인제대학교 의과대학
INJE UNIVERSITY COLLEGE OF MEDICINE

Molecular Therapy Lab

인제대학교부산백병원
INJE UNIVERSITY BUSAN PAIK HOSPITAL

Gene Therapy for Diabetes Mellitus

Jeong H. Park, M.D., Ph.D.

Department of Internal Medicine, Pusan Paik Hospital,
College of Medicine, Inje University, Busan, Korea

Disclaimer

1. I received only official lecture fee from Korean Diabetes Association for this talk

2. I do not own venture corporations, and hold any stocks of pharmaceutical companies

3. Because my concerns are concentrated in the non-viral gene delivery system, my talk might include biased opinions for the gene therapy researches using viral gene carriers

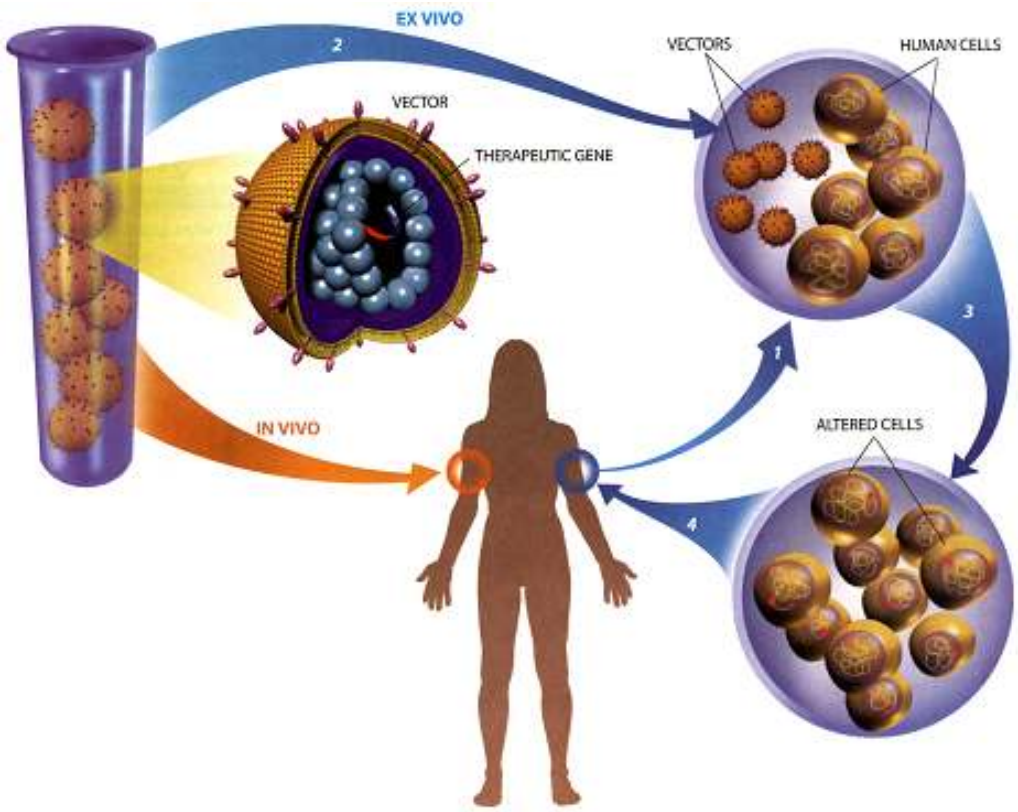
Contents

1. Definition & Current Status of Gene Therapy Researches
2. Gene Therapy for Type 2 Diabetes Mellitus
3. Gene Delivery Systems & Potential for the Commercial Novel Therapies
4. UTMD (Ultrasound Micro-bubble Destruction) Gene Therapy
5. Our Previous & Current Works
6. Future Perspectives

Gene Therapy

Therapeutic
Nucleic Acids

Gene Delivery Techniques



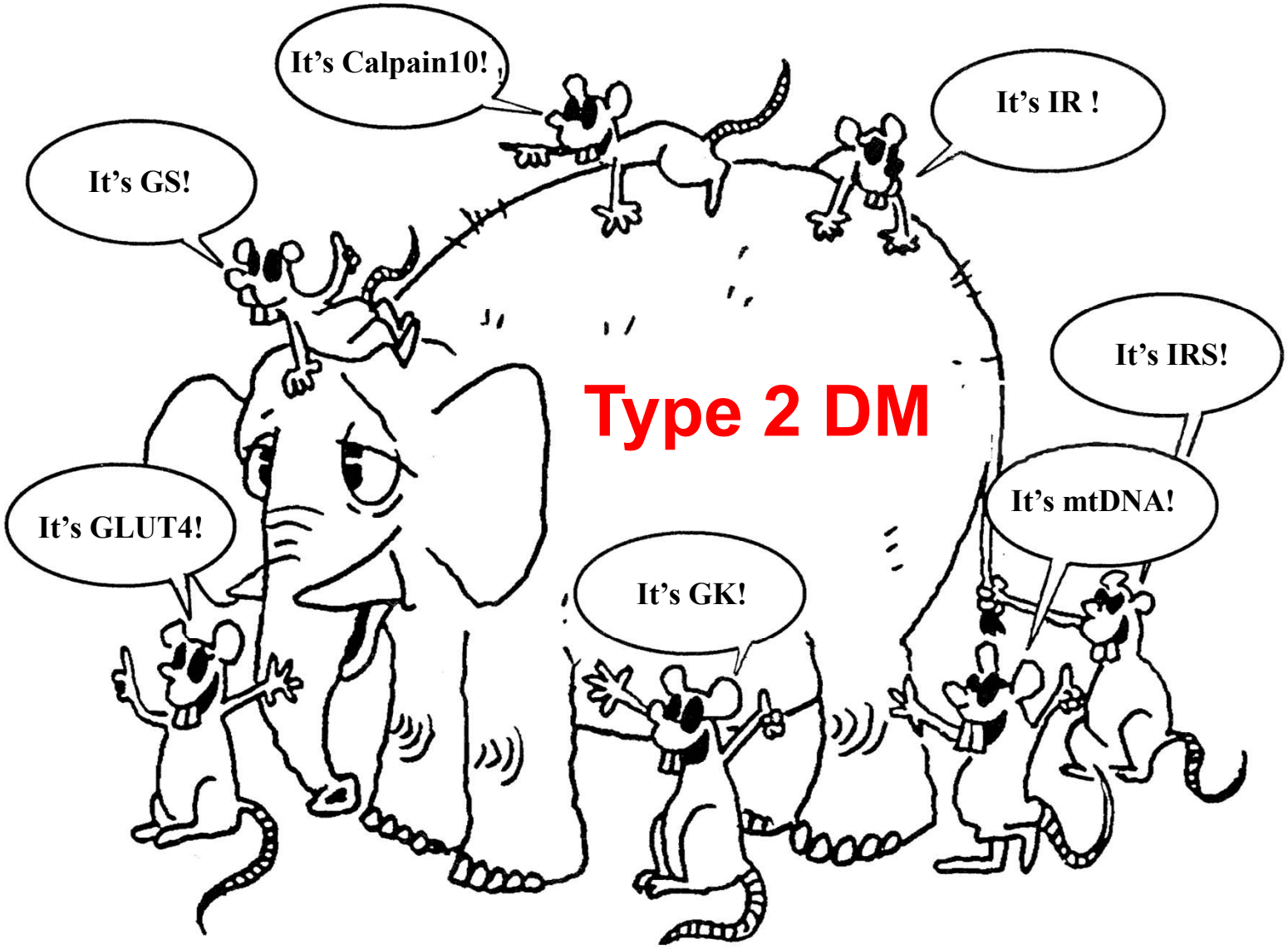
Country	Indication	Gene	Vector	Commencement	Current status	Reference
China	Cancer	*p53*	Adenovirus	1998	Commercialized	—
	Cancer	Replication-competent adenovirus	Adenovirus	2000	Commercialized	—
	Cancer	Selective oncolytic adenovirus	Adenovirus	2003	Phase II ongoing	*
	Cancer	TK	Adenovirus	2004	Phase I ongoing	10
	Cancer	IL-2	Adenovirus	2003	Phase I/II ongoing	11
	Ischemic disease	Endostatin	Adenovirus	2004	Phase I ongoing	*
	Ischemic disease	HGF	Adenovirus	2005	Phase I ongoing	*
	Cardiovascular disease	VEGF	Adenovirus	2001	Phase I completed	*
	AIDS	Adeno-vaccine + DNA vaccine	Adenovirus	2004	Phase I ongoing	*
	Hepatitis B	HBV antigen	DNA vaccine	2005	Phase I ongoing	*
	Leukemia	Cytokine-activated lymphocyte	Retrovirus (*ex vivo*)	1997	Phase I completed	*
	Cancer	Activated dendritic cell	Retrovirus (*ex vivo*)	2001	Phase I completed	*
	Late stage gastric cancer	IL-2-modified allogenic gastric cancer cell line vaccine	Retrovirus (*ex vivo*)	2001	Phase I	11
	Hemophilia	Factor IX	AAV-2	1994	Phase I completed	48
	Hemophilia	Factor IX	AAV-2	2003	Phase I ongoing	*
	Hemophilia	Factor IX	Retrovirus (*ex vivo*)	1991	Phase I completed	49
	Glioma	pLTKcSN/VPC (HSV-tk/GCV)	Retrovirus (*ex vivo*)	1996	Phase I completed	10,50
Japan	Lung cancer (NSCC)	*p53*	Adenovirus	1998	Phase I/II completed	18
	Esophageal cancer	*p53*	Adenovirus	2000	Phase I/II completed	19
	Lung cancer (NSCC)	*p53*	Adenovirus	2000	Phase I/II completed	18
	Lung cancer (NSCC)	*p53*	Adenovirus	2000	Phase I/II completed	18
	Prostate cancer	HSV-tk	Adenovirus	2000	Phase I/II completed	51
	Lung cancer (NSCC)	*p53*	Adenovirus	2000	Phase I/II completed	18
	Lung cancer (NSCC)	*p53*	Adenovirus	2000	Phase I/II completed	18
	Prostate cancer	HSV-tk	Adenovirus	2003	Phase I/II ongoing	—
	ADA deficiency	ADA	Retrovirus (*ex vivo*)	1995	Phase I/II completed	15
	ADA deficiency	ADA	Retrovirus (*ex vivo*)	2002	Phase I/II on going	—
	Renal carcinoma	GM-CSF	Retrovirus (*ex vivo*)	1998	Phase I completed	52
	Mammary cancer	MDR1	Retrovirus (*ex vivo*)	2000	Phase I/II ongoing	—
	Leukemia	HSV-tk	Retrovirus (*ex vivo*)	2002	Phase I/II ongoing	—
	Glioma	IFN-β	Liposome	2000	Phase I/II ongoing	21
	Melanoma	IFN-β	Liposome	2003	Phase I/II ongoing	—
	ASO/Burger	HGF	Naked DNA	2001	Phase III ongoing	16
	ASO/Burger	FGF-2	Sendai virus	2006	Phase I/II ongoing	—
	Parkinson's disease	Aromatic l-amino acid decarboxylase (AADC)	AAV-2	2007	Phase I/II ongoing	—
Korea	Melanoma	HLA-B7/β2 microglobulin	Liposome	1994	Phase I completed	28
	Melanoma, breast cancer, head-and-neck cancer	Skin fibroblasts transduced with retroviral vectors expressing IL-12	Retrovirus (*ex vivo*)	1998	Phase I completed	29
	Ischemic limb disease	VEGF165	Naked DNA	2001	Phase II ongoing	30
	Hepatitis B	HBV antigen, IL-12	DNA vaccine	2006	Phase I	31
	Liver cancer	Oncolytic vaccinia virus expressing GM-CSF	Vaccinia virus	2006	Phase I	32
	Chronic granulomatous disease	*gp91*	Retrovirus (*ex vivo*)	2007	Phase I/II	—
	Coronary artery disease	HGF	Naked DNA	2006	Phase I started	—
	HIV	HIV antigen, IL-12	DNA vaccine	2006	Phase I	—
	Osteoarthritis	TGF-β	Retrovirus (*ex vivo*)	2006	Phase I	—
	Prostate cancer	TK, CD	Adenovirus	2005	Phase II	—

Abbreviations: AAV-2, adeno-associated virus-2; ADA, adenosine deaminase; AIDS, acquired immunodeficiency syndrome; ASO, arteriosclerosis obliterance; CD, cytosine deaminase; FGF-2, fibroblast growth factor-2; GCV, ganciclovir; GM-CSF, granulocyte-macrophage colony-stimulating factor; HBV, hepatitis B virus; HGF, hepatocyte growth factor; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; HSV, herpes simplex virus; IFN-β, interferon-β; IL-2, interleukin-2; MDR1, multi-drug resistance 1; NSCC, non-small cell cancer; TGF-β, transforming growth factor-β; TK, thymidine kinase; VEGF, vascular endothelial growth factor.
* http://www.sfda.gov.cn.

