, MA 02114

Edited by A. James Hudspeth, The Rockefeller University, New York, NY, and approved September 17, 2003 (received for review July 17, 2003)

The increase in life expectancy is accompanied by the growing burden of chronic diseases. Hearing loss is perhaps the most prevalent of all chronic diseases. In addition to age-related hearing loss, a substantial number of cases of audiological impairment are either congenital in nature or acquired during childhood. The permanence of hearing loss is mainly due to the inability of the cochlear sensory epithelium to replace lost mechanorecep or hair cells. Generation of hair cells from a renewable s progenitors that can be transplanted into damaged inner

factors that have been shown to mitogenically or trophically promote inner ear progenitors, such as EGF, IGF-1, and basic fibroblast growth factor (bFGF), could be used to selectively enrich the cell population for inner ear progenitor cells. After withdrawal of mitogenic supplements, these progenitors should be able to differentiate without external factors (1, 4, 11, 12) into



- 2003년 하버드 의대의 연구 결과 배아 줄기 세포를 유모 세포로 분화시키는 데에 성공함으로써 줄기 세포로 청각 손상 회복의 전망

줄기 세포 이용한 세포 치료의 문제점

- 줄기세포의 대량 증식
- 원하는 세포로의 분화
- 면역학적 거부반응
- 주입 시 목적 기관 위치
- 이식 후 환자에서의 기능 유지
- 기형 종 (암세포 등) 으로 발전

세계 각국의 줄기 세포 연구 현황

- 미국
  - 1998년 인간 줄기 세포 배양 세계 최초 성공
  - 2002년 인간 줄기 세포를 혈관 조직으로 분화 성공
  - 줄기 세포 분화 분야에서 선두적
  - 배아 줄기 세포 연구에 비교적 소극적이었으나, 캘리포니아 주에서 Proposition 71 통과(2005)로 매년 3조 투입
- 영국
  - 1997년 세계 최초로 포유류(돌리 양) 복제 성공
  - 2004년 인간 복제배아 연구 승인
  - 세계 최초로 세계 줄기 세포 은행 설립
  - 줄기세포를 국가 중점 연구개발 과제에 포함
- 일본
  - 복제 배아보다 임상에 활용할 연구에 주력
  - 2004년 줄기세포로 혈관 분화 성공
  - '복제 배아를 줄기세포나 신경세포로 분화해 만든 새로운 약의 효과 시험', '복제 배아에 특정한 유전자를 삽입 혹은 제거해 질병의 발생 원리 규명' 등 두 연구 분야에 대해 한해 100억원 투입

선진국과의 기술 격차

- 전반적 기술은 선진국의 60~70%수준
- 배아 줄기 세포 획득이나 배양 기술은 세계적
- 지속적인 투자가 필요한 생명 공학 분야의 특성상 모든 분야에서 선진국을 따라잡기는 힘들 것
- 특히 세포 분화와 동물 실험 연구에서 협력 연구가 필요

ㅡ과학 기술부ㅡ

세포치료요법과 관련 기술에 관한 시장규모

단위 : 억달러

기 술	2005년	2010년	2015년
줄기세포	20	20	109
제대혈	5	10	23
조직공학	69	135	232
Blood transfusion products	128	224	350
세포기반 유전자요법	15	30	59
Encapsulated cell therapy	4	19	31
세포기반 암 백신	9	16	29
이종이식	6	19	32
요소 기술(세포주, 세포배양액, 세포운반체)	20	57	98
총 계	266	562	963

자료 : **A Jain PharmaBiotech Report 2005**

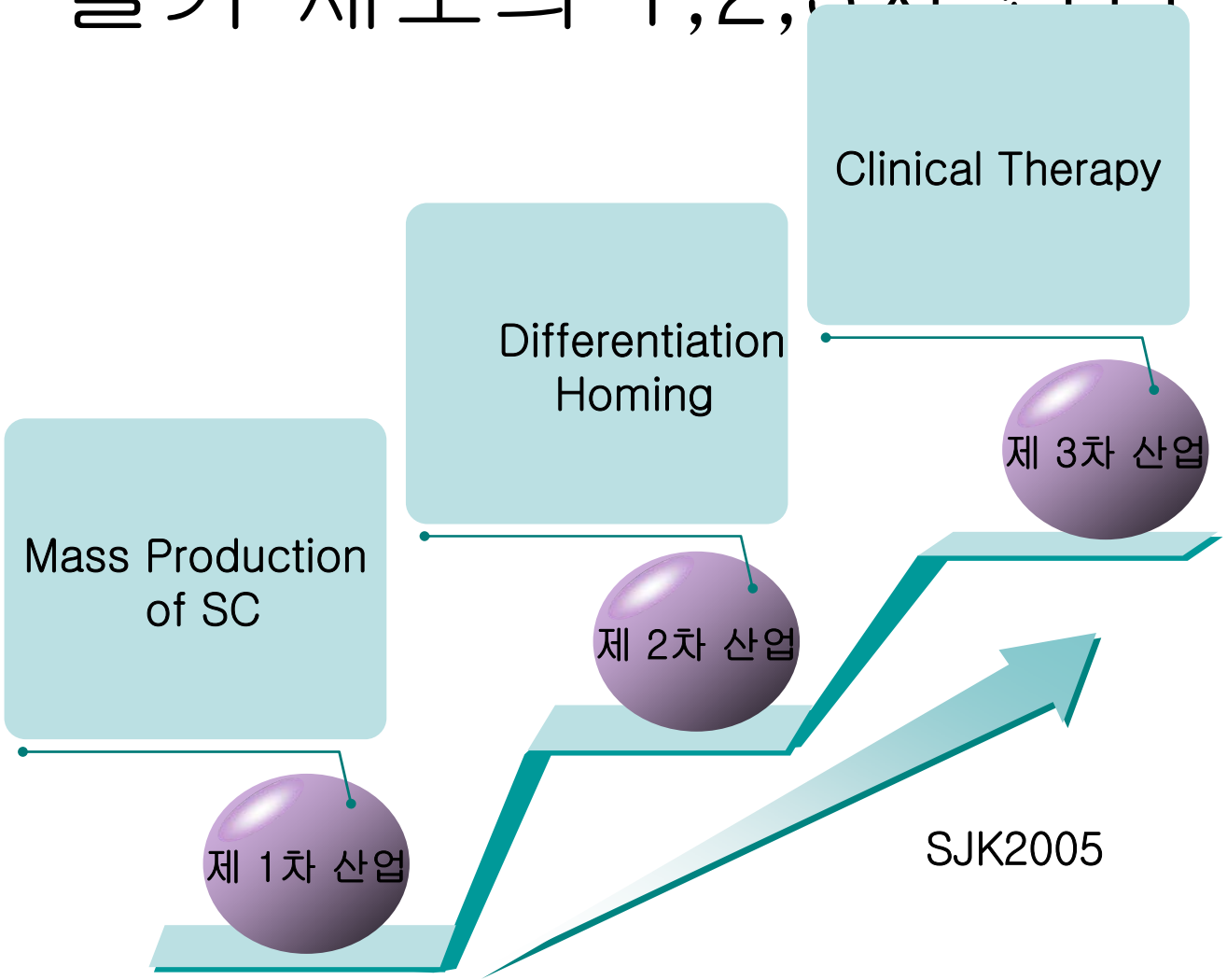
산업 전망

- 세포 치료 수요 증가
  - 2002년 세포이식 건수는 세계적으로 대략 50,000건으로 추정되며 2007년에는 159,000건으로 약 3배정도 증가할 전망
  - 현재 줄기세포가 차지하고 있는 비중은 전체 세포치료시장의 7.5%인 20억 달러에 불과하나 향후 10년간 18.5%의 고성장 예측
- 기업 환경
  - 막대한 연구 개발 비용이 필요한 관련 사업의 특성상 취약한 재무구조와 수익 모델을 확보하지 못한 벤처 기업들의 인수와 합병이 이루어 질 것으로 기대. 결국은 글로벌제약기업의 주도아래에서 이루어질 것.
  - 줄기 세포를 이용한 세포 치료 이외에 신약 후보 물질 테스트에 시장이 새롭게 떠오를 것임 (연간 10억 달러 시장 형성 기대)

기업 현황

- 국제 기업 현황
  - 세계적으로 85개의 줄기세포 관련 세포치료 회사가 존재
  - Aastrom, Stem-cell, Osiris Therapeutics, Lexion Therapeuticals 등의 바이오 벤처기업의 상업화 임상이 활발
  - Baxter, Novartis, Johnson & Johnson, Smith and Nephew, Boston Scientific, Medtronic, Wyeth, Schering, Becton Dickinson, Stryker, Genzyme과 같은 다국적 제약기업 및 의료기기회사의 참여 확대
  - 연구가 상용화 되어 투자를 회수하기 위해서는 장기간이 소요되나 열악한 재정환경을 가진 기업들은 감당하기 어려워 관련 기업들의 폐업 (Advanced Tissue Sciences, Artecel, StemRon 등), 자산매각 (Nexell Therapeurtics는 Baxter에 자산 매각), 합병(Diacrin와 GenVec과의 합병), 인수(Neurotech의 StemCell 인수) 등이 급증
- 국내 기업 현황
  - 줄기 세포주를 제외한 줄기 세포 분화 및 기전 연구 및 기술 확보 매우 취약하며 관련된 지적 재산권 확보도 미약
  - 취약 분야에 대한 공동 연구 부족: 세포치료제기술 특허동향 분석 자료에 따르면 한국특허에서 국제공동 연구에 의한 특허 점유율은 2.6%에 불과(특허청, '05. 5)
  - 분자 발생조절, 마커 및 항체개발, 세포신호전달, 생분자, 생물정보학 등의 전문 인력 부족
  - 줄기세포은행, 제대혈 네트워크, 전임상 및 임상시험 등의 인프라 미흡
  - 국내 줄기 세포 기업의 자본 구조는 매우 취약

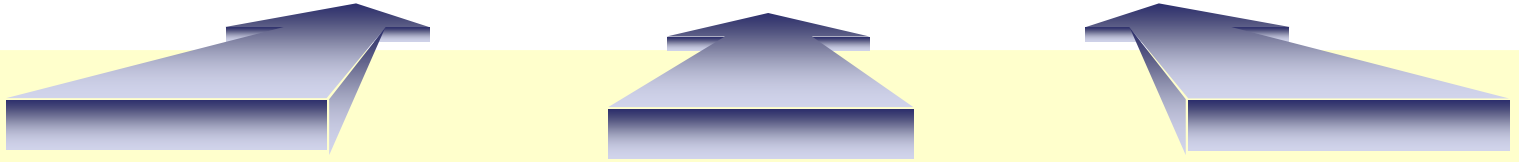
Where are we?
줄기 세포의 1,2,3차 산업



CAN WE MAKE MONEY?

