

Topical Application of CD362⁺ Human Mesenchymal Stem Cells (Cyndacel-M) Seeded in Excellagen™ Scaffold Augments Wound Healing and Increases Angiogenesis in a Diabetic Rabbit Ulcer Model

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Introduction

- Diabetic foot ulcers (DFU) is one of the most common complication of diabetes
- 15-25% of all patients with diabetes will develop DFU
- 15-fold higher risk of lower-extremity amputation with DFU
- cell-based therapy provides a novel therapeutic strategy for increasing wound closure and augmenting angiogenesis.



Objectives

1. To compare the efficacy of **topical administration of CD362⁺, CD362⁻ and PA-SSC** in wound healing in the alloxan-induced rabbit model of diabetic ulceration
2. To determine if **combined (topical + IV) delivery of cells** is more effective in wound healing and will improve glycemic control
3. To understand the **mechanism** of SSC mediated wound healing in diabetes mellitus.

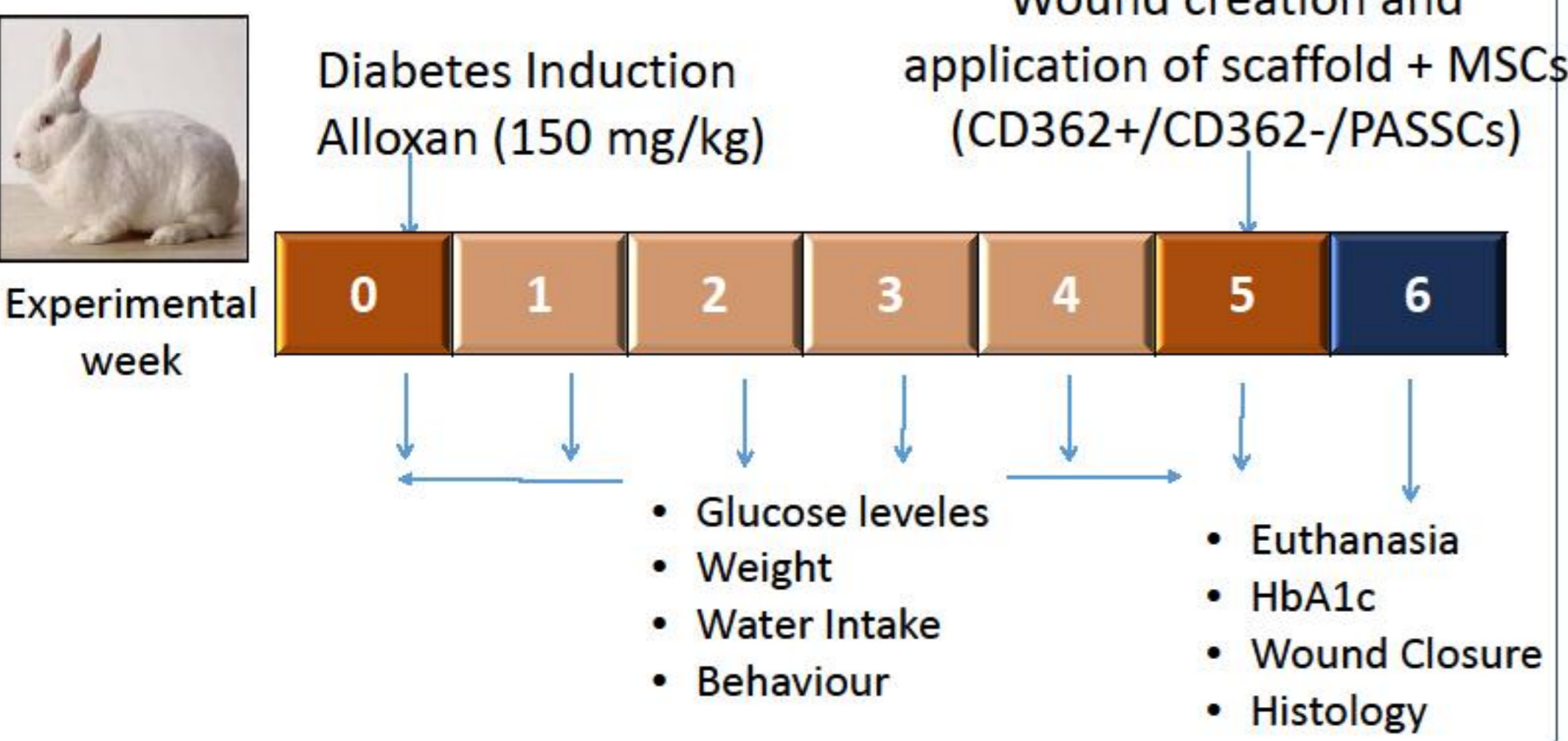
Methods

Induction of hyperglycemia: Diabetes was induced in rabbits by using 150mg/kg alloxan (Sigma-Aldrich) in 10ml saline and administered via an ear vein using an intravenous cannula. Blood glucose was checked daily using a glucometer from a pinprick of the marginal ear vein.

Surgical procedure-After 5 weeks of hyperglycemia, wounds were created by using sterile, disposable 6-mm punch biopsies. Each wound was treated with one of the randomized treatment groups

Diabetic Rabbit Ear Ulcer Model

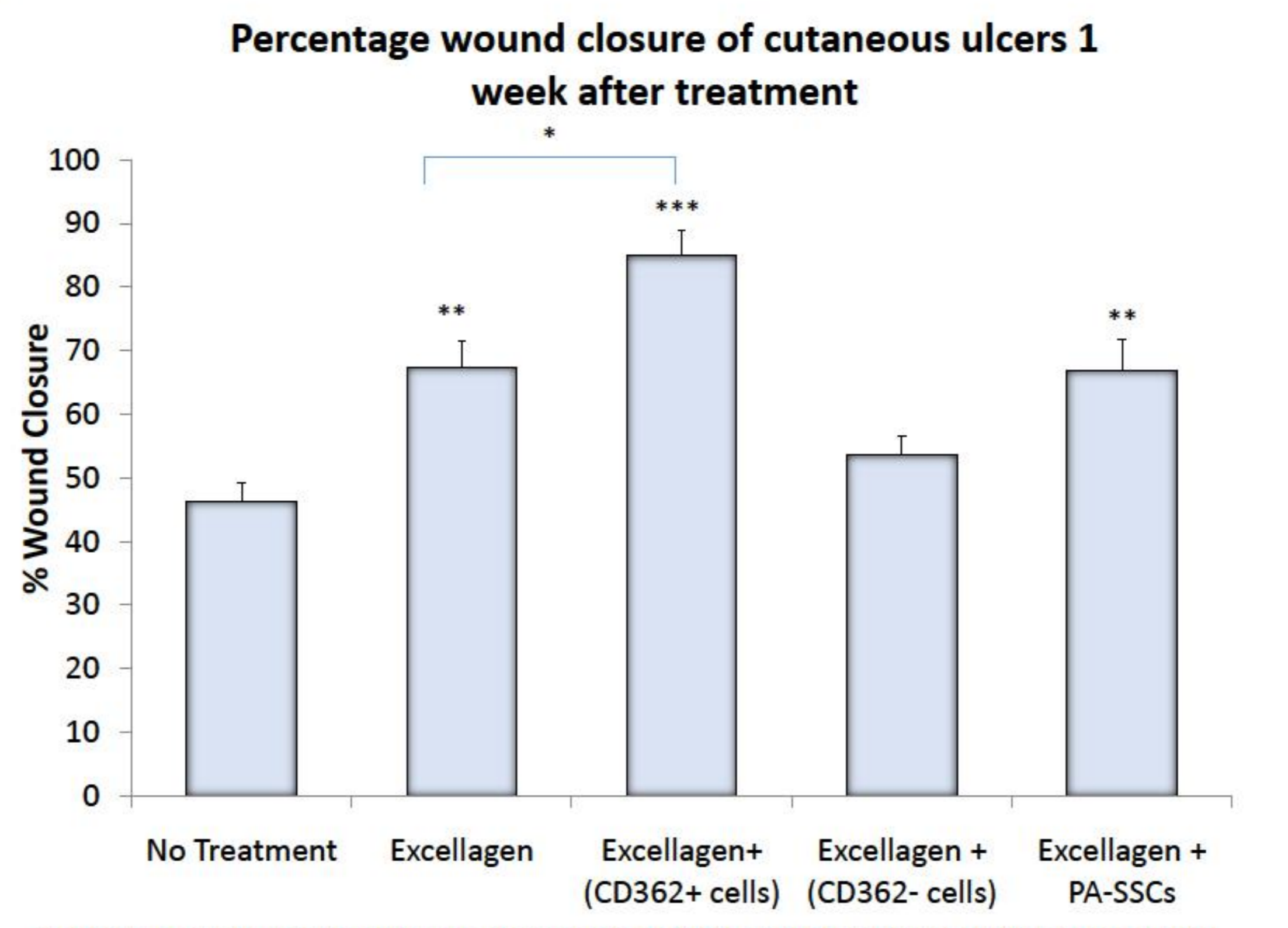
New Zealand white rabbits (3-3.5 Kg)



