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

Human ear grown on mouse

Tissue Engineering

- How were scientists able to get a human ear on a mouse?
- The experiment in which a human ear was attached to a mouse's back was perfomed in the laboratory of Dr. Charles Vacanti at the University of Massachusetts. He was assisted by Dr Linda Griffith-Cima from MIT. The study was done at the suggestion of a plastic surgeon who was interested in developing techniques for attaching ears in children who had external ear deformities or had lost ears in accidents. It was designed to serve as a model for tissue engineering. The mouse used for the study had a defective immune system so it was unable to reject the human tissue. The scientists created a ear-like scaffold of porous, biodegradable polyester fabric and then distributed human cartilage cells throughout this form. The entire construct was then implanted onto the back of the nude mouse. The mouse nourished the ear as the cartilage cells grew to replace the fiber. The mouse remained healthy and alive throughout. While the cartilage grew in the shape of an ear, it was not a functional ear since it lacked any connection to the nervous system or internal structure of the ear.
http://www.madsci.org/posts/archives/2000-06/961007439.Ge.r.html

Tissue Engineering

http://www.youtube.com/watch?v=0taE4F0Wkhg
CBS report on Tissue Engineering

http://www.youtube.com/watch?v=uIM0sSTwU9Q
Awarded Bob Langer

Objective

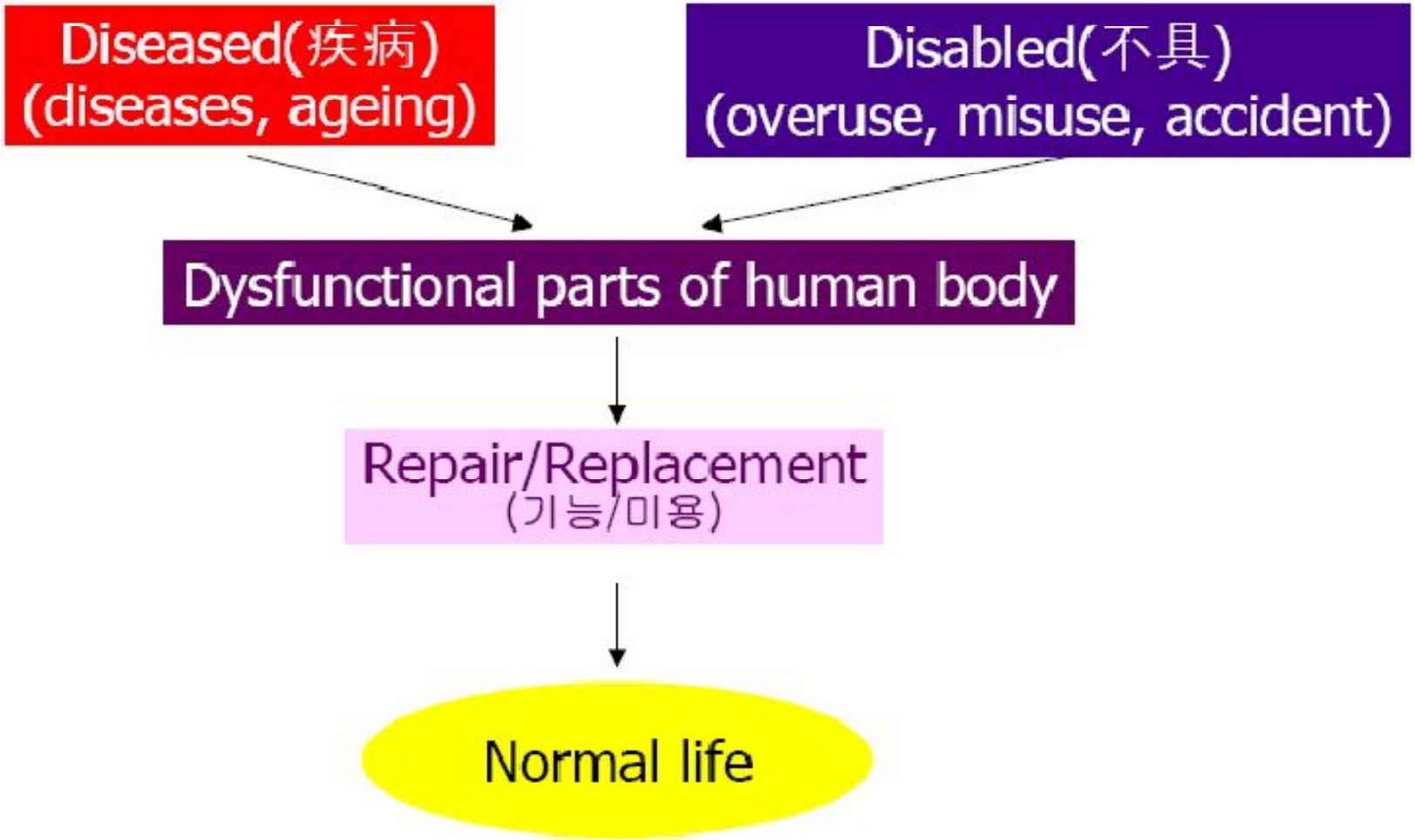
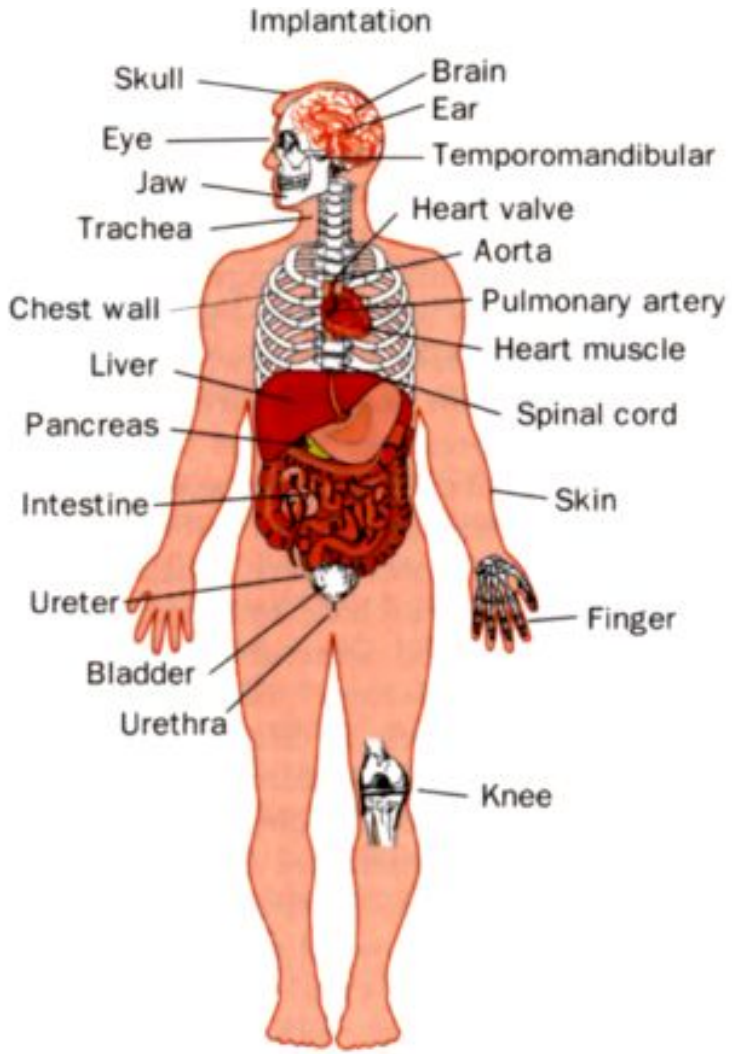

Diseased(疾病)
(diseases, ageing)
Disabled(不具)
(overuse, misuse, accident)
Dysfunctional parts of human body
Repair/Replacement
(기능/미용)
Normal life

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.



Two Methods for Artificial Organ Development

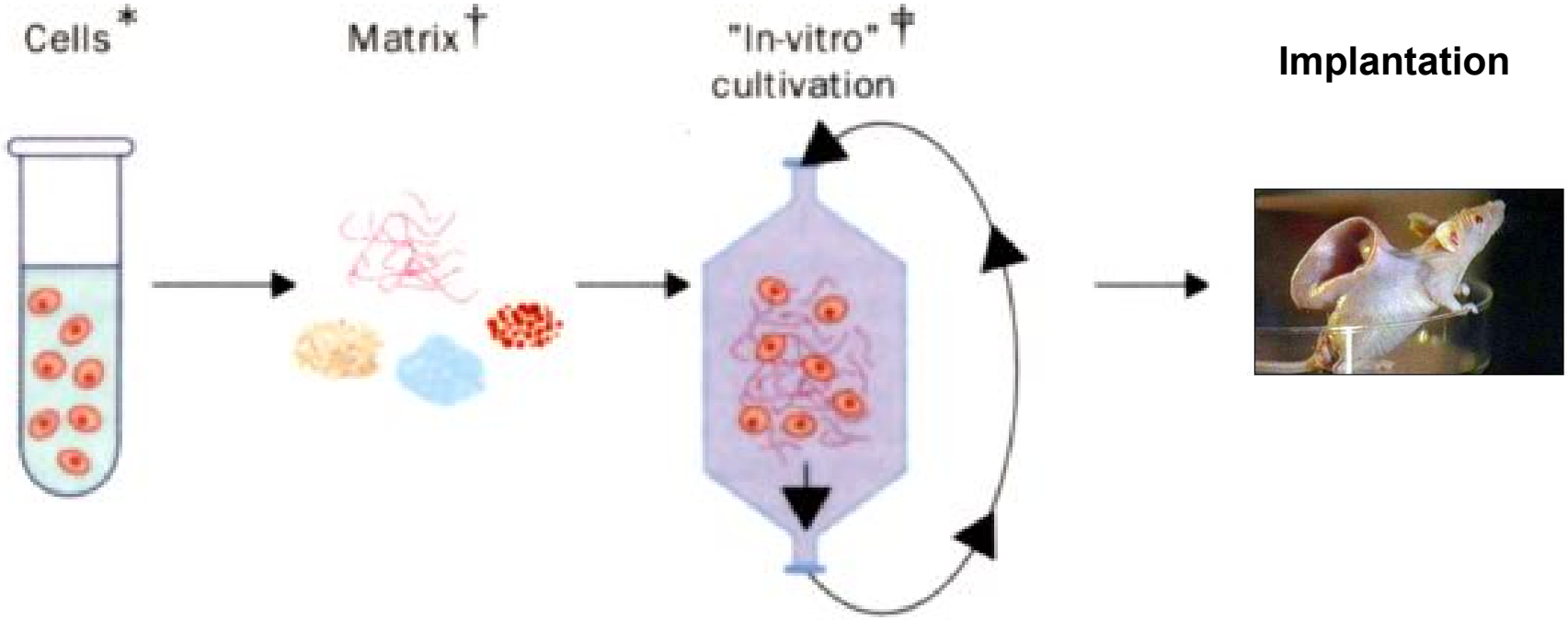
Organ Transplantation (장기이식)

- **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)

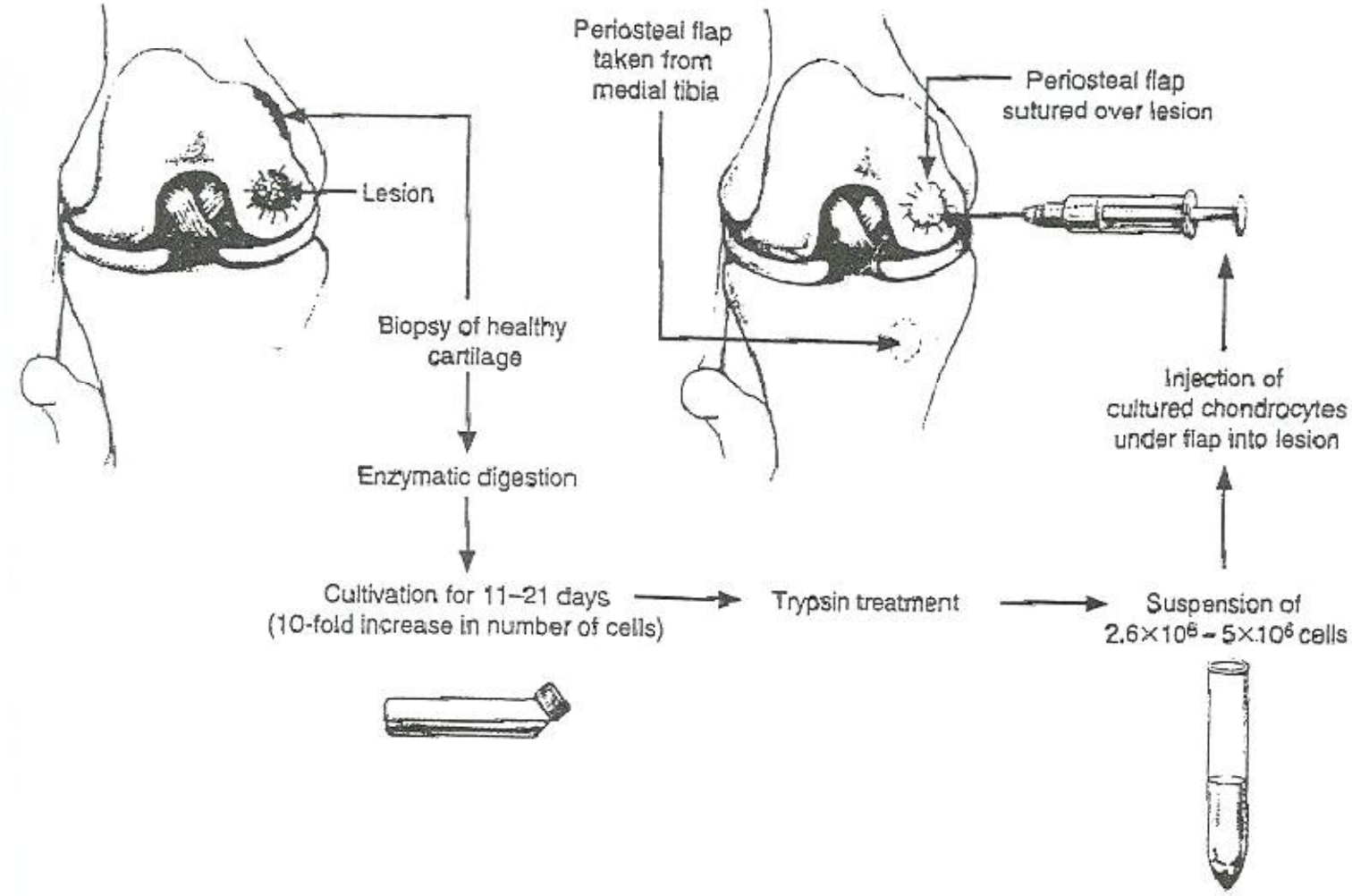


Fig. 12.3 Diagram of chondrocyte transplantation in the right femoral condyle (from Brittberg, 1994).

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

- Tissue Function (homeostasis)
- Tissue Formation (developmental biology)
- Tissue Repair (wound healing)

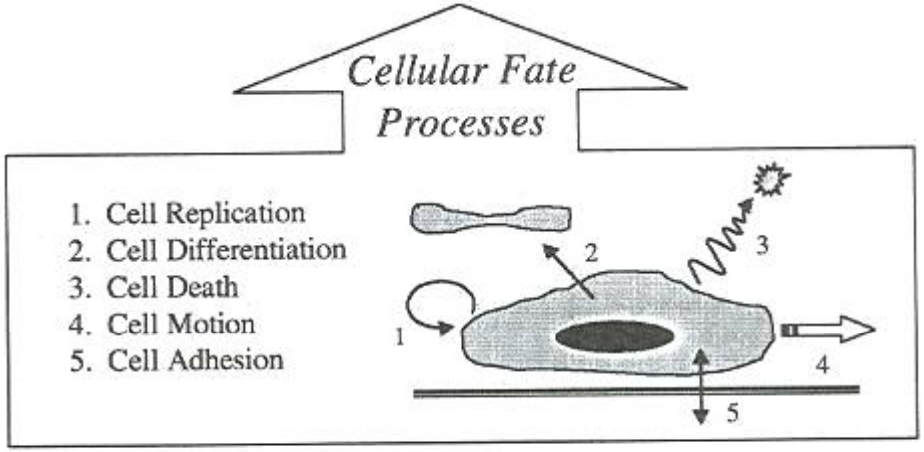


Fig. 12.5 Tissue dynamics. The three dynamic states of tissues and the underlying cellular fate processes.

