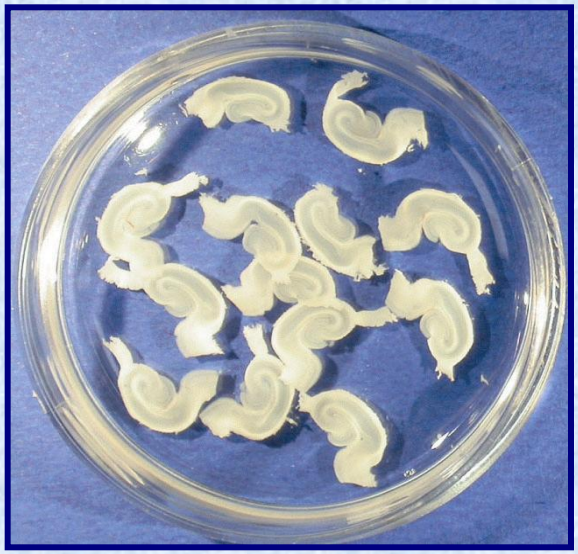
SİNİR DOKUSU VE GLIOMA KÜLTÜRLERİ



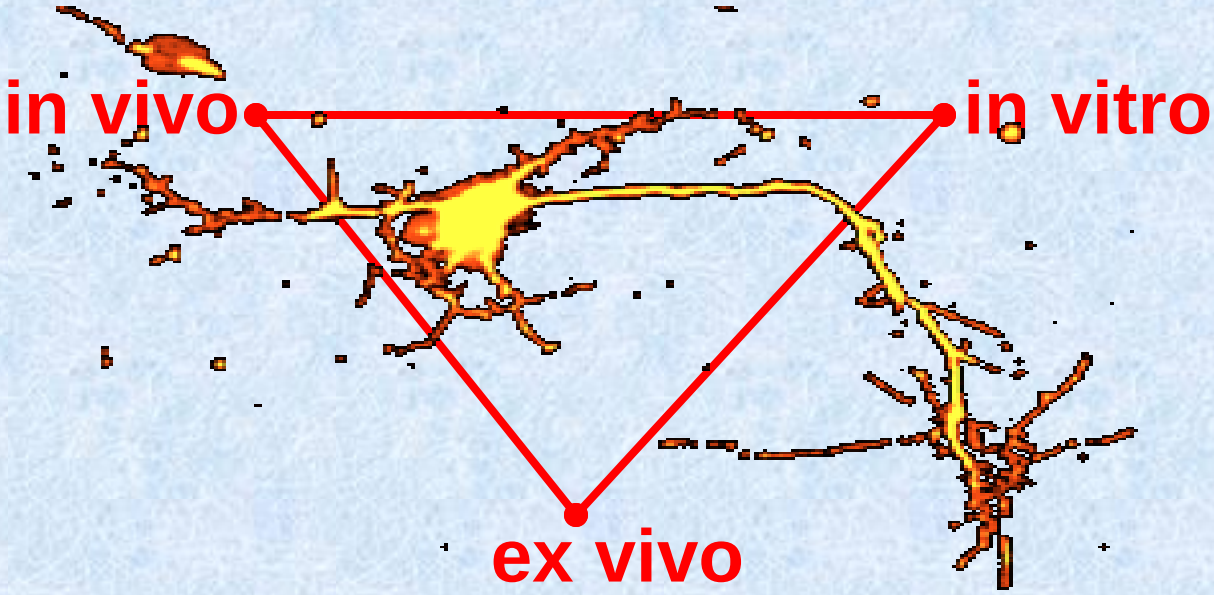
Prof. Dr. Ali Savaş

Ankara Üniversitesi Tıp Fakültesi
Beyin ve Sinir Cerrahisi Anabilim Dalı

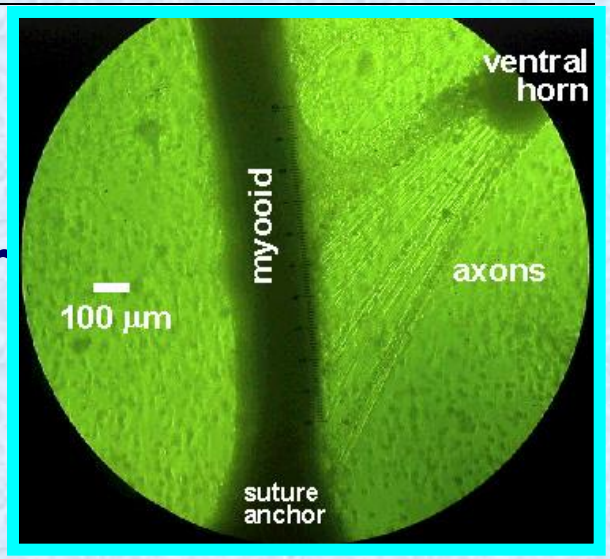
DOKU VE HÜCRE KÜLTÜRLERİ

Harrison RG : Observations on a living developing nerve fiber.
Proc Soc Exp Biol Med 4: 140-143, 1907

Canlı biyolojik materyallerin in vitro kultivasyonu



DOKU VE HÜCRE KÜLTÜRLERİ

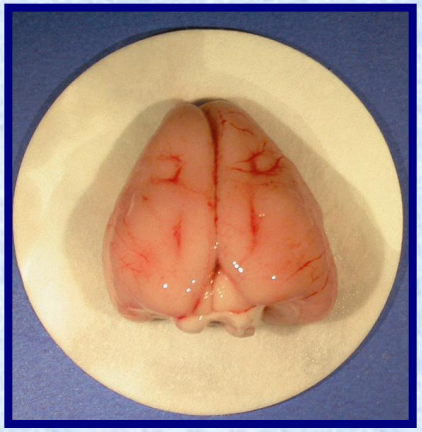


1- Hücre kültürleri-Ayrılmış hücreler
Tek kat veya suspansiyon

2- Reagregate kültürler

3- Organotipik doku kültürleri: Ayrılmamış hücreler doku
hücre ilişkileri korunmuş- sinir dokusu
3 boyutlu nöronal ilişki

SİNİR DOKUSU KÜLTÜRLERİ



- Hayvan: Memeliler, tavşan ve sıçan
- Yaş: Embrional, Neonatal veya Mature
- Neoplastik hücreler, kök hücreler, transfekte hücre serileri
- Seçilmiş – klonlanmış spesfikhücre serileri
- Sinir dokusu: Hippocampus, caudate nucleus, ganglion hücreleri

SİNİR DOKUSU KÜLTÜRLERİ

Cell lines & clones

Purified homogenious

Dissociated

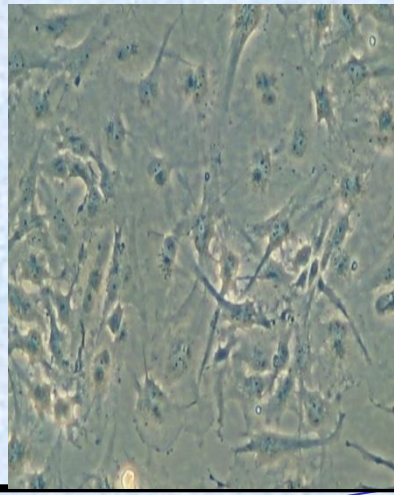
Reagregate

Organotypic

In vivo

Doku organizasyonu

Deneysel manipulasyon



MATURE

NEONATAL

EMBRIONAL

NEOPLASTIC

saatler

haftalar

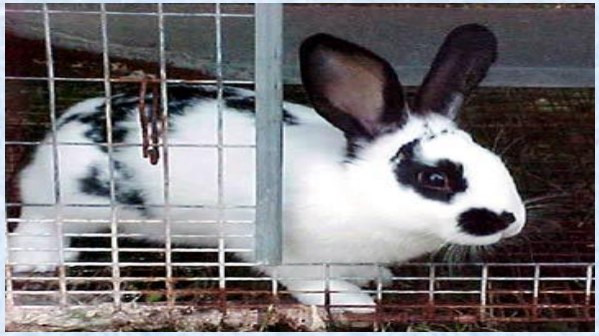
aylar

ORGANOTİPİK SİNİR DOKUSU KÜLTÜRLERİ

Materials & Methods

- 17 Watanabe rabbits

2 x 17 hippocampi ⟶ > 490 slices (data)



Chemicals:

- Choline chloride [methyl-^{3}H] (79.2 Ci/mmol) & hemicholinium-3
- Amino acids:

alanine, arginine, citrulline, cysteine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, serine, taurine, threonine, tryptophan, tyrosine, valine

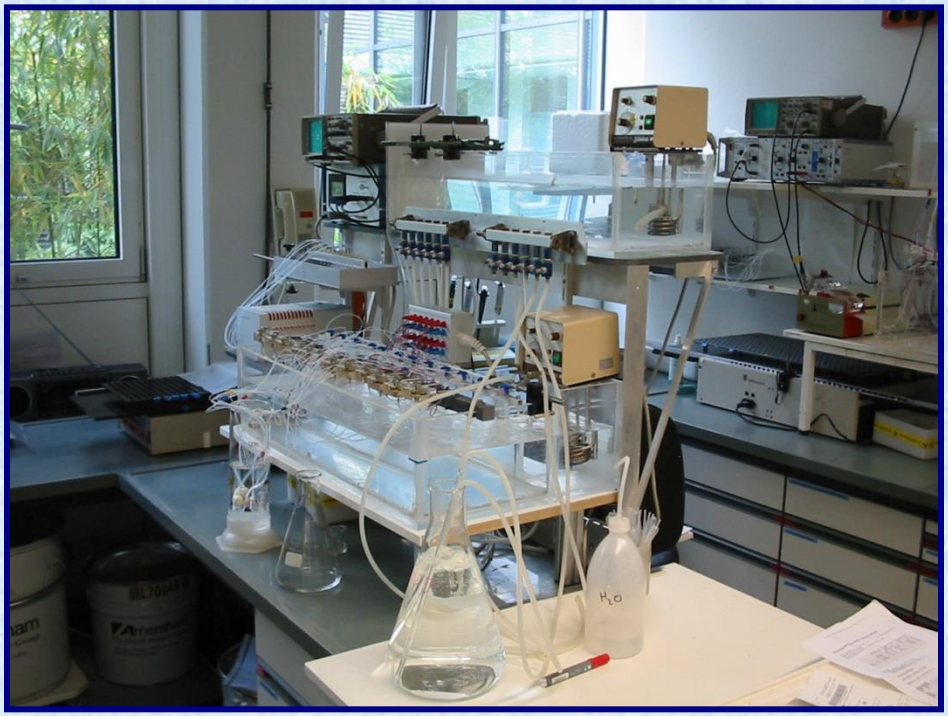
- Bovine serum albumin (Chon V fraction)
- NaCl, KCl, KH_2PO_4, $NaHCO_3$, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, NaOH,
- Glucose
- L-ascorbic acid
- Penicillin/streptomycin

Materials & Methods

- Culture Medium & Solutions:
  Enviromental Influences

1- Modified Krebs-Henseleit buffer

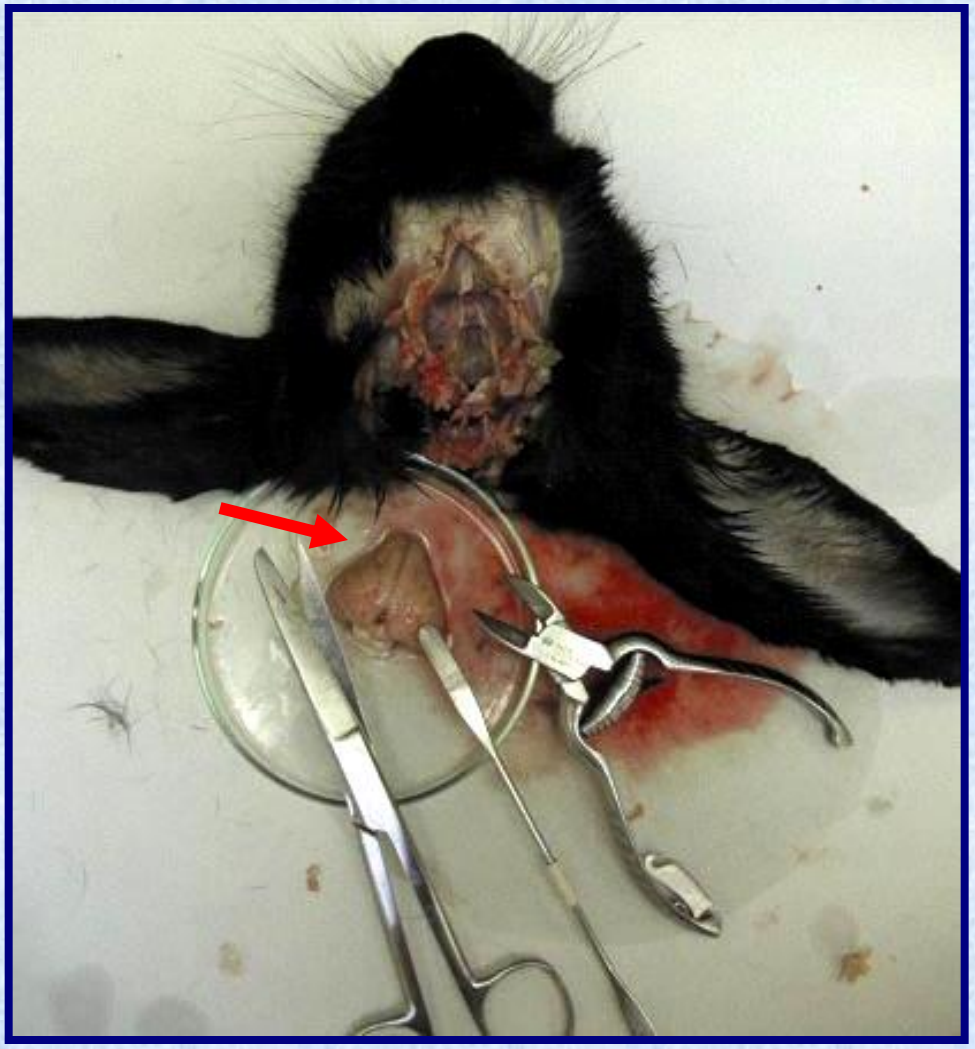
2- CSF-like culture medium- fresh



Chemicals	Molecular Weight	Concentration	
NaCl	58.44	121.0 mM	7.07 g/l
KCl	74.56	1.8 mM	0.13 g/l
KH_2PO_4	136.09	1.2 mM	0.16 g/l
$NaHCO_3$	84.01	25.0 mM	2.10 g/l
$MgSO_4.7H_2O$	246.48	1.2 mM	0.30 g/l
$CaCl_2.2H_2O$	147.02	1.3 mM	0.19 g/l
Glucose	198.17	20.0 mM	3.96 g/l
L-Ascorbic acid	176.13	11.0 mM	0.10 g/l
Bovine serum albumine		3.00 g/l	
Penicillin-Streptomycin		200U/l-200mg/l	
Amino acids:			
alanine	89.0	34.0 mM	3.03 mg/l
arginine	210.7	22.0 mM	4.64 mg/l
aspartate	133.1	0.13 mM	0.02 mg/l
citrulline	175.2	5.70 mM	1.00 mg/l
cystine	240.3	0.12 mM	0.03 mg/l
histidine	209.6	13.00 mM	2.73 mg/l
isoleucine	131.2	3.8 mM	0.5 mg/l
leucine	131.2	11.4 mM	1.5 mg/l
lysine	182.6	27.70 mM	5.06 mg/l
methionine	149.2	3.40 mM	0.51 mg/l
ornithine	168.6	3.80 mM	0.64 mg/l
phenylalanine	165.2	9.10 mM	1.50 mg/l
serine	105.1	28.50 mM	3.00 mg/l
taurine	125.1	8.00 mM	1.00 mg/l
threonine	119.1	32.00 mM	3.81 mg/l
tryptophan	204.2	1.50 mM	0.31 mg/l
tyrosine	181.2	8.30 mM	1.50 mg/l
valine	117.1	12.80 mM	1.50 mg/l

