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Development in vitro of a glycated tissue-engineered wound healing model to mimic diabetic ulcers

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Introduction

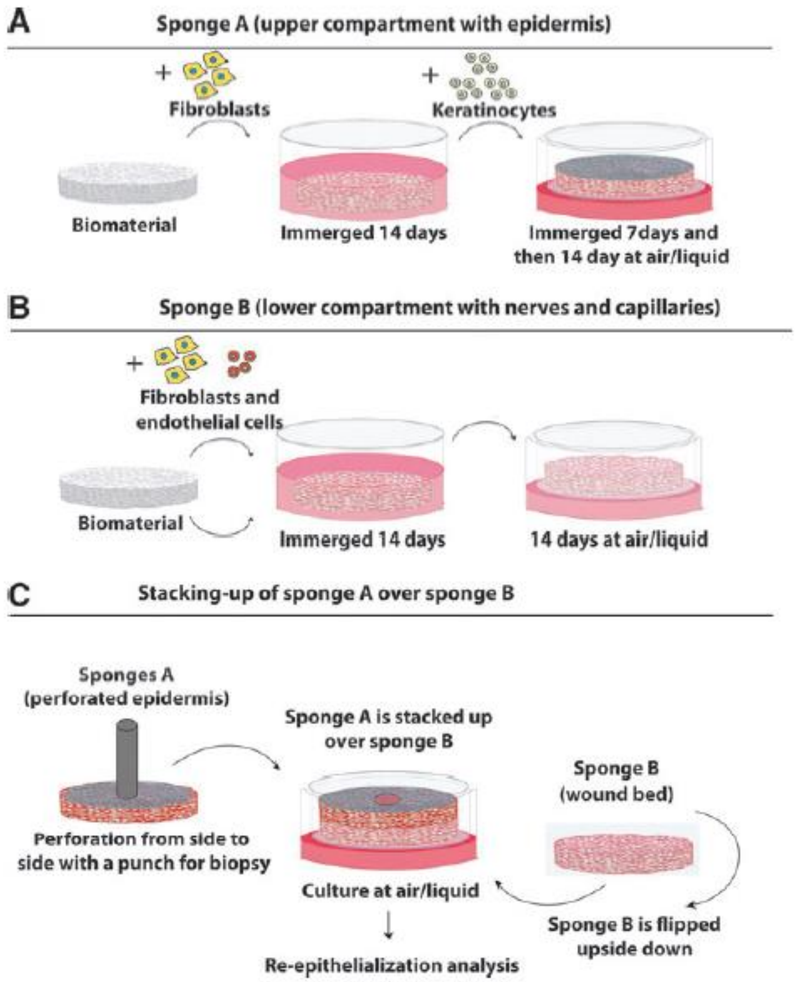
Diabetic ulcer (**DU**) is a major complication of diabetes. The formation of DU is notably caused by the accumulation of advanced glycation end-products (AGE) which induces diabetic neuropathy. To advance on a cure to DU, we developed a tissue-engineered skin treated with glyoxal, an glycation inducer, to mimic the diabetic skin environment. We studied the effect of aminoguanidine, an anti-glycation compound, on this wound healing model (WHM). Our objective is to assess the potential of our WHM to adequately mimic DU and to be used to develop new treatments.