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.

Current status and prospects for Cell Engineering

: roles and applications of stem cell in cell-theraphy

Objects

- Intractable disease treatment – the present ?
- Present and future in stem cell theraphy
- Economical efficiency of stem cell
- What is important ?

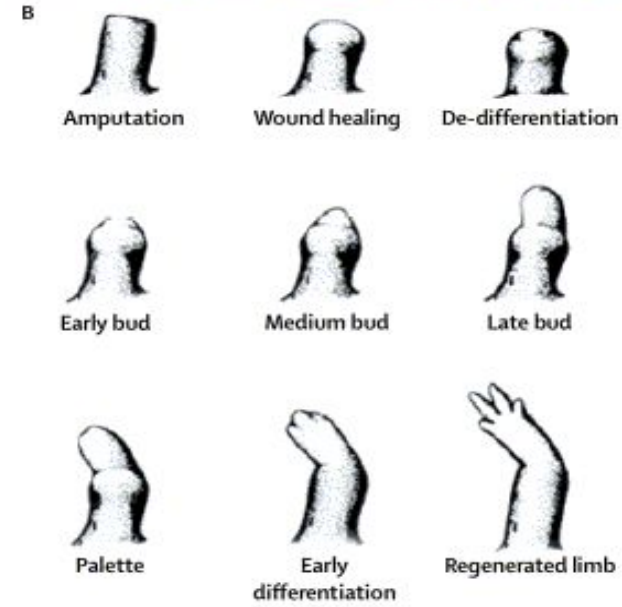
Cell theraphy

- Cell theraphy
  - Cell therapy is the transplantation and/or the enhancement of proliferation and differentiation of human or animal cells to replace or repair damaged organ, tissue, and/or cells.

- The first cell theraphy
  - German physicians attempted to treat children with hypothyroidism, or an underactive thyroid, with thyroid cells in 1912.

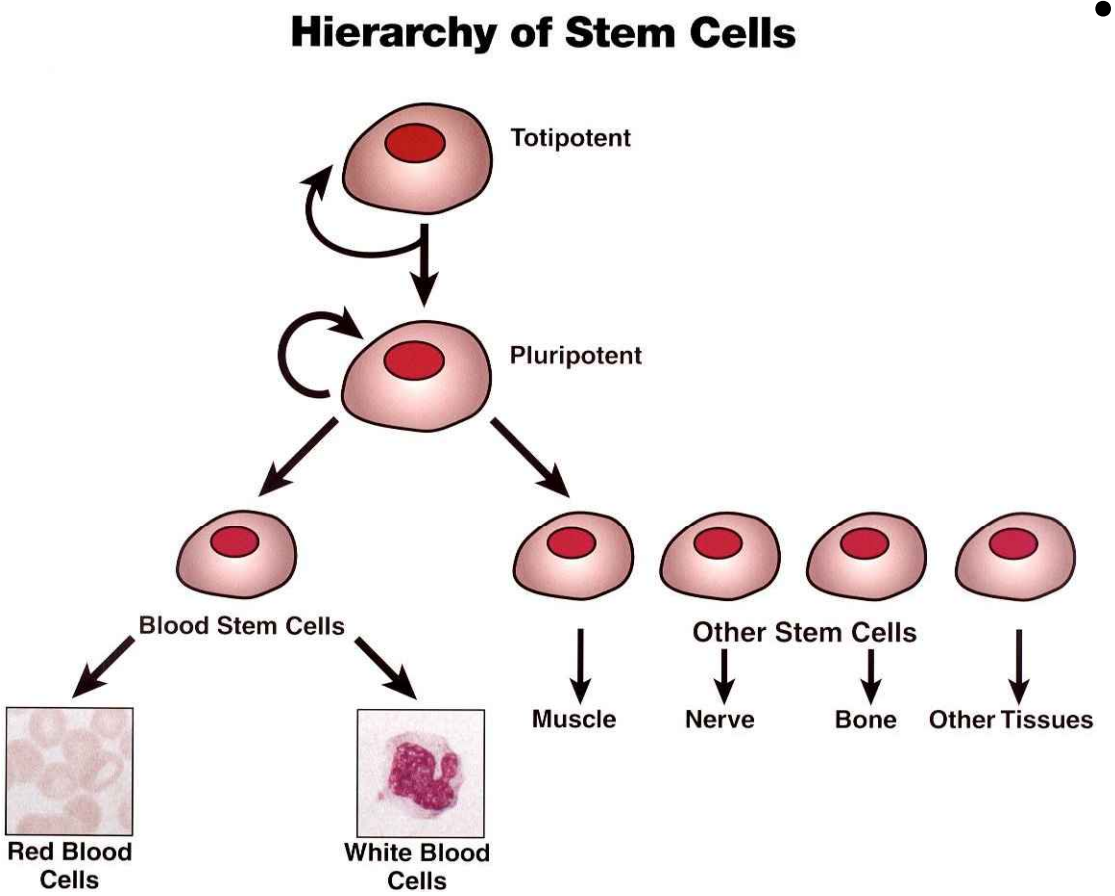
- Bone-marrow transplantation awarded a Nobel prize in 1971 is a good example in cell theraphy.

Stem Cell



- In the case of Amphibia, it is reported that severed limbs can be regenerated by stem cells.

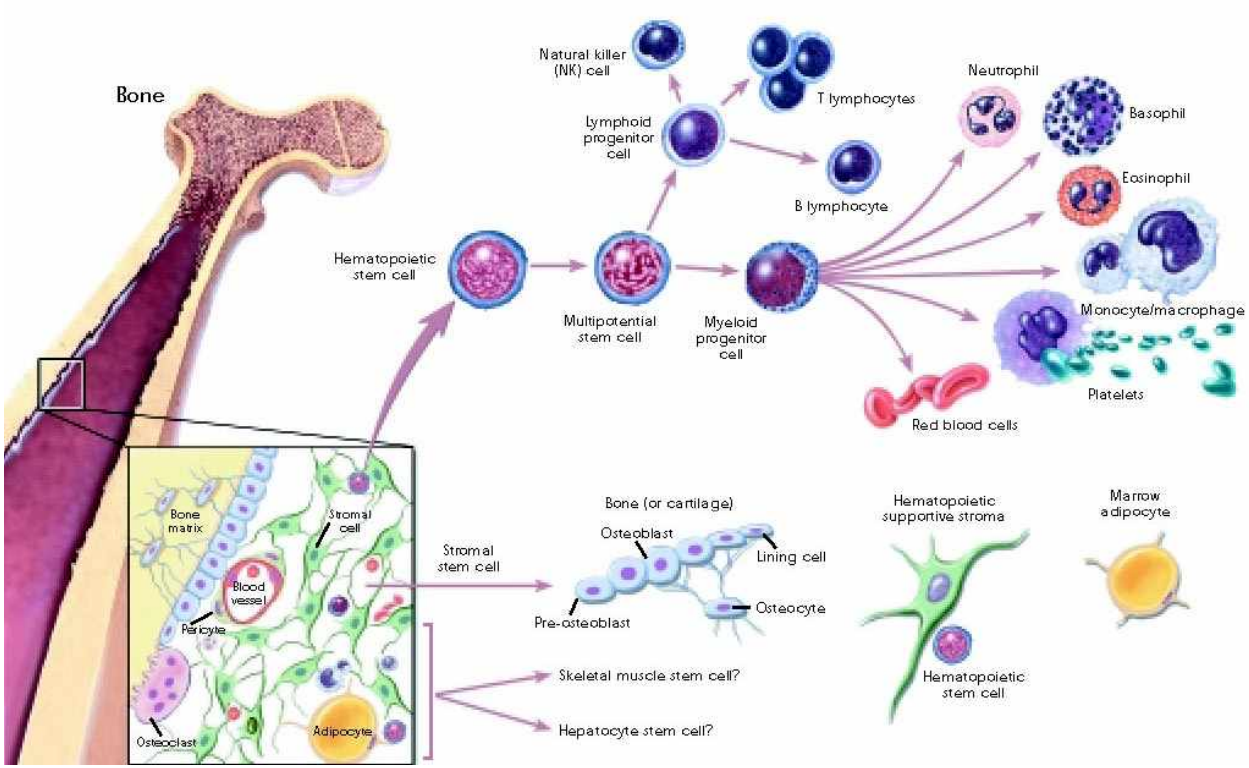
Stem cell



- classification of stem cell
  - Embryonic stem cell : Pluripotent cells that can give rise to all tissue types
  - Adult stem cell : Multipotent cells that have restrictions in differentiative potential

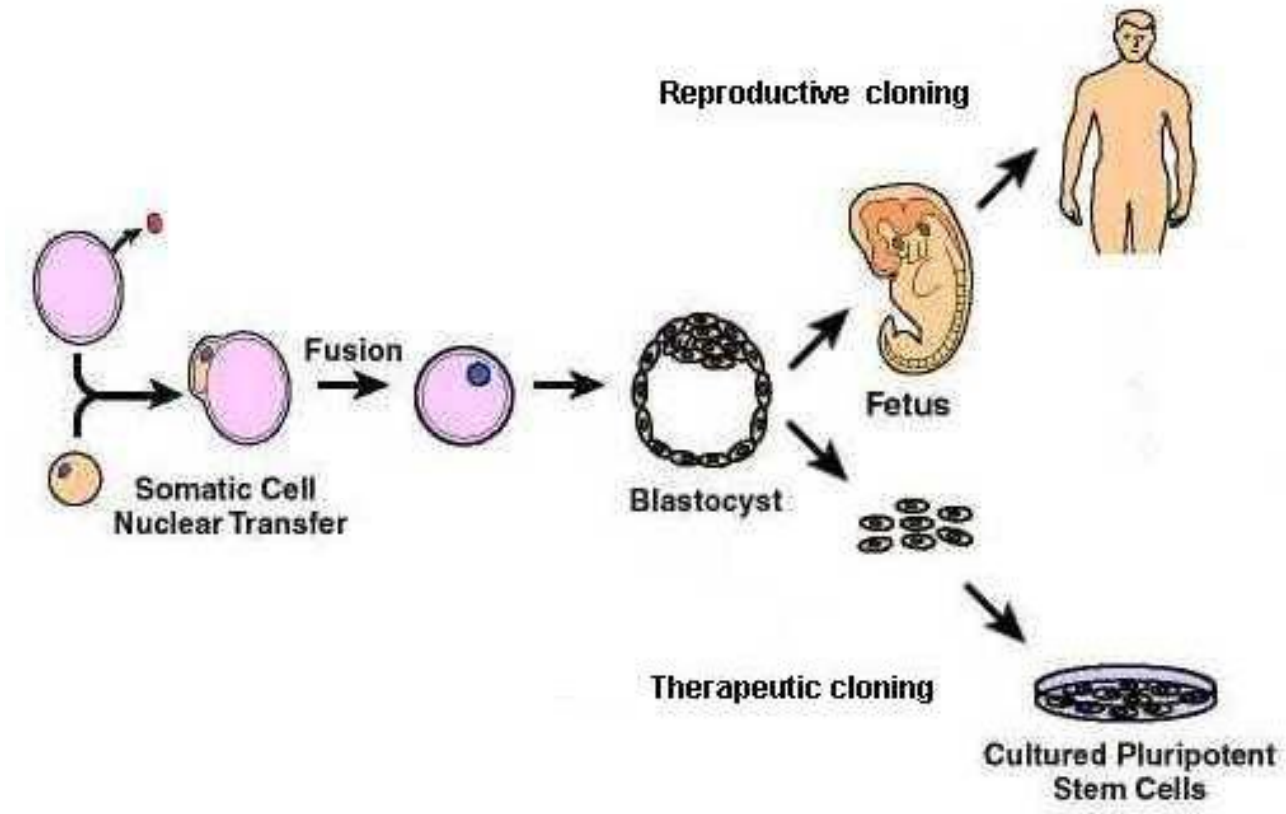
- Stem cell acquisition methods
  - Fetal stem cell : aborted fetus
  - Adult stem cell : bone marrow, cord blood, tissue
  - Embryonic stem cell : fertilized egg, somatic cell nuclear transfer

Adult stem cells



- can be acquired at almost parts of body
  - bone marrow, cord blood, brain, fat, bowel, epidermis, retina, pancreas and so on
- can avoid issues of immune rejection by autologous stem cell donation
- have restrictions in differentiative potential

Embryonic stem cells

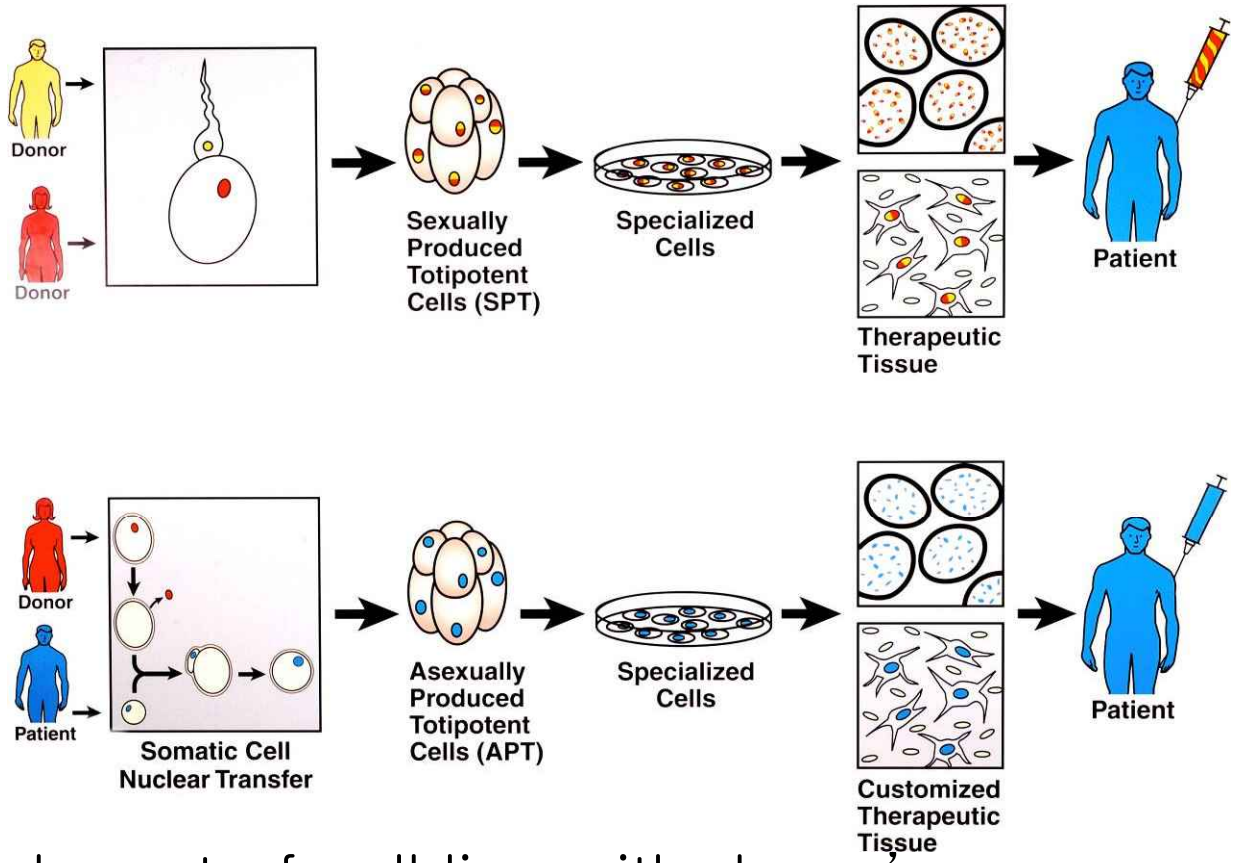


- separated from blastocyte by Thomson in 1998 for the first time.
- ethical issues of the destruction of a human life by usage of fertilized egg.
- Pluripotent stem cells that can give rise to all tissue types

