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

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INTRODUCTION

Currently, 3D skin models fail to adequately represent the cellular microenvironment of the dermis. This is partly due to the artificial materials scaffolds used, which lack essential components of the dermis. A scaffold generated from decellularized human dermal extracellular matrix will enable the preservation of native dermal components and generate 3D skin models that are closer to reality.

OBJETIVE

To reproduce an accurate dermis within a skin on chip model by embebbing human fibroblasts within a hydrogel generated from extracted human dermal extracellular matrix.

EXPERIMENTAL METHODS

Extraction

FROZEN SKIN SAMPLE
↓
EPIDERMIS-DERMIS SEPARATION
↓
HOMOGENIZATION AND DECELLULARIZATION
↓
LYOPHILIZATION
↓
DIGESTION
↓
NEUTRALIZATION AND DERMAL ECM HYDROGEL

Characterization

DNA quantification: DNA was extracted using the AllPrep® DNA/RNA MicroKit (Quiagen), quantified and visualized by agarose gel electrophoresis.

Immunofluorescence: Hydrogel samples fixed in 4% paraformaldehyde (PFA) were stainned with antibodies against human collagen I, collagen III, collagen IV and fibronectin.

Scanning electron microscopy (SEM): Fixed hydrogels in 2,5% glutaraldehyde overnight and 2% osmium tetraoxide were dehydrated with ethyl alcohol at increasing concentrations and coated with Au/Pb. The images were acquired with the JSM 6360-LV scanning microscope.

Collagen, sulfated glycosaminoglycan (sGAG), elastin and lipid quantification: Collagen content was determined by quantifying the hydroxyproline present in the hydrogels with Hydroxyproline Assay (Sigma). sGAGs were quantified with the chromotropic agent DMMB. Elastin were quantified using Fastin assay kit (Biocolor). Lipids present in the hydrogel were stained with oil red and extracted with isopropanol. Lipid content was quantified using spectrophotometry at 490 nm.

Rheology: 4mg/mL ECM hydrogel formation was monitored using a rheometer (Haake Mars 40^{TM}) with a plate-plate geometry (25 mm) and a gap of 0.5 mm. The gelation kinetics and amplitude sweep were studied.

Cell viability: The viability of keratinocytes and fibroblasts on top and within ECM hydrogels respectively was quantified using an MTT assay. Viable cells were also observed under confocal microscopy after calcein ethidium staining.

Statistical analysis: Statistical analysis was performed using Excel, calculating the standard deviations of the results from at least three independent experiments. To determine the significance between individual measurements, Student's t-tests were conducted.