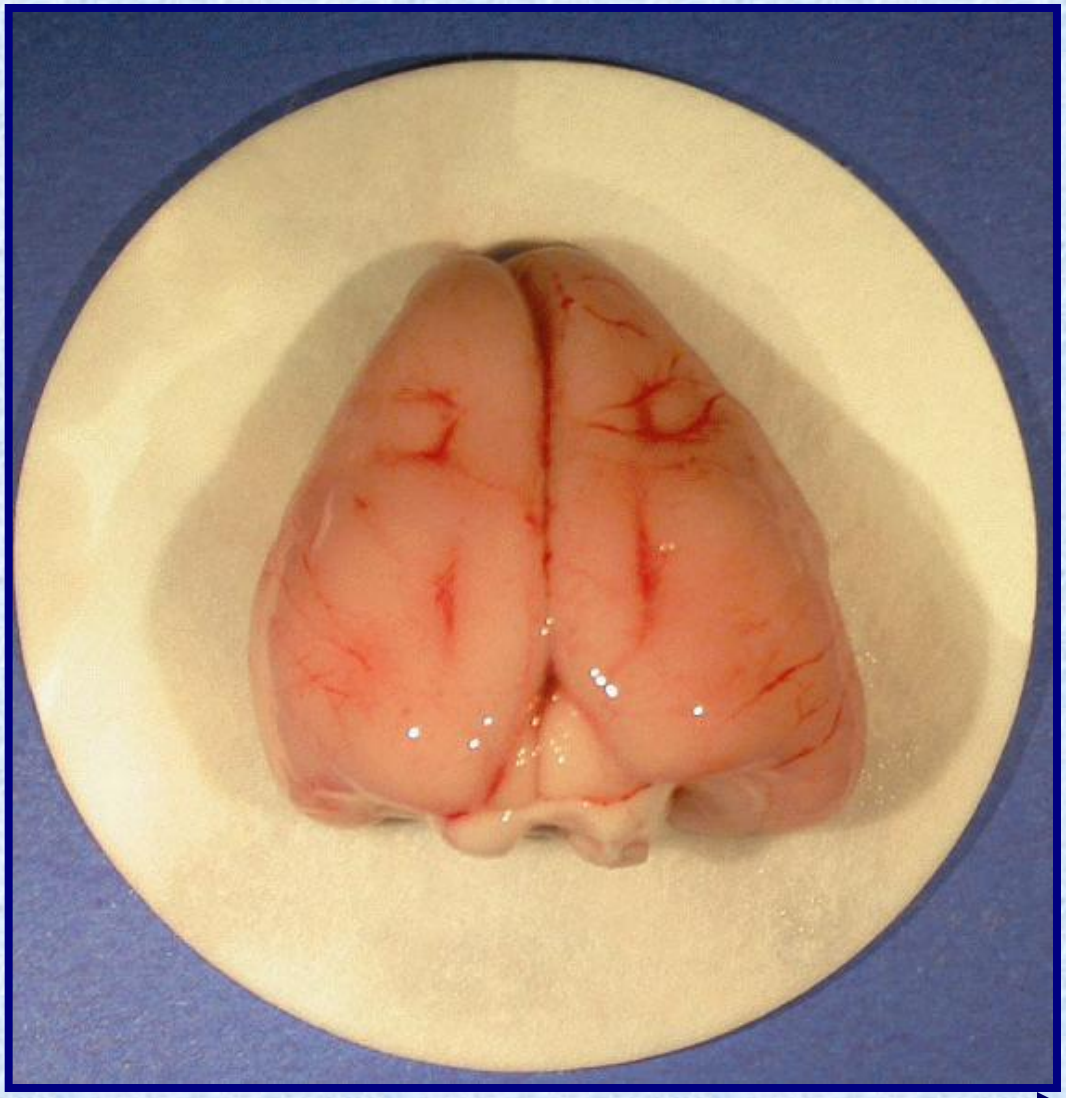
Materials & Methods

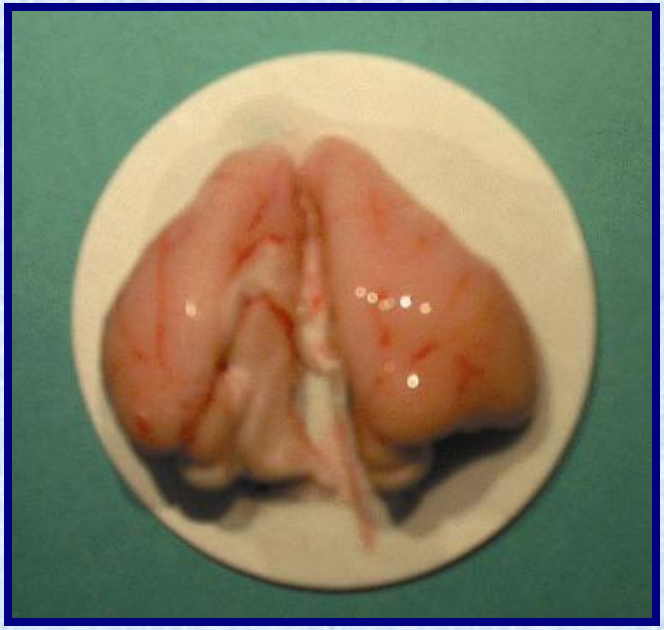
- Decapitation and removal of the rabbit brain in ice-cold conditions

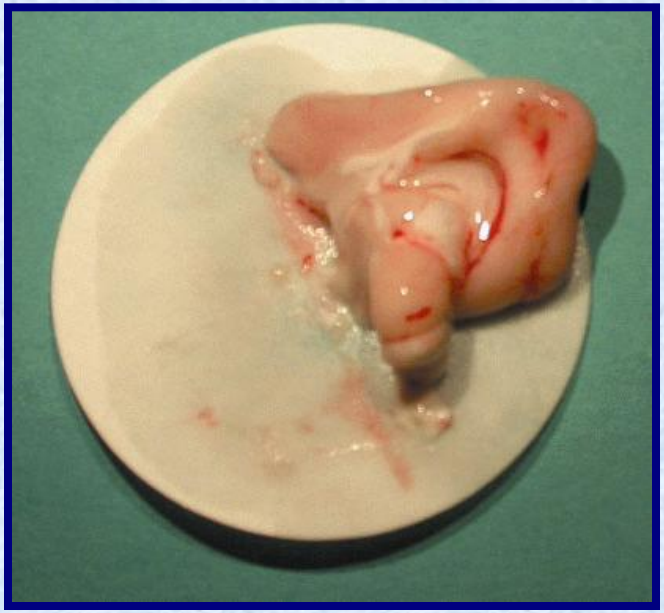


Materials & Methods

- Dissection & removal of the hippocampus



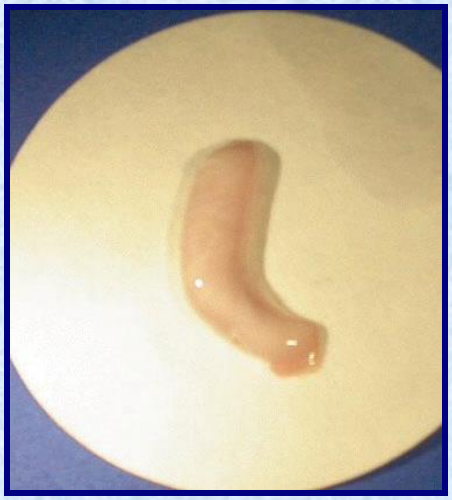


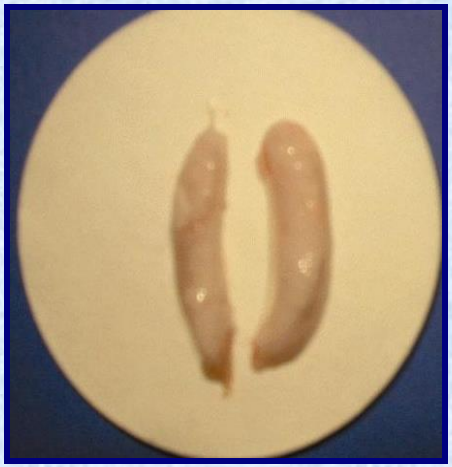


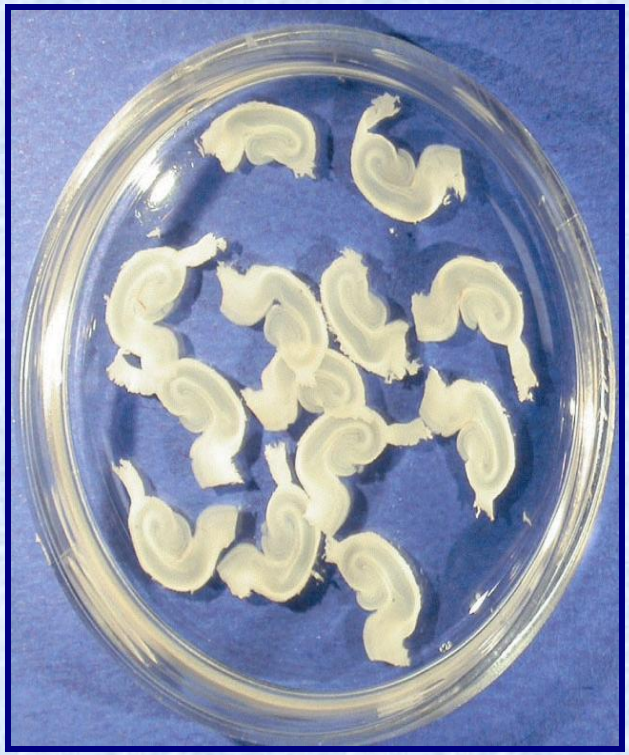
Materials & Methods

- Two hippocampi → 350 micrometer slices

Tissue Chopper



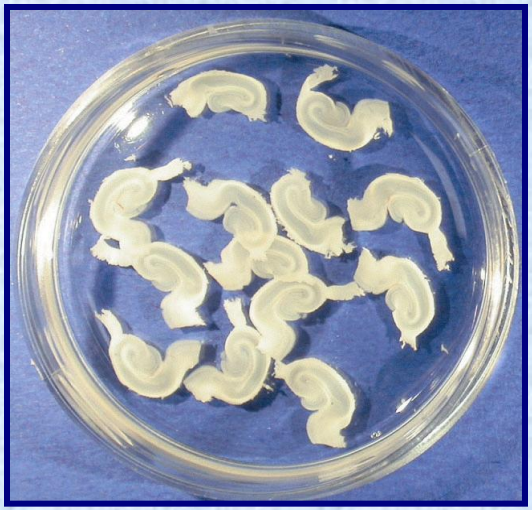




10 min

Materials & Methods

- Incubation of the slices

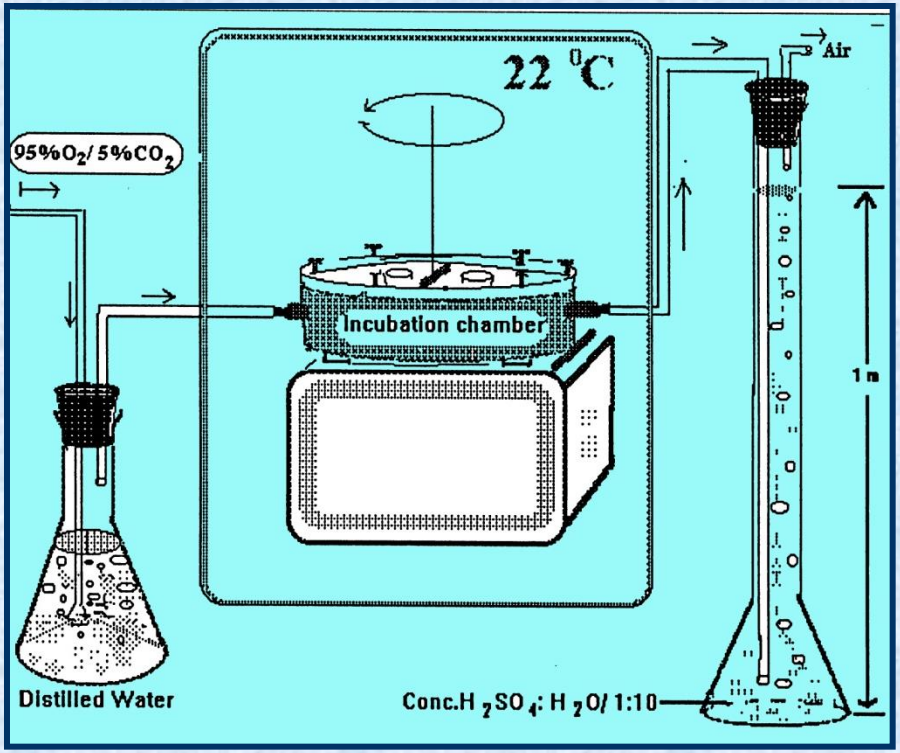


Dynamic incubation chamber

Incubation of the slices **72 hours**

Materials & Methods

- **Dynamic Incubation of the slices**



Incubation of the slices **72 hours**
(O_2 + rotation + culture medium change)

SAFLAŞTIRMA İŞLEMLERİ

Dissection

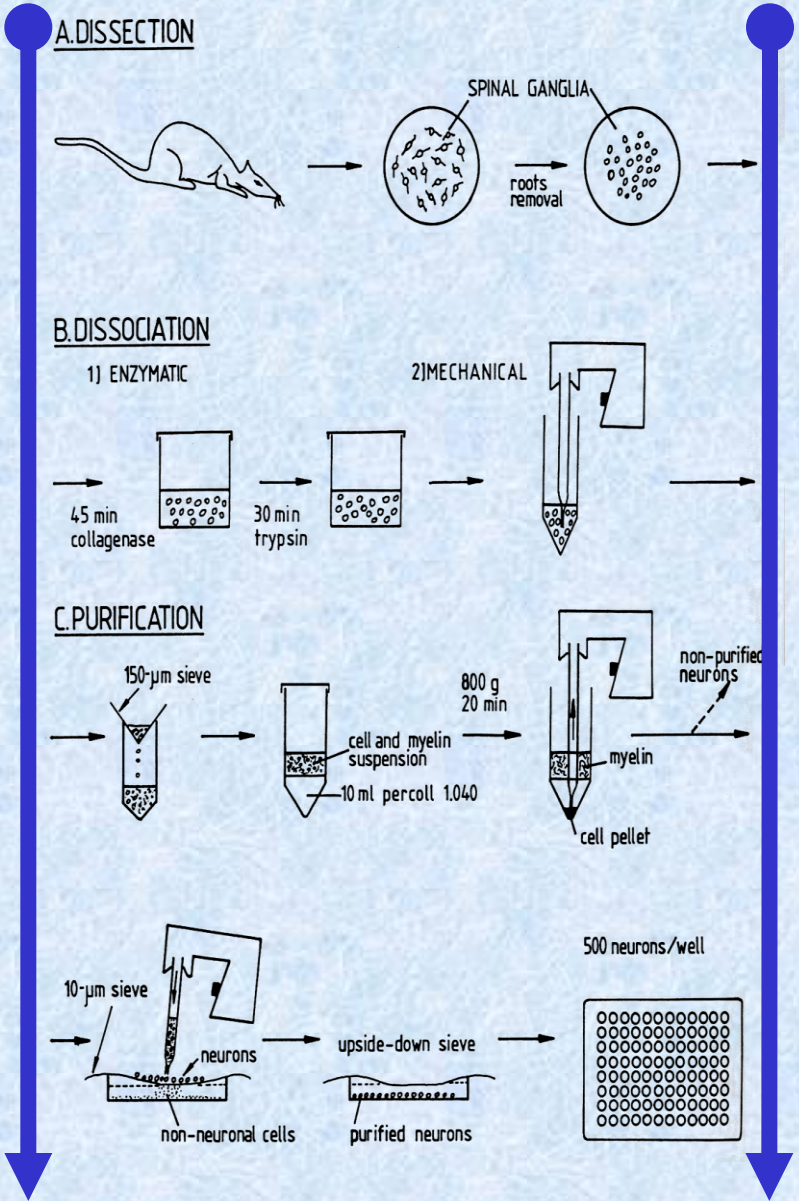
Dissociation

Enzymatic (Collegenase & Trypsin)
Mechanical

Purification

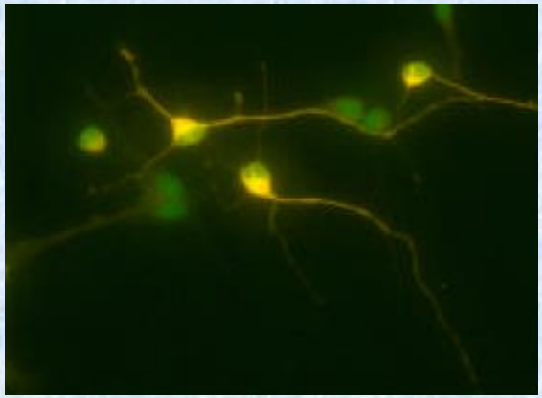
Sieve (150 μm) Myelin + cell
Isoosmotic Percoll sol.

