

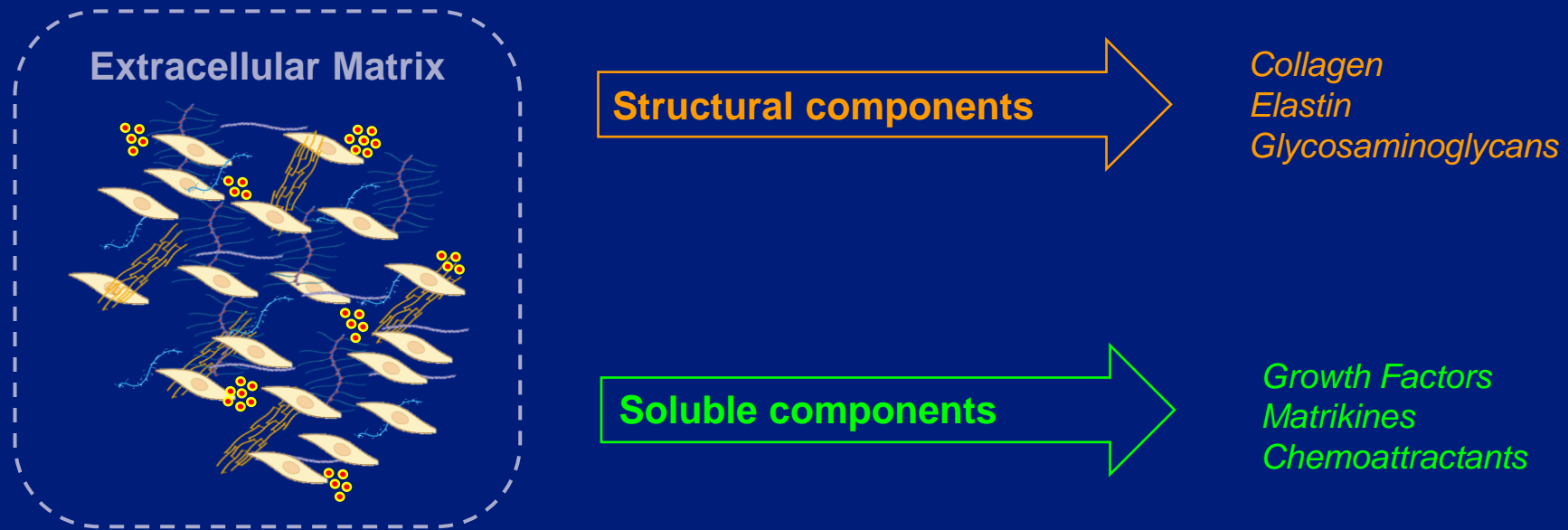
Dermal extracellular matrix extracts for wound healing: *a pleiotropic trigger*

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Extracellular Matrix in Tissue Homeostasis & Repair

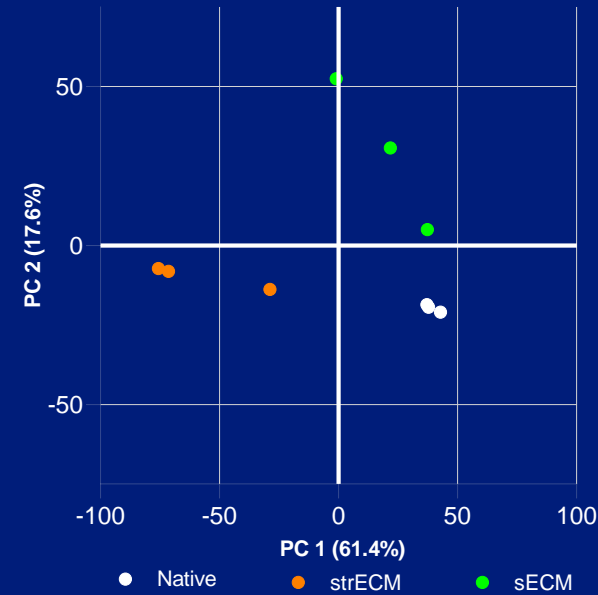
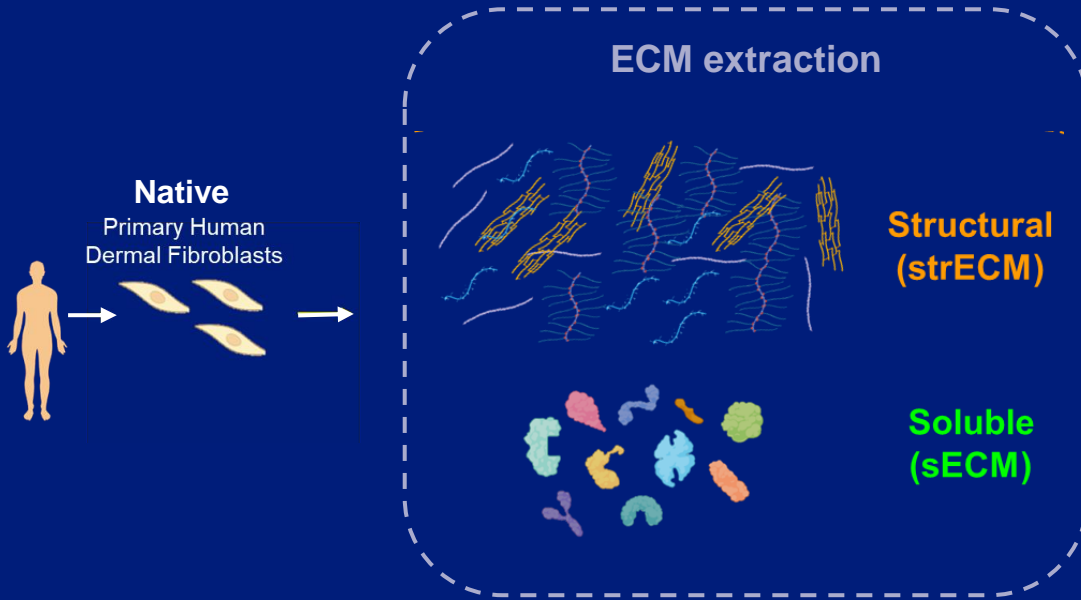