Results

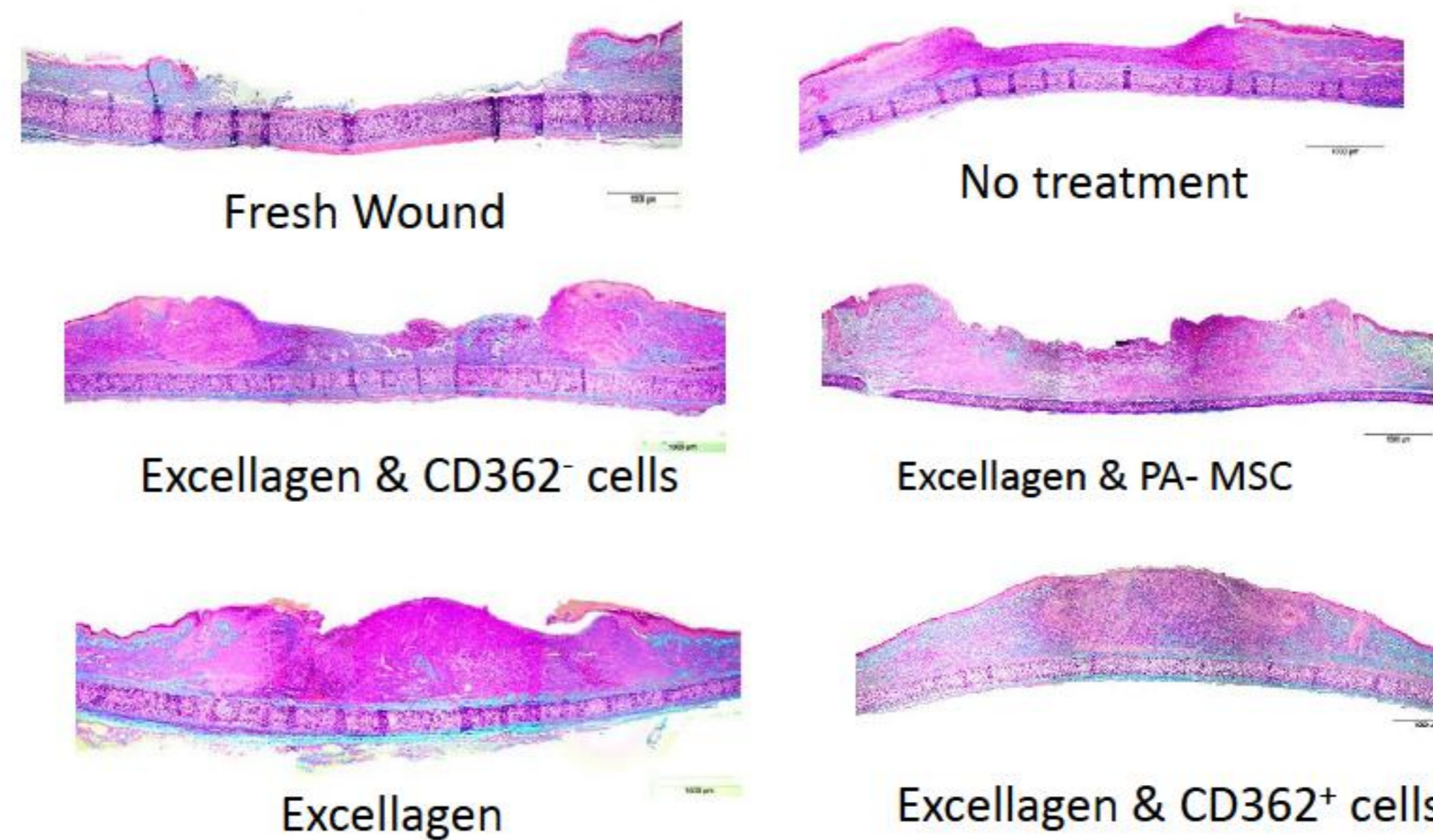
Study 1- Comparison of **topical administration of CD362⁺, CD362⁻ and PA-SSC** in diabetic wound healing