Comparison between adult stem cell and Embryonic stem cell

	Embryonic stem cell	Adult stem cell
Differentiative potential	almost all cell types	the limited number of cell types
Possibility of teratoma	High	Low
Life-span	Unlimited. unlimited proliferation	Limited. limited proliferation
Ethical issues	Holding	Avoidable
Problems to be solved	Issues of immune rejection Guidance of differentiation into a desired cell type	Acquisition and proliferation of stem cells maintenance of cell properties

Somatic cell nuclear transfer stem cells

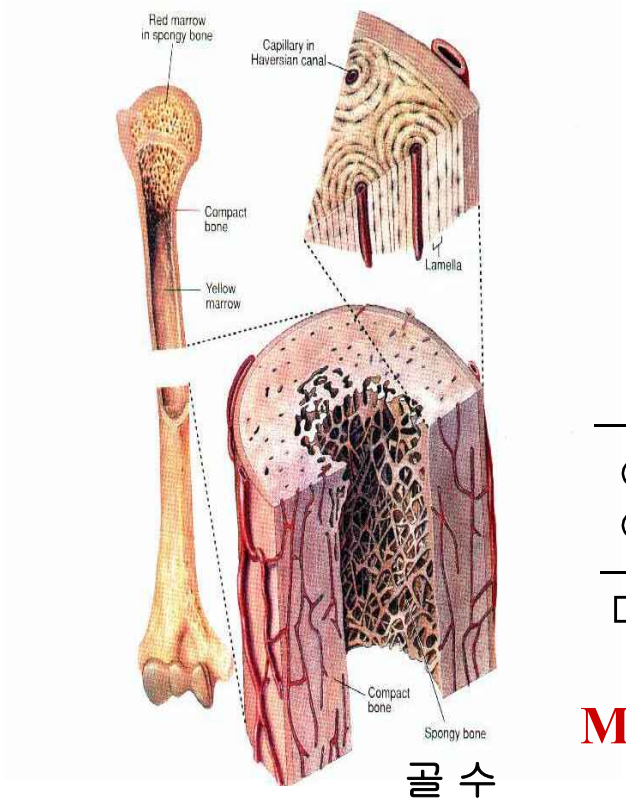


- establishment of cell line with donor's own genetic information by transferring a somatic cell nucleus of patient to a human ovum.
  - no immune rejection, genetically matched to the donor organism.

Examples of clinical applications

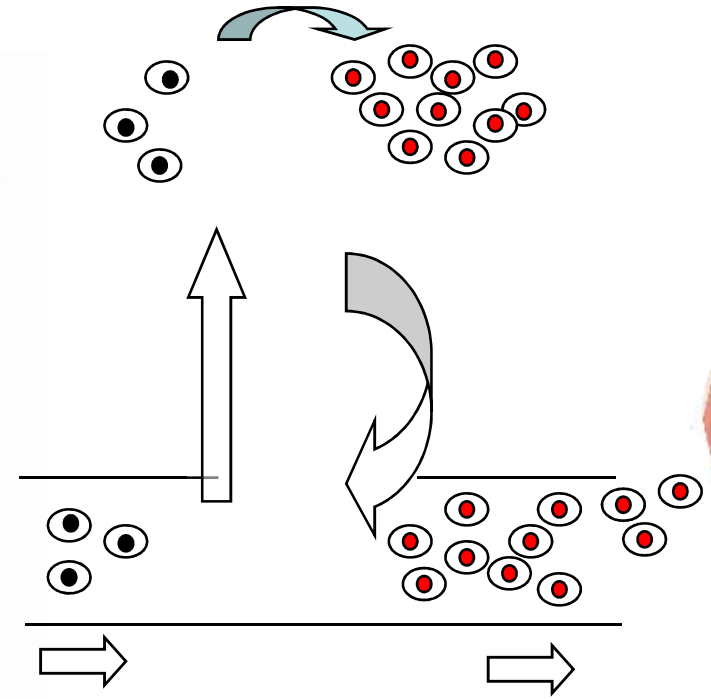
Cardiovascular regeneration using bone marrow stem cells

Three aspects of modification in stem cell therapy



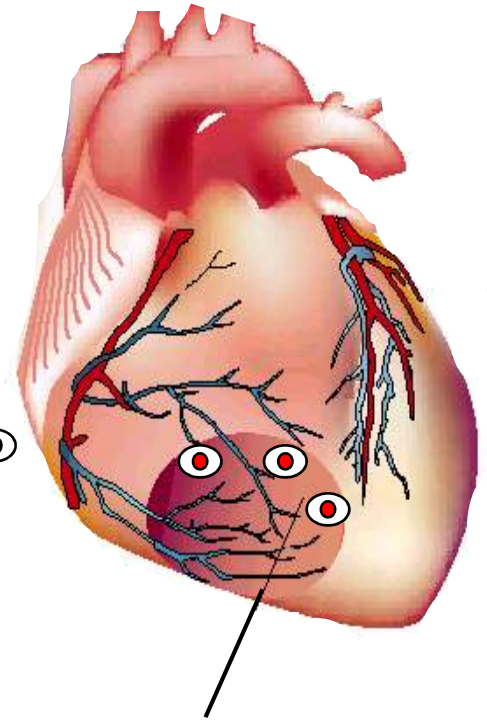
골 수

Ex vivo expansion



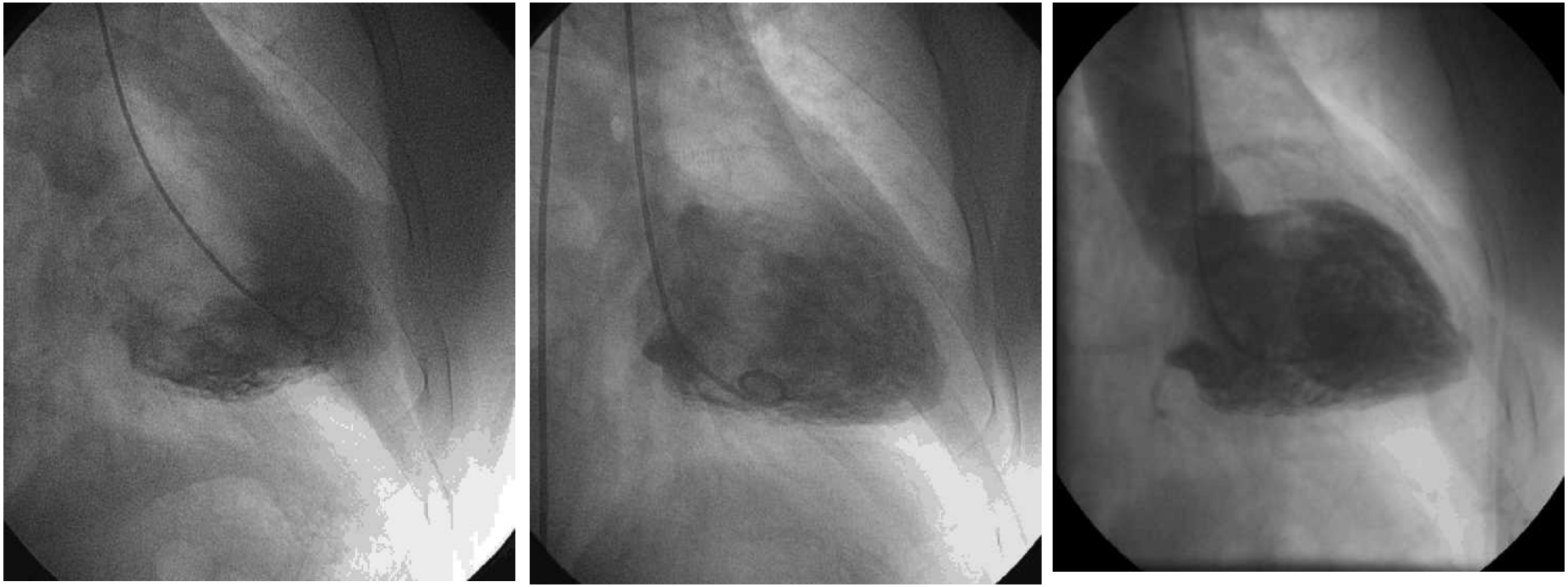
Mobilization

Homing



허혈 심근

Direct injection of bone marrow stem cells into Cardiovascular system



Baseline	6months follow up	1 year follow up

- recovery of heart palpitation of a myocardial infarction patient.

Spinal cord injury

- injection of cord blood stem cells into the spinal cord of a waistdown paralysis patient for 19 years
- reported the partial recovery of a sense
- need additional researches on the stability and effectiveness

Auditory sense injury

Generation of hair cells by stepwise differentiation of embryonic stem cells

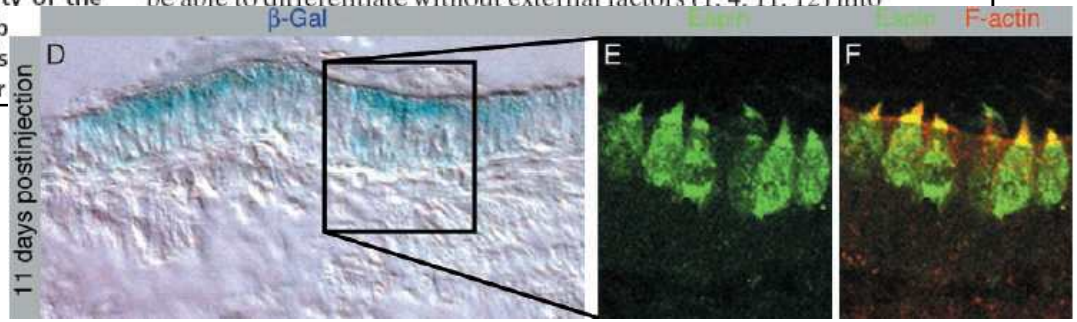
Huawei Li, Graham Roblin*, Hong Liu, and Stefan Heller†

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



- prospects of the recovery of auditory sense injury encouraged by success in generation of hair cells from embryonic stem cells by Harvard research group in 2003.

Problems in stem cell theraphy

- mass proliferation of stem cells
- guidance of differentiation into the desired cell type
- immune rejection
- positioning at the targeted location after injection
- functional maintenance in patient body after the transplantation
- development into teratoma (Ex. Cancer,…)

Stem cell research status of countries of the world

- 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

Technological gap from developed countries

- 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

Cell Theraphy Market

단위 : 억달러

기 술	2005년	2010년	2015년
Stem cell	20	20	109
Cord blood	5	10	23
Tissue engineering	69	135	232
Blood transfusion products	128	224	350
Gene -theraphy based on cell	15	30	59
Encapsulated cell therapy	4	19	31
Cancer vaccine based on cell	9	16	29
Xenograft (이종이식)	6	19	32
요소 기술(세포주, 세포배양액, 세포운반체)	20	57	98
총 계	266	562	963

자료 : **A Jain PharmaBiotech Report 2005**

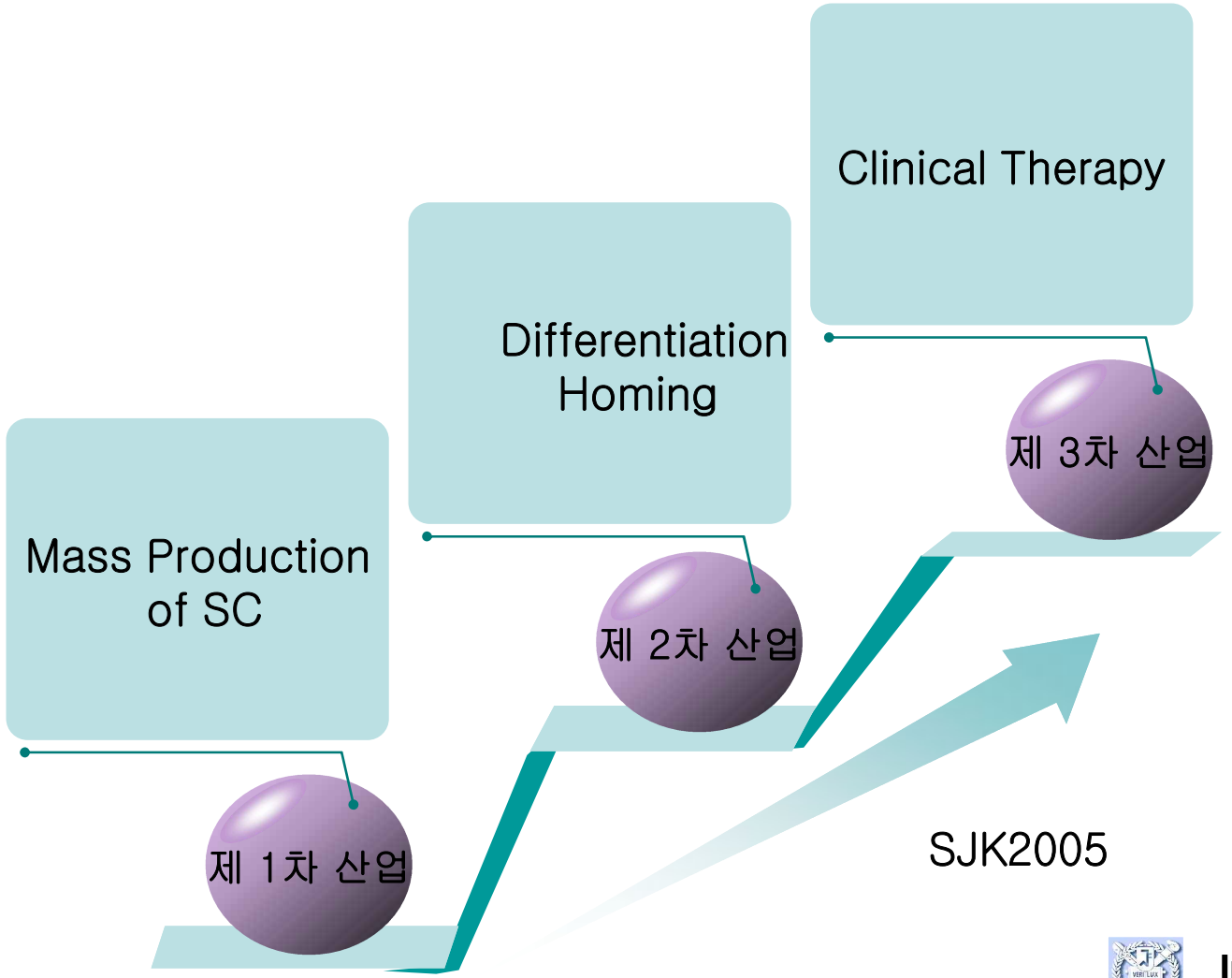
Industrial prospects

- 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)

Present business status

- 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

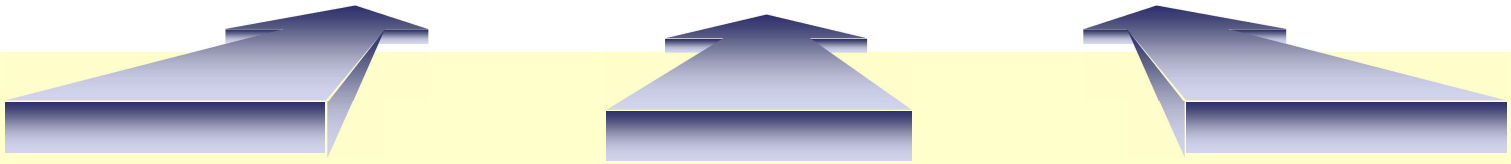
Where are we?
1st,2nd,3rd industry in stem cell