Tissue multifunctionality ascribed to the combination of structural elements decorated with a myriad of dwelling soluble factors

ECM Extraction Approach

Panacean Approach

Two-fraction method allows the retainment of components usually rinsed through extraction processes

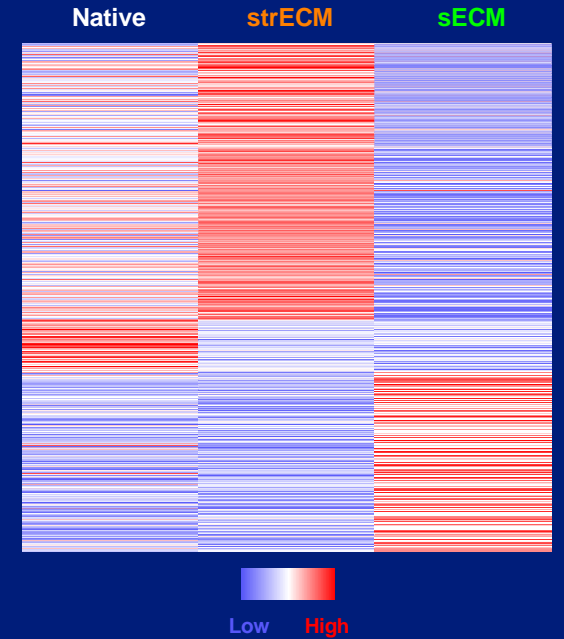


Principal Component Analysis (PCA) score plot of protein composition (n=3)

PCA grouping indicates:

- Low batch-to-batch variability (Native)
- Robustness of extraction process (strECM & sECM)

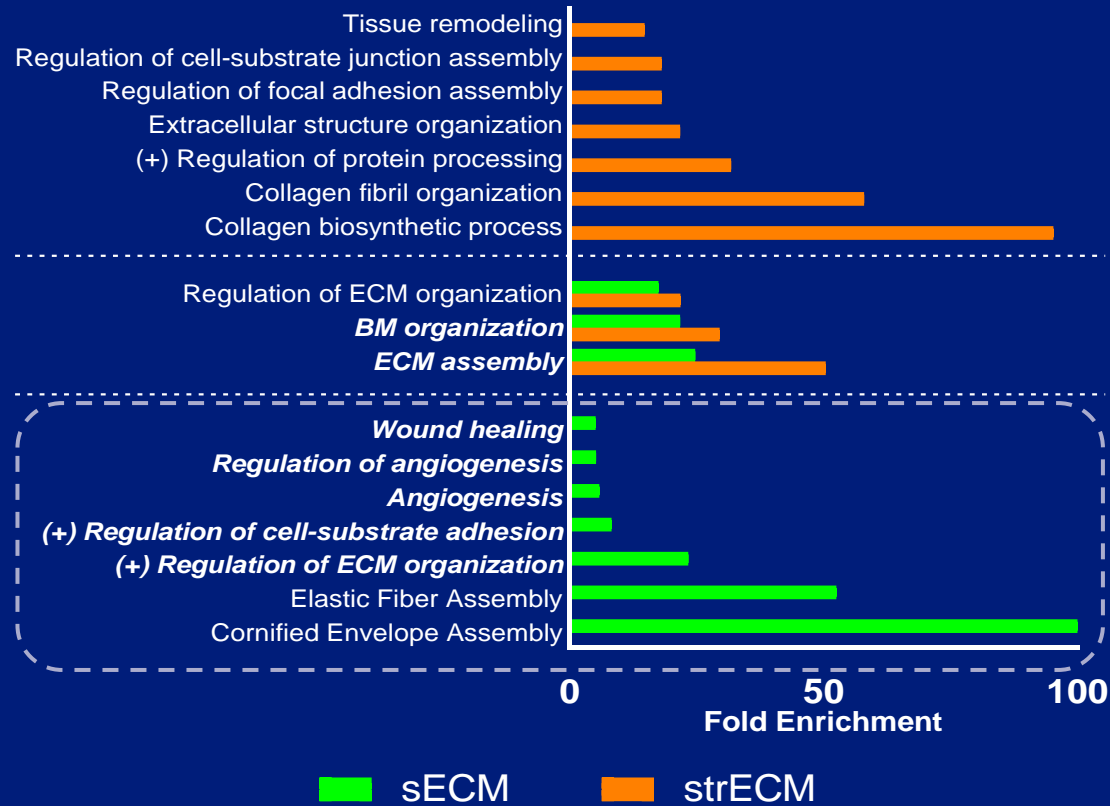
Heatmap revealed complementarity in protein composition between strECM & sECM



