

# Cell and Tissue Therapies: Current Trend and Challenges



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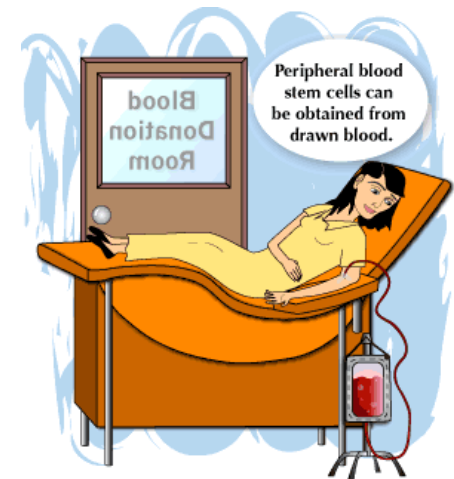
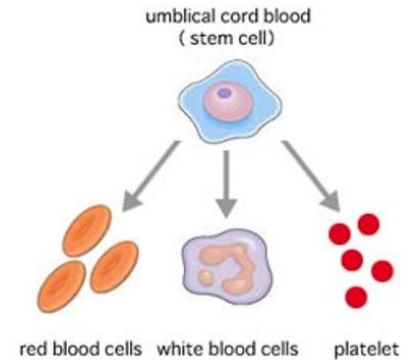
# What is Cell-based therapy?

-the process of introducing new cells into a tissue in order to treat a disease

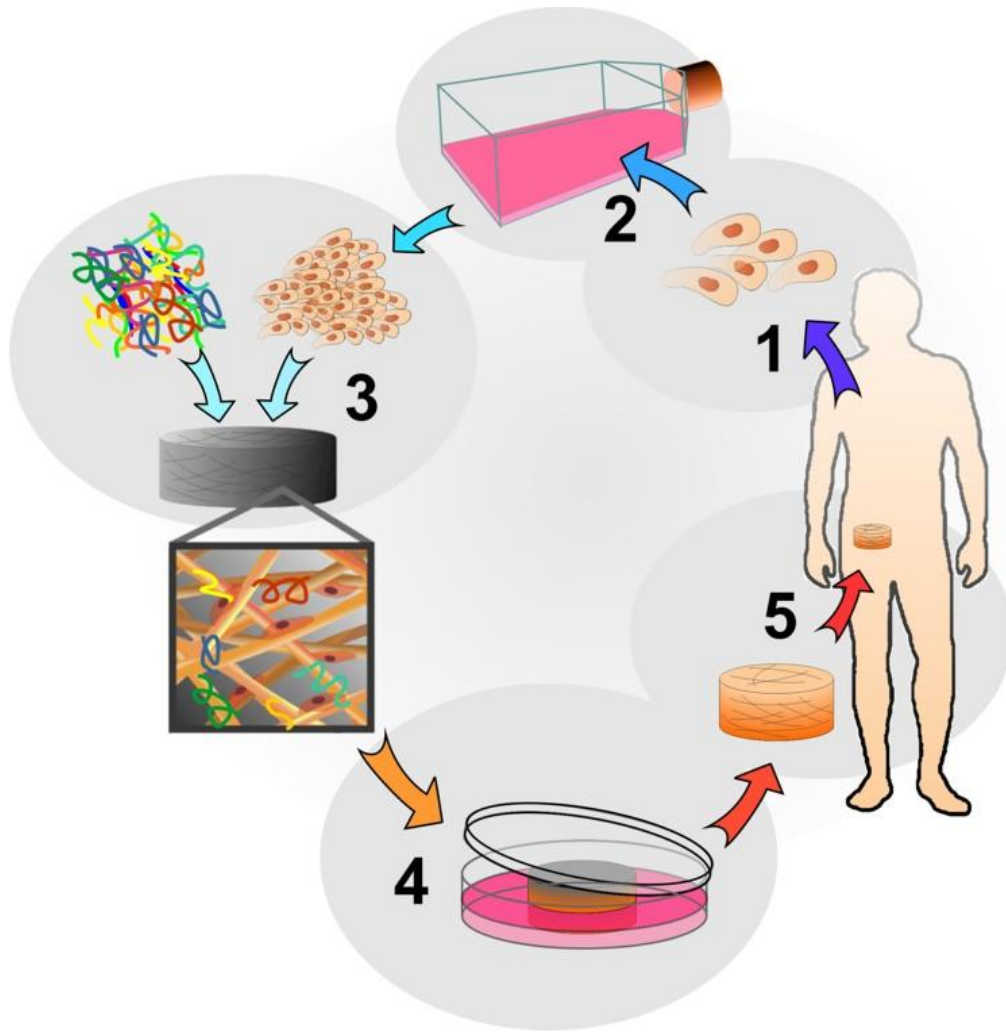
-uses stem cells

- Autologous (implanted cells comes from the same individual)
- Allograft (different individual)
- Xenograft (animal origin)

- ESC, Fetal origin: CB, WJ, Adult: BM, AD, PB



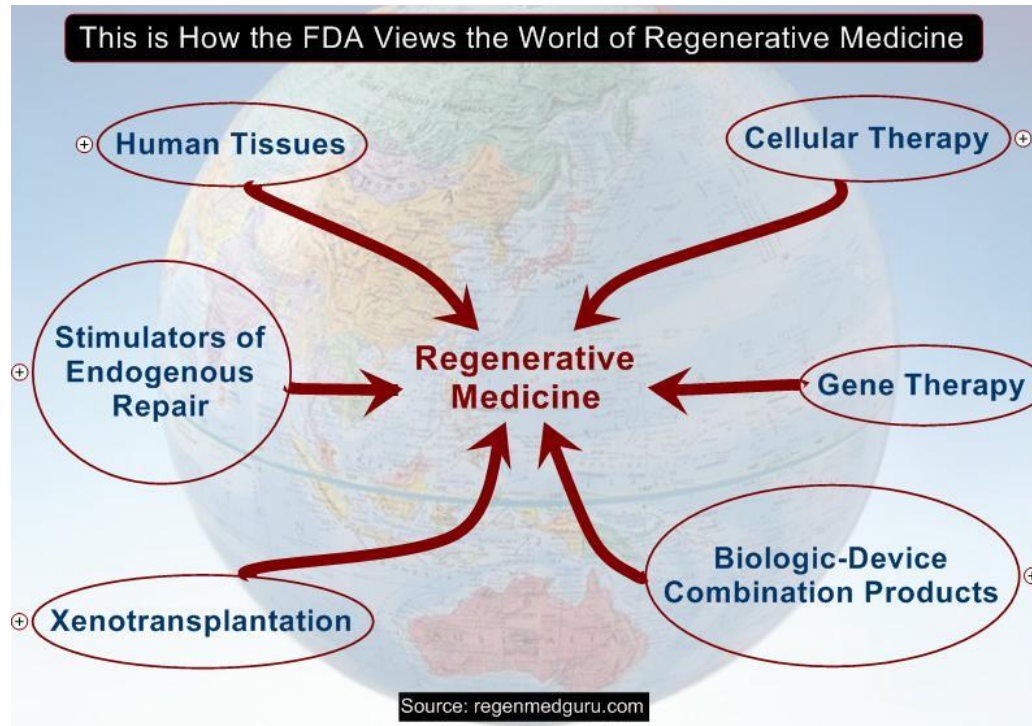
# What is Tissue engineering?