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

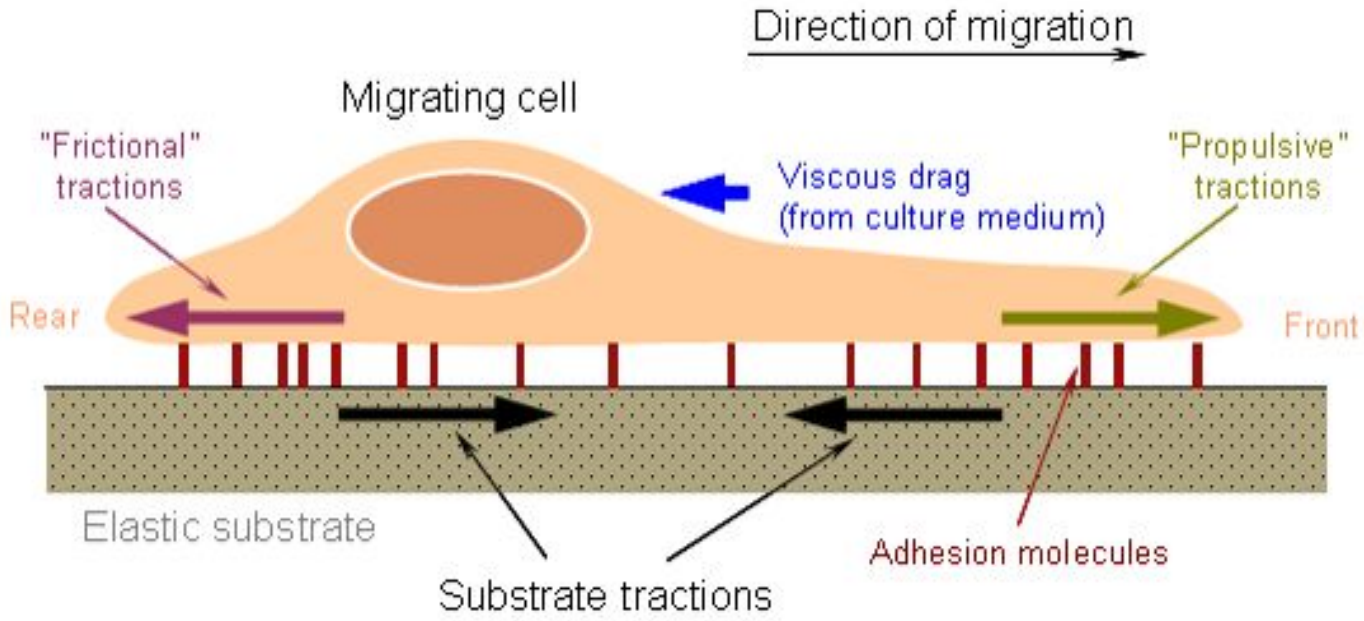
Be Wise

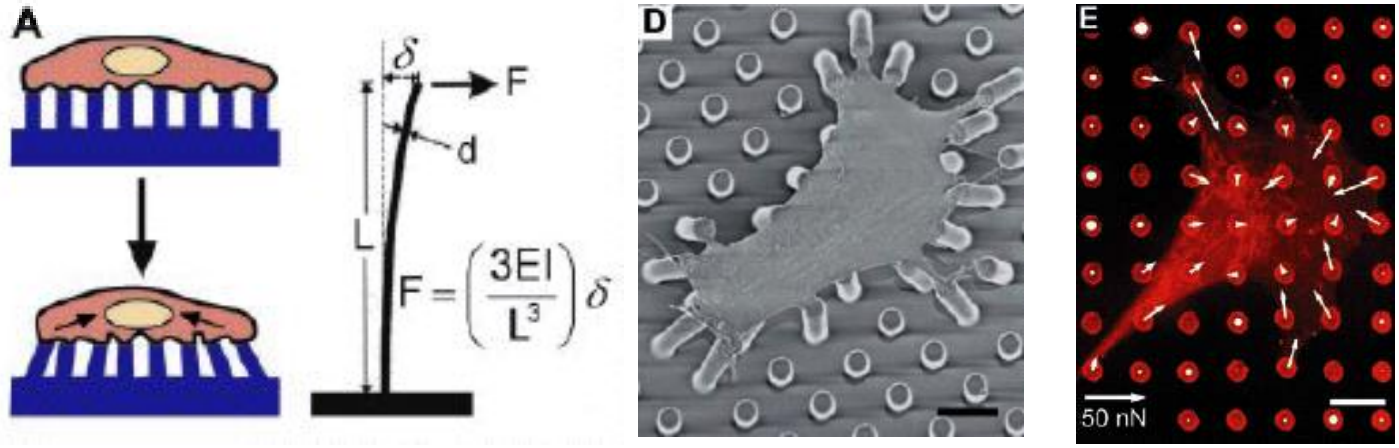


Biotech Economist group	Biotech Legal Advice group	Biotech Ethics monitoring group
●경제적 가치 평가 ●산업 모델 분석 ●산업 지원	●법적 제도적 장치 마련	●사회적 합의 도출 ●민간 사회 단체, 종료 단체

SJK 2005

A Cell Motility Study
Guidance of Cellular Growth

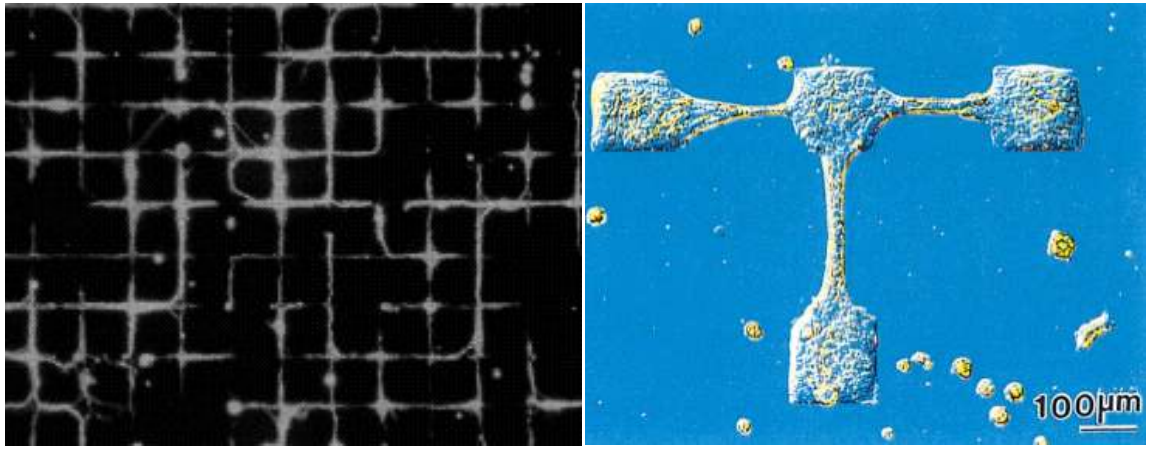




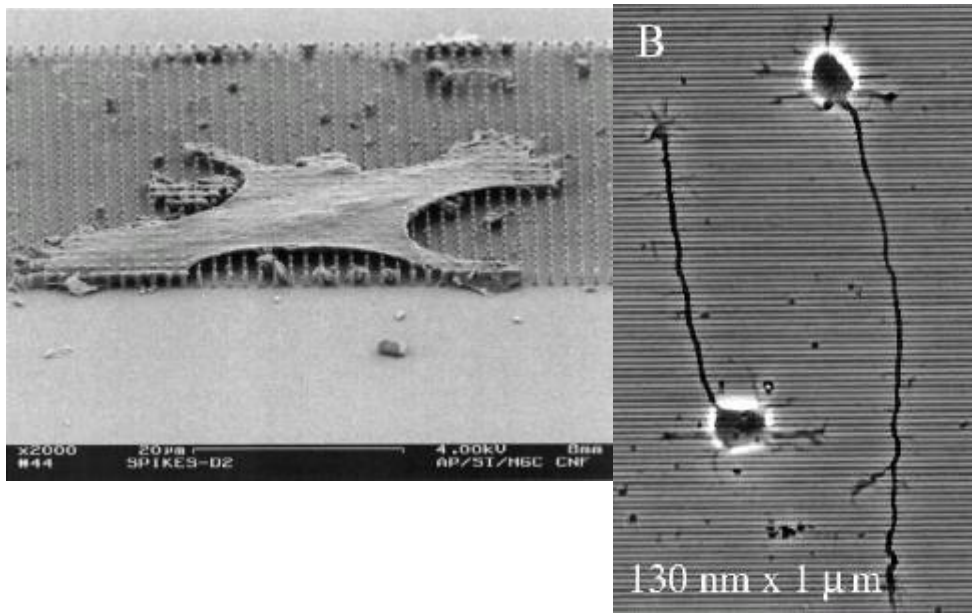
- *Tan J. et. al., Proc Natl Acad Sci. (2003)*
- The tractions can be calculated from the recorded deflection of the posts
- Ability to control the compliance of the substrate geometrically instead of chemically
- No need for recording the unstressed state of the substrate, because the posts were manufactured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

Building Methods of Cellular Network

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



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



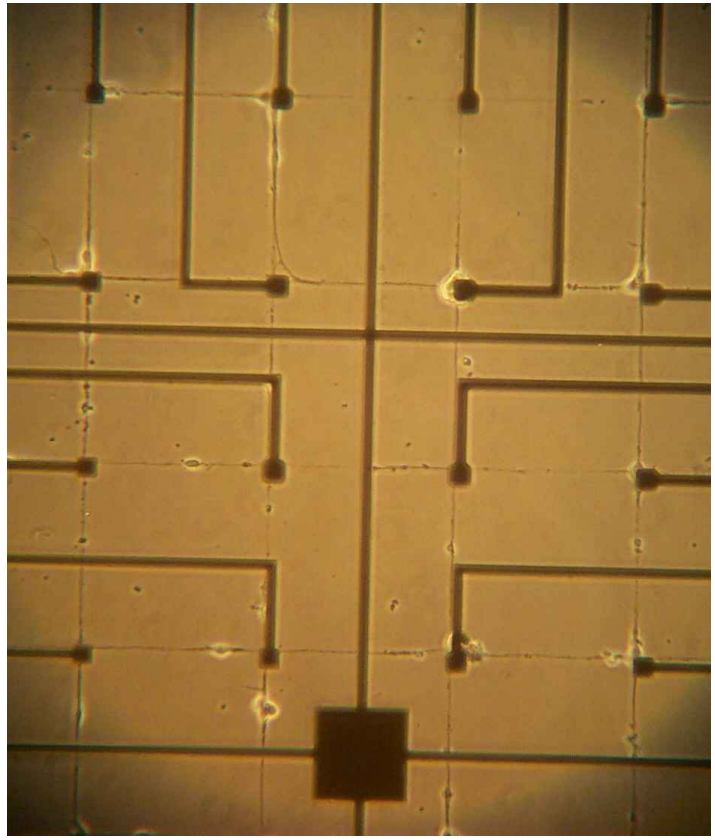
Research Goal

- 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.

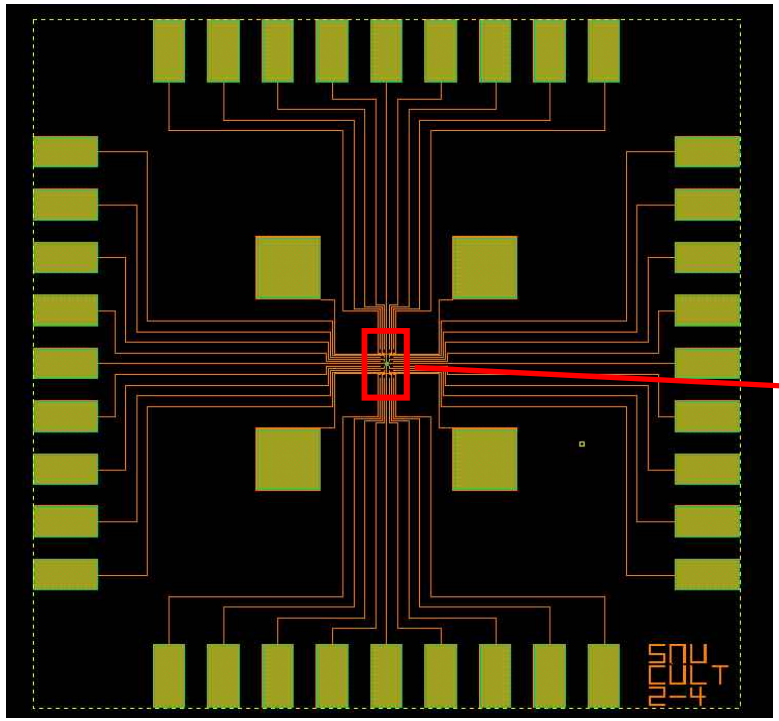
Why Microelectrode Array?

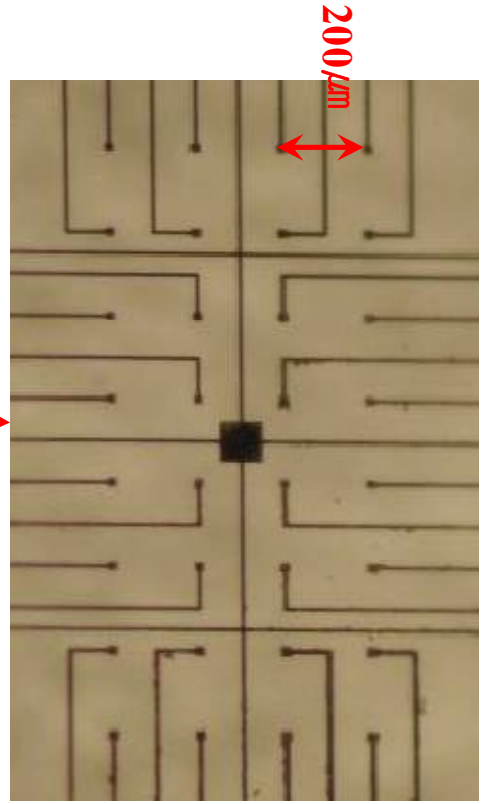
- Culture neurons for the long term
- Measuring neuronal responses simultaneously over a long period of time
- Precise Control of the environmental conditions around the neurons
- Direct visualization



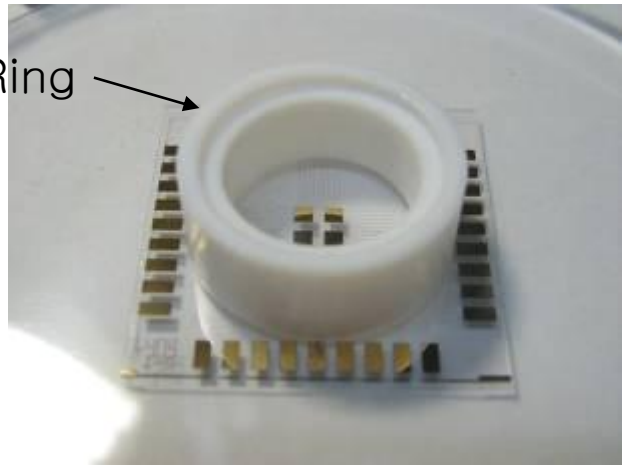
Hippocampal neurons at 5 days after culture

