

WCNC 2017
2nd International Conference on
ADVANCES IN SKIN WOUND CARE AND TISSUE SCIENCE
November 9-10, 2017 // Frankfurt, Germany

Efficacy of Umbilical Cord Lining Stem Cells for Wound Healing in Diabetic Murine Model

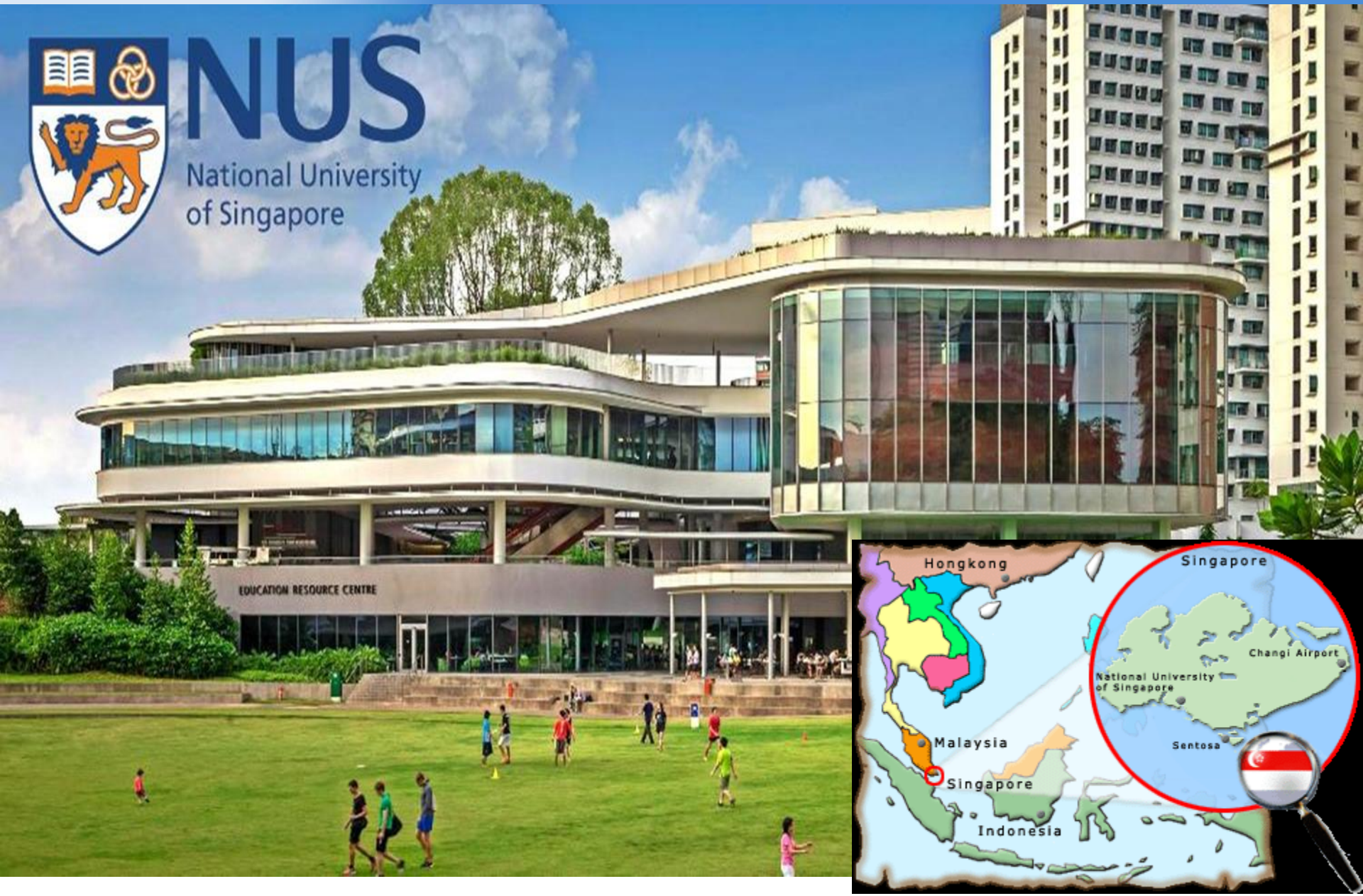
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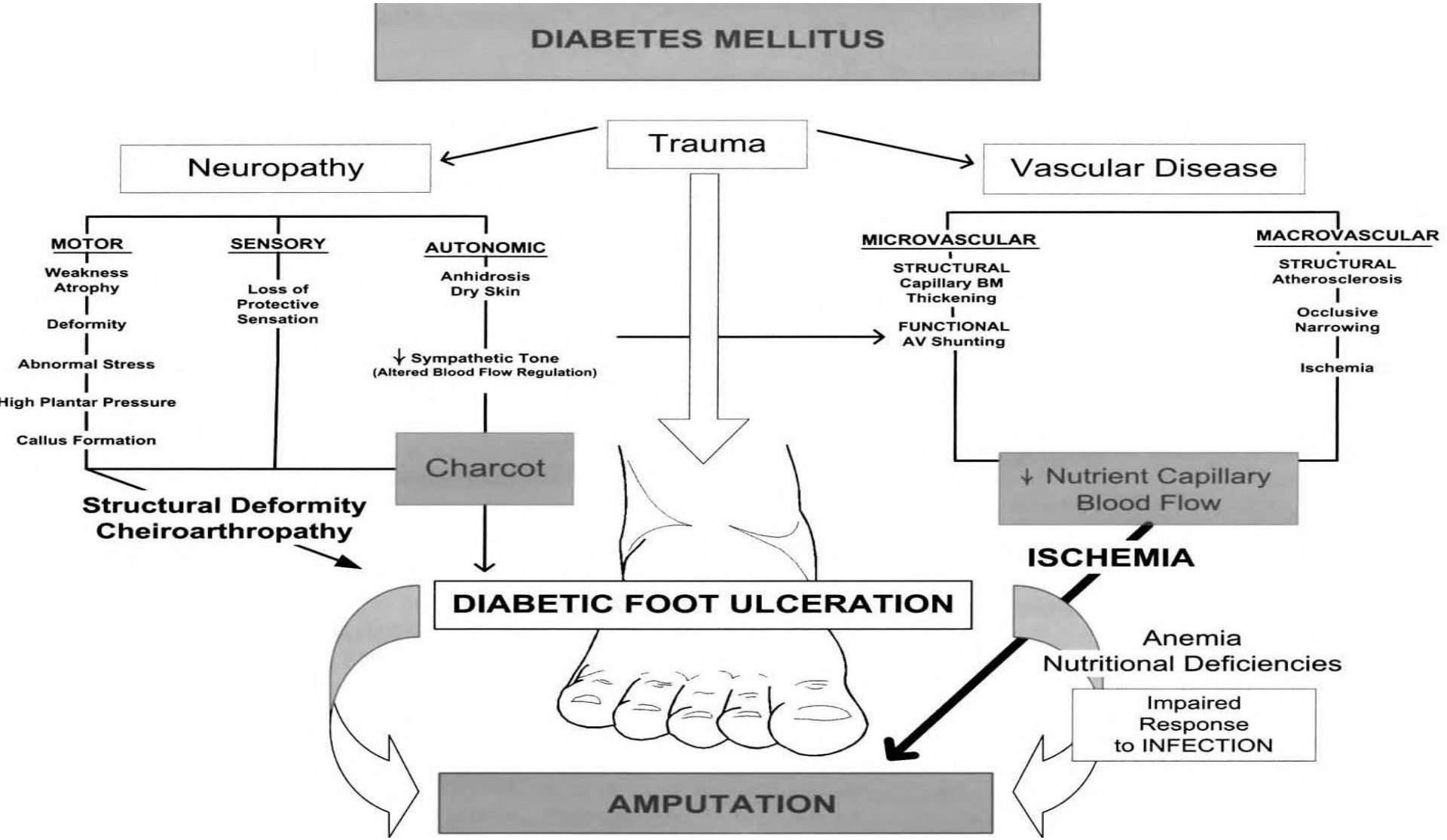


THE DIABETIC FOOT: FACTS AND FIGURES

- One limb is lost to diabetes every thirty seconds[1]
- Each year, more than 82,000 amputations are performed among people with diabetes[1]
- One in five diabetic patients (22 per cent) who underwent lower extremity amputation (LEA) dies within a year due to multiple complications[3]
- After an amputation, the chance of another amputation within 3 to 5 years is as high as 50 percent[1]
- The 5 year mortality rate after amputation ranges from 39 to 68 percent[1]

1. International Diabetes Federation. *IDF Diabetes, 7 ed.* Brussels, Belgium
2. The Straits Times, Apr 26, 2016

The Diabetic Foot

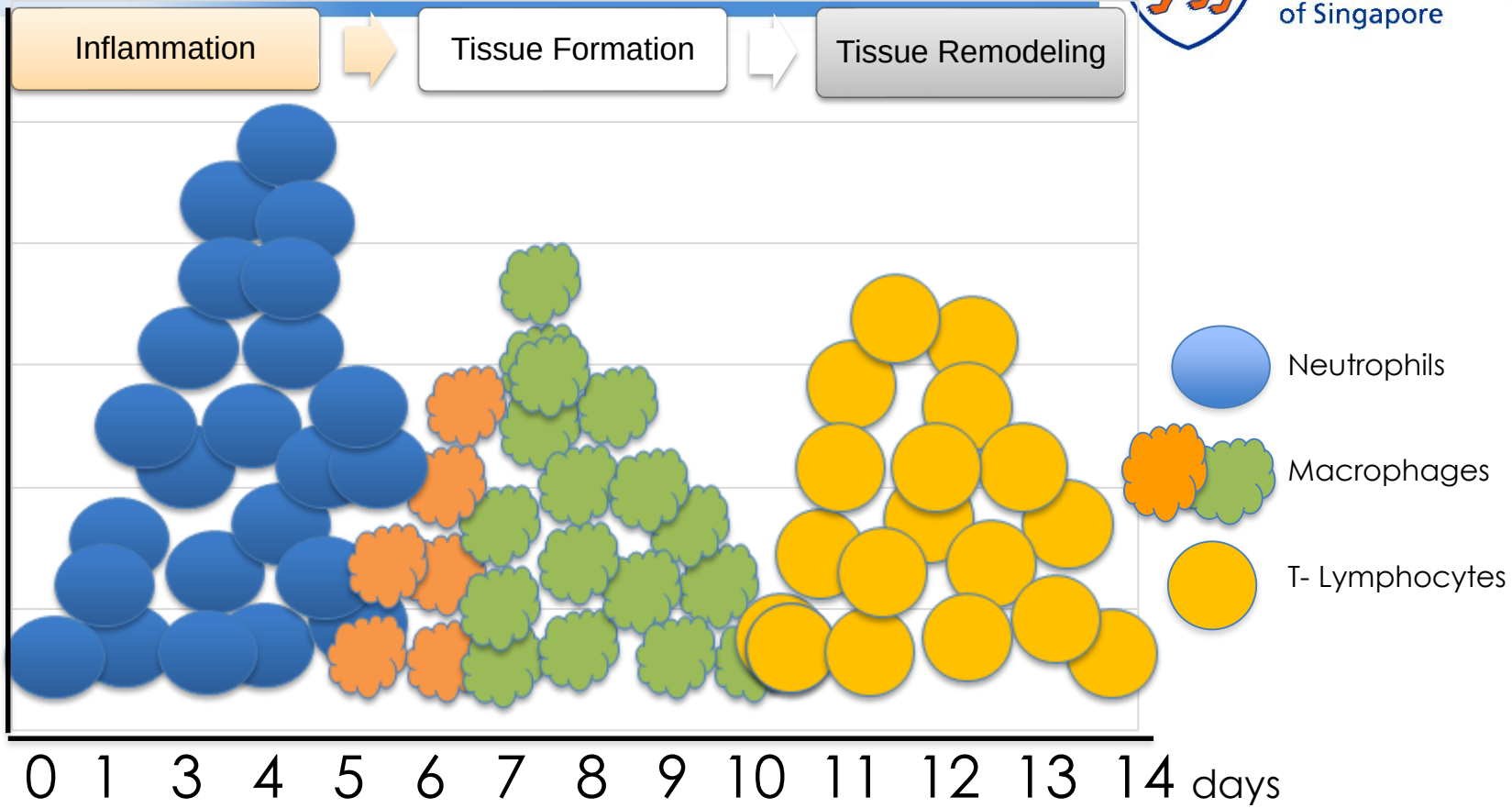




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IMMUNE DYSFUNCTION IN DIABETIC WOUND HEALING

Normal Wound Healing Processes

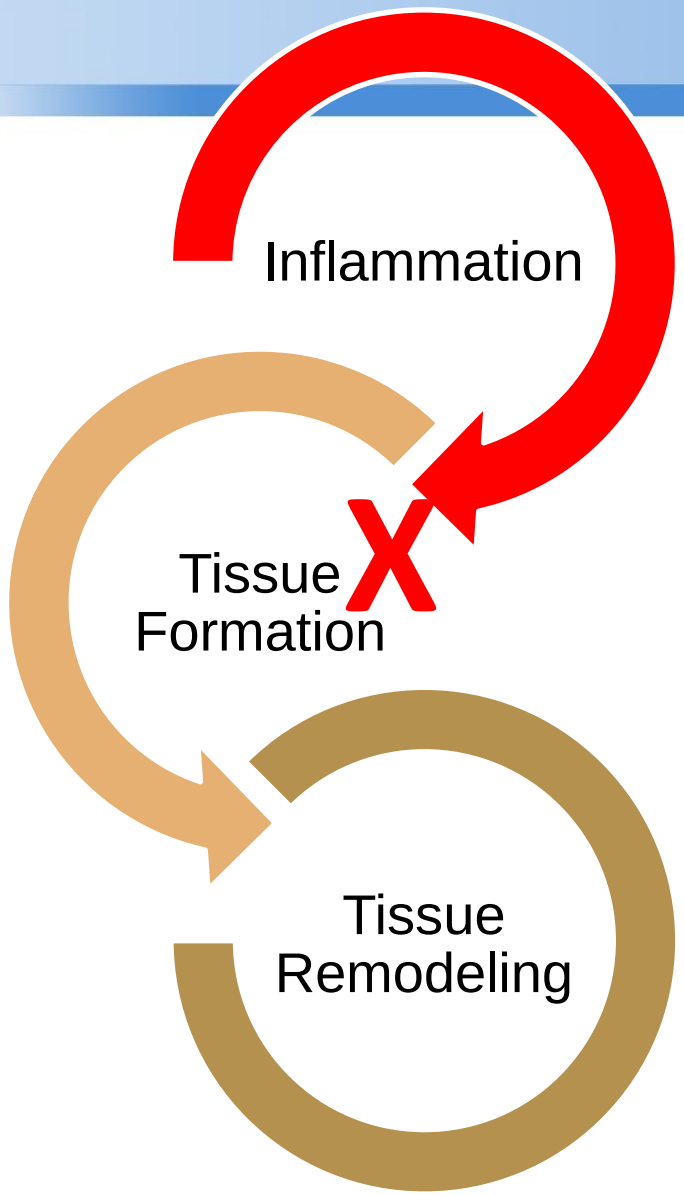


Neutrophils
- Kill foreign microbes
- Damage surrounding tissue

Macrophages
-Clear the wound site of debris
-Initiate fibroblast activity and angiogenesis

