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# Natural and Synthetic lipid membrane nanovesicles to repair and regenerate skin

Sai Kiang Lim, PhD

# Research Focus of Lab

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- ▶ Nano Lipid membrane vesicles

- ▶ Natural

- Extracellular Vesicles: microvesicles, exosomes, apoptotic bodies

- Mesenchymal stem cell exosomes

- ▶ Synthetic

- ▶ Liposomes (exosome-like)



# Introduction

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- ▶ Mesenchymal stem cell exosomes
  - ▶ Mesenchymal stem cells
    - ▶ Most used cell type in cell therapy trials
      - 400 registered clinical trials at clinicaltrials.gov
      - 3-fold increase in IND submissions to FDA : 2006-2012 (Mendicino et al, 2014. Cell stem cell 14(2):141-145)
    - ▶ MOA: paracrine and not differentiation
      - “Notably, all corporately sponsored trials in cell therapy for heart disease are currently testing cell types hypothesized to have a paracrine mechanism of action.” Proceedings of the California Stem Cell Agency July 22, 2015
      - Extracellular vesicles are the prime paracrine candidates

▶ Timmers (2008). Stem Cell Research 1:129–137.

Chen (2010). Nucleic Acid Research 38 (1): 215–224.

Lai (2010) Journal of Molecular Cardiology 48:1215-1224.

Lai (2010) Stem Cell Research 4: 214-222.

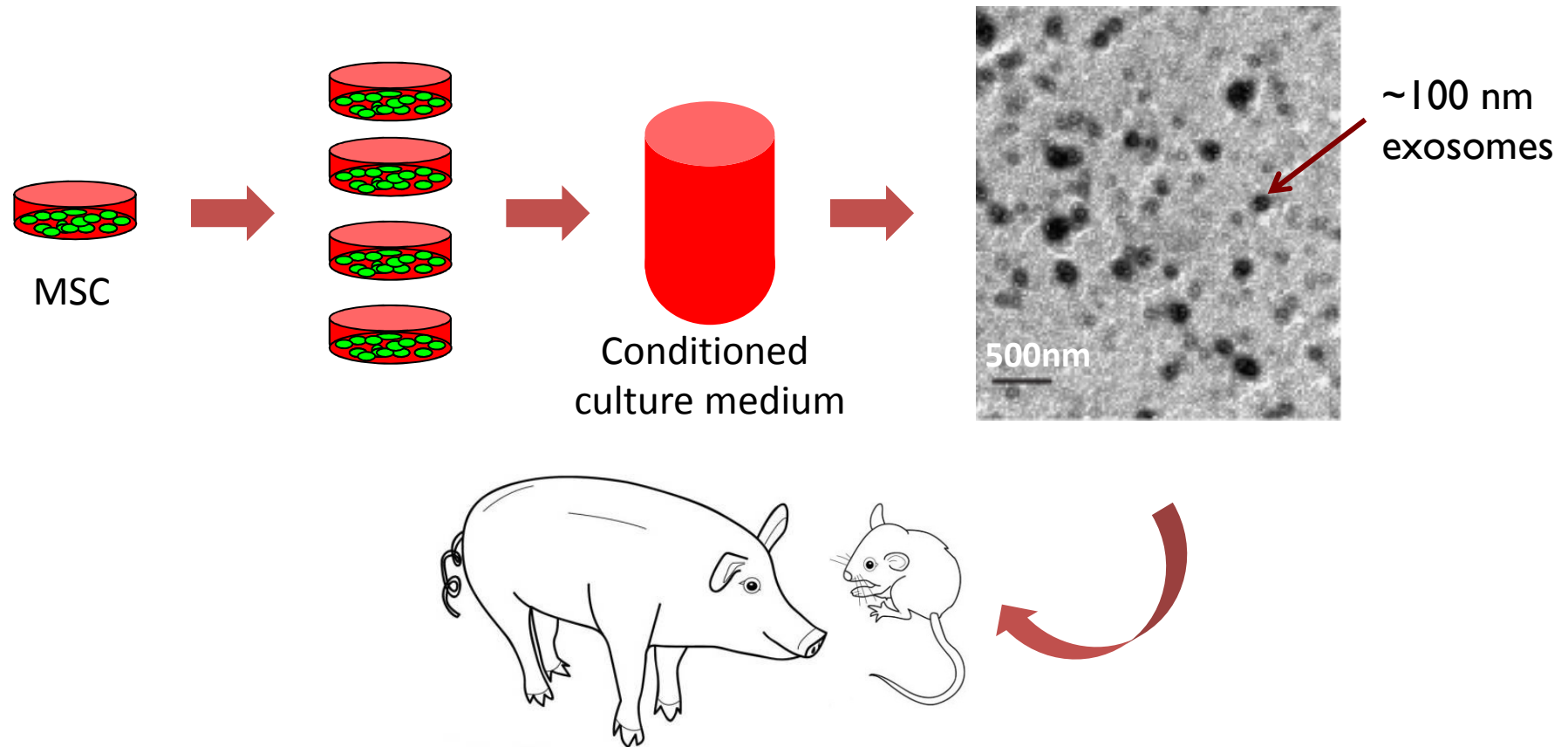
Lai (2012) Int J Proteomics 2012, 971907