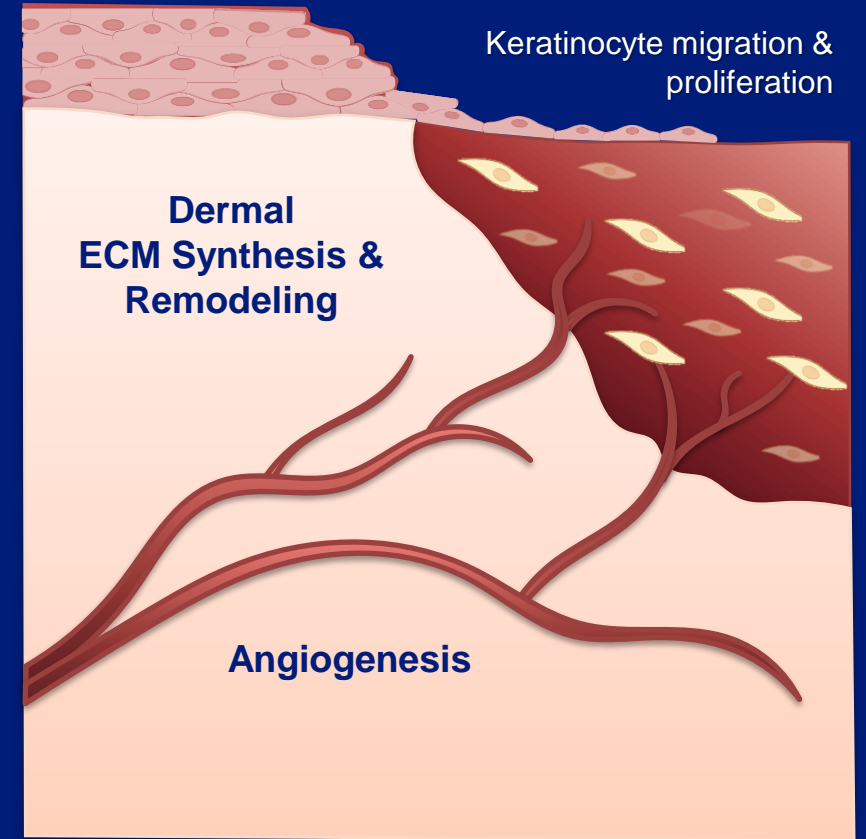
Heatmap of relative protein abundances in: Native (Source material), sECM and strECM

sECM role as a Biological Trigger

Top 10 GO Biological Functions

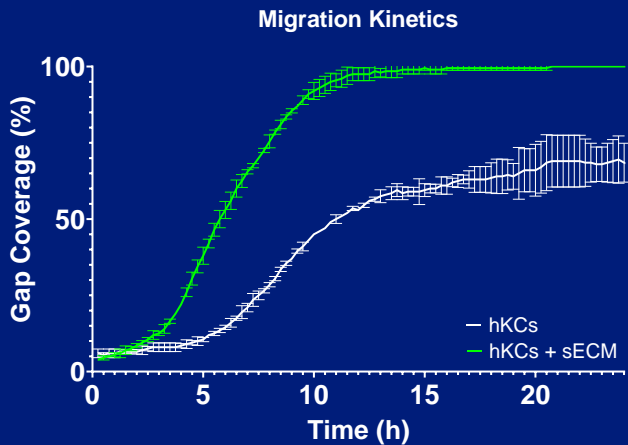
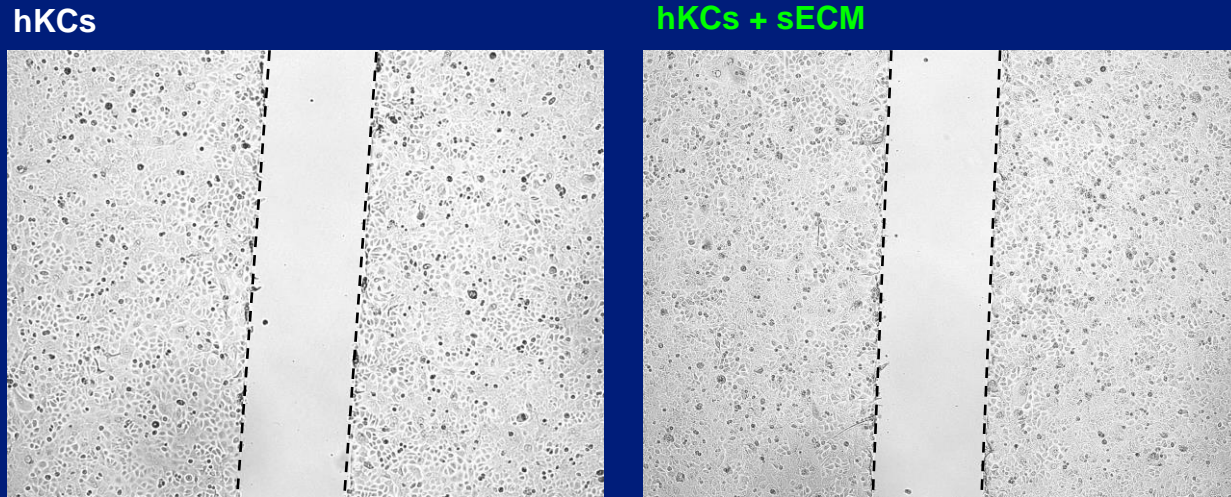