RESULTS

Descelullarization

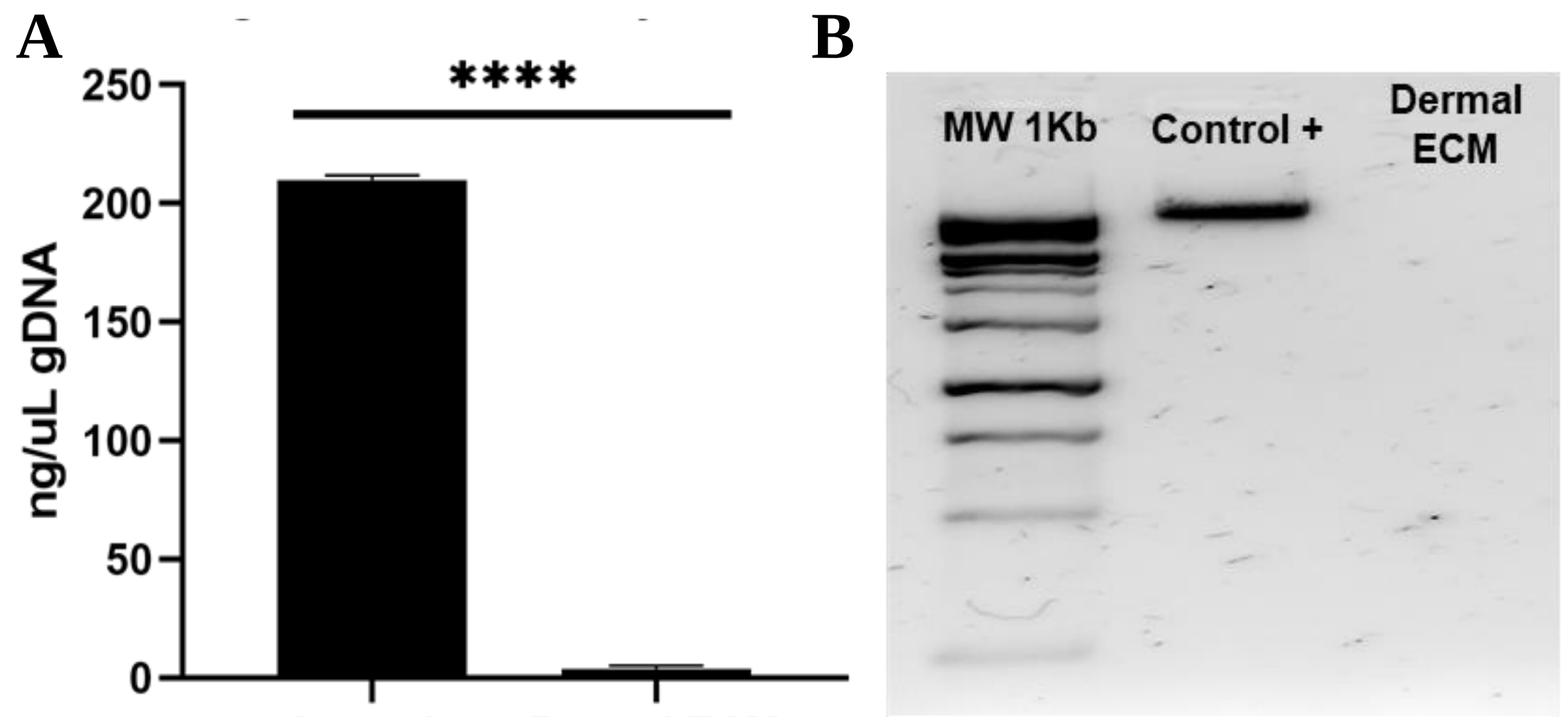

Figure 1. Absence of genomic DNA by (A) DNA quantification by spectrophotometry and (B) 2% agarose gel electrophoresis. ****$p < 0.0001$ compared with a known number of cells.