Li (2012) J. Proteome Res. 6;11(4):2331-46.22614

Lai (2013) Mesenchymal Stem Cell Therapy pp. 39-62.

Arslan (2013). Stem Cell Research 10, 301-312.

# Exosome as a therapeutic MSC paracrine factor



Reduce infarct size and improve cardiac function in pig and mouse model of AMI

# Efficacy of MSC exosomes in diseases

EV source	Disease studied	Therapeutic effect
BM-MSC (human)	Glycerol-induced acute kidney injury (Mouse)	Reduced tubular lesions and enhanced renal function
ESC-, Fetal-, UC-MSC (human)	Myocardial ischemia/reperfusion injury (Mouse)	Reduced infarct size
UC-MSC (human)	Hypoxia-induced pulmonary hypertension (Mouse)	Reduced inflammation, pulmonary vascular remodeling and enhanced pulmonary function
UC-MSC (human)	CCl <sub>4</sub> -induced liver fibrosis (Mouse)	Inhibited hepatocyte apoptosis, alleviated fibrosis
BM-MSC (rat)	Middle cerebral artery occlusion (Rat)	Promoted neurite outgrowth of neurons and astrocytes
BM-MSC (human)	Graft-versus-host disease (Human)	Reduced diarrhea volume, cutaneous and mucosal GVHD severity
ESC-MSC (human)	CCl <sub>4</sub> -induced liver injury (Mouse)	Reduced hepatic necrosis
UC-MSC (human)	Skin deep second degree burn wound (Rat)	Promoted skin cell proliferation and re-epithelialization

