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Aged human dermal extracellular matrix as an innovative scaffold for aging on chip model

Estibaliz Fernandez-Carro[1], Ana Rosa Remacha[1], Alberto Garcia-Barrios[2], Irene Orera[3] , Giuseppe Lattanzio[3], Jesús del Barrio[4], Clara Alcaine[1] and Jesús Ciriza[1,2]

[1] Tissue Microenvironment Lab (TME). Aragón Institute of Engineering Research (I3A) University of Zaragoza, 50009 Zaragoza, Spain.
[2] Department of Anatomy and Histology, Faculty of Medicine, University of Zaragoza, 50009 Zaragoza, Spain
[3] Proteomics Research Core Facility, Instituto Aragonés de Ciencias de la Salud (IACS), 50009 Zaragoza,
[4] Aragón Institute of Nanoscience and Materials (INMA) University of Zaragoza, 50009 Zaragoza, Spain

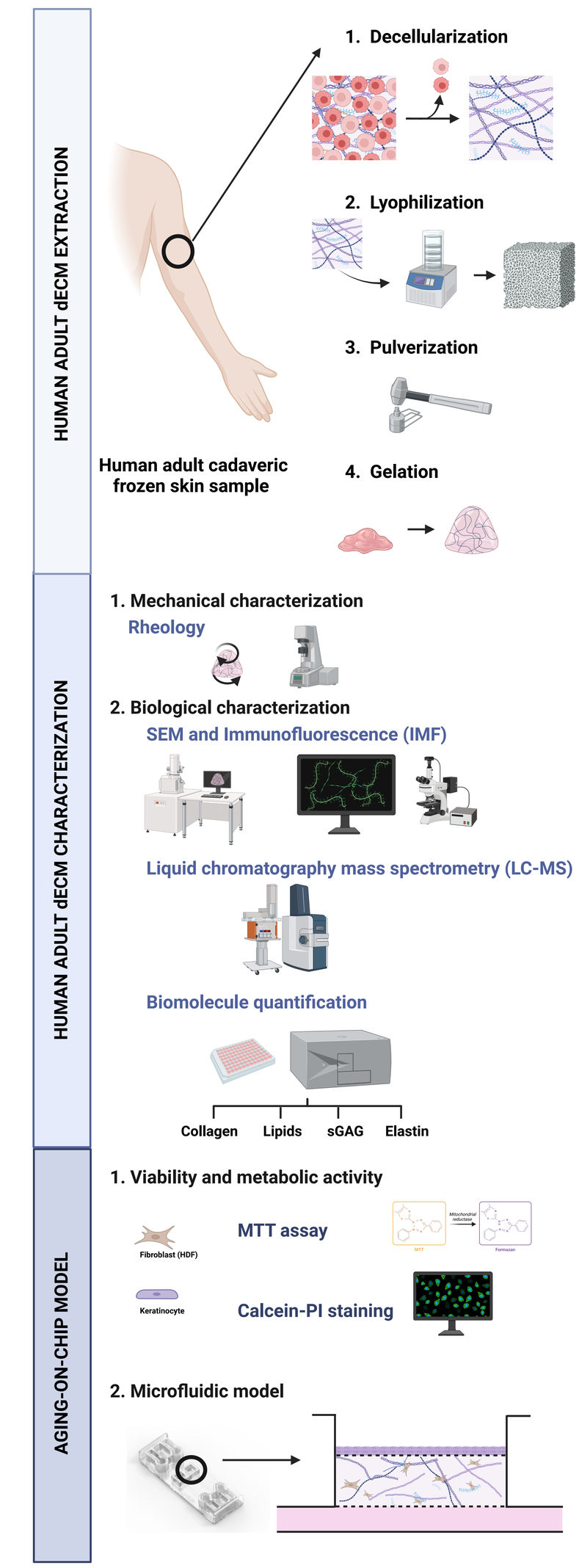
Correspondence:
808895@unizar.es (E.F.)