Microelectrode Electrode





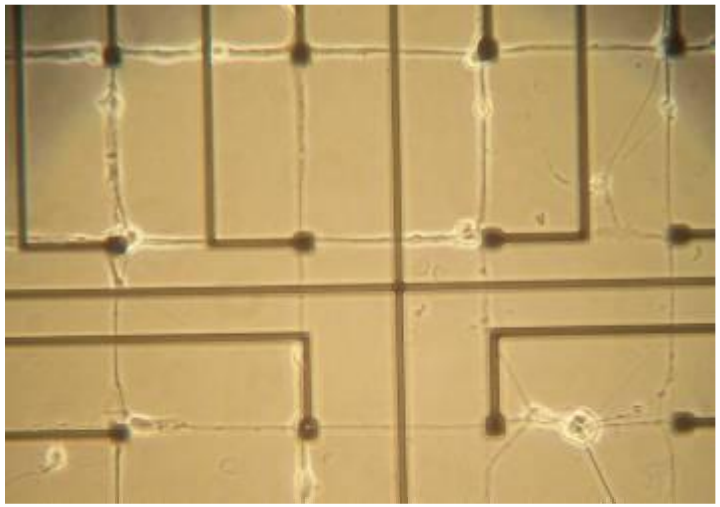
Teflon Ring

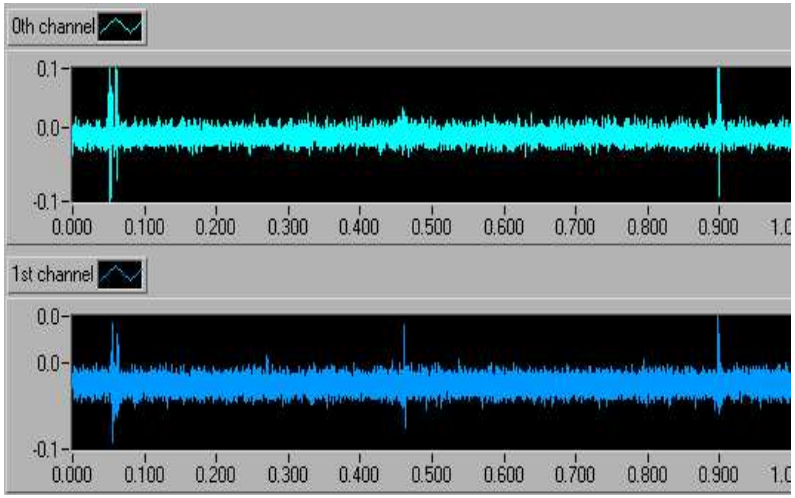


- 8 × 4 array
- Each electrode size : 10×10μm^2
- Interelectrode Spacing : 200 μm

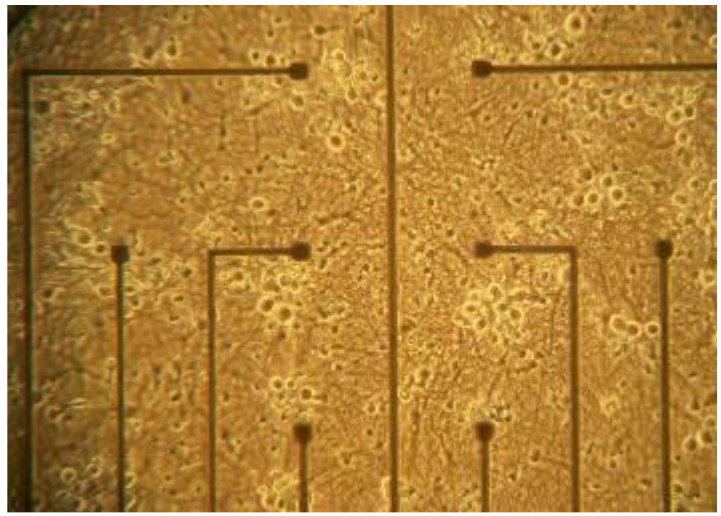
Reason to use patterned networks

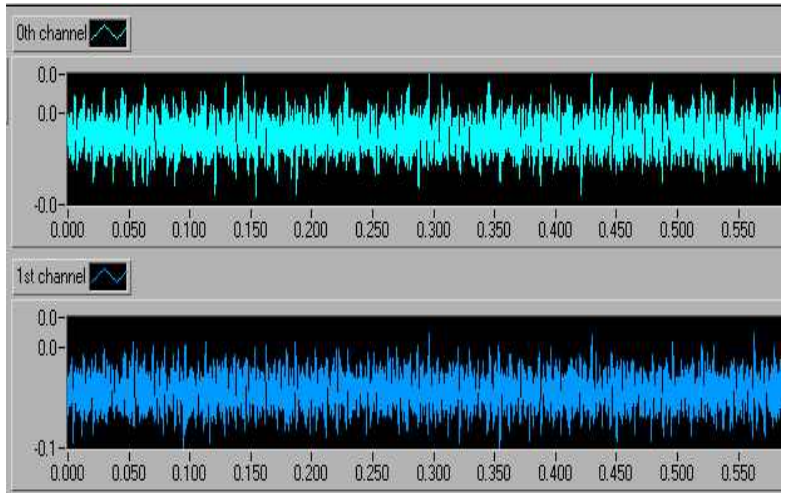
Patterned





Random



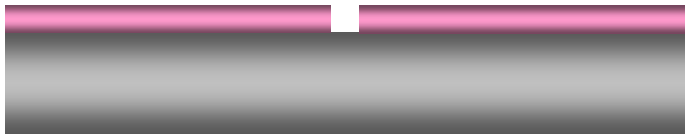


In order to get action potential from randomly cultured neurons, much more neurons should be plated than patterned neurons. Patterning neurons enhances activities of the network.

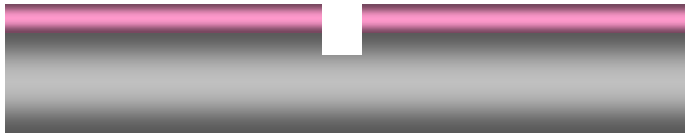
Fabrication of stamp master

Method 1

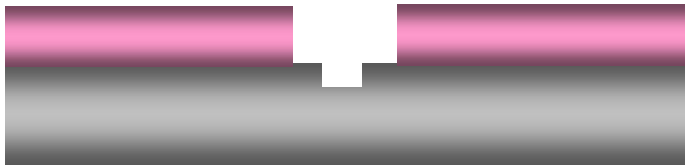
1) 1st Photo Lithography



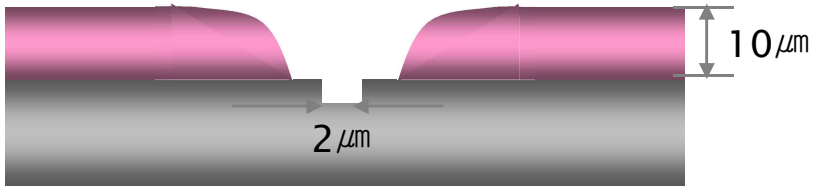
2) Silicon RIE-dry etching

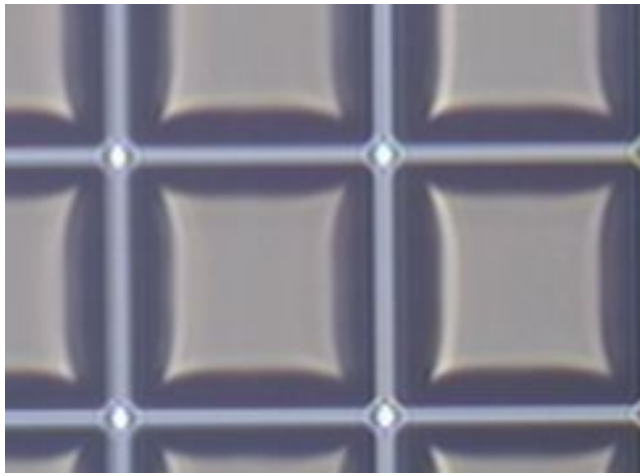


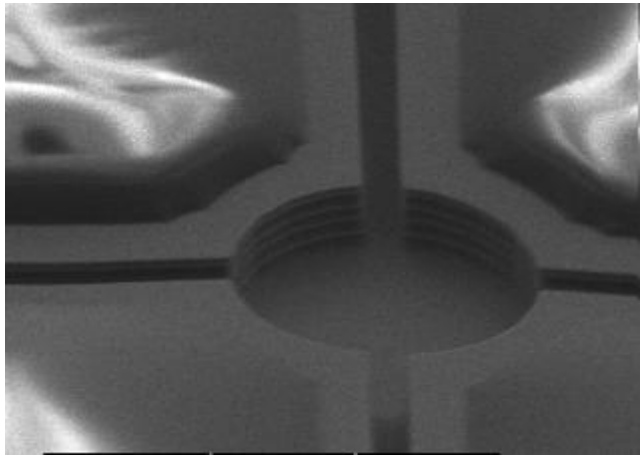
3) 2nd Photo Lithography



4) Photo Resist Reflow





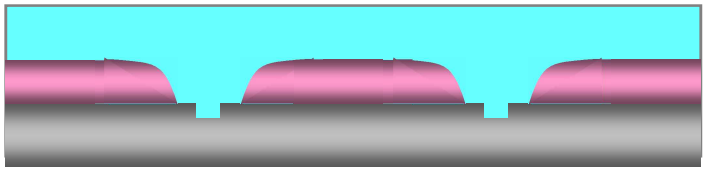


Shallow Si etch(3um)
and PR reflow(10um)

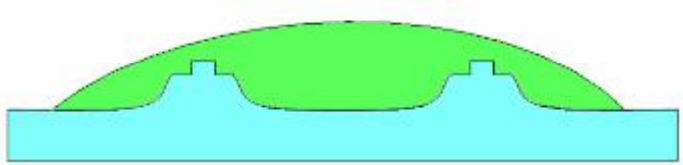
The supportive structure is needed for preventing the fine stamp pattern from collapsing especially for the very fine pattern like several microns.

Microstamping Procedure

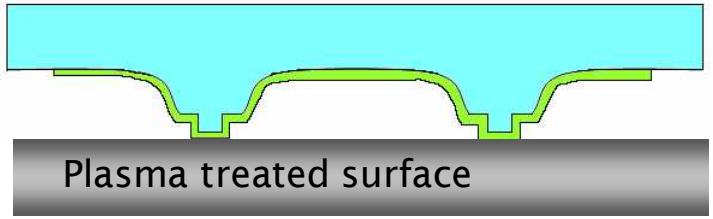
1) Sonication in 50% ethanol for 10 min
2) PDMS stamp molding with stampmater

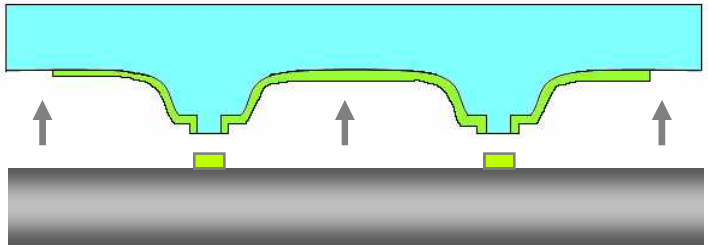


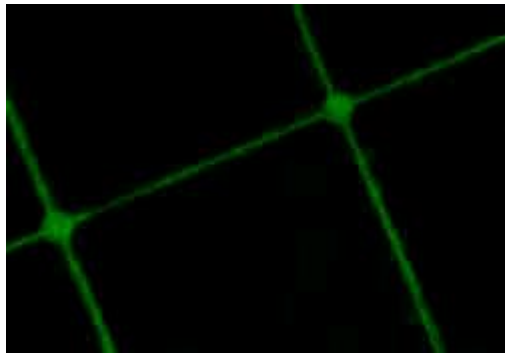
3) Poly-L-lysine (diluted in BBS) Inking for 2 hr



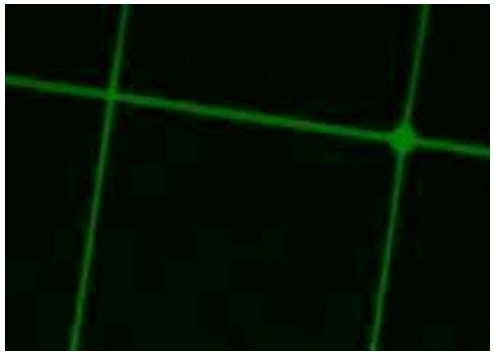
4) Stamping for 1 hr (50 g/cm^2)