# Hypothesis for the MOA of MSC exosomes

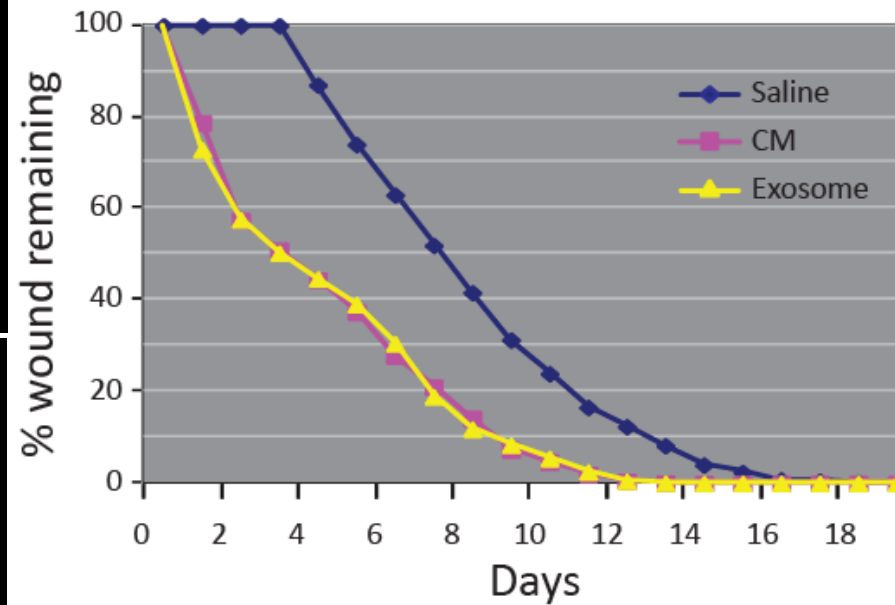
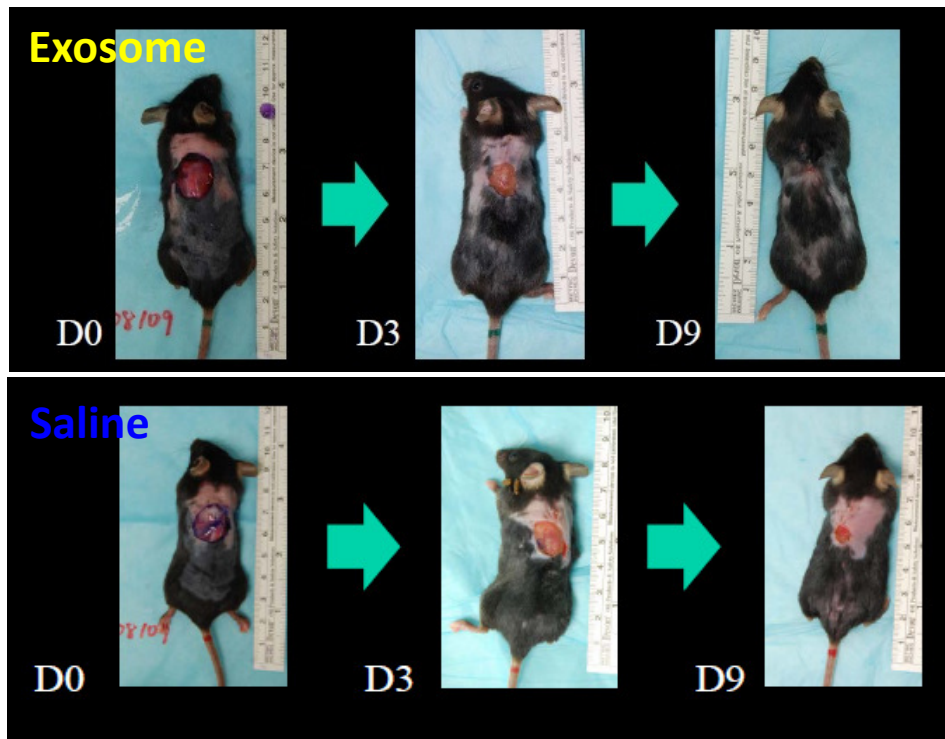
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- ▶ Extensive biochemical and biophysical characterization
  - ▶ >15 publications in the last 5 years
- ▶ Current working hypothesis
  - ▶ MSC exosomes mediate the stromal support function of MSC in maintaining the integrity and homeostasis of tissue stromal for optimal cell and tissue biochemistry and function
    - ▶ *Rationale and evidence for hypothesis:*
      - *Lai et al., (2015). Seminars in Cell & Developmental Biology 40, 82-88.*
      - *Lai et al., (2013). Regenerative Medicine 8 (2), 197-209*
      - *Yeo et al., (2013). Exosomes Microvesicles, 1:7. doi: 10.5772/57460.*
      - *Zhang et al., (2014). Frontiers in Immunology, 5, 518. doi:10.3389/fimmu.2014.00518*
      - *Tan et al., (2015). Chapter 2 in "Mesenchymal Stem Cell Derived Exosomes.*
      - *Yeo and Lim Book Chapter in "Advances in cell therapy" (WorldScientific) in press*