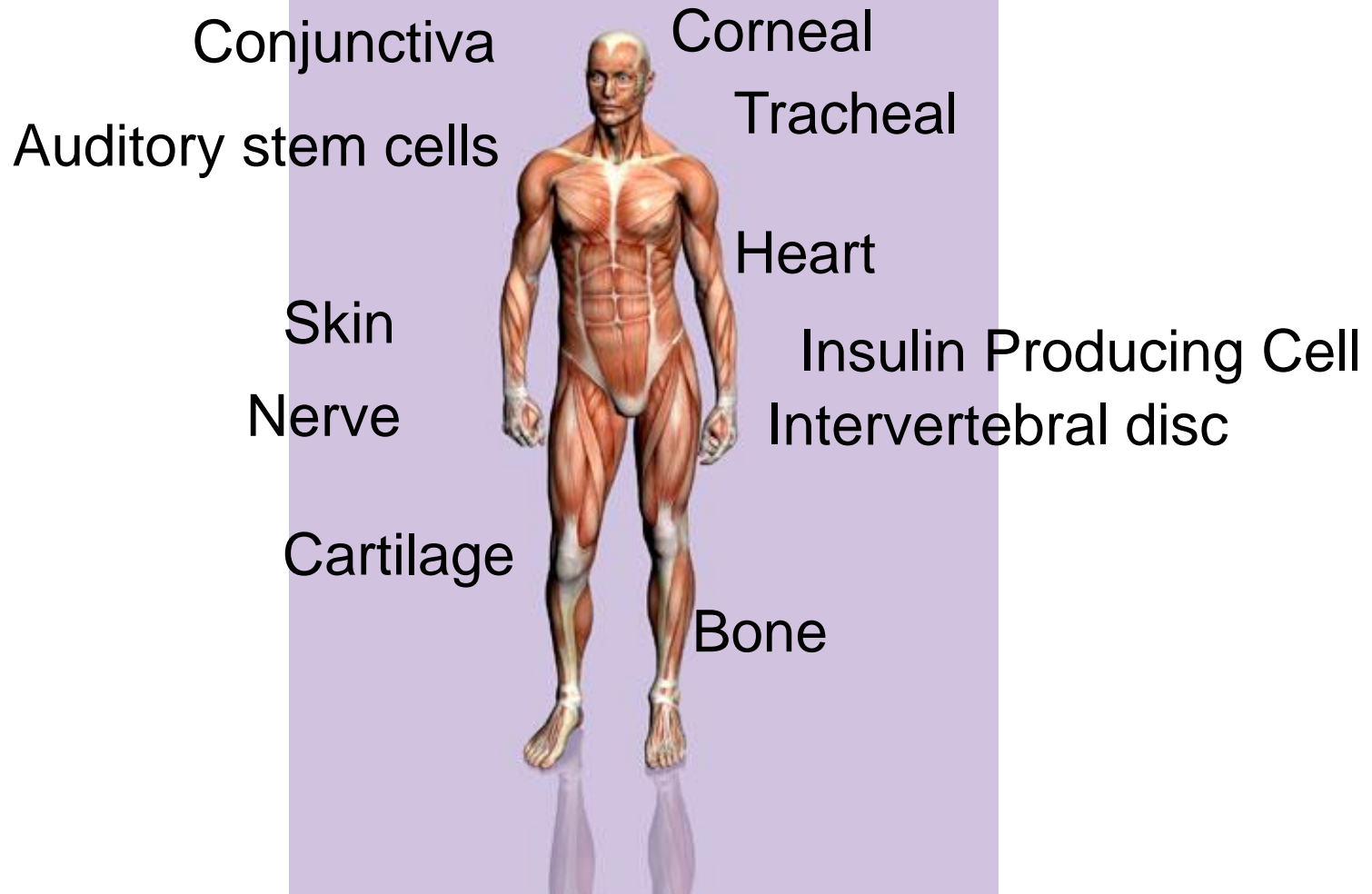


-associated with applications that repair or replace portions of or whole tissues (i.e., bone, cartilage, blood vessels, bladder, etc...)

# regenerative medicine

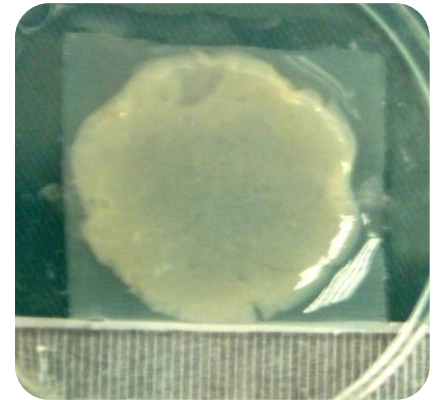
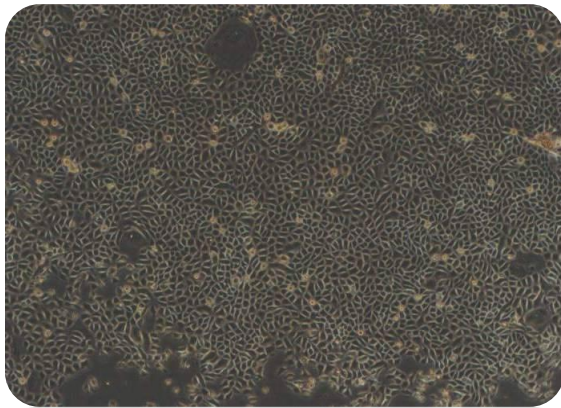
The term **regenerative medicine** is often used synonymously with tissue engineering, although those involved in regenerative medicine place more emphasis on the use of **stem cells** to produce tissues.

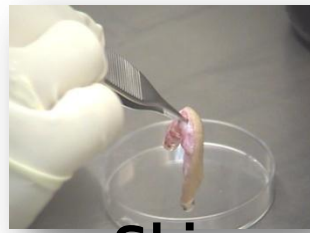






# SKIN TISSUE ENGINEERING





**Skin**

Digested with  
Dispase

**Epidermis**

**Dermis**

Trypsinized

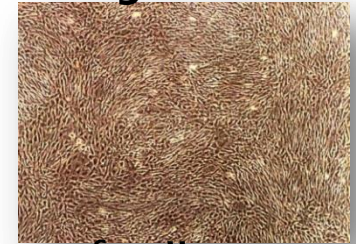
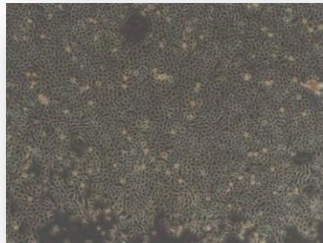
Cultured

Cultured

Digested with  
Collagenase

**Keratinocytes**

**Fibroblasts**



Keratinocytes ( $1-5 \times 10^6$   
cells / ml) + human fibrin  
matrix

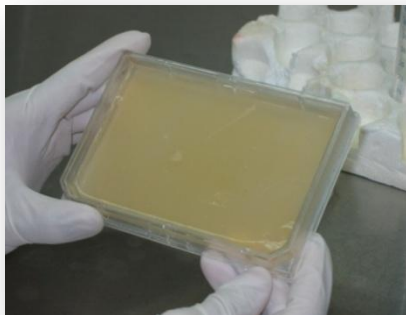
Dermal fibroblasts ( $1-5 \times 10^6$   
cells / ml) + human fibrin matrix

Poured on top

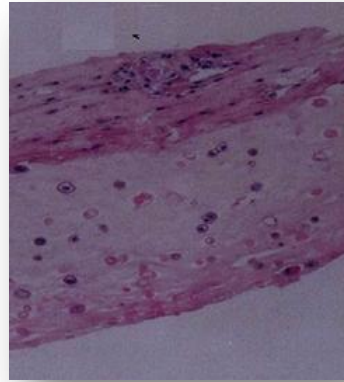
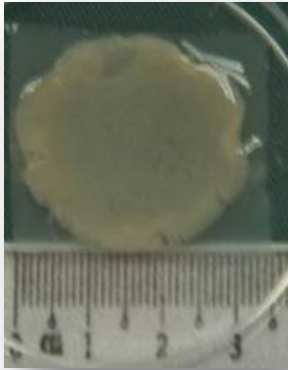
Poured into a plate.  
Add  $\text{CaCl}_2$   
to polymerize



