3D bi-layer skin construct fabricated using recombinant spider silk-based non-woven meshes and foams

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Spider silk is an exceptional biomaterial exhibiting excellent biocompatibility, low immuno-reactivity [1], microbial repellence [2], and remarkable mechanical properties [3]. Thus, it holds great potential for biomedical applications. The recombinant spider silk protein eADF4(C16) has been shown to be suitable for tissue engineering [4,5], i.e., for bioink development or 3D scaffold generation. In this study, eADF4(C16) and eADF4(C16)-RGD, a cell-adhesion peptide-functionalized variant, were fabricated into porous foams representing the dermal skin layer and non-woven meshes resembling the basement membrane. Good cyto-compatibility, cell adhesion and proliferation was demonstrated.

bi-layer skin construct from recombinant spider silk

engineered ADF4 (eADF4) production

sequence extracted from spider silk: "C-module"

GSSAAAAAAAASGPGGYGPENQGPSGPGGYGPGGP

dragline silk

C-module ↓ 16x

HOOC – eADF4(C16) – NH_2

Araneus diadematus

cytocompatibility, bacterial repellance, low immunoreactivity

HOOC – eADF4(C16)-RGD – RGD (cell adhesion peptide)

enhanced cell proliferation and adhesion

epidermis

scaffold-free epidermis

↓

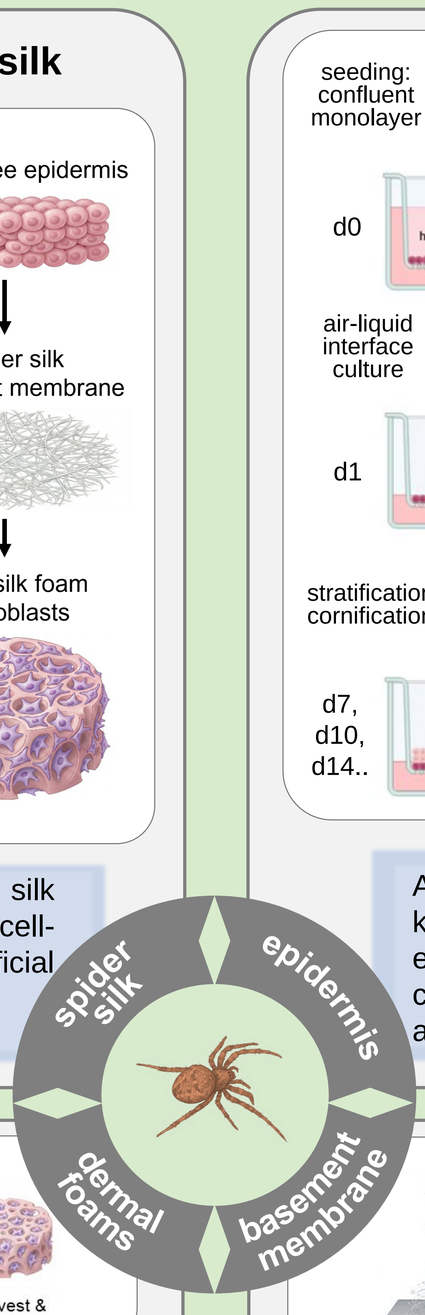
spider silk basement membrane

↓

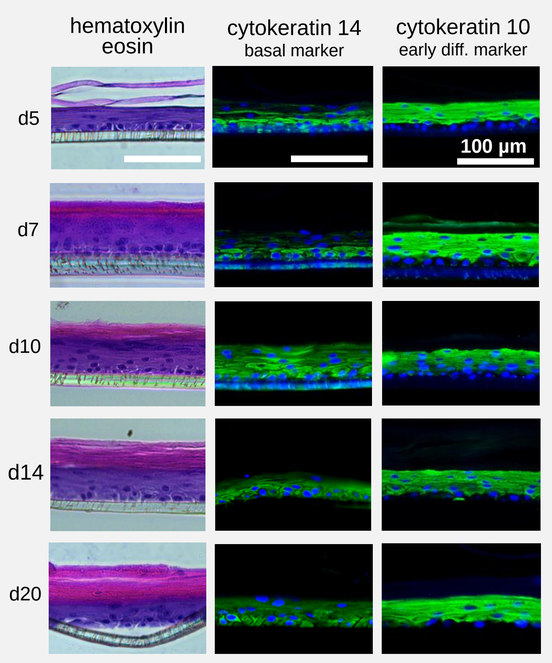
dermis

spider silk foam + fibroblasts

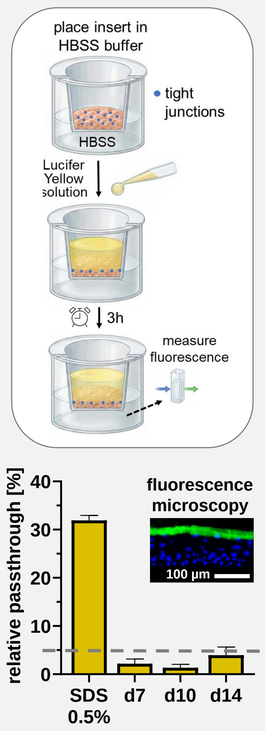
A bi-layer skin construct is fabricated using the recombinant spider silk protein eADF4(C16)-RGD, a variant of eADF4(C16) that carries the cell-adhesion peptide RGD. Primary keratinocytes are seeded onto an artificial spider silk basement membrane, while the dermal layer is represented by a spider silk-derived foam colonized with human fibroblasts.



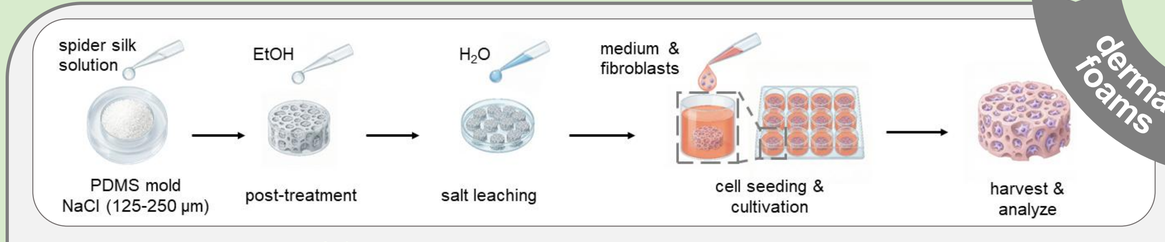
keratinocyte differentiation and cornification



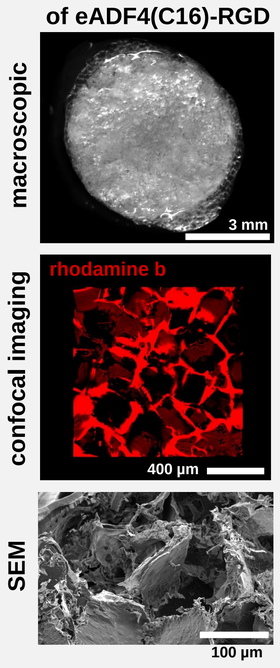
barrier function



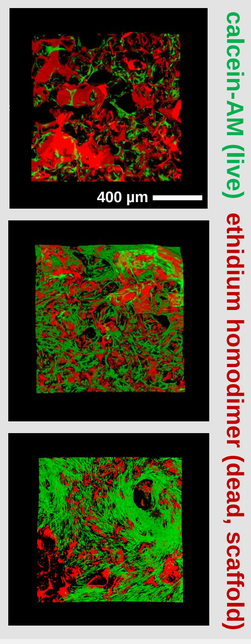
A stratified 3D epidermis is successfully obtained by cultivating primary keratinocytes in commercially available inserts. While hematoxylin and eosin (H&E) staining shows increased cornification over time, cytokeratin expression reveals *in vivo*-like maturation. Lucifer yellow assay shows good barrier function on day 7, 10 and 14.