Sieve (10 μm)- Dorsal goot ggl. cells-

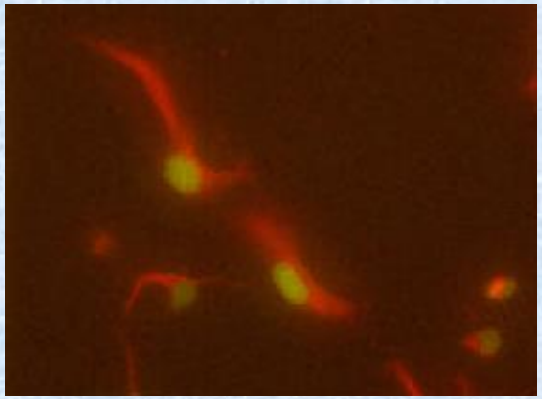


SİNİR DOKUSU KÜLTÜRLERİ

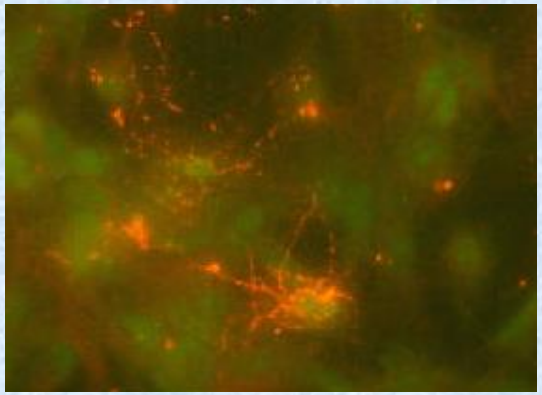
ÖZEL MEDUM ORTAMINDA 7-10 GÜN



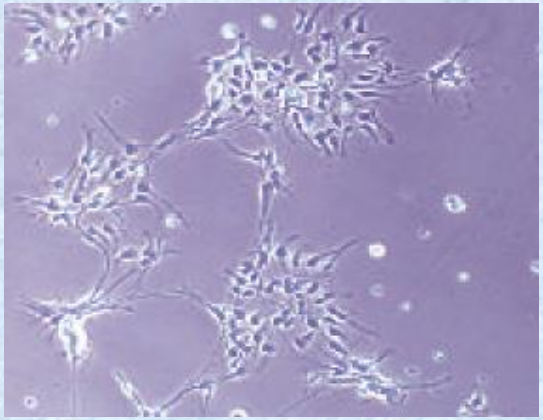
Neurons
(Neuron specific ß-III tubulin)



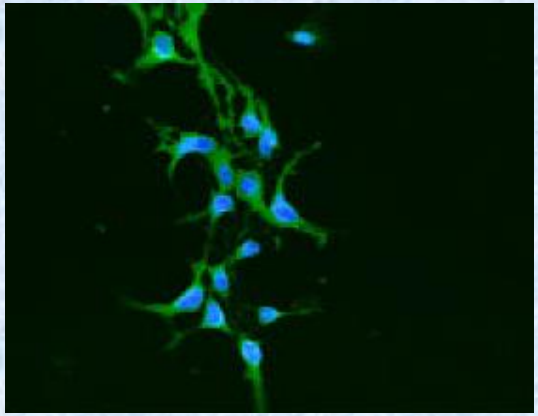
Astrocytes
(GFAP)



Oligodentdrocytes
(Oligodendrocyte marker O4)



Neural Stem Cells

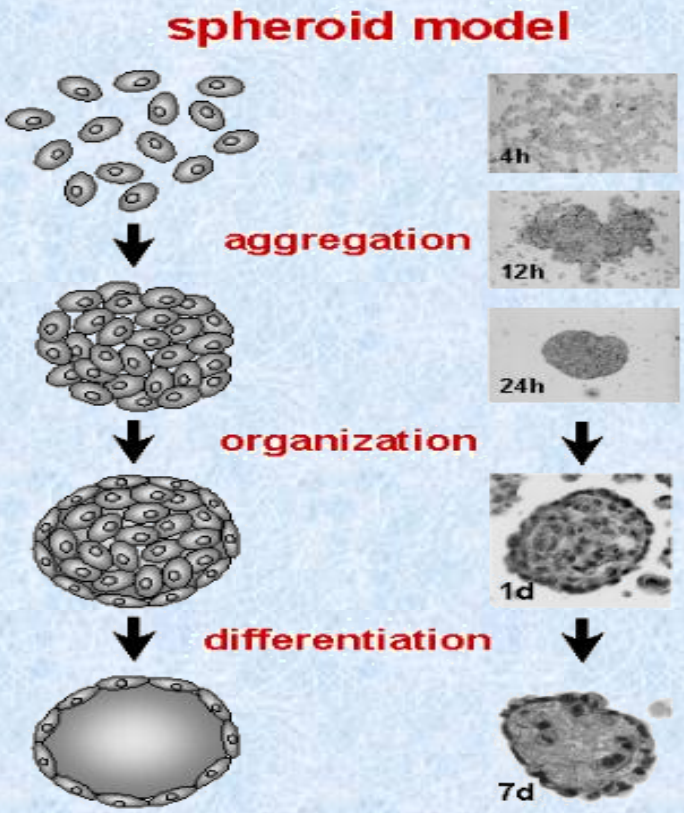


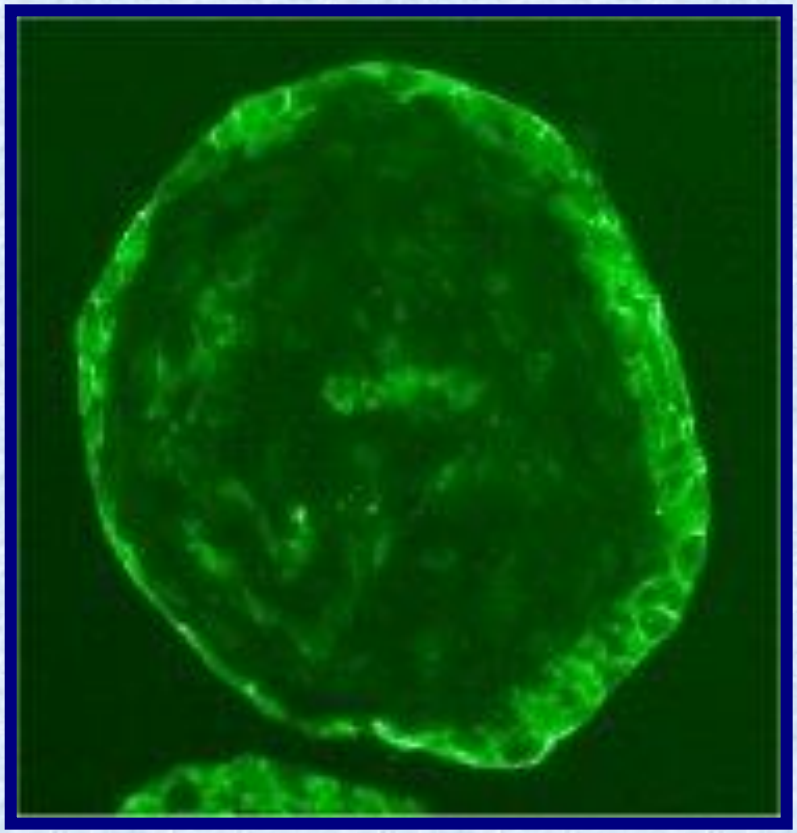
Neural Stem Cells
(Nestin)

GLİOMA KÜLTÜRLERİ

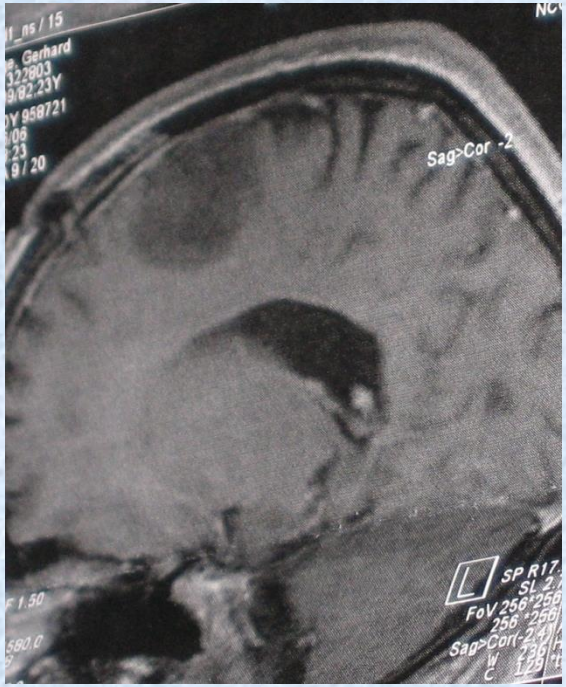
- Spheroids: hücrelerin spontan agregasyonu

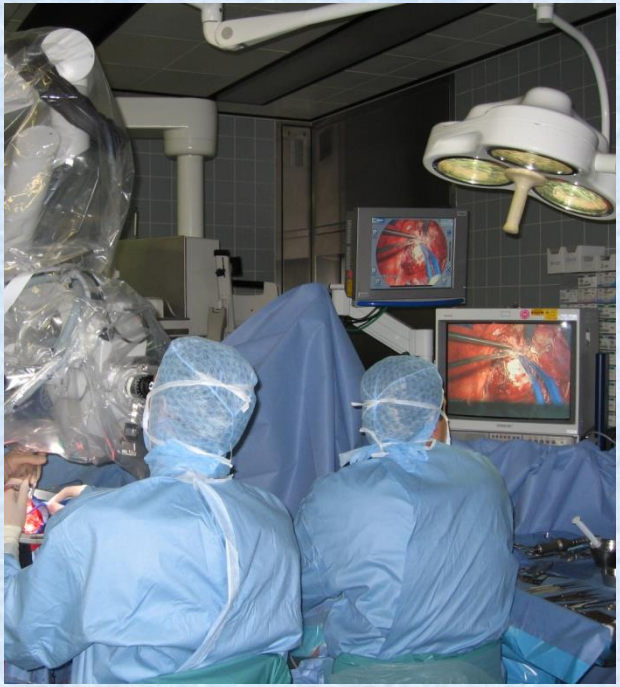
growth – organization - differentiation

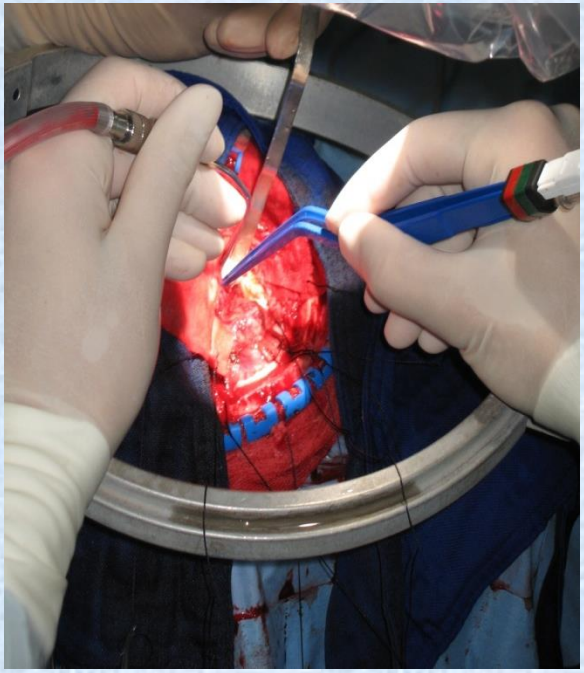




PRİMER HÜCRE KÜLTÜRLERİ VE TÜMÖR İZOLASYONU







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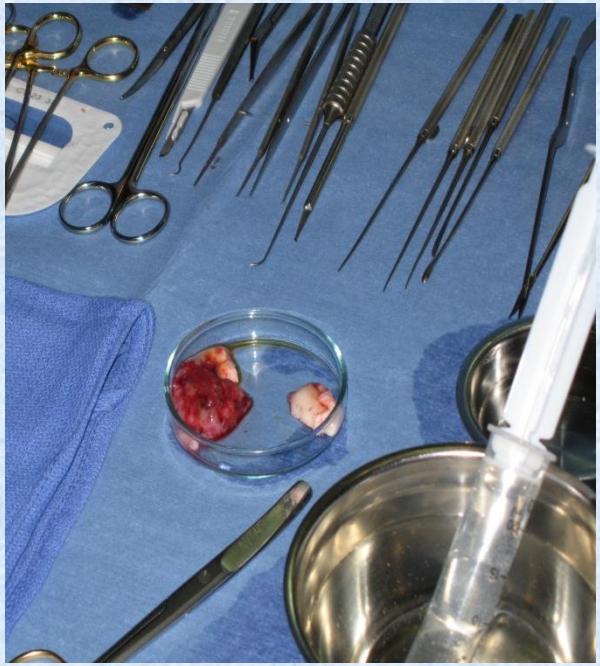
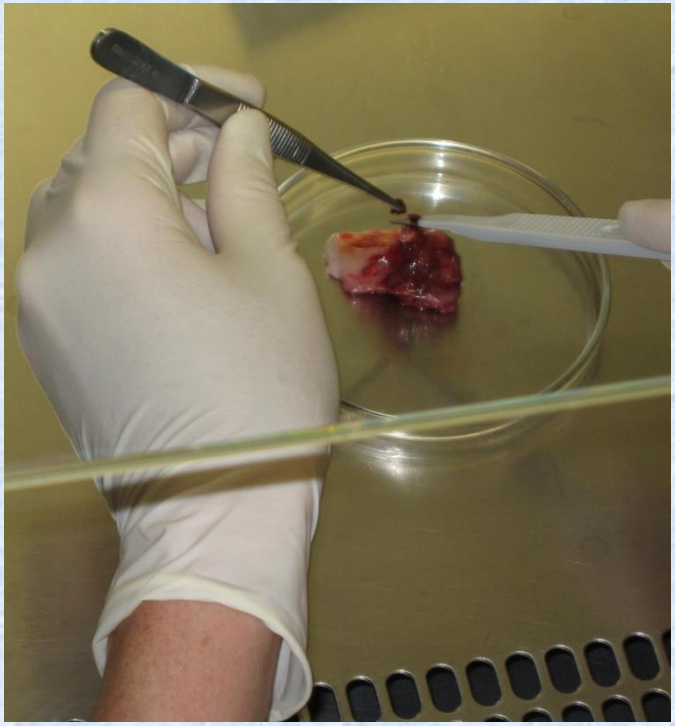
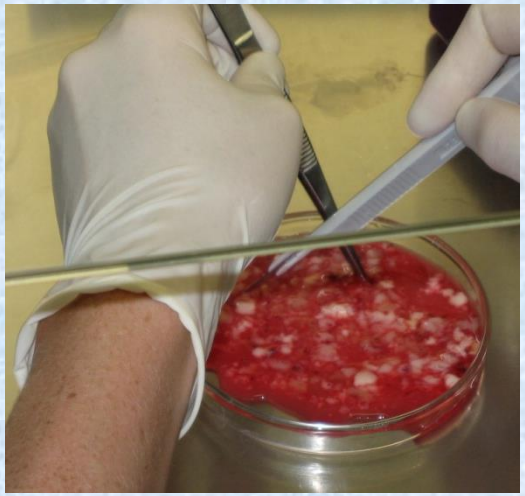
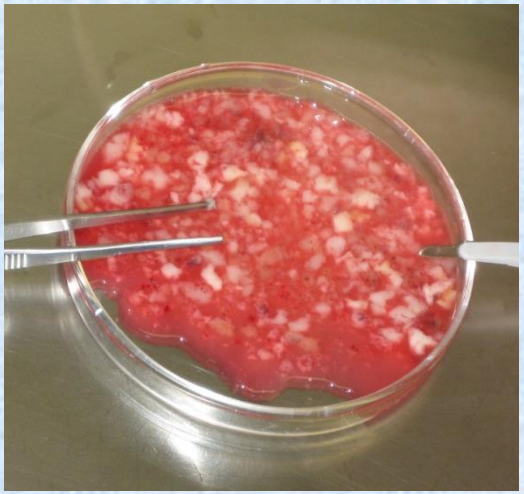


PRİMER HÜCRE KÜLTÜRLERİ VE TÜMÖR İZOLASYONU

- Hızlı mekanik diseksiyon: bıçak, penset (at 4 C^0)
-

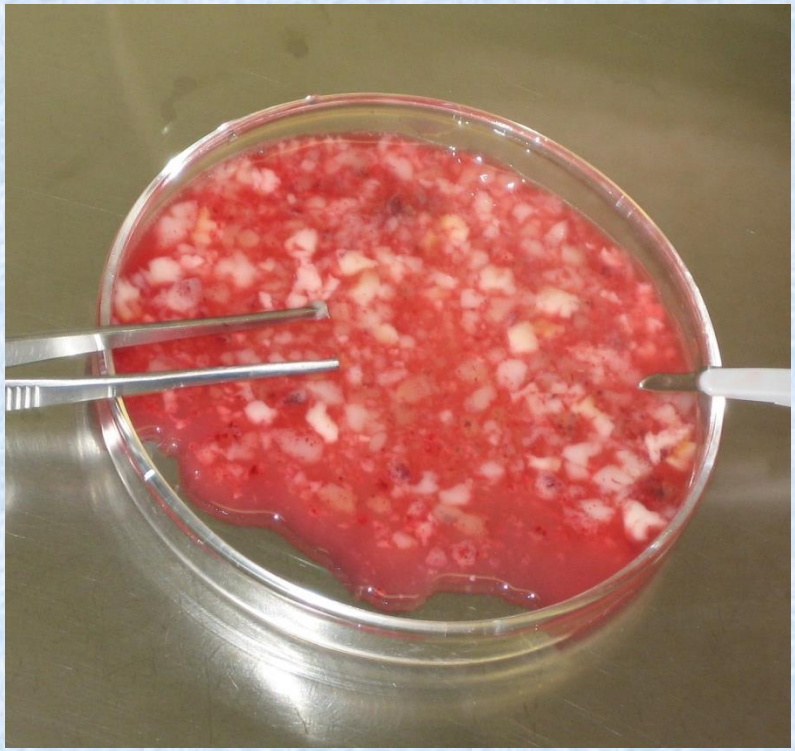






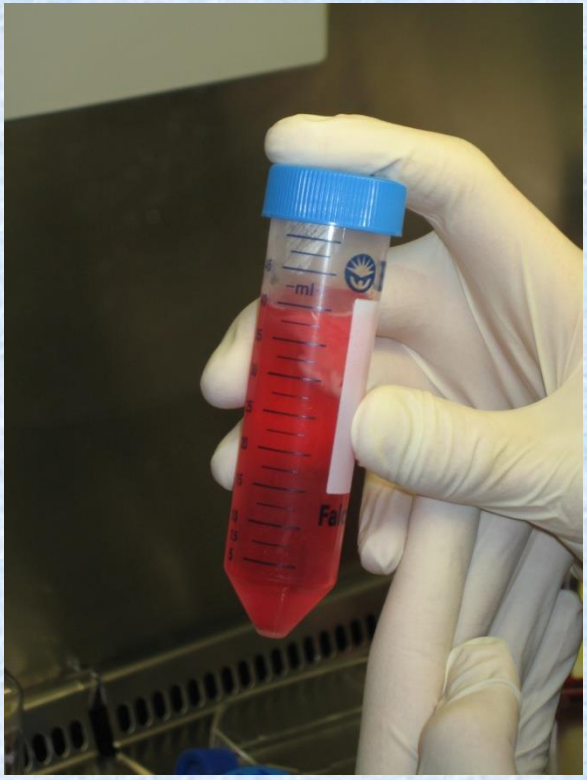
PRİMER HÜCRE KÜLTÜRLERİ VE TÜMÖR İZOLASYONU