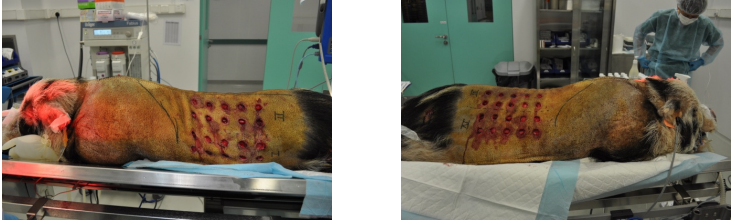
# Potential therapeutic applications for skin (1)

## Mouse

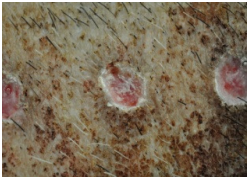


# Potential therapeutic applications for skin (2)

Pig



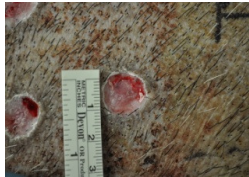
saline



exosome



exosome



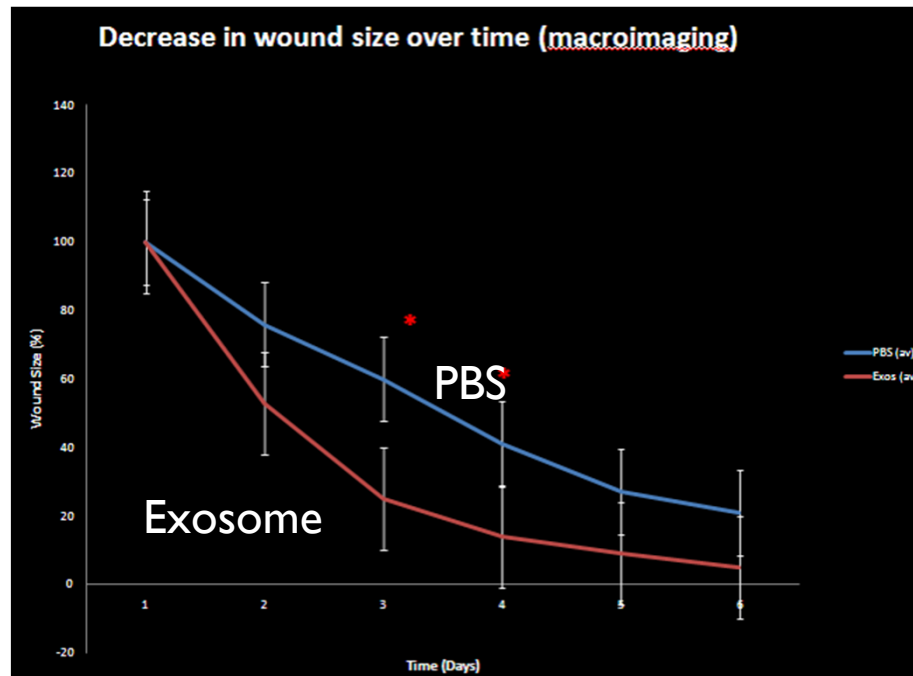
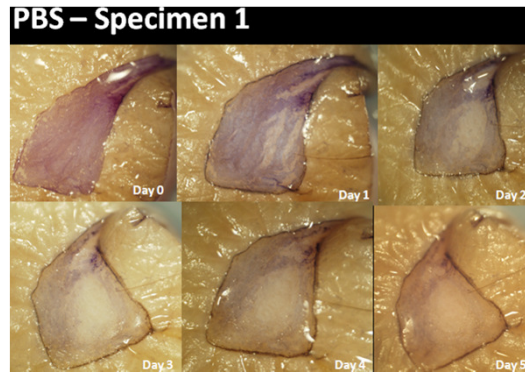
saline