Methods

Preparation of collagen-chitosan sponges

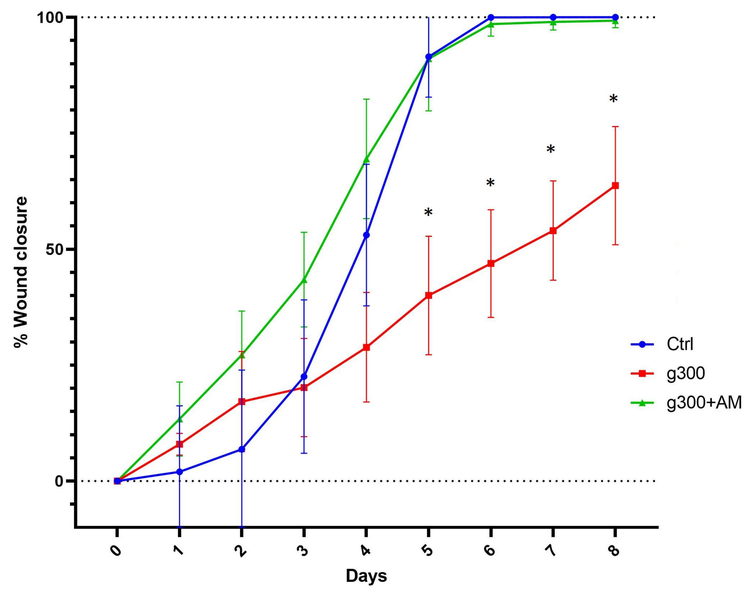
Types I and III bovine collagen and chitosan were dissolved in 0.1% acetic acid. Then, 1.3 mL/well of the final solution was poured in six-well plates frozen at -80°C, and lyophilized in a vacuum lyophilizer. We then obtain sponges suitable for cell culture.

Preparation of skin substrates



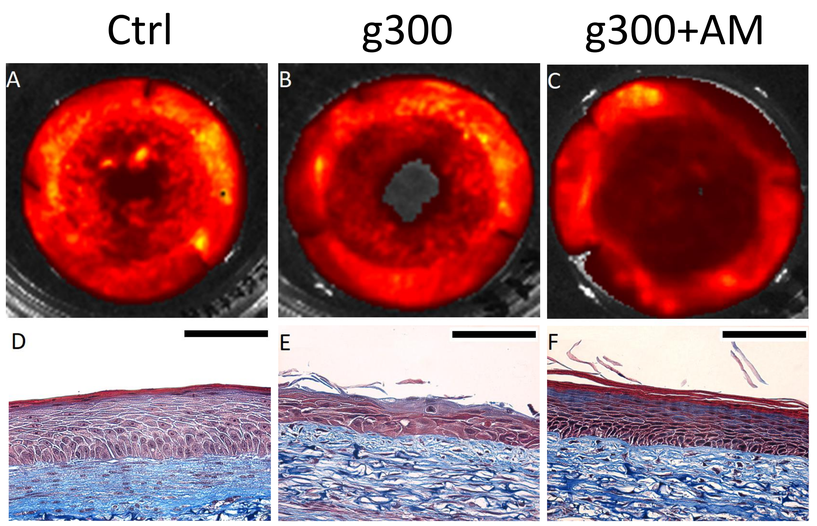
A week before perforation, 300µM of glyoxal (**g300**) or 300µM of glyoxal and 1,5 mM aminoguanidine (**g300+AM**) was added to the culture medium of both sponges and then every two days until the end of the experiment.

Results



Rate of wound closure

The addition of glyoxal in the culture medium successfully **impaired the reepithelialization** of the wound. The effects of glycation were canceled with addition of aminoguanidine witch demonstrate a **reversibility** of the reepithelialization impairment.
(* p <0,05, n = 7)



A,B,C: IVIS observation of WHM at the end of the eight days of wound closure. Red-yellow : keratinocytes

D,E,F: The histology of the WHM was assessed on 4 µm thick tissue cross-sections stained with Masson's Trichrome. The epithelium was disrupted in presence of glyoxal (E). However, with the addition of aminoguanidine (F), we obtained a similar structure to the control (D).
Scale bar: 100µm

Conclusion

The glycation of the skin substrates successfully impaired reepithelization in a manner similar to DU. The addition of an anti-glycation compound, the aminoguanidine, reversed the effect of glyoxal meaning that the model is able to respond positively to a potential treatment. Therefore, this model could be a step toward a successful care of DU that could lower the number of amputation due to chronic wound infection.