Fibroblasts +  
fibrin matrix  
Keratinocytes  
+ fibrin matrix  
Silk



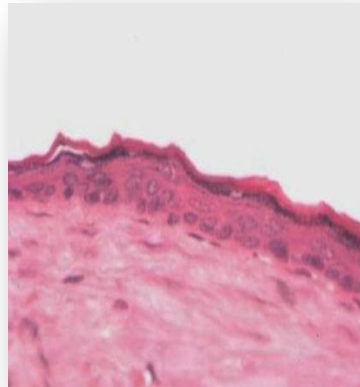
invitro



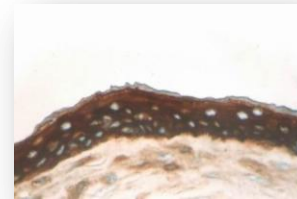
H&E

Mazlyzam AL, Aminuddin BS, Fuzina NH, Norhayati MM, Fauziah O, Isa MR, Saim L and **Ruszymah BHI**. Reconstruction of living bilayer human skin equivalent utilizing human fibrin as a scaffold. *Burns* **33**: 355-363. 2007.

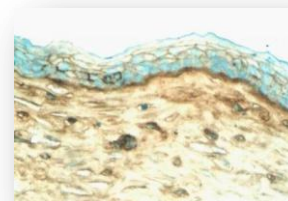
invivo



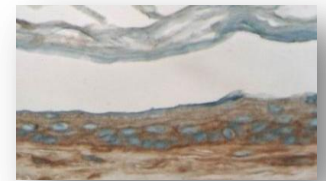
H&E



cytokeratin

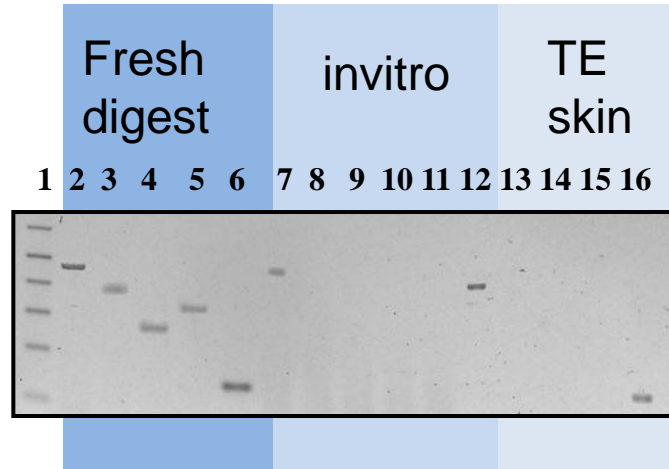
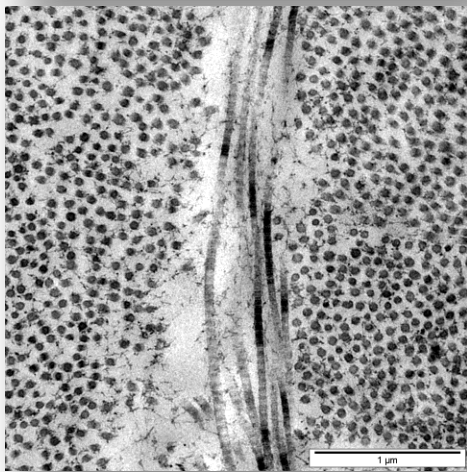
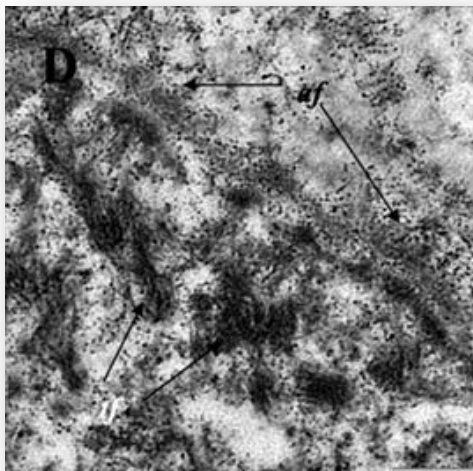


collagen type I



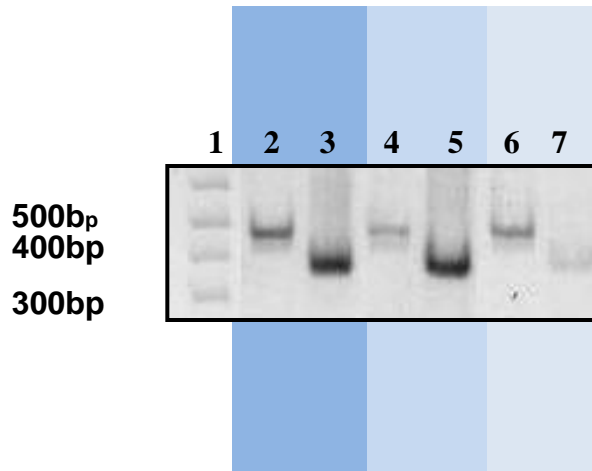
ivolucrin





500bp  
400bp  
300bp  
200bp  
100bp

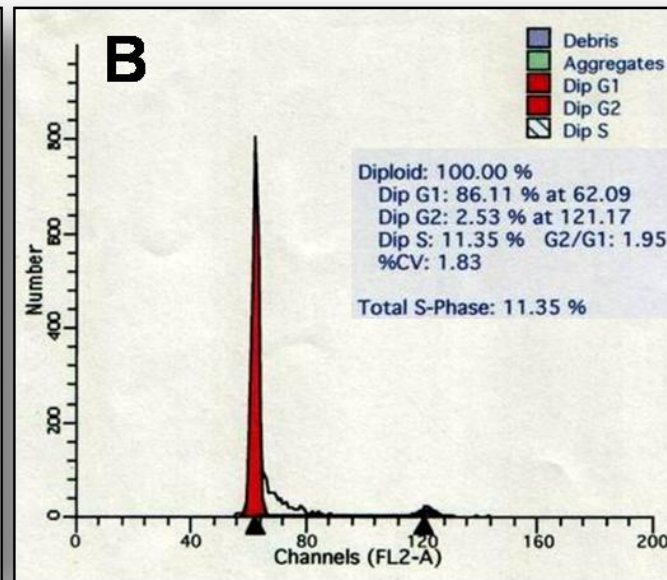
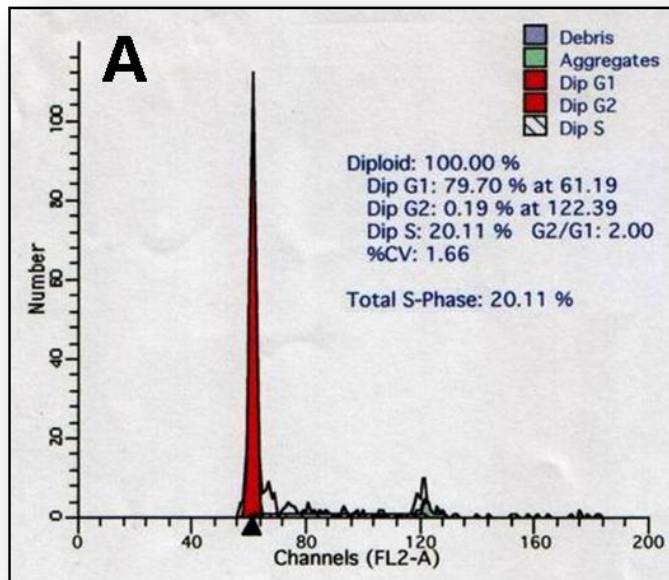
**Lanes:**  
 1 – 100bp DNA ladder  
 2,7,12 –  $\beta$ -actin  
 3,8,13 – keratin I  
 4,9,14 – keratin 5  
 5,10,15 – keratin 10  
 6,11,16 – keratin 14