- Cam tüpte mekanik homojenizasyon



PRİMER HÜCRE KÜLTÜRLERİ VE TÜMÖR İZOLASYONU

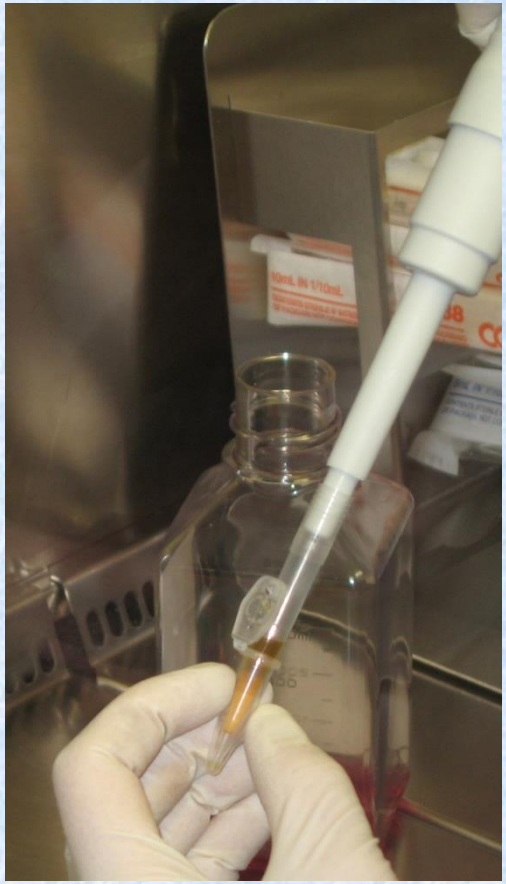
- Myelinin ayrılması- Dextran 15%

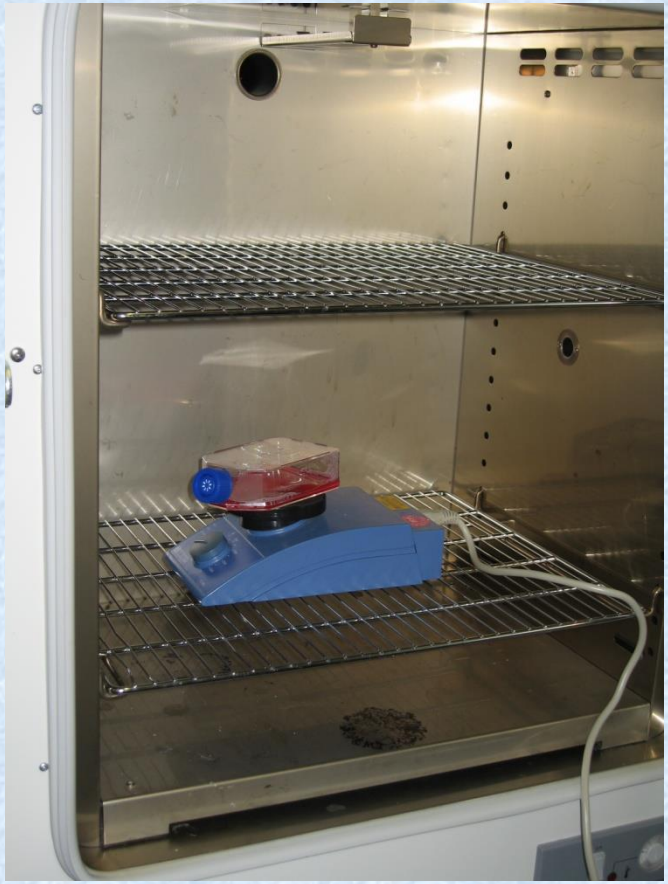


PRİMER HÜCRE KÜLTÜRLERİ VE TÜMÖR İZOLASYONU

- Dinamik enzimatik disagregasyon

  Collogenase (200 U/ml) /Dispase at 37 C^0





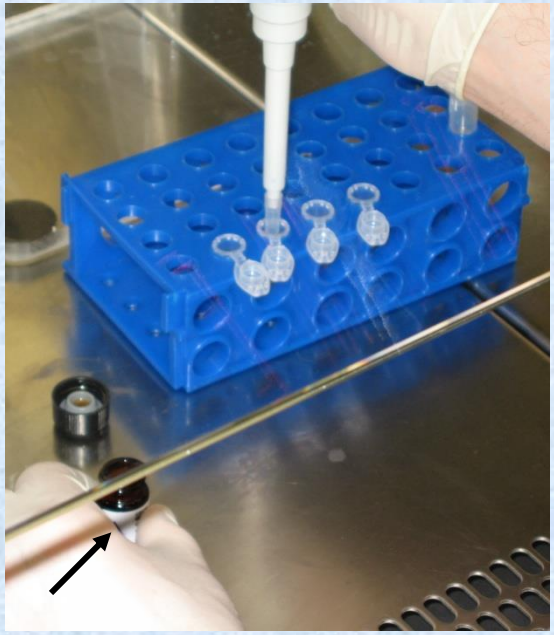
PRİMER HÜCRE KÜLTÜRLERİ VE TÜMÖR İZOLASYONU

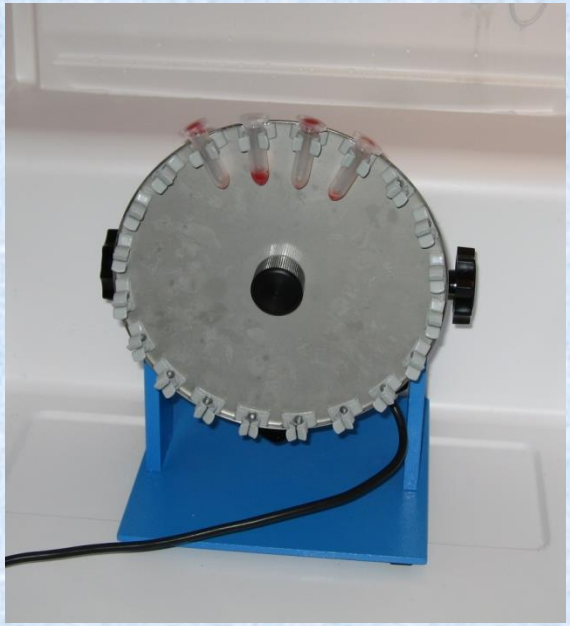
- Filtrasyon-100 µm and 40 µm – santrifüj
- Pellet (5-10 saat)

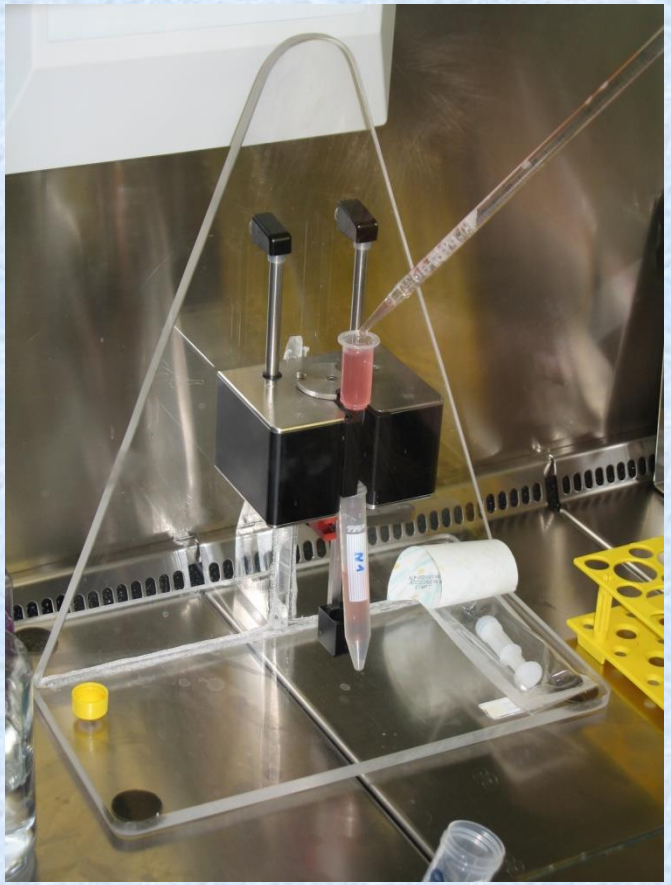
PRİMER HÜCRE KÜLTÜRLERİ VE TÜMÖR İZOLASYONU

- Nöral kanser kök hücre izolasyonu

  MACS-system – CD133 antibody+magnet marker
  Magnetic cell separation

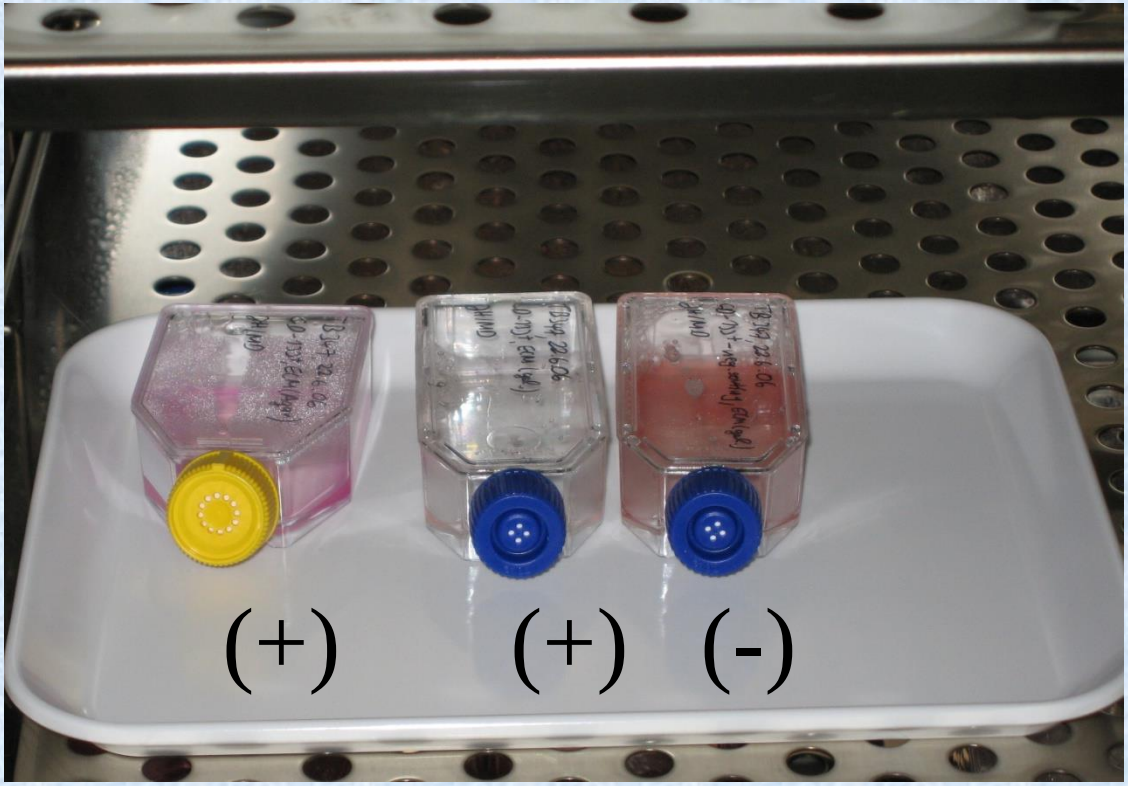






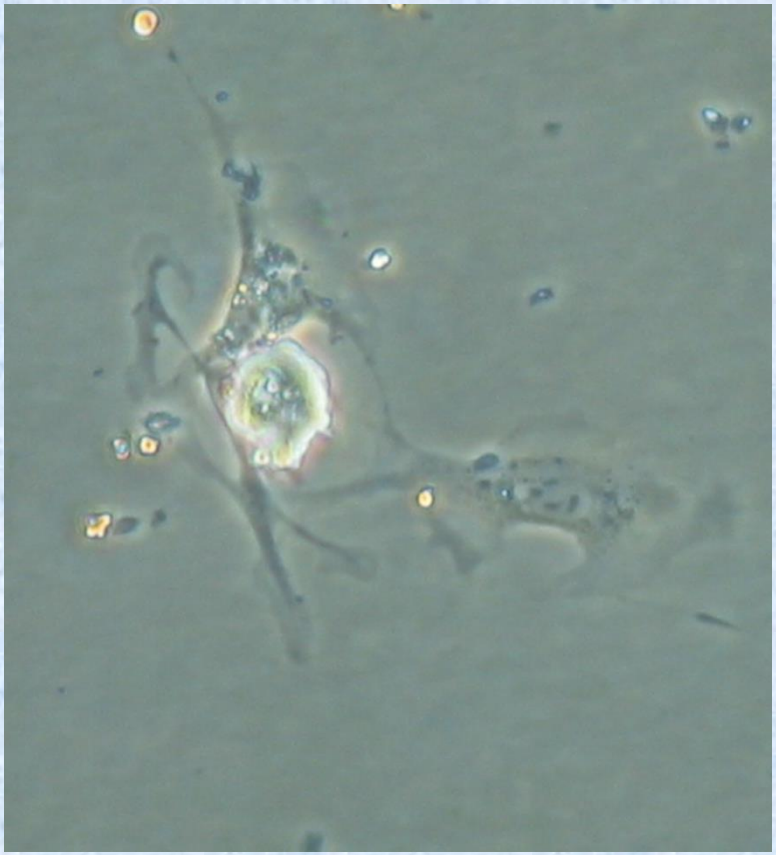
PRİMER HÜCRE KÜLTÜRLERİ VE TÜMÖR İZOLASYONU

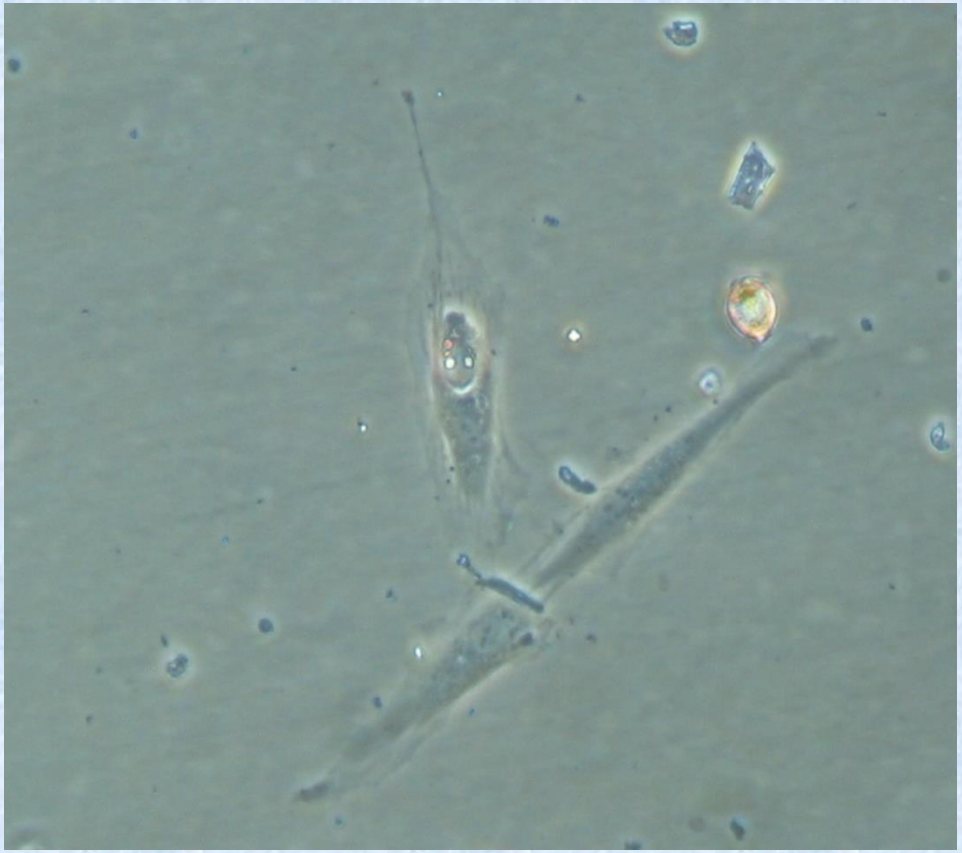
- Agar and gelatine culture flasks
  + (stem cells) and - solution cultures
- Monolayer- DMEM or Neural stem cell media
- Medium change- 48 hours – 37 C^0