- 치료의 불 특허성
- 그러나 상업적 기술을 이용한 치료에는 기술료 부과 가능
- 줄기 세포주도 특허가능
- 20년 동안만 보장: 상업화에 걸리는 시간+그 동안 유사기술이 나올 가능성
- 장비 등에 특허가능

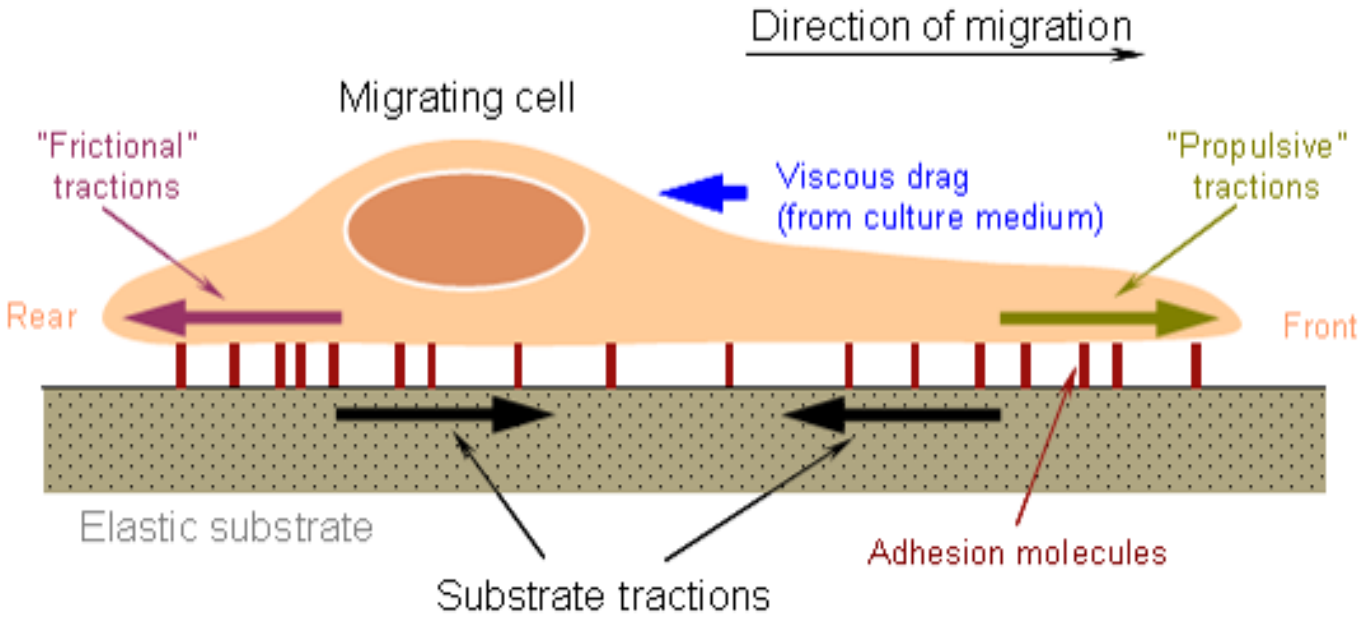
Be Wise

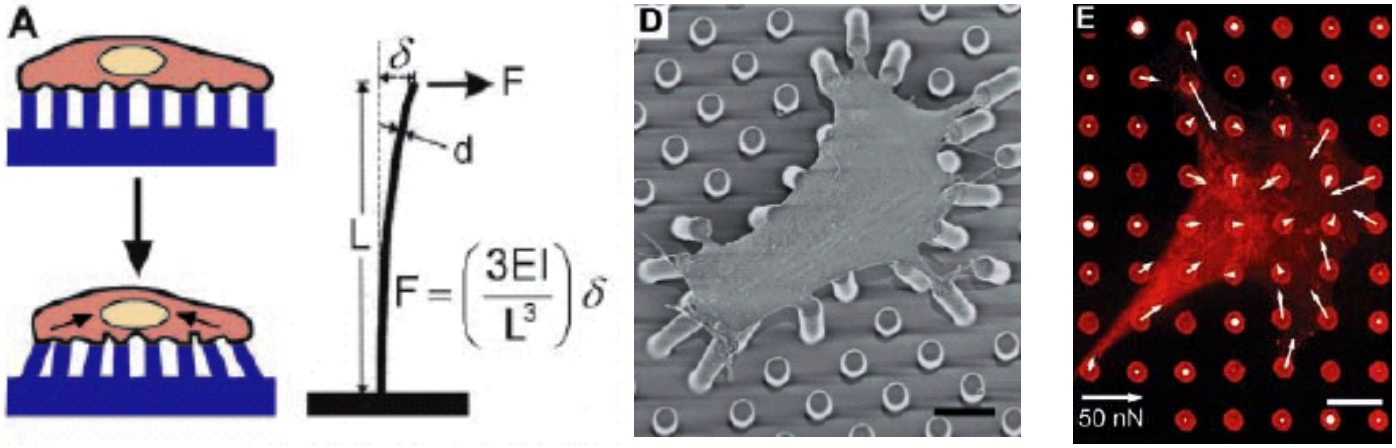


Biotech Economist group	Biotech Legal Advice group	Biotech Ethics monitoring group
●경제적 가치 평가 ●산업 모델 분석 ●산업 지원	●법적 제도적 장치 마련	●사회적 합의 도출 ●민간 사회 단체, 종료 단체

SJK 2005

A Cell Motility Study
Guidance of Cellular Growth

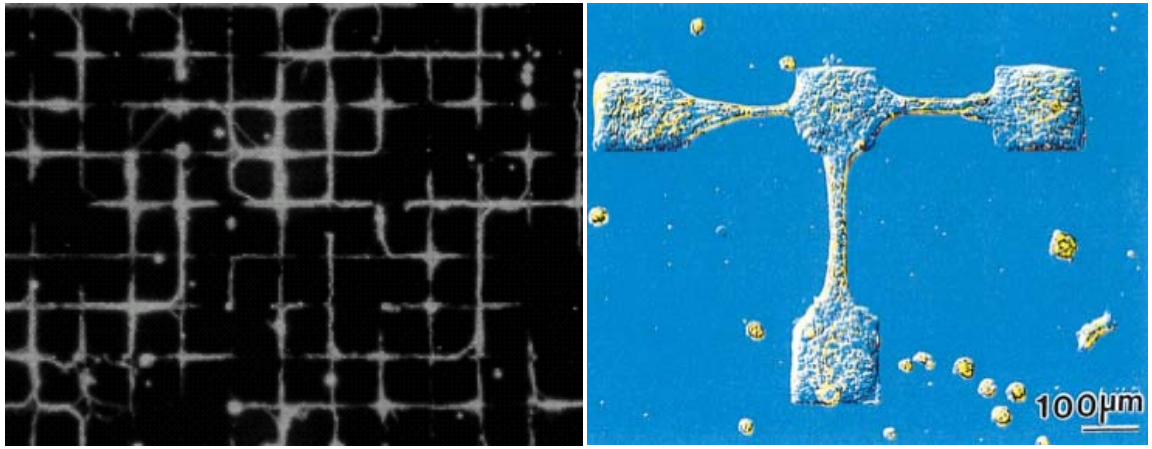




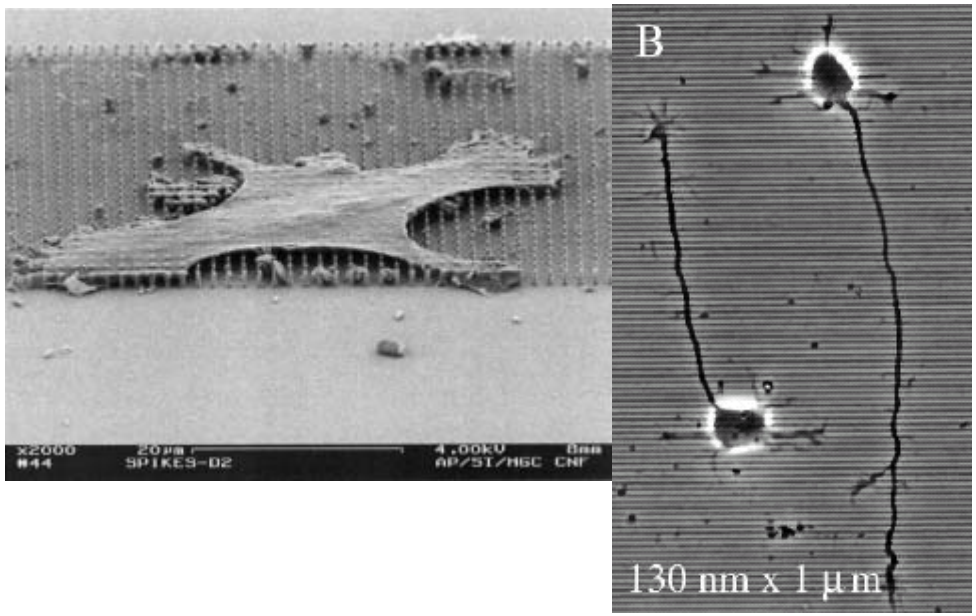
- *Tan J. et. al., Proc Natl Acad Sci. (2003)*
- The tractions can be calculated from the recorded deflection of the posts
- Ability to control the compliance of the substrate geometrically instead of chemically
- No need for recording the unstressed state of the substrate, because the posts were manufactured with sufficient precision

Building Methods of Cellular Network

- **Chemical cue**
  - **Non-biological**
  - **Biological proteins**



- **Topographical cue**
  - **Cliffs, grooves, spikes, tubes, mesh and random roughness**



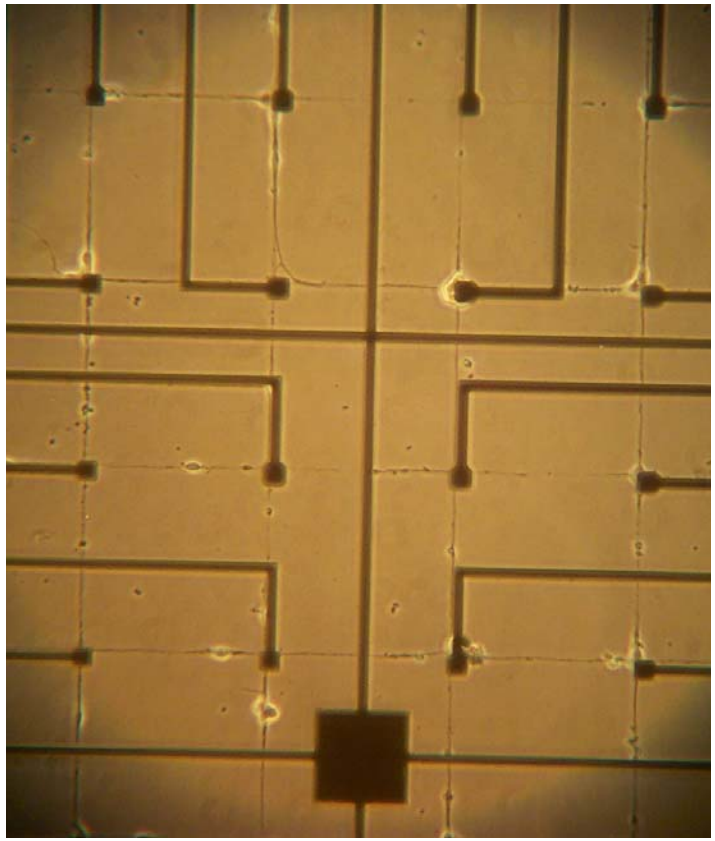
Research Goal

- Patterning neuronal network with the small number of cells
  - Previous studies on patterned neuronal network have focused on high density of cells and the collective characteristics such as firing rates from lots of cells.
  - In order to investigate the interaction between neurons in the network, patterning the small number of neurons is necessary.
  - The electrical connectivity should be maintained.

- What are the challenges?
  - Patterning polylysine on the electrode with very small dimension
  - Maintaining small number of neurons for long time.
  - Analysis of the patterned network in the respect of a single cell.