Fold Enrichment of Major Biological Functions of Proteins enriched in sECM and strECM



Re-epithelialization-related processes

Gap-closure assay, Human Keratinocytes (hKCs), 24h timelapse

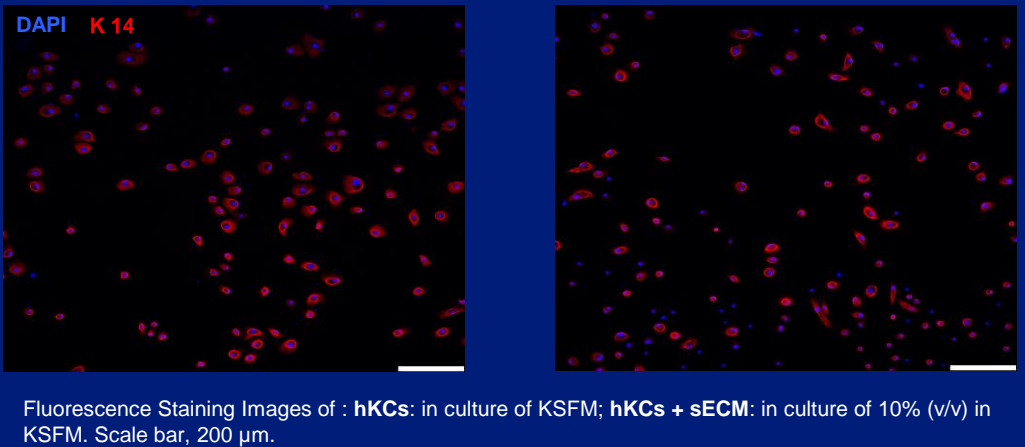
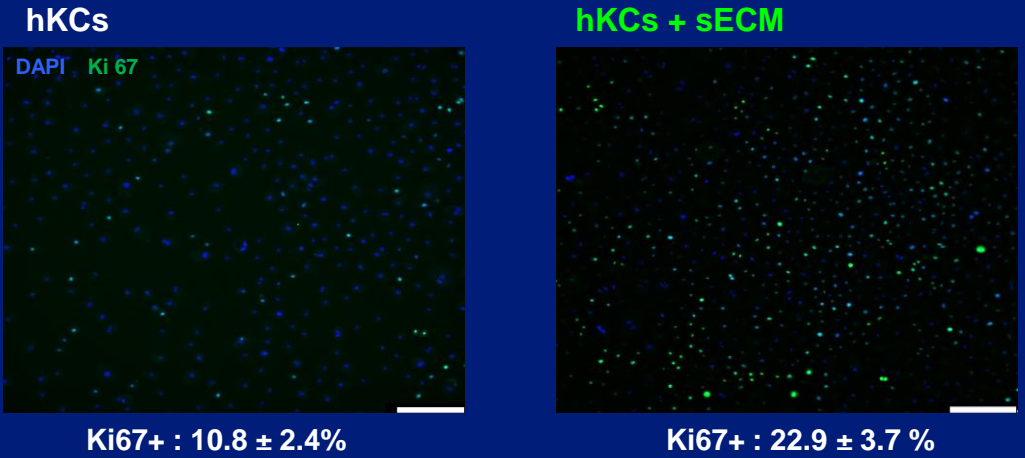


Migration Rate

- hKCs + sECM: $6.2 \pm 2.1\%/h$
- hKCs: $3.0 \pm 0.5\%/h$

sECM enhances hKCs Migration

Human Keratinocytes (hKCs), 3 days in culture



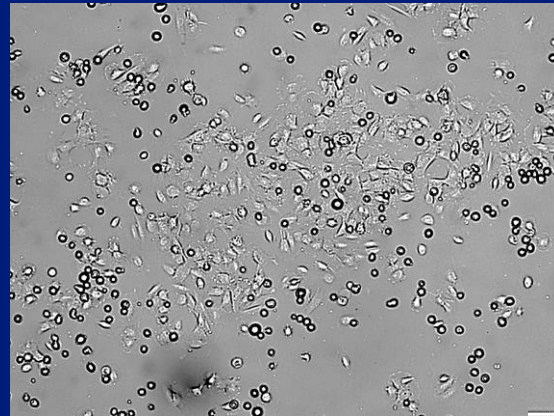
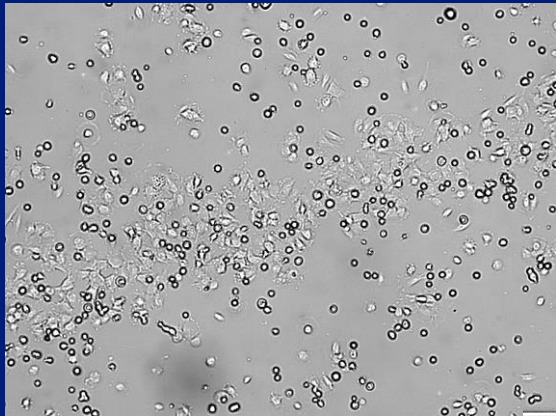
hKCs cultured with sECM have enhanced proliferative phenotype