Kim et al. Mol Therapy 2007;16:237-243

Comparisons with Conventional Therapy

	Gene Therapy	Conventional Therapy
Materials	Nucleotide Acid, DNA, RNA;etc. Cells, Tissues, Or Organs.	Small molecules, Peptide, Proteins.
Delivery	Usually required to be delivered into cells (antisense ODN) or Nucleus (genes).	Effect on the cell membrane or diffuse into cells
Mechanisms	Usually cure the causes of the diseases	Usually relieve the symptoms or signs
Duration of Effect	Can be permanent and also can be passed down to next generation in germline gene therapy.	Usually stop the effect once stop taking it.
Ethics	**Major Issues**	**Usually Not**


It's Calpain10!
It's IR !
It's GS!
It's IRS!
Type 2 DM
It's mtDNA!
It's GLUT4!
It's GK!

Gene Therapy for type 2 Diabetes ?

- Polygenic disorder : Gene – Environmental Interaction

- What is the canditate gene ?

- Causes ? or just Risk Factors ?

- **Insulin deficiency :** Insulin GT, Beta cell rejuvenation, regeneration

- **Insulin resistance :** Hepatic glucose production, adipocytokines

- **CNS, neural regulation :** leptin signaling

Diabetic Symptom Palliation rather than CURE

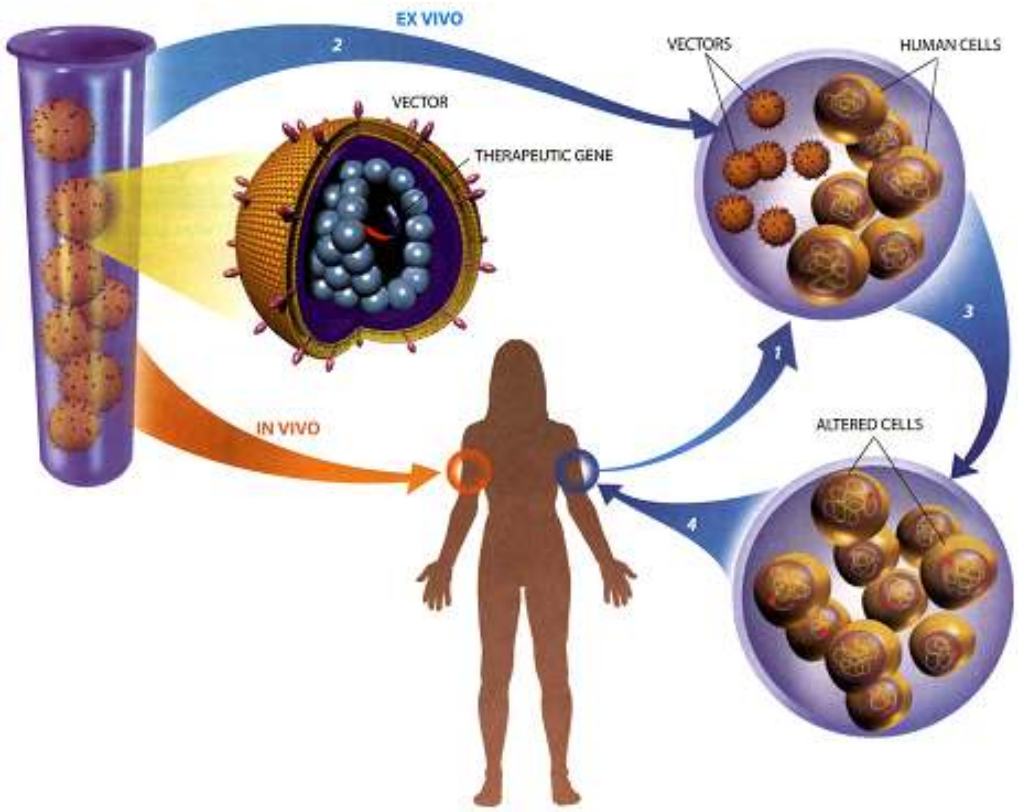
Gene Therapy for type 2 Diabetes ?

Lancet 2009;6736(09):60448-7

Gene Therapy

Therapeutic
Nucleic Acids

Gene Delivery
Techniques



Gene Delivery Methods

Viral Vectors

- Retroviral Vectors
- Lentiviral Vectors
- Adenoviral Vectors
- AAV
- HSV
- Baculovirus
- Vaccinia virus

Non-viral Vectors

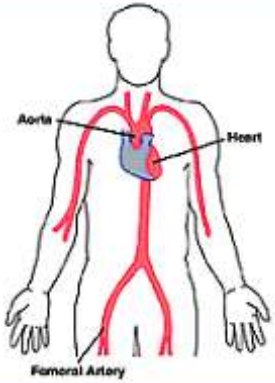
- Naked DNA
- Lipoplex
- Polyplex
- Lipopolyplex
- Sonoporation
- electroporation
- Gene gun

	Transduction efficiency	Integration efficiency
Chemical		
Calcium-phosphate transfection	**Low**	**Low**
DEAE-dextran transfection	**Low**	**Low**
Physical		
Electroporation	**Low**	**Low**
Microinjection	**High**	**Low**
Particle bombardment	**High**	**Low**
Fusion		
Liposomes	**Low**	**Low**
Receptor-mediated endocytosis		
DNA-protein complexes	**High**	**Low**
Viral envelope/capsid-DNA complexes	**High**	**Low**
Recombinant viruses		
Adenovirus	**High**	**Low**
Adeno-associated virus (AAV)	**High**	**High**
Herpes simplex virus	**Low**	**Low**
Human immunodeficiency virus (HIV)	**High**	**High**
Moloney murine leukemia virus	**High**	**High**
Vaccinia virus	**High**	**Low**

Barriers for Gene Therapy

Extracellular:

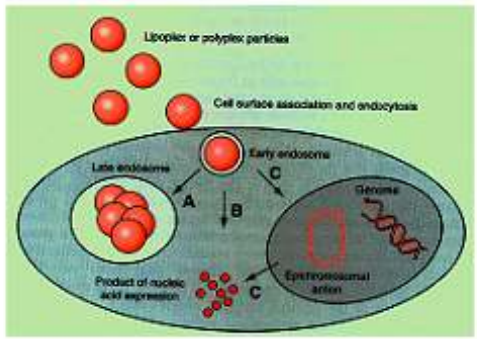
1. Epithelial Barriers;
2. Circulation and The Blood Components such as RBC, serum proteins, Enzymes, etc.
3. RES cleaning system;
4. Etc.



Require inert surface and targeting molecules for specific transportation of complex in the blood.

Intracellular:

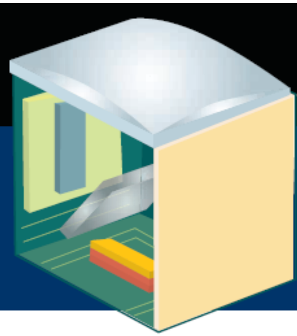
1. Cell Membrane
2. Endosomal Membrane
3. Nuclear Membrane
4. DNA Releasing at Right Site and Right Time



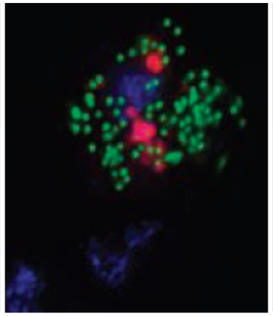
Require cationic groups for successful gene delivery.

Tragedies in Early Clinical Trials

NEWS>> THIS WEEK



A record-breaking solar cell 583



Amoeba immunity 584

CLINICAL RESEARCH

Death Prompts a Review of Gene Therapy Vector

The death last week of a patient receiving experimental gene therapy for arthritis has triggered a federal review of all trials using the same vector. Few details have been made public; if it turns out that the therapy is to blame, it

tumor necrosis factor α (TNF-α). Although Enbrel and similar drugs are effective, they don't always penetrate all joints, and they have to be injected regularly. Targeted Genetics uses a modified virus, called an adeno-associated

using AAV, including 21 active studies.

The tragedy has stirred speculation about the cause. One suspect is the gene product, because Enbrel, which suppresses one immune response, has been linked to sepsis and bacterial infections, suggests gene therapy expert Terry Flotte, dean of the University of Massachusetts Medical School in Worcester. But Carter says the protein is "not necessarily the issue" because the protein has not been detected in serum from nonhuman primates or patients.

country last winter while investigating vector toxicity.

www.sciencemag.org SCIENCE VOL 288 12 MAY 2000

come as a further blow. And given recent history, it's understandable that authorities in France, directly responsible for the affected trial, and the United States, where the Gelsinger debacle has left painful memories, should move quickly to suspend such trials.

However, a closer examination of the French case reveals important differences to the Gelsinger case that should convince regulators to proceed once more with SCID trials, albeit more cautiously.

risk. Regulators must also reconsider the wisdom of using retroviral vectors, particularly where genes are introduced to mark populations of cells for study, rather than for their intrinsic therapeutic effects.

The challenge for gene therapists and regulators is to show that the field can respond appropriately to a serious adverse event in an otherwise successful clinical trial. It is unlikely to be the last: such setbacks are inherent to the development of new medical treatments. ■

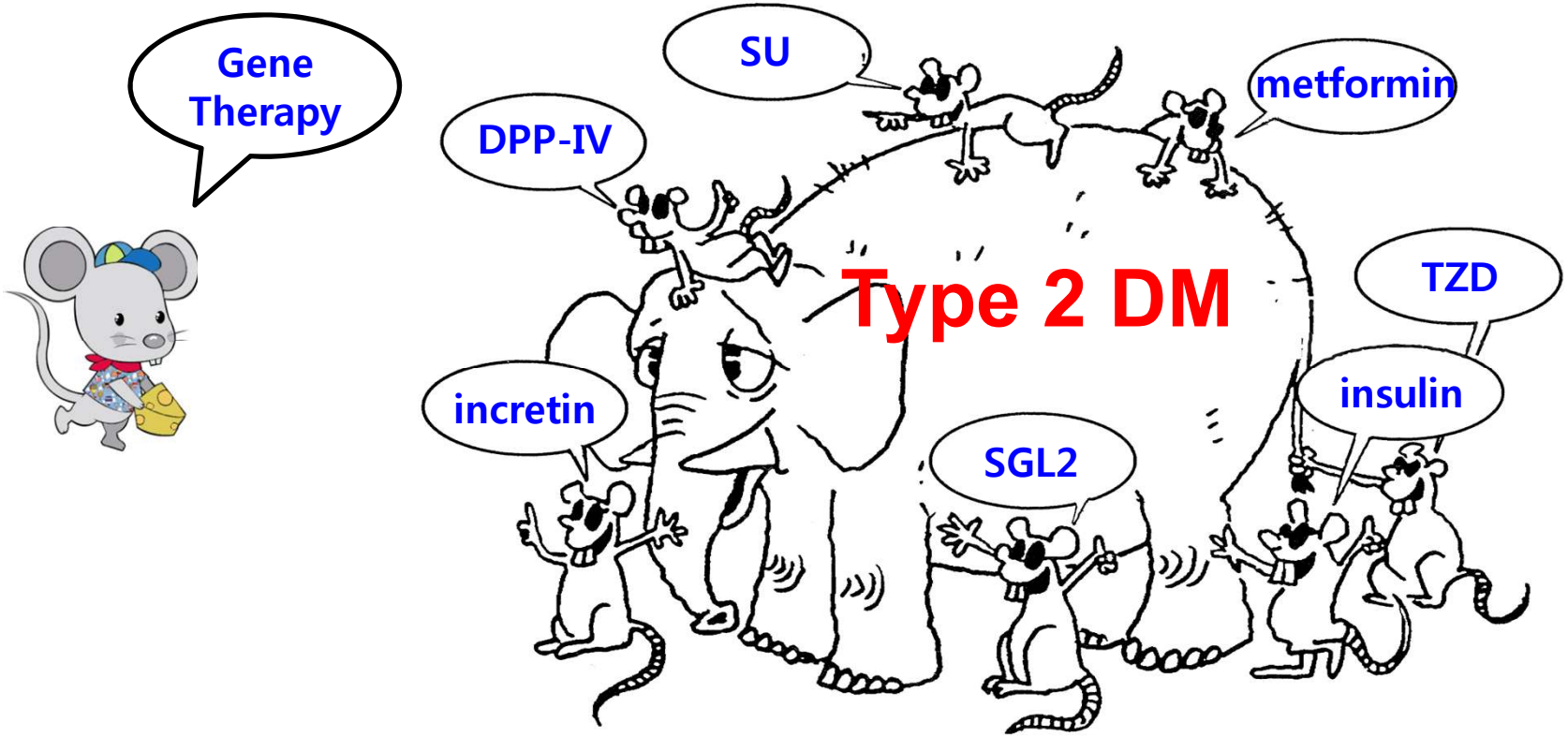
Requirements for the Success in Real World

Only desired cells or tissue Be Delivered.

