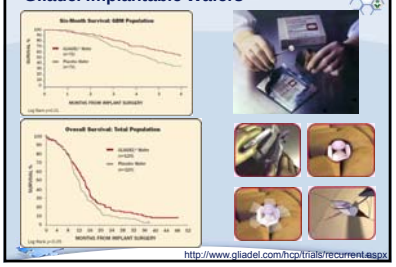
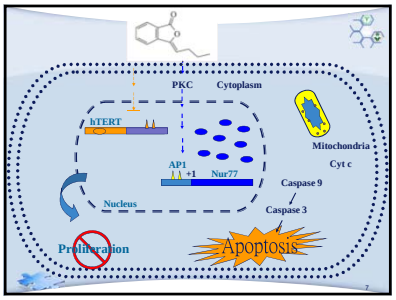
Applications of Biomaterials in Neurological Diseases

林 欣 榮
Shinn-Zong (John) Lin, MD. PhD. CPI.
Professor of Neurosurgery
Superintendent, Tainan Municipal An-Nan Hospital-China Medical University
Superintendent, China Medical University Beigan Hospital
Vice superintendent, Center for Neuropsychiatry
China Medical University Hospital
Taichung, Taiwan

Gliadel Implantable Wafers



http://www.gliadel.com/hcp/trials/recurrent.aspx



Gliadel™ Wafer implant

- A sterile, off-white to pale yellow wafer approximately 1.45 cm in diameter and 1 mm thick.
- Each wafer contains 192.3 mg of a biodegradable polyanhydride copolymer and 7.7 mg of carmustine [1,3-bis (2-chloroethyl)-1-nitrosourea, or BCNU].
- Polyanhydride copolymer (polifeprosan 20) consists of poly[bis(p-carboxyphenoxy) propane: sebacic acid] in a 20:80 molar ratio.
- More than 70% of the copolymer degrades by three weeks

BP

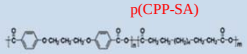
Angelica sinensis 當歸

- Lipid soluble
- Low toxicity
- Small molecule

Polymer: p(CPP-SA)

- 癸二酸 (sebacic acid；SA) 和1,3-雙羧基對苯丙醇 (1,3-bis(4-carboxyphenoxy)propane；CPP)

p(CPP-SA)



Domb and Langer, 1987

Gliadel™ Wafer implant

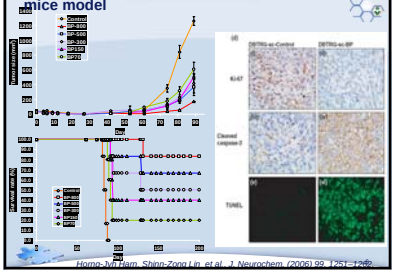
- GLIADEL is the only FDA-approved chemotherapeutic implant Median survival of 13.8 mos (Gliadel) vs 11.6 mos (Placebo) in initial high-grade malignant glioma (hazard ratio, 0.73; 95% CI: 0.56-0.95).
- Six-month survival rate of 56% (40/72) (Gliadel) vs 36% (26/73) (Placebo) in recurrent GBM.
- Complication:

  Seizures, Brain edema,

  Healing abnormalities,

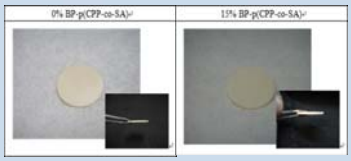
  Intracranial infection

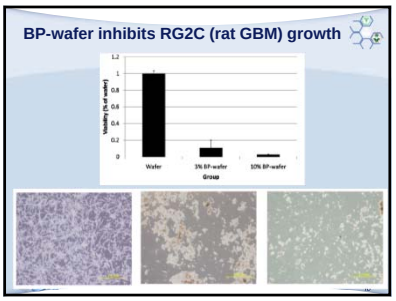
BP inhibits human GBM tumor growth in nude mice model

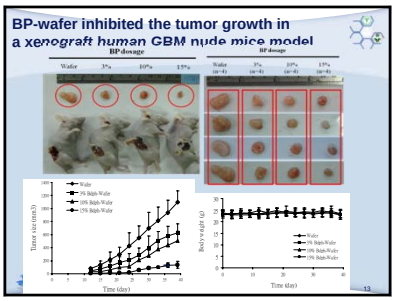


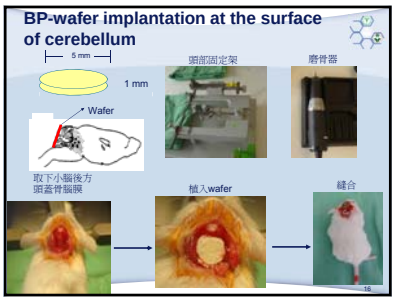
Homg-JVI Tsern, Shinn-Zong Lin, et al., J. Neurochem. (2006) 99, 1251–1265.