INTRODUCTION

Currently, there is a growing demand for developing innovative biomaterials that represent skin microenvironment more realistically. In the majority of reported models, the dermis is represented by a natural or synthetic material, which is unable to accurately reflect the complex network present in dermal extracellular matrix (dECM). Furthermore, the majority of these scaffolds have been observed to exhibit excessive shrinkage as a result of fibroblast contractility, which has the effect of reducing the lifespan of these models.

AIM: Extract and characterize adult human dECM for use as scaffolds in aging-on-chip model.

METHODS



RESULTS

Descelullarization and gelation test

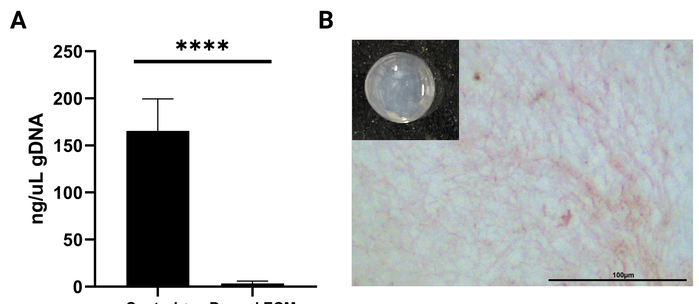


Figure 1. (A) DNA quantification by spectrophotometry and **(B)** haematoxylin-eosin stain where no cell nuclei were observed in human adult dECM hydrogel at 4 mg/mL. ****p< 0.0001 compared with a known number of cells.

Biological characterization
SEM and IMF

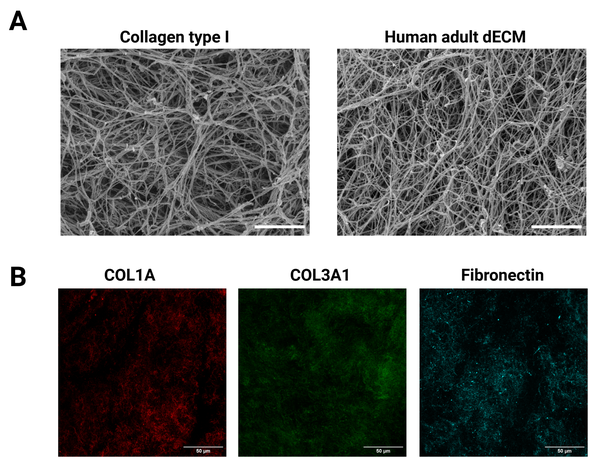
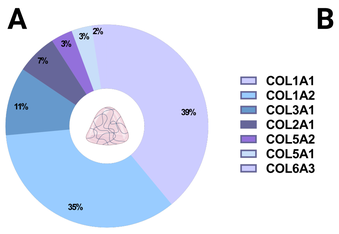


Figure 2. (A) SEM images of collagen type I and human adult dECM hydrogles. **(B)** IMF of human adult dECM hydrogels at 4mg/mL stained with antibodies against collagen type I, collagen type 3 and fibronectin. SEM scale bar: 5µm.

LC-MS : Protein identification



B

Gene name	Description	Function
ACTB	Actin, cytoplasmic 1 OS=Homo sapiens OX=9606 GN=ACTB PE=1 SV=1	Collagen synthesis regulation
ACTG1	Actin, cytoplasmic 2 OS=Homo sapiens OX=9606 GN=ACTG1 PE=1 SV=1	Collagen synthesis regulation
DPT	Dermatopontin OS=Homo sapiens OX=9606 GN=DPT PE=1 SV=2	Collagen fibrillogenesis Cell–cell adhesion
ELN	Elastin OS=Homo sapiens OX=9606 GN=ELN PE=1 SV=4	Elasticity Stretch resilience
FBN1	Fibrillin-1 OS=Homo sapiens OX=9606 GN=FBN1 PE=1 SV=4	Elasticity
DCN	Decorin OS=Homo sapiens OX=9606 GN=DCN PE=1 SV=1	Collagen fibrillogenesis

Figure 3. Identification of **(A)** collagen types and **(B)** other essetial proteins present in human extracellular matrix by mass spectrometry.