SEM

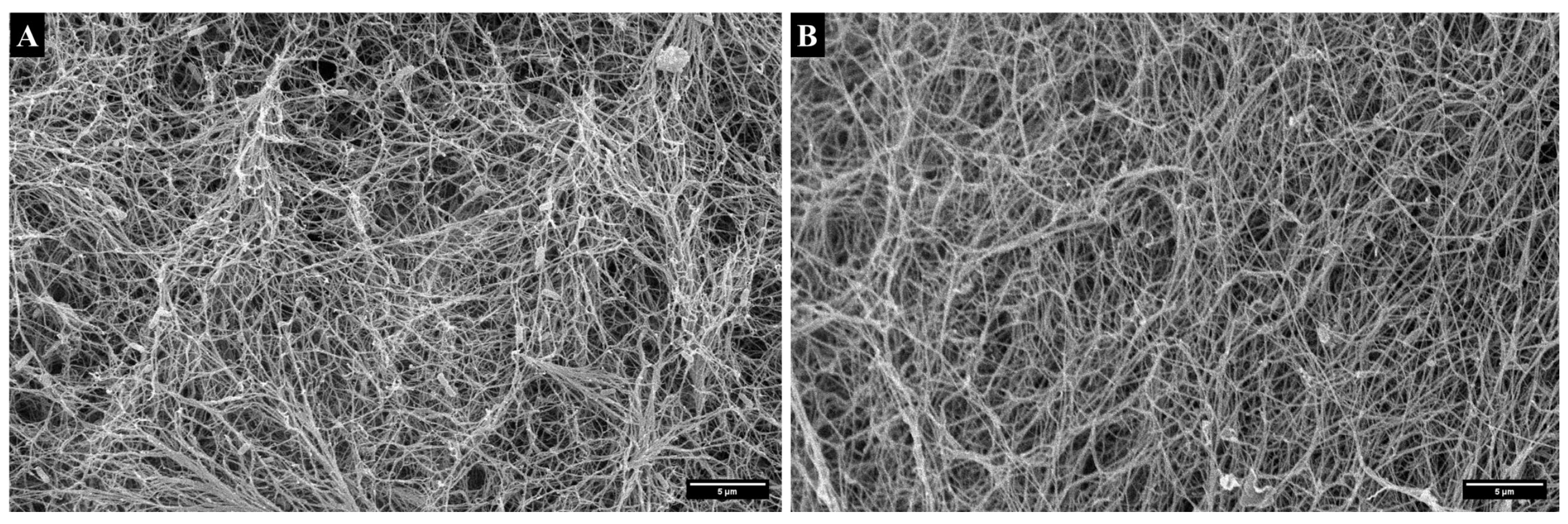

Figure 3. SEM images of (A) collagen and (B) dermal ECM hydrogels.

Rheology

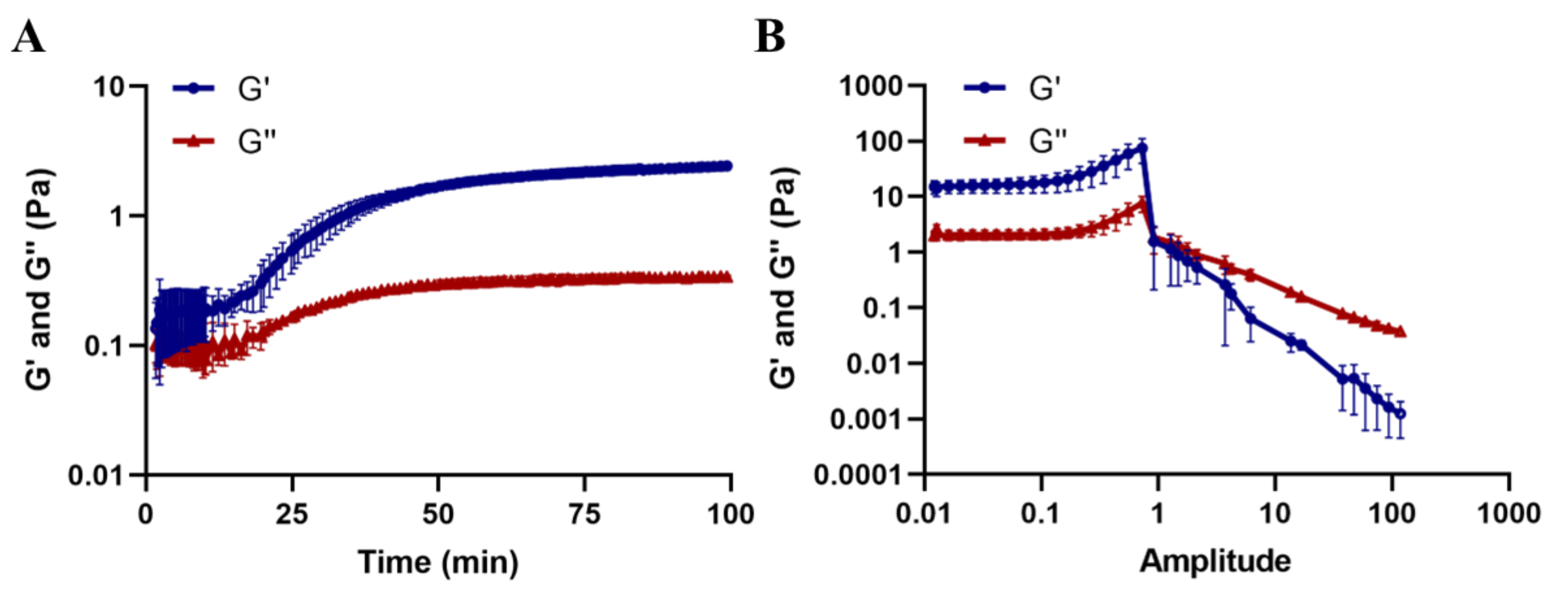

Figure 5. Rheological characterization of ECM hydrogels. Representative curves of the (A) gelation kinetics and (B) amplitude sweep.