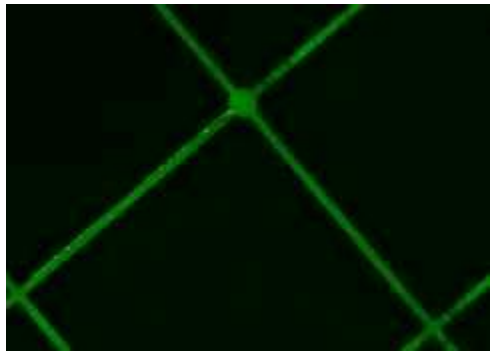




Line width = 2

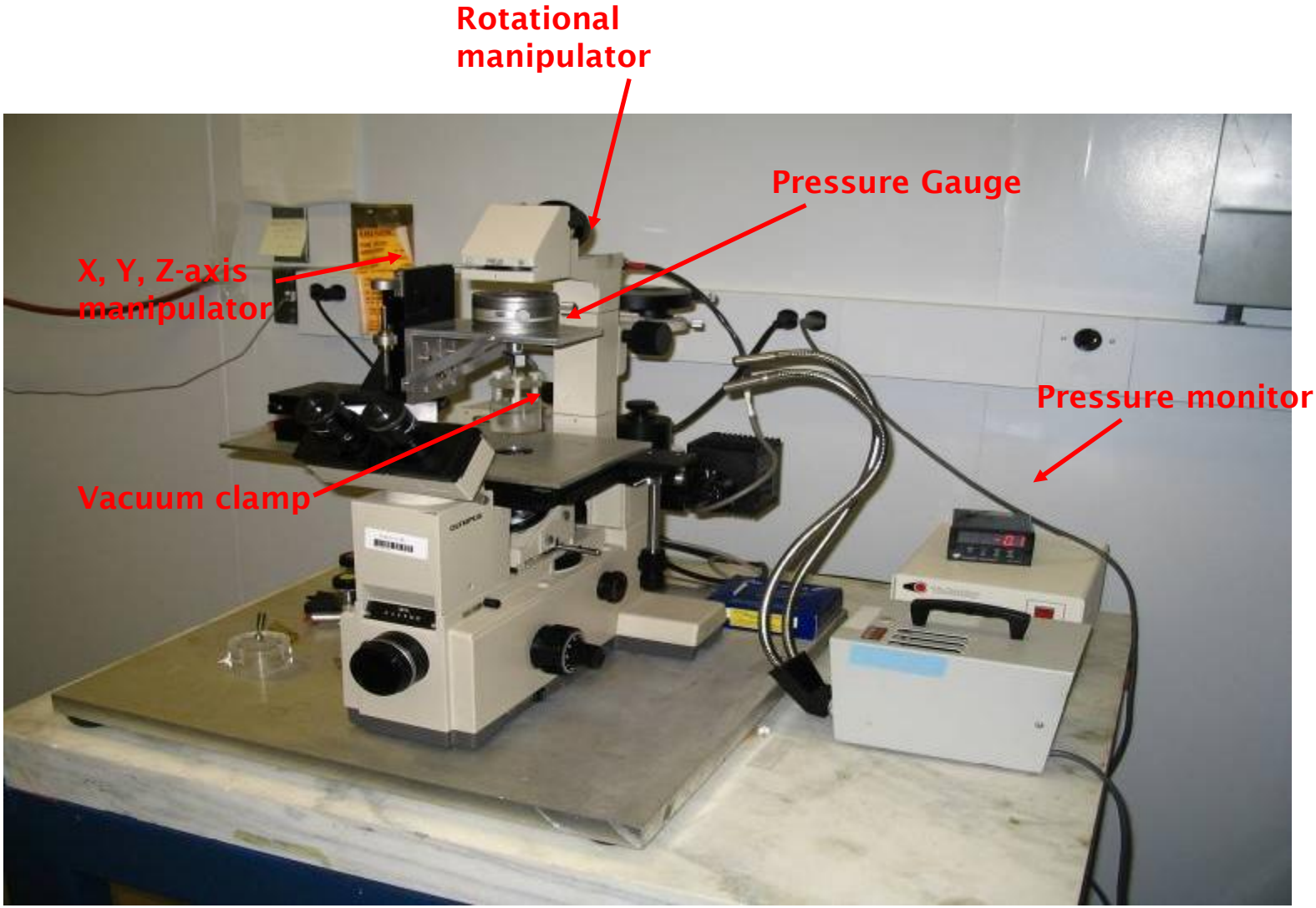


Line width = 4㎛

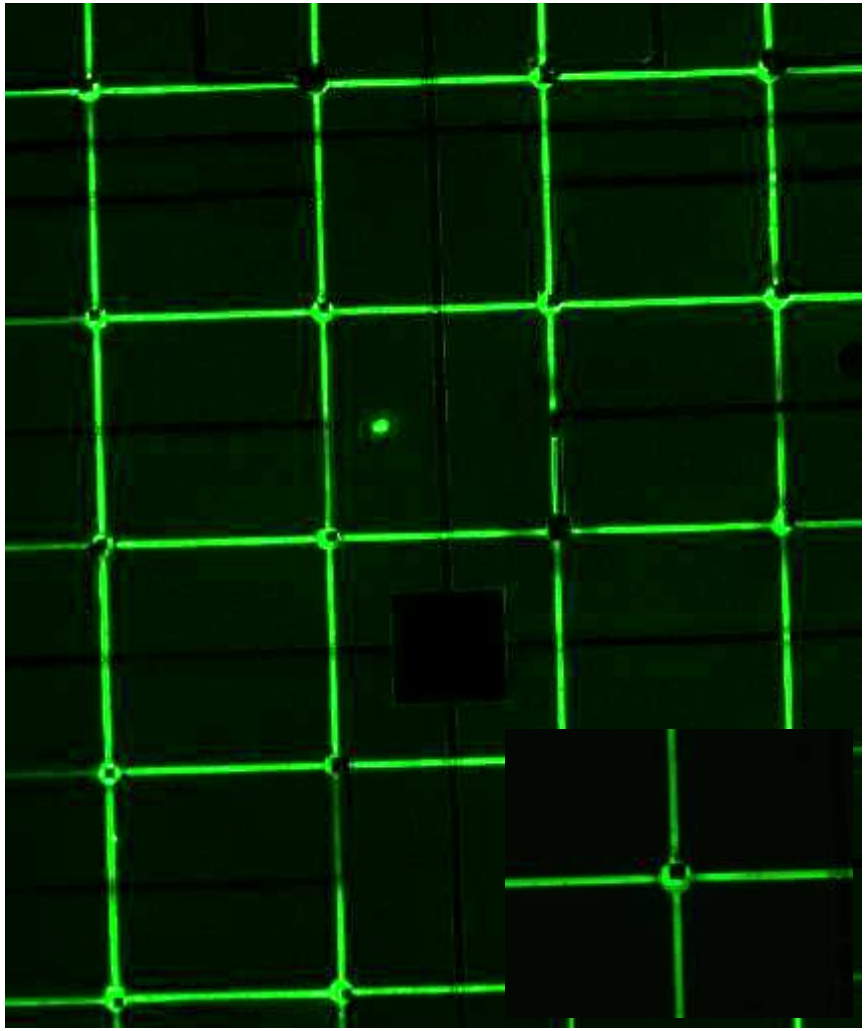


Line width = 6㎛

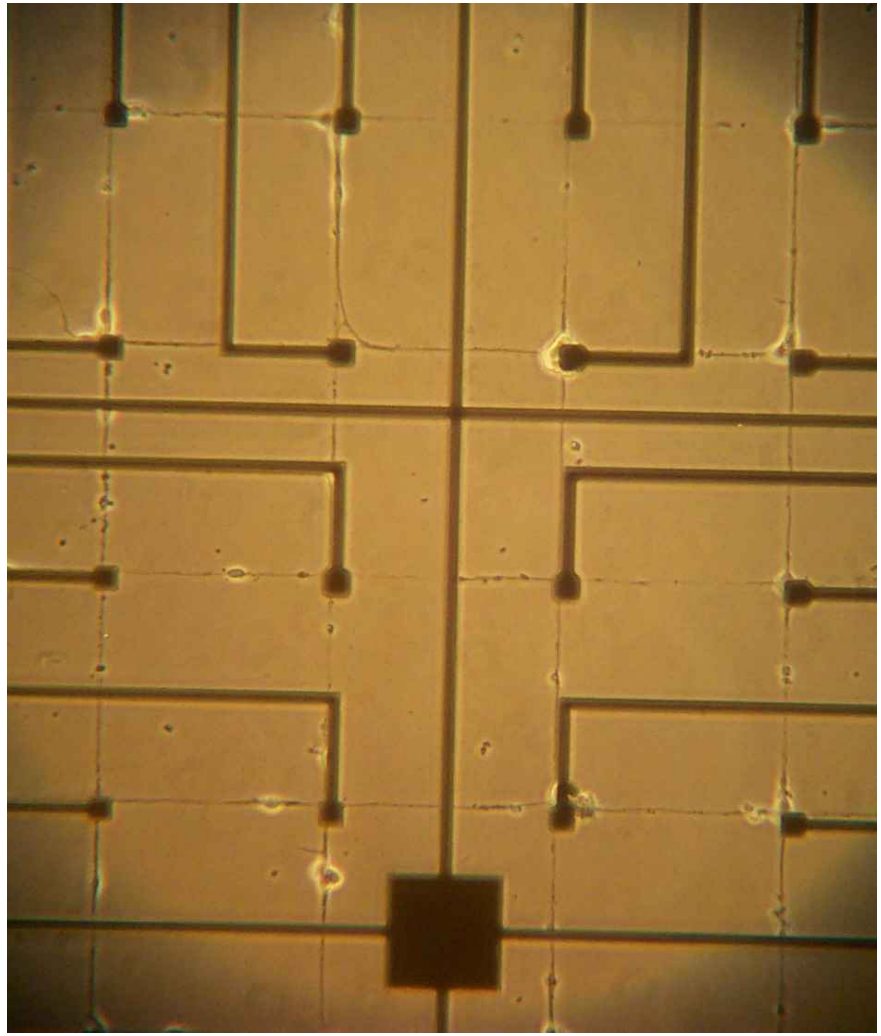
Aligner for Chemical Stamping



Stamped Surface



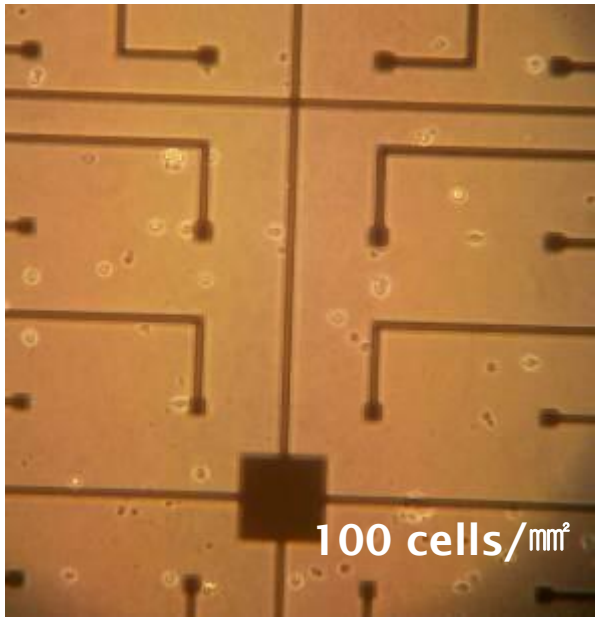
Stamped pattern on the microelectrode array
(FITC-labeled Poly-L-lysine)

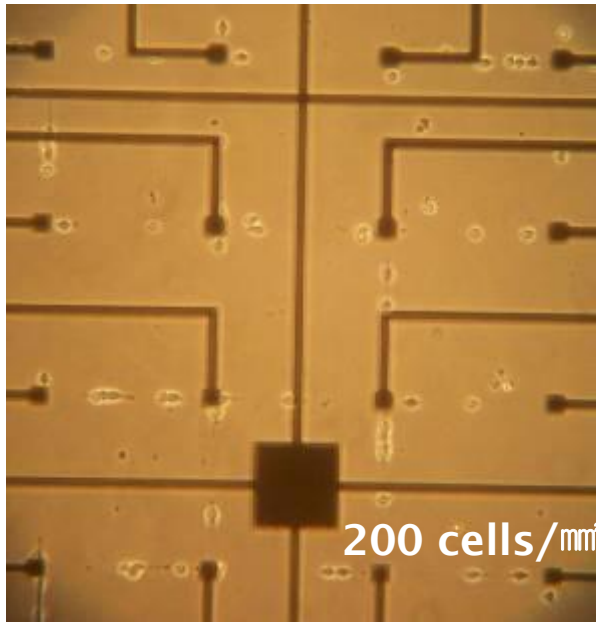


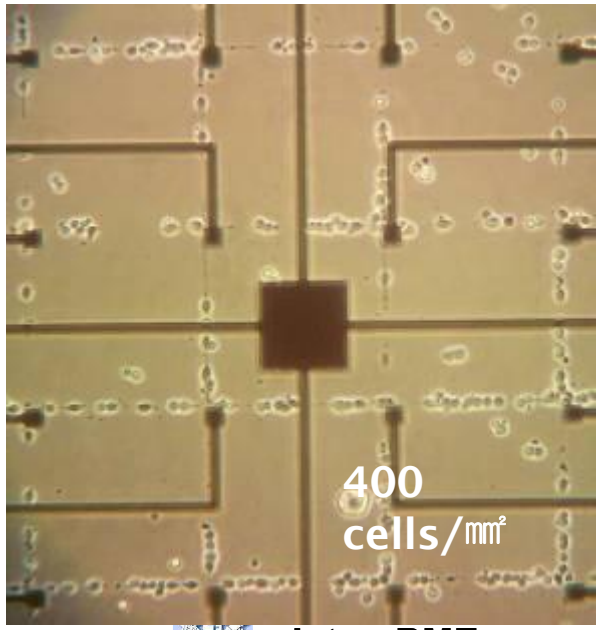
Hippocampal neurons at 5 days after culture