Biomolecule quantification

Figure 4. Human dECM **(A)** hydroxyproline, **(B)** lipid, **(C)** sulfated GAG and **(D)** elastin content. ****p< 0.0001 compared with commercial collagen type I.

RESULTS

Mechanical characterization
Rhelogy

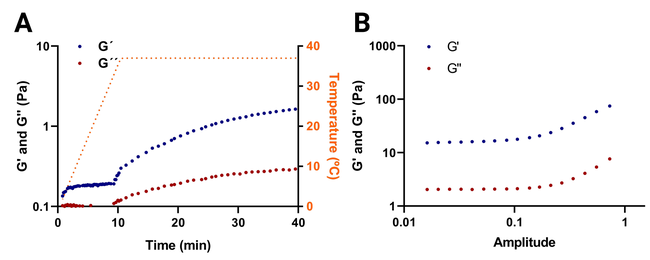


Figure 5. Rheological characterization of human adult dECM hydrogels. **(A)** Gelation kinetics and **(B)** amplitude sweep.

Viability and metabolic activity

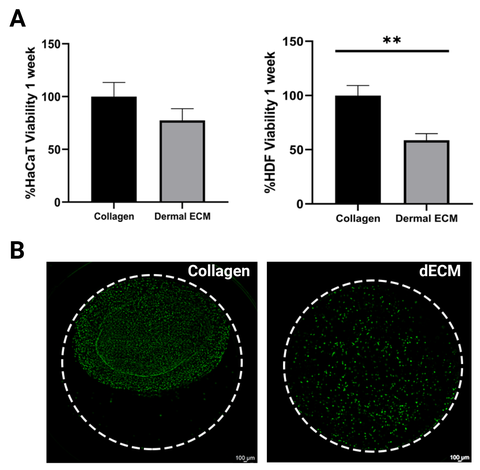


Figure 6. (A) Metabolic activity of HaCaT keratinocytes and HDF fibroblasts after 1 week. **(B)** Fibroblast viability staining embedded in collagen type I and human adult dECM hydrogels .

Aging-on-chip model

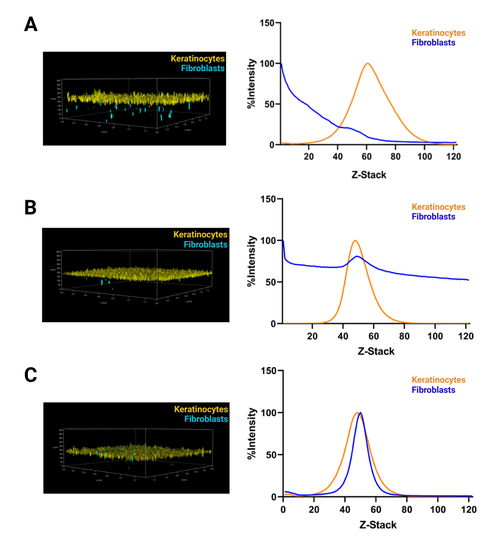


Figure 7. Aging on chip model composed of keratinocytes on top of dECM hydrogel with embedded fibroblasts and study of the migration of the cells of the model at **(A)** day 1, **(B)** 5 and **(C)** 12 .

CONCLUSION

- Human dECM hydrogels from adult cadaveric frozen skin are promising scaffolds for use in advanced aging skin models.
- Hydrogels preserve essential components of native adult dermis, offering a realistic microenvironment, and exhibit similar mechanical behavior to collagen type I.
- Fibroblast behavior within human dECM resembles natural adult skin, and no shrinkage of the scaffold is observed, increasing the lifespan of the model.
- Human adult dECM is presented as an innovative alternative for the development of advanced *in vitro* skin ageing models.

ACKNOWLEDGEMENTS

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