Immunofluorescence

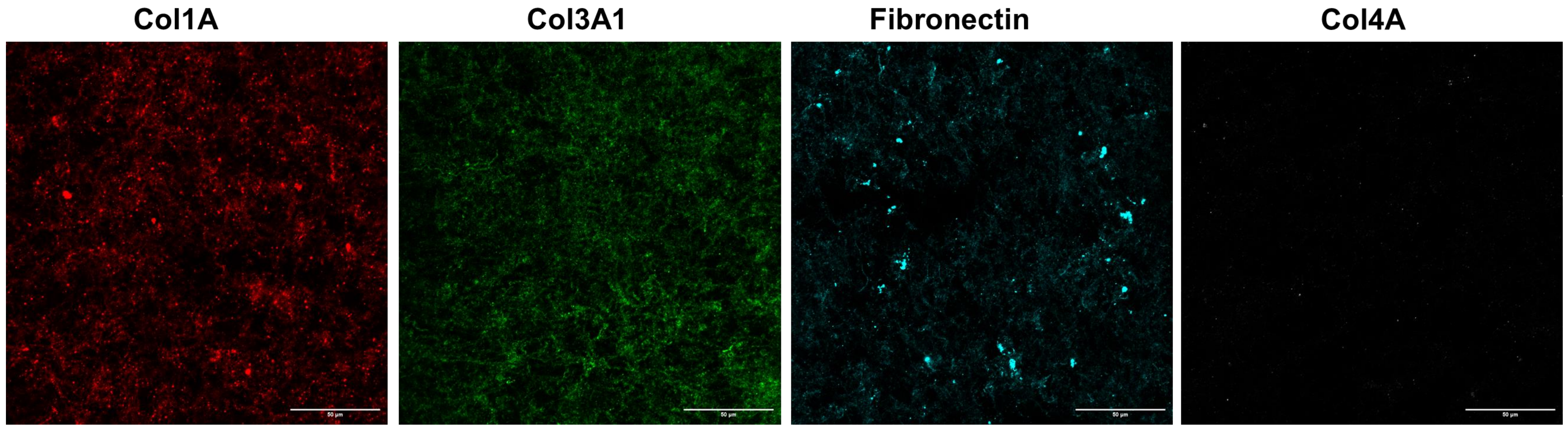

Figure 2. IMF of dermal ECM hydrogels at 4 mg/mL stained with antibodies against collagen I, collagen III, fibronectin and collagen IV.