500bp  
400bp  
300bp

**Lanes:**  
 1 – 100bp DNA ladder  
 2,4,6 –  $\beta$ -actin  
 3,5,7 – collagen type I

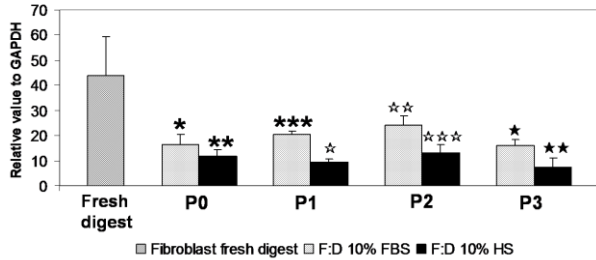
Monzai MN, Mazlyzam AL, Sharida F, Asmah R, Aminuddin BS, Ruszymah BHI and Fauziah O. 2005. Morphological changes of cytoskeletal proteins in monolayer cells of tissue engineered skin. Malaysia Journal of Microscopy. 1: 90-93.



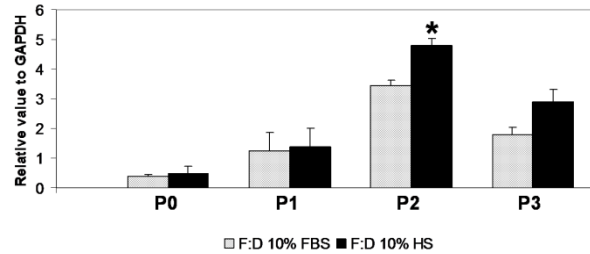
	G0/G1	S	G2+M
F:D 10% FBS(n=6)	92.34 ± 1.36	5.71 ± 1.18	1.96 ± 0.3
F:D 10% HS (n=6)	82.96 ± 3.66 *	17.12 ± 3.77 *	3.49 ± 0.34

Human dermal fibroblasts cultured in HS demonstrated higher expanding capability and still maintained normal cell cycle.

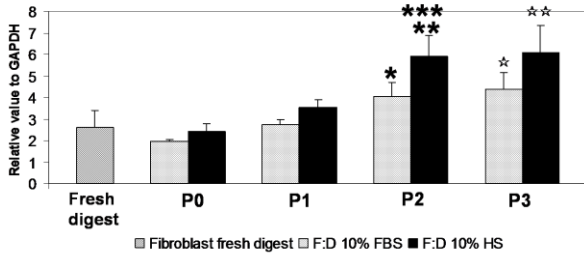
Collagen type I gene expression



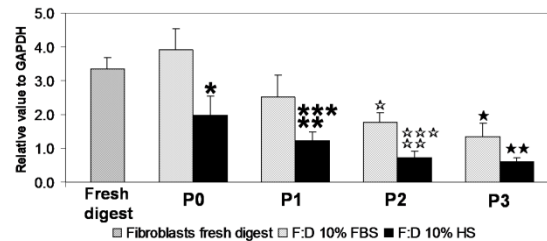
Collagen type III gene expression



Fibronectin gene expression



Alpha smooth muscle actin gene expression

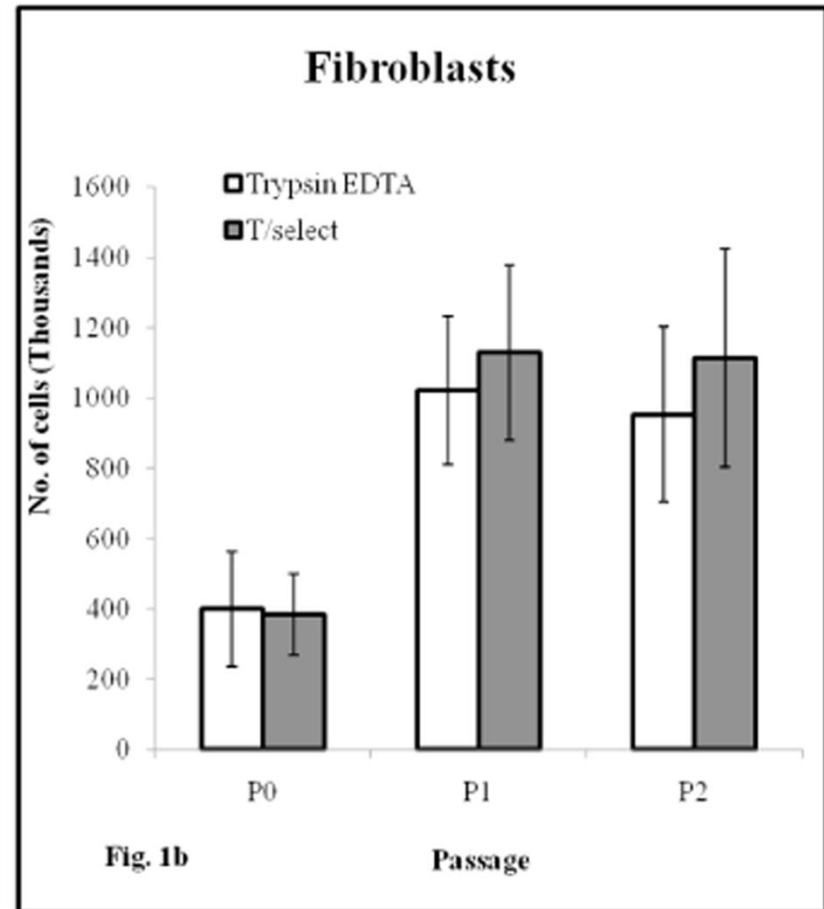
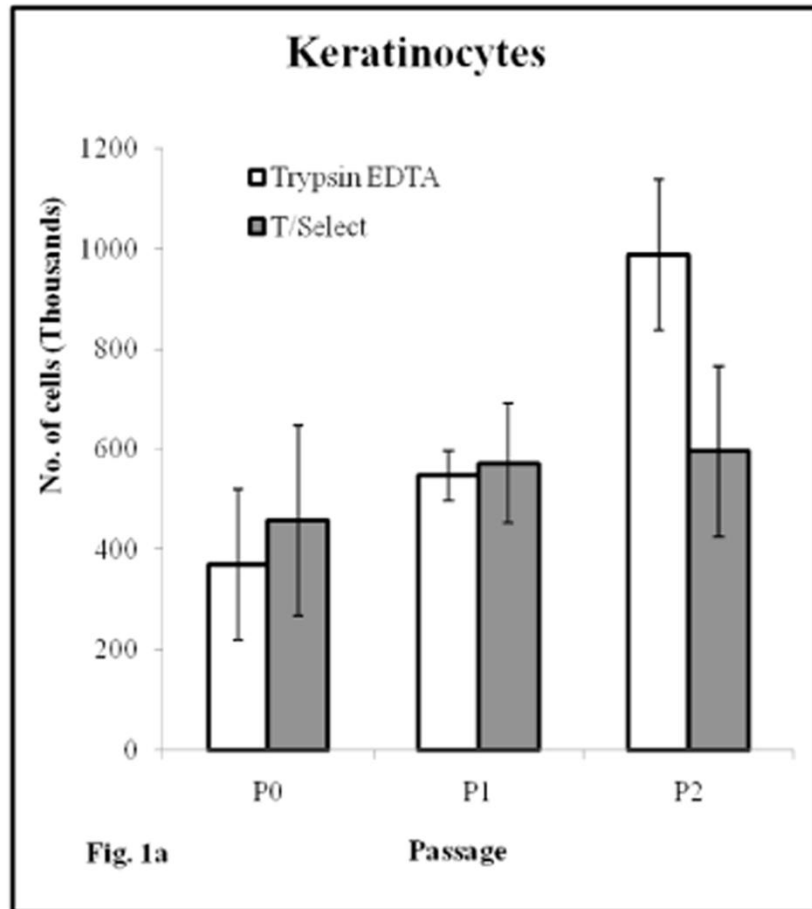


Mazlyzam AL, Aminuddin BS, Saim L, Ruszymah BH. Human serum is an advantageous supplement for human dermal fibroblast expansion: clinical implications for tissue engineering of skin.. Arch Med Res. 2008 Nov;39(8):743-52.