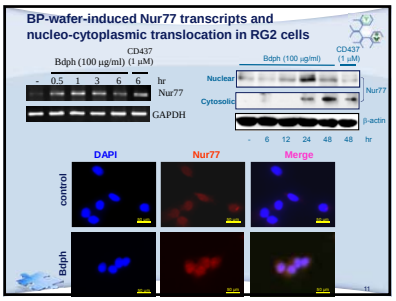
BP -wafer

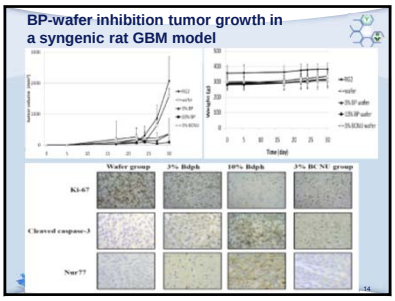


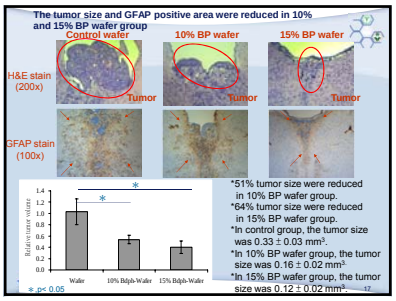

BP-wafer inhibits RG2C (rat GBM) growth
Wafer
3% BP-wafer
10% BP-wafer
Group

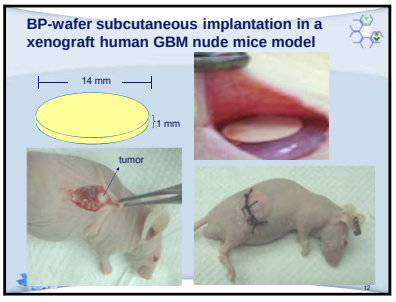

BP-wafer inhibited the tumor growth in a xenograft human GBM nude mice model
BP dosage
Wafer
3%
10%
15%
Time (day)

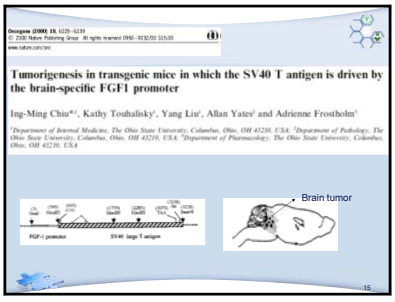

BP-wafer implantation at the surface of cerebellum
5 mm
1 mm
Wafer
頭部固定架
磨骨器
取下小腦後方
頭蓋骨腦膜
植入wafer
縫合

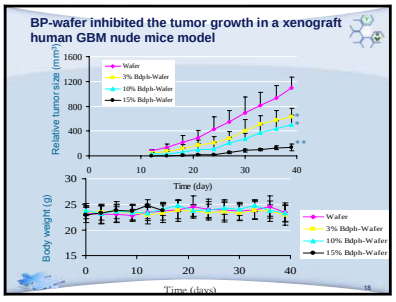

BP-wafer-induced Nur77 transcripts and nucleo-cytoplasmic translocation in RG2 cells
CD437
Bdph (100 μg/ml) (1 μM)
hr
Nur77
GAPDH
Nuclear
Cytosolic
β-actin
DAPI
Merge
control
Bdph


BP-wafer inhibition tumor growth in a syngenic rat GBM model
Wafer group
3% Bdph
10% Bdph
3% BC NE group
Ki-67
Cleaved caspase-3
Nur77

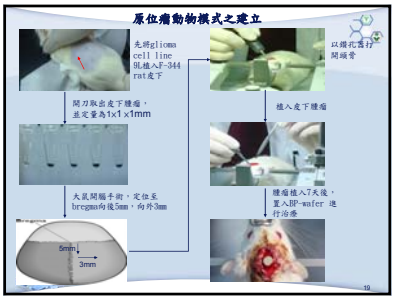

The tumor size and GFAP positive area were reduced in 10% and 15% BP wafer group
Control wafer
10% BP wafer
15% BP wafer
H&E stain (200x)
GFAP stain (100x)
Tumor
*51% tumor size were reduced in 10% BP wafer group.
*64% tumor size were reduced in 15% BP wafer group.
*In control group, the tumor size was 0.33 ± 0.03 mm³.
*In 10% BP wafer group, the tumor size was 0.16 ± 0.02 mm³.
*In 15% BP wafer group, the tumor size was 0.12 ± 0.02 mm³.
* p< 0.05