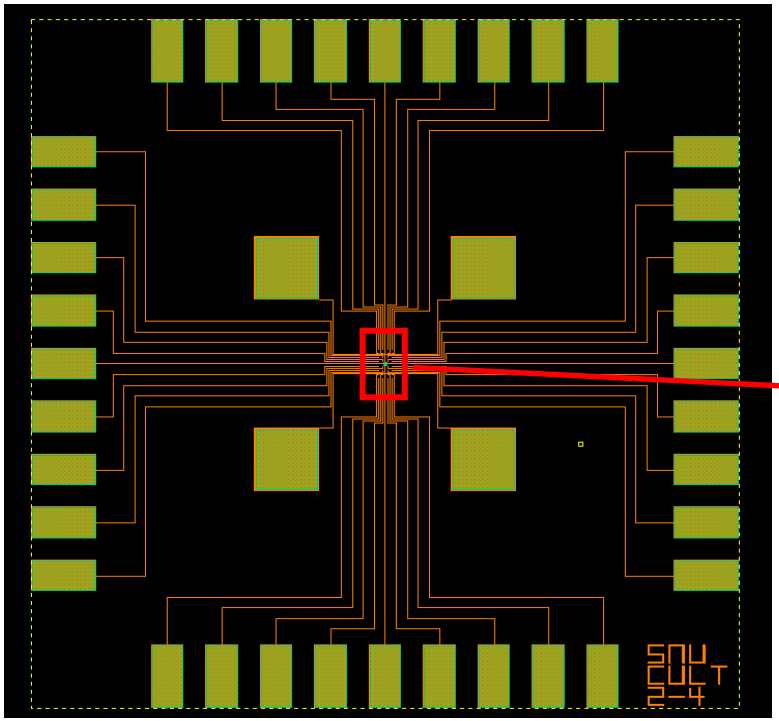
Why Microelectrode Array?

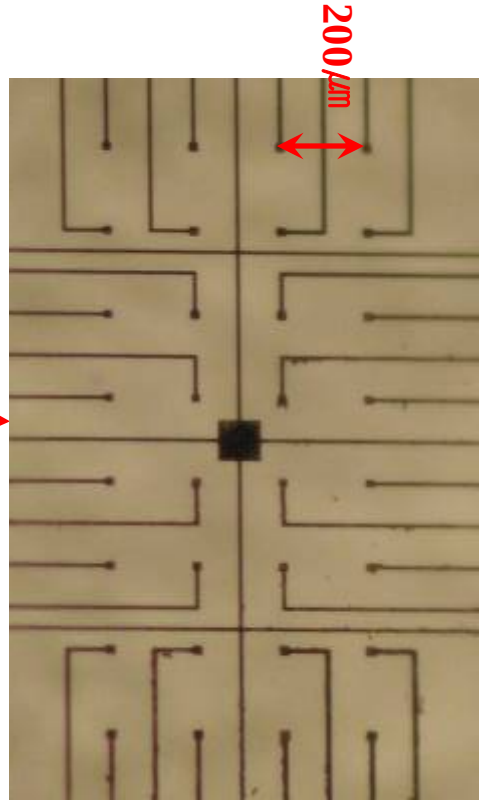
- Culture neurons for the long term
- Measuring neuronal responses simultaneously over a long period of time
- Precise Control of the environmental conditions around the neurons
- Direct visualization

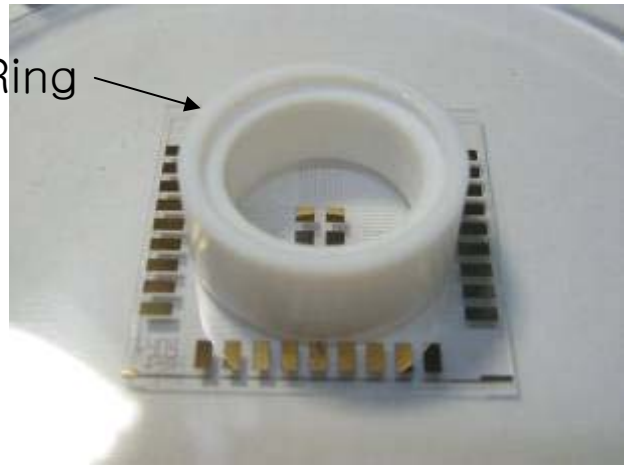


Hippocampal neurons at 5 days after culture

Microelectrode Electrode



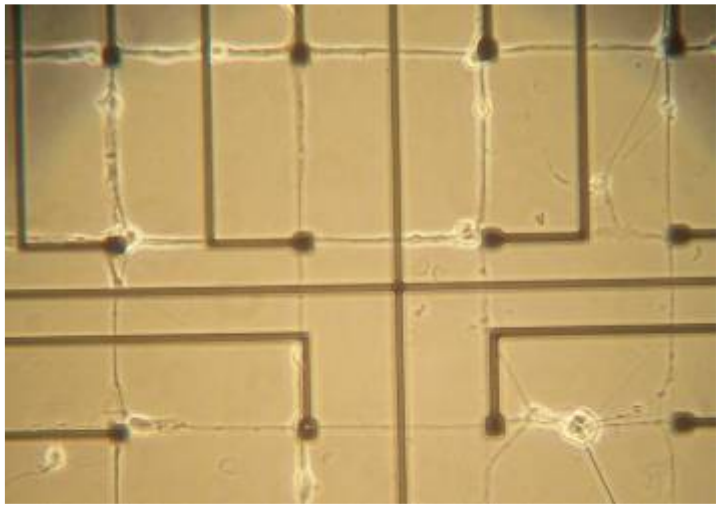


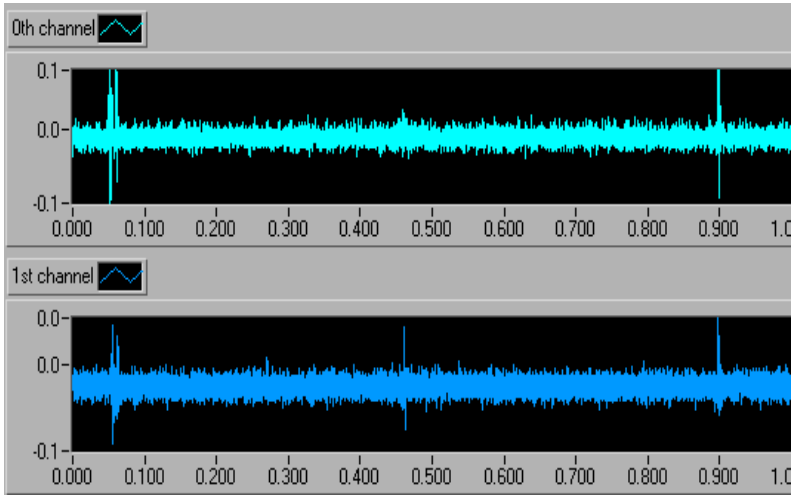


- 8 × 4 array
- Each electrode size : 10×10μm^2
- Interelectrode Spacing : 200 μm

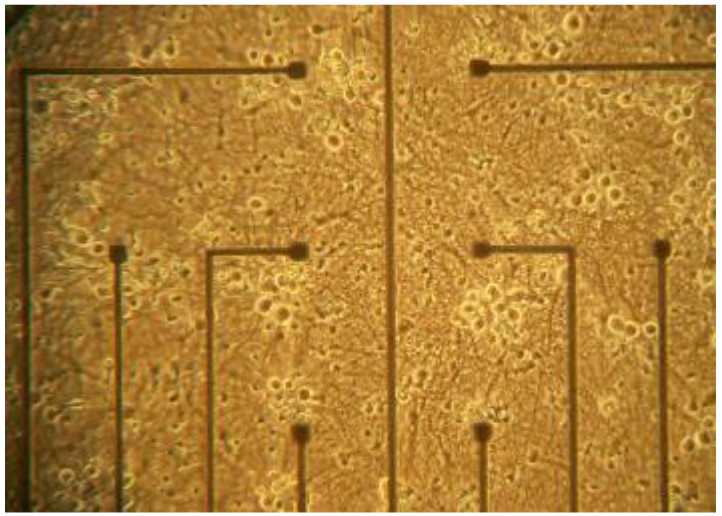
Reason to use patterned networks

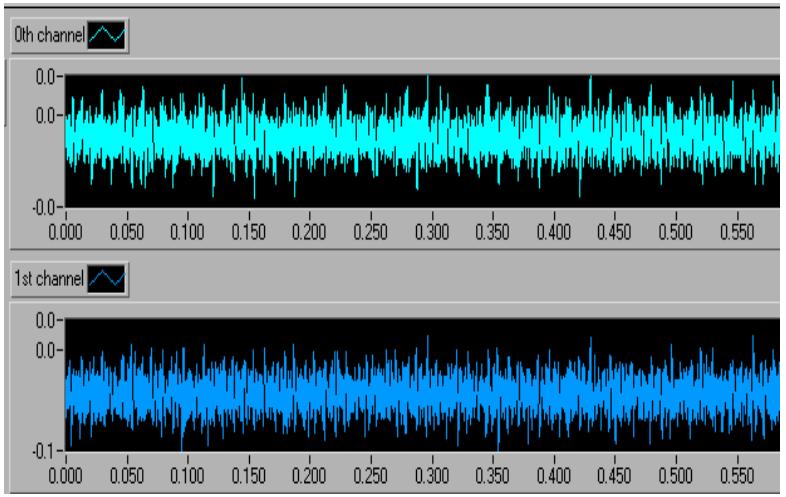
Patterned





Random



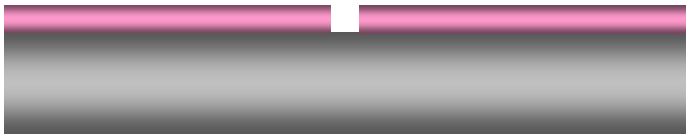


In order to get action potential from randomly cultured neurons, much more neurons should be plated than patterned neurons. Patterning neurons enhances activities of the network.

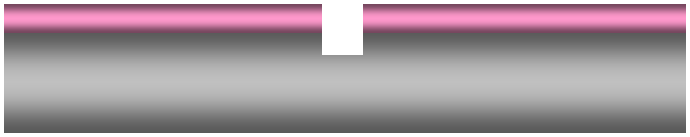
Fabrication of stamp master

Method 1

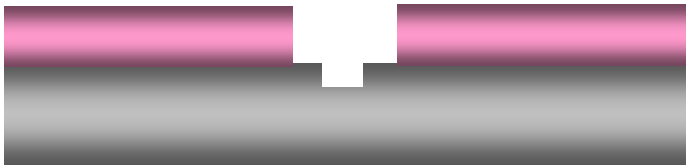
1) 1st Photo Lithography



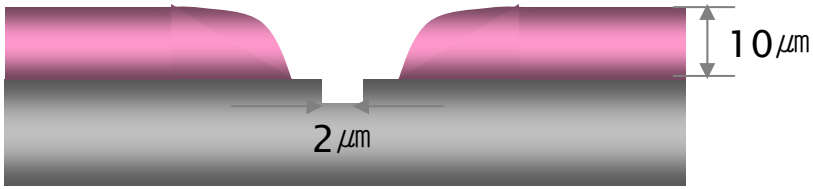
2) Silicon RIE-dry etching

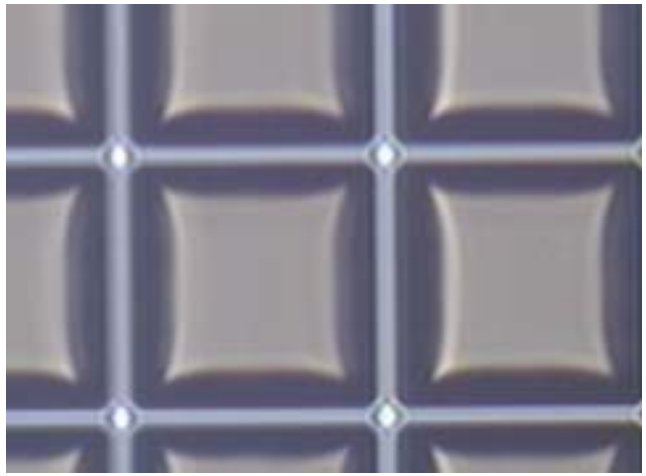


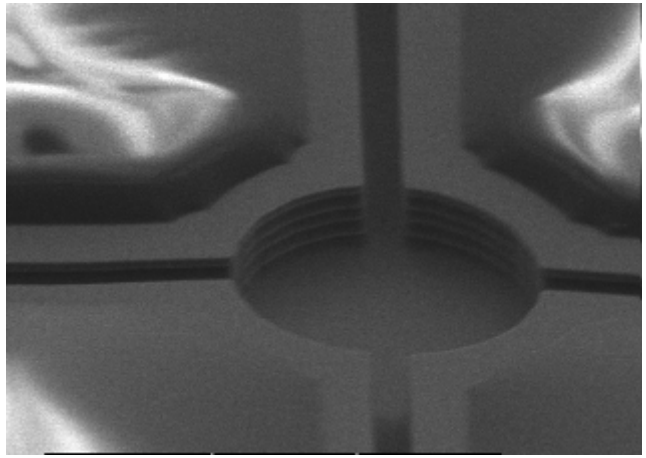
3) 2nd Photo Lithography



4) Photo Resist Reflow





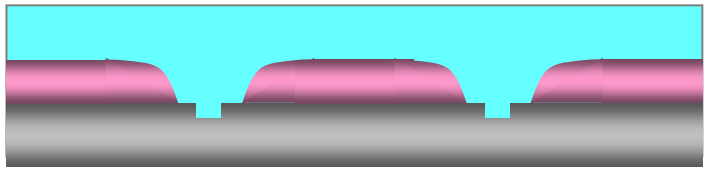


Shallow Si etch(3um)
and PR reflow(10um)