The delivery efficiency Dependent on the disease requirements

Biocompatible
Non-cytotoxicity
Non-immunogenecity
Non-inflammation
Non-Tumor Generation

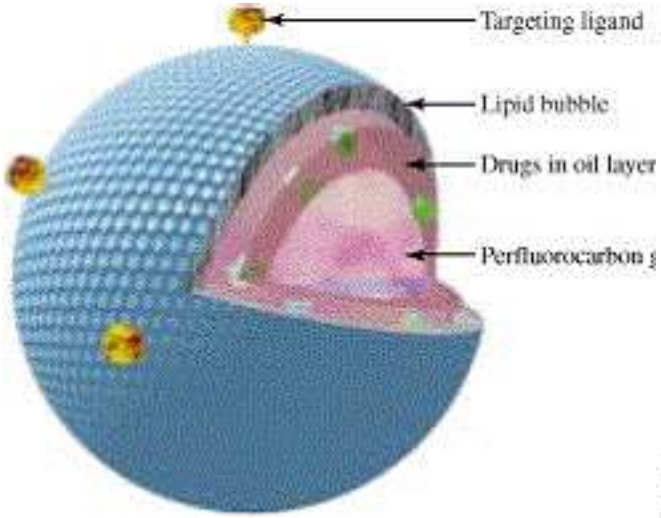
Requirements for the Success in Real World

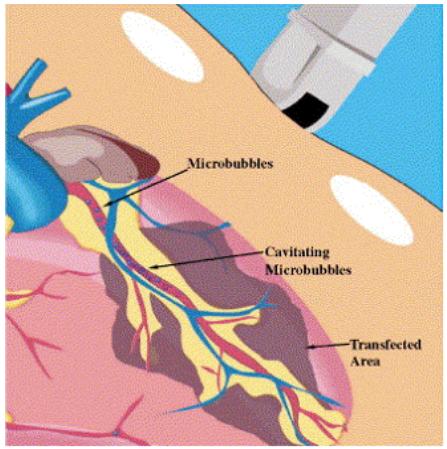


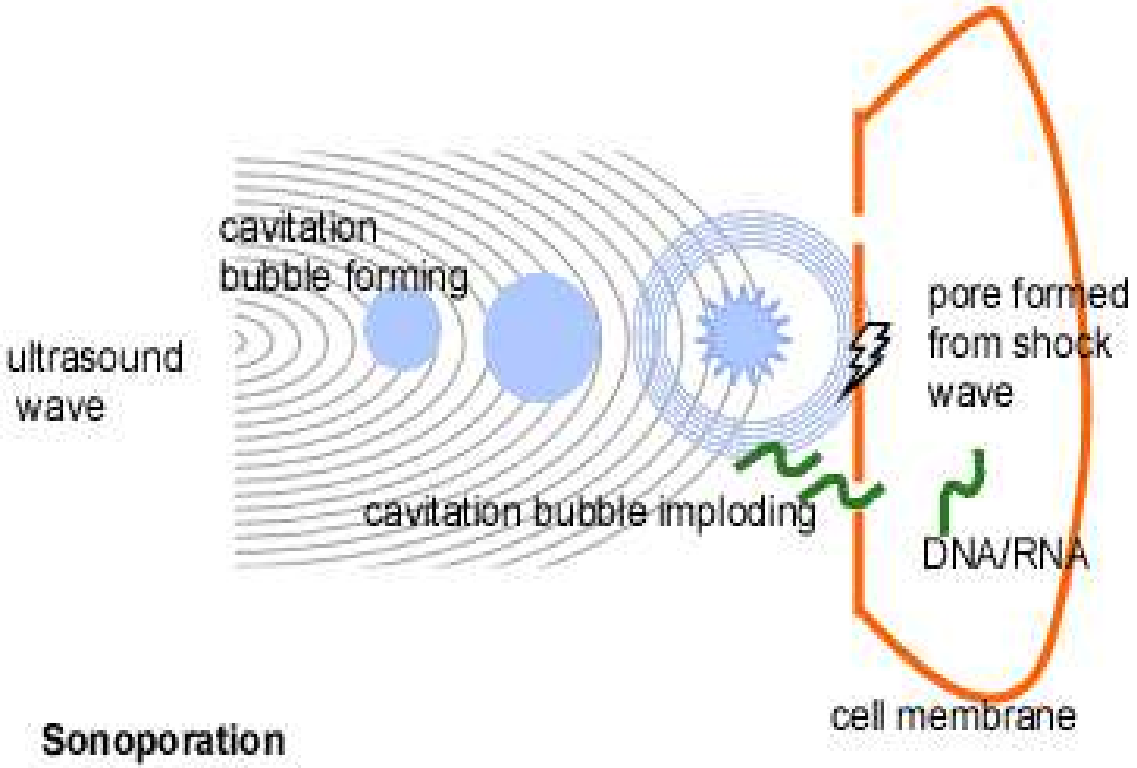
superiority → investment → practicalize

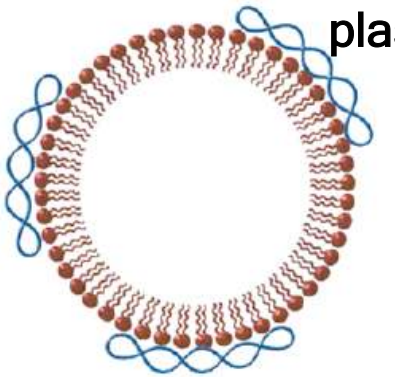
Safest method in Gene Therapy ?

UTMD: Sonoporation

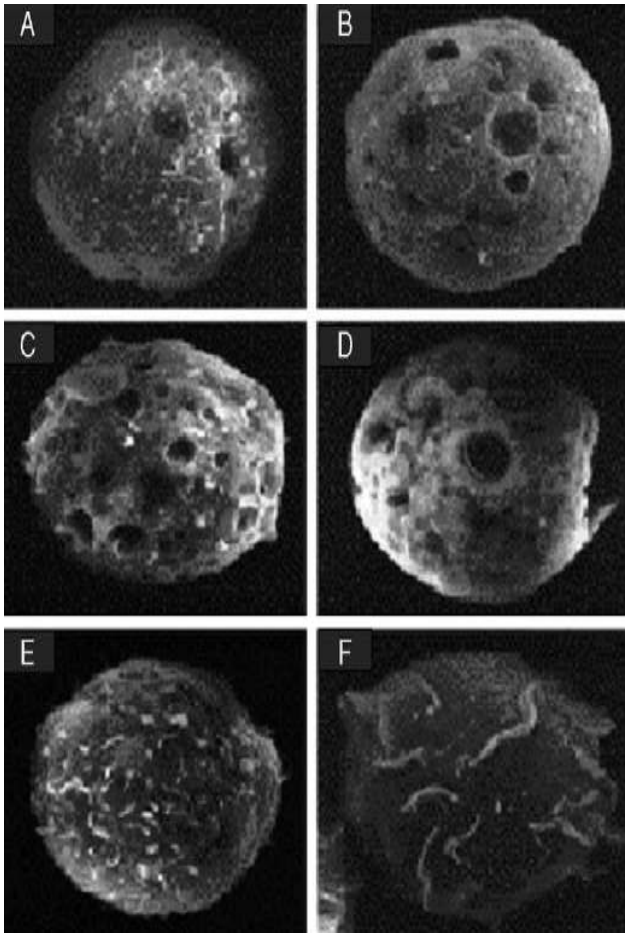








micro-bubble



Y Liu et al, J Cont Release, 2006

Efficiency vs Toxicity.. Sonoporation

Journal of Drug Targeting, December 2008; 16(10): 773–779

informa
healthcare

Comparison of the efficiency and toxicity of sonoporation with branched polyethylenimine-mediated gene transfection in various cultured cell lines

CHANG S. YOON[1], HYE S. JUNG[1], TAE K. KIM[1], MIN J. KWON[1], MI K. KIM[1,2], MINHYUNG LEE[3], KYUNG S. KOH[1], BYUNG D. RHEE[1], & JEONG H. PARK[1]

[1]*Molecular Therapy Laboratory, Department of Internal Medicine, College of Medicine, Paik Institute for Clinical Research, Inje University, Busan, South Korea,* [2]*Department of Internal Medicine, Maryknoll General Hospital, Busan, South Korea, and* [3]*Department of Bioengineering, Hanyang University, Seoul, South Korea*

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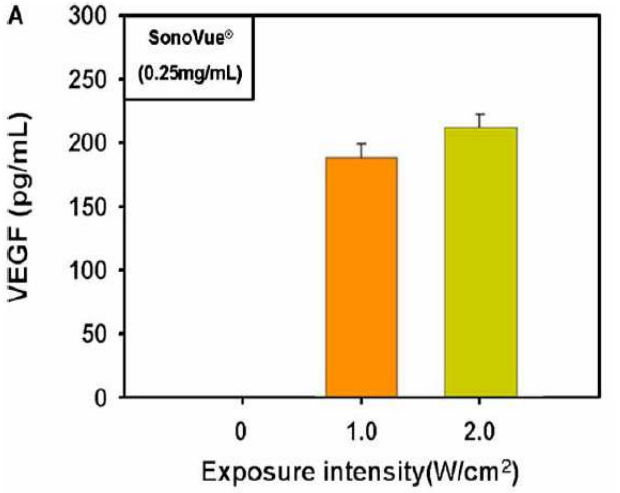
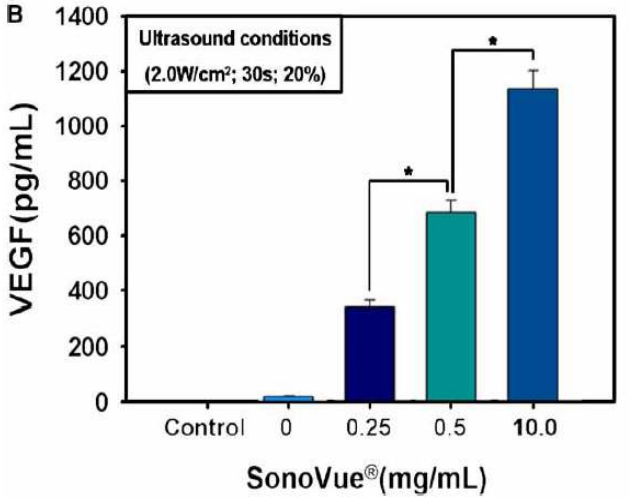
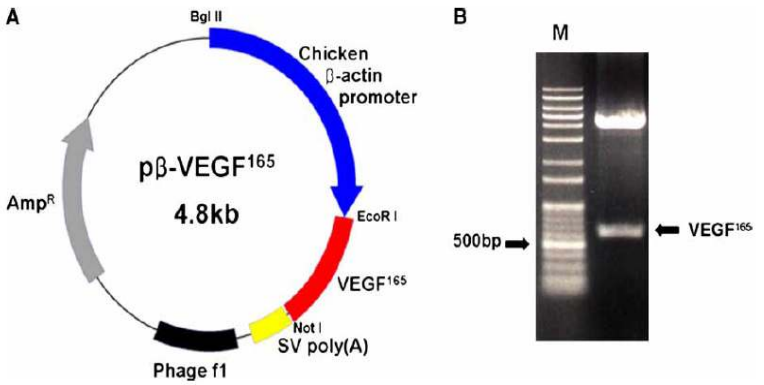
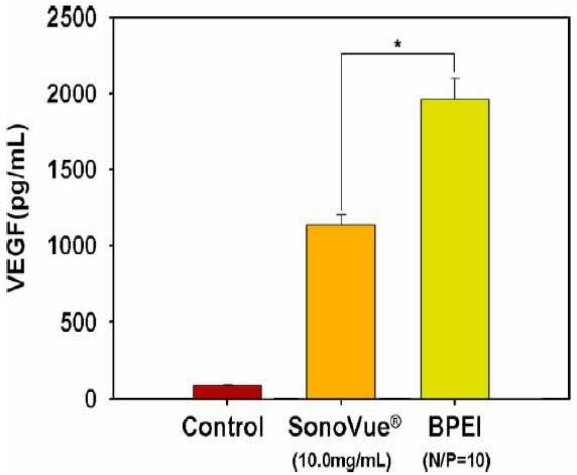
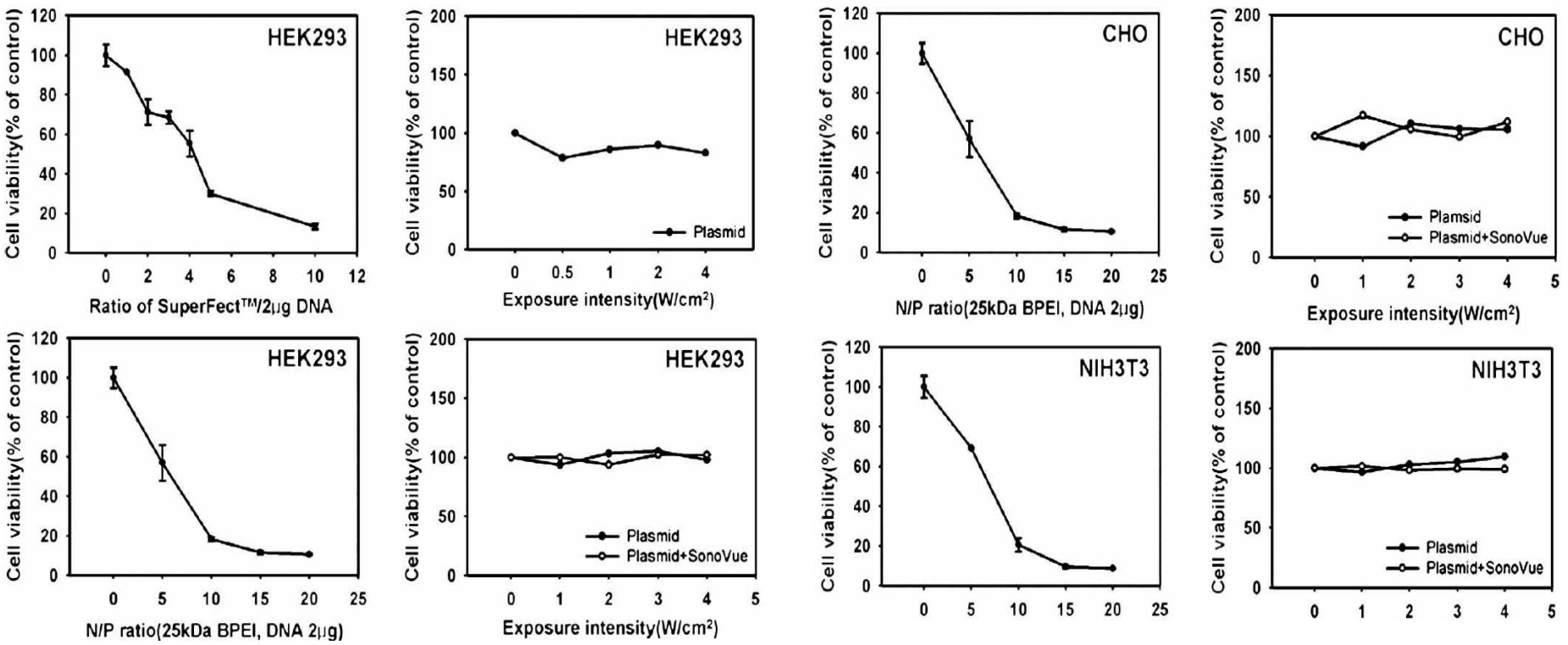