The cells expressed higher level of Collagen type III and Fibronectin which are important in wound healing. The expression of  $\alpha$ -Smooth muscle actin is lower indicating less wound contraction which can result in excessive scarring.

Thus, HS is a better supplement compare to FBS. This is a very important finding for the future of autologous tissue engineered skin.

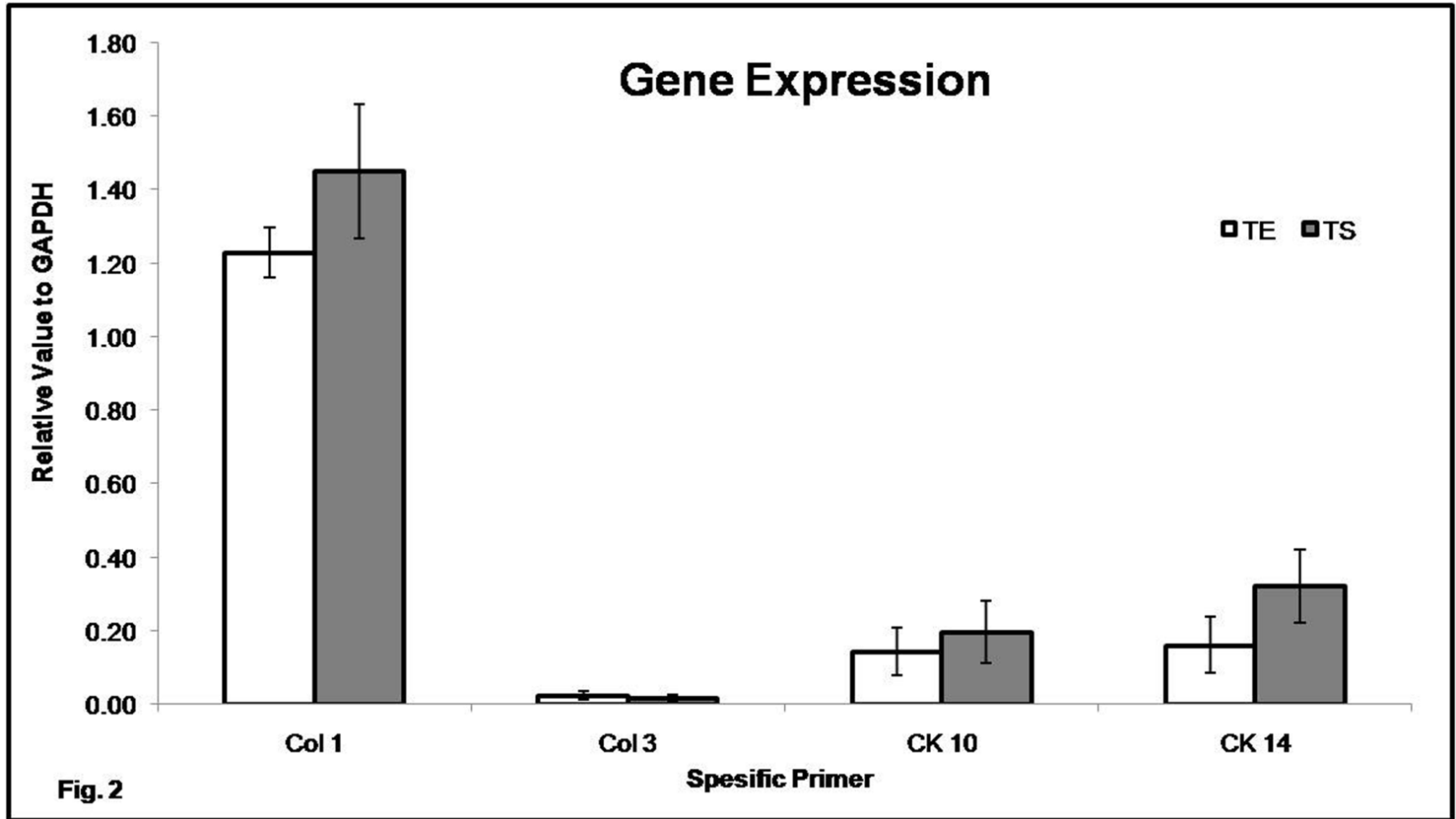
- ✓ Enzyme used to dissociate cells & detach cells from culture vessels
  
- ✓ **Trypsin EDTA (1x):**
- ✓ Originated from porcine
- ✓ 0.05% Trypsin, 0.53 mM EDTA (liquid) in HBSS without sodium bicarbonate, calcium and magnesium
- ✓ Mediatech Cellgro, USA
  
- ✓ **Recombinant Trypsin – Tryple Select (1x) :**
- ✓ Derived from microbial fermentation
- ✓ Formulated in DPBS with 1mM EDTA.
- ✓ GIBCO, USA