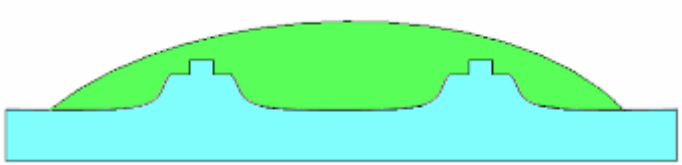
The supportive structure is needed for preventing the fine stamp pattern from collapsing especially for the very fine pattern like several microns.

Microstamping Procedure

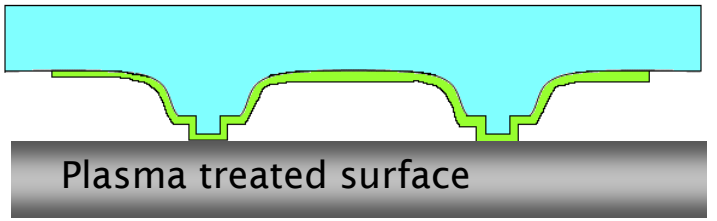
1) Sonication in 50% ethanol for 10 min
2) PDMS stamp molding with stampmater

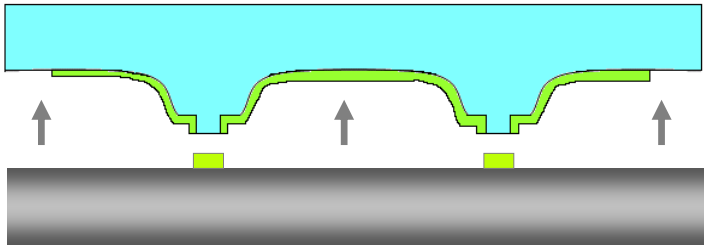


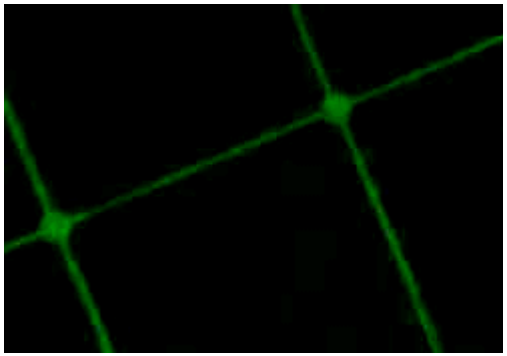
3) Poly-L-lysine (diluted in BBS) Inking for 2 hr



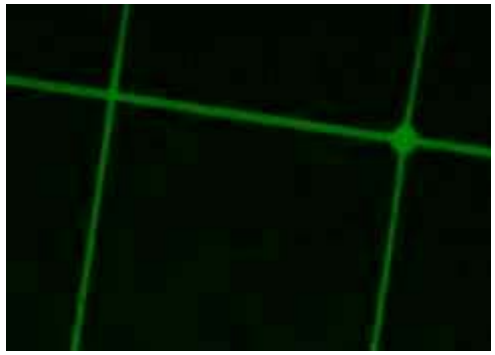
4) Stamping for 1 hr (50 g/cm^2)