Proliferative stage-related processes: Fibroblast Adhesion

Human Dermal Fibroblasts (hDFBs), 30 min sECM incubation, 2h seeding in basal medium (w/o Fetal Bovine Serum), Non-adhesive plates

hDFBs

hDFBs + sECM

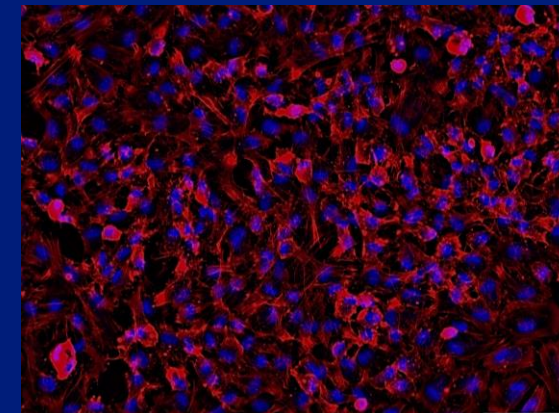
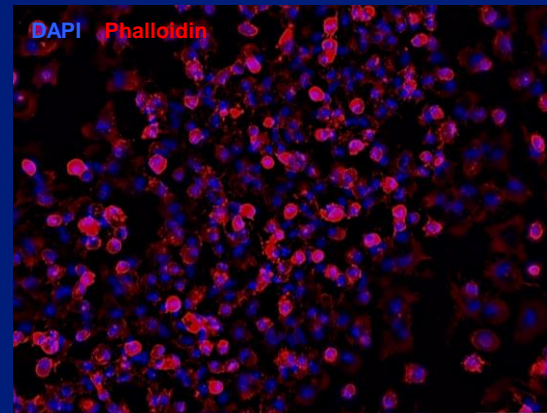
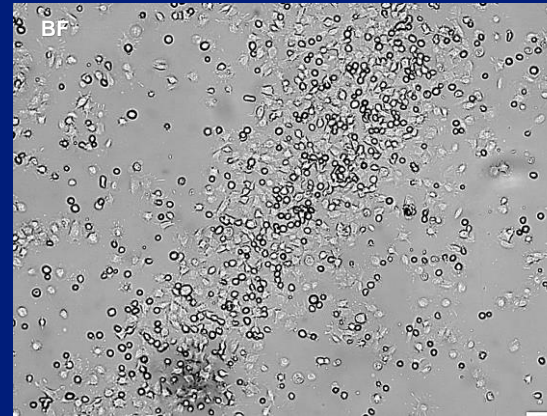


Pre-seeding sECM incubation period did not influence cell adhesion

Human Dermal Fibroblasts (hDFBs), 2h seeding in sECM-supplemented medium (w/o Fetal Bovine Serum), Non-adhesive plates

hDFBs

hDFBs + sECM

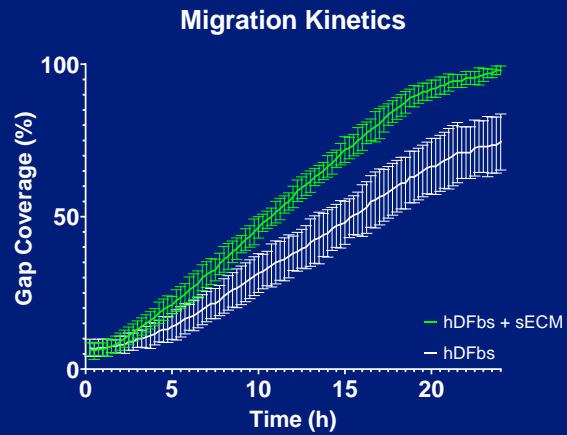
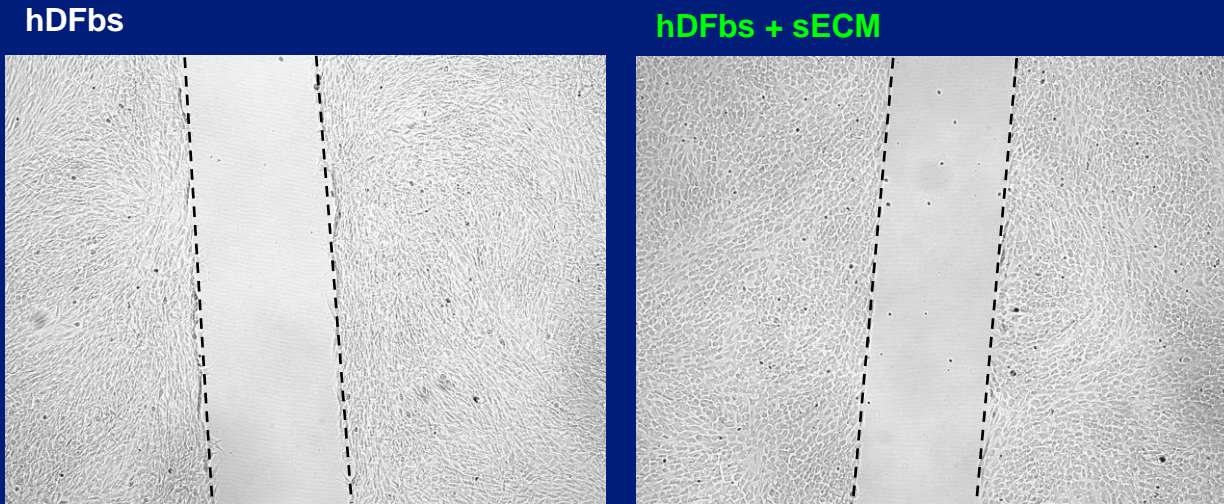