Group	Treatment
1	Control (No treatment)
2	Excellagen scaffold alone
3	Excellagen scaffold + 1,000,000 CD362 ⁺ cells
4	Excellagen scaffold + 1,000,000 CD362 ⁻ cells
5	Excellagen scaffold + 1,000,000 PA-SSC



Analysis in comparison with no treatment group using ANOVA and Tukey-Kramer Multiple Comparisons Test. N= *p<0.05, ** p<0.01, ***p<0.001. Error bars= SEM.

Masson's trichrome staining of rabbit ear ulcer wounds

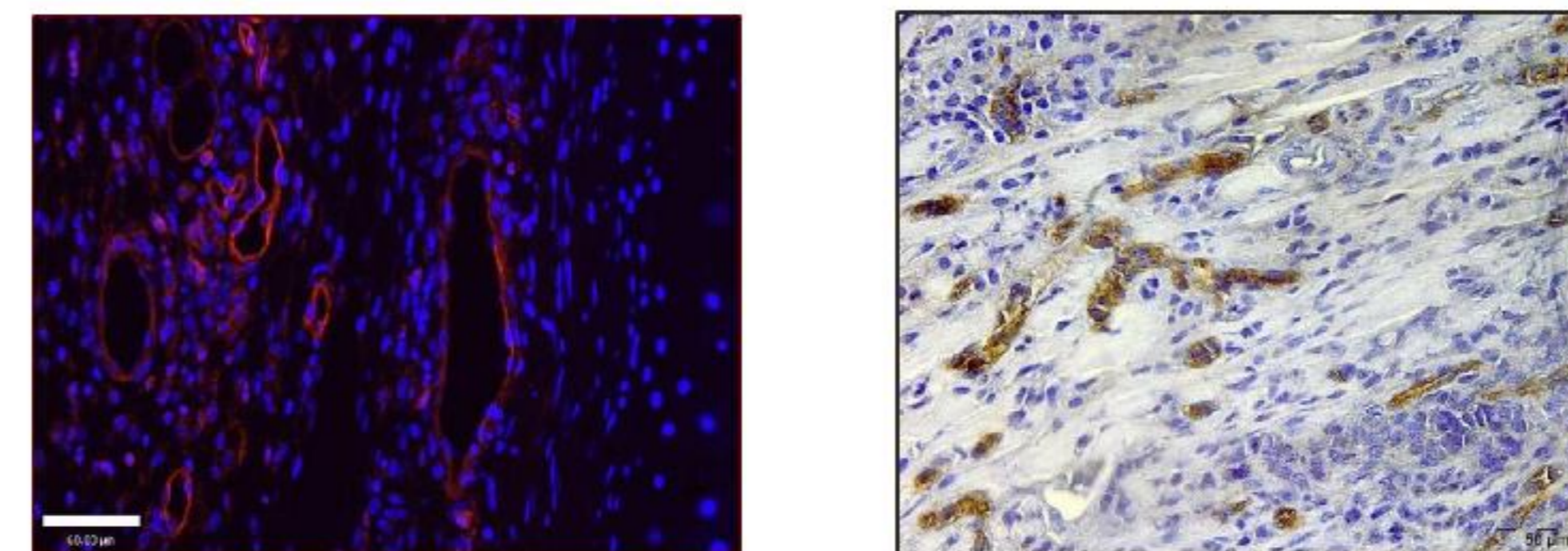


Stereological analysis of wounds in diabetic rabbits

	No Treatment	Excellagen	Excellagen+ (CD362 ⁺ cells)	Excellagen+ (CD362 ⁻ cells)	Excellagen + PA-SSCs
Wound volume (mm ³)	10.0±3.9	6.7±2.0	5.8±2.6	9.0±3.9	7.5±3.6
Surface density of blood vessels in wound (1/mm)	10.4±1.9	20.3±2.9 [#]	24.3±3.5 [#]	11.3±2.5	15.9±3.9