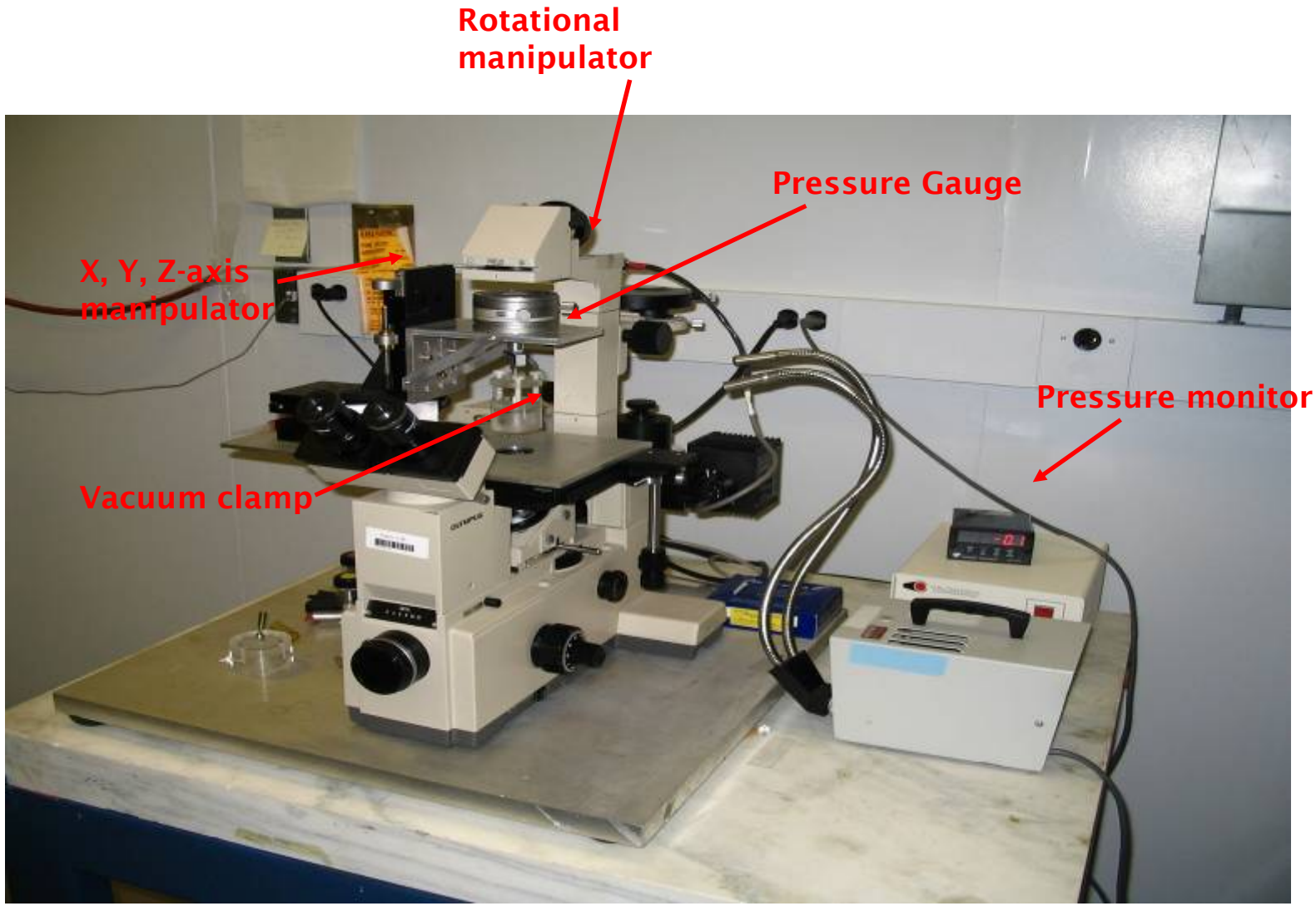
Line width = 2



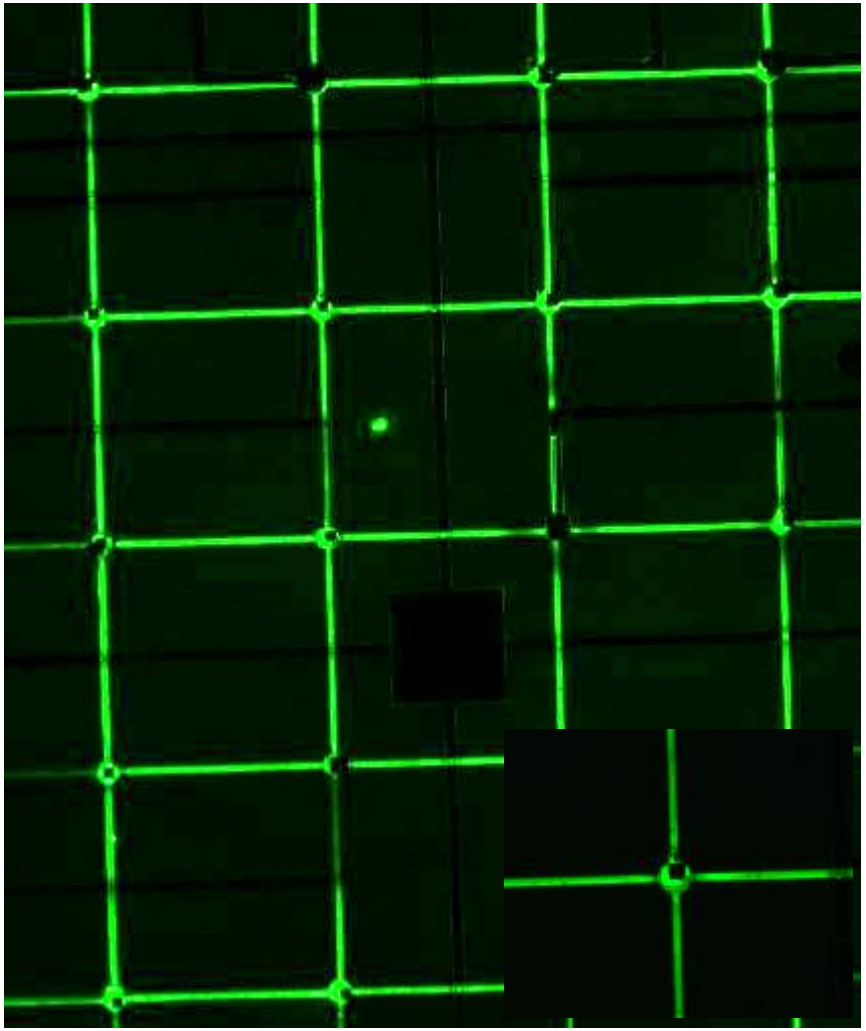
Line width = 4㎛

Line width = 6㎛

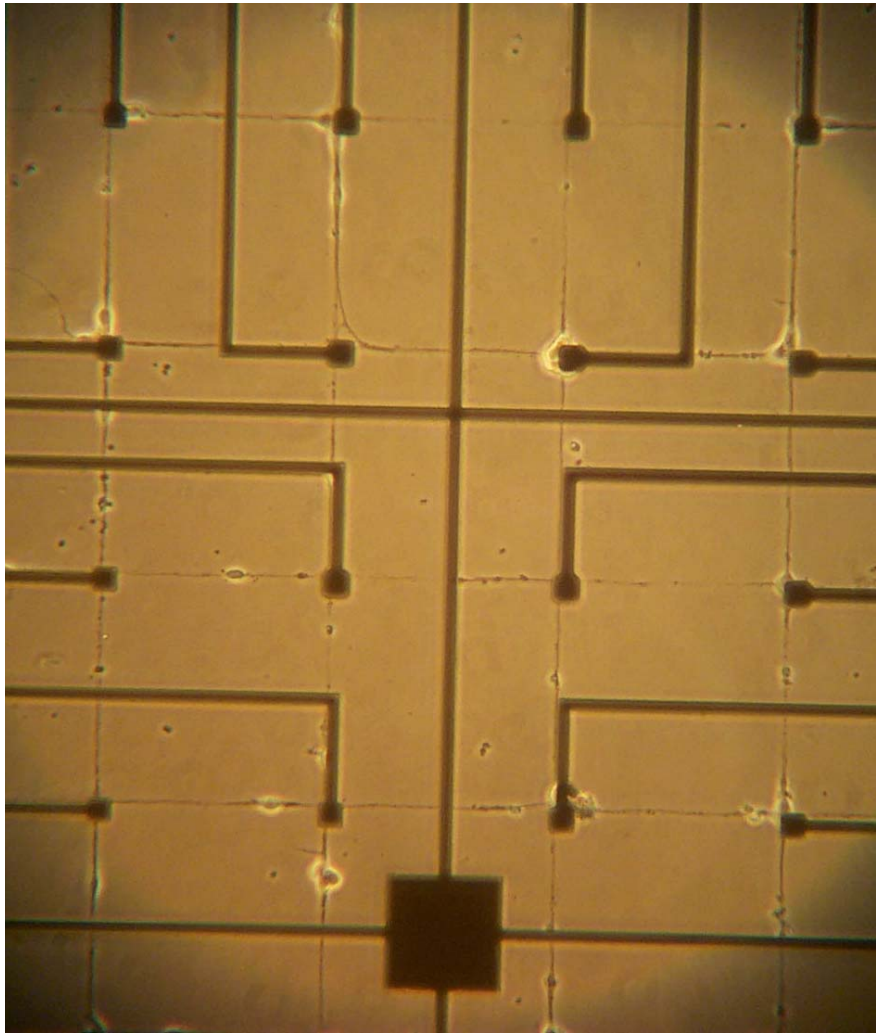
Aligner for Chemical Stamping



Stamped Surface



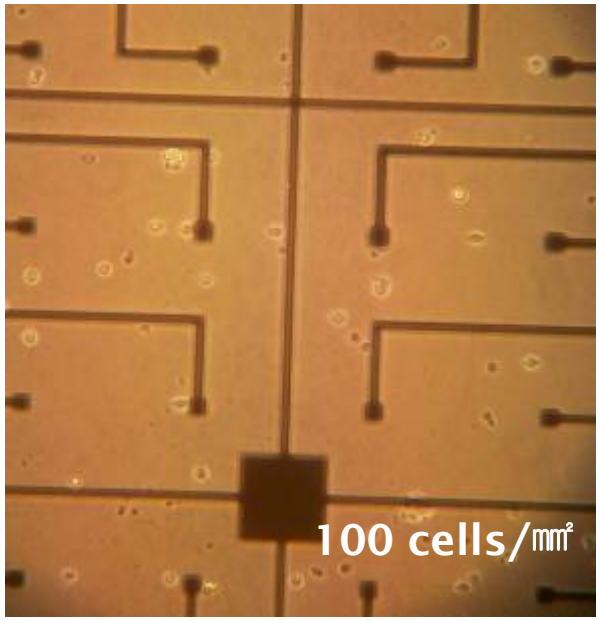
Stamped pattern on the microelectrode array
(FITC-labeled Poly-L-lysine)

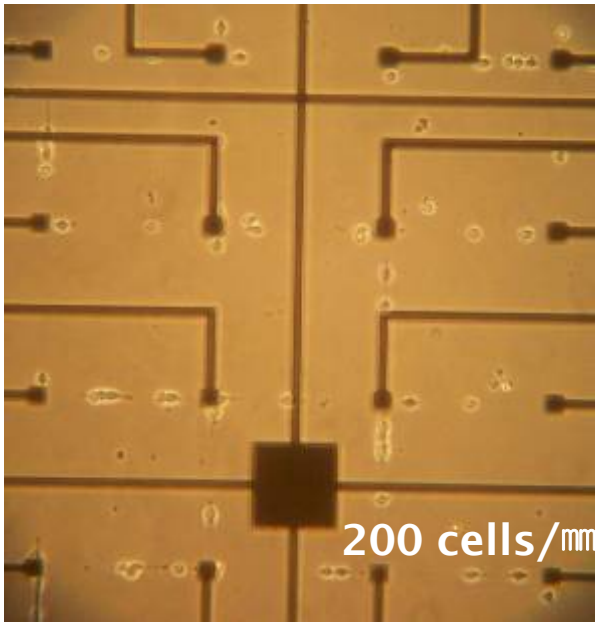


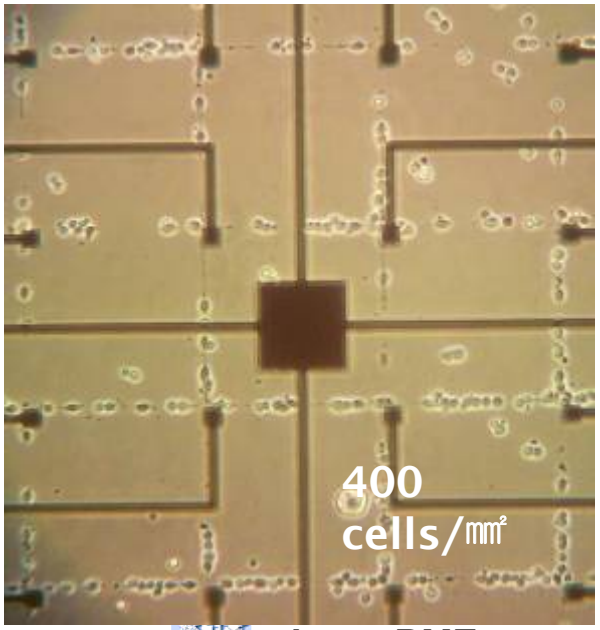
Hippocampal neurons at 5 days after culture

Neuronal Culture conditions

- Hippocampal neuron culture
  - Collected from 18-day gestation Sprague-Dawley rat embryos
  - Cultured in serum-free B27/neurobasal medium (25mM L-glutamine)
    - For culturing neurons only
  - Plating Cell density
    - 100 cells/㎟, 200 cells/㎟, 400 cells/㎟