Journal of Drug Targeting 2008;16(10):773-779



Journal of Drug Targeting 2008;16(10):773-779

Expert Opinion

Ultrasound-mediated gene delivery

Chang S Yoon & Jeong H Park†
†*Paik Memorial Institute for Clinical Research, Department of Internal Medicine, College of Medicine, Inje University, Busan, South Korea 614-735*

Importance of the field: The use of ultrasound with microbubbles raises the possibility of an efficient and safe gene delivery.
Areas covered in this review: This review summarizes the current state of the art of gene delivery by sonoporation under the following topics. First, the basic ultrasound parameters and the characteristics of microbubble in biological systems are discussed. Second, the extensions of sonoporation to other fields of gene delivery such as viral and non-viral vector are briefly reviewed. Finally, recent applications in an animal model for various diseases are introduced.
What the reader will gain: Information and comments on gene delivery by sonoporation or enhanced cell membrane permeability by means of ultrasound.
Take home message: Ultrasound-mediated gene delivery combined with microbubble agents provides significant safety advantages over other methods of local gene delivery.

Keywords: drug/gene delivery, microbubbles, sonoporation, ultrasound

Expert Opin Drug Deliv 2010;7(3):321-330

UTMD: Sonoporation

. One of the main advantages of drug or gene delivery by sonoporation is to achieve site specificity with negligible local and systemic toxicities, by the optimization of parameters of ultrasound and microbubbles.

. The dynamic characteristics of ultrasound that induce cell membrane porosity can be controlled by ultrasonic factors such as frequency, intensity and duration.

. The induction of microbubble cavitation by ultrasound has been considered a major mechanism of delivery, which carries non-permeable macromolecules across the cell membrane.

. Ultrasound can act synergistically with other vector systems in order to have several advantages, such as low cytotoxicity, high target selectivity, low immunogenicity and repeatable application.

Expert Opin Drug Deliv 2010;7(3):321-330

UTMD: Sonoporation

. Gene delivery by sonoporation could be a possible therapeutic alternative in current cancer treatment.

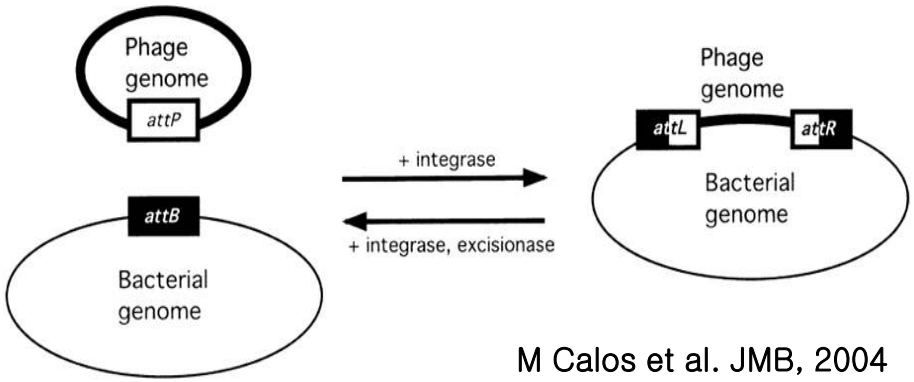
. Non-invasive specific gene transfection into the deep seated internal organ is very difficult. Sonoporation might be used for this purpose and the possibility for the application of this technique to the various kinds of diseases would be promising.

. Particularly suited for the various localized diseases and the diseases requiring limited transfection into the deep-seated organs or tissues, sonoporation could be used successfully in clinical practice in the near future.

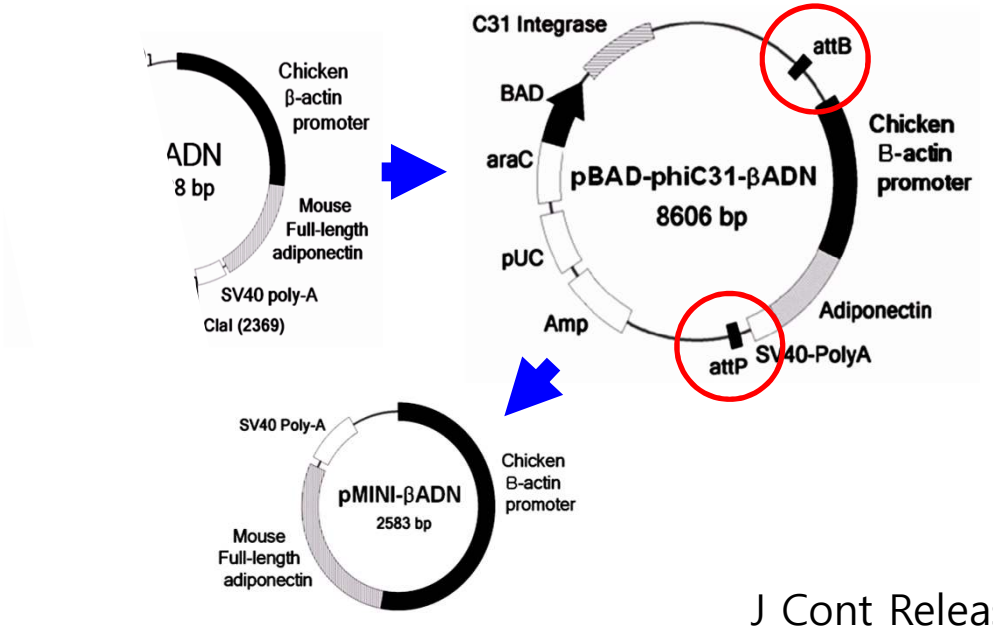
. Along with its superior safety profile, sonoporation might be regarded as a pioneering technique that could move gene therapy a step closer to clinical medicine.