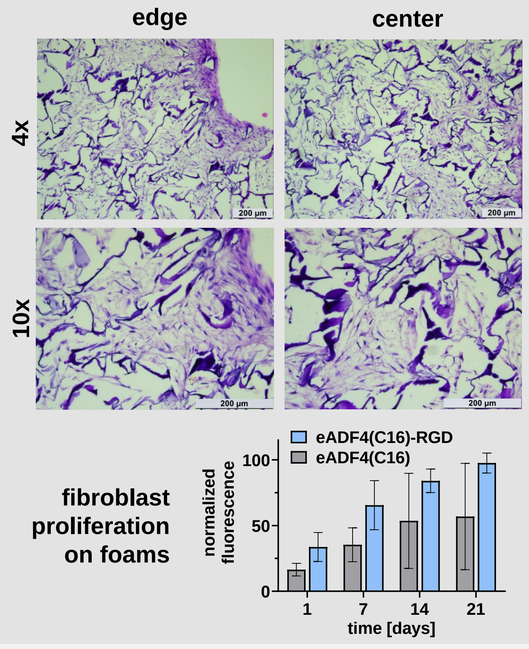
foam structure of eADF4(C16)-RGD



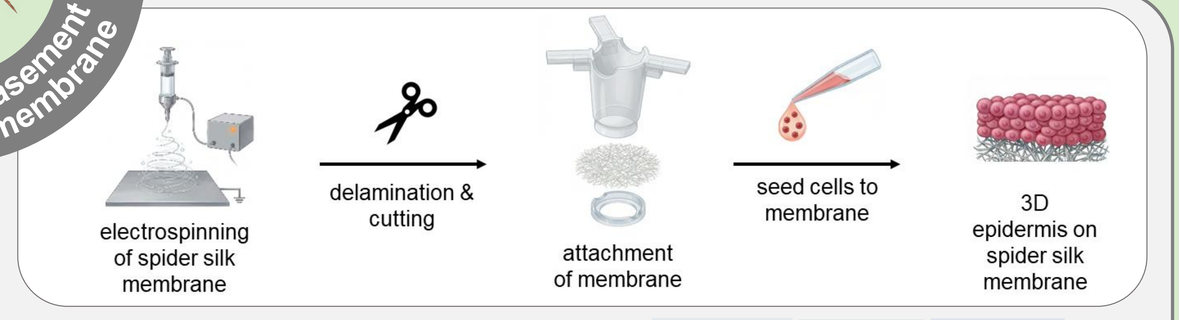
fibroblast viability



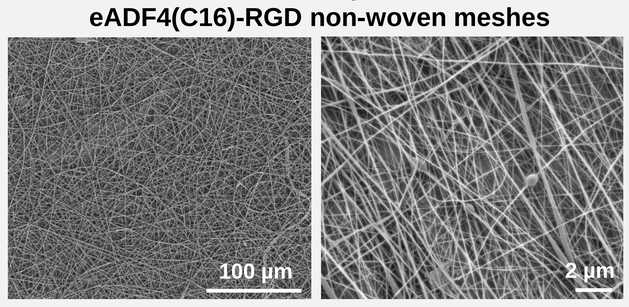
H&E staining: fibroblasts on foams

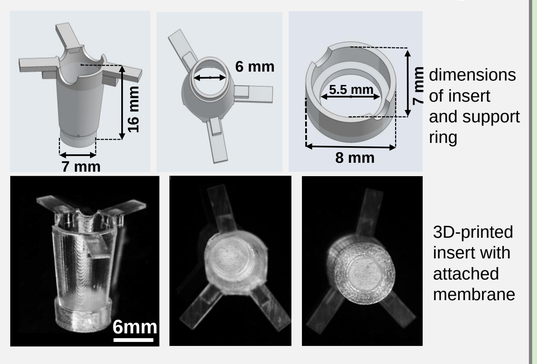


eADF4(C16)-RGD foams with a pore size of 125-250 µm are fabricated by salt-leaching. Fibroblasts cultivated on eADF4(C16)-RGD foams display high cell viability and homogenous distribution throughout the scaffold. Cell proliferation on eADF(C16)-RGD was higher compared to eADF4(C16).



electro-spun eADF4(C16)-RGD non-woven meshes





Spider silk fibers with an average diameter of 50-200 nm are electro-spun into thin non-woven meshes representing the basement membrane. Subsequently, non-woven meshes are attached to customized 3D-printed cell culture inserts. An epidermis will be cultivated on such spider silk basement membrane.

Conclusion. Dermal foams made from eADF4(C16)-RGD are tunable porous scaffolds showing high cytocompatibility and good fibroblast adhesion and proliferation. Additionally, non-woven meshes fabricated from eADF4(C16)-RGD fibers, which mimic the basement membrane, are promising constructs for subsequent epidermal cultivation at the air-liquid interface.

References

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