Decellularized human dermal extracellular matrix-derived scaffolds: compositional, mechanical, and *in vitro* biological characterizations

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Abstract

Currently, there is a growing demand for *in vitro* skin models. In search of a more realistic approximation of the tissue microenvironment, here, we present an optimized protocol for extracting and characterizing hydrogels obtained from decellularized dermal extracellular matrix from human skin to generate scaffolds for subsequent use in 3D skin models.

Introduction

The skin is the largest organ of the body and plays a key role in various bodily functions (van den Broek et al. , 2017). To understand the intricate dynamics of normal tissue physiology, disease progression, and the development of therapeutics and consumer health products, it is imperative to employ experimental models that faithfully recapitulate the fundamental functions of the skin.

Existing *in vitro* models of human skin predominantly comprise a dermis composed of purified type I collagen scaffolds and dermal fibroblasts, supplemented by epidermal keratinocytes on the surface. However, these scaffolds have several drawbacks. Excessive shrinkage of the hydrogel and its rapid degradation shorten its lifetime and hinder its long-term applicability. In addition, they do not adequately represent the cellular microenvironment of the dermis (Risueno et al. , 2021).

To improve existing *in vitro* skin models, in this work we present scaffolds generated from human dermal extracellular matrix (dECM). The extracted dECM retains numerous components found in the skin, thus representing the cellular microenvironment more accurately.

Matherial and methods

Following and modifying the protocol of Wolf *et al*. (Wolf et al. , 2012), we decellularized and extracted the dECM from frozen human skin samples. After digesting the dECM, correct decellularization was corroborated by the quantification of genomic DNA following the protocol of the AllPrep® DNA/RNA MicroKit (Quiagen) and by 2% agarose gel electrophoresis.

To determine the composition of the dECM obtained, the amount of lipids, collagen, sulphated glycosaminoglycans (sGAG) and elastin was quantified by oil red staining, Hydroxyproline Assay Kit (Sigma-Aldrich), DMMB chromotropic agent and Fastin™ Elastin Assay Kit (Biocolor), respectively. Immunofluorescence was performed on hydrogels fixed in 4% parafomaldein (PFA) against type 1A collagen (SC-59772, Santa Cruz Animal Health), type 3A1 collagen (SC-271249, Santa Cruz Animal Health), type 4A collagen ((SC-59814, Santa Cruz Animal Health) and fibronectin (SC-8422, Santa Cruz Animal Health), and the hydrogels were also observed by scanning electron microscopy (JSM 6360-LV). Mechanical properties of hydrogels were determined by rheology (Haake Mars 40^{TM}) operating with a 25mm parallel plate geometry and a gap of 0.5mm. Studies of gelation kinetics, frequency sweeps and amplitude sweeps were performed to obtain the elastic modulus (G') and viscoelasticity modulus (G'').

Finally, to determine the biocompatibility, dECM hydrogels with embedded dermal fibroblasts (HDF) or with monolayers of keratinocytes (HaCaT) on the surface were prepared for an MTT assays (Sigma-Aldrich) after 1 week of culture.

Results

dECM hydrogels were successfully prepared at 2 and 4 mg/mL. 4 mg/mL hydrogels were stiffer than 2 mg/mL hydrogels and at both concentrations the hydrogels were manipulable, and the edges were defined. DNA could be removed from the tissue and components of dECM were quantified. As expected, the amount of collagen, sGAG and elastin in the dECM hydrogel was higher than in commercial collagen hydrogels (Fig. 1). Furthermore, the extraction process effectively removed lipids. This elimination is crucial as lipids hinder the gelation of hydrogels (Sackett et al. , 2018). Moreover, lipids are not a natural constituent of the dermis but rather belong to the hypodermis.