PRİMER HÜCRE KÜLTÜRLERİ VE TÜMÖR İZOLASYONU

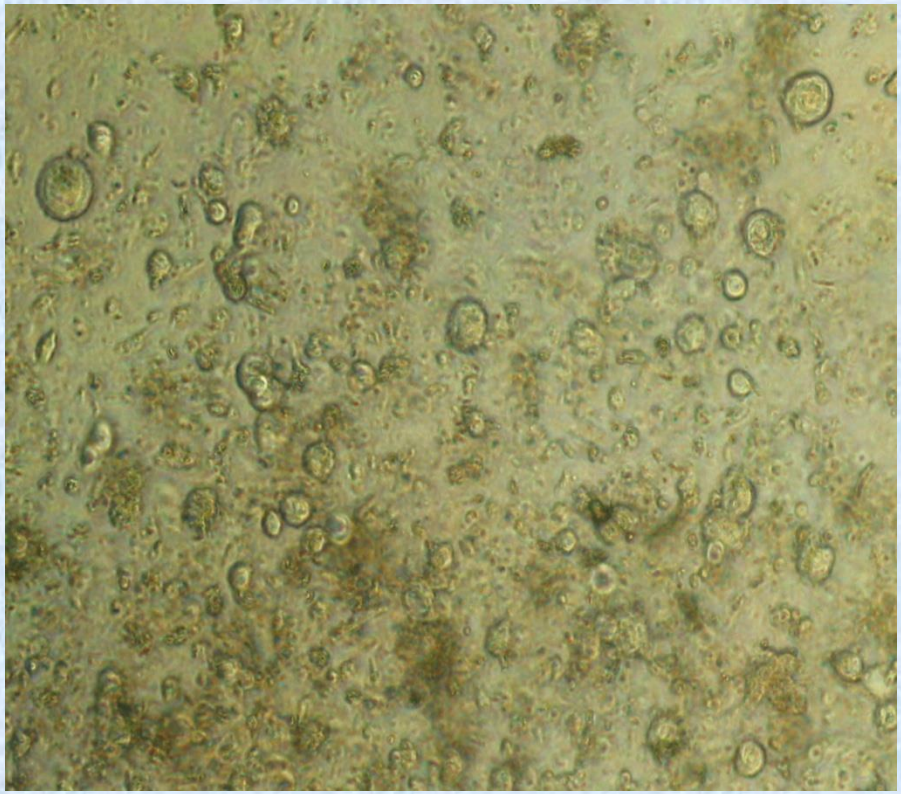
- 3. gün
- Neural-Cancer stem cells

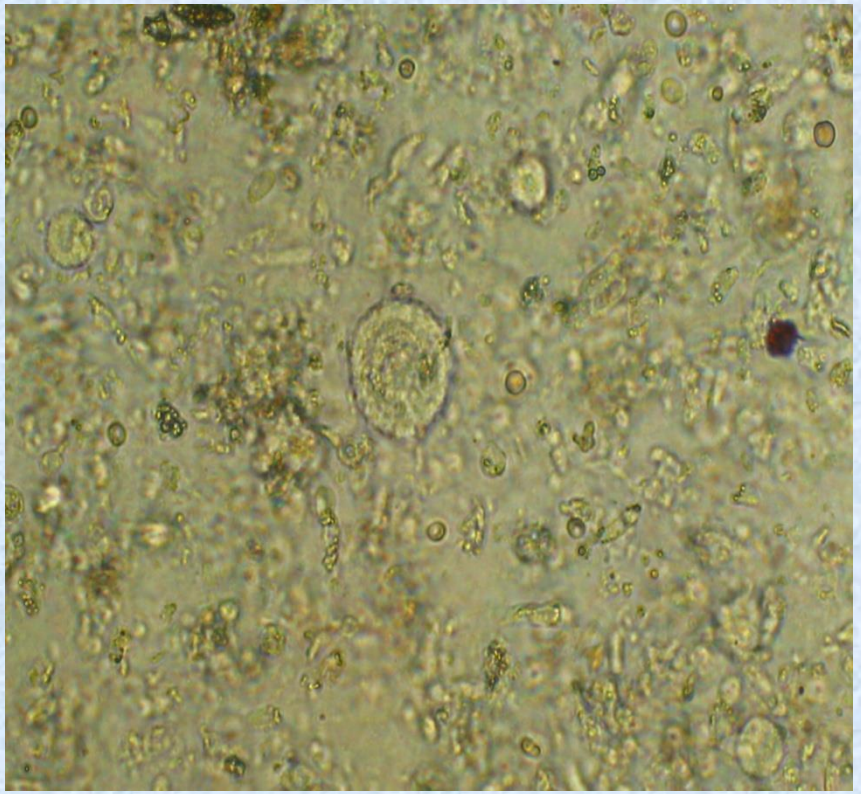




PRİMER HÜCRE KÜLTÜRLERİ VE TÜMÖR İZOLASYONU

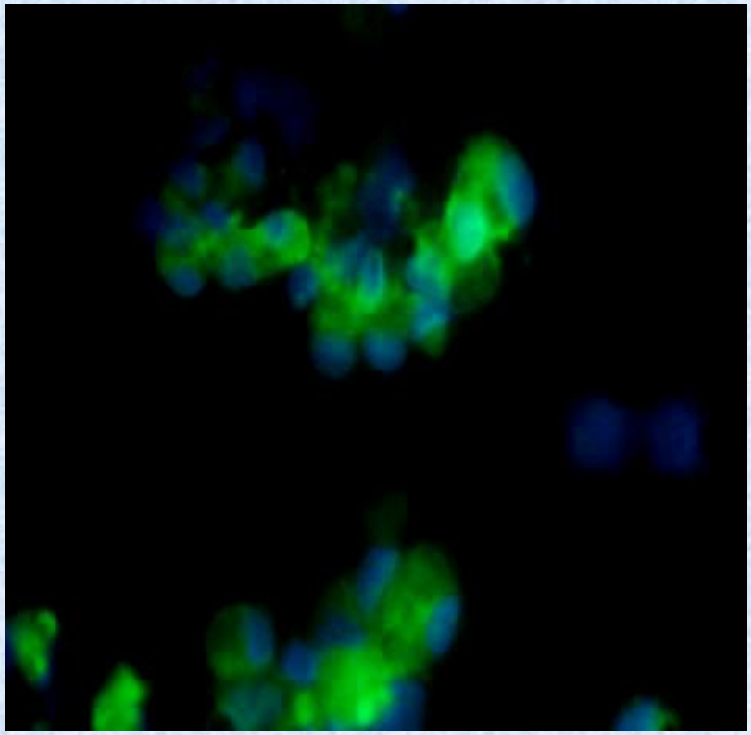
- 1 hafta
- Neural-Cancer stem cells



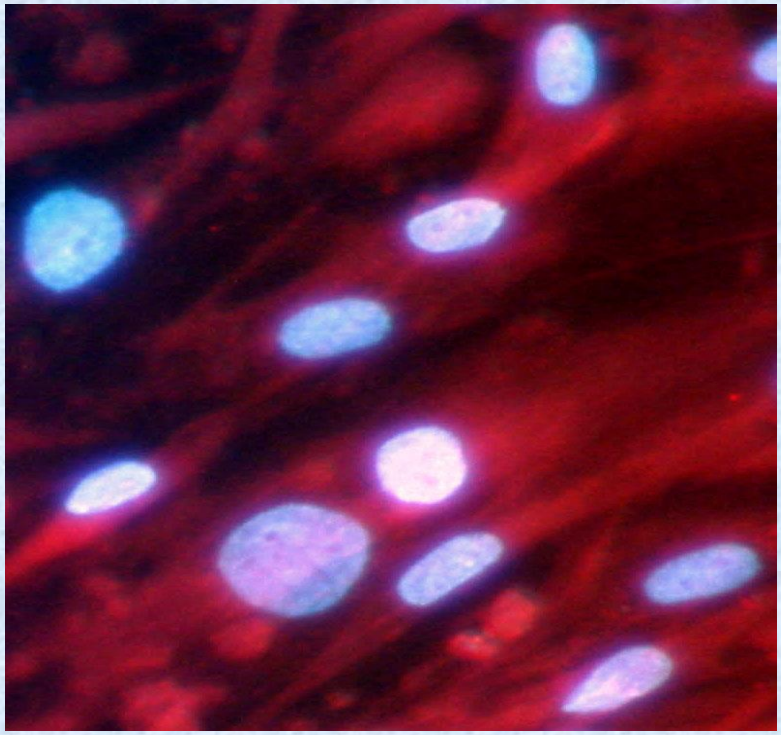


PRİMER HÜCRE KÜLTÜRLERİ VE TÜMÖR İZOLASYONU

- 10. gün
- Neural-Cancer stem cells + DMEM



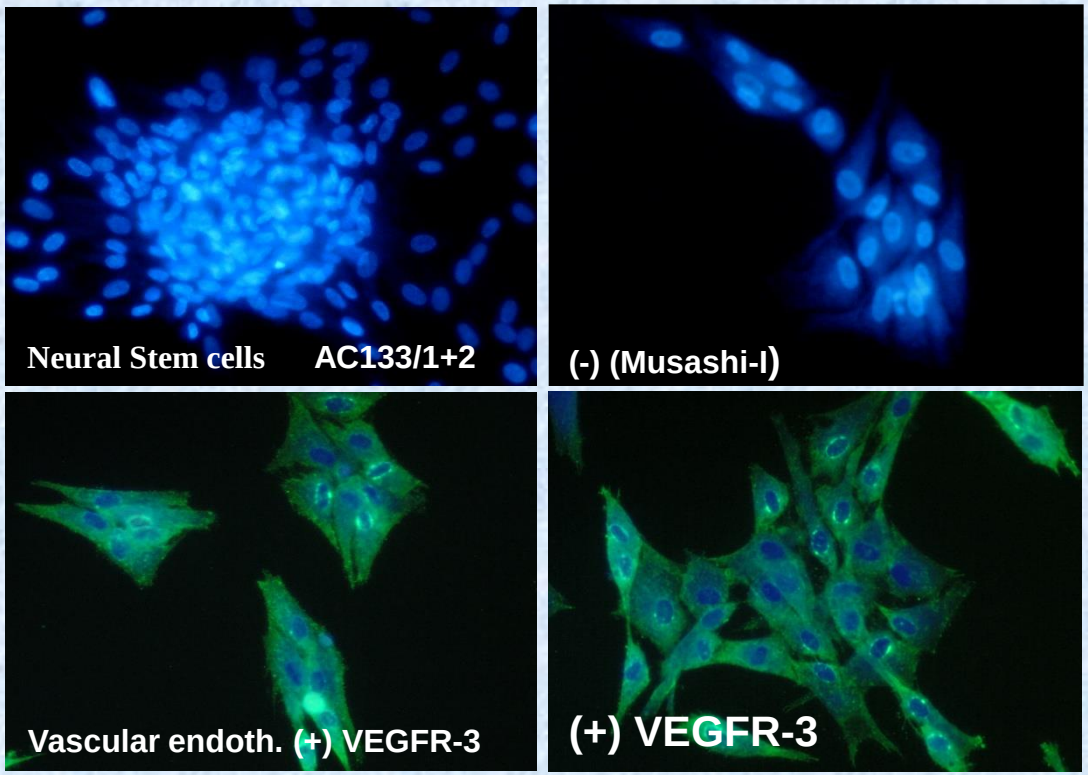
CD133 (+)



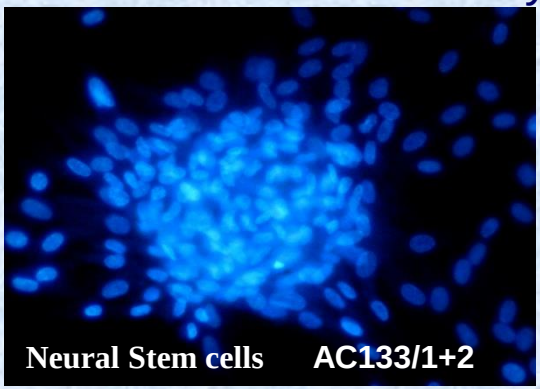
Musashi (+)

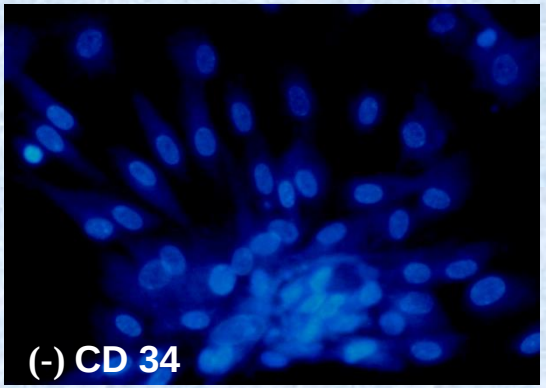
PRİMER HÜCRE KÜLTÜRLERİ VE TÜMÖR İZOLASYONU

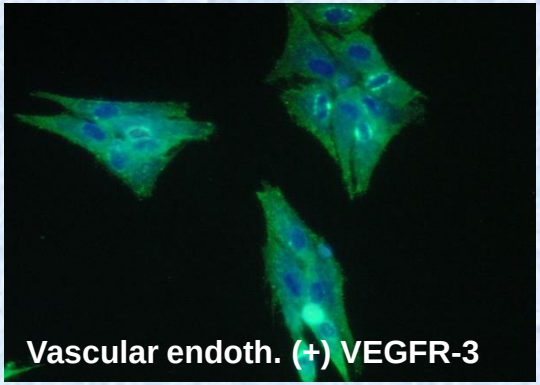
- Culture Medium
  (EGF, VEGF, bFGF, IGF-I)

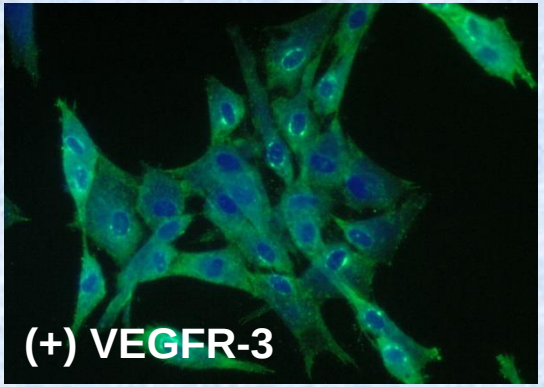


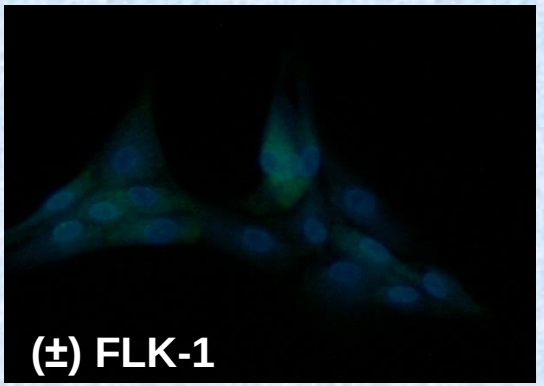
- CD133 from Cells (Grade-III) after 16 days in EGC-Medium
- Immunohistochemistry