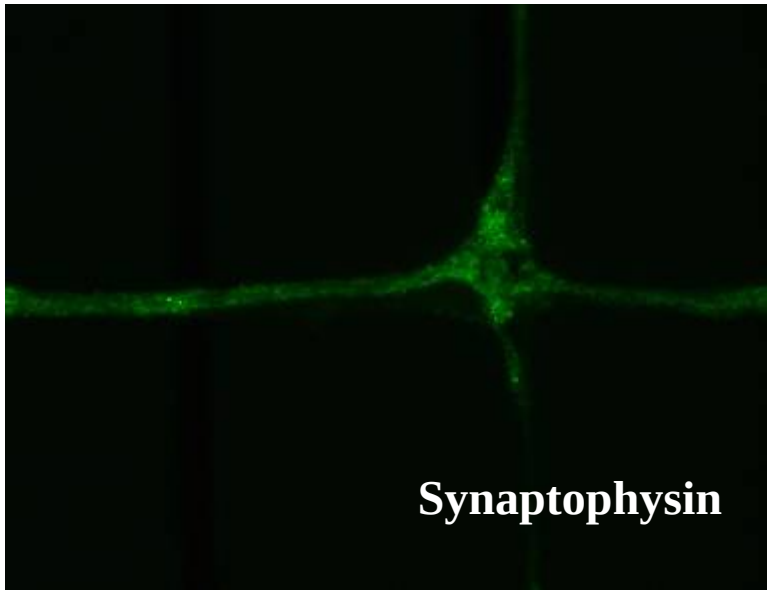


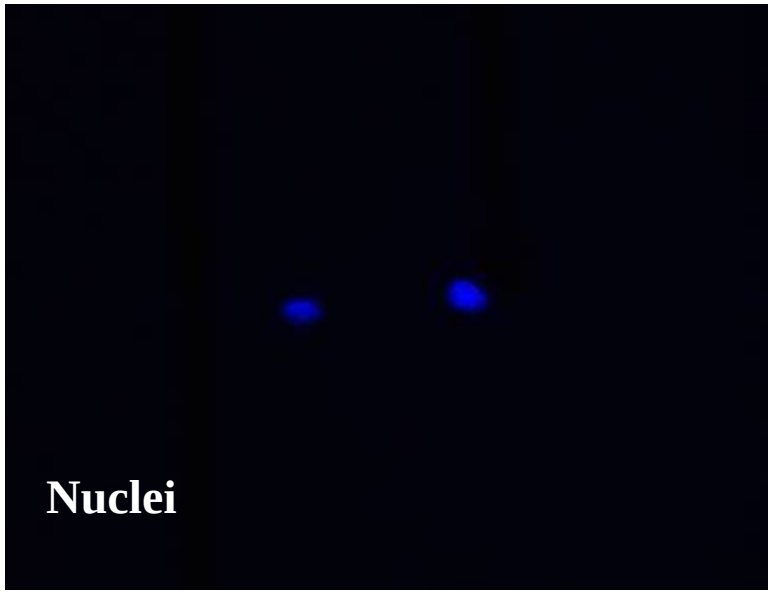


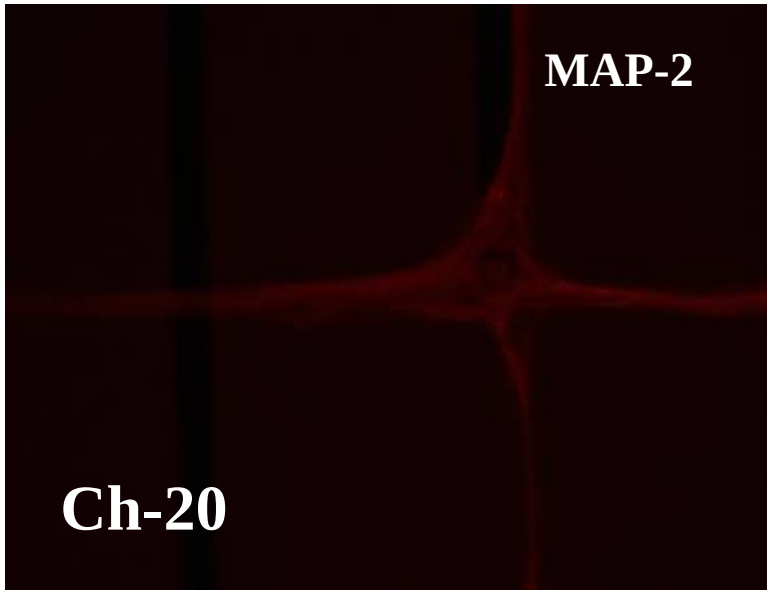


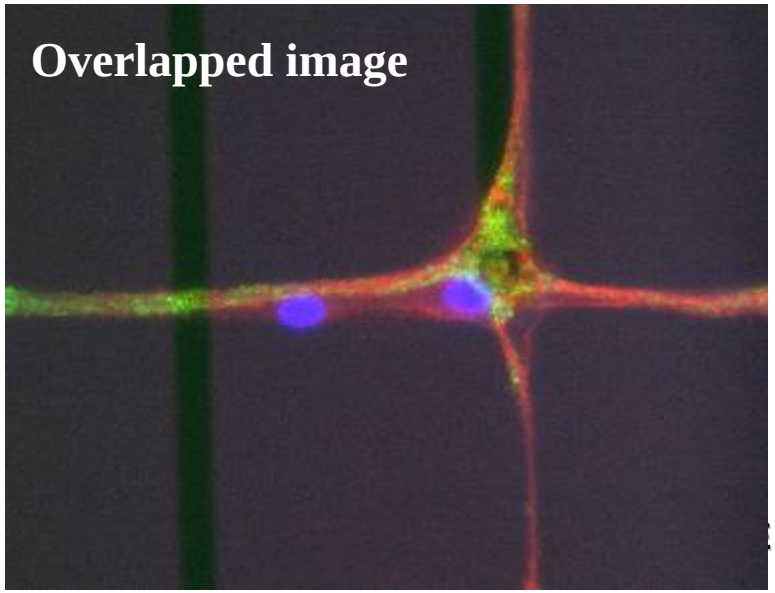
4 hours after plating cells

Immunocytochemical descriptions of cells in networks

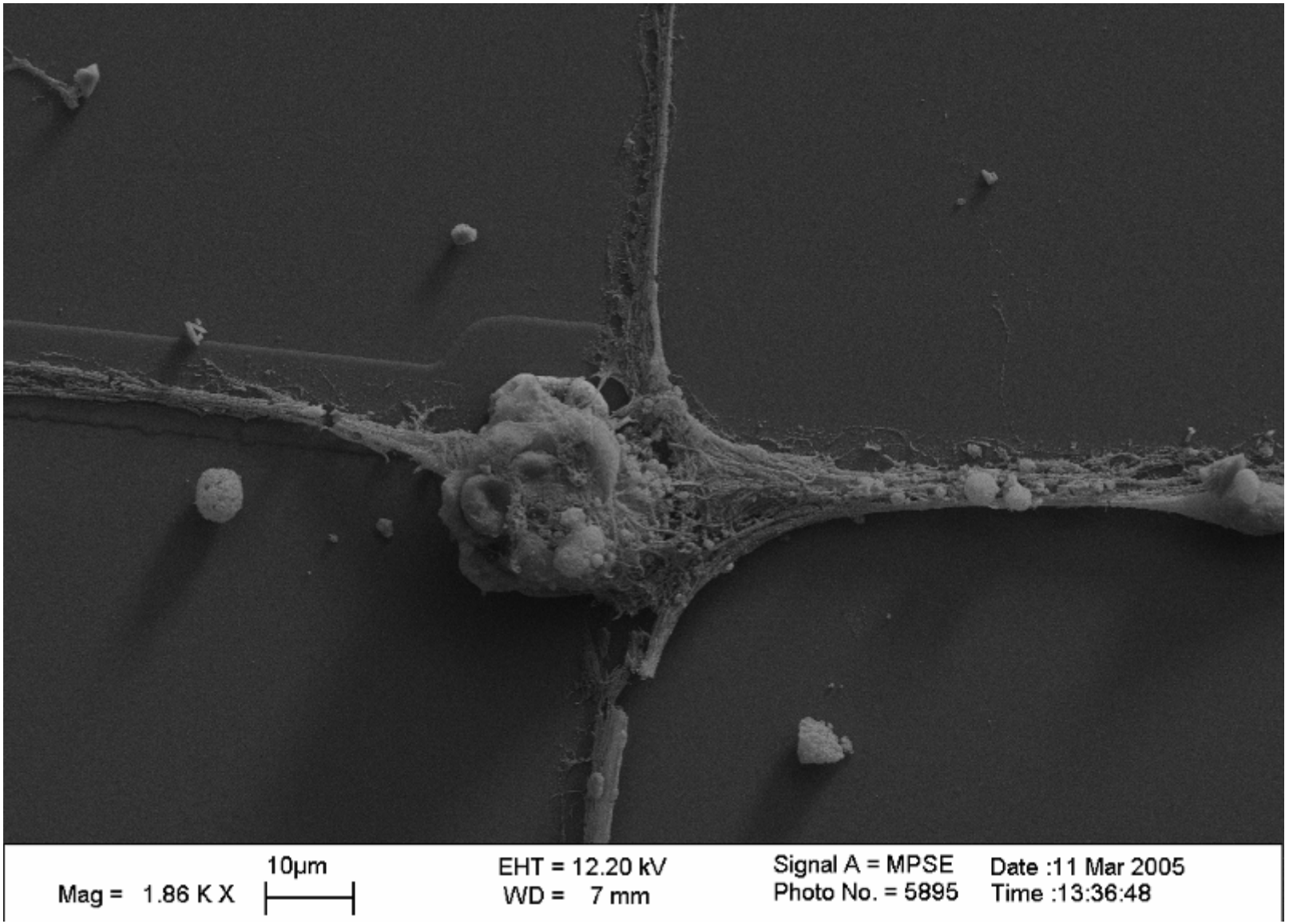








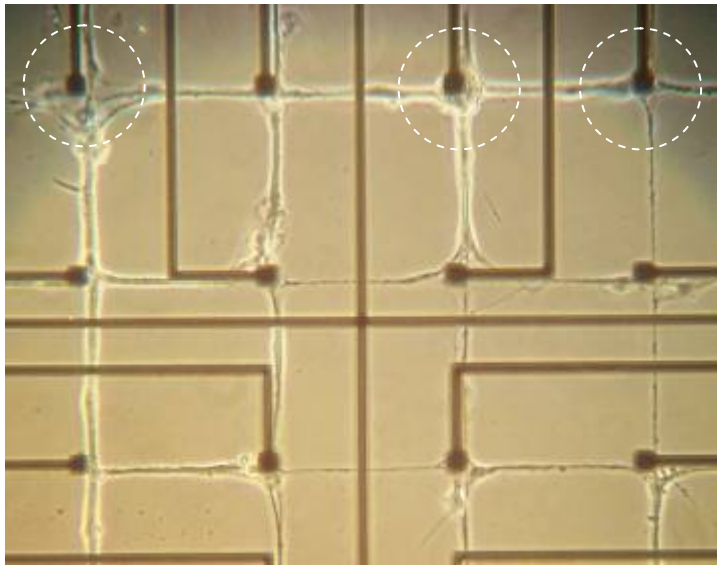
SEM image


Mag = 1.86 K X
10µm
EHT = 12.20 kV
WD = 7 mm
Signal A = MPSE
Photo No. = 5895
Date :11 Mar 2005
Time :13:36:48

Evoked Action Potential

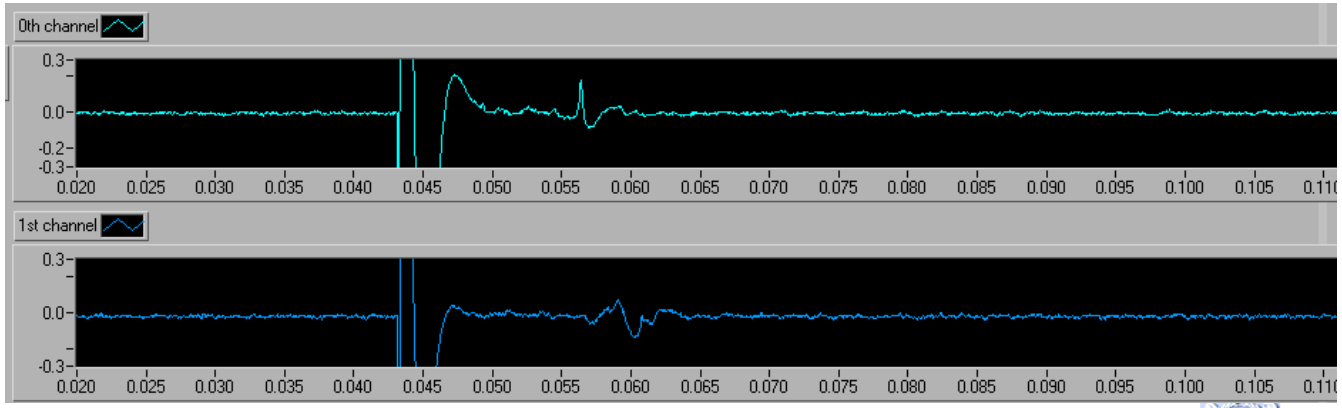
Stimulation electrode
I_{stim} = 100μA, 50ms

Ch 22 **Ch 20**



Ch22

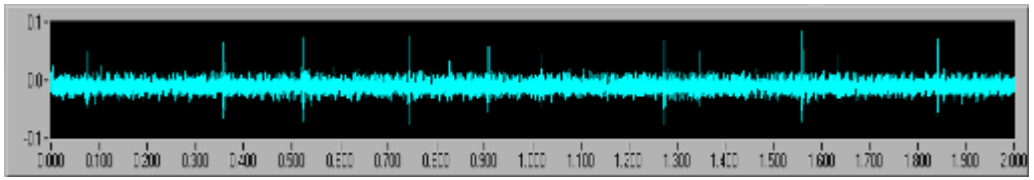
Ch20



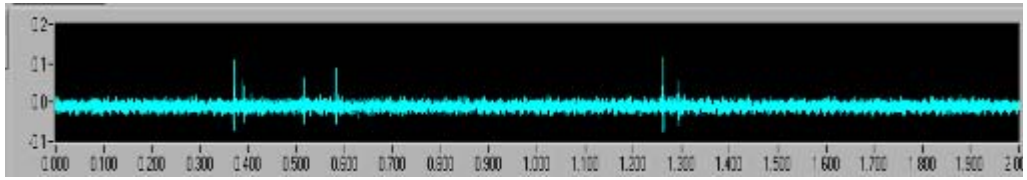
Pharmacological Study

- The spontaneous activities showed the dose-dependency to DNQX(6,7-dinitroquinoxaline-2,3-dione), AMPA receptor antagonist.