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/㎟

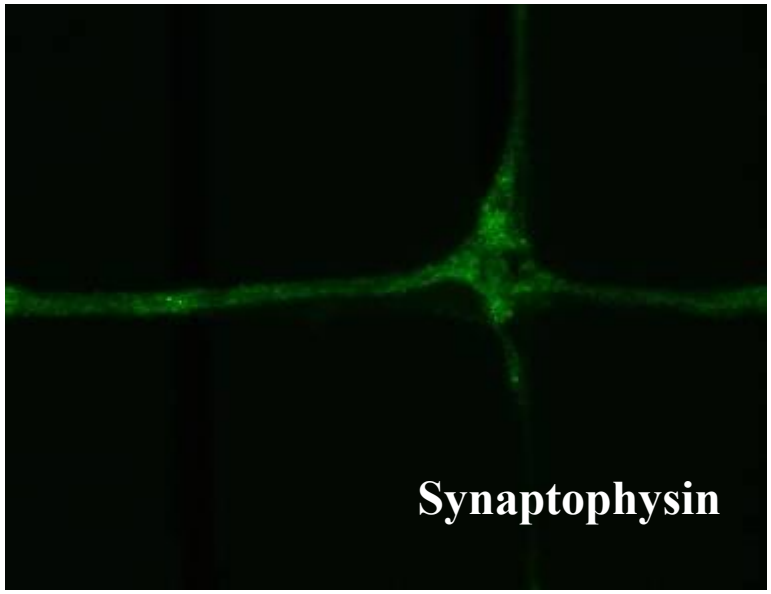


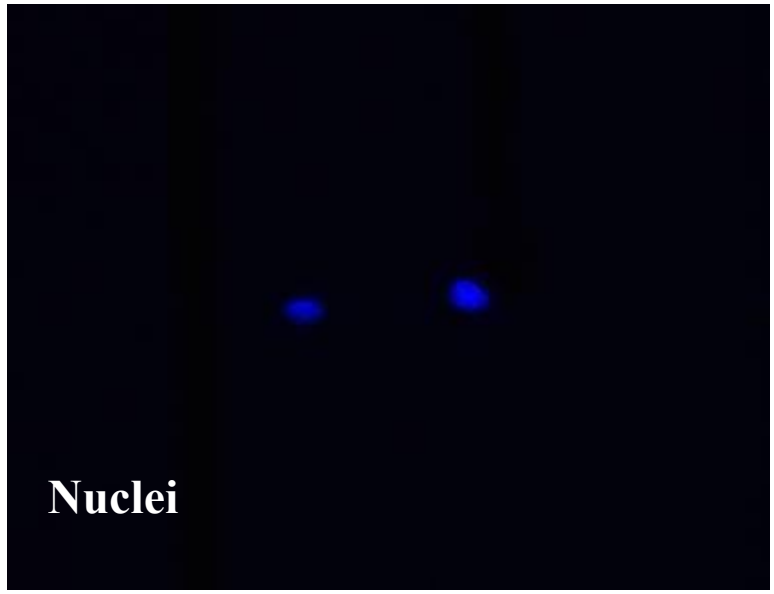


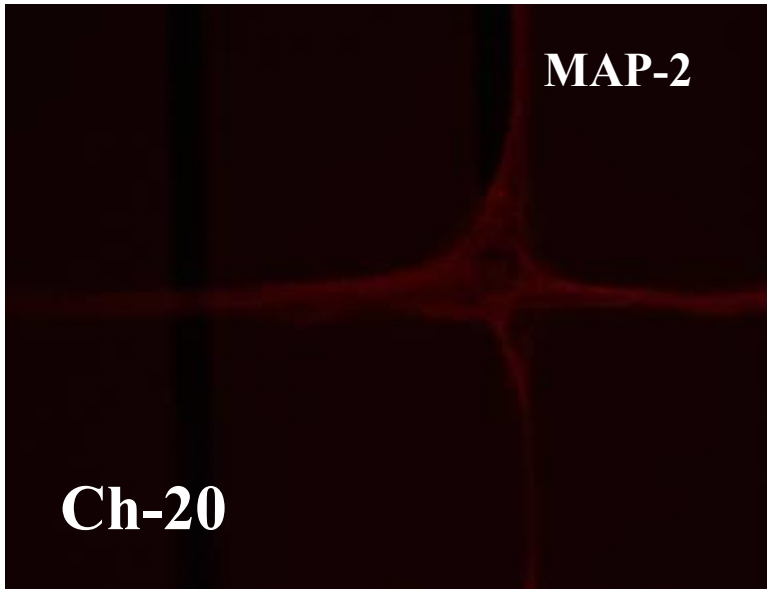


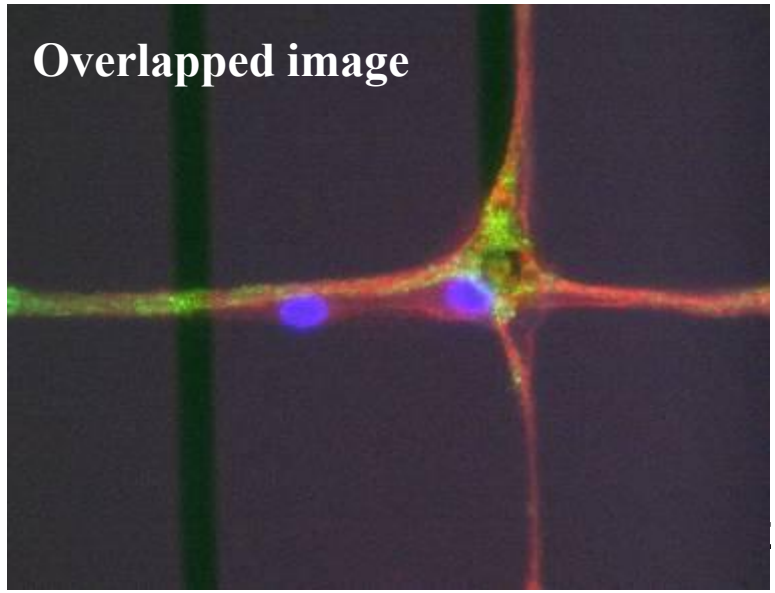
4 hours after plating cells

Immunocytochemical descriptions of cells in networks

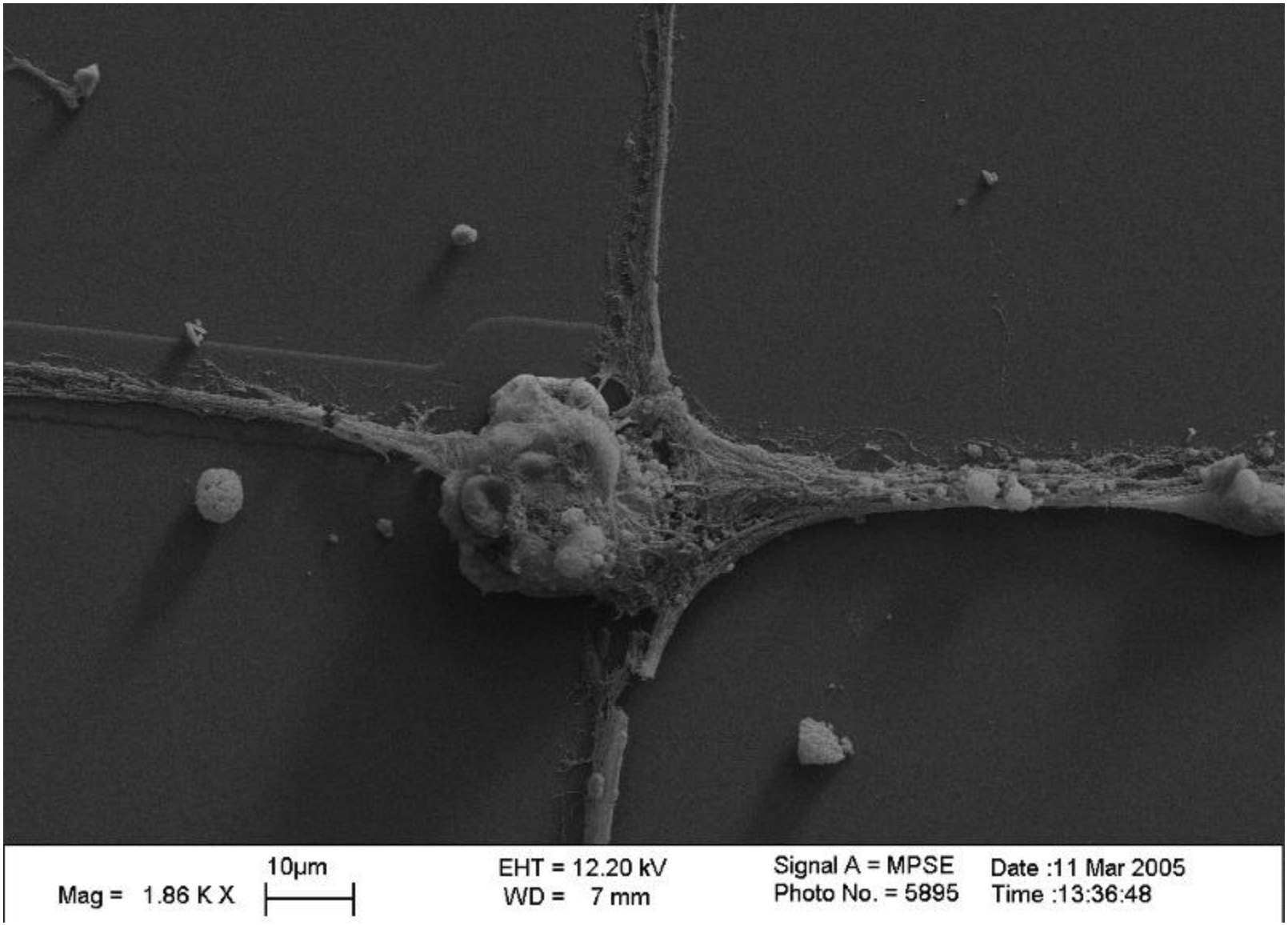








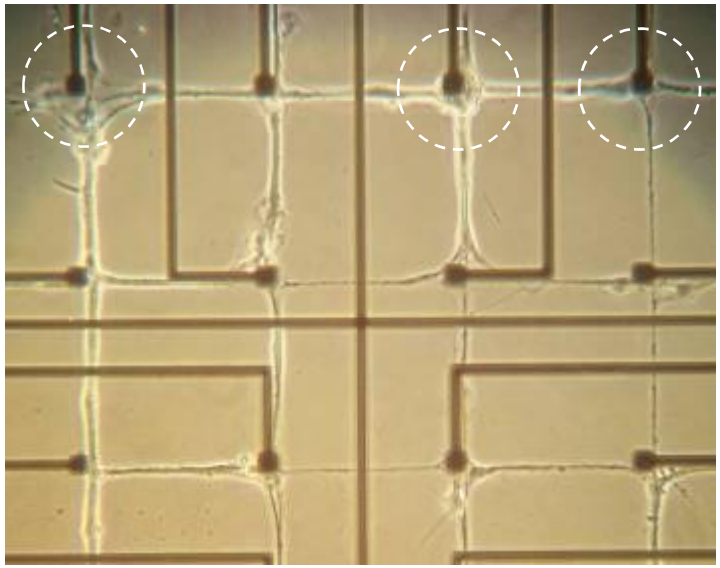
SEM image


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

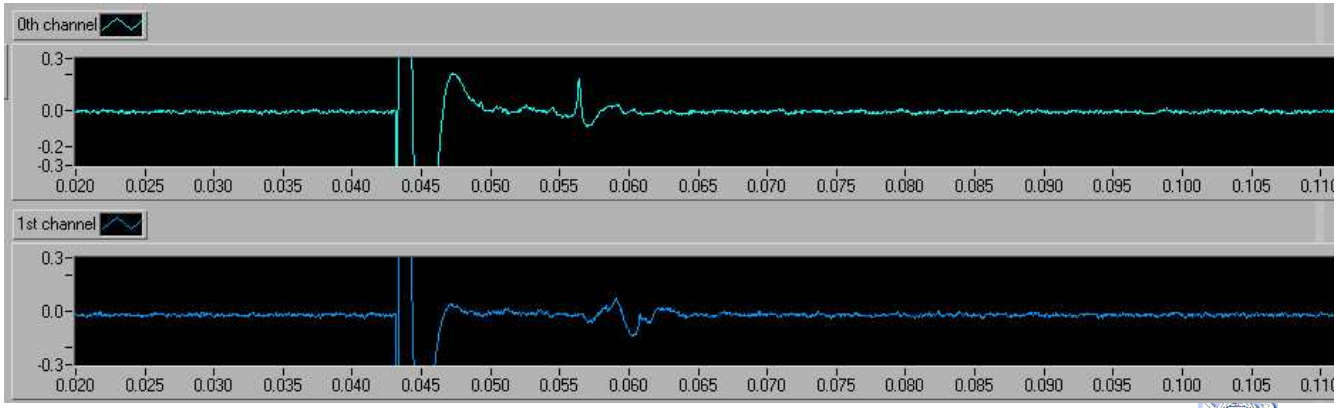
Stimulation electrode
I_{stim} = 100μA, 50ms