T-Lymphocytes
- Tissue remodeling

Remodelling of collagen fibre

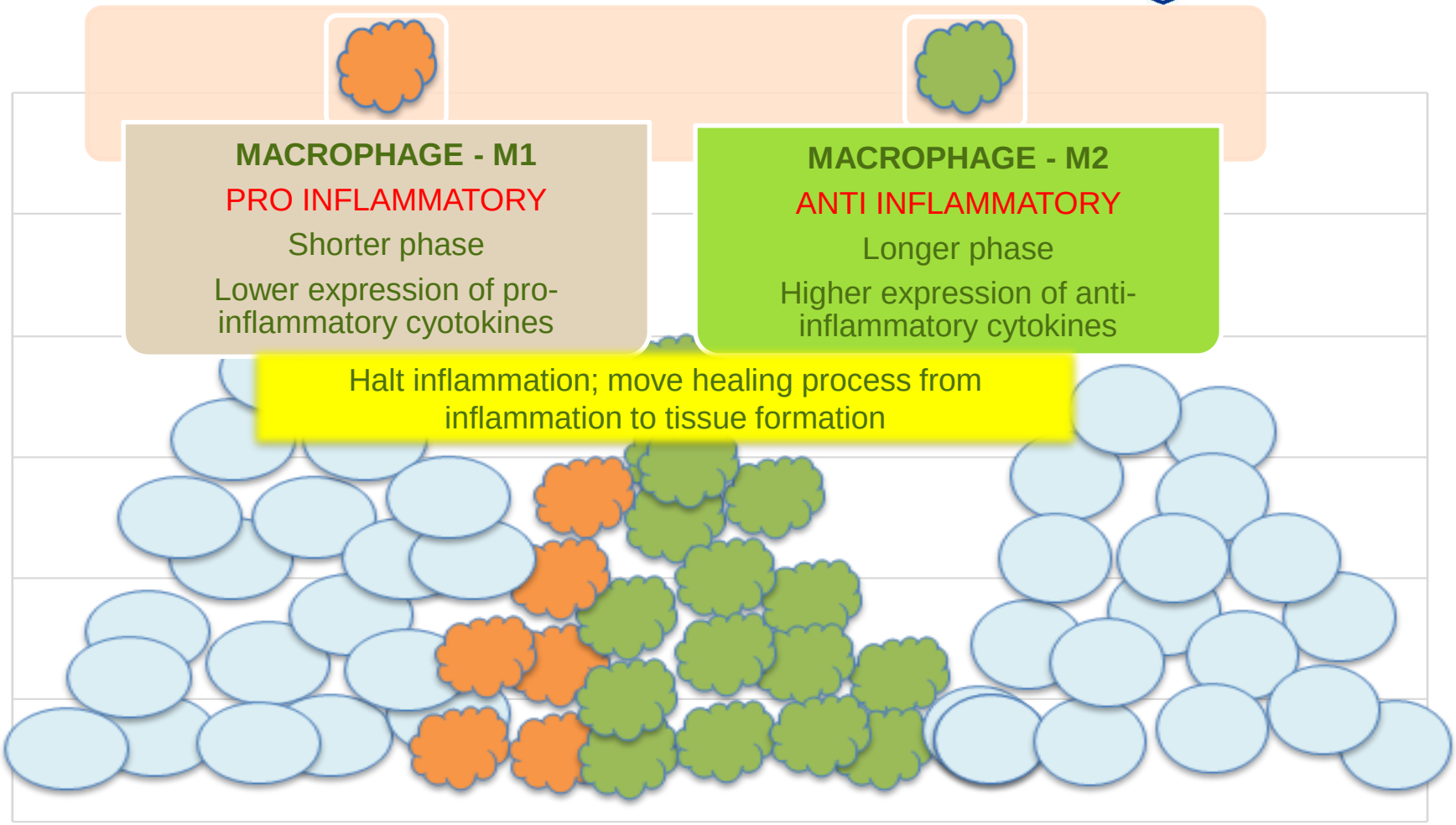


Neutrophil response continues

Macrophage response does not take over

Wound becomes chronic, prone to infection, difficult to heal

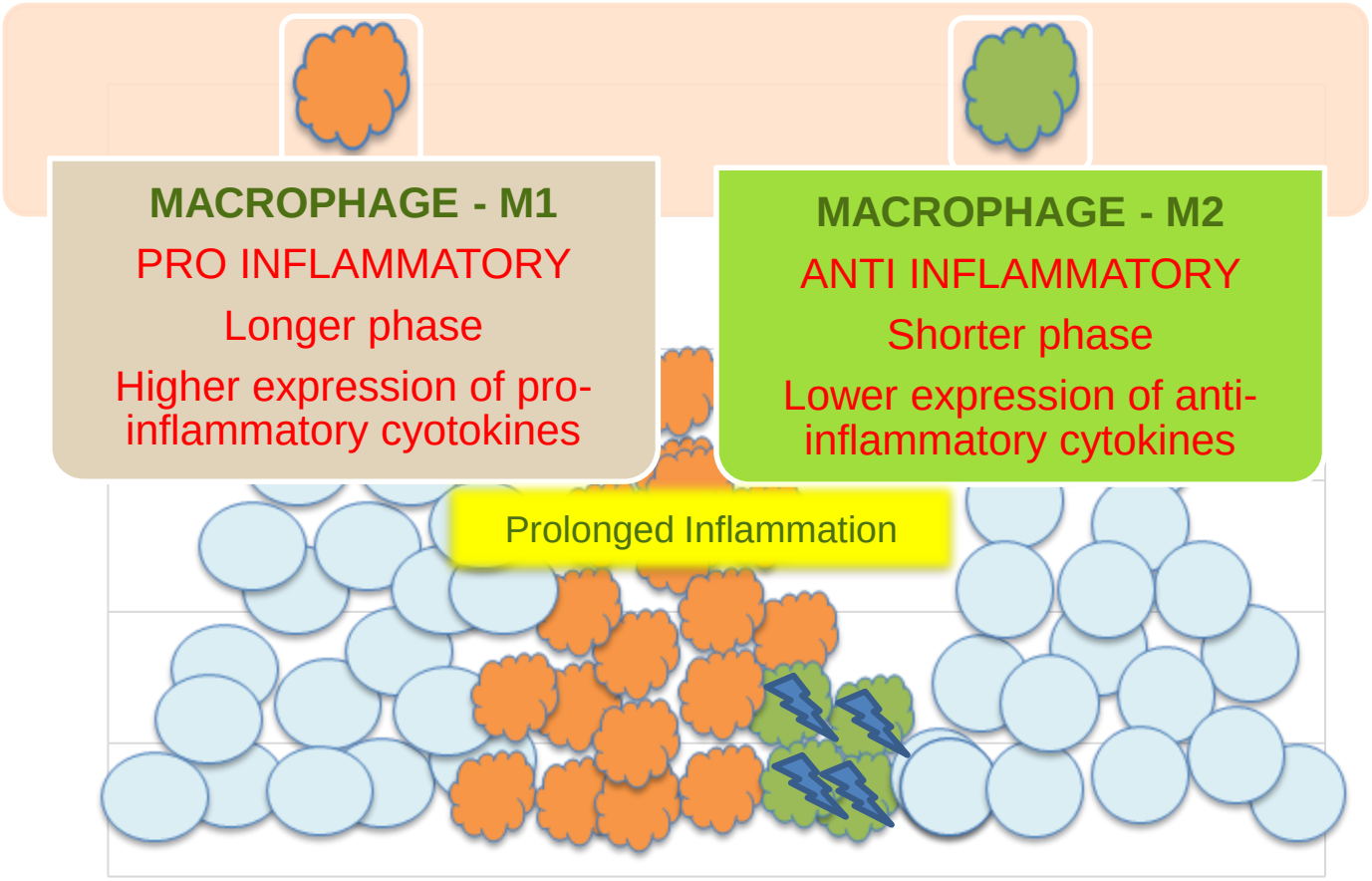
THE INFLAMMATORY CELLS ~ MACROPHAGES



MACROPHAGE DYSFUNCTION IMPAIRS RESOLUTION OF INFLAMMATION IN THE WOUNDS OF DIABETIC MICE

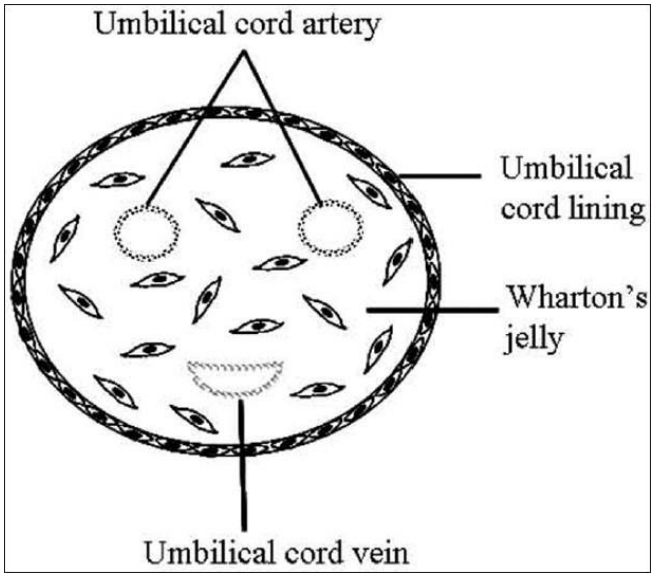
by Khanna et al. Published in PLoS One 5(3) 2010

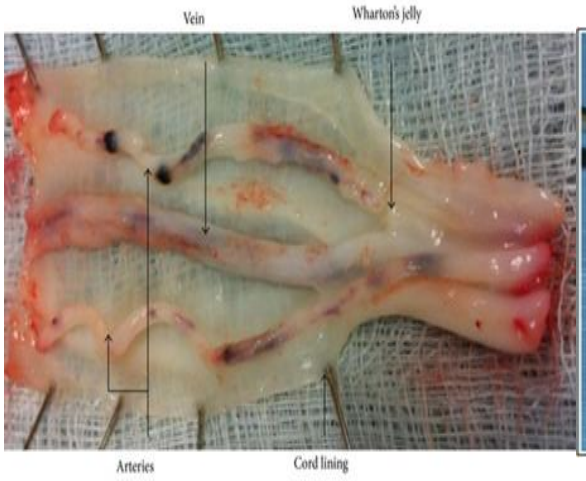
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CORD LINING MESENCHYMAL STEM CELLS (CL-MSC): ITS POTENTIAL IN TISSUE REPAIR

Cold Lining Mesenchymal Stem Cells: Characteristics



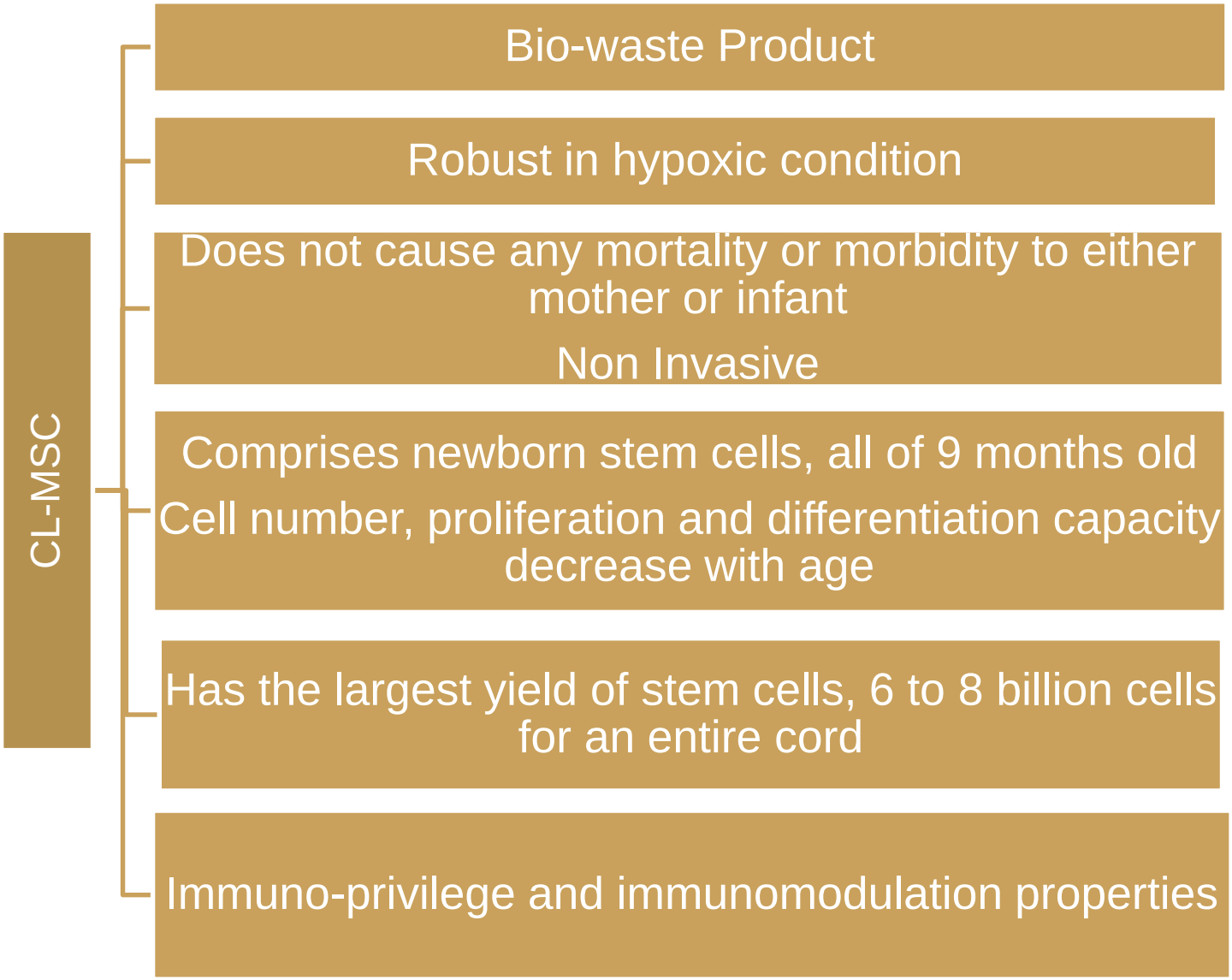




Rich source of two strains of stem cells: mesenchymal stem cells (from the sub-amniotic layer) epithelial stem cells (from the amniotic layer)

- Mesenchymal Stem Cells markers CD 73, CD90, and CD105
- Negative for hematopoietic stem cell marker CD34, CD45, and CD117

