The hurdles of Collagen shrinkage: A promising GelMA-based organotypic skin approach

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INTRODUCTION: Collagen I (Col I) is the gold standard material to generate many *in vitro* tissue models, including organotypic skin. Col I remodeling by fibroblasts incorporated in the dermal part of the model leads to significant dimensional changes associated with additional hurdles when cultured within dynamic culture systems. These systems are relevant for the biofunctionality mimicry of the skin due to its multilayered nature and by providing the necessary fluid flow conditioning. Gelatin methacrylate (GelMA) is proposed in this study to validate a dimensionally stable organotypic skin model developed and maintained in a custom-made bioreactor [1], generating a dynamic *in vitro* testing platform.

METHODS: Human dermal fibroblasts (hdFbs)-laden 7.5% GelMA hydrogels were prepared with a 2.3×10^5 cell/ml density and crosslinked for 30 seconds under 7.2 mW/cm^2 UV light. After 14 days in culture, uniaxial compression test and dynamic oscillatory tests were performed. Mechanical properties were compared with standard Col I-based hydrogels. The organotypic skin model was prepared by coating a 0.26% Col I solution onto the GelMA hydrogels immediately after the encapsulation of hdFBs. The Col I was let to polymerize for 1 hour at 37°C and the whole construct was cultured for 7 days. After this, 5×10^4 keratinocytes (KCs) were seeded on top, and constructs were cultured for more 7 days before being lifted to air-liquid interface (ALI) for KCs stratification. The model was characterized histologically regarding cell differentiation (epidermis), basement membrane (dermal-epidermal interface) and dermal extracellular matrix deposition.

RESULTS: The storage modulus of GelMA presented a mean value of 1kPa throughout the culture, whereas the mean value of Col I, increased from 0.5 to 1.2kPa. Likewise, the compression modulus of GelMA of 18kPa was maintained, in contrast to the 2-fold stiffer value of Col I at day 14. The shrinkage ratio of GelMA and Col I was circa 20% and 83%, respectively. The obtained organotypic model was characterized by a fully differentiated epidermis and extensive proliferation of hdFbs coupled with significant Col I and Fibronectin deposition.

CONCLUSION: So far, the generated GelMA-based organotypic skin seems to be a suitable candidate for surpassing the struggles associated with collagen shrinkage observed in the standard organotpic skin model. Future experiments will focus on the long term dynamic culture of the skin equivalent in the recently developed sandwich-like bioreactor.

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REFERENCES

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