BP-wafer subcutaneous implantation in a xenograft human GBM nude mice model
14 mm
1 mm
tumor


Tumorigenesis in transgenic mice in which the SV40 T antigen is driven by the brain-specific FGF1 promoter
Brain tumor


BP-wafer inhibited the tumor growth in a xenograft human GBM nude mice model
Relative tumor size (mm³)
Wafer
3% Bdph-Wafer
10% Bdph-Wafer
15% Bdph-Wafer
Time (day)
Body weight (g)
Time (days)

原位癌動物模式之建立

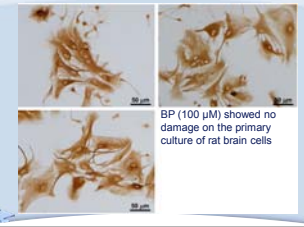


Stability test: ZBP-CPPSA only storage condition test

Purity of ZBP-CPPSA

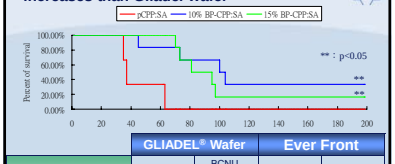
Entry	Temp	Condition	1 week		2 week		3 week		4 week	
			ZBP (%)	EBP(%)	ZBP (%)	EBP(%)	ZBP (%)	EBP(%)	ZBP (%)	EBP(%)
1	8℃	Ar	99.66	0.15	99.51	0.18	99.41	0.16	99.41	0.17
2	25℃	Ar	98.91	0.16	98.88	0.17	94.64	0.14	96.07	0.16
3	40℃	Ar	97.43	0.26	97.65	0.24	66.29	-	59.27	-

BP is not toxic to neurons and glias



BP (100 μM) showed no damage on the primary culture of rat brain cells

The survival time of Bp-wafer is 1.8-fold increases than Gliadel-wafer



Group	GLIADEL® Wafer		Ever Front	
	PCPP:SA	BCNU-CPP:SA	PCPP:SA	BP- CPP:SA
Medium survival (days)	50	11	37	100
Mean Survival (days)	11.6 ±0.7	62.3 ±9.9	44 ±16	135.4 ±37
Prolong survival (days)		50.7		91.4

Z-BP stability

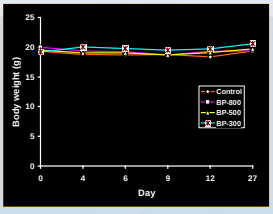
The Blank: (Z)-BP, purity is 99.96%, 0.3mg/1mL of ACN solution. The retention time is 5.336 min in UPLC (PDA detector 254nm)

The retention time of (E)-BP is 4.716min.

The table of decomposition condition of (Z)-BP

Irradiation	Heating	Oxidation	Acidic condition	Basic condition
1hr, (Z)-BP(85.06%), (E)-BP(8.01%)	Neat, 60℃, 1 day, (Z)-BP (57.49%)	30% H_2O_2, 70min, (Z)-BP (99.73%)	2.0N $HCl_{(aq)}$, 70min, (Z)-BP (99.50%)	0.1N $NaOH_{(aq)}$, 30min, (Z)-BP (1.39%)
	Solution, 60℃, 3 day, (Z)-BP (99.86%)			

No systemic toxicity to rats



相較於控制組，BP治療組並未有造成明顯的體重下降

(Tsai NM et al., 2006)

Stability test: Z-BP only storage condition test

Purity of neat ZBP

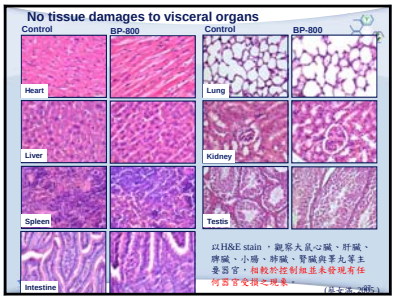
Entry	Temp	Condition	1 week		2 week		3 week		4 week	
			ZBP (%)	EBP (%)	ZBP (%)	EBP (%)	ZBP (%)	EBP (%)	ZBP (%)	EBP (%)
1	8℃	Ar	99.74	0.16	99.7	0.16	99.69	0.16	99.72	0.16
2		air	99.72	0.16	99.72	0.17	99.67	0.16	99.71	0.16
1	25℃	Ar	99.72	0.16	99.68	0.17	99.56	0.18	99.65	0.18
2		air	99.65	0.16	99.63	0.16	98.58	0.16	99.15	0.17
1	40℃	Ar	99.39	0.25	99.19	0.27	97.91	0.33	97.82	0.45
2		air	99.07	0.21	98.64	0.24	97.93	0.33	98.13	0.45