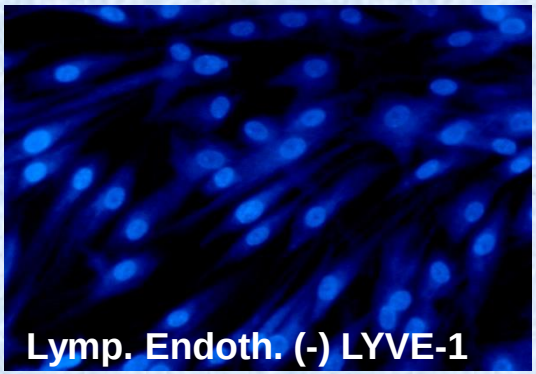


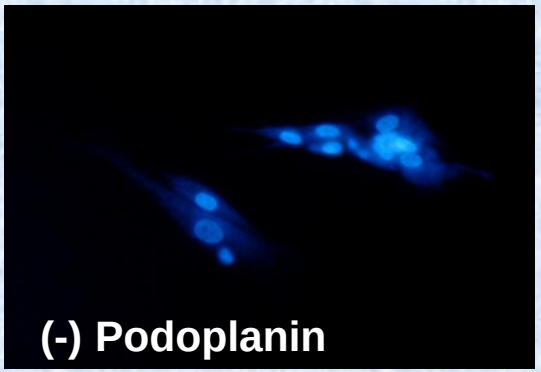




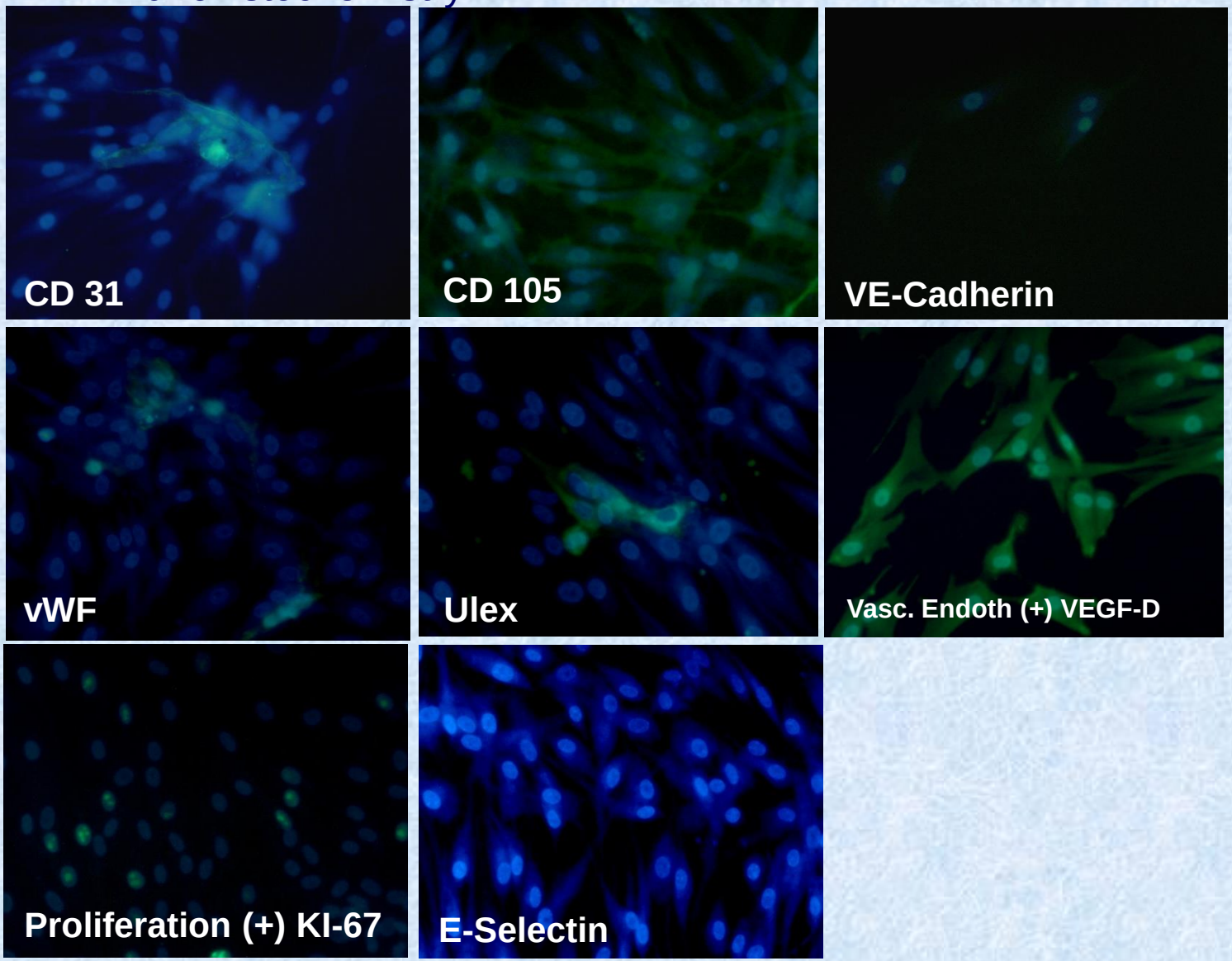




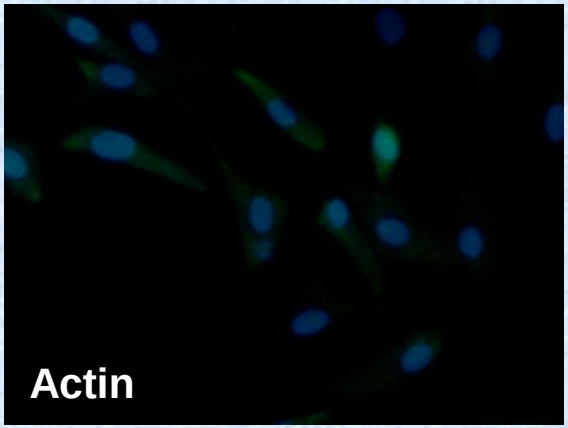


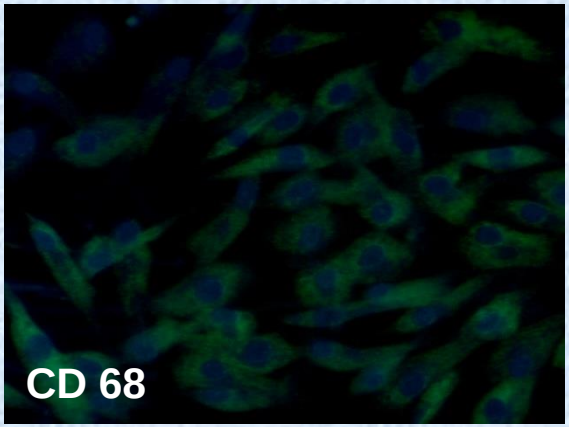


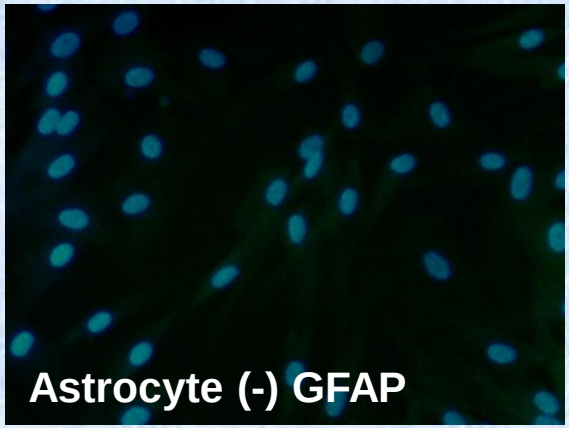
- CD133 from Cells (Grade-III) after 16 days in EGC-Medium
- Immunohistochemistry

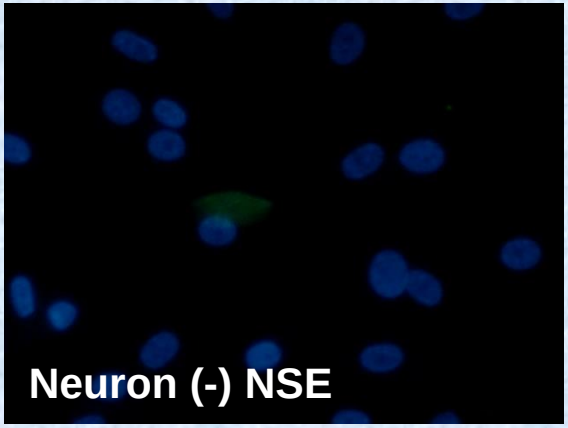


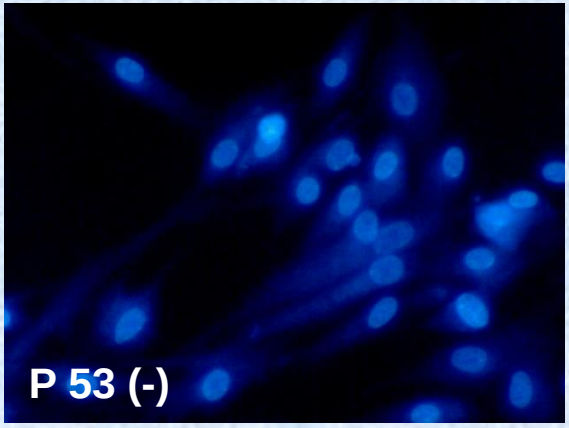
- CD133 from Cells (Grade-III) after 16 days in EC-Medium
- Immunohistochemistry

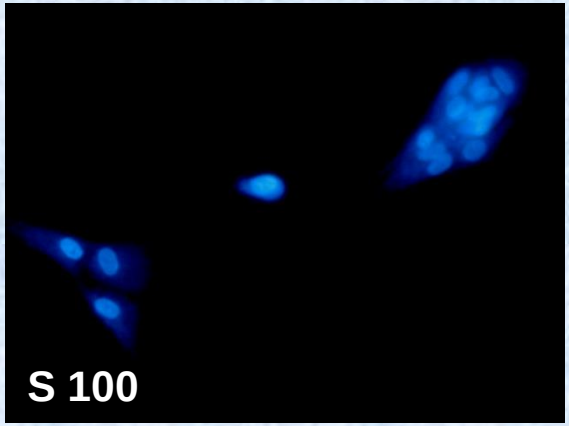












GLİOMA HÜCRELERİNİN SEKONDER KÜLTÜRÜ

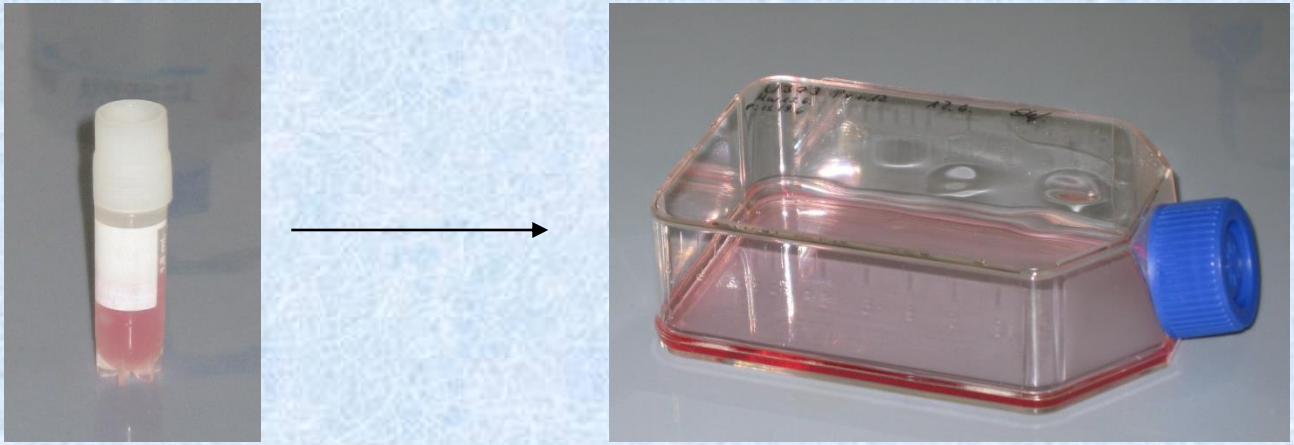
Klonlanmış glioma hücreleri (U373)- immortal

- Frozen – Tumor bankası

GLİOMA HÜCRELERİNİN SEKONDER KÜLTÜRÜ

- Monolayer-Cultivation of the frozen (U373) glioma cells after revival in the culture flask

  No gelatine or agar surface- only DMEM culture medium



- Cultivation in the Eagle‘s Medium (DMEM)

  + Glutamine + Penicilline-streptomicine

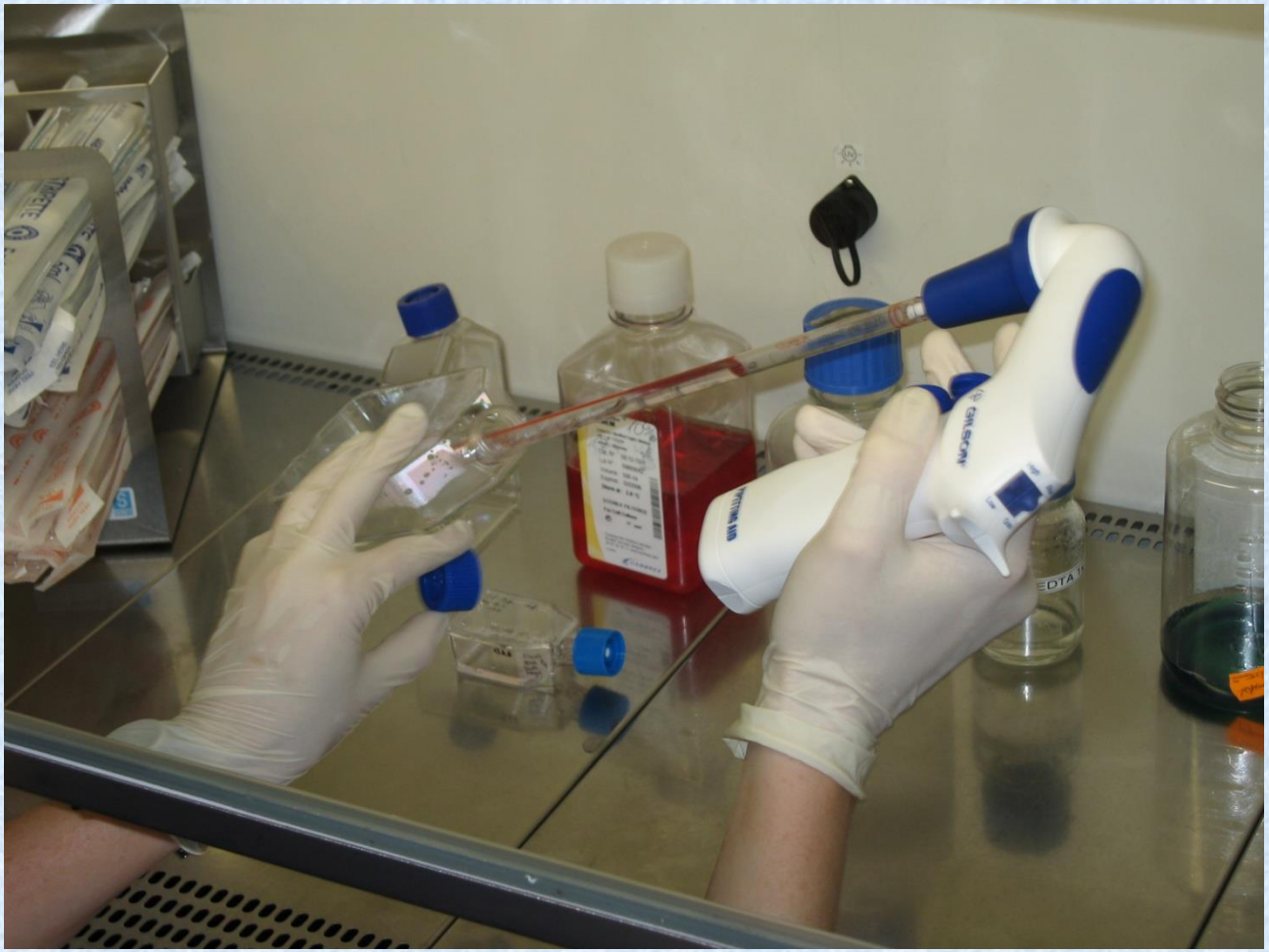
  Cell density: 105-106 cells / ml

GLİOMA HÜCRELERİNİN SEKONDER KÜLTÜRÜ

- Incubation and Culture at 37^0 C; 95% O^2 + 5% CO^2

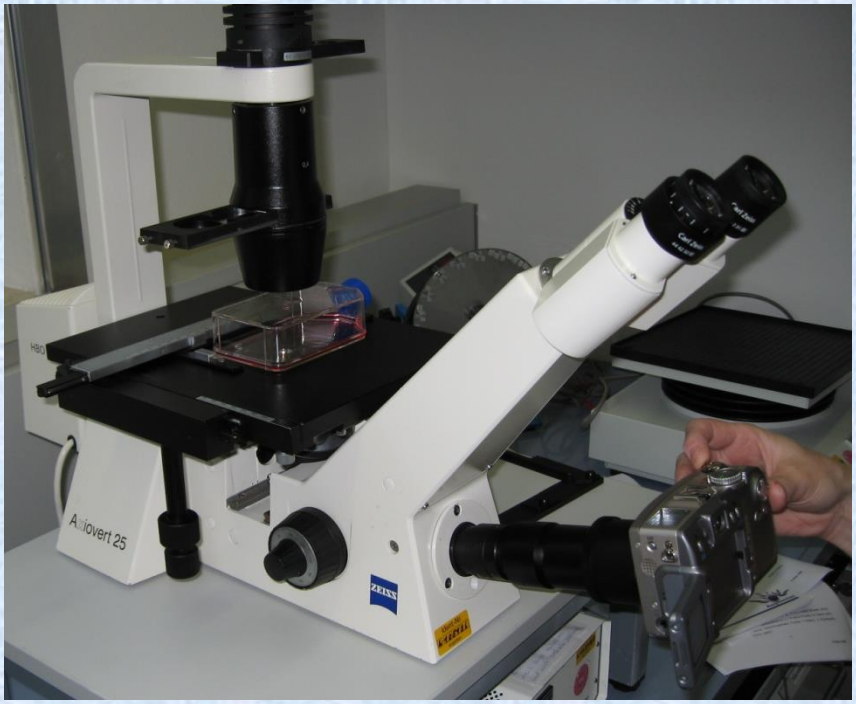
GLİOMA HÜCRELERİNİN SEKONDER KÜLTÜRÜ

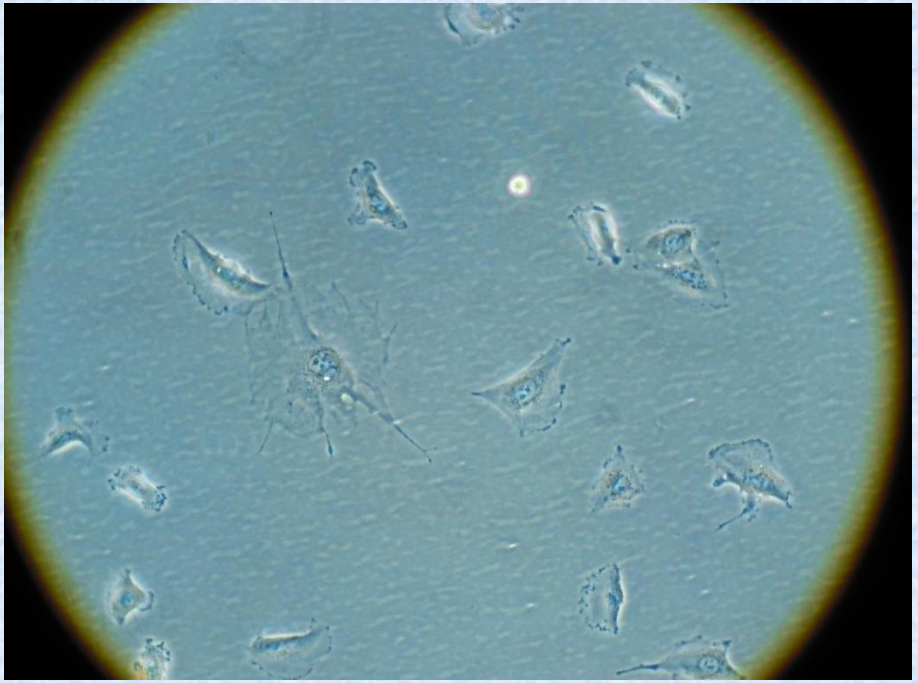
- Chage of the culture medium– 48 hours



GLİOMA HÜCRELERİNİN SEKONDER KÜLTÜRÜ

- (U373 Glioma cells)

