Development of a wound healing model produced from diabetic patient cells

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Introduction

Diabetic patients suffer from frequent formation of sores on the foot, called diabetic ulcers (DU), that are struggling to heal and subject to frequent infections that may require amputation of the lower legs. Thus, diabetes is the leading cause of non-traumatic amputations in the US. However, the link between diabetes and mechanisms compromising skin wound healing is not yet fully understood. To advance on a cure to DU, we developed a tissue-engineered skin produced from diabetic patient cells to reproduce the diabetic skin environment. We then studied the effect of substance P (**SP**), a neuropeptide involved in neurogenic inflammation, on this diabetic wound healing model (**dWHM**). We also conceived an endothelialized reconstructed connective tissue (**ERCT**) in order to study the effect of diabetic fibroblasts on angiogenesis and explore the effect of a treatment of Calcitonin Gene Related Peptid (CGRP) on this model.

Hypothesis

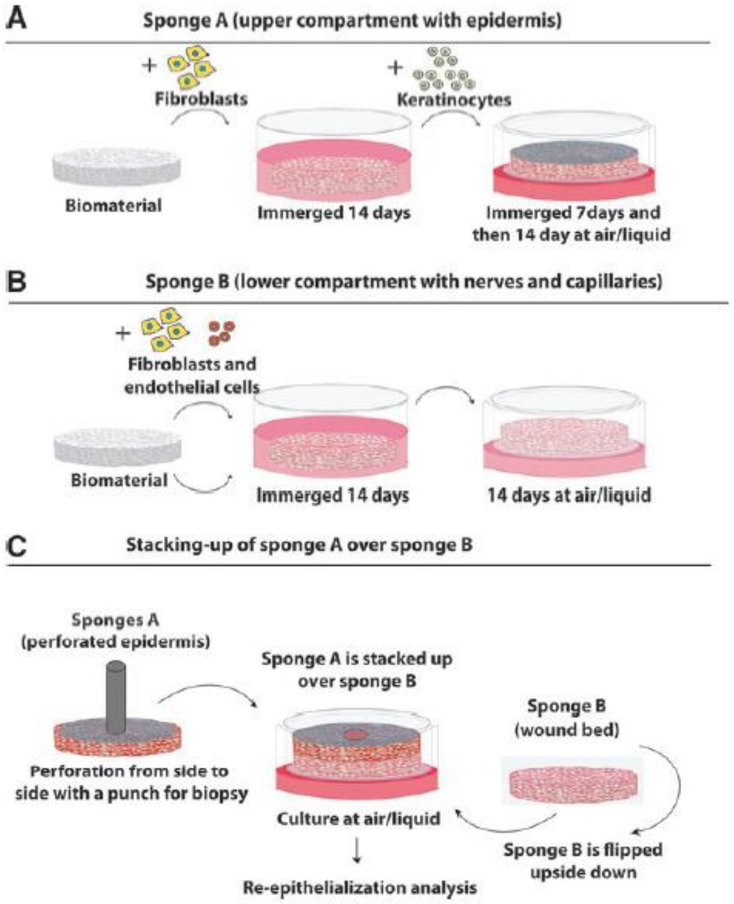
Our main hypothesis is that the lack of innervation induced by diabetic neuropathy in the skin of the patient is partly responsible for the impairment of the wound closure. Indeed, sensory neurons are involved in the regulation of the inflammation and reepithelialization through the release of neuropeptides such as substance P (SP) and Calcitonin-Gene Related Peptide (CGRP)[1,2]. These neuropeptides could be a lead for a potential treatment of DU[3].

Methods

Preparation of collagen-chitosan sponges

Types I and III bovine collagen and chitosan were dissolved in 0.1% acetic acid. Then, 1.3 mL/well of the final solution was poured in twelve-well plates frozen at -80°C, and lyophilized in a vacuum lyophilized. We then obtain sponges suitable for cell culture.