sECM supplemented media enhanced cell adhesion

Scale bar , 100 μ m

Proliferative stage-related processes: Fibroblast Migration & Proliferation

Gap-closure assay, Human Dermal Fibroblasts (hDFbs), 24h timelapse



Migration Rate

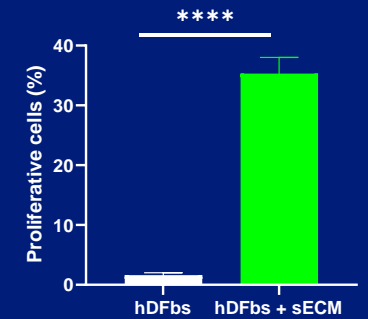
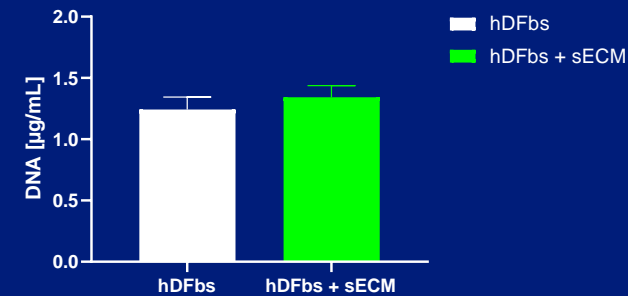
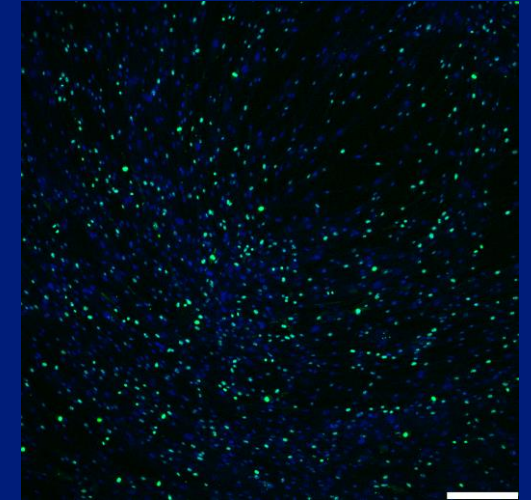
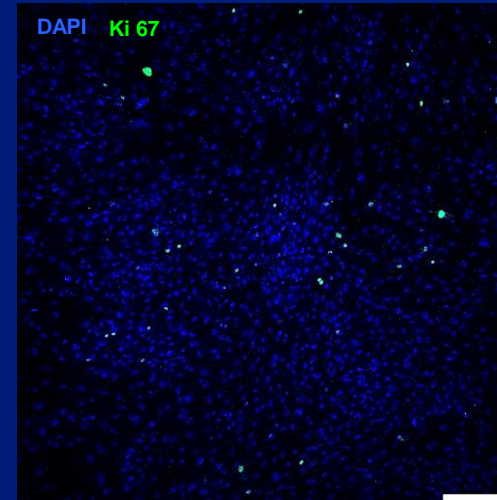
- hDFbs + sECM: $5.1 \pm 0.4\%/h$
- hDFbs: $3.4 \pm 0.8\%/h$