Cord Lining Mesenchymal Stem Cells



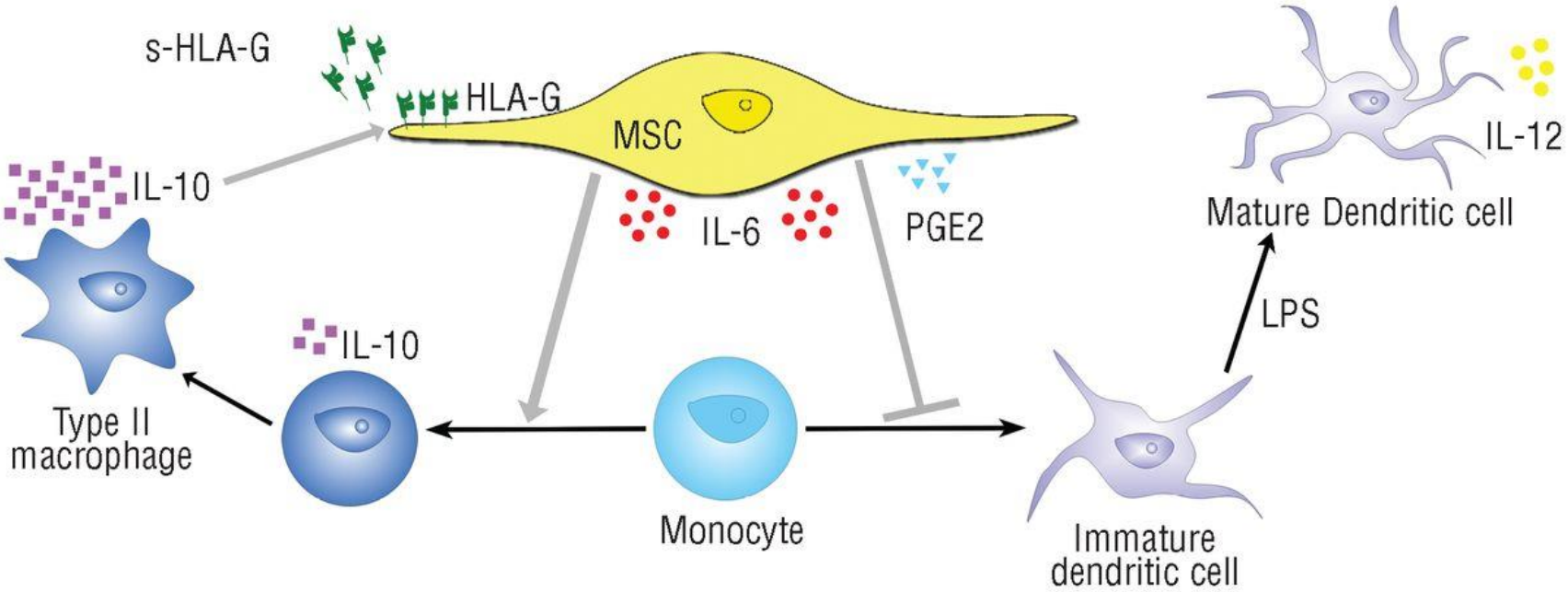
Why a mother's immune system does not reject a developing fetus as foreign tissue?





The cord lining tissues express the atypical MHC class 1 isotypes HLA-E and HLA-G

- downregulate the immune response and helps in the maintenance of pregnancy



Immunomodulation by MSC

- MSC express IL-6 and prostaglandin E2 (PGE2) that skew monocyte differentiation toward the formation of IL-10-expressing monocyte-derived cell population (MDC)

Preliminary Study



Aim 1

To investigate the healing potential of cord lining stem cells in full thickness dermal wounds of db/db induced diabetes mice

Hypothesis 1: The CL-MSC will contribute to the healing of full thickness diabetic wound

Aim 2

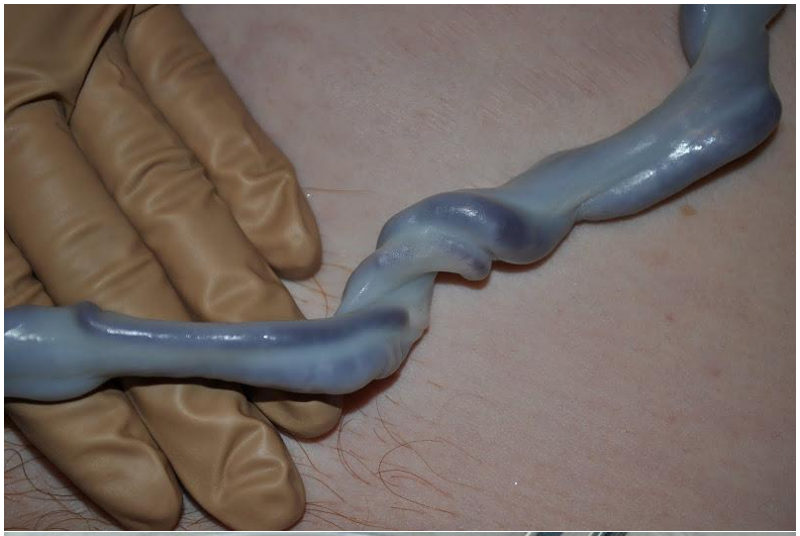
To determine the expression of wound healing related cytokines in CL-MSC

MATERIALS AND METHODS

Cultivation of CL-MSC

15cm of the umbilical cord

- Cord lining membranes are separated from umbilical cord
- Cell culture medium specific for the developing of stem cells
- The nutrients in these media will stimulate stem cells to grow and move from cord lining membrane to the culture dish.





Cultivation of CL-MSC

Harvest into cryotubes with preservatives and then stored in liquid nitrogen.



Control Rate Freezers system to help them reduce temperature gradually
Stem cells are preserved by deep freezing technology (at -196 degree C)



Murine Model of Diabetes – db/db mice

- Genetically diabetic db/db mice from Jackson Laboratories
- 12-20 week old adult diabetic mice
- Represent a model of type 2 diabetes healing, characterized by hyperglycemia, hyperinsulinemia, obesity and impaired wound healing
- Demonstrated the most critical impairment in wound healing
  - significant delay in wound closure, decreased formation of granulating tissue, decreased wound bed vascularity, diminished proliferation



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EXPERIMENTAL DESIGN

Aim 1

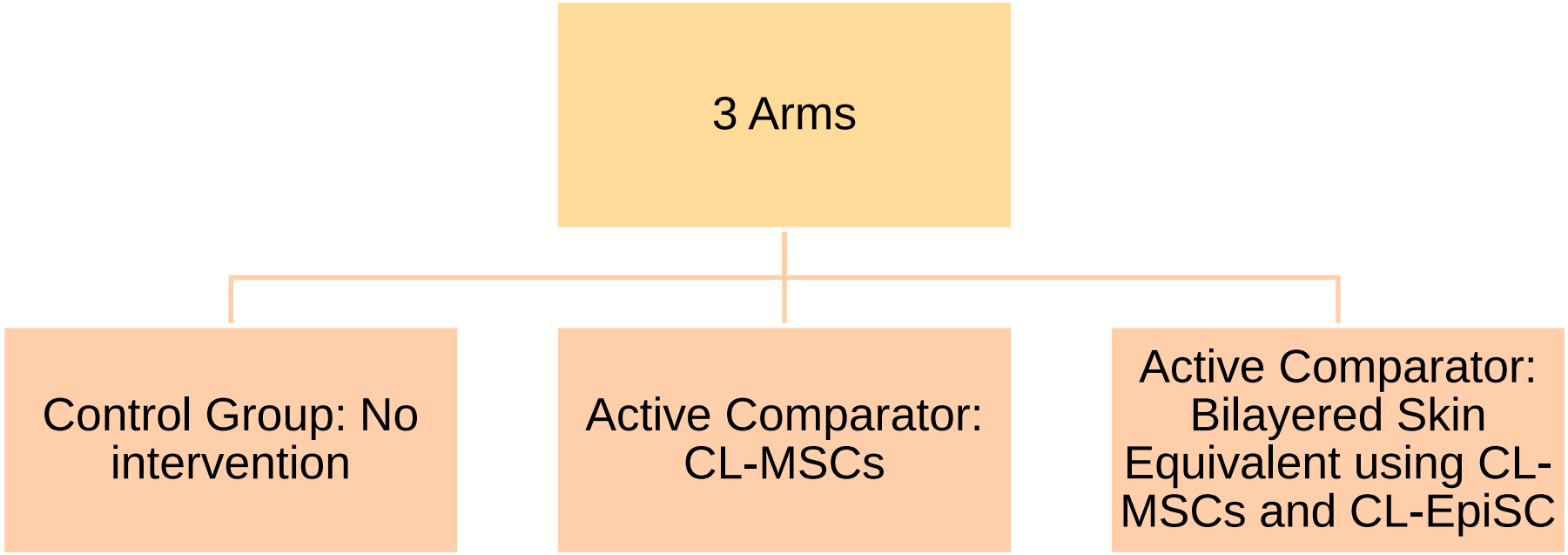
To investigate the healing potential of cord lining stem cells in full thickness dermal wounds of db/db induced diabetes mice

Hypothesis 1: The CL-MSC will contribute to the healing of full thickness diabetic wound

Aim 2

To determine the expression of wound healing related cytokines in CL-MSC

EXPERIMENTAL DESIGN

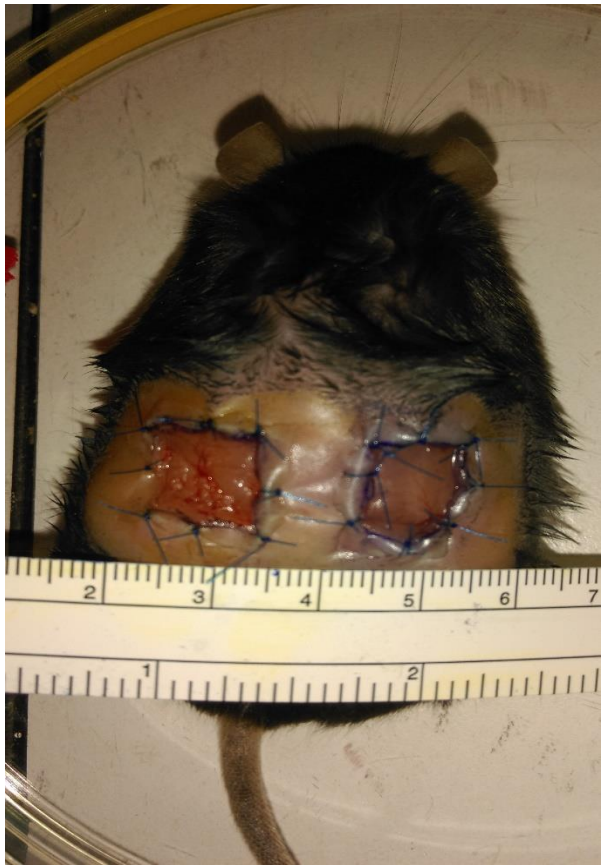


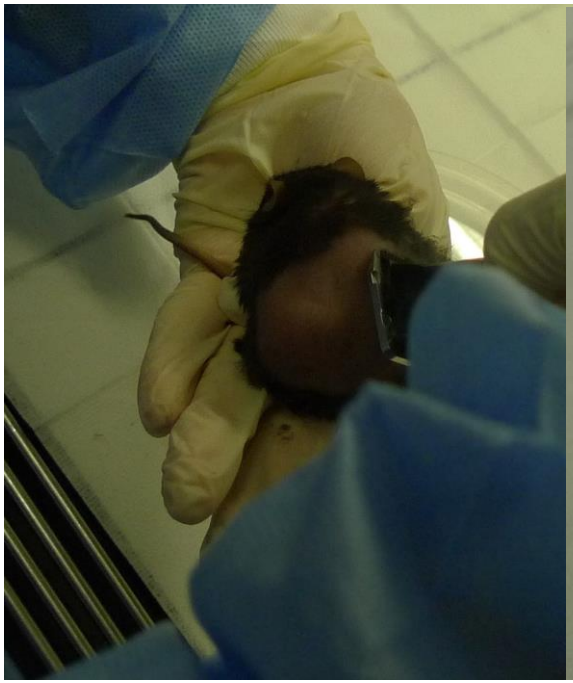
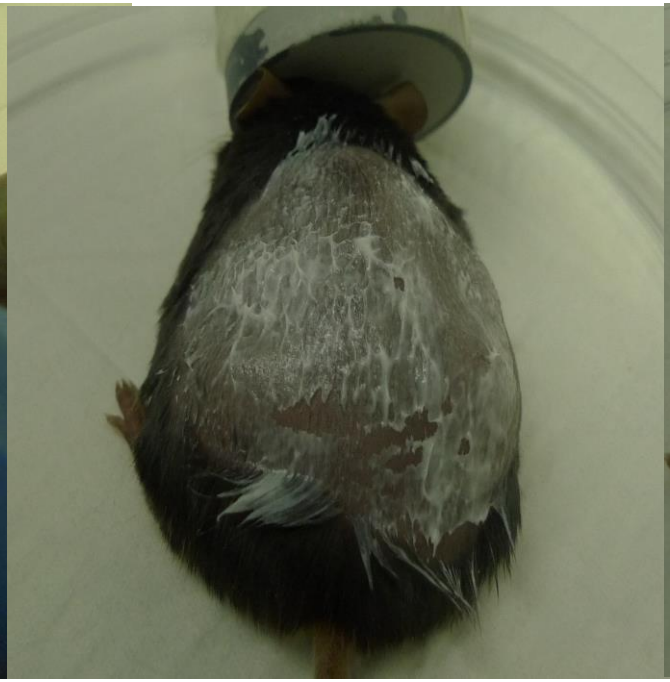
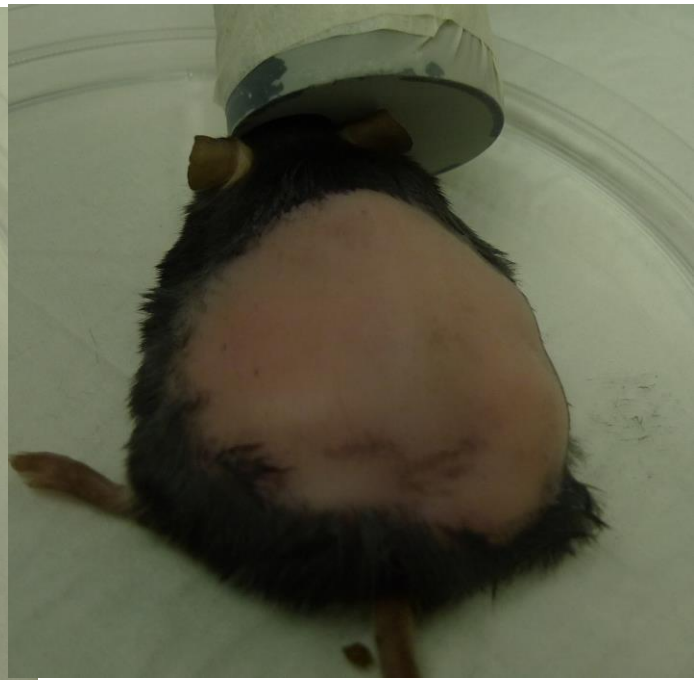
Arms	Assigned Intervention
Control Group: No intervention	Placebo treatment: Sham media (Cells delivery material only) - Dulbecco's Modified Eagle's Medium (DMEM) Mode of delivery: Intraperitoneal (IP) injection only (systematically) Wound Dressing: Jelonet, Gauze, Coban Bandage N= 10
Active Comparator: CL-MSCs	Treatment: CL-MSC Dosage: 1 x 10^6 cells/kg only (1ml per mouse) Mode of delivery: Intraperitoneal (IP) injection only (systematically) Wound Dressing: Jelonet, Gauze, Coban Bandage N= 10
Active Comparator: Bilayered Skin Equivalent using CL-MSC and CL-EpiSC	Treatment: CL-EpiSC (as epidermis) and CL-MSCs (as dermis) Mode of delivery: Topical Application (locally) Wound Dressing: Jelonet, Gauze, Coban Bandage N=6