Exosome enhances wound closure over saline,  $p= 2.4E-06$



# Potential therapeutic applications for skin (3)

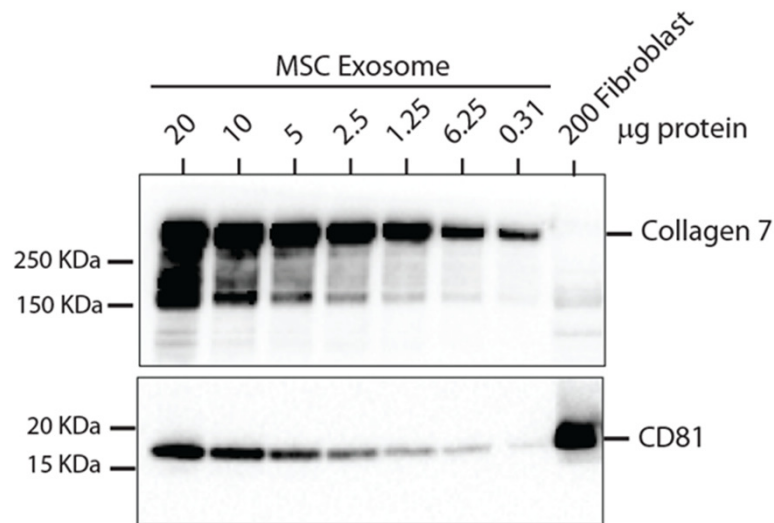
Human  
(Birgit Lane)



# Rationale for use of MSC exosomes in skin wound

## ► MSC exosomes

- Rich in Extracellular matrix (ECM) proteins, Cell Adhesion Molecules (CAMs), ECM enzymes



ECM proteins				CAMs		ECM enzymes
AGRN	FBN1	KRT15	NID1	ALCAM	ITGAV	ADAM9
ARMS	FBN2	KRT16	VCAN	CD44	ITGB1	ADAM10
COL1A1	FGA	KRT17	SDC1	CDH13	ITGB4	ADAMTS12
COL1A2	FGB	KRT18	SDC2	CEACAM8	ITGB5	ENTPD4
COL2A1	FGG	KRT19	SDC4	CLSTN1		MMP1
COL3A1	FN1	KRT27	SPARC	CNTN1		MMP1
COL4A2	KRT1	KRT28	TGFBI	CTNNA1		MMP10
COL4A3	KRT2	KRT72	THBS1	CTNNB1		MMP2
COL5A1	KRT3	KRT73	THBS2	CTNND1		MMP2
COL6A1	KRT4	KRT74	TNC	FAT		MMP3
COL6A2	KRT5	KRT76	VTN	FAT2		PCOLCE
COL7A1	KRT6A	KRT77		FAT4		PLAU
COL12A1	KRT6B	KRT78		ICAM1		PLOD1
COL14A1	KRT6C	KRT79		ICAM5		PLOD2
COL18A1	KRT7	KRT80		ITGA11		PLOD3
ECM1	KRT8	KRT84		ITGA2		SERPINE1
EFEMP2	KRT9	LAMA3		ITGA3		TIMP1
FBLN1	KRT10	LAMA4		ITGA4		TIMP2
FLG2	KRT13	LAMB1		ITGA5		TIMP3
FBLN1	KRT14	LAMC1		ITGAL		

# MSC exosomes for RDEB?

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## Potential of Systemic Allogeneic Mesenchymal Stromal Cell Therapy for Children with Recessive Dystrophic Epidermolysis Bullosa

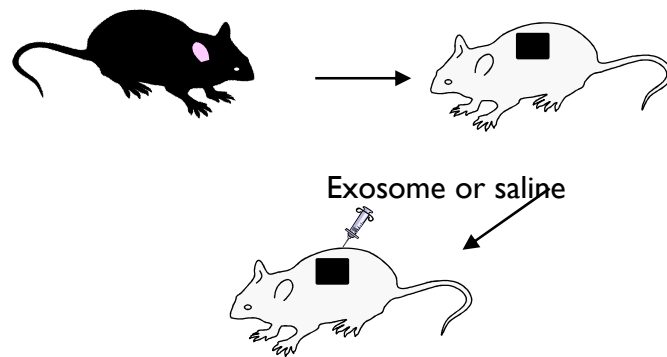
*Journal of Investigative Dermatology* (2015) 135, 2319–2321; doi:10.1038/jid.2015.158; published online 14 May 2015

- ▶ Improved healing, reduced wounding (transient, ~6 months)
- ▶ Skin biopsies
  - No increase in C7
  - MOA
    - Immunomodulatory activity of MSC