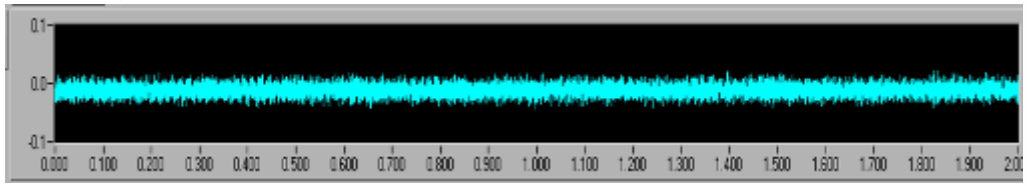
Before the treatment of DNQX



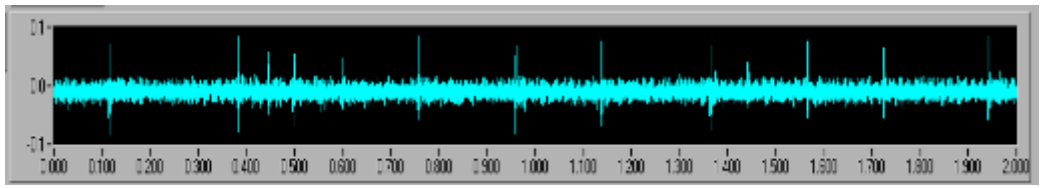
1μM DNQX

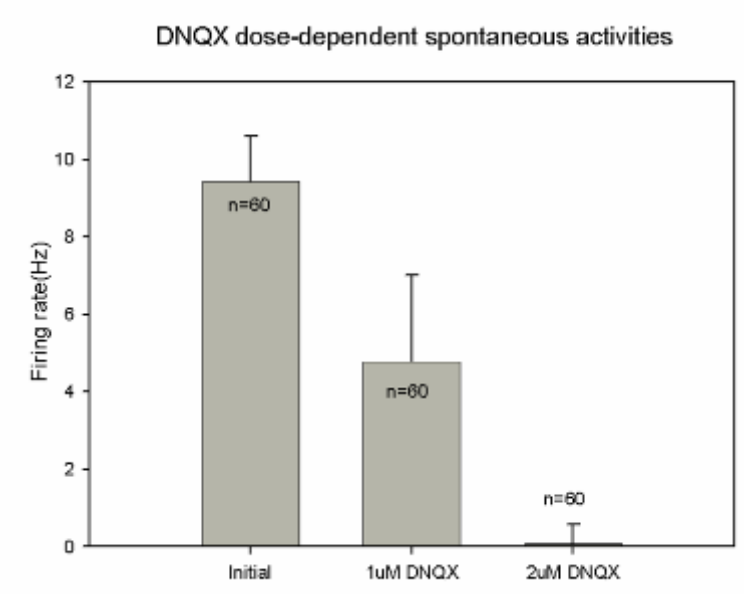


2μM DNQX



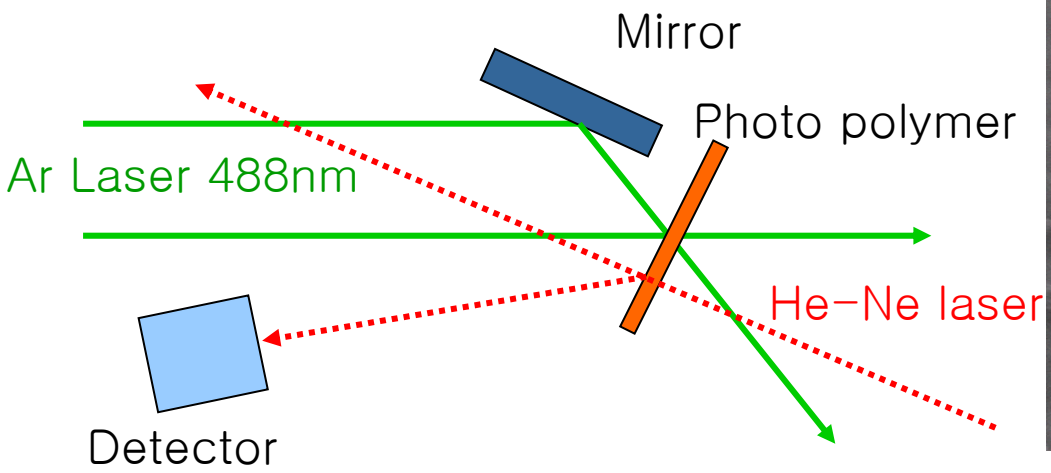
After washing out DNQX



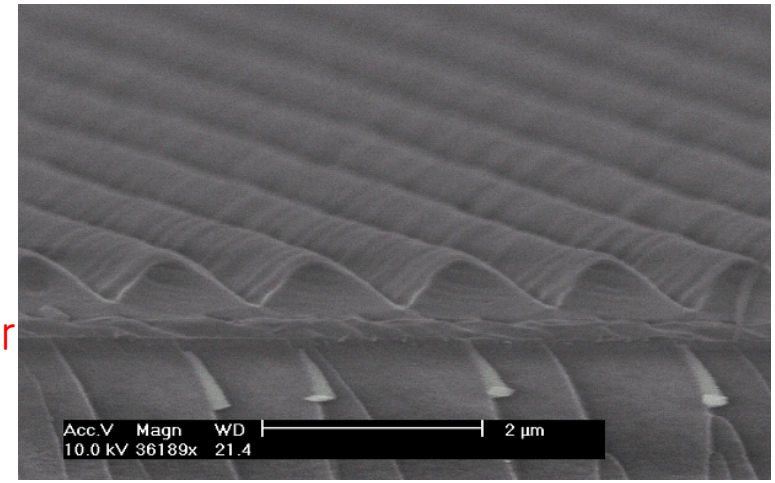


Topological Method for the Guidance of Cellular Growth

Surface Relief Grating Technique

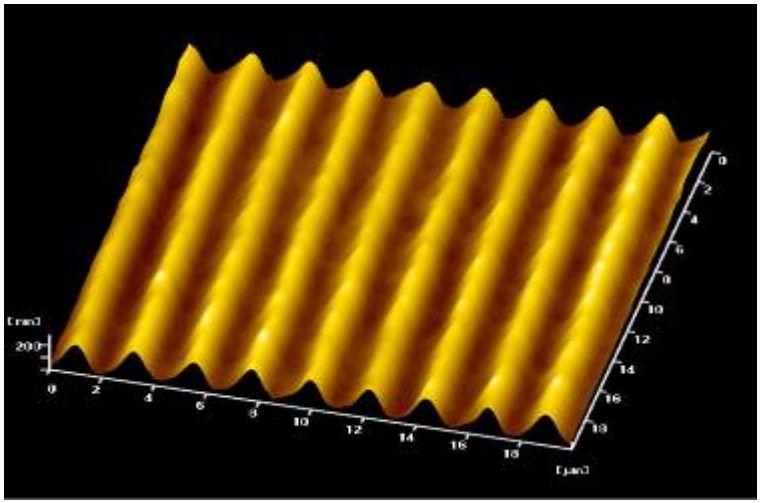


〈Optical SETUP〉



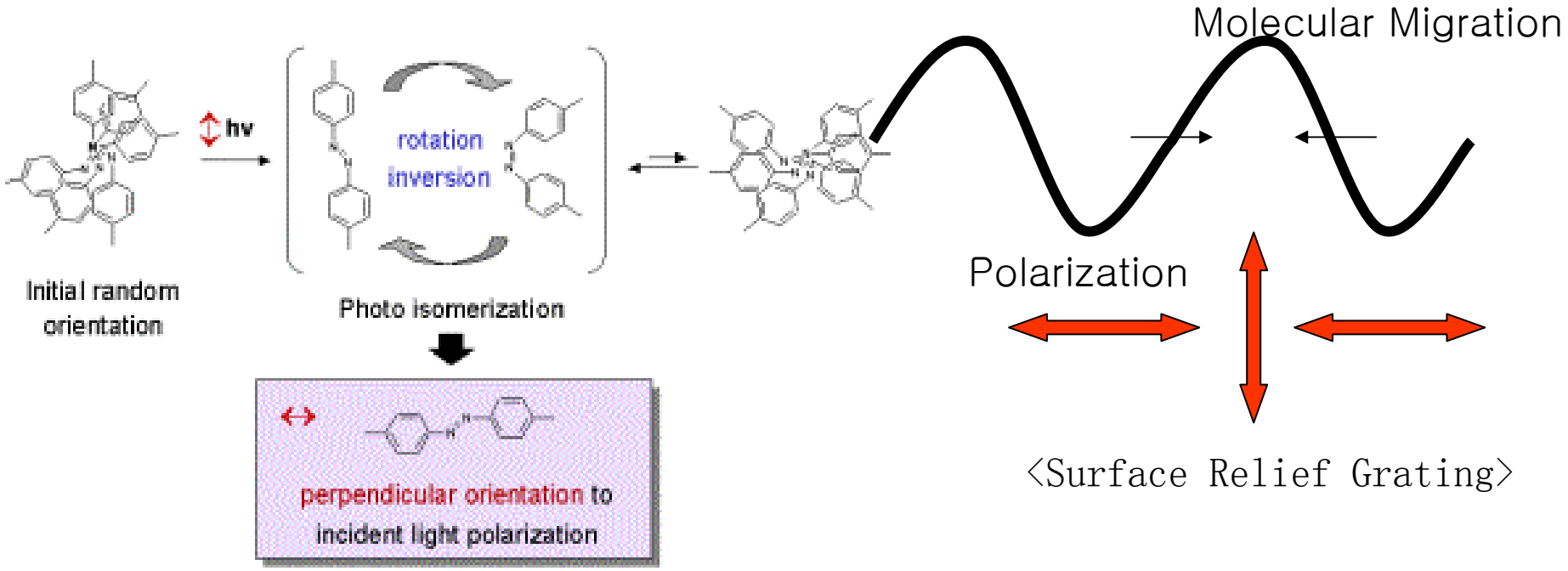
〈SEM Image〉

- **Phase Interference by Lloyd's mirror setup**
- **Forming regular sinusoidal grooves**
  - **SRG (Surface Relief Grating)**
- **Control of depth and width**
  - **Depth : Time of Beam Irradiation**
  - **Width : Incident angle of Beam**



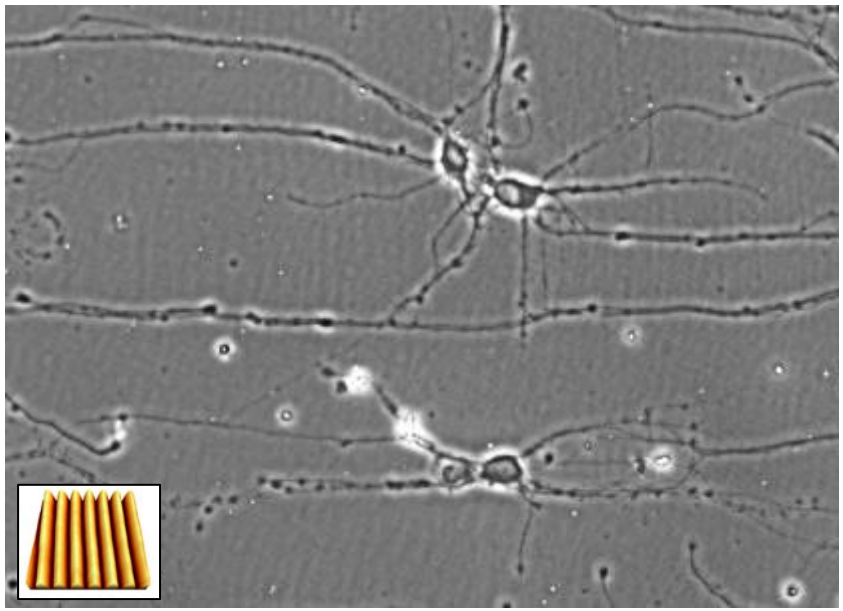
〈AFM Image〉

Photo-Responsive Polymer

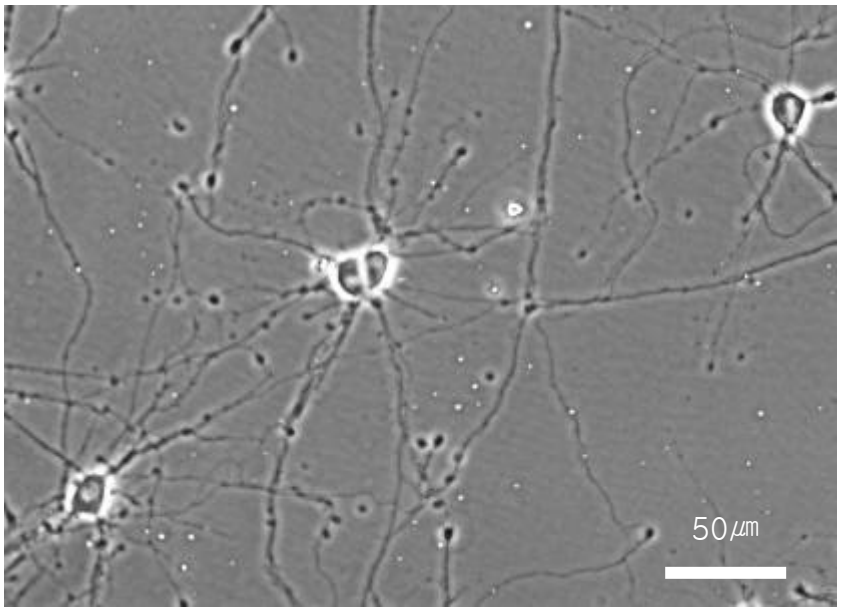


- **Photo-Responsive Azo-dye benzene copolymer**
- **$C_{12}H_{10}N_2$**
- **Cis-trans isomerization**
  - **Aligned parallel to axis of beam polarization**
- **Molecular Migration by modulation of polarization**