sECM enhances hDFbs Migration

Human Dermal Fibroblasts (hDFbs), 5 days in culture

hDFbs

hDFbs + sECM

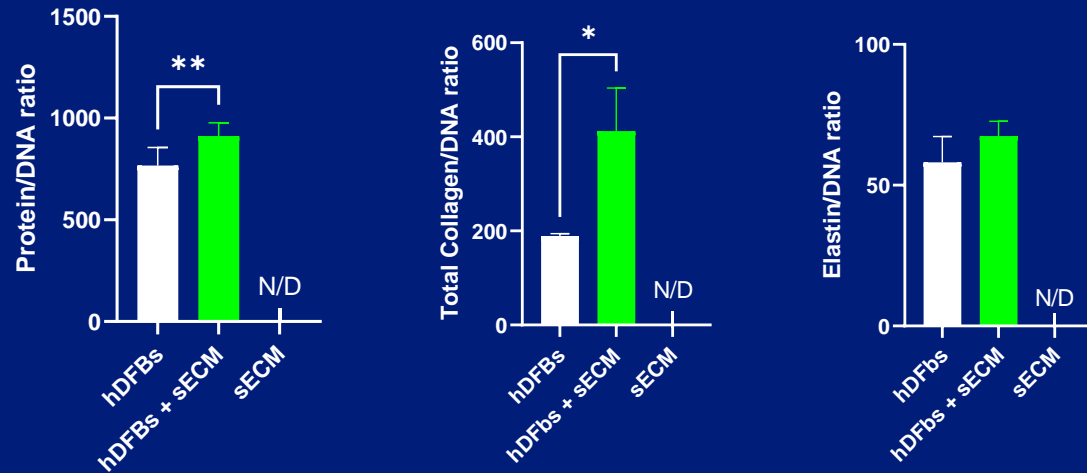


sECM-cultured hDFbs show enhanced proliferative phenotype

Remodeling stage-related processes

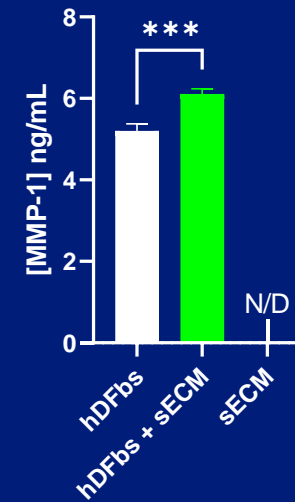
Human Dermal Fibroblasts (hDFbs), 5 days in culture

ECM Production

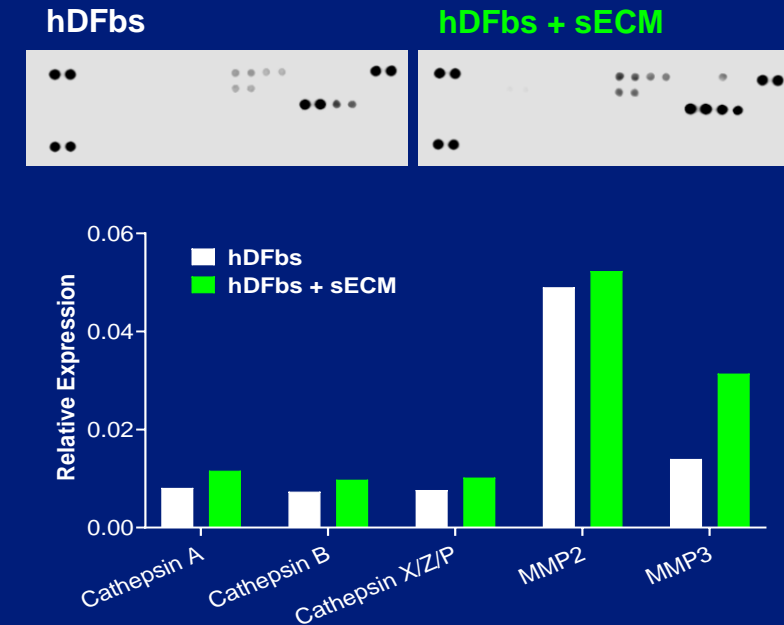


Quantification of DNA, total protein, collagen and elastin: **hDFbs**: cells cultured in basal α -MEM; **hDFbs + sECM**: cells cultured in 10% v/v in basal α -MEM; N/D: non-detected

ECM Degradation



Quantification of MMP-1 content: **hDFbs**: cells cultured in basal α -MEM; **hDFbs + sECM**: cells cultured in 10% v/v in basal α -MEM; N/D: non-detected.

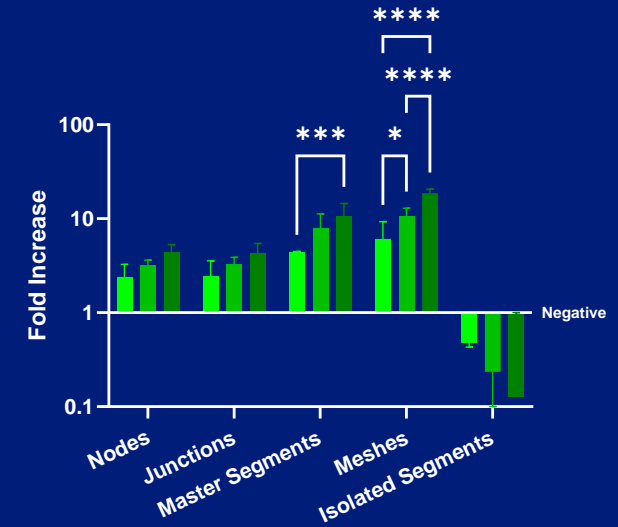
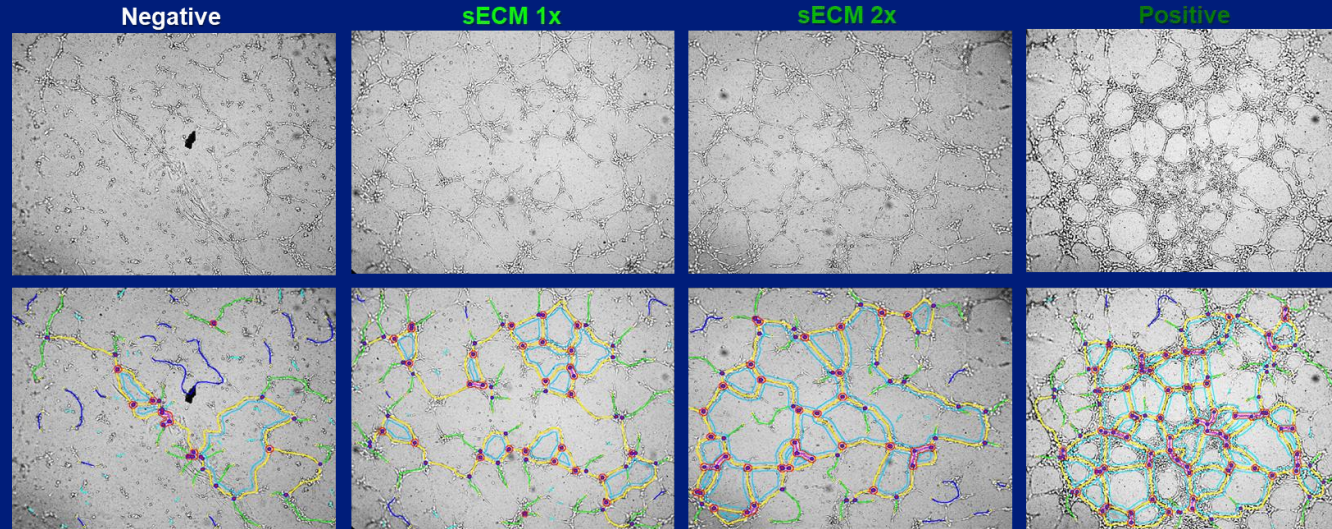