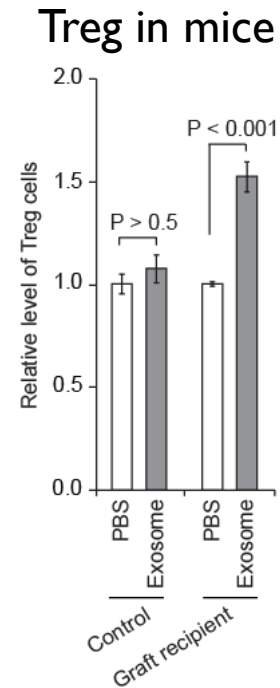


# Immunomodulatory activity of MSC

- ▶ MSC approved for GVHD
- ▶ Paracrine mechanism
  - ▶ MSC exosomes
    - ▶ modulate immune system in GVHD patients (Giebel's lab)



B Zhang, Y Yin, RC Lai, SS Tan, ABH Choo, SK Lim (2014). Mesenchymal stem cell secretes immunologically active exosomes- Stem Cells and Development 23(11):1233-44  
Zhang, B., Yin, Y., Lai, R. C., & Lim, S. K. (2014). Immunotherapeutic Potential of Extracellular Vesicles. Frontiers in Immunology, 5, 518. doi:10.3389/fimmu.2014.00518



## Future Work

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1. Clinical trial: MSC exosomes for wound healing? RDEB?
2. Elucidation of MOA:
  - a. Proteomic complementation
  - b. Structural ECM support
  - c. Immune modulatory



# Research Focus of Lab

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## ▶ Nano Lipid membrane vesicles

### ▶ Natural

- Extracellular Vesicles: microvesicles, exosomes, apoptotic bodies
  - Mesenchymal stem cell exosomes

### ▶ Synthetic

- ▶ Liposomes
  - Egg lecithin
  - Efficient, high through-put and automated closed production system



# Exosome-like liposomes

Lipid (class) in liposome
Phosphatidylcholine
Lyso-phosphatidylcholine
Phosphatidylethanolamine
Lyso-phosphatidylethanolamine
Ceramides
Glucosylceramide
Sphingomyelin
Cholesterol
Triglyceride
Diglyceride
Monoglyceride
Free fatty acids

## ▶ Features

### ▶ Physiological

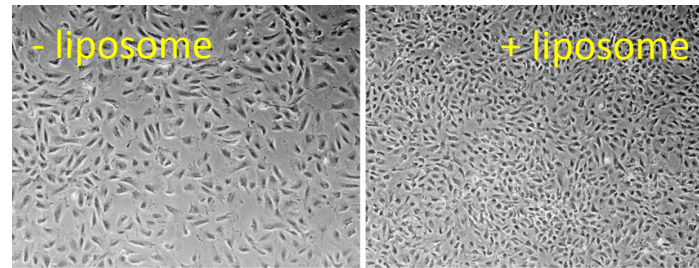
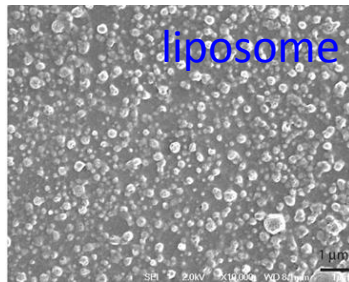
#### ▶ Lipid composition of a mammalian cell/exosome membrane

- Appropriate ratio of lipid classes
- Undetectable level of PIs**
- High level of sphingomyelin**