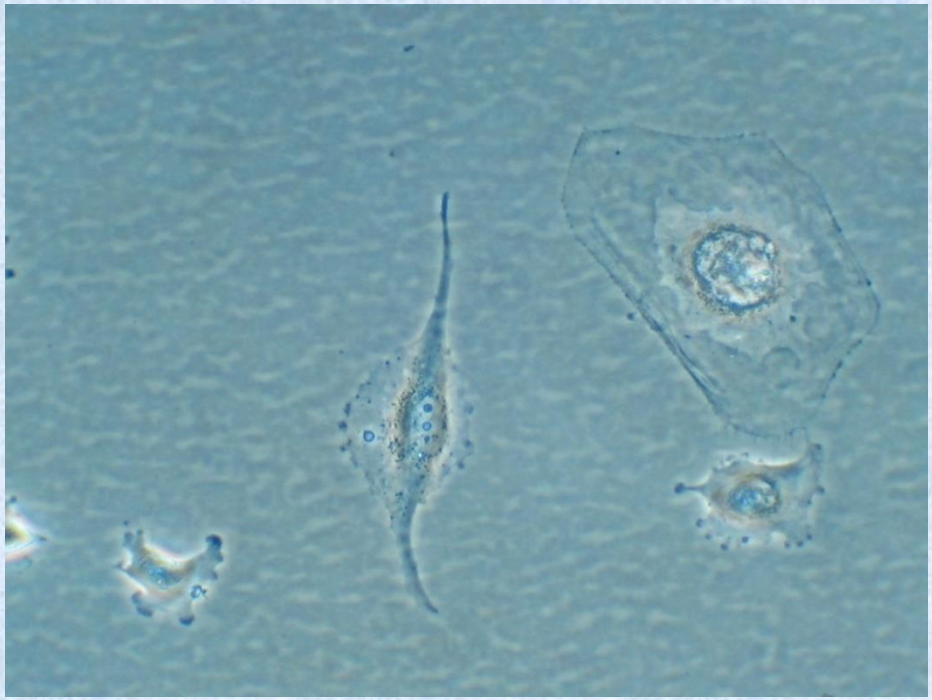


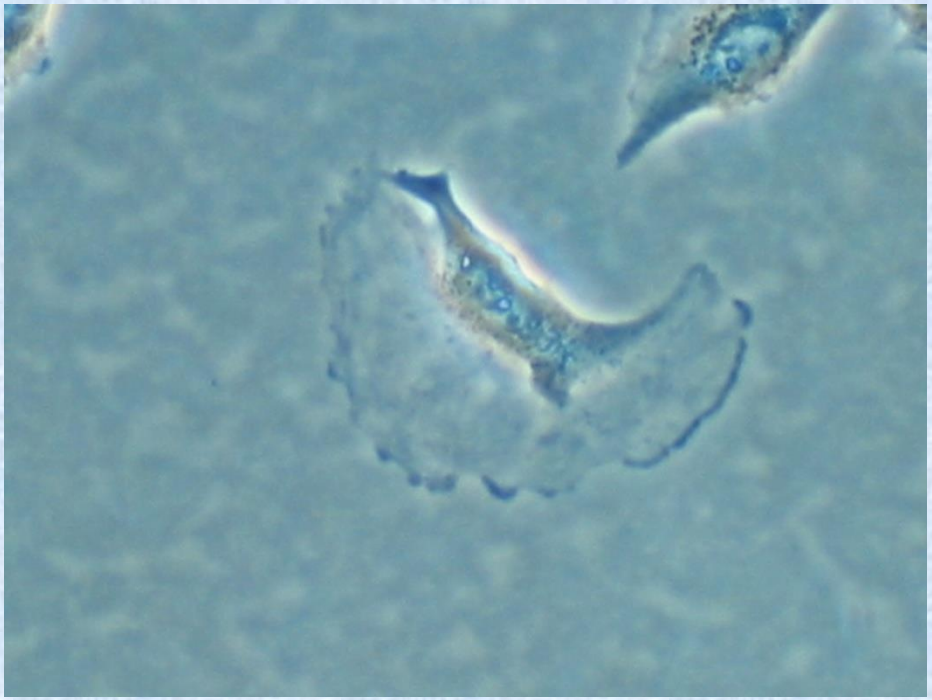


24 hours

GLİOMA HÜCRELERİNİN SEKONDER KÜLTÜRÜ

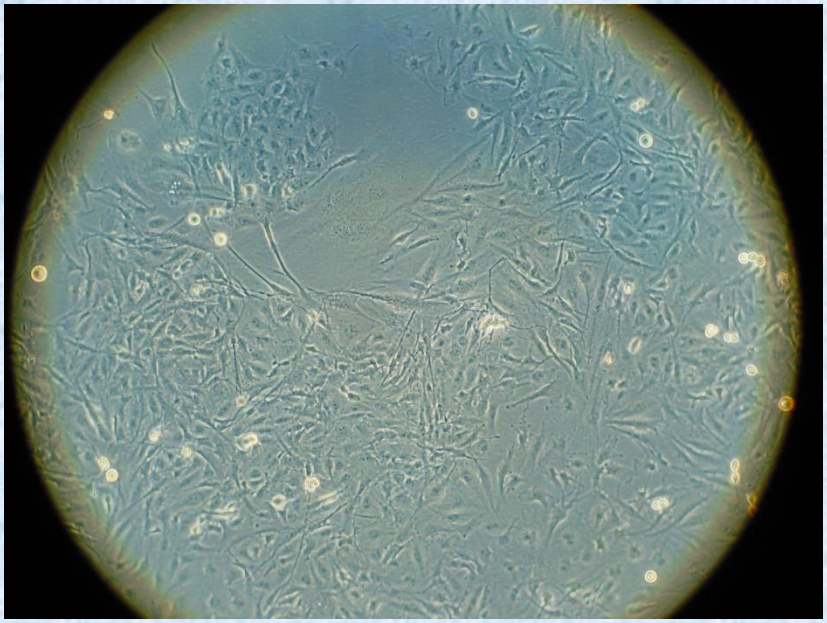
- (U373 Glioma cells) (48 hours)

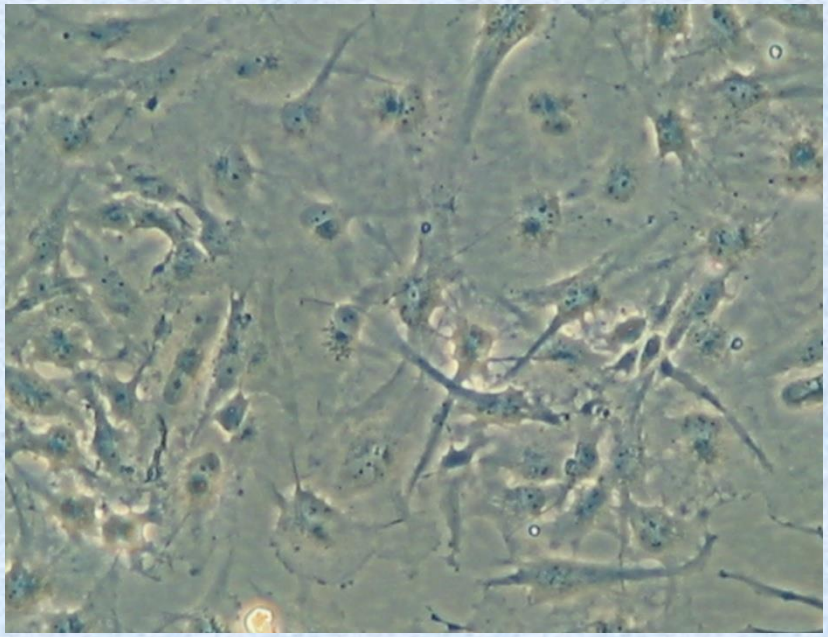




GLİOMA HÜCRELERİNİN SEKONDER KÜLTÜRÜ

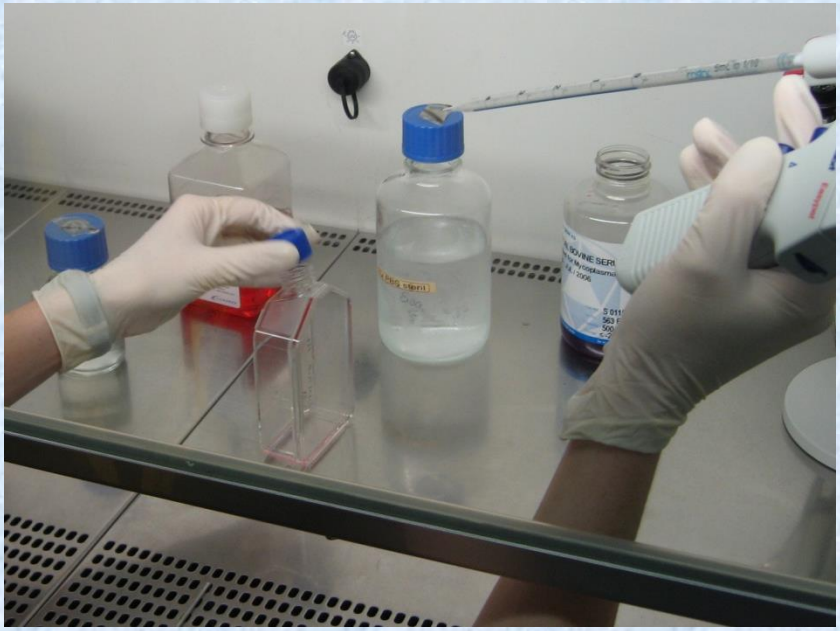
- (U373 Glioma cells) (7 days)

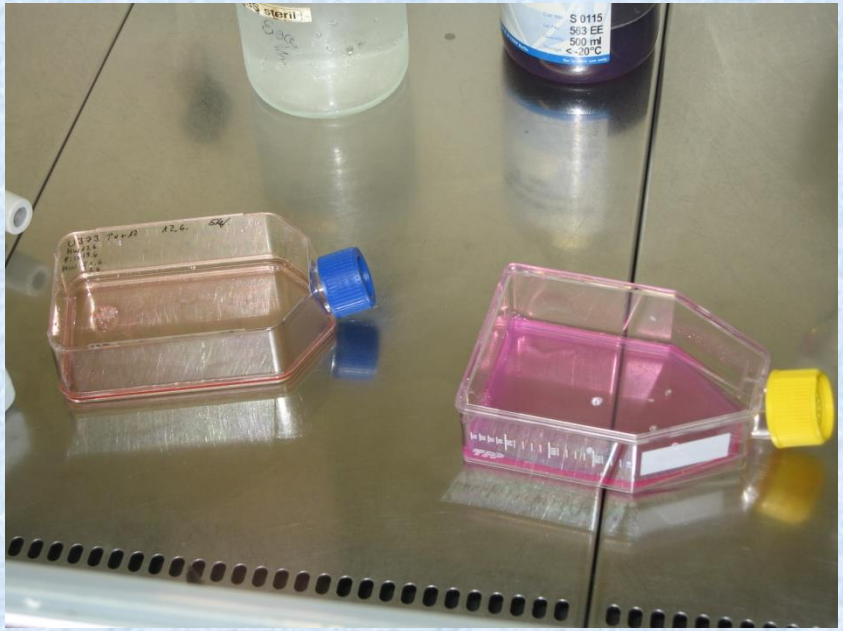




GLİOMA HÜCRELERİNİN SEKONDER KÜLTÜRÜ

- Formation of spheroids (after 7 days)
- Trypsin (remove cells from plastic)
- Culture flask with agar + DMEM
- Cultivation 24 hours more