Therapeutic Index of BP

	GBM LD_{50}	GBM ED_{50}	Therapeutic index (LD_{50}/ED_{50})
mg/kg	7500	80	94

EC_{50}:Concentration required to product 50% inhibition.
LD_{50}:50% Lethal Dose

No tissue damages to visceral organs



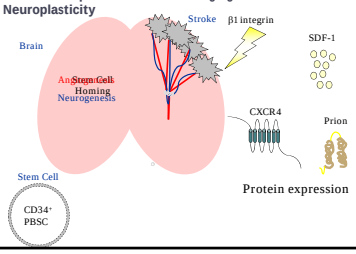
以H&E stain，觀察大鼠心臟、肝臟、脾臟、小腸、肺臟、腎臟與睪丸等主要器官，相較於控制組並未發現有任何器官受損之現象。

(基文進, 2005)

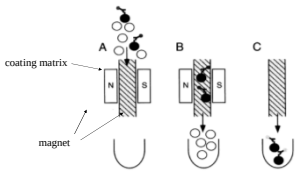
Conclusion

- BP-wafer has a potential to become a new drug for treating GBM
- BP-wafer is filing IND to FDA
- Plan to do pilot clinical trial in 6 patients

Stem Cell Implantation Induces Angiogenesis and Neuroplasticity



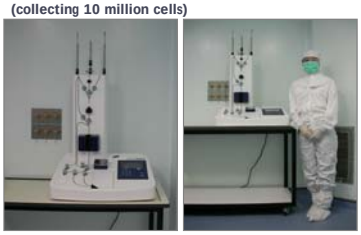
High Gradient Magnetic Separation: (HGMS)



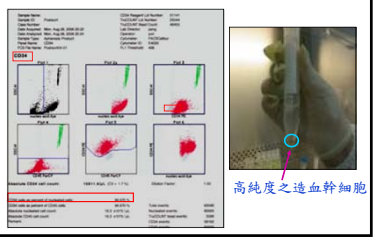
US Patent: Stefan Miltenyi ; 1995, 1996 and 2002

Cell Therapy in Neurological Diseases

CD34+ cells are isolated by CliniMACS (collecting 10 million cells)



Assessment of stem cell purity and viability



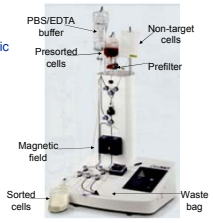
高純度之造血幹細胞

Stroke induced hemiplegia

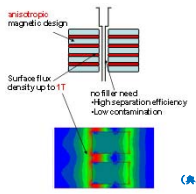
- Stem cell therapy — autologous CD34 stem cell implantation in injured corticospinal tract
- (A) P.I. initialted clinical trials
  (B) Founded by government, hospital and companies

The CliniMACS® Instrument

a closed, sterile magnetic cell separation system

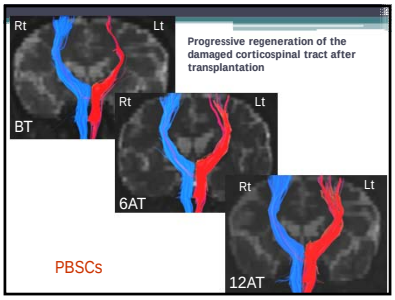


Cell separation kit design of this project



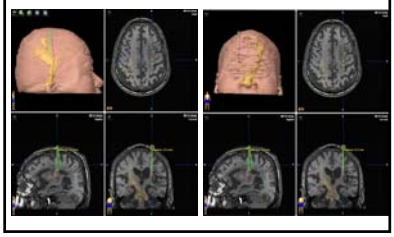
（與工研院共同合作開發）

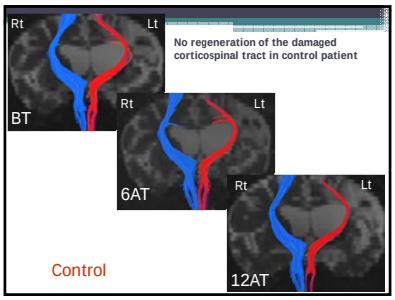
CD34⁺ stem cell were implanted into the brain targets



6 months after O.P.

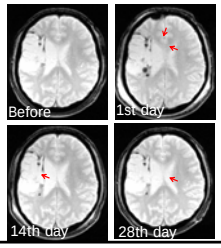
Injured pyramidal tract was identified





Improvement in hand fine movement
Teeth Brushing

Transplanted cells retained at the injection sites



Before O.P.
(Mr. Huang stroke for 1.5 years)

Patient lives independently one year after transplantation


Thank You !