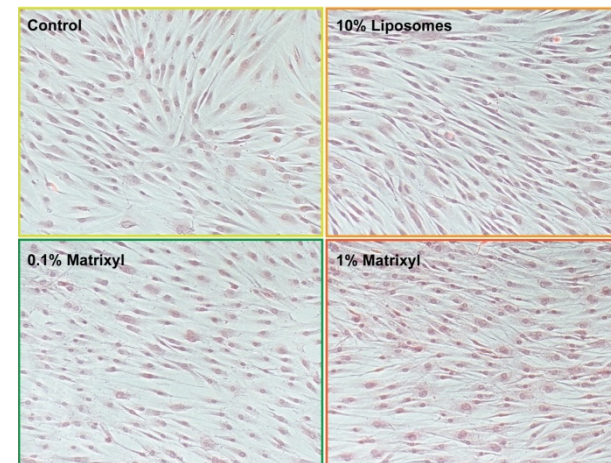
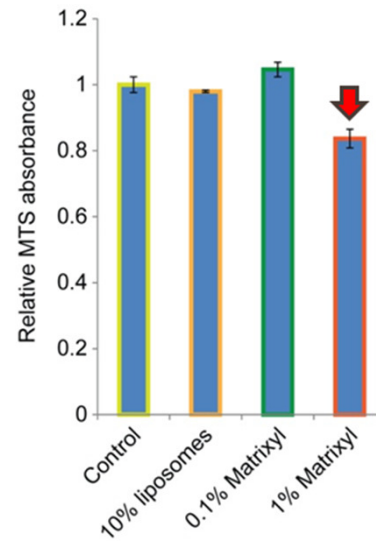
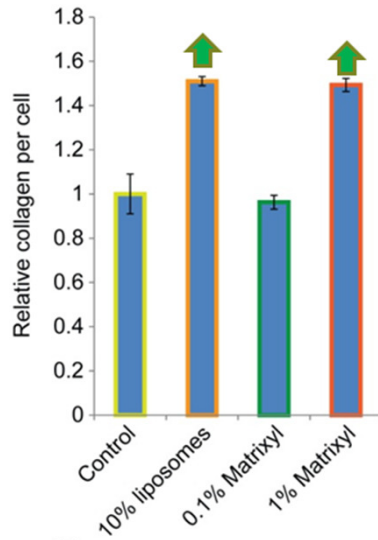
### ▶ Safe for cosmetic applications

- Cosmetic Ingredient Review Expert Panel, International Journal of Toxicology, 20 (suppl 1) 21-45 (2001)