Expert Opin Drug Deliv 2010;7(3):321-330

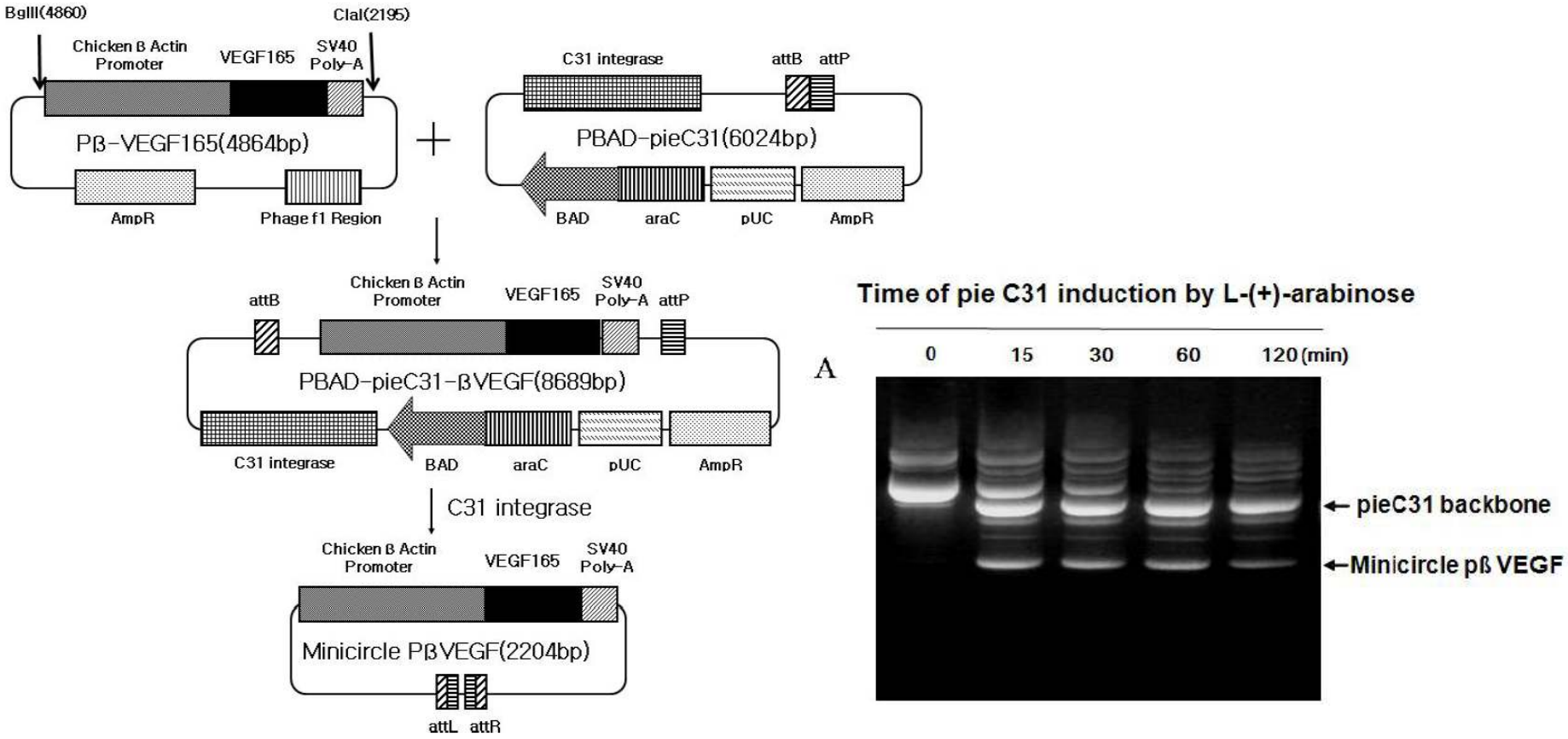
Improving Gene Expression - Minicircle



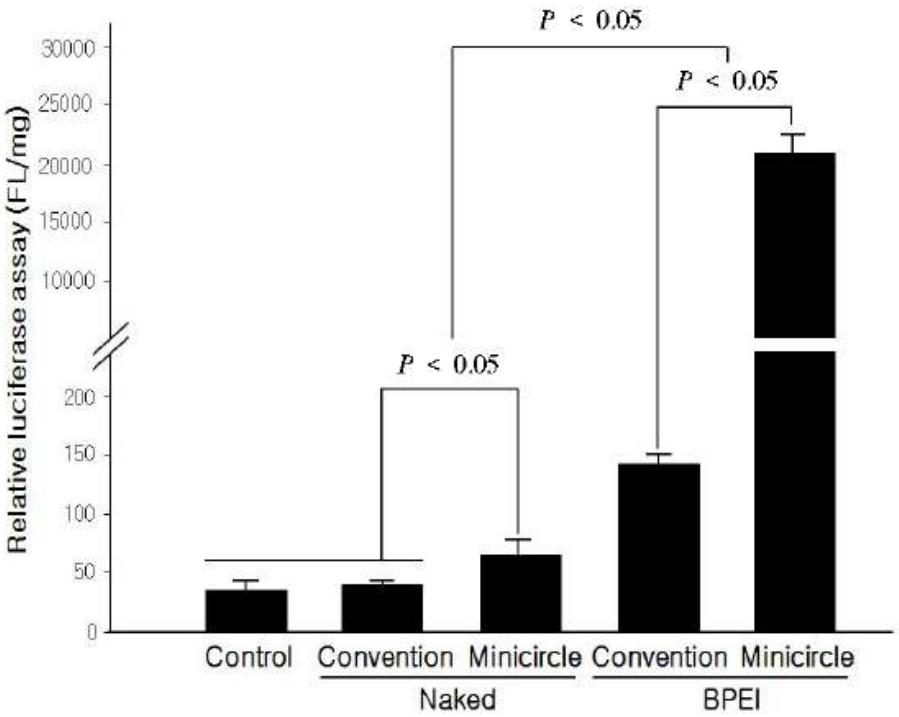
M Calos et al. JMB, 2004

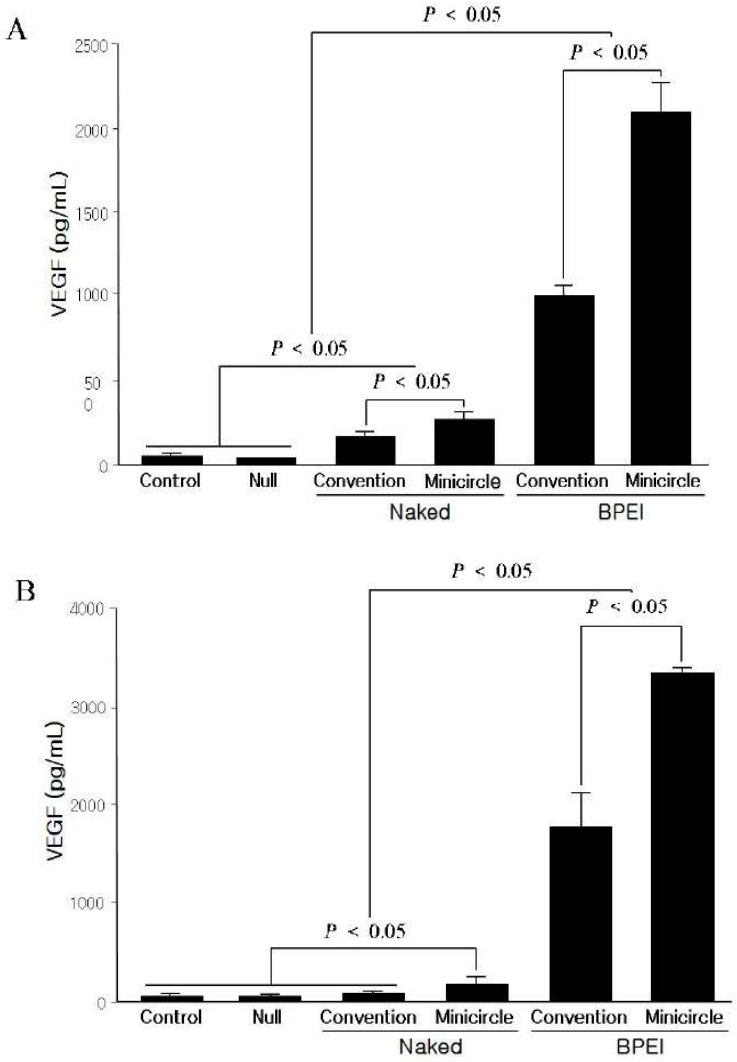


J Cont Release 2006;114:118-125



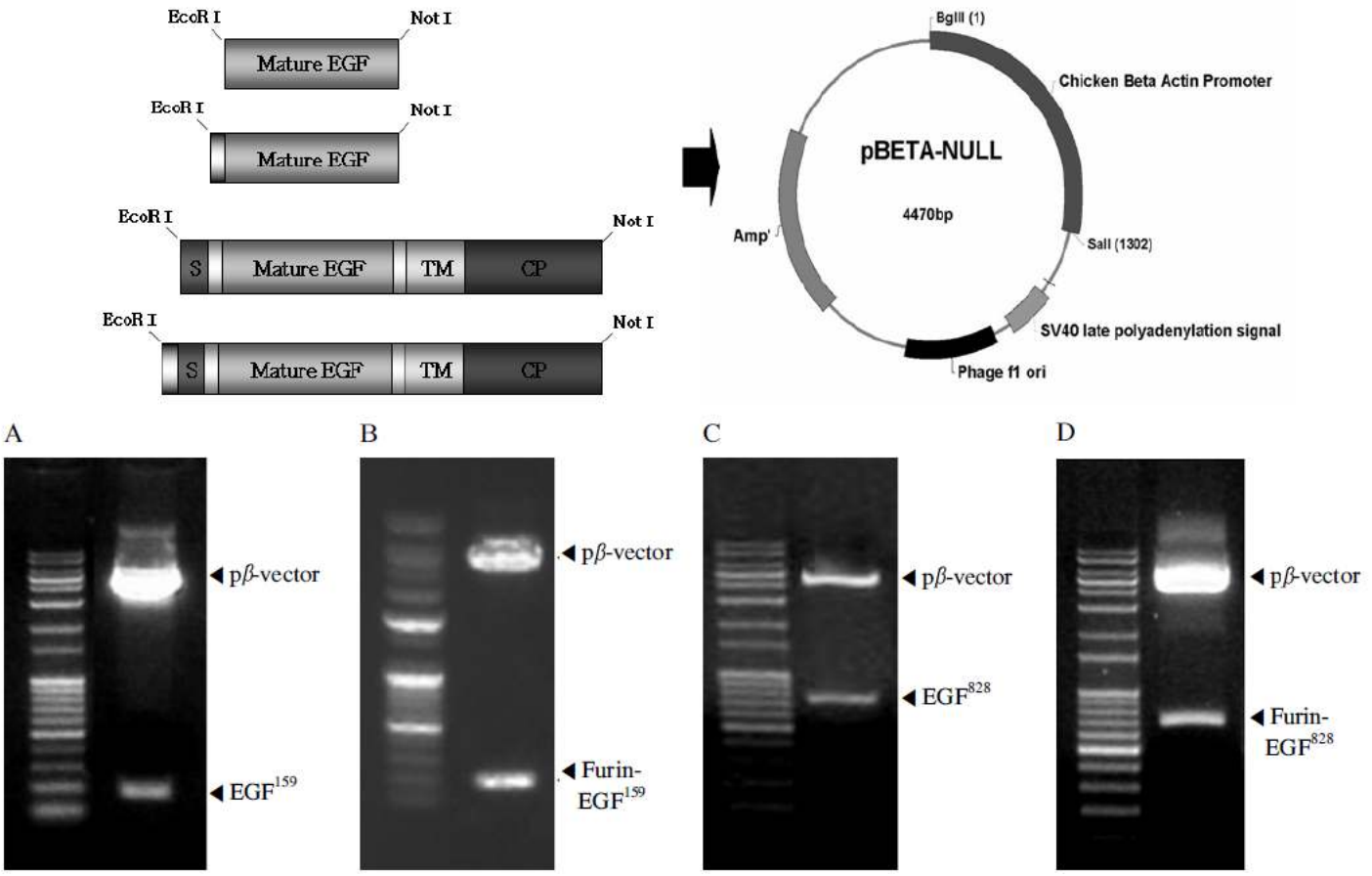
J Kor Diabetes Assoc 2007;31:465-471



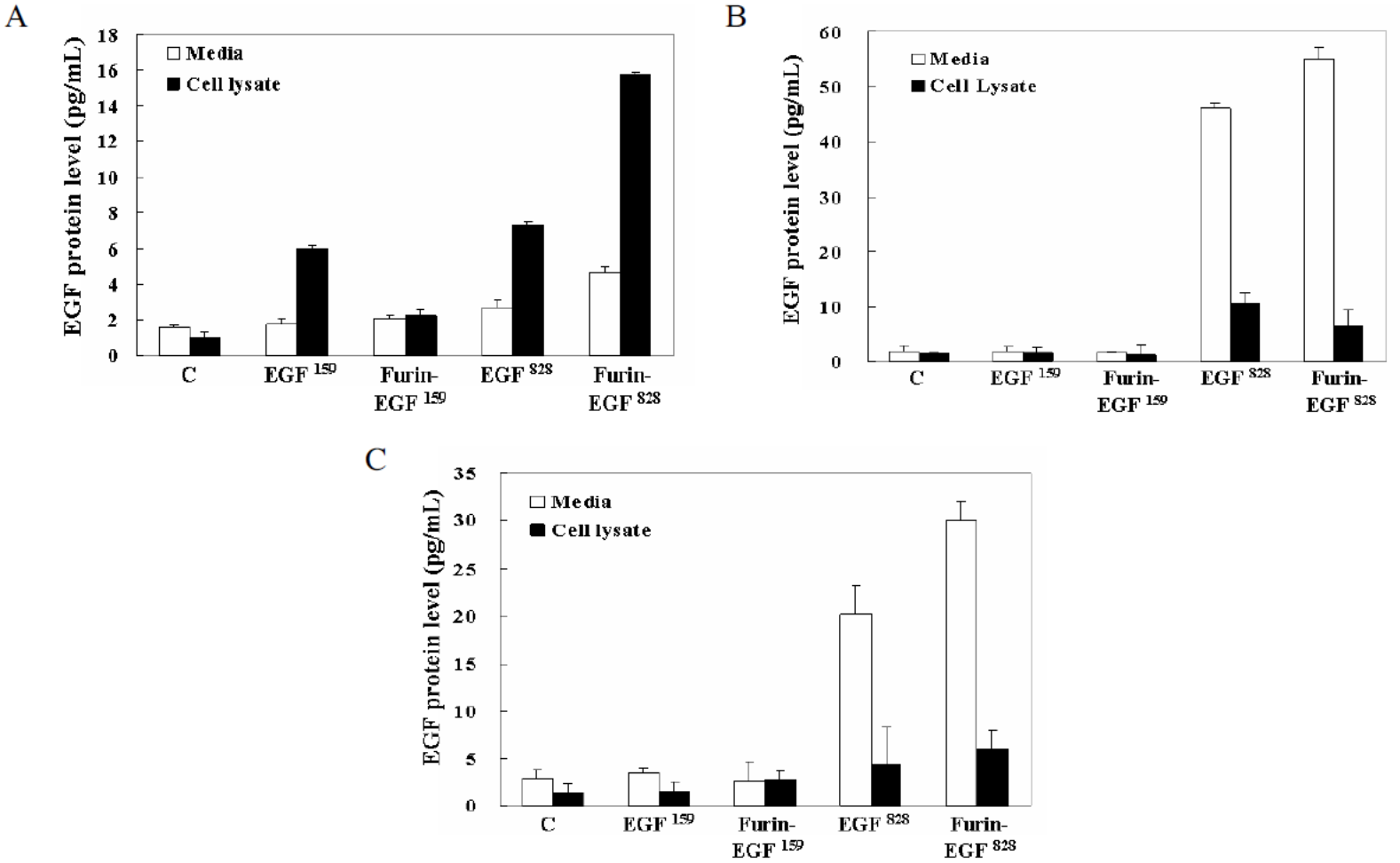


J Kor Diabetes Assoc 2007;31:465-471

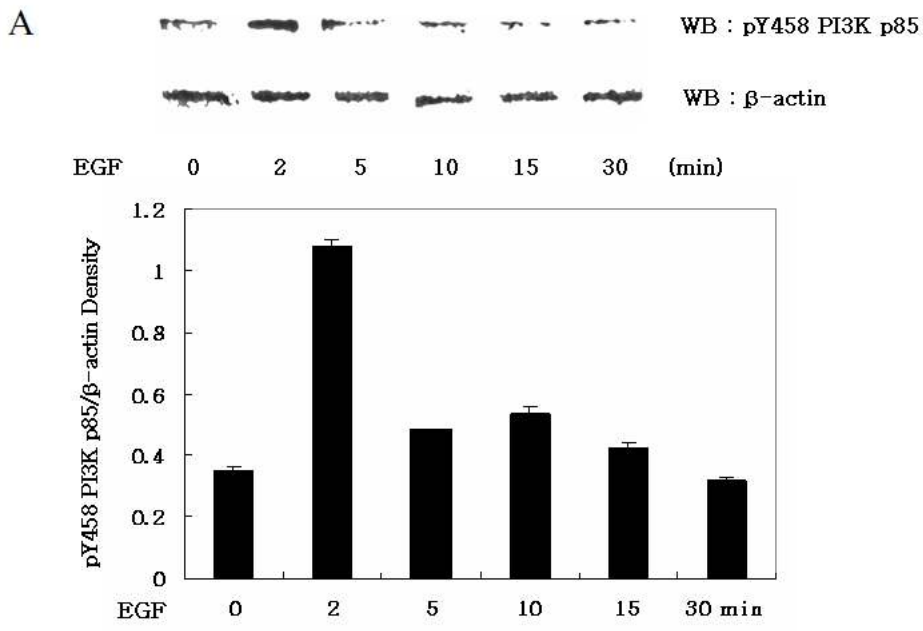
Improving Gene Expression - Modification