Reaction of Neuron to Grating



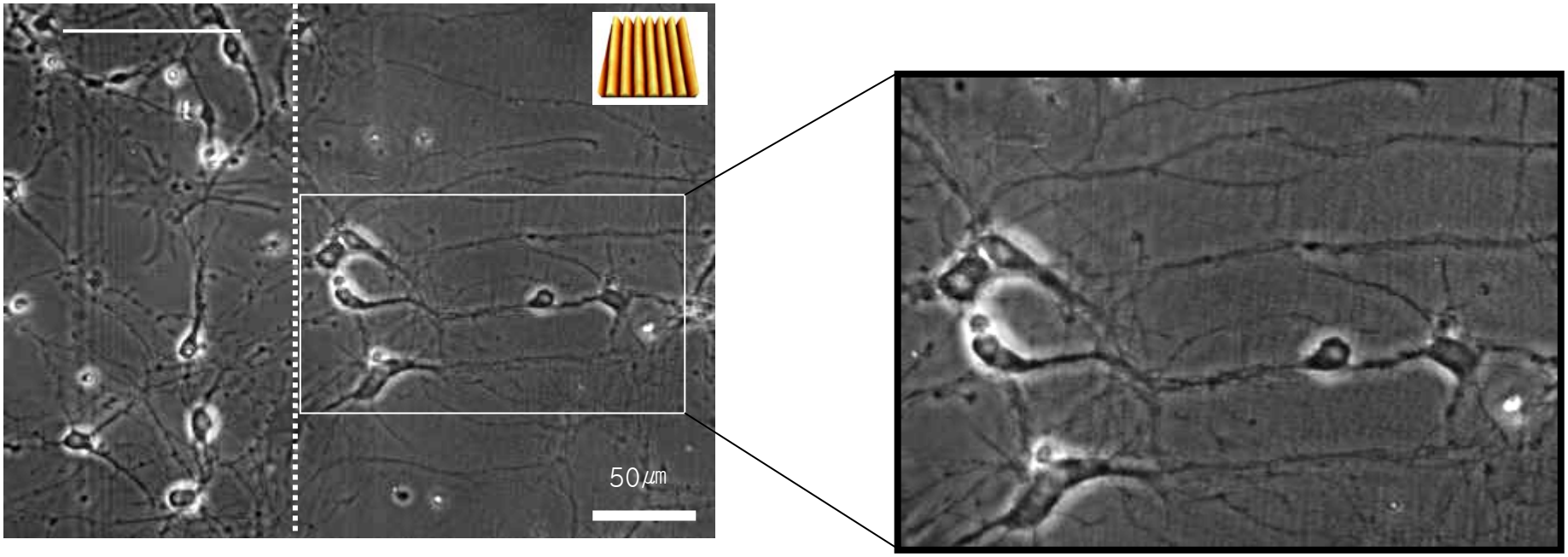
<Hippocampal neuronal cells On polymer with Grating >



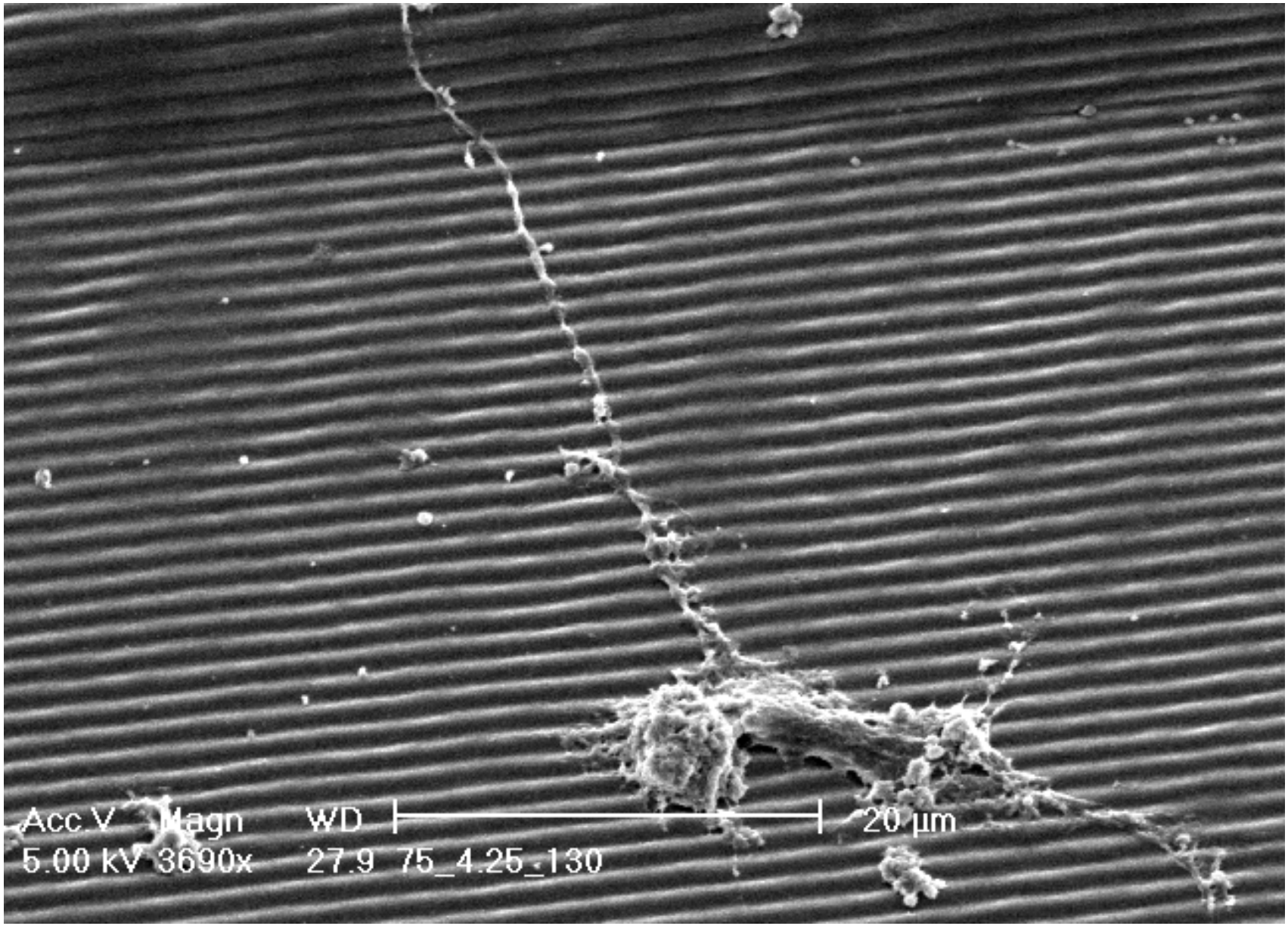
<On polymer without grating>

- **Cultured neurons on polymer with Grating**
- **Extending neurites perpendicular to groove direction**

Contact Guidance of Neuron

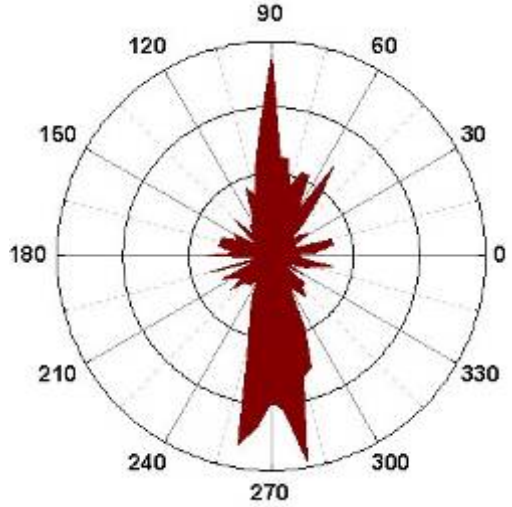


- **Random growth on smooth surface**
- **Perpendicular growth on the grooved surface**
- **Turning neurites on grating**

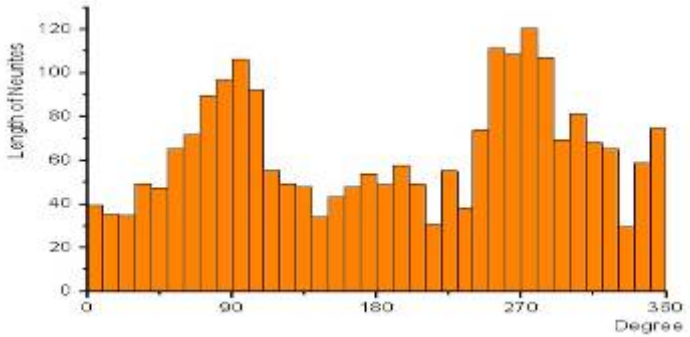

Acc.V Magn WD
5.00 kV 3690x 27.9 75_4.25_130
20 µm

Quantification of Cell Elongation

- **On Surface Relief**

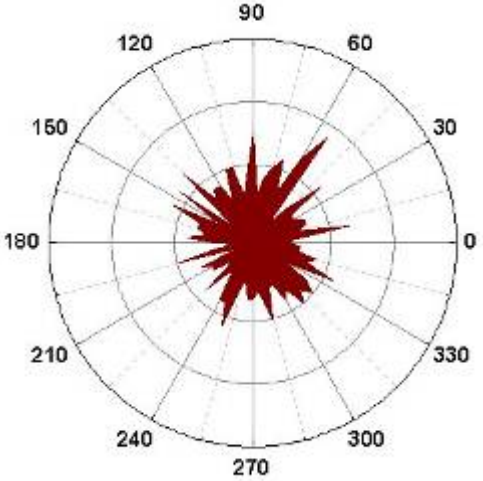


Frequencies of Neurites Orientation

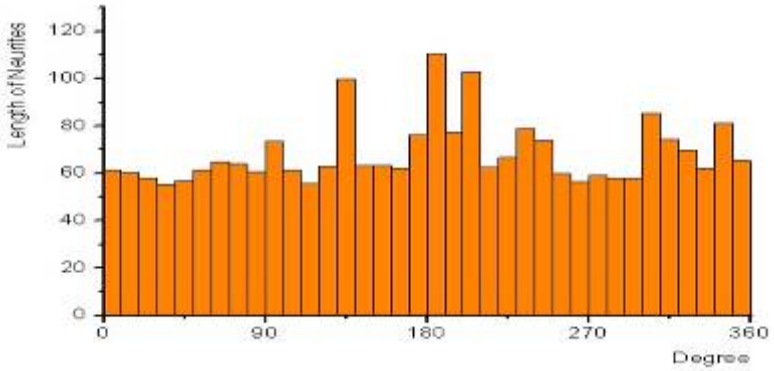


Lengths of Neurites Vs Angle

- **On Smooth Surface**



Frequencies of Neurites Orientation



Lengths of Neurites Vs Angle

- **Neurons show the best alignment on 1.4um wide, 450nm deep grooves**