Immunofluorescence microscopy observation allowed the detection of type 1A, type 3A1 collagen and fibronectin fibers, characteristic of the dermis. Type 4A collagen was not detected indicating the correct separation of epidermis and dermis in the extraction. The fibers showed random orientation generating a three-dimensional network similar to scaffolds generated from commercial material.

Rheological analysis of hydrogels showed that storage modulus (G´) was higher than loss modulus (G´´), indicating the dominance of elastic properties. In addition, dECM hydrogels exhibited strain-stiffeness behavior, similar to biomaterials used to generate scaffolds (Xu et al. , 2023).Finally, the scaffold proved to be biocompatible with skin cells (Fig 2).

Conclusions

Hydrogels generated from decellularised human dermal ECM maintain components of the demis, generating a cellular microenvironment that adequately mimics native skin. The adequate survival of keratinocytes and fibroblasts in the scaffold indicates that it may be a good candidate for 3D skin models. dECM hydrogels are presented as an alternative to the use of commercial materials for the generation of scaffolds and in future work will be incorporated into advanced skin models in microfluidic systems.

REFERENCES

1. VAN DEN BROEK, L. J.*, et al*. ISSN 2629-3277. 2017. Progress and Future Prospectives in Skin-on-Chip Development with Emphasis on the use of Different Cell Types and Technical Challenges. *Stem cell reviews and reports.* 13(3), pp 418-429.

2. RISUENO, I.*, et al*. ISSN 2473-2877. 2021. Skin-on-a-Chip Models: General Overview and Future Perspectives. *APL bioengineering.* 5(3), pp 030901.

3. WOLF, M. T.*, et al*. ISSN 1878-5905. 2012. A Hydrogel Derived from Decellularized Dermal Extracellular Matrix. *Biomaterials.* 33(29), pp 7028-7038.

4. SACKETT, S. D.*, et al*. ISSN 2045-2322. 2018. Extracellular Matrix Scaffold and Hydrogel Derived from Decellularized and Delipidized Human Pancreas. *Scientific reports.* 8(1), pp 10452-1.

5. XU, J., Y. JIANG, and L. GAO. ISSN 2050-7518. 2023. Synthetic Strain-Stiffening Hydrogels Towards Mechanical Adaptability. *Journal of materials chemistry.B.* 11(2), pp 221-243.

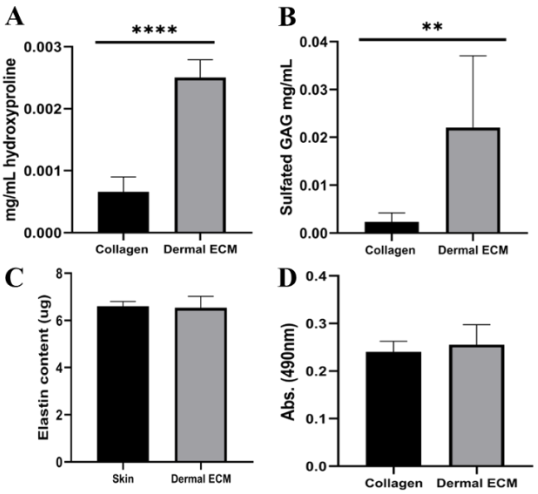

Figure 1. Quantification of **(A)** collagen, **(B)** sGAG, **(C)** elastin and **(D)** lipids in dECM.

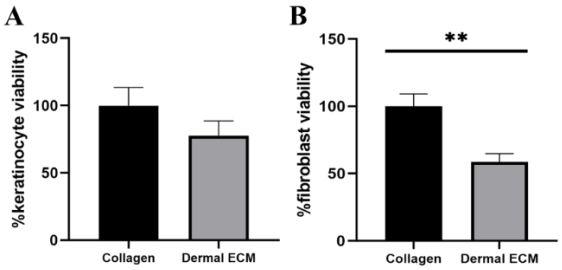

Figure 2. Viability of **(A)** keratinocytes on the hydrogel surface and **(B)** fibroblast embeebed in hydrogels.