Presence of sECM boosts production of collagen and ECM-degradation enzymes

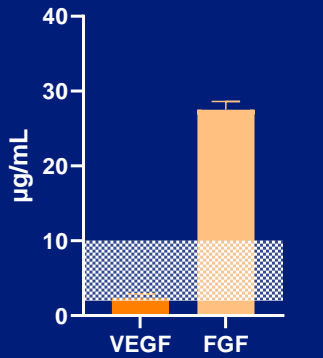
Angiogenic potential

2D

Human Dermal Microvascular Endothelial Cells seeded on Matrigel, 24 hours



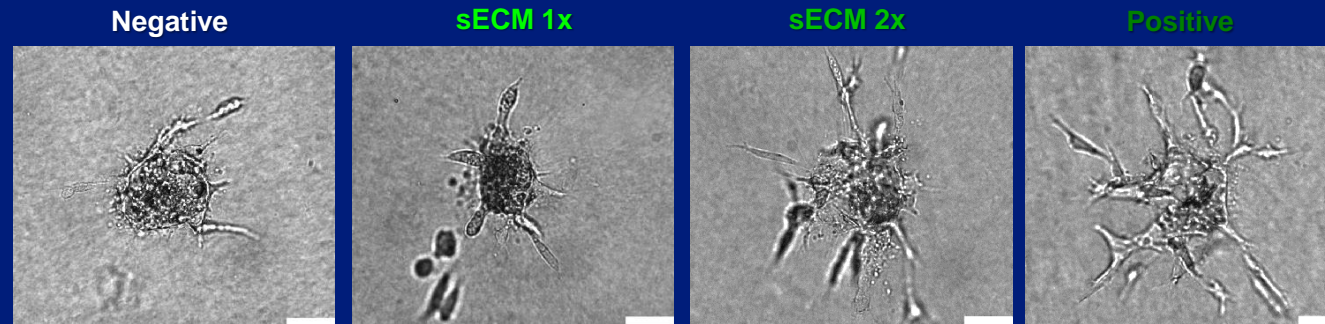
sECM Composition



Quantification of angiogenic factors VEGF & FGF detected in sECM by ELISA. Grid range represents reference values for endothelial cell culture media supplementation (2-10µg/mL)

3D

Human Dermal Microvascular Endothelial Cell Spheroids embedded in Collagen, 24 hours



sECM enriched in angiogenic factors enhances hDMECs sprouting

Endothelial Cell Sprouting

Take Home Messages

- ✓ ECM extraction protocol was capable of retaining most of the complex composition of Dermal ECM:
 - Yielded extracts with distinct Proteomic Profiles: Structural Features (strECM) and Functional Cues (sECM)
- ✓ sECM acts, *in vitro*, as an enhancer in several key biological processes involved in wound healing:
 - Keratinocyte Migration & Basal Phenotype – Re-epithelialization
 - Fibroblast Adhesion Migration & ECM Remodeling – Pro-repairing microenvironment
 - Endothelial Cell Tube formation & sprouting – Angiogenic Potential

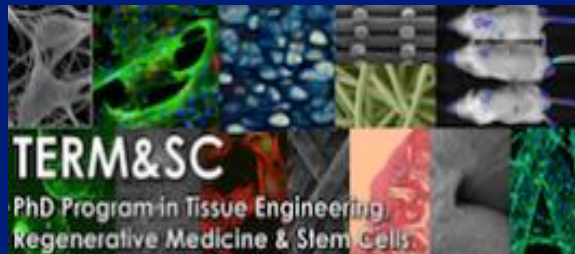
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Thank you all for your attention!