Ch 22 **Ch 20**



Ch22

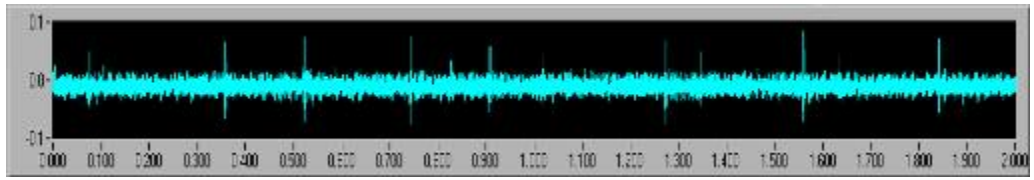
Ch20



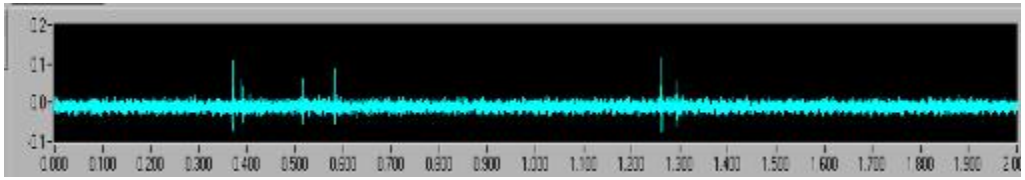
Pharmacological Study

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

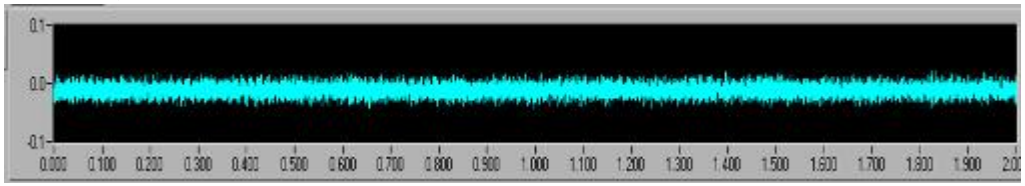
Before the treatment of DNQX



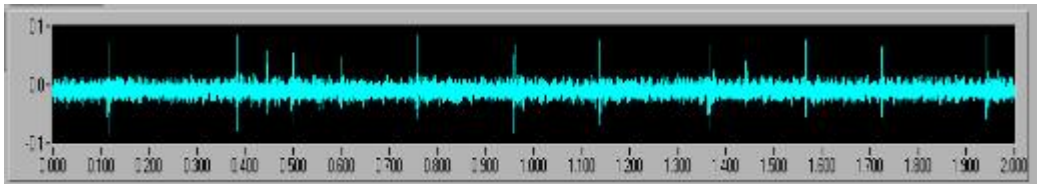
1μM DNQX

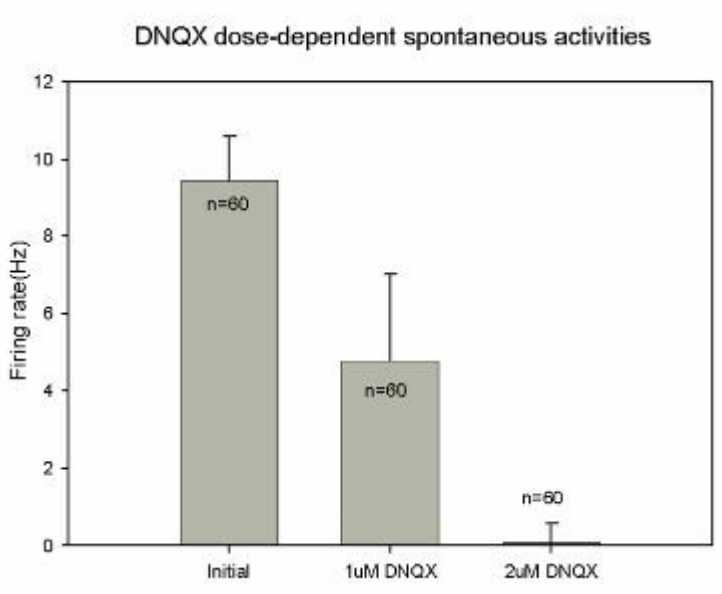


2μM DNQX



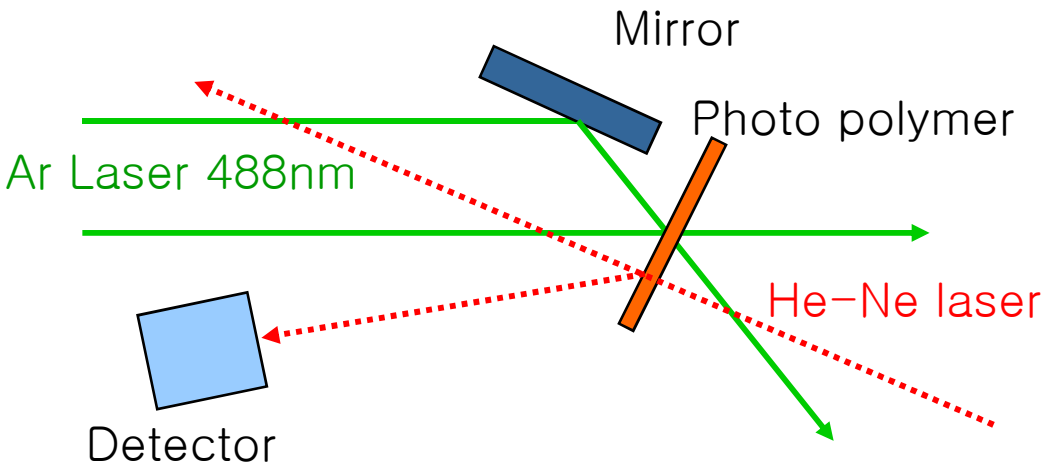
After washing out DNQX



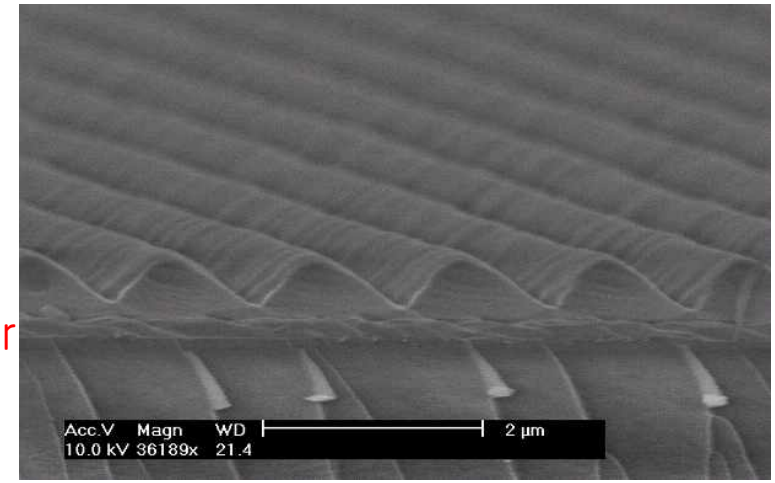


Topological Method for the Guidance of Cellular Growth

Surface Relief Grating Technique

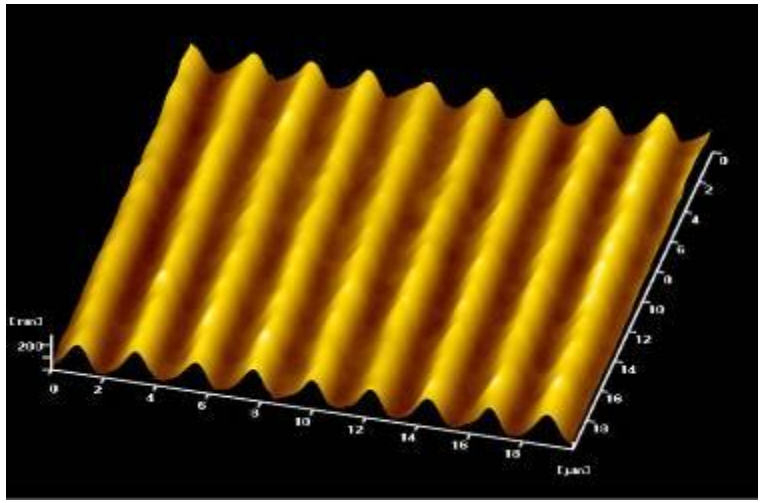


<Optical SETUP>



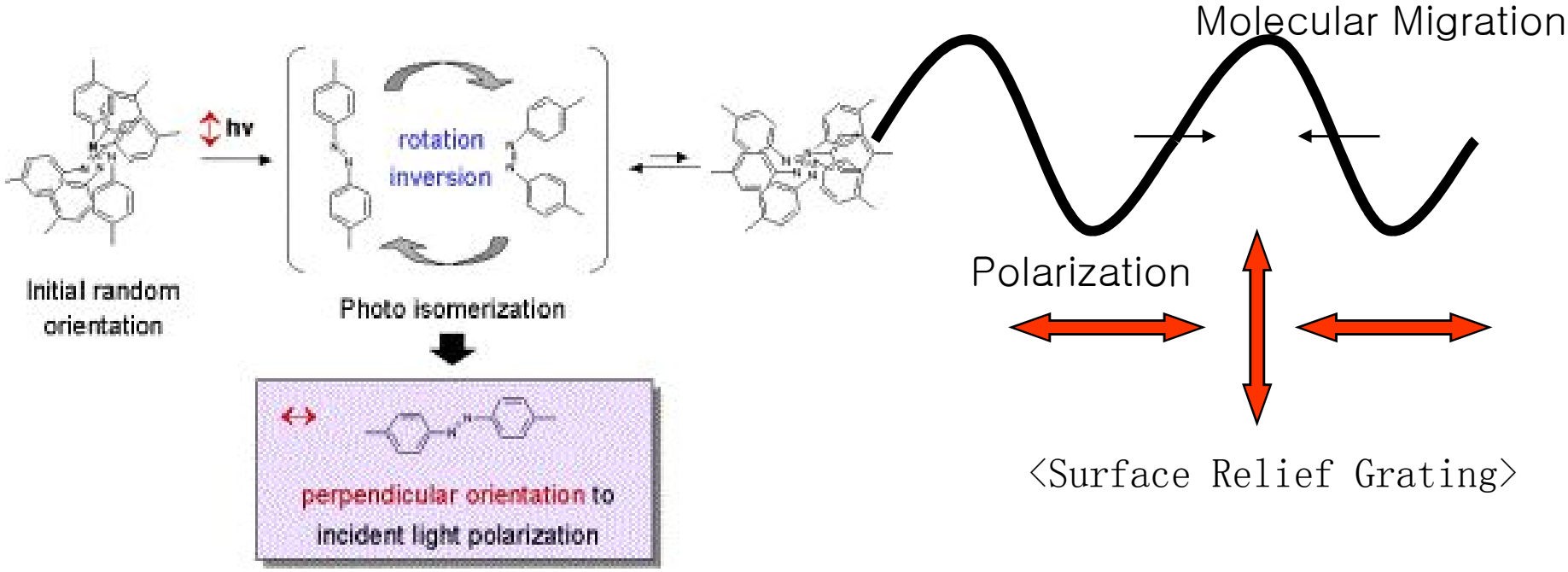
<SEM Image>

- **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**



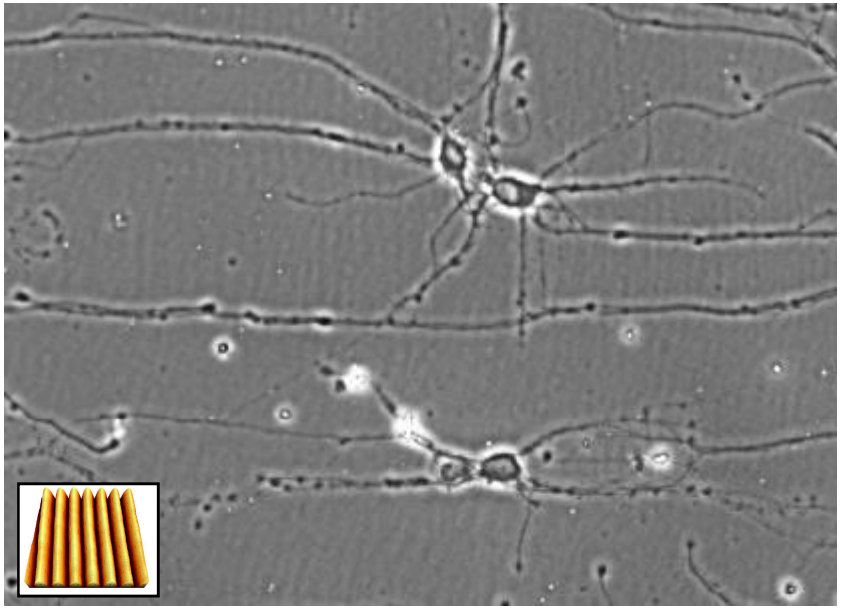
<AFM Image>

Photo-Responsive Polymer

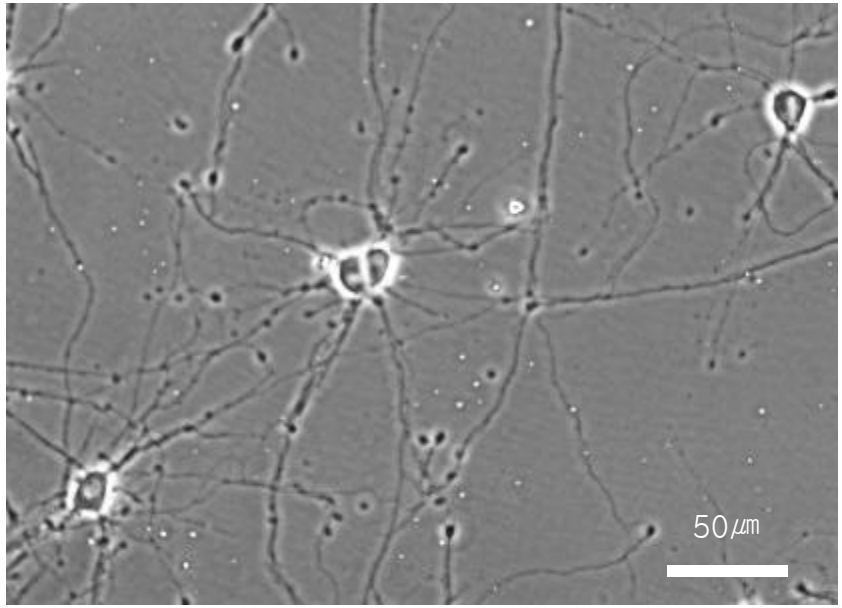


- **Photo-Responsive Azo-dye benzene copolymer**
- **$C_{12}H_{10}N_2$**
- **Cis-trans isomerization**
  - **Aligned parallel to axis of beam polarization**
- **Molecular Migration by modulation of polarization**

Reaction of Neuron to Grating



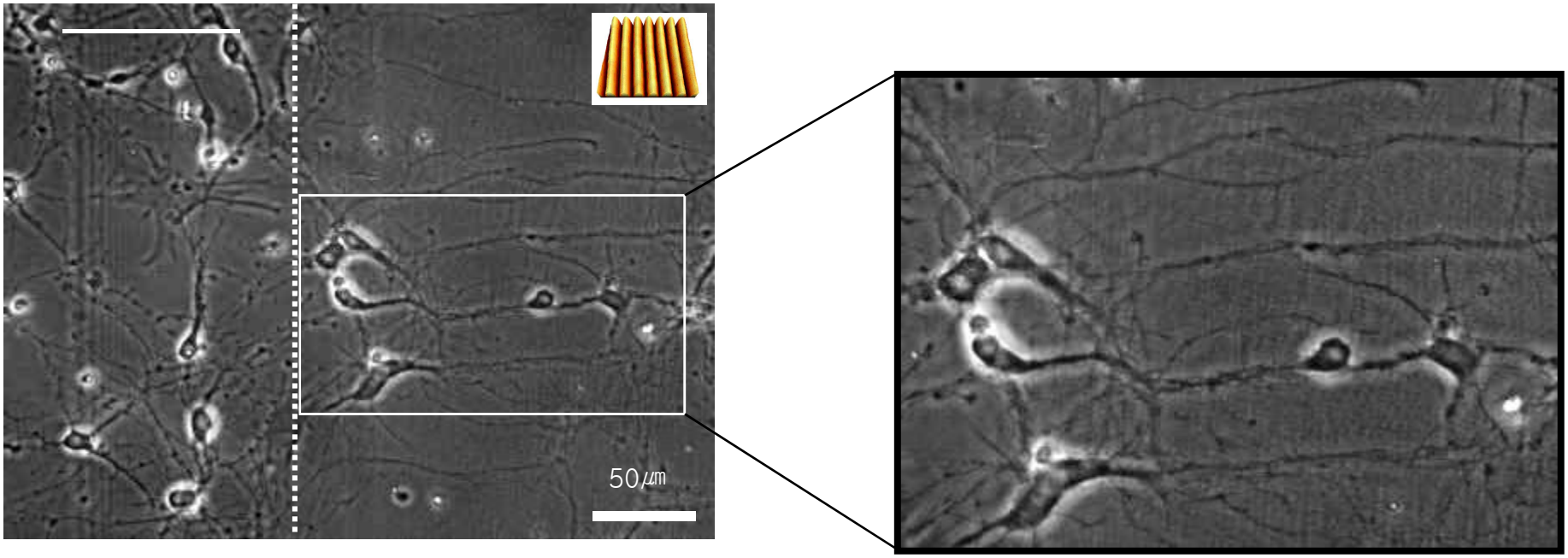
<Hippocampal neuronal cells On polymer with Grating >



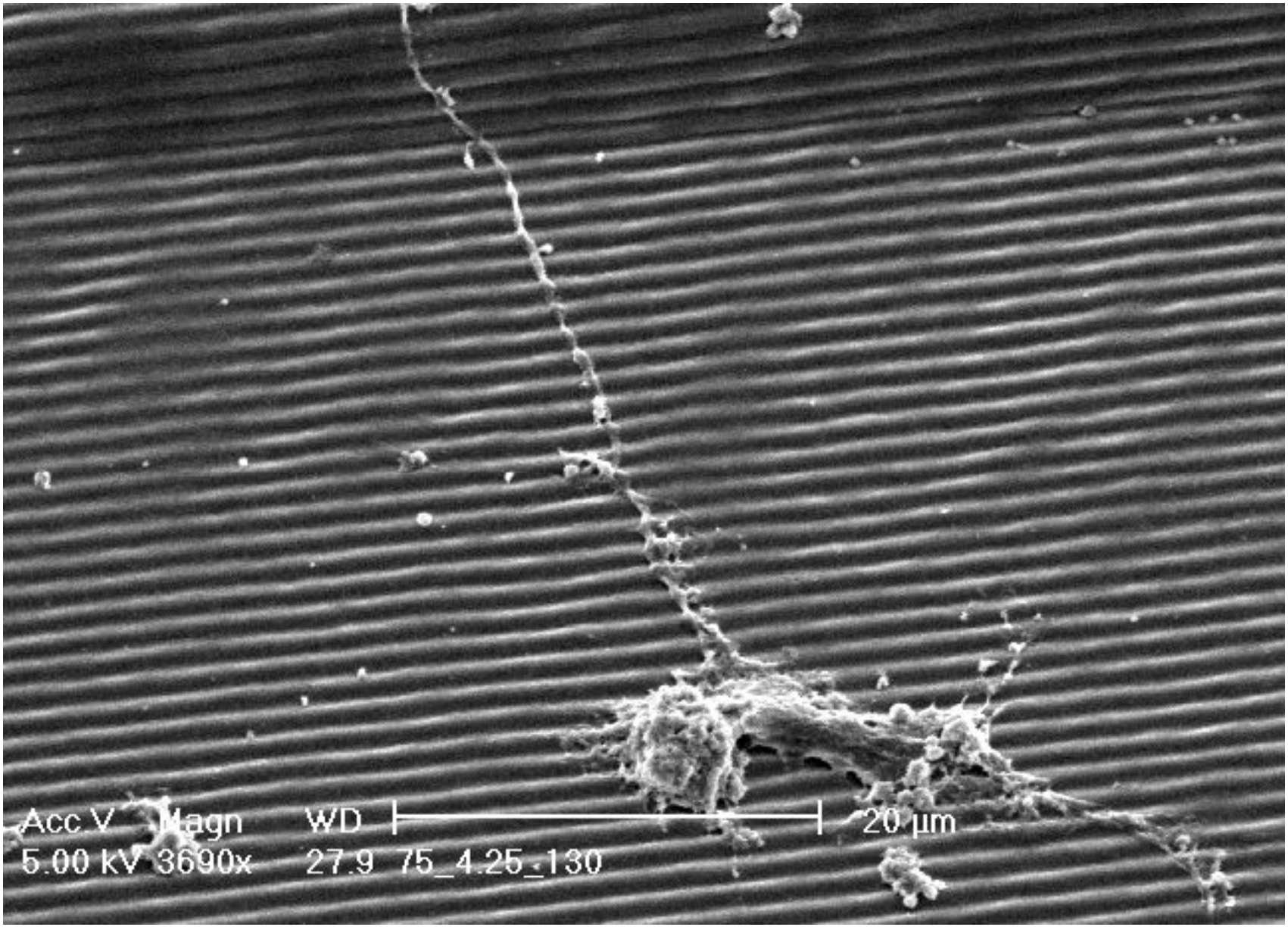
<On polymer without grating>

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

Contact Guidance of Neuron

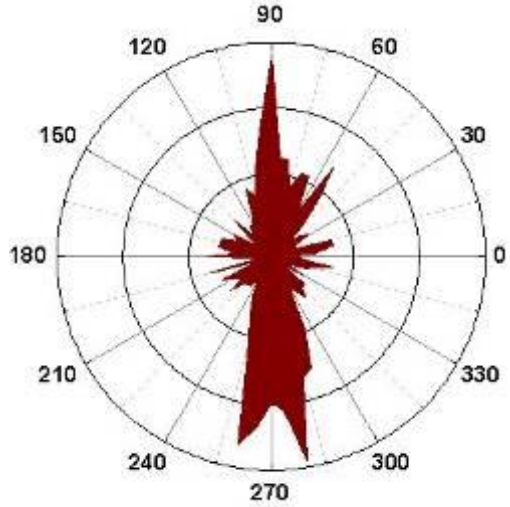


- **Random growth on smooth surface**
- **Perpendicular growth on the grooved surface**
- **Turning neurites on grating**

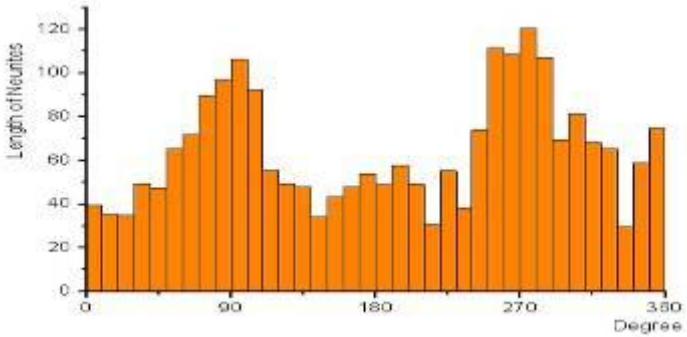

Acc.V Magn WD
5.00 kV 3690x 27.9 75_4.25_130
20 µm

Quantification of Cell Elongation

- **On Surface Relief**

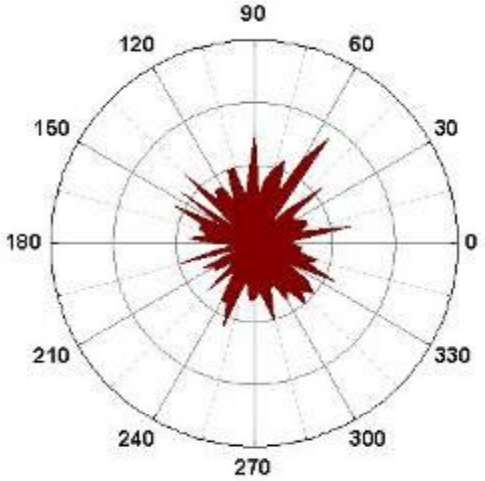


Frequencies of Neurites Orientation

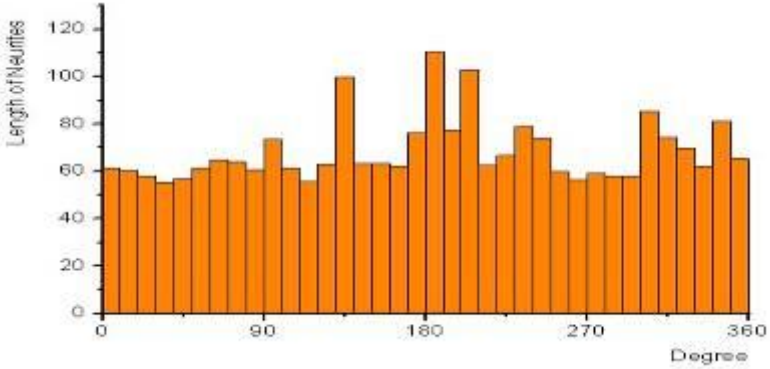


Lengths of Neurites Vs Angle

- **On Smooth Surface**



Frequencies of Neurites Orientation



Lengths of Neurites Vs Angle

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