MURINE WOUND MODEL

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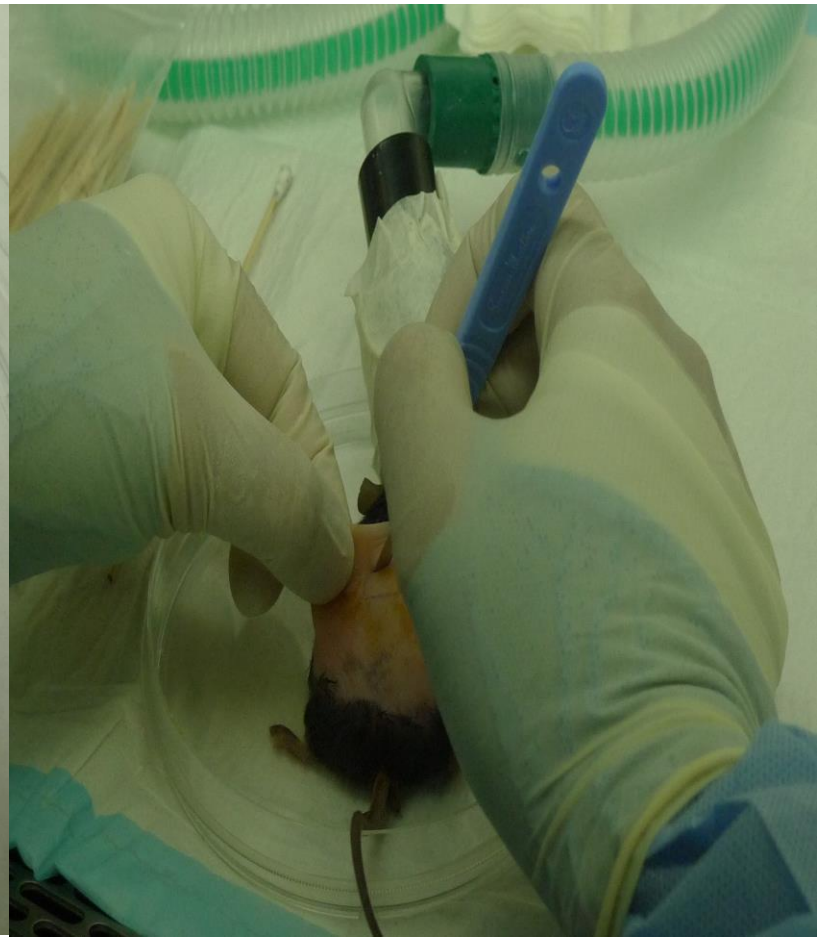
1. 1 X 1 cm wound will be traced on the mouse dorsum, one on each side of the midline.
2. Two full-thickness wounds are created extending through the panniculus carnosus.
3. A silicone splint is used and centered on the wound, fixed with 6-0 nylon sutures applied to ensure splint position.
4. Covered with an non adherent dressing. To prevent self-mutilation, usually to the wound created, we use padded gauze and Coban over the wound dressing to secure the wound sites



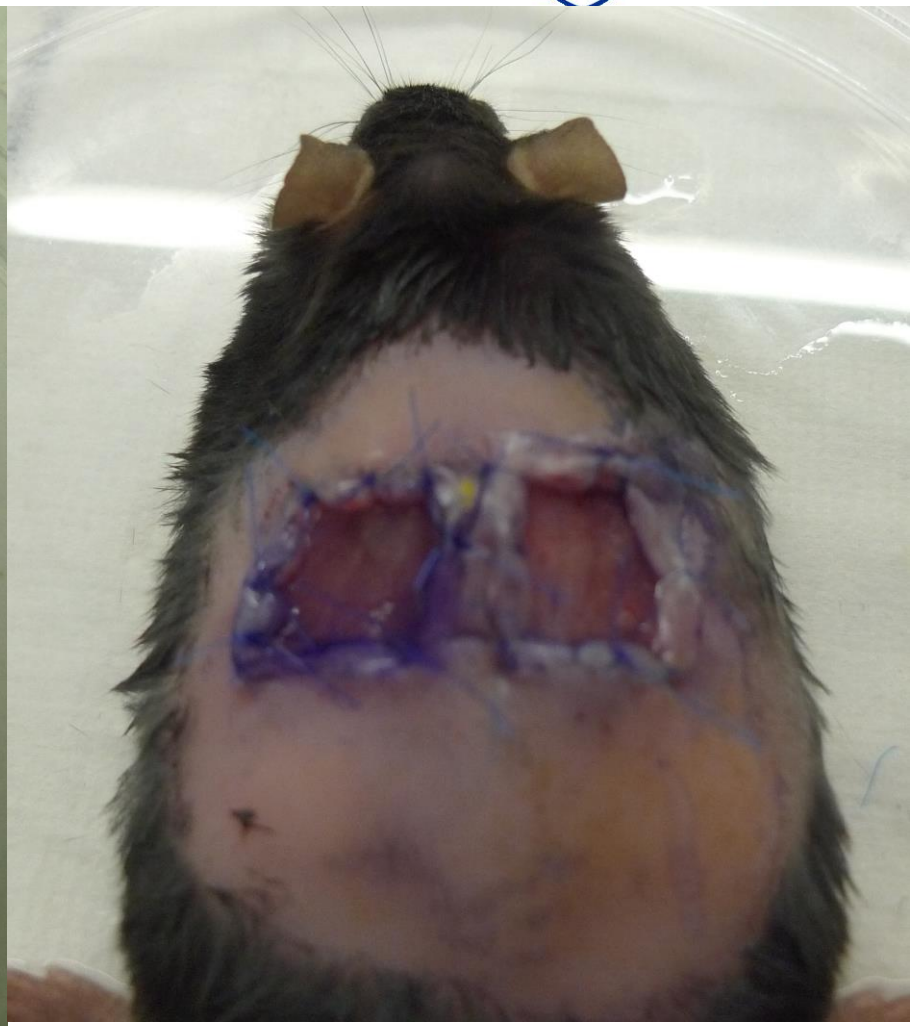
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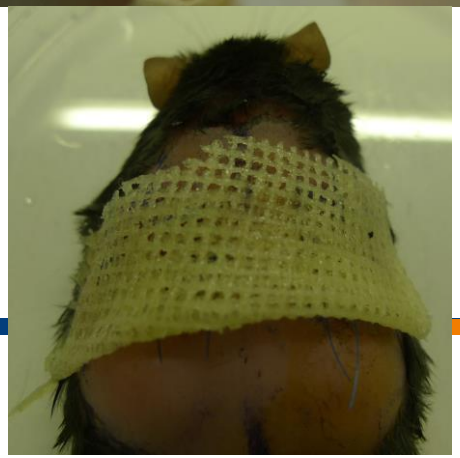
Every 7 days

Treatment: CL-MSC
Dosage: 1 x 10^6 cells/kg only (1ml per mouse)
Route: IP injection

Placebo treatment: Sham media (Cells delivery material only) - Dulbecco's Modified Eagle's Medium (DMEM)
Route: IP Injection



Treatment: CL-EpiSC (as epidermis) and CL-MSCs (as dermis)
Route: Topical Application



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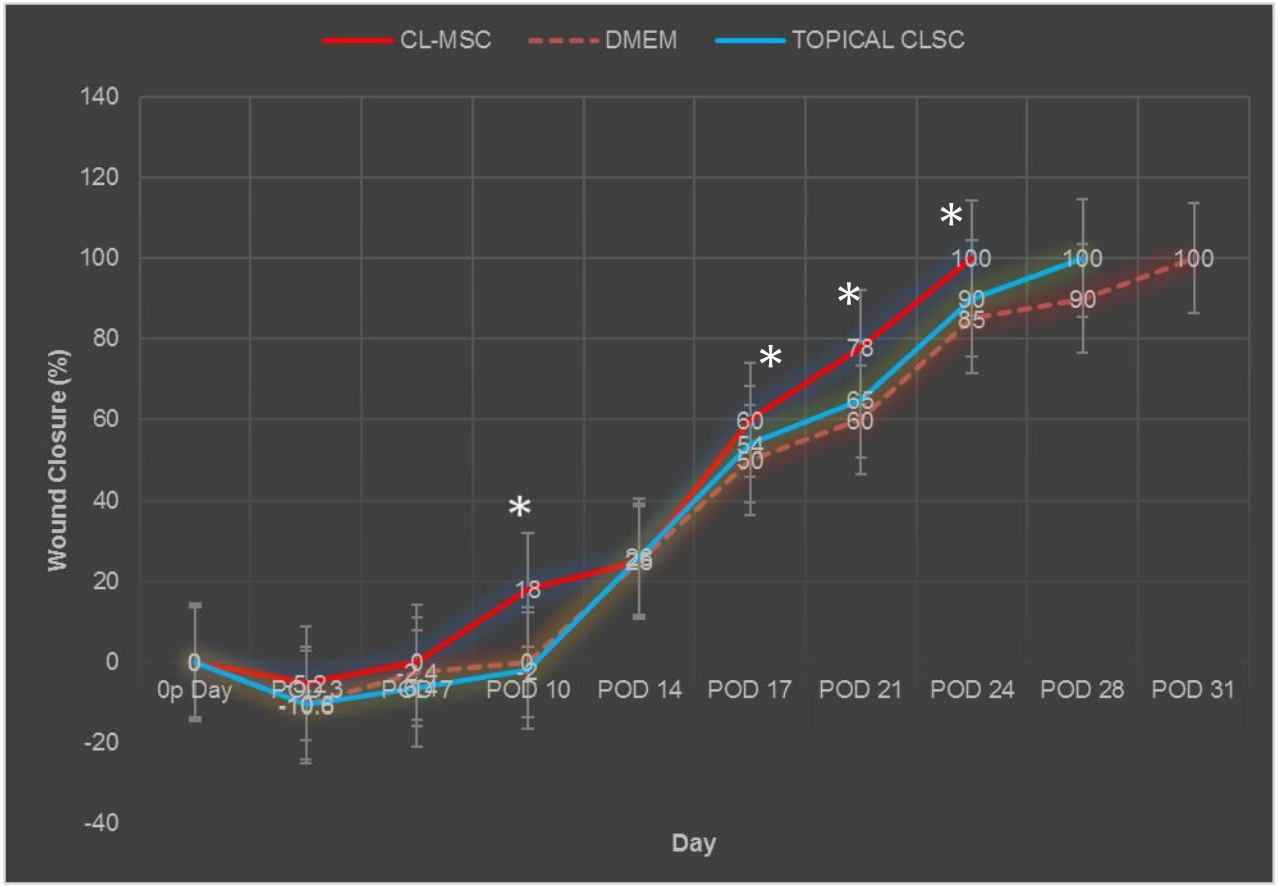
PLANIMETRY ANALYSIS

- Digital pictures will be taken on day 1, 3, 7, 10, 14, 17, 21, 24, 28….. and till wound achieve full epitheliazation
- Wound area will be analyzed with ImageJ ™ software and calculated as percentage of the original wound.

$$\text{Percentage of wound closure} = \frac{(\text{Wound area at day 0} - \text{wound area at day n}) \times 100}{\text{Wound area at day 0}}$$

- Time to closure will be defined as the day the wound bed is completely epithelized and filled with new tissue.

Measurement of Wound Closure Percentage between DMEM (n=10), CL-MSCs (n=10) and Topical CL-MSC;CL-EpiSC groups (n=6)



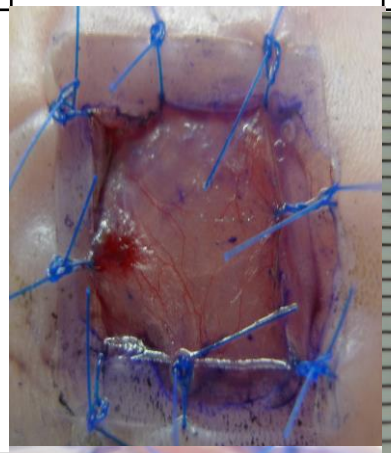
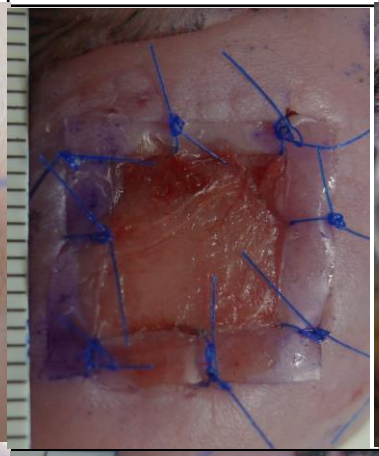
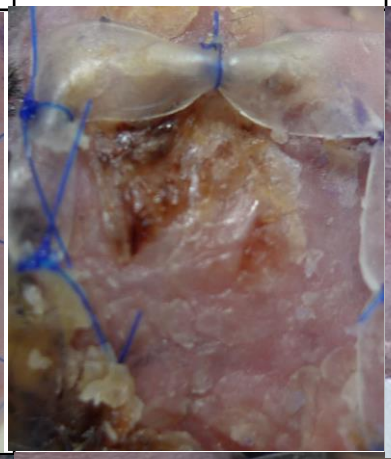
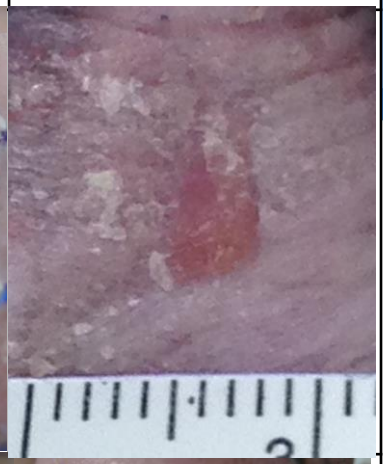
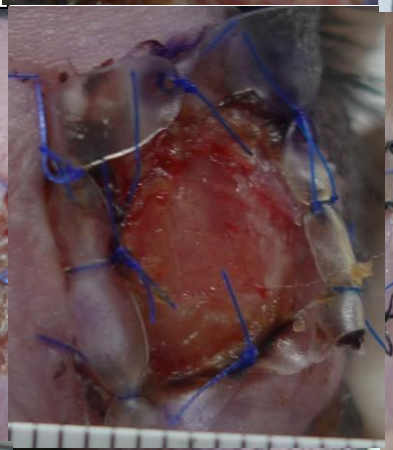
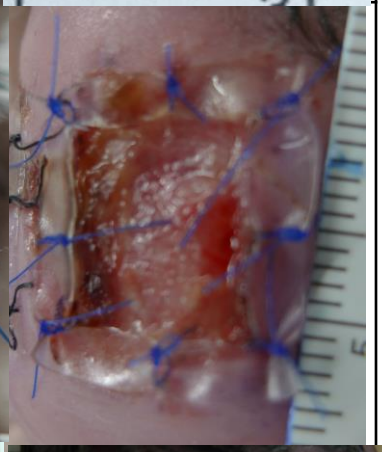
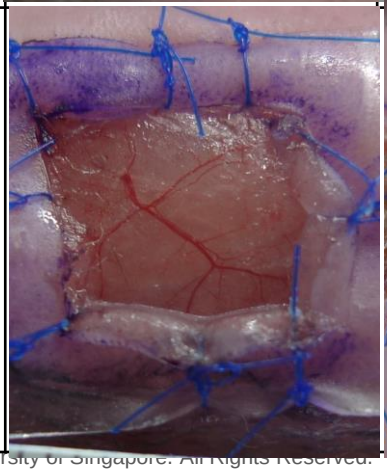
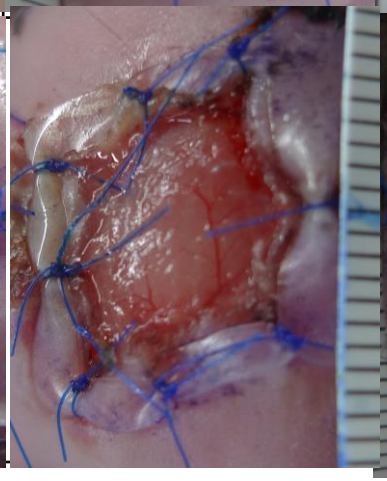
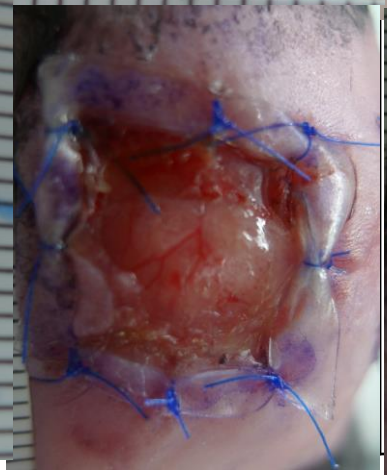
1-tailed t-test: $P < 0.05$ (*)