# Exosome-like liposomes – nourishing property

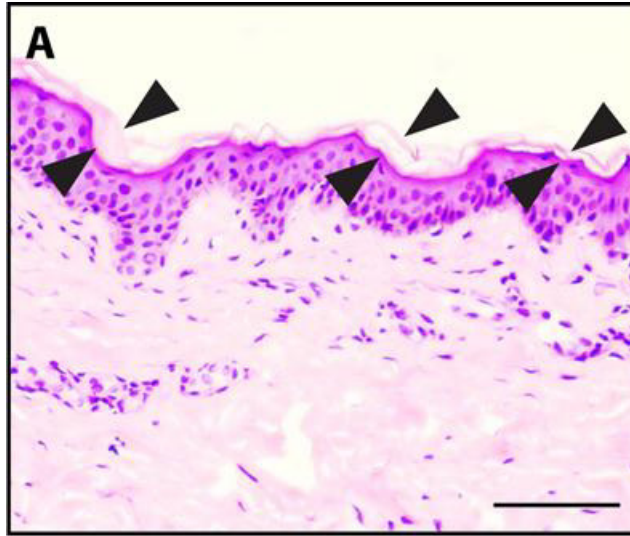


MSCs cultured in serum-free medium

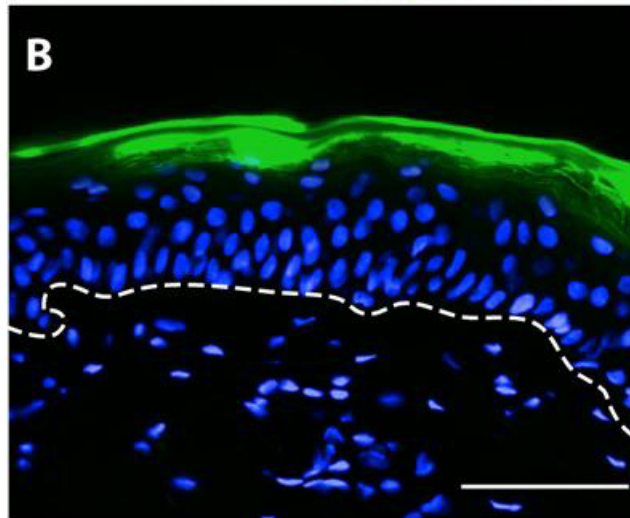




# Application of liposomes on human skin



← Intact Stratum Corneum

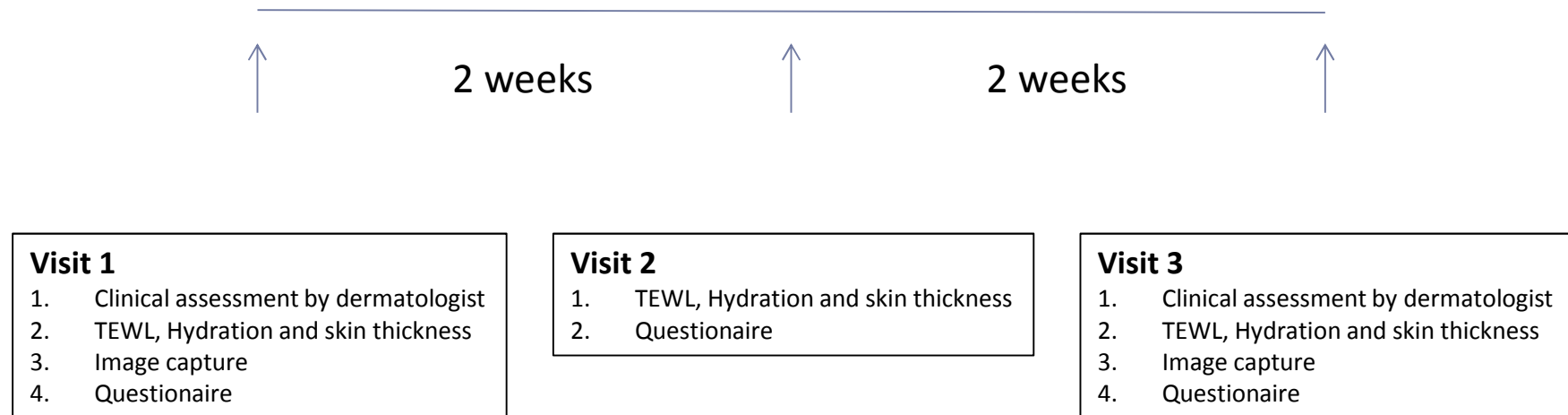


← Fluorescence-labelled liposome

# Clinical Study: liposomes to reduce skin dryness of menopausal women (Mark Koh, KKH)

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## ▶ Study design



### Protocol

1. Test leg (randomly assigned by clinical co-ordinator)
  - a. Lotion twice a day
  - b. No lotion at least one hour before V2 and V3
2. Control leg
  - a. As per participant's normal routine

### Preliminary results:

Significant increase in skin hydration,  
2/3 patients have >0.1 mm increase in dermis thickness

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# The Relationship Between Wrinkle Depth and Dermal Thickness in the Forehead and Lateral Canthal Region

*Kazue Tsukahara, PhD; Yuichi Tamatsu, DDS, PhD;  
Yasushi Sugawara, MD, PhD; Kazuyuki Shimada, DDS, PhD*

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ARCH DERMATOL/VOL 147 (NO. 7), JULY 2011    WWW.ARCHDERMATOL.COM

A wrinkle of between 0.25 and 0.62 mm deep is associated with a 0.2 mm decrease in dermal thickness.



## Future Work

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- ▶ Test efficacy in alleviating skin dryness and disease severity for skin diseases e.g. eczema
- ▶ Develop liposomes as drug delivery vehicles for topical applications of fat soluble drugs



# Contributors

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Zhang Bin

Lai Ruenn Chai

Tan Soon Sim

Ronne Yeo

Yin Yijun

Tan Kok Hian  
(KKH)

(Eugene Sim)

## Our collaborators



Andre Choo



Newman Sze



Dominique de Kleijn



Kok Hian Tan  
Mark Koh



Chuen Neng Lee  
Mark Richards

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