Surface area of blood vessels (mm ²)	99.3±35.4	141.8±62.1	134.1±48.7	102.6±53.8	112.2±56.2
Length density of blood vessels in wound (mm ²)	5246±775	10775±1588 [#]	13427±1431 [#]	6249±973	9014±1829
Total Length of Blood Vessels in Wound (mm)	49879±17226	72738±28719	74507±28485	52376±22351	63769±37226
Radial diffusion distance (µm)	8.01±0.58	5.59±0.42 [#]	4.94±0.25 [#]	7.37±0.57	6.33±0.71

Analysis in comparison with No treatment group using ANOVA and Tukey-Kramer Multiple Comparisons Test. * p<0.01, #p<0.001. Error bars= SD.

Specific staining's for endothelial cells/blood vessels



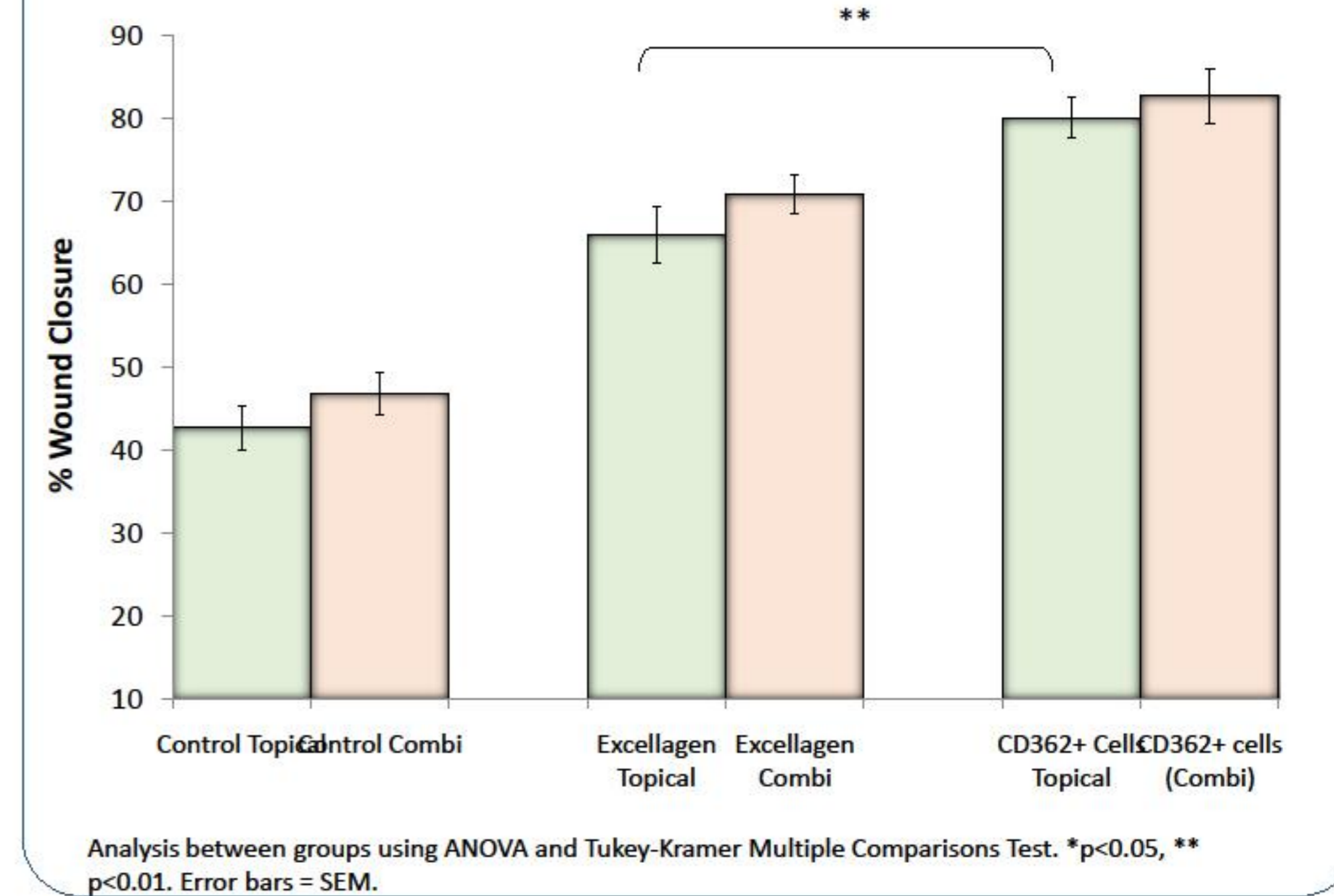
• Griffonia simplicifolia Lectin (GSL)-I isolectin B₄