Preparation of skin substrates diabetic Wound Healing Model (dWHM)



Populations of fibroblasts and keratinocytes were extracted from the diabetic patient skin harvested after a foot amputation. The dWHM was prepared by seeding these diabetic fibroblasts and keratinocytes on a collagen-chitosan sponge and cultured at the air/liquid interface. Fibroblast and healthy endothelial cells were seeded on a second sponge. Once the epidermal layer was mature, a wound was performed with au biopsy punch on the sponge featuring an epidermis. The reepithelialization was then monitored during 8 days, with or without the addition of **10^{-7}M of SP** in the culture medium.
Controls were produced the same way using age-matched healthy fibroblasts and keratinocytes. Populations of keratinocytes and fibroblasts were extracted from skin from two diabetic patients and one healthy subject. 5 replicates were produce for each condition.

Preparation of Endothelialized Reconstructed Connective Tissue (ERCT)

4 population of human umbilical vein endothelial cell (HUVEC) were seeded on sponges with 4 populations of healthy or diabetic fibroblasts. After 10 days, the sponges are lifted to air/liquid interface for 7 days. **10^{-7}M of CGRP** in then added to the culture medium of treated ERCT seven days before fixation of the tissue.

Results

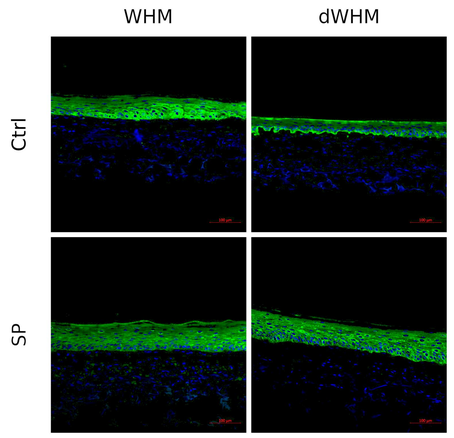


Fig.1 Immunofluorescence staining of K14 (green) and nuclei (blue) in WHM and dWHM at the end of the eight days of wound closure.

The epithelium is thinner in dWHM compare to WHM. However, the addition of SP has restored a morphology similar to the healthy model in the dWHM.
Scale bar: 100µm

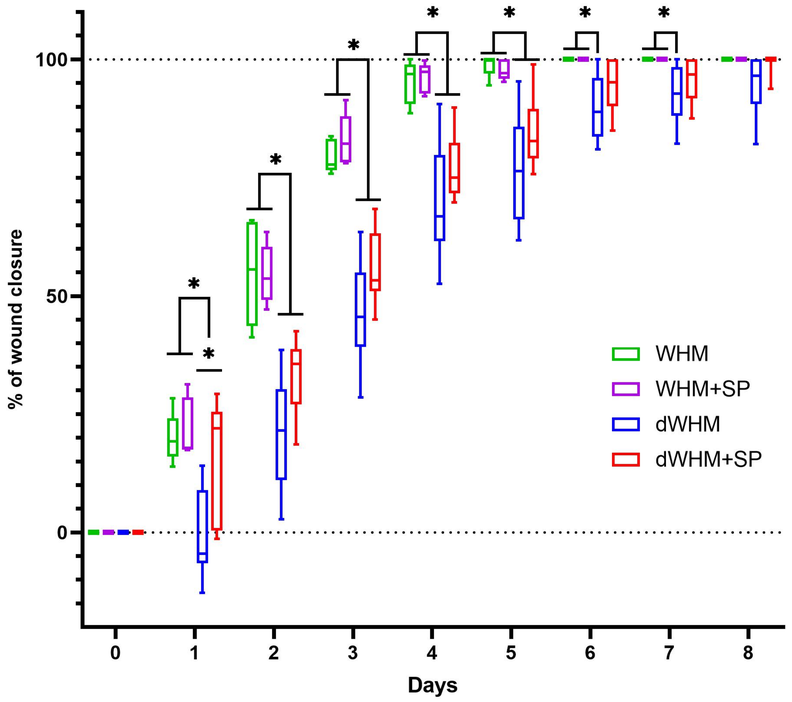


Fig. 2: Rate of wound closure

The reepithelialization of the wound is impaired in the diabetic wound healing model. The initiation of the wound closure is accelerated in the dWHM treated with SP.
Statistical significances were tested by an ANOVA with Tukey's post-hoc test (* p <0,05, n = 1 for WHM and 2 for dWHM)