The percentage of wound closure, either treated or not, present with an uptrend pattern starting from day 10; however the CLMSCs treated wounds, compared with the control group, showed a significant increase in the percentage of wound closure on day 10, 17 and day 21



Time to closure = the day the wound bed is completely epithelised and filled with new tissue

The CLMSCs treated mice have a shorter wound closure time (mean closure day: 22.8 days), as compared to topical application of CL-MSC and CL-EpiSC (mean closure day 27.3) and the control group (mean closure day 30.7)

	Day 0	Day 7	Day 14	Day 21
IP CL-MSC				
Topical CL-MSC and CL-EpiSC				
IP Control (DMEM)				

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Aim 1

To investigate the healing potential of cord lining stem cells in full thickness dermal wounds of db/db induced diabetes mice

Hypothesis 1: The CL-MSC will contribute to the healing of full thickness diabetic wound

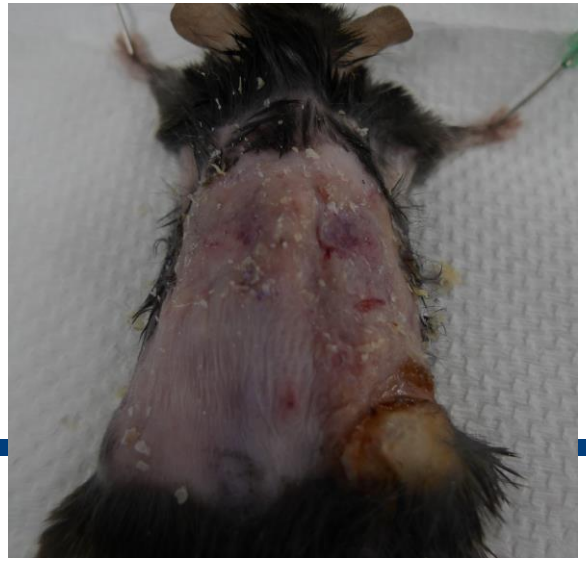
Aim 2

To determine the expression of wound healing related cytokines in CL-MSC

	Day 1	Day 7	Wound Healed
CLMSC treated mice	n = 3	n = 3	n = 3
Control group	n = 3	n = 3	n = 3

Skin were harvested at day 1, day 7 and when wound bed is completely epithelized

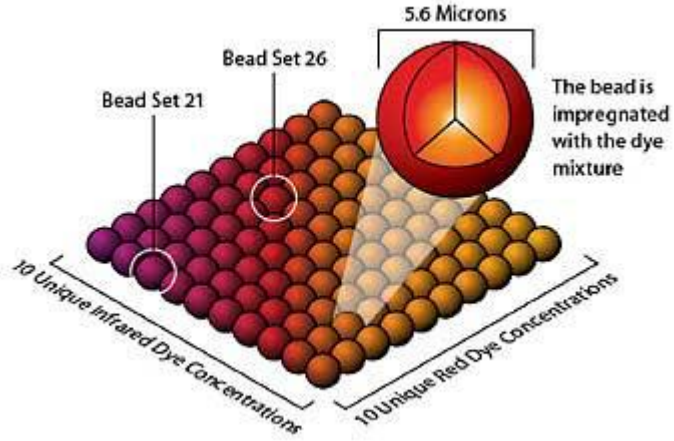
Blood (2mls) obtained via cardiac puncture when wound bed is completely epithelized

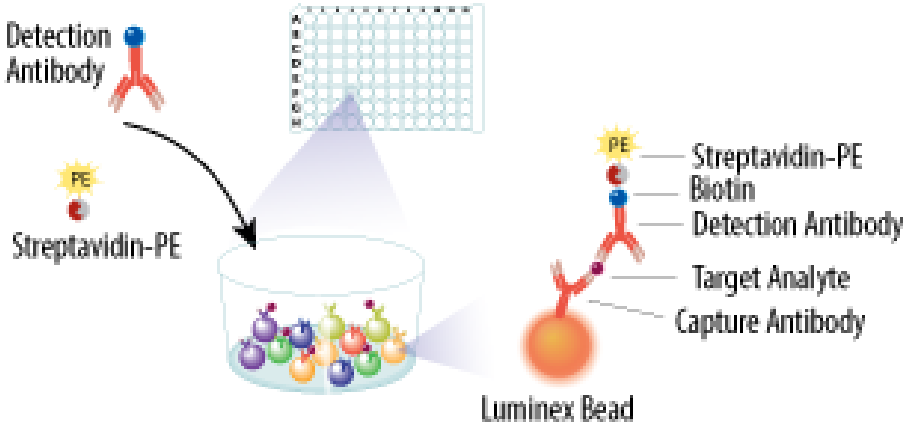




Blood serum analysis

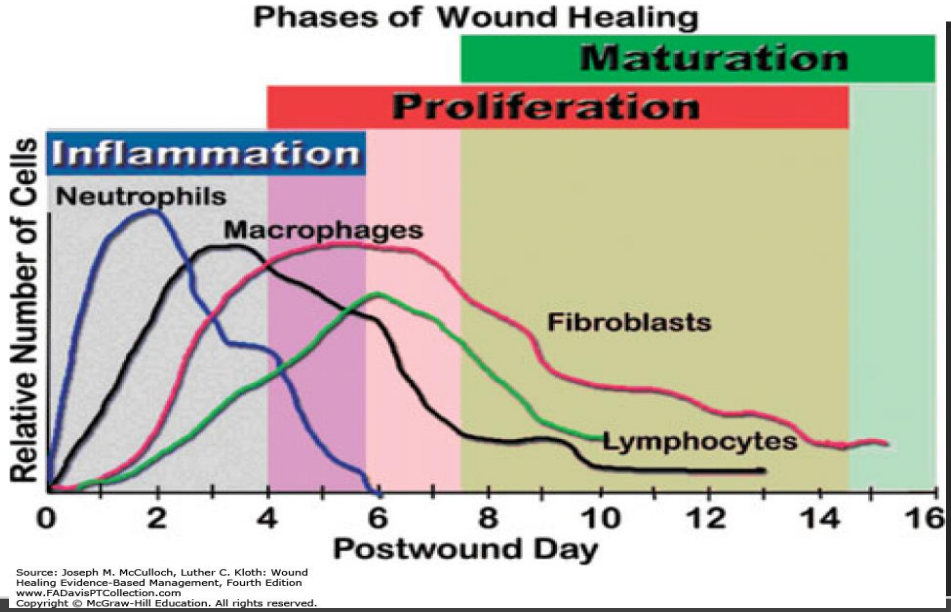
- Miliplex cytokine arrays – Milliplex murine cytokine/chemokine magnetic bead panel kit (EMD Millipore Corporation, USA)

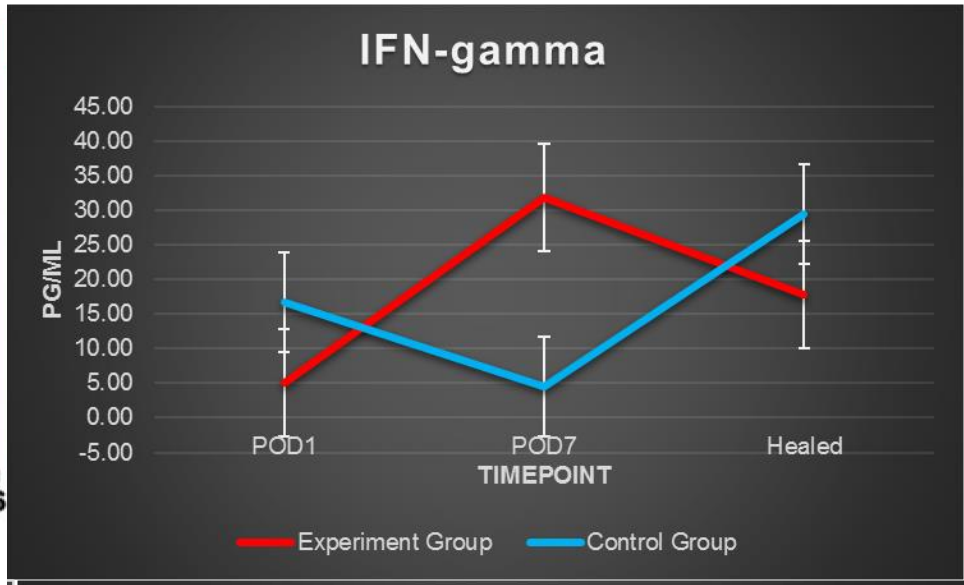


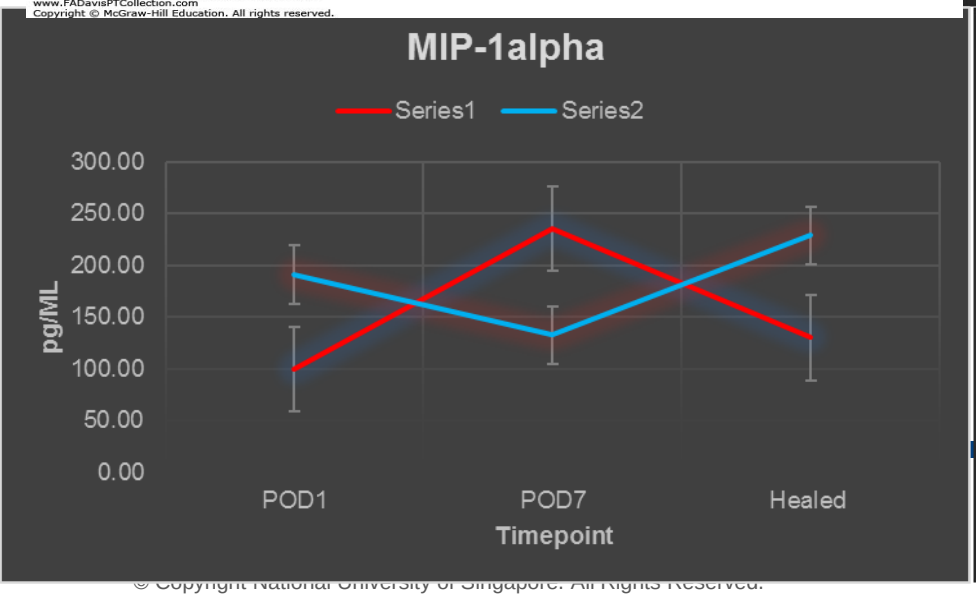


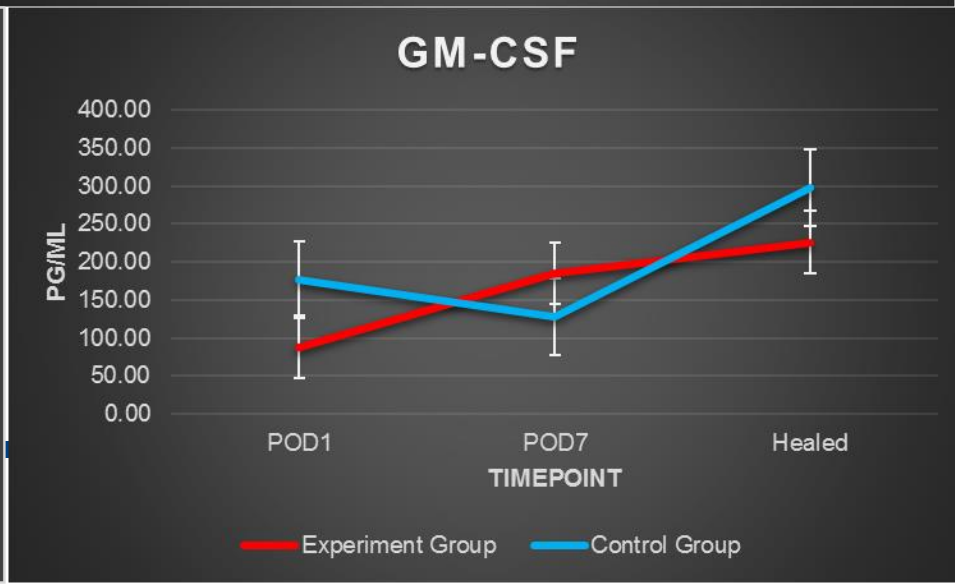
Antibody attached to a bead

Level of systemic pro-inflammatory murine serum cytokines in POD1, POD 7, and when wound achieved complete healing between CL-MSCs treated mice (red graph line) and control group (blue graph line)