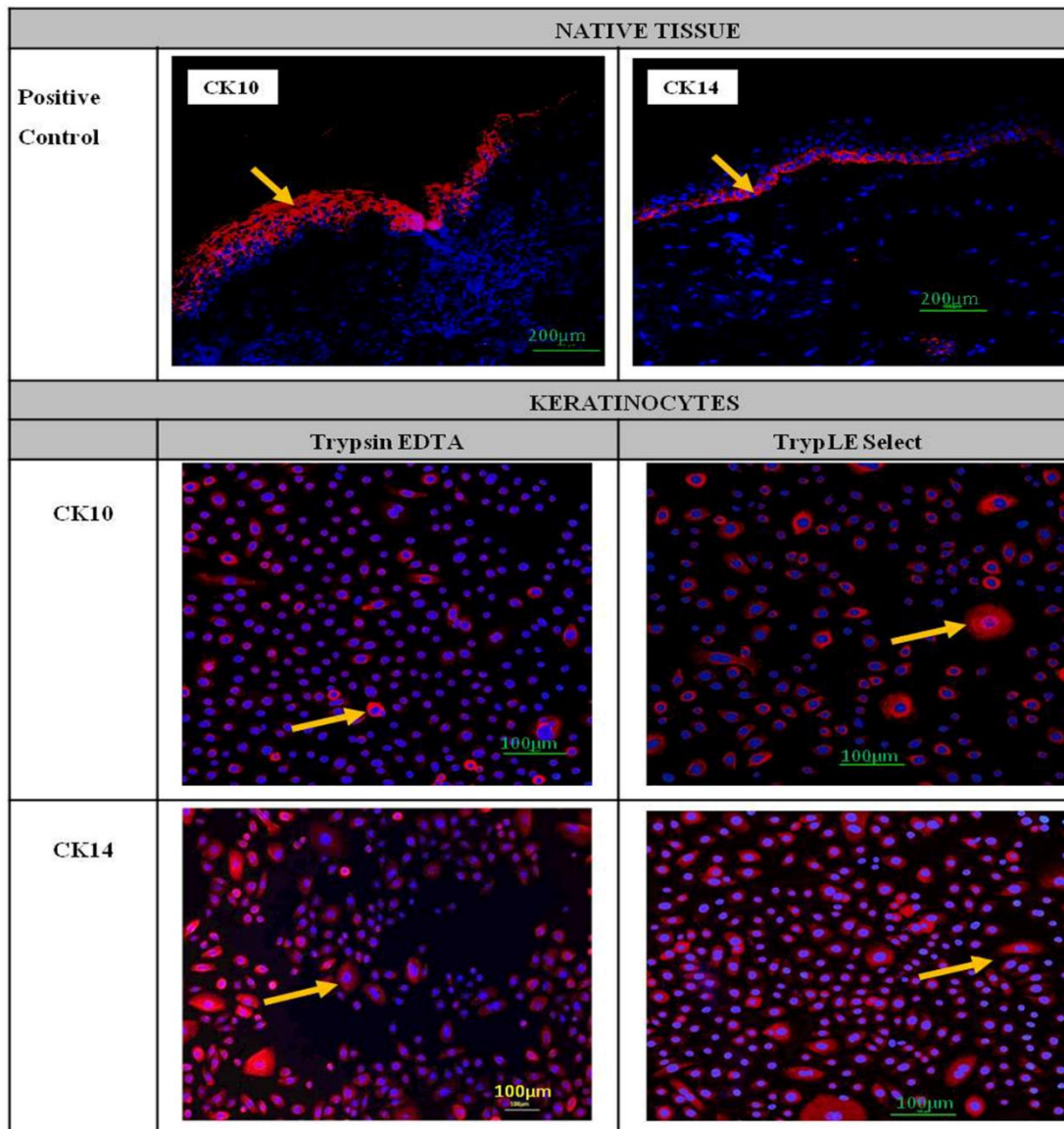
**Keratinocytes:** No significant difference at P0 and P1 (P0:  $p=0.546$ ; P1:  $p=0.951$ ) for TE and TS. Total cell in TE group was significantly higher compared to TS at P2 ( $p=0.008$ ).

**Fibroblasts:** No significant differences between both groups (P0:  $p=0.762$ ; P1:  $p=0.217$ ; P2:  $p=0.148$ ).



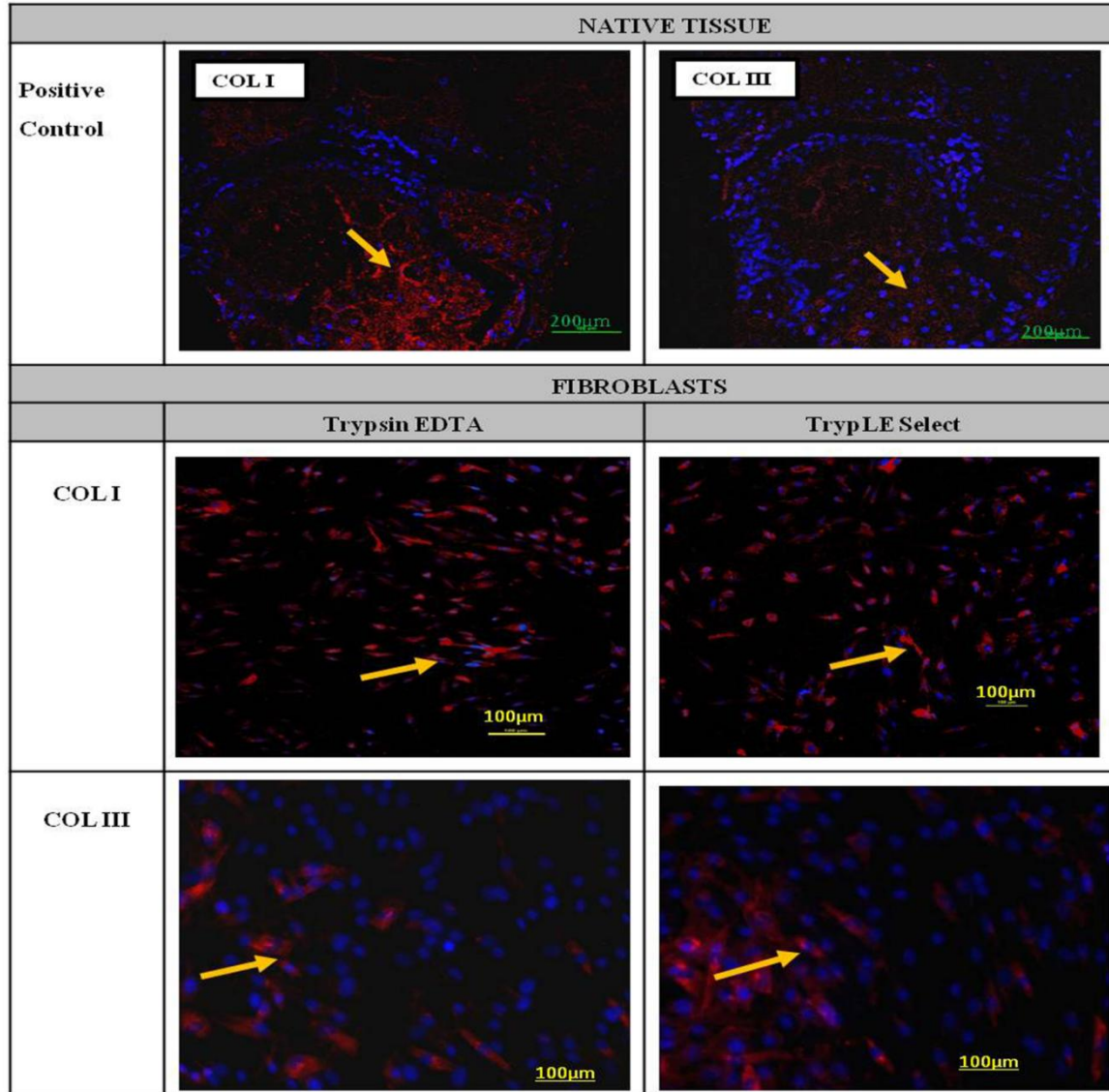


No significant difference between TS and TE groups for all specific genes



Both groups positively expressed CK10 & CK14 antibody

Fig. 3a



Fibroblasts from both groups positively expressed COL I & COL III antibody

Fig. 3b

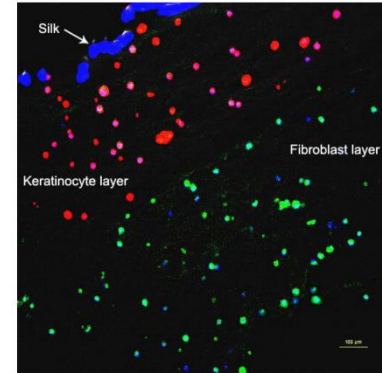
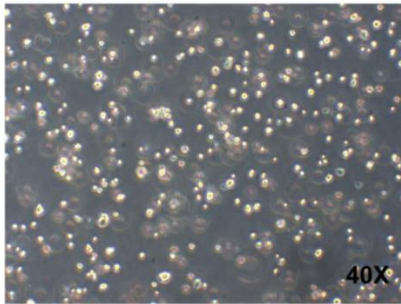
- The performance of recombinant trypsin (TS) is comparable with the well-established animal-derived trypsin (TE)
- The recombinant trypsin support similar cell proliferation, and produce similar results in total cell yield, functional gene and protein expression levels for trypsinization of cultured keratinocytes and fibroblasts
- Recombinant trypsin (TS) can be used for human skin cells culture for clinical applications