Lipid, collagen and sulfated GAG quantification of dermal ECM hydrogels

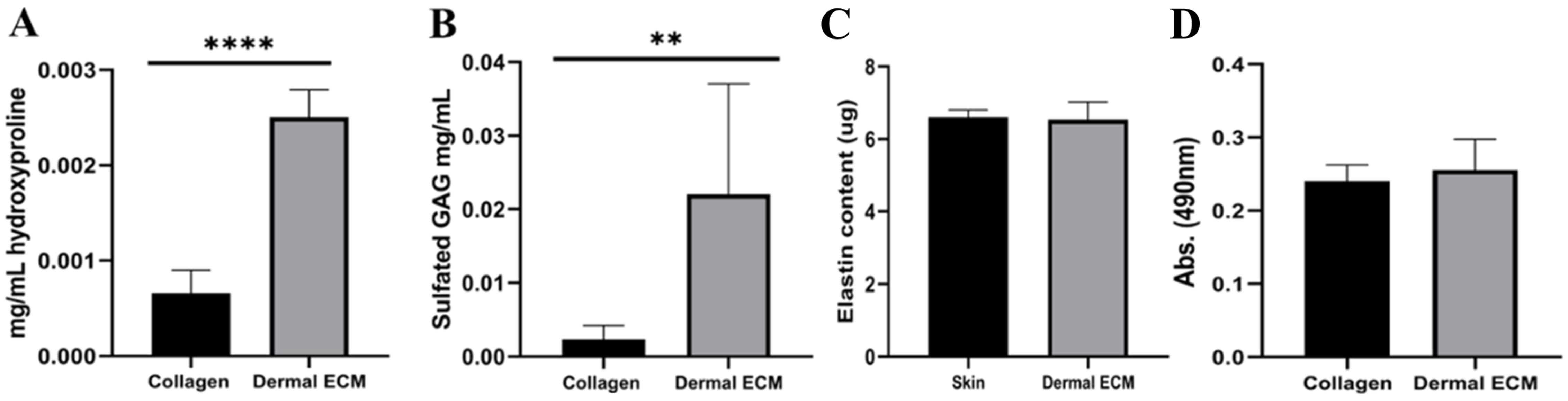

Figure 4. Biochemical composition of dermal ECM hydrogels. Quantification of (A) collagen, (B) sGAG, (C) elastin and (D) lipids. **$p < 0.01$, ****$p < 0.0001$ compared with type I collagen hydrogel or native skin.

Cell viability

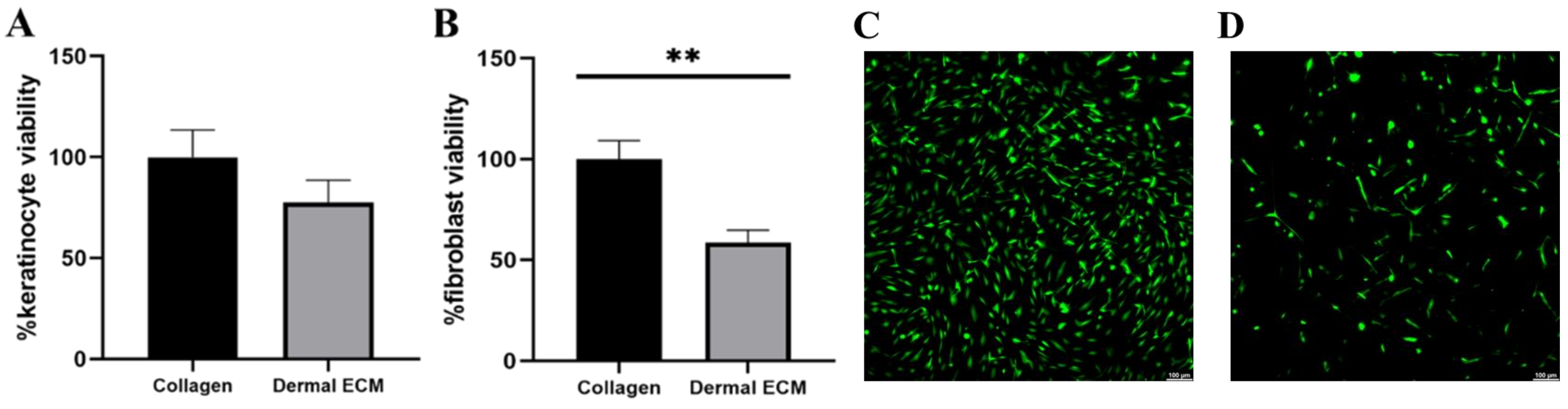

Figure 6. Quantification of metabolic activity of (A) keratinocytes on the hydrogel surface and (B) fibroblast embeebed in hydrogels. Calcein staining of fibroblasts embeebed in hydrogels of collagen (C) and dermal ECM (D).

CONCLUSIONS

1. Dermal extracellular matrix hydrogels preserve the native components of the human dermis, providing closer cellular microenvironment to the human skin.
2. Hydrogels exhibit reproducible behavior with strain-stiffness behavior similar to commercial and other ECM scaffolds.
3. Keratinocytes can be growth on top of ECM hydrogels with similar survival to commercial hydrogels, while constraining the characteristic embedded fibroblasts uncontrolled growth responsible of ruining hydrogel architecture.
4. Dermal ECM scaffolds can be a promising candidate to replace commercial materials for representing the dermal layer in 3D skin models.

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