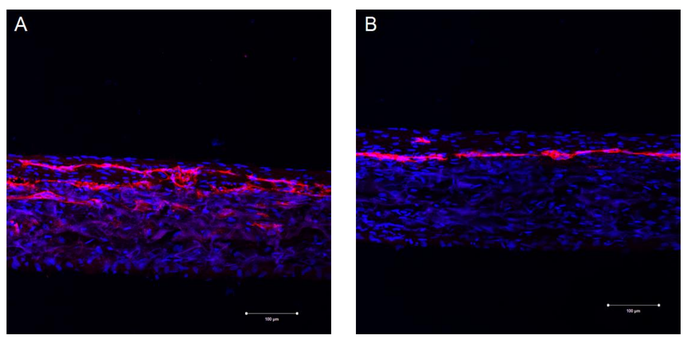


Fig. 3: Immunostaining of healthy ERCT (A) and diabetic ERCT (B) with PECAM-1 (red) and the nuclear marker DAPI (blue).

The capillary network in healthy endothelialized reconstructed connective tissue were able to form deep in the sponge whereas the capillary network on diabetic fibroblast formed in the superficial layer.

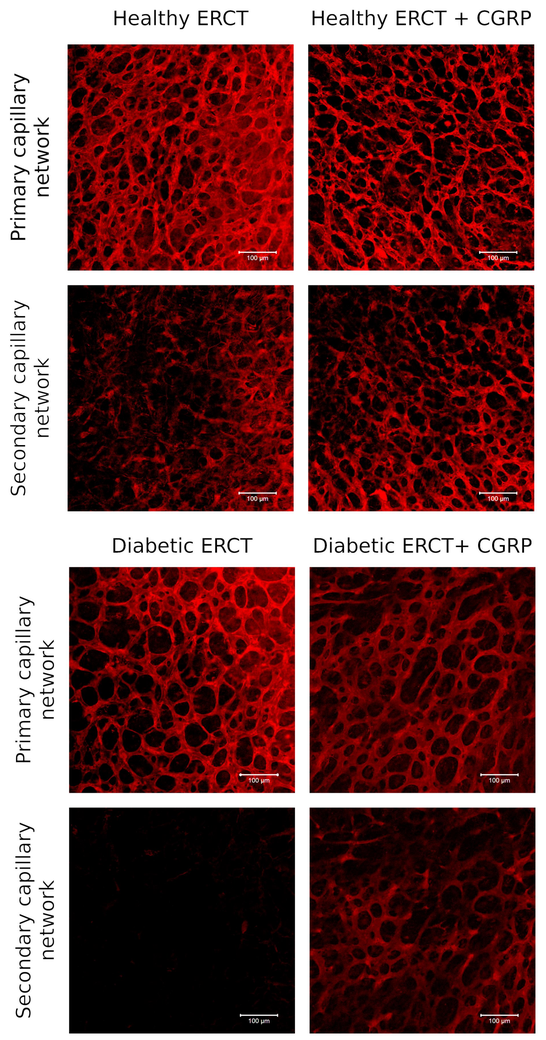


Fig. 4 : Whole tissue immunostaining of PEACM-1 in healthy and diabetic ERCT, with or without treatment with CGRP

Capillary network were able to form in each condition. However, it failed to form in the depth of the sponge in dERCT compare to healthy control. In addition, treatment with CGRP induced the formation of more capillary with larger diameter in dERCT.

Conclusion

- We produced a wound healing model able to mimic the condition of a diabetic ulcer, which is suitable to screen new therapies for DUs.
- Substance P enhance the initialization of reepithelialization in dWHM and the formation of a thicker epidermis.
- Capillary network fail to form in the depth of a reconstructed connective tissue produce with diabetic fibroblasts
- Treatment with CGRP induce the formation of a more developed capillary network in the dERCT and hERCT.

References

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