Transient epigenetic reprogramming by mRNA for skin rejuvenation

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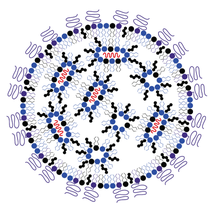
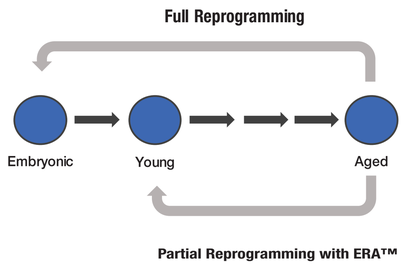
Introduction

In recent years, epigenetic reprogramming has come to the forefront of tissue regeneration and is based on the concept that as cells age, their nuclear regulation of gene expression becomes aberrant, leading to senescence and disruption of cellular homeostasis[1]. In aged skin, this manifests in many ways, such as loss of extracellular matrix (ECM) deposition and increased oxidative stress, which are the molecular hallmarks underlying poor skin quality[2-4].

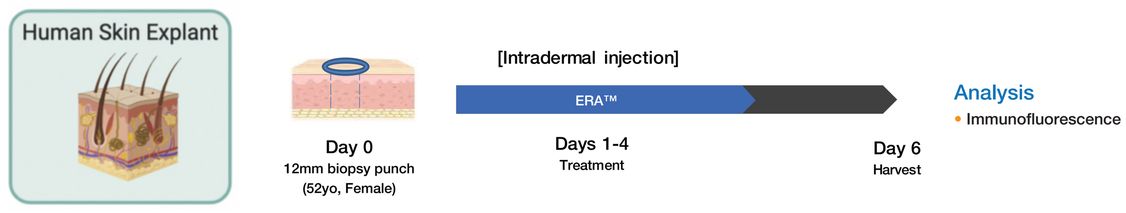
Epigenetic Reprogramming of Aging (ERA™) is a proprietary cocktail of mRNA encoding transcription factors that when delivered transiently, restores cellular gene expression back to a more youthful state without changing the cell's identity. ERA™ has the potential to disrupt the practice of aesthetic medicine by complementing and/or possibly replacing many of the skin rejuvenation treatments used today. By reprogramming cells at the epigenetic level to a more youthful state without changing their identity, ERA™ has the potential to address multiple aspects of aged skin in a more natural, non-invasive manner, with long-lasting effects.

ERA™: solid lipid nanoparticle containing mRNA that encodes reprogramming factors





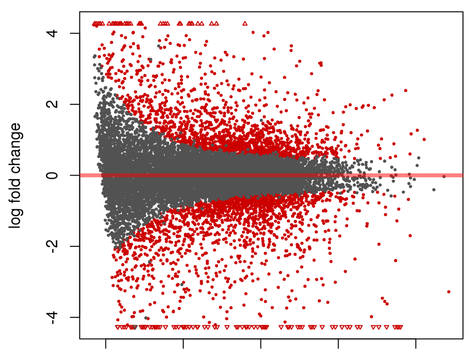
Methods



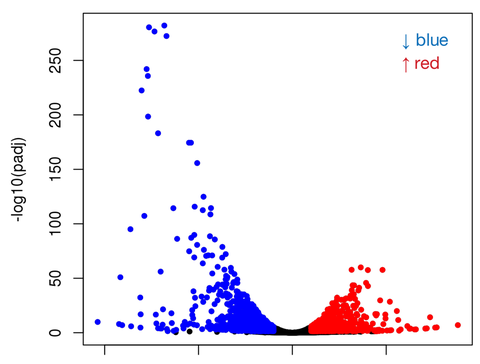
Results

FIGURE 1

A) MA Plot: genes in red are differentially expressed



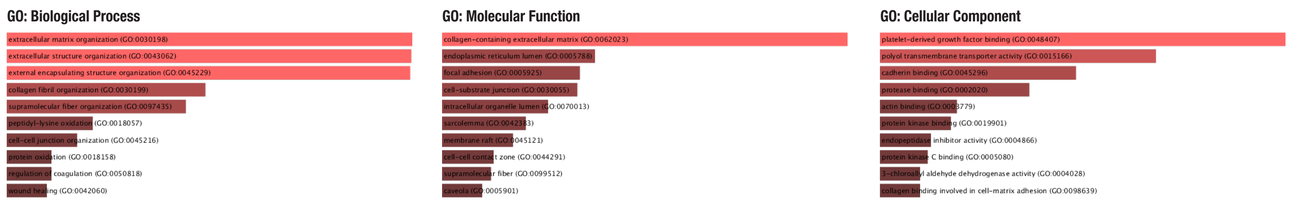
B) Volcano Plot: distribution of significant (2-fold) genes