Cell Tissue Banking  
DOI 10.1007/s10561-013-9368-y  
Springer 2013

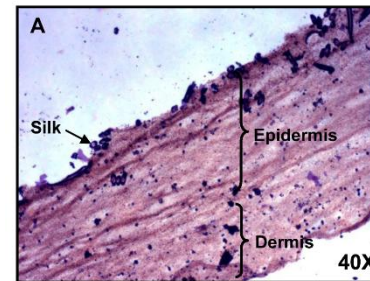
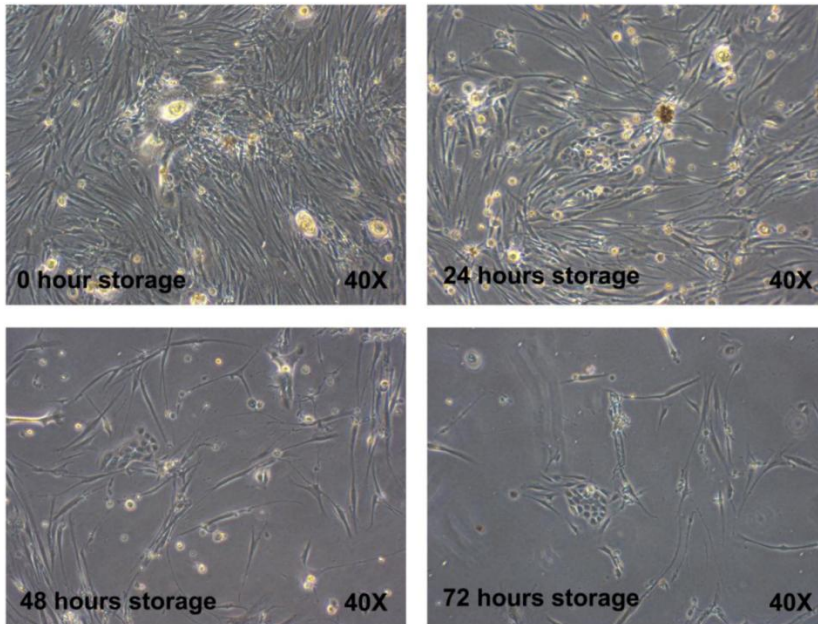


# Shelf life evaluation

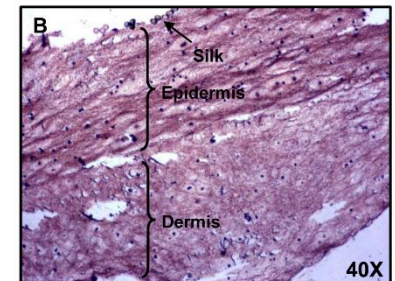
Morphology of keratinocytes and fibroblasts immediately after liberation from MyDerm™



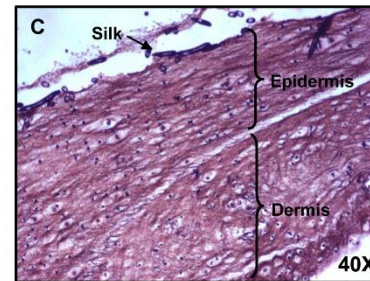
Morphology of fibroblasts and keratinocytes after liberation from MyDerm™ and cultivation for 144h in monolayer culture