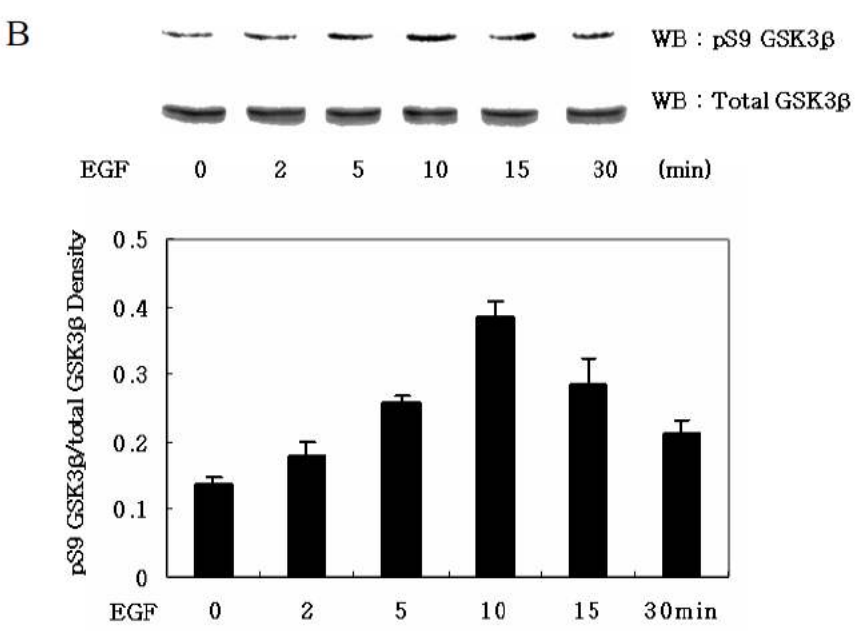


Kor Diabetes J 2008;32:131-140



Kor Diabetes J 2008;32:131-140





Kor Diabetes J 2008;32:131-140

Pharmaceutical Research, Vol. 26, No. 4, April 2009 (© 2008)
DOI: 10.1007/s11095-008-9778-x

Research Paper

Sonoporation of the Minicircle-VEGF^{165} for Wound Healing of Diabetic Mice

C. S. Yoon,[1] H. S. Jung,[1] M. J. Kwon,[2] S. H. Lee,[2] C. W. Kim,[3] M. K. Kim,[4] M. Lee,[5] and J. H. Park[2,6]

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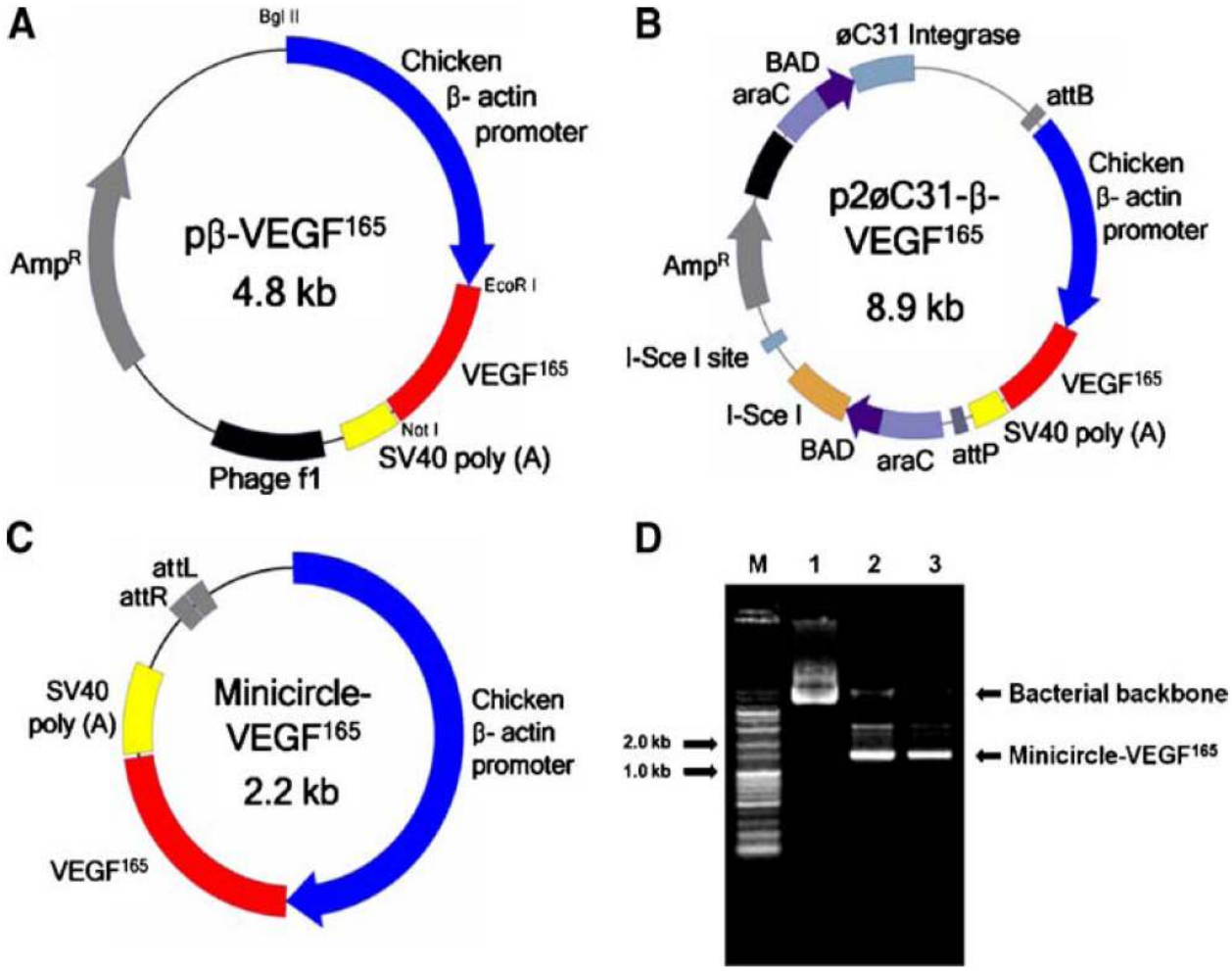
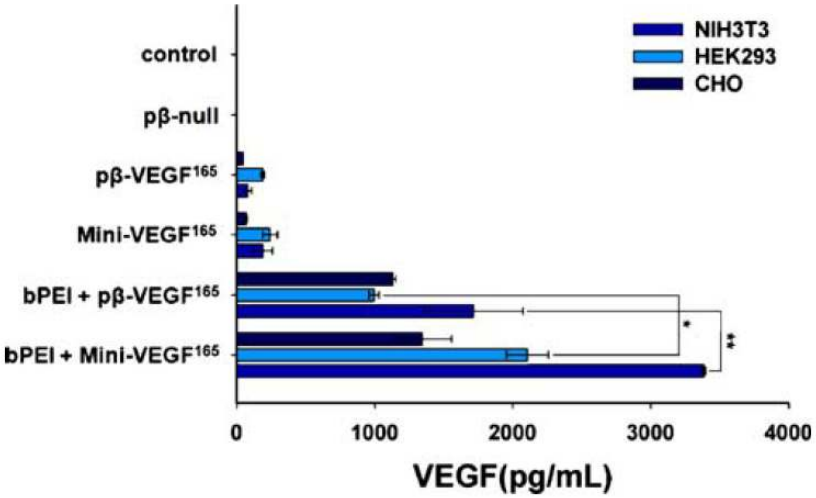
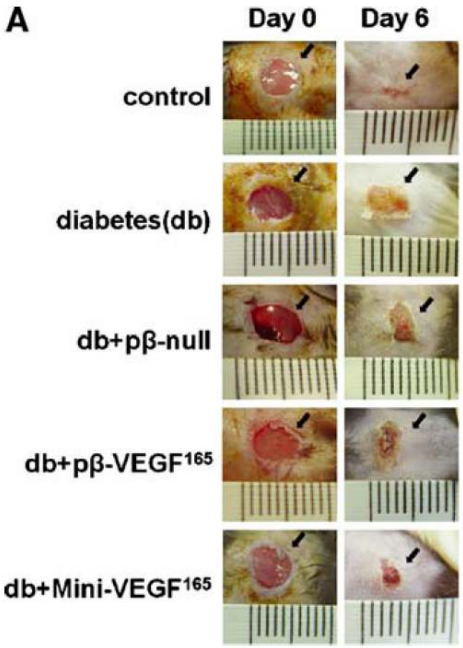
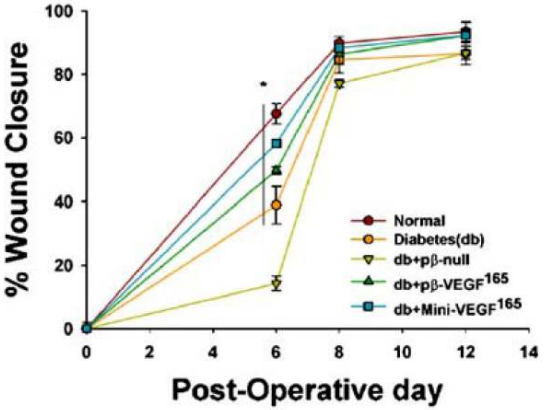
Pharm Res 2009;26(4):794-801



Pharm Res 2009;26(4):794-801







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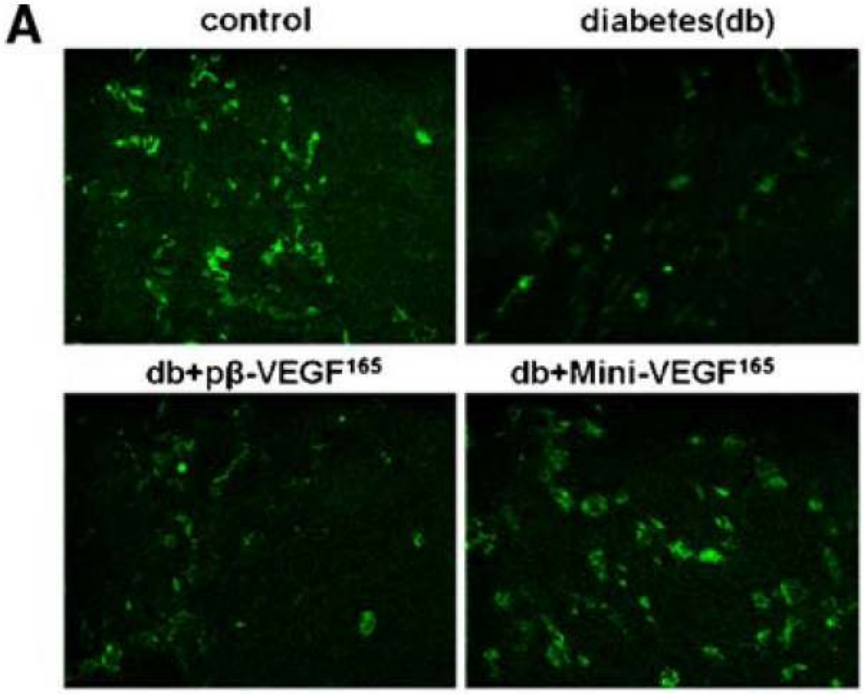