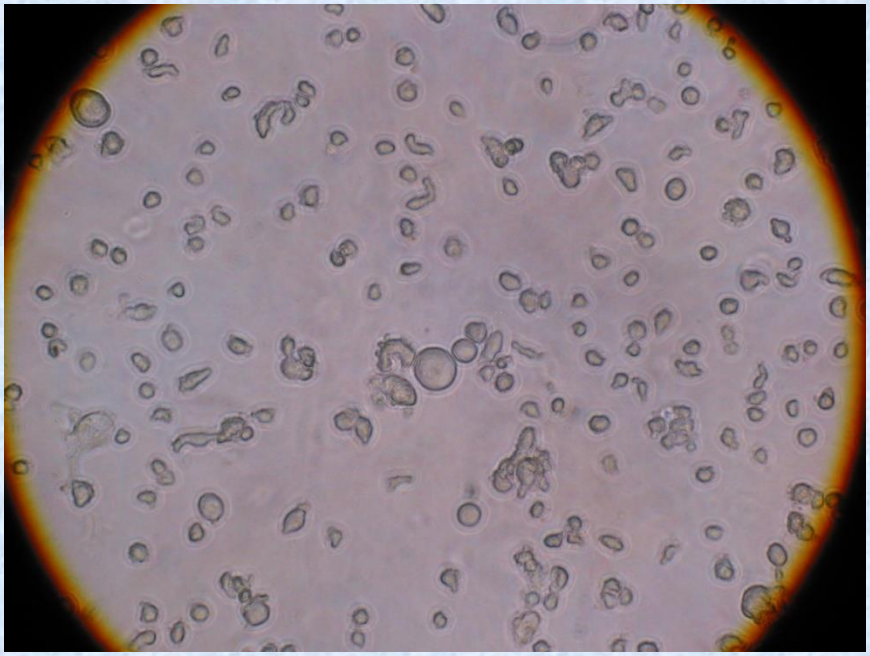


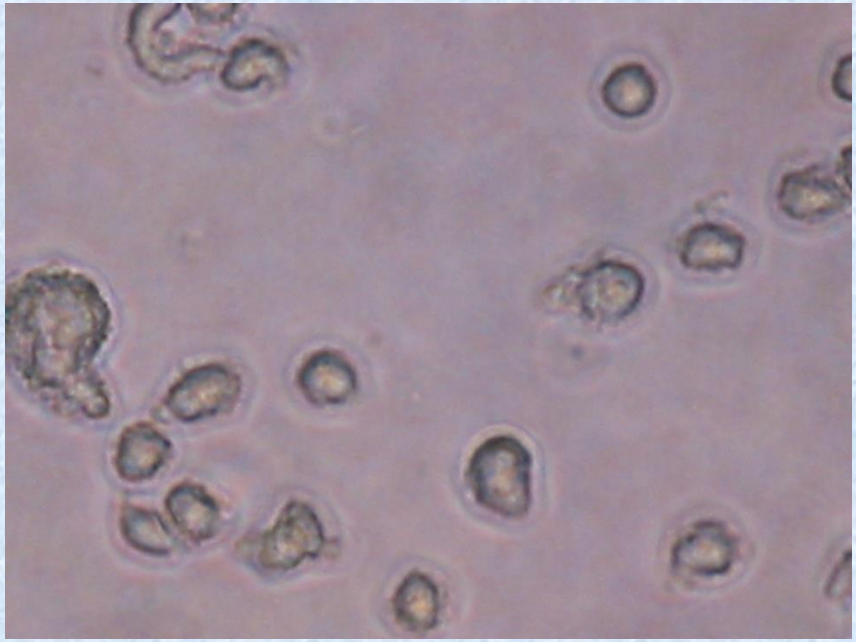


GLİOMA HÜCRELERİNİN SEKONDER KÜLTÜRÜ

- (U373 Glioma cells)
- - Formation of spheroids

7 days + 0 day

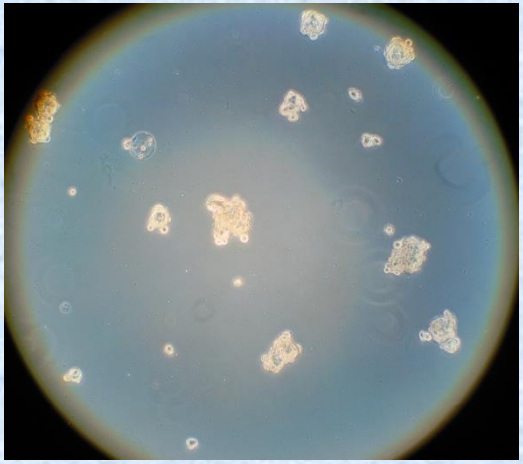


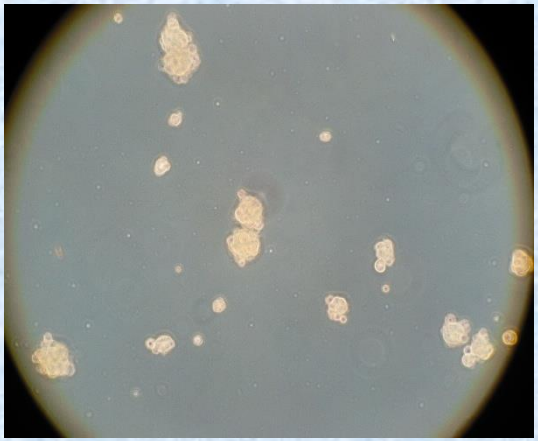


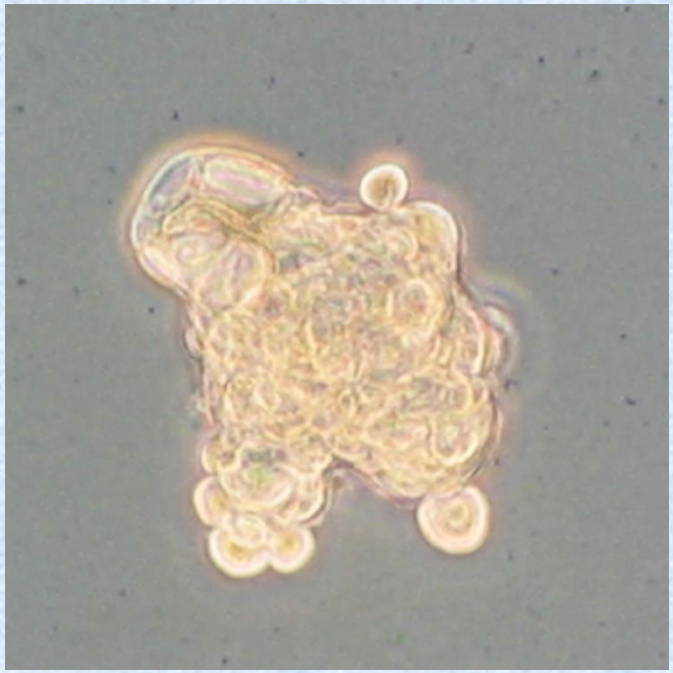
GLİOMA HÜCRELERİNİN SEKONDER KÜLTÜRÜ

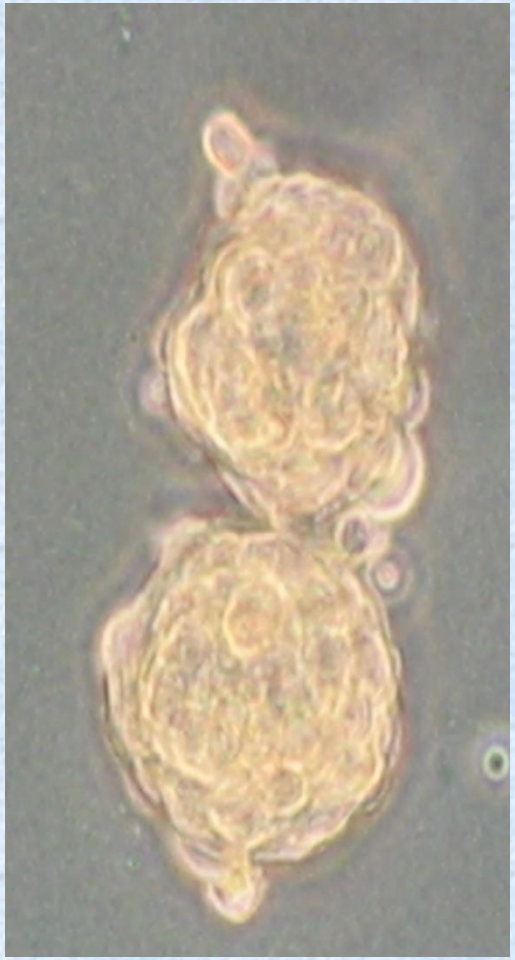
spheroid

7 days + 1 day





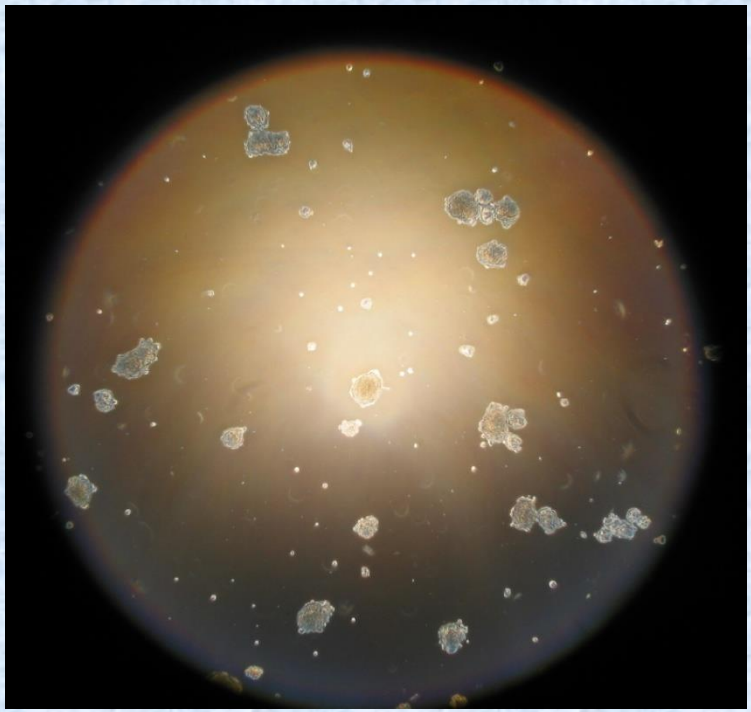


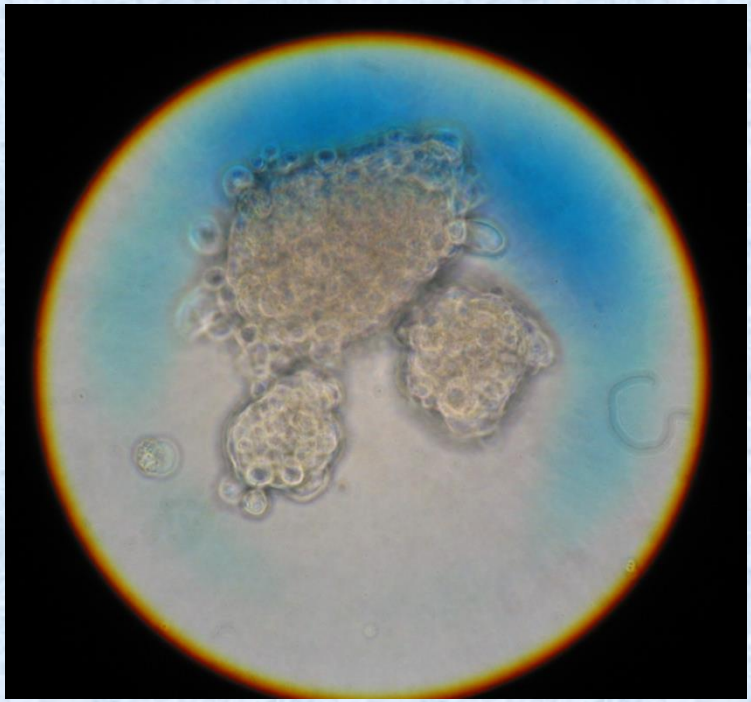


GLİOMA HÜCRELERİNİN SEKONDER KÜLTÜRÜ

- (U373 Glioma cells)
- Formation of spheroids

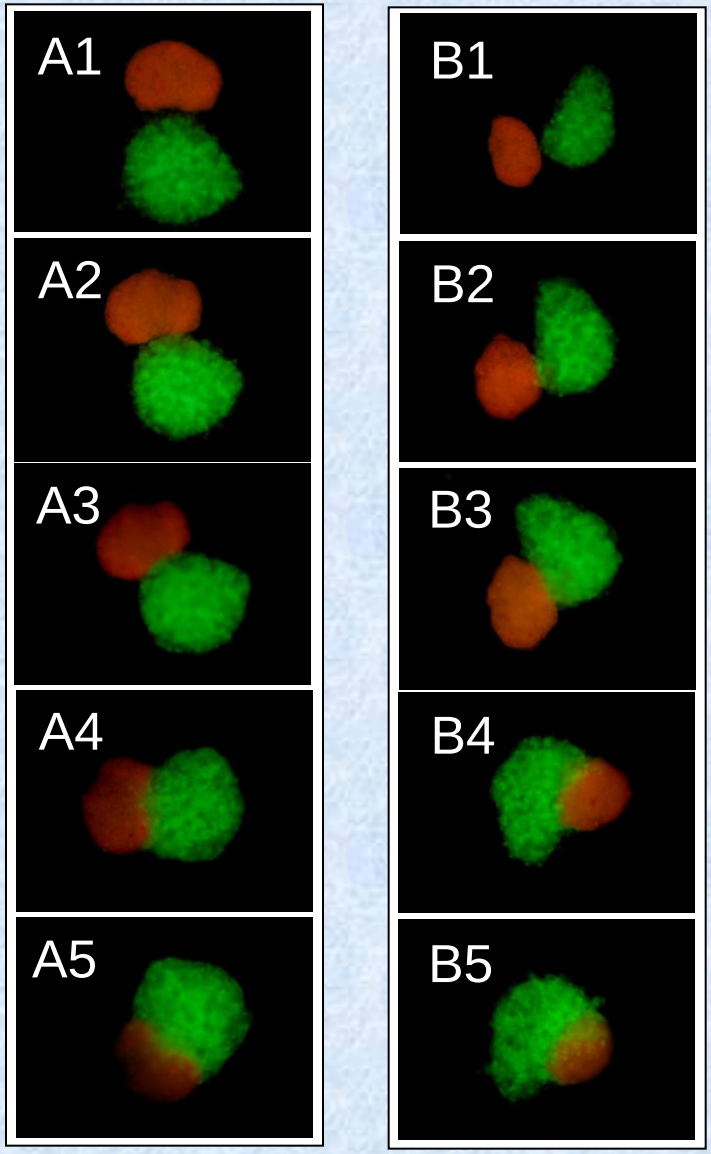
7 + 2 days





Konfrontation-Assay: ECM

CD133- Stem cell Spheroid (Grade-III; red) / U373-GFP- Glioma cell Spheroid (green)



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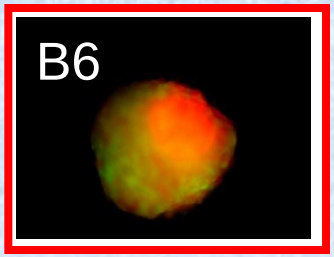
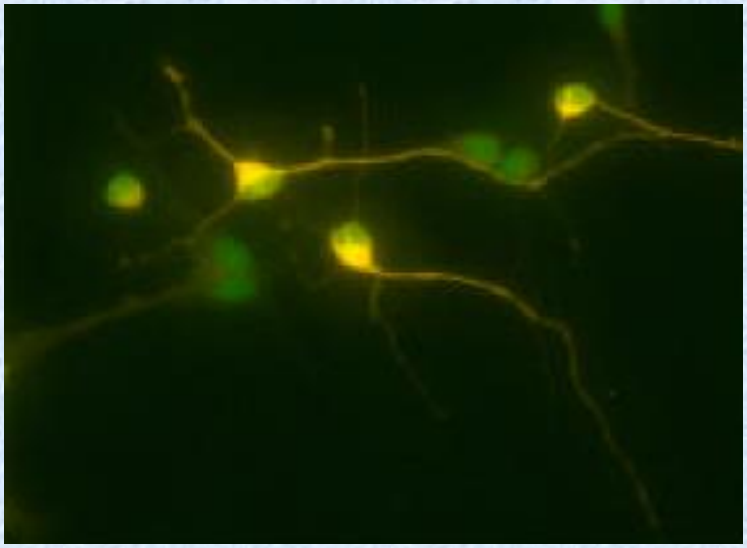
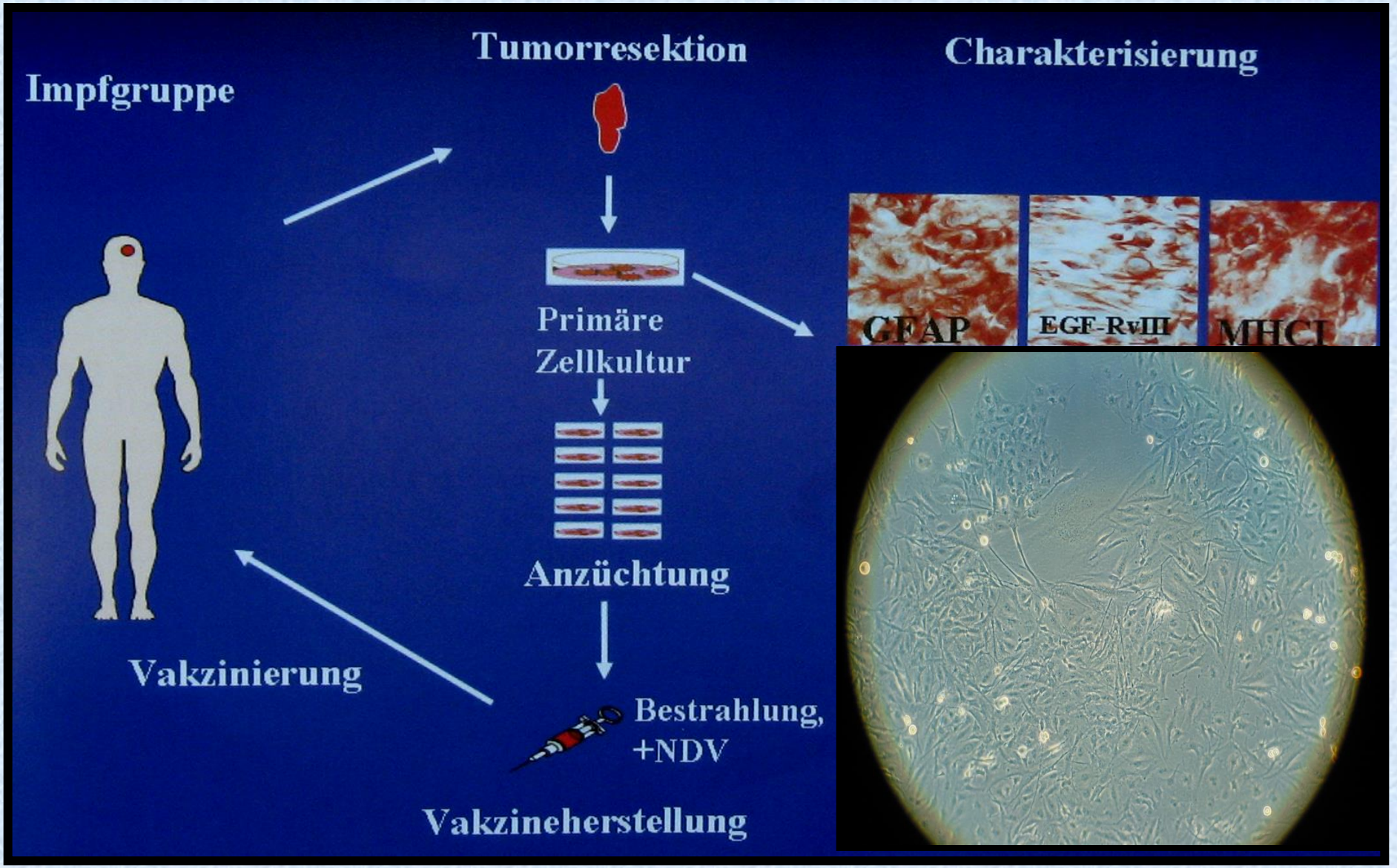


AVANTAJLAR

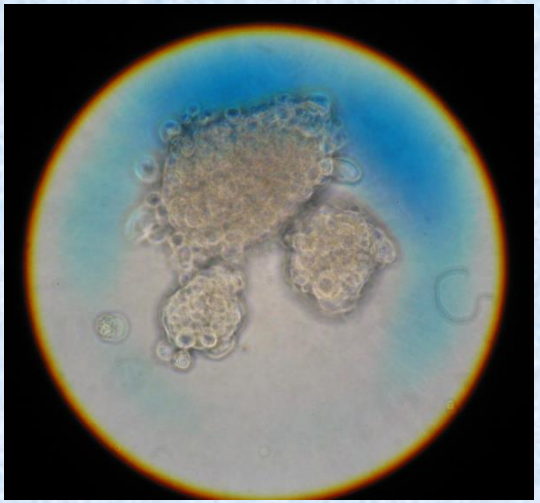
- Doku kültürlerinde hücresel ilişkiler korunabiliyor
- Homojen izole tümör hücreleriyle çalışma
- Avaskuler izole ortamda manipulasyon
- Çok sayıda veri az sayıda hayvandan elde edilebilir



TEDAVİ


Tumorresektion
Charakterisierung
Impfgruppe
Primäre
Zellkultur
GFAP
EGF-RvIII
MHCI
Anzüchtung
Vakzinierung
Bestrahlung,
+NDV
Vakzineherstellung

DEZAVANTAJLARI



- Deneyim gerekli
- Gelişmiş laboratuar ve işgücü
- Ufak doku ornekleriyle çalışma
- Genetik instabilite
- Heterojenik hücreler, büyüme hızı farklılıkları

Nörolojik Bilimlerde

dinamik araştırma modelleri
&
Sinir Bilimi Kavramlarının Gelişimi