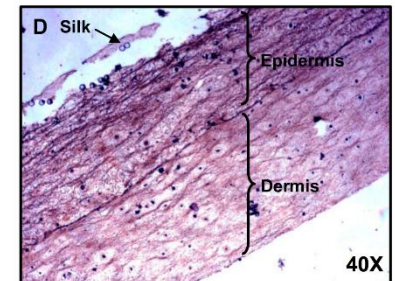
0 hour storage



24 hours storage



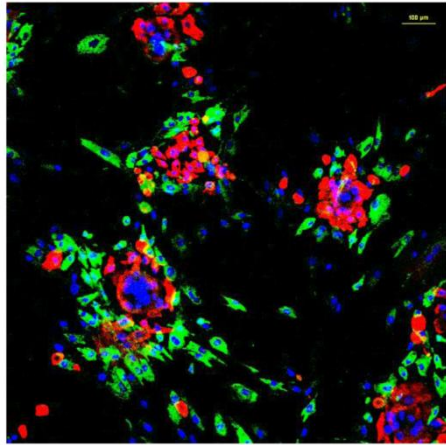
48 hours storage



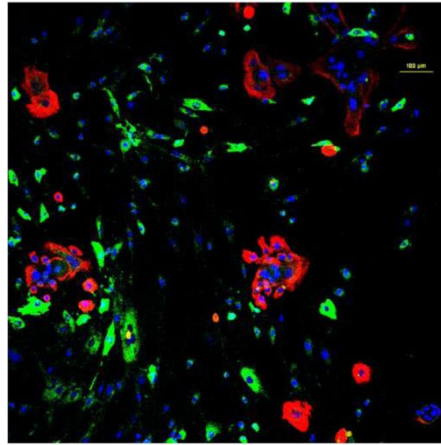
72 hours storage



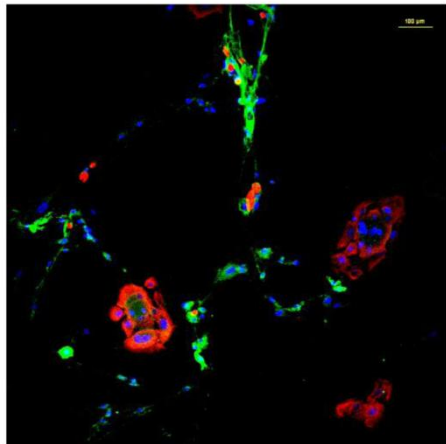
# Shelf life evaluation



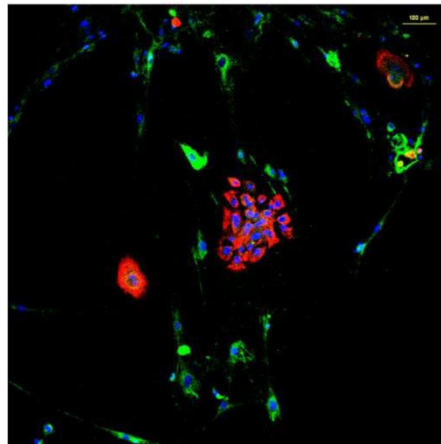
0 hour storage



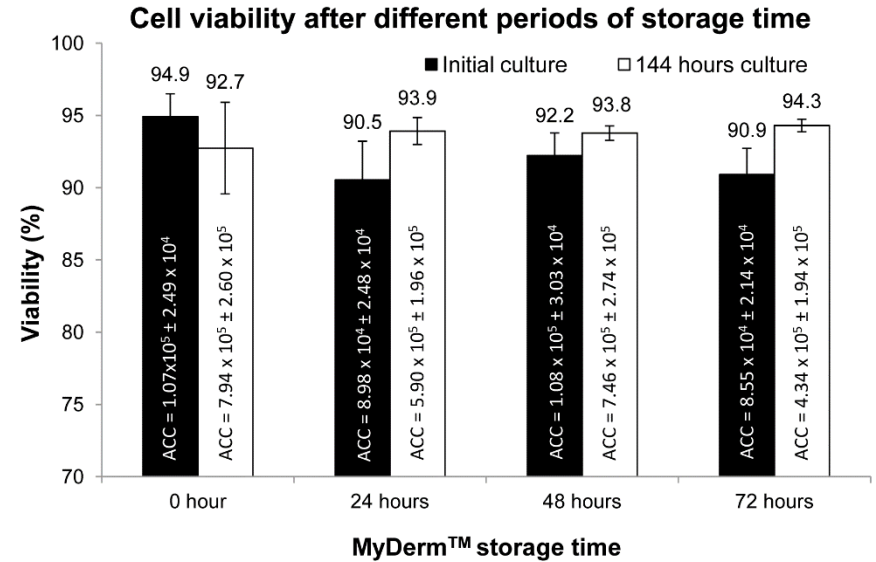
24 hours storage



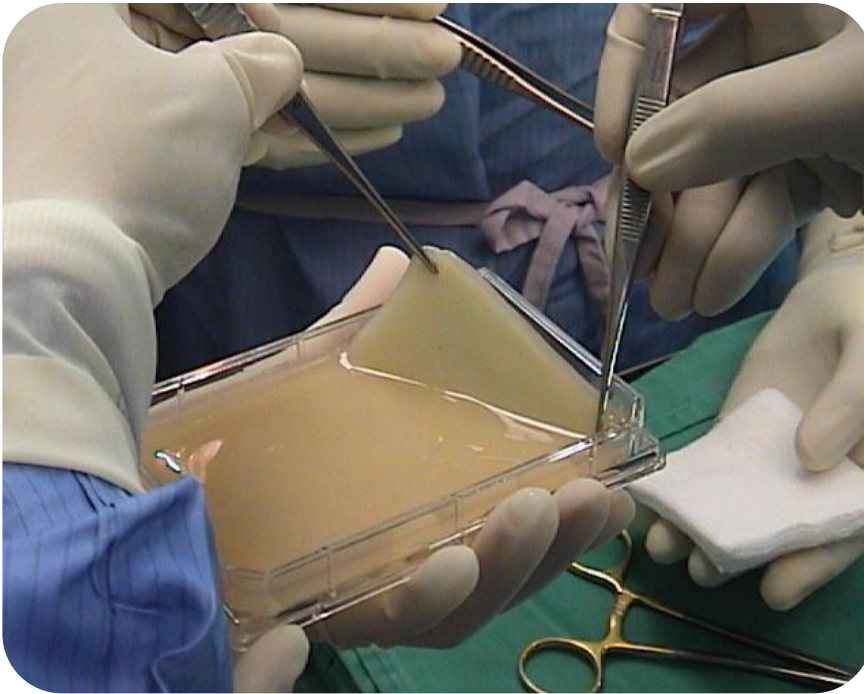
48 hours storage



72 hours storage



PLoS ONE 2012



# KULITKU

## MyDerm™

Malaysian Patent Application No PI20042556- Filing Date : 29 June 2004, granted 2011

***Geneva Gold Medal Award***

32rd International Exhibition of Invention, Geneva, 2004, Geneva, Switzerland.

### **Clinical Translation**

**-Proof of Concept**

**-Clinical Trial**

**TISSUE ENGINEERING CENTER UKMMC**



## Benefits of GMP?

- ✓ Documented standard procedures.
- ✓ Staff trained against standard procedures.
- ✓ Process Development.
- ✓ Process Control.
- ✓ Traceability throughout processes.
- ✓ Consistent product quality.
- ✓ Products manufactured with emphasis on Quality, Safety & Efficacy.

## Location and Site Infrastructure

- Located at the 12<sup>th</sup> Floor of the Clinical Block, UKM Medical Centre (UKMMC)
- Total floor area is approximately 550 m<sup>2</sup> with 3 clean rooms of 25-36 m<sup>2</sup> in sizes and 2 gowning rooms (grade B)
- Dedicated Grade A, B, C and D zone/room
- Graded area is maintained by 3 AHUs, located at 13<sup>th</sup> floor
- Supported by unclassified lab area and general office/utility area
- Access controlled to all area, with CCTVs and intercom
- Monitored with Building Monitoring System (BMS) and Equipment Monitoring System (EMS)

# FACILITY PICTURES



External View from TEC, Common Corridor



Main Entrance from Common Corridor



The visitor viewing panel



Common Corridor with Emergency Exit



# FACILITY



Facility Corridor Leading to Utility, Storage and Cryopreservation Room.



Storage Room (non temperature controlled)



Generator Set of the Facility in Utility Room



Cryo-room

# FACILITY



Entry to the Unclassified Area



Receiving Counter / Pre Quarantine Room



Entry to Classified Area



Unclassified Corridor

# FACILITY



Grade D Change Room



Grade D Change Room



Grade C Change Room



Grade C Corridor which connects the 3 Cleanrooms, Post Quarantine Room & Entrance



# FACILITY



Door to Gowning Room 1 and CR 1



Door to Gowning Room 2 and CR 2 / 3



Post Quarantine Room



Cleaners' Sluice

# FACILITY



Door to CR1 from Gowning Room1



Cleanroom 1



Equipments in CR1



Equipments in CR1



# FACILITY

Phase I Clinical Trial: GMP certified lab for cell & tissue therapy



# FACILITY



Biro Pengawasan Farmaseutikal Kebangsaan  
National Pharmaceutical Control Bureau  
KEMENTERIAN KESIHATAN MALAYSIA  
MINISTRY OF HEALTH MALAYSIA

**LC No. 032/12**

**Our Ref** : ( 14 ) dlm.BPFK/30/12/2120  
**Date** : 28<sup>th</sup> September 2012

**MAKMAL TEKNOLOGI SEL TISU UKM-MTDC**

Pusat Kejuruteraan Tisu,  
Tingkat 12, Pusat Perubatan Universiti Kebangsaan Malaysia,  
Jalan Yaacob Latif,  
Bandar Tun Razak,  
56000 Cheras Kuala Lumpur.

**Letter of Conformation**

This is to confirm that your manufacturing premises **Tingkat 12, Pusat Perubatan Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras Kuala Lumpur** conforms to the requirement of Good Manufacturing Practices (GMP) in accordance to the current Pharmaceutical Inspection Co-operation Scheme (PIC/S) GMP Guides and its relevant Annexes for:-

- 1 Processing, Construct Formation And Storage Of Human Cells & Tissues For The Purpose Of Clinical Trial

The premises was last inspected on **23<sup>rd</sup>-24<sup>th</sup> July 2012** and is subjected to inspections at suitable interval.

**(SULAIMAN HJ. AHMAD)**

Head of Centre for Compliance and Licensing  
for Director of Regulatory Pharmacy  
National Pharmaceutical Control Bureau  
Ministry of Health Malaysia

***This document is valid until 22<sup>nd</sup> July 2014.  
(2 years from the date of last inspection unless otherwise specified).***

Jalan Universiti, P. O. Box 319, 46730 Petaling Jaya, Selangor, Malaysia  
Tel.: +603 7883 5400 Fax: +603 7958 2924/7958 1312  
<http://www.bpfk.gov.my>

# CLINICAL TRIAL

## Proposed Phase I Clinical Trial

### **Full Title:**

A Prospective, Single-center, non-randomized, Phase I, Clinical Investigation of “MyDerm™” as Skin Replacement in Treatment of Patients with Diabetic Ulcers, Burn and Trauma Injuries

### **Funding Mechanism:**

UKM-MTDC (Malaysian Technology Development Corporation)

### **Primary Objective:**

To treat diabetic ulcers, burn and trauma injuries using MyDerm™

### **Secondary Objective:**

To evaluate the safety and efficacy of MyDerm™

# Thank you



For further information, please contact Prof. Dr. Ruszymah bt Hj Idrus at [ruszyidrus@gmail.com](mailto:ruszyidrus@gmail.com) or [ruszy@medic.ukm.my](mailto:ruszy@medic.ukm.my) , Phone: +603-9145 7670 (ext: 7669)