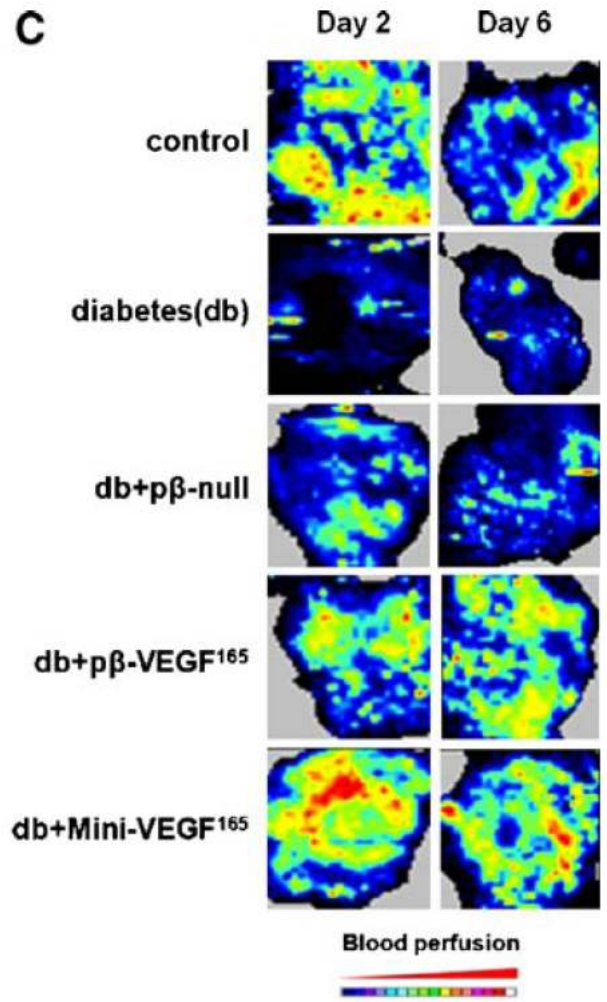

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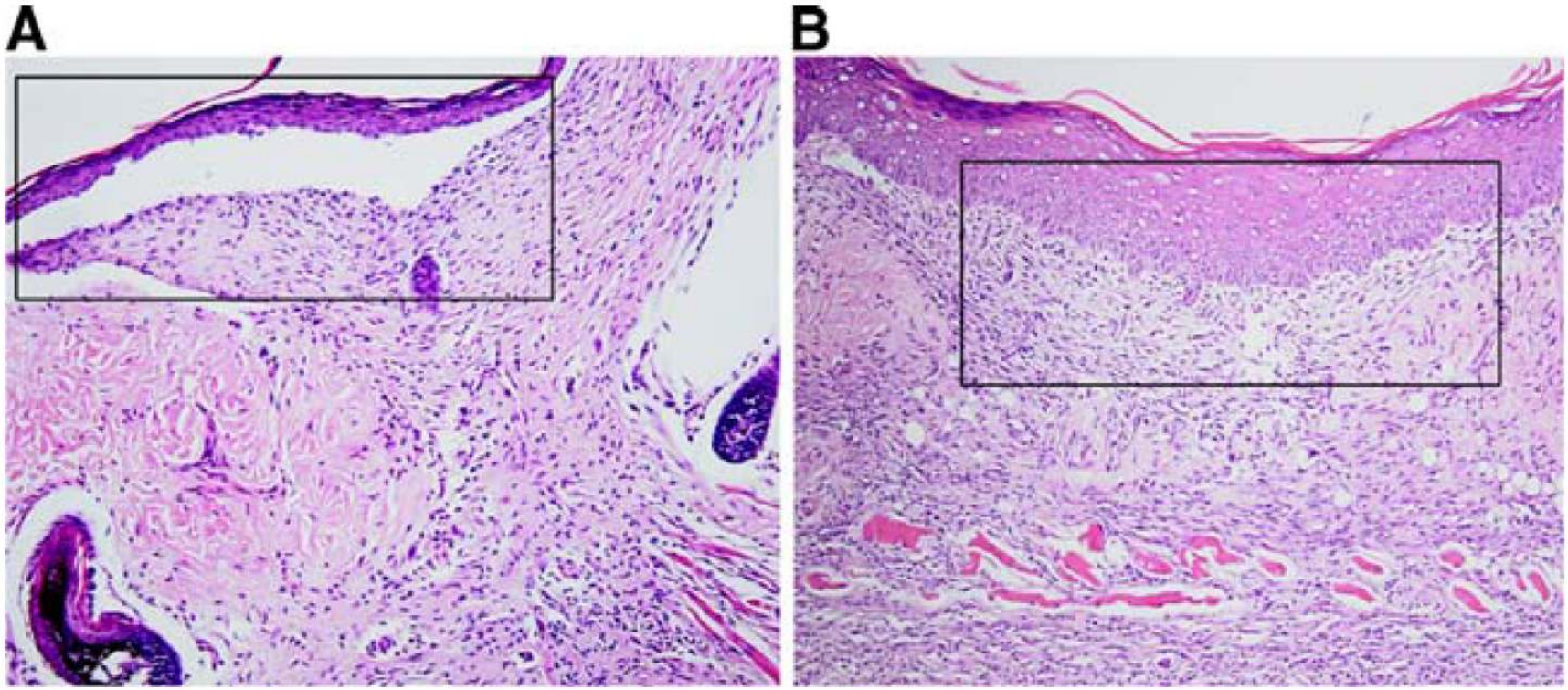

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J Gene Med 2012; **14**: 272–278.
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RESEARCH ARTICLE

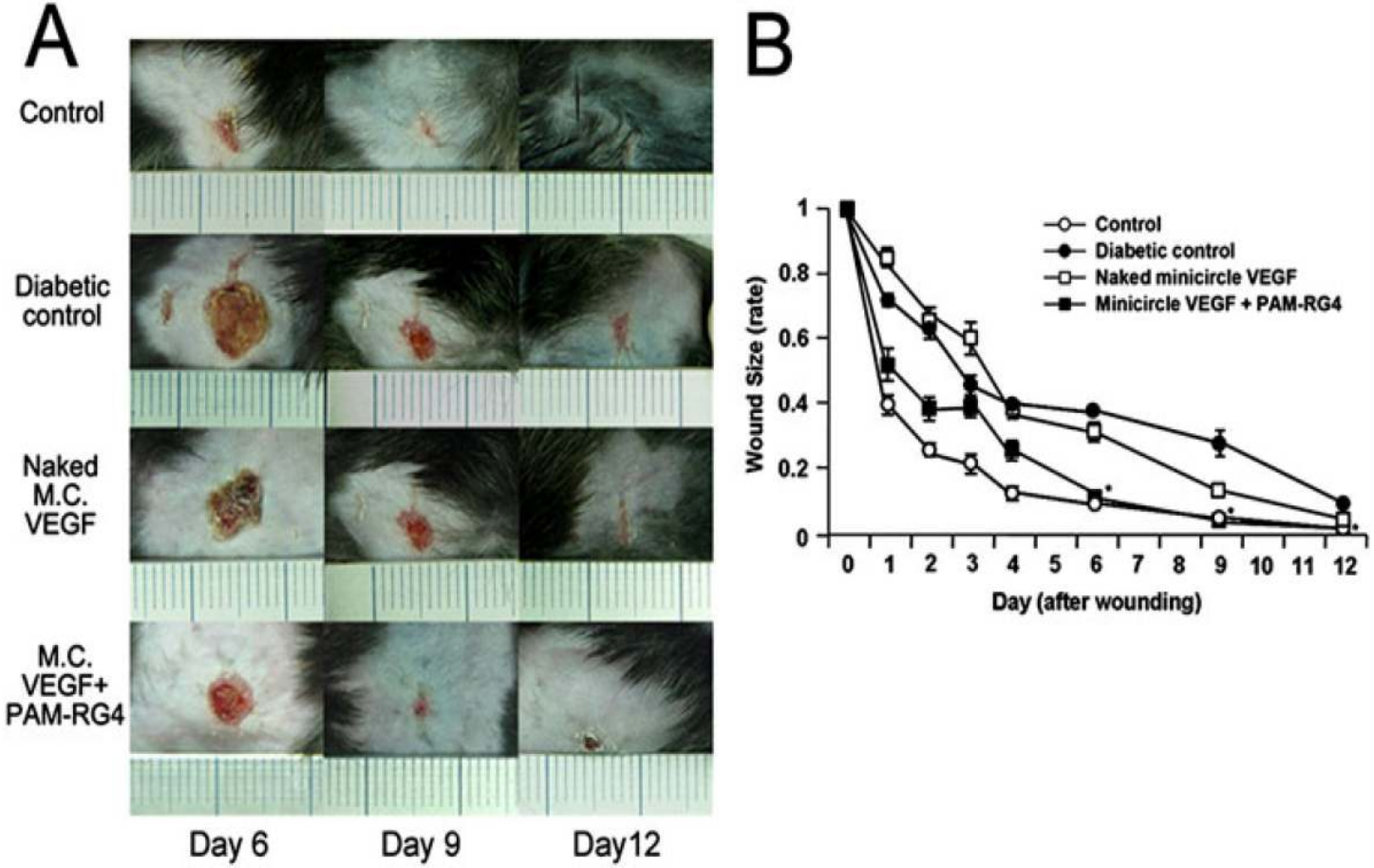
Effective healing of diabetic skin wounds by using nonviral gene therapy based on minicircle vascular endothelial growth factor DNA and a cationic dendrimer

J Gene Med 2012;**14**:272-278

인제대학교 의과대학 INJE UNIVERSITY COLLEGE OF MEDICINE

Molecular Therapy Lab

인제대학교부산백병원 INJE UNIVERSITY BUSAN PAIK HOSPITAL



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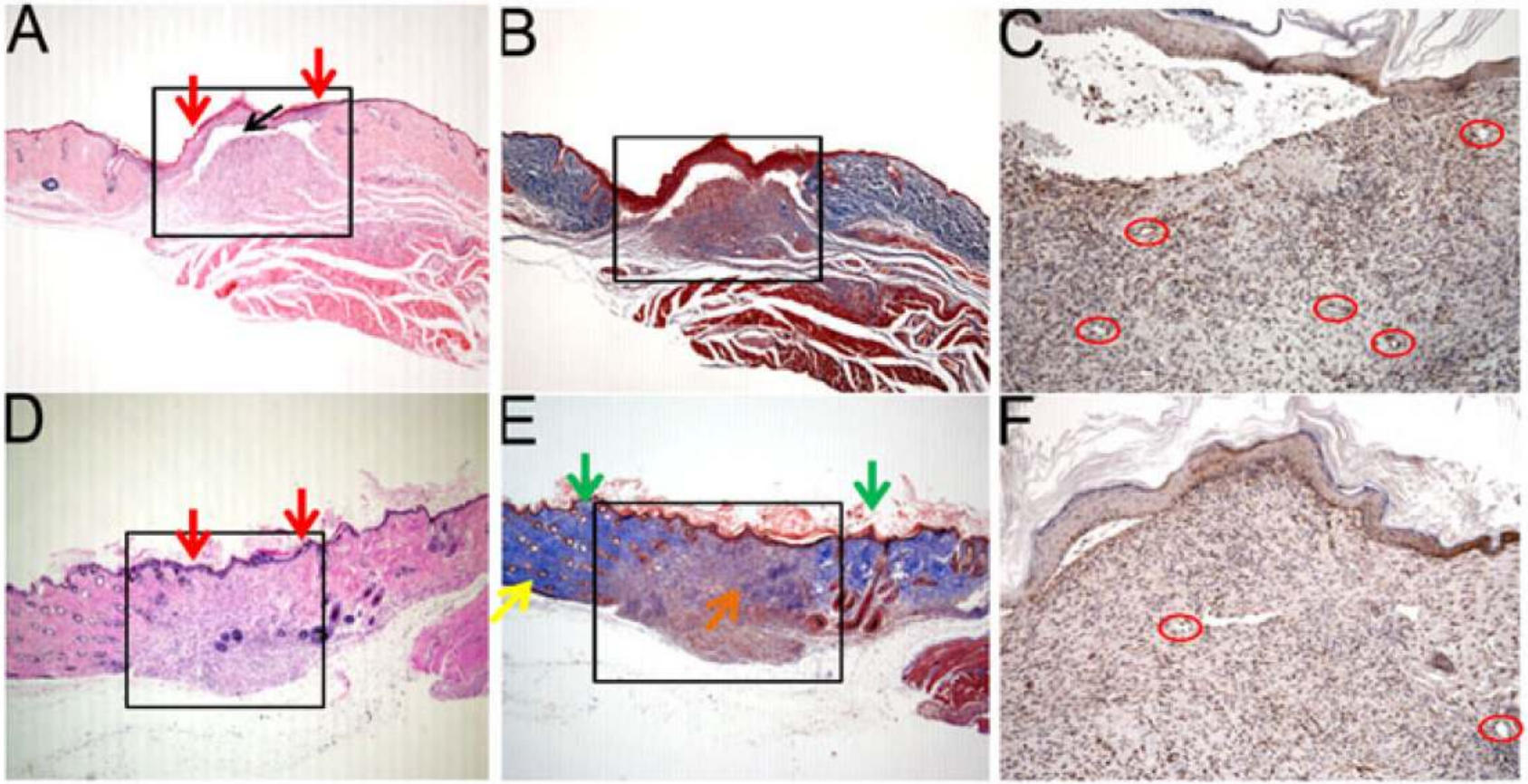