IHC staining- CD31

Study 2- To determine if combined topical & IV delivery is more effective in wound healing and will improve glycemic control

Group	Treatment
1	Topical administration of Excellagen + 1x10 ⁶ optimal cells
2	Topical treatment with Excellagen + 1x10 ⁶ cells and intravenous delivery of 2x10 ⁶ cells/Kg body weight

Study 2- Percentage wound closure of cutaneous ulcers 1 week after combinatorial treatment



Analysis between groups using ANOVA and Tukey-Kramer Multiple Comparisons Test. *p<0.05, ** p<0.01. Error bars = SEM.

Summary and conclusion

Study 1: Effect of topical administration of CD362⁺, CD362⁻ and PA-SSC in diabetic wound healing

1. Excellagen accelerates the wound healing rate as compared to untreated wounds
2. Wounds treated with **1 x 10⁶ (CD362⁺ cells) in an Excellagen scaffold showed highest and most significant % wound closure** when compared with the untreated group at 1 week period
3. Increased percentage wound closure may be associated with more efficient neovascularization-Angiogenesis
4. A significantly increased surface density (S_v), length density (L_v) and reduced radial diffusion distance (R_{diff}) is observed in Excellagen + (CD362⁺ cells) treated wound groups in comparison to untreated wounds

Study 2 :Topical vs Combination study

1. Effect of combination (topical & IV) treatment- slight increase in % wound healing is observed in combination versus topical treated animals but this difference is not significant
2. Topical treatment alone- The wounds treated with Excellagen + (CD362⁺ cells) showed increased % wound closure compared to wounds treated with Excellagen alone and confirms the results seen with objective 1.

Thus, the application of CD362⁺ cells in an Excellagen matrix may lead a new therapeutic product with improved wound healing potential and in a less healing time.

References

1. O'Loughlin A et al. 2013 Topical administration of allogeneic mesenchymal stromal cells seeded in a collagen scaffold augments wound healing and increases angiogenesis in the diabetic rabbit ulcer. Diabetes 62(7):2588-94
2. REDDSTAR, Repair of Diabetic Damage by Stromal Cell Administration (www.reddstar.eu)

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