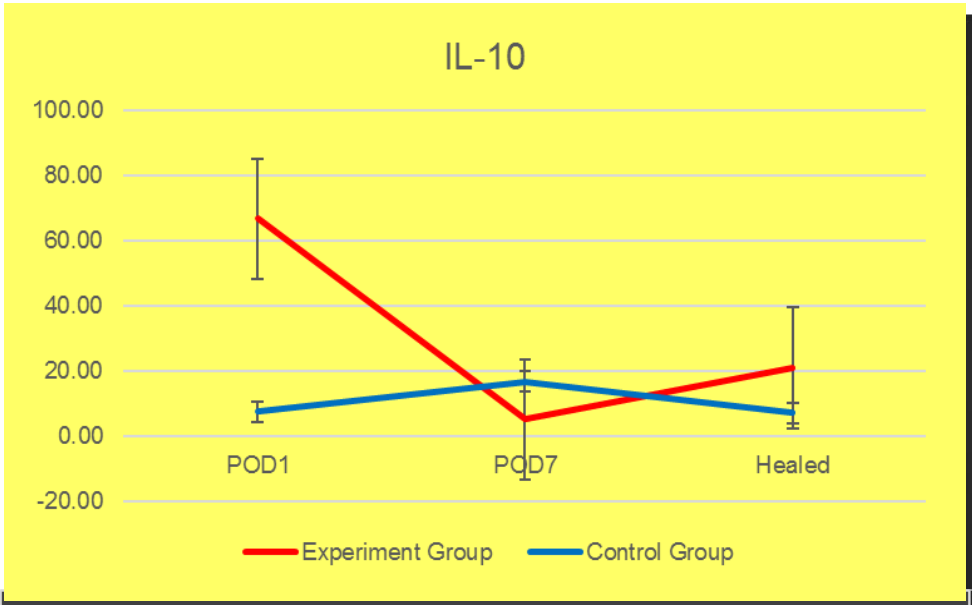


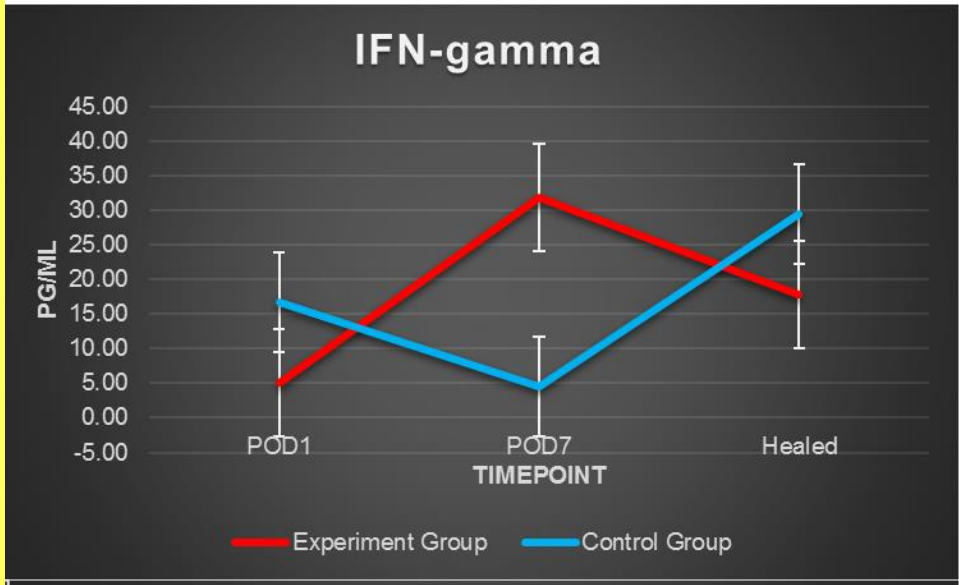


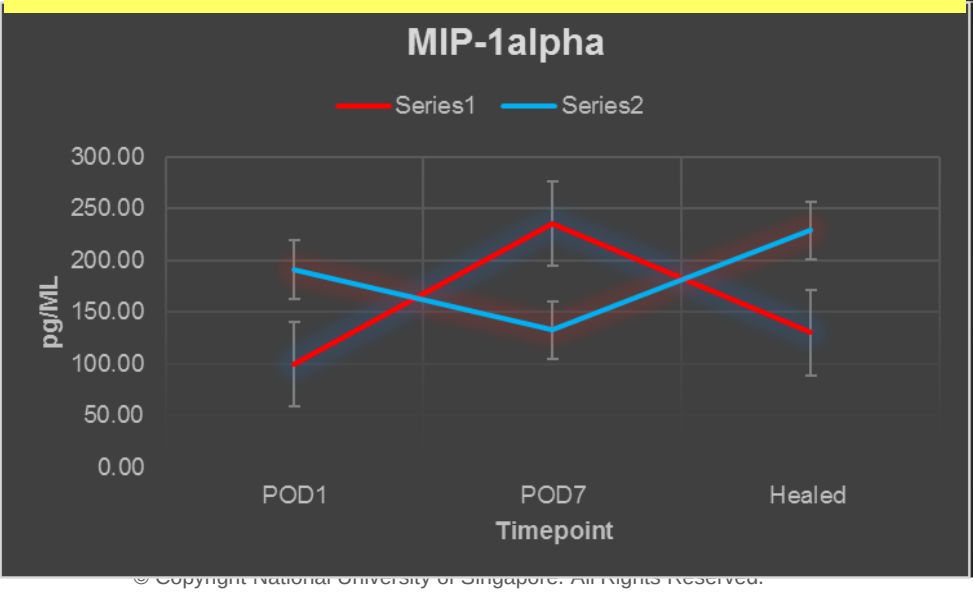


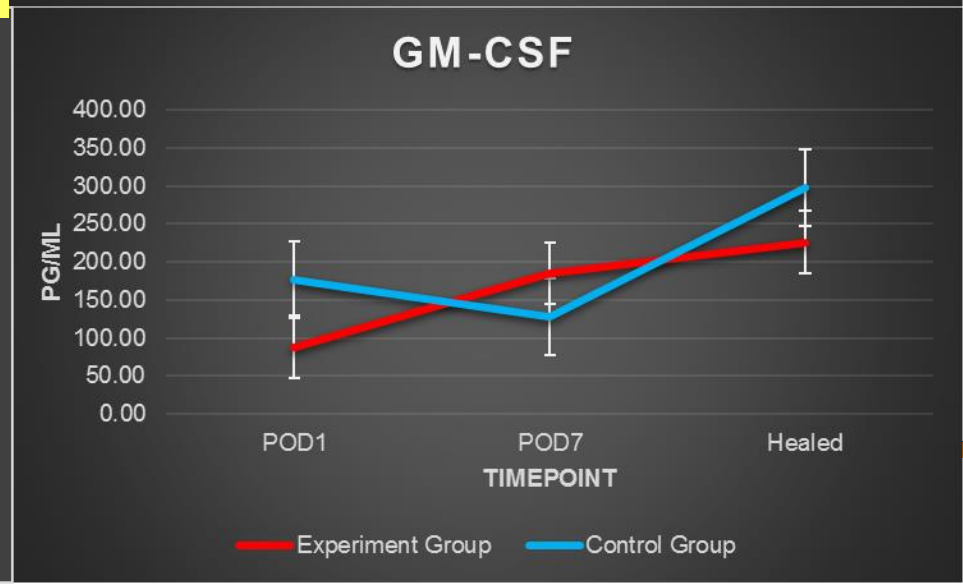
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Level of systemic anti-inflammatory murine serum cytokines in POD1, POD 7, and when wound achieved complete healing between CL-MSCs treated mice (red graph line) and control group (blue graph line)









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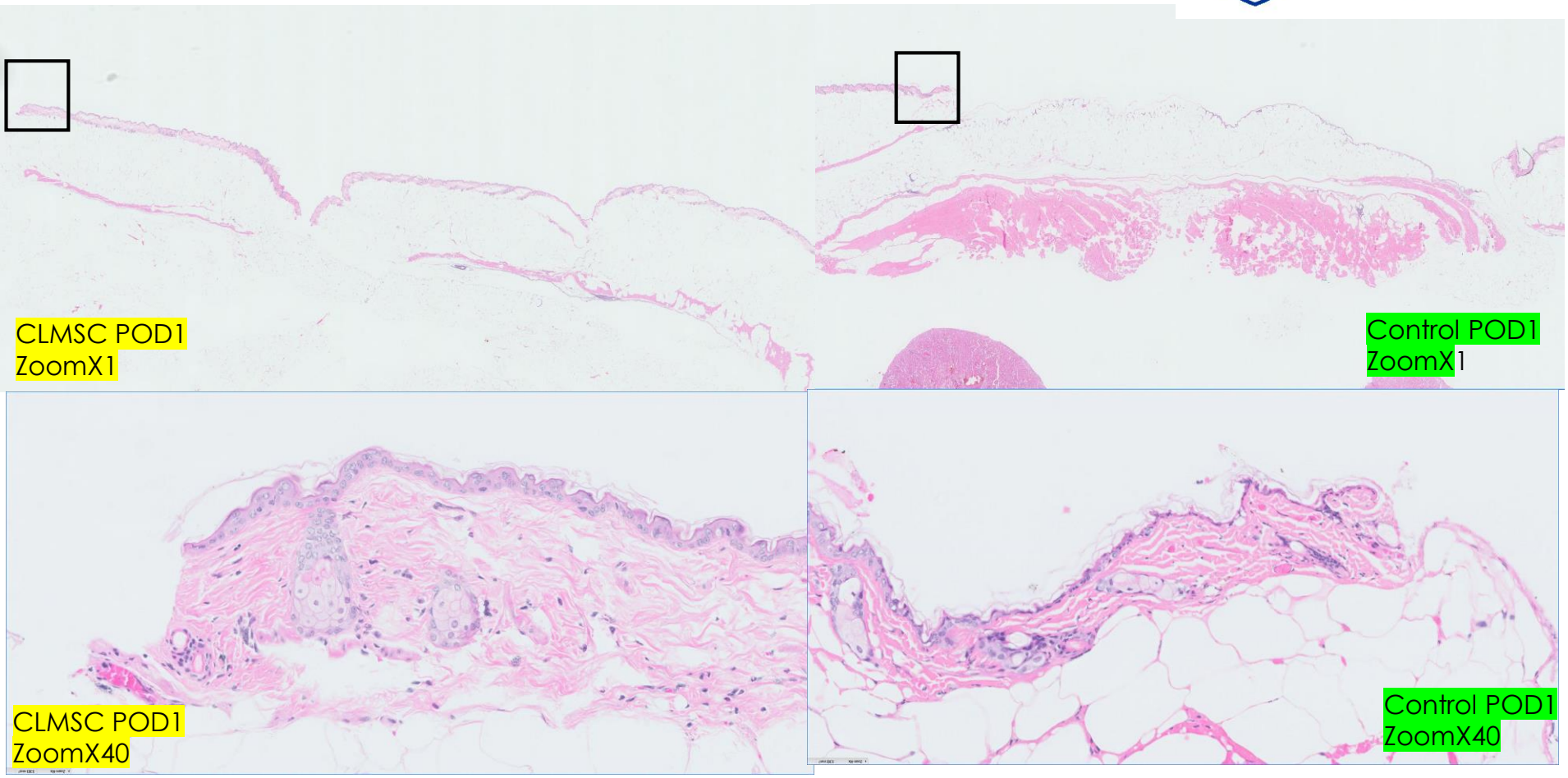
Tissue Analysis

- The morphology of the tissues will be assessed by a standard hematoxylin and eosin (H&E) stain of paraffin embedded samples

- Immunohistochemistry tissue analysis:
  - INOS (pro-inflammatory markers);
  - IL-10 (anti-inflammatory marker),
  - F4-80 (macrophage),
  - co-localization of IL-10 and F4-80

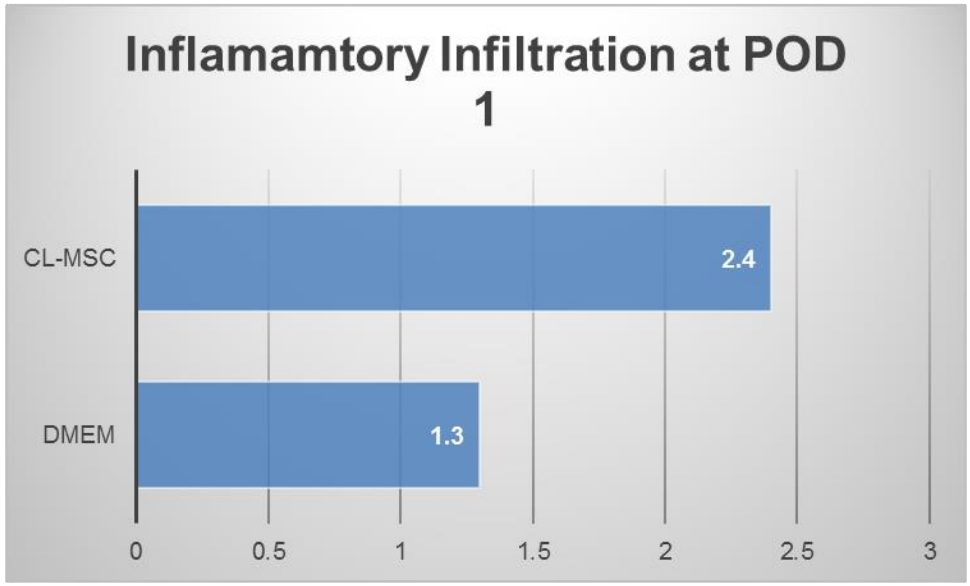
Post wounding Day 1: CLMSC treated mice elicit a higher level of tissue inflammatory infiltration compared to control group

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POST WOUNDING DAY 1: SEMI QUANTITATIVE RATING SCALE FOR INFLAMMATORY INFILTRATION

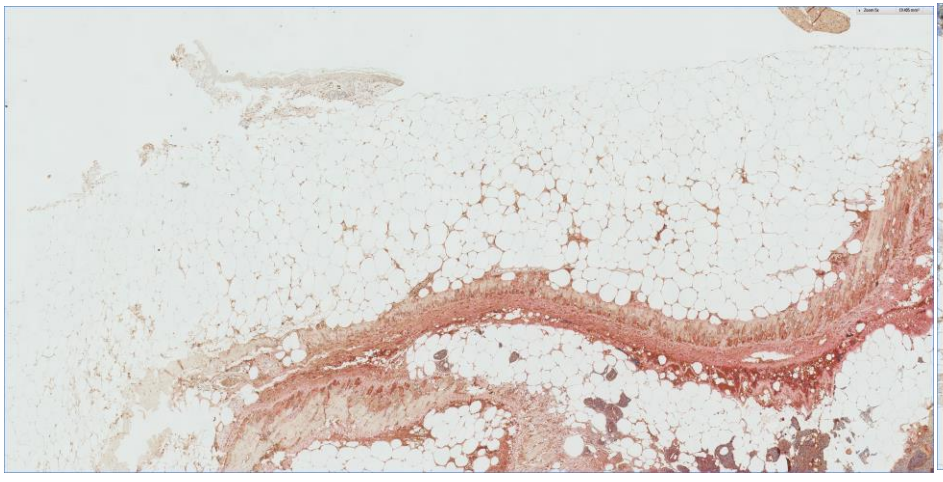
	0	1	2	3
Inflammatory Infiltration	Minimal	Mild	Moderate	Severe



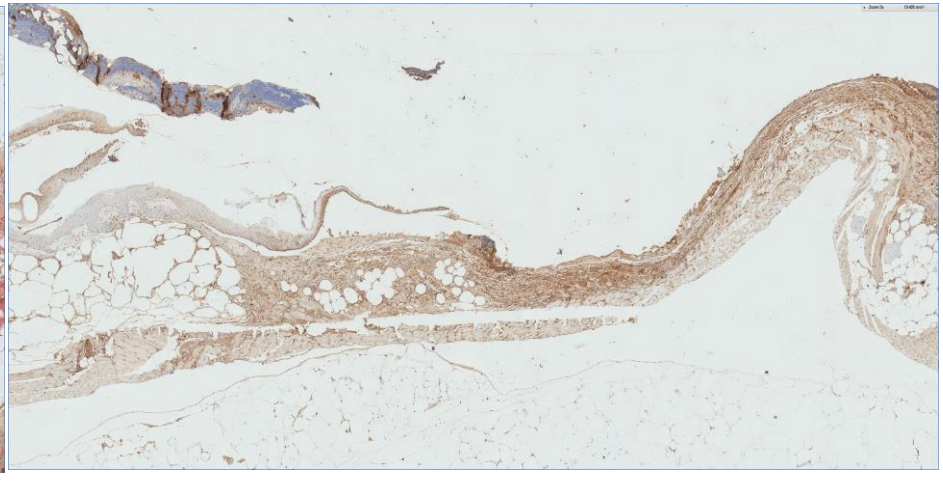
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Post Wounding Day 7: Upregulation of anti-inflammatory marker IL-10 (Red) Downregulation of pro-inflammatory marker- iNOS (brown) for **CL-MSC treated mice**; Control group persist with the presence of proinflammatory marker – iNOS (brown) with no presence of anti-inflammatory marker

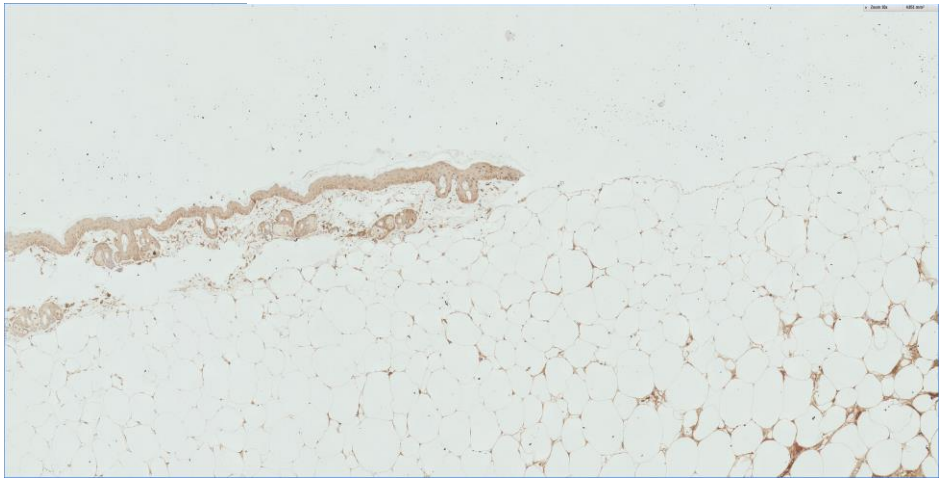
CLMSC POD 7 IL-10 RED X5



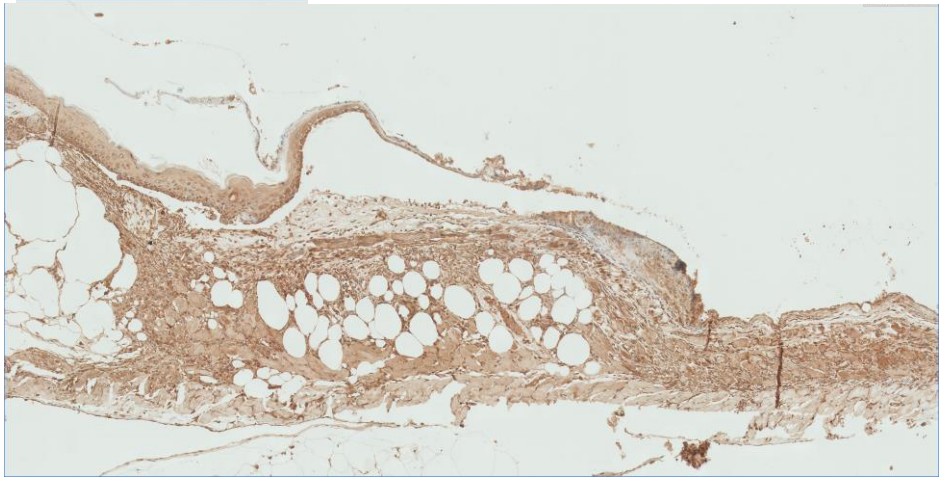
CTRL POD7 IL-10 RED



CLMSC POD 7 INOS- BROWN x10

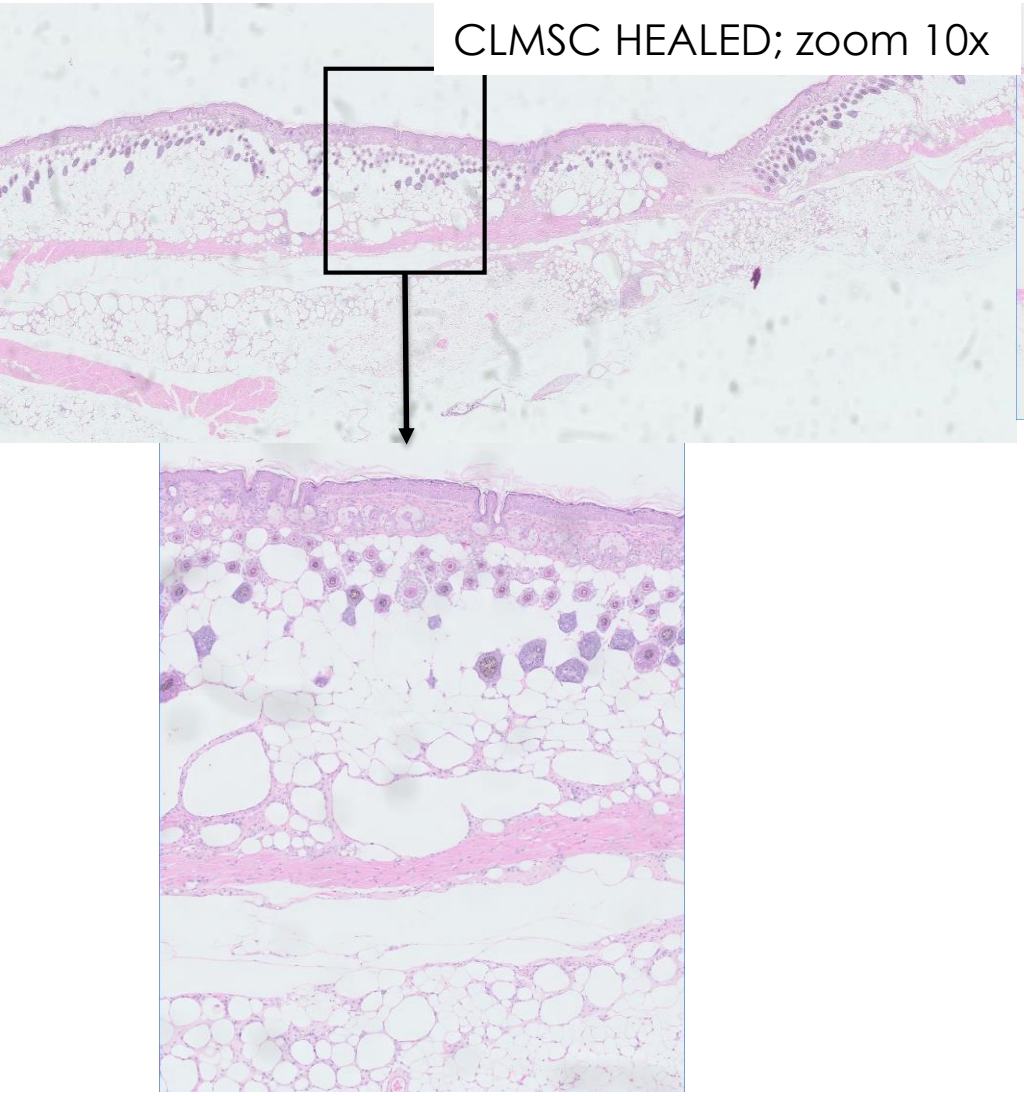


CTRL POD7 INOS- BROWN x10

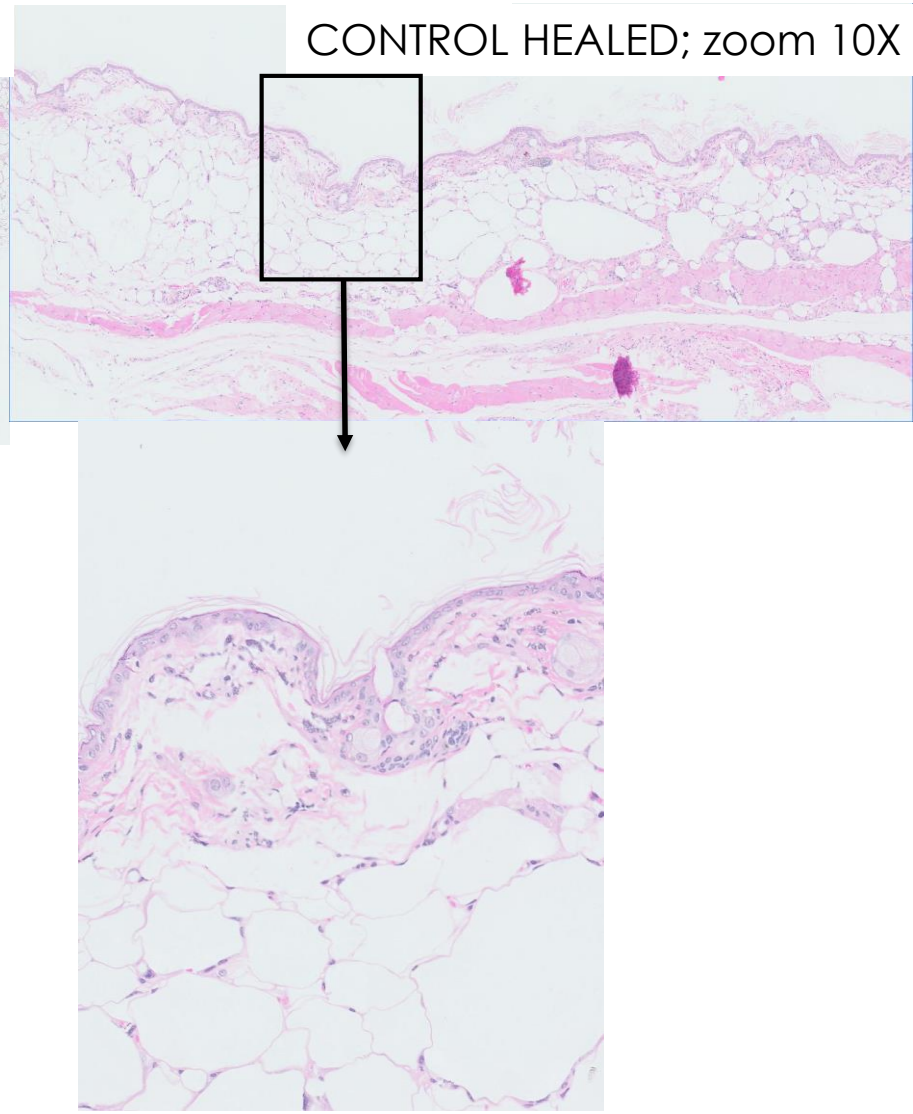


Wound Healed Timepoint: A fully differentiated multi layered epithelium (with stratum corneum) was developed in CL-MSC treated mice , while the epithelium regenerated in control group was not fully differentiated

CLMSC HEALED; zoom 10x



CONTROL HEALED; zoom 10X



CLMSC HEALED; zoom 20x at day 22

CONTROL HEALED zoom 20X at day 30

KEY FINDINGS

- The CLMSCs treated mice have a shorter wound closure time (mean closure day: 22.8 days), as compared to the control group (mean closure day 30.7)
- Post wounding Day 1: CLMSC treated mice elicit a higher level of tissue inflammatory infiltration compared to control group
- Post Wounding Day 7: Upregulation of anti-inflammatory marker IL-10. Downregulation of pro-inflammatory marker- iNOS for CL-MSC treated mice; Control group persist with the presence of proinflammatory marker – iNOS with no presence of anti-inflammatory marker
- Wound Healed Timepoint: A fully differentiated multi layered epithelium (with stratum corneum) was developed in CL-MSC treated mice , while the epithelium regenerated in control group was not fully differentiated

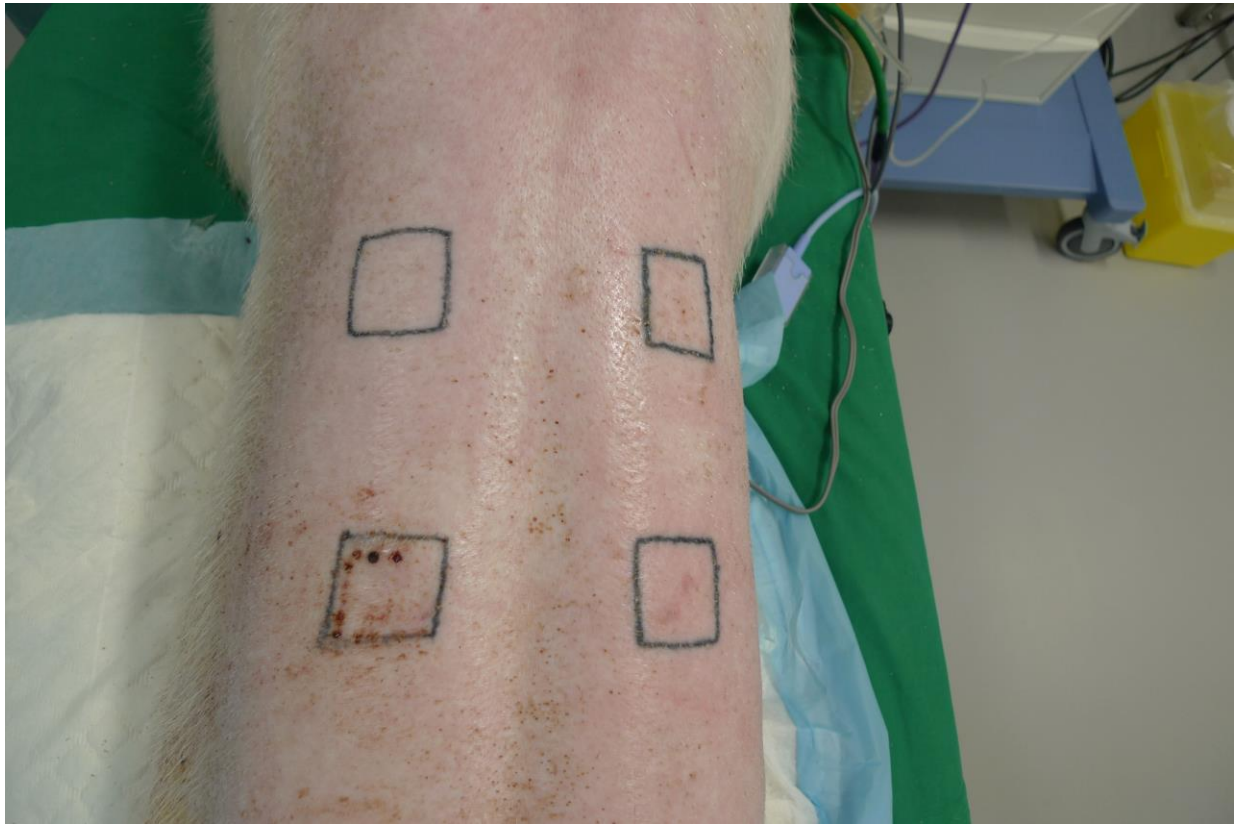
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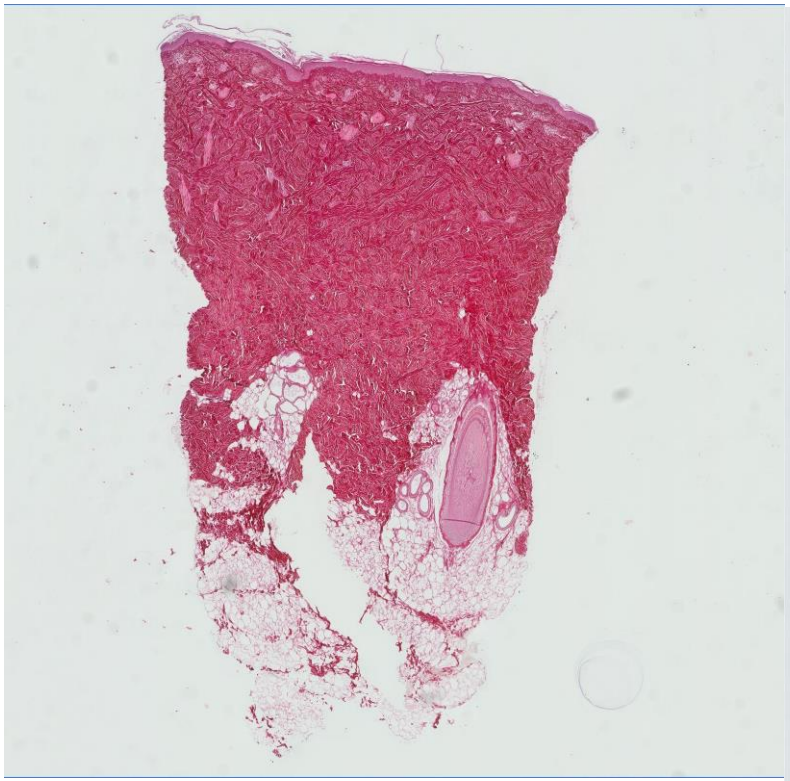
FUTURE WORK

ADVANCED WOUND MODEL IN DIABETIC PIG TO BE USED TO STUDY THE ROLE OF STEM CELL THERAPY IN DIABETIC WOUND HEALING

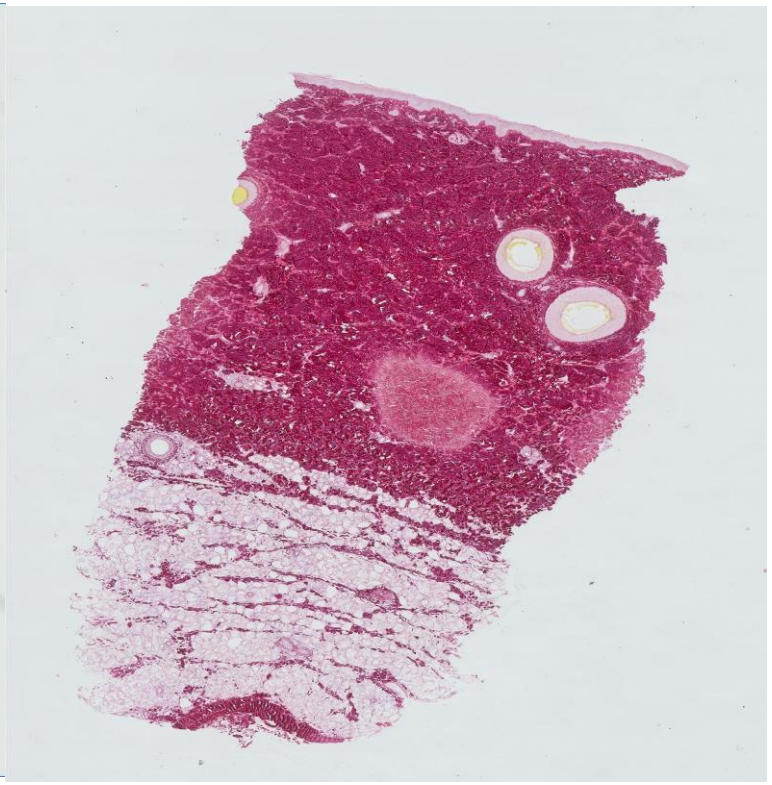


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STZ INDUCED DIABETIC PORCINE MODEL

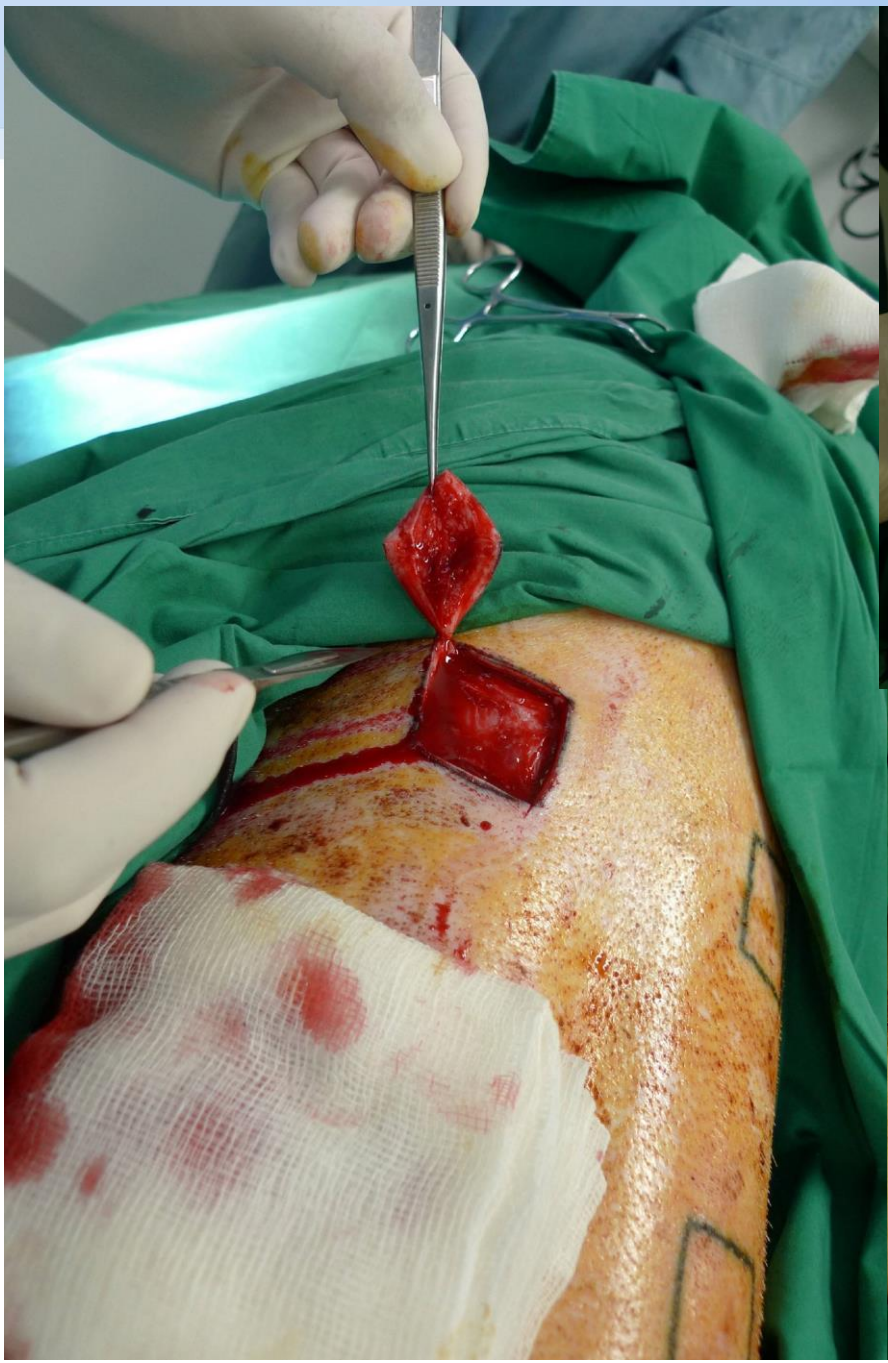


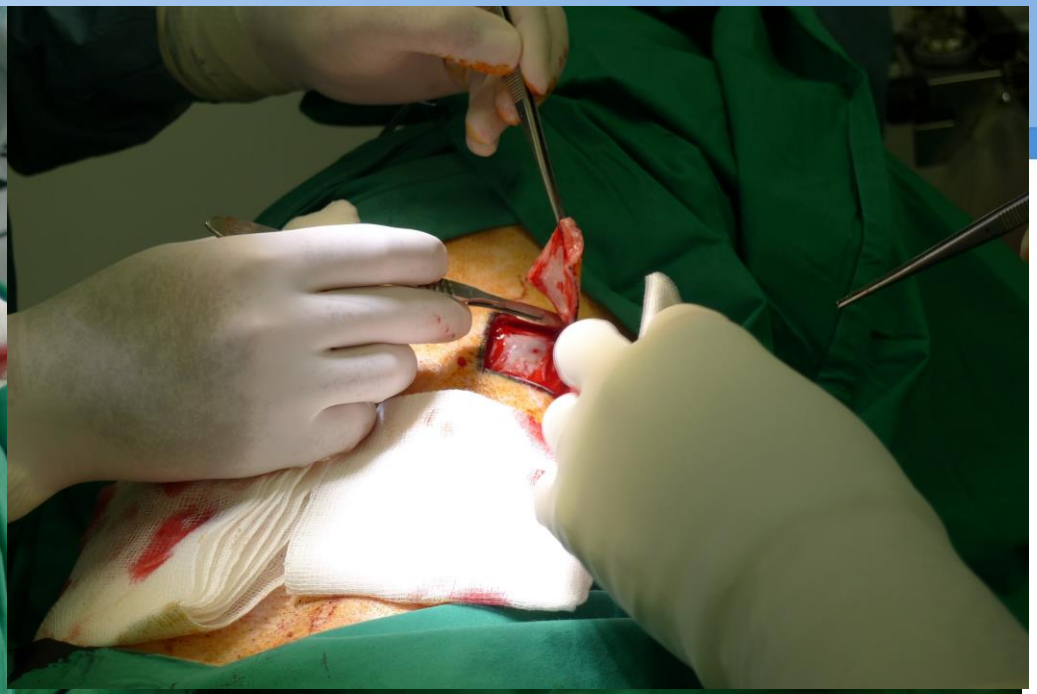
Pre STZ Induction



Post STZ Induction Day 42

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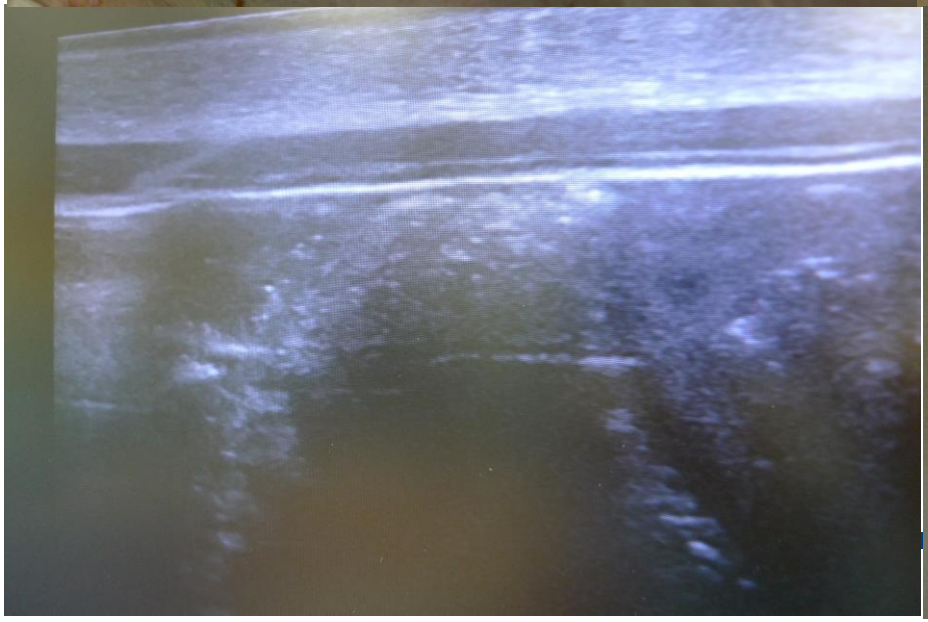


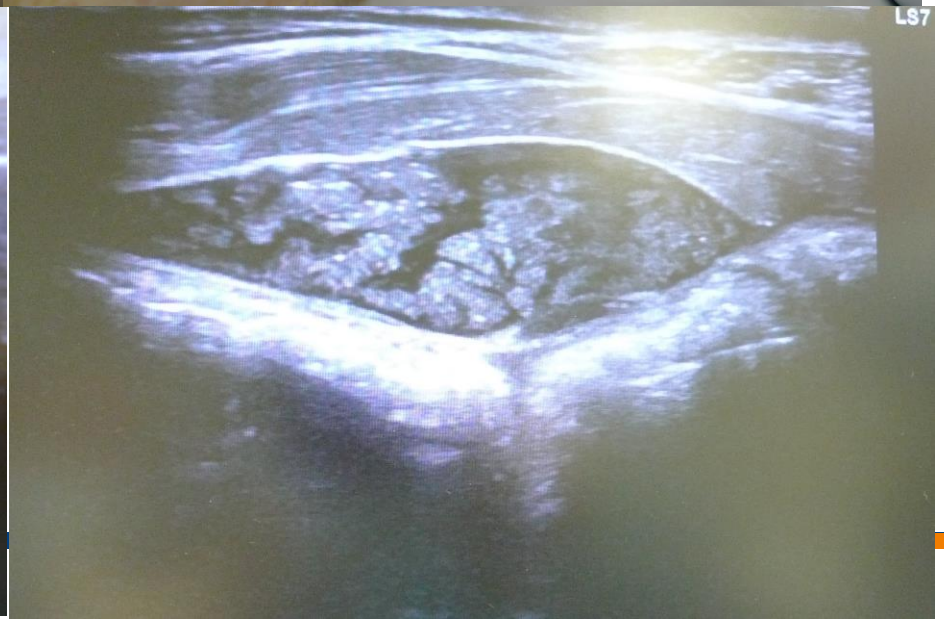


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THANK YOU

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