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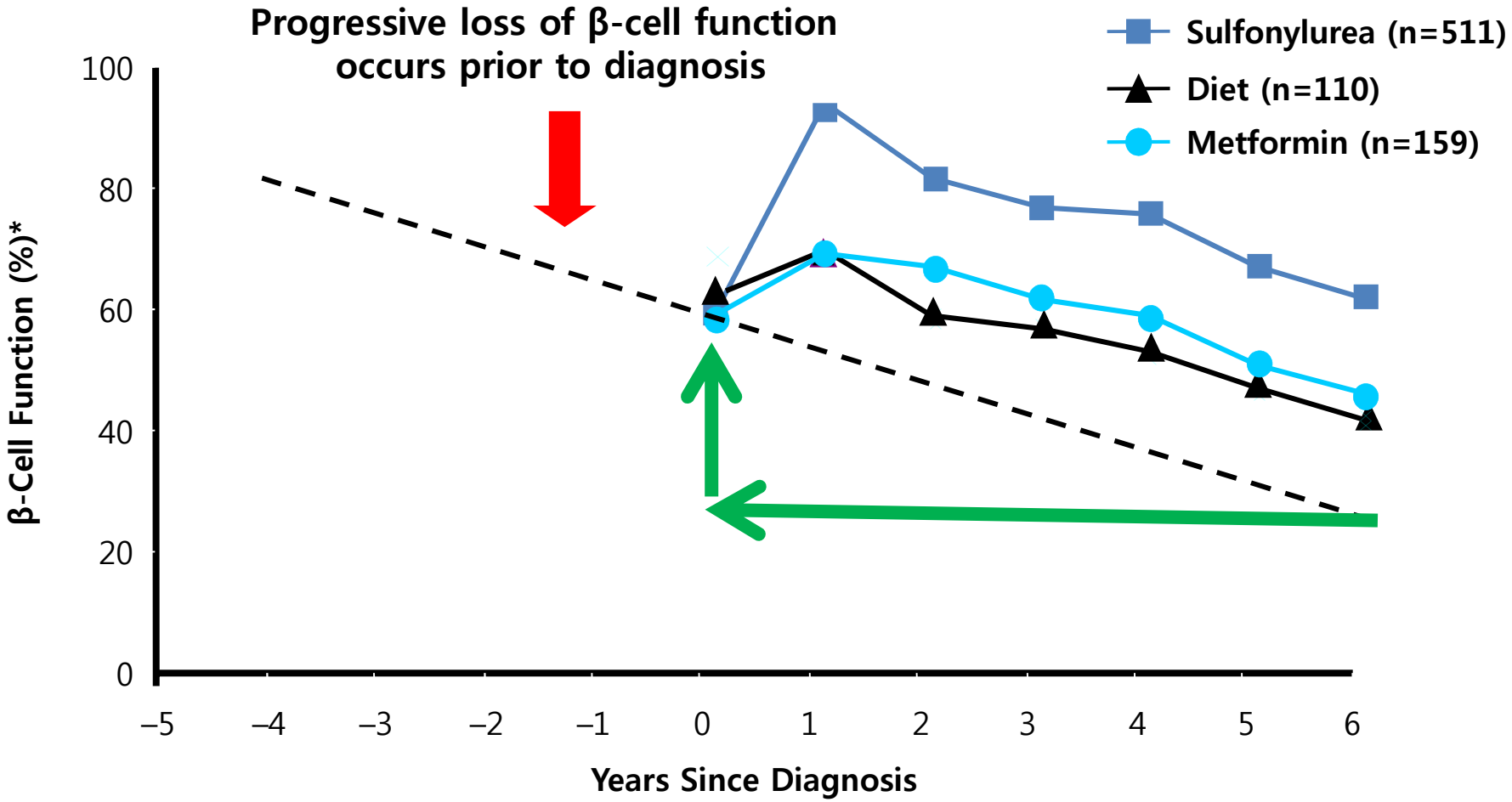
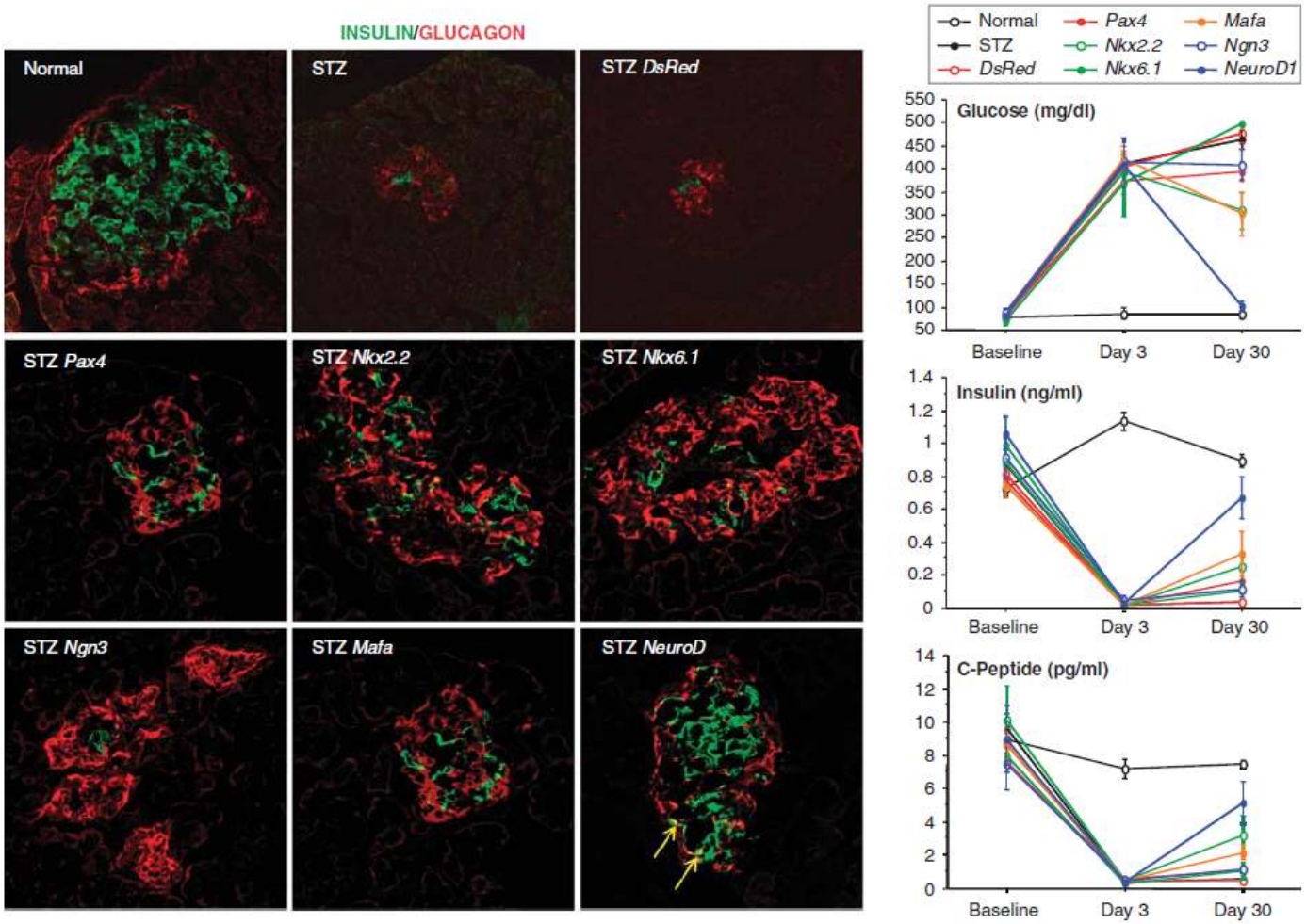
The Last Topic – Type 2 Diabetes Remission

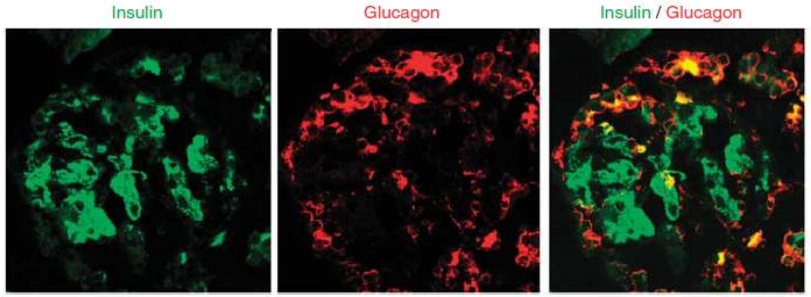


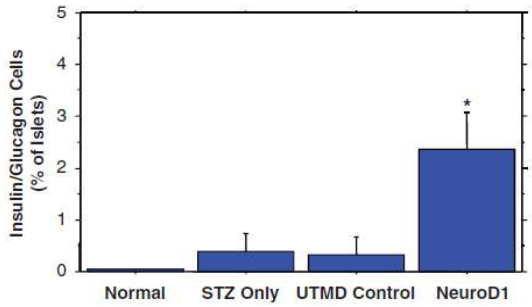
UKPDS Group. *Diabetes*. 1995; 44: 1249–1258.

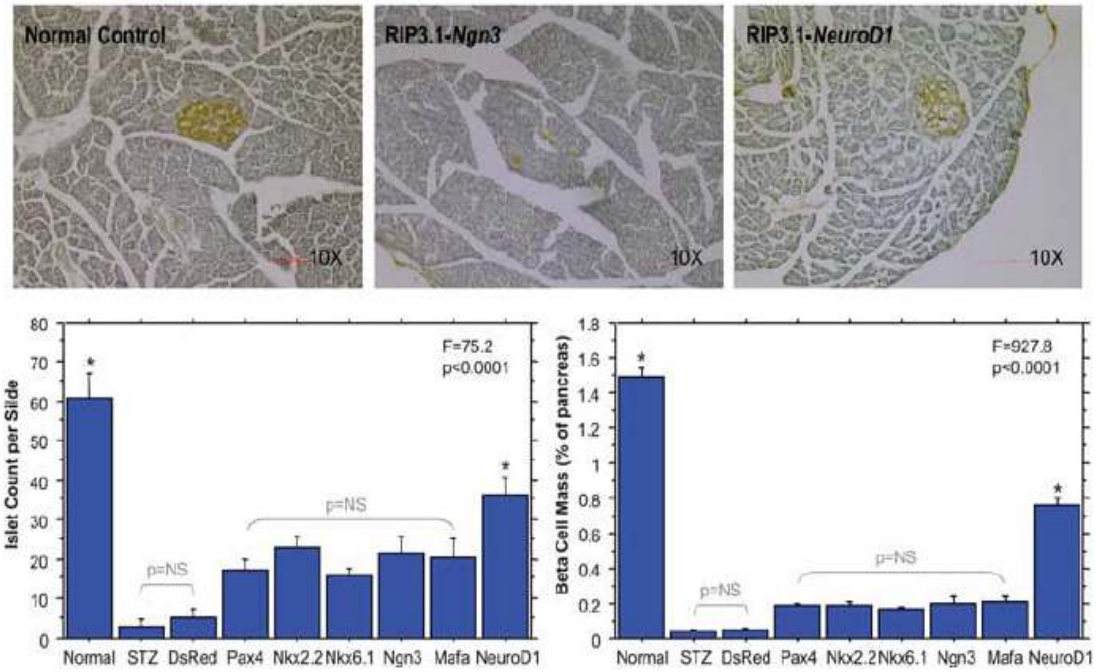
UTMD-Pancreatic β cell targeting



PA Grayburn et al. Gene Therapy 2010







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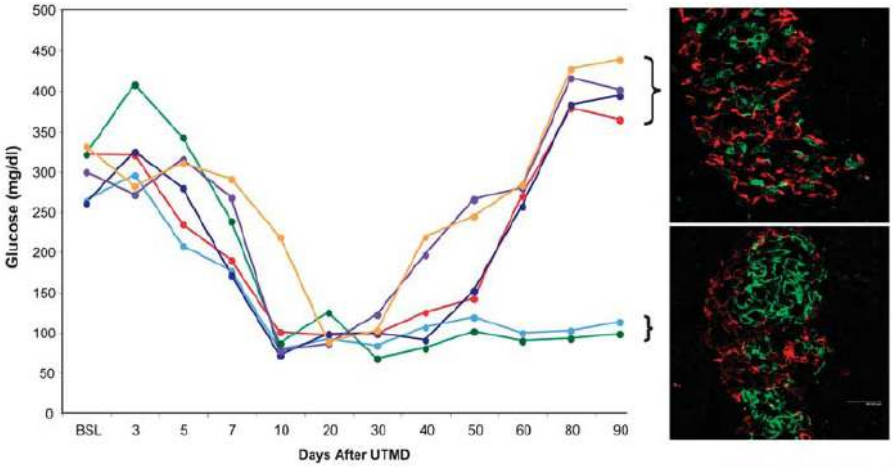

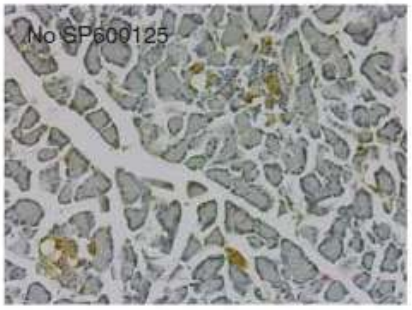

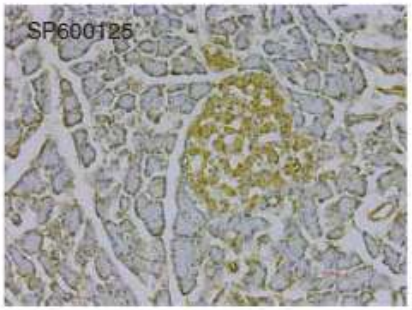

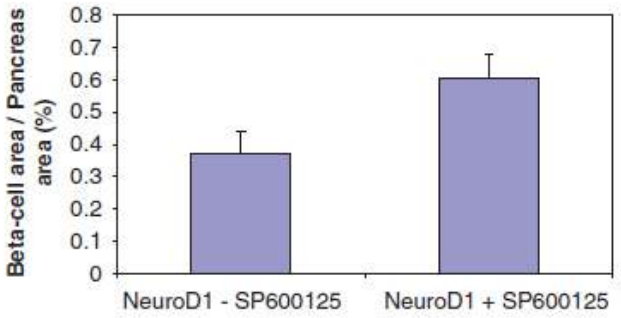

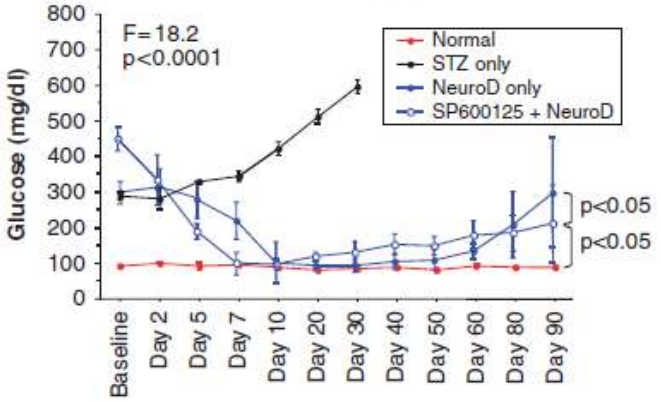

PA Grayburn et al. Gene Therapy 2010

Summary & Conclusion

1. Gene Therapy for Diabetes

- Researchers, Government, Corporation, Investor, Patency, Market etc.

2. Scanty Possibility for Glorious Victory

- Genetics Researches, Competition with many available modalities

3. State of the Art

- From dreaming to design, based on valid technologies

4. Safety, rather than efficacies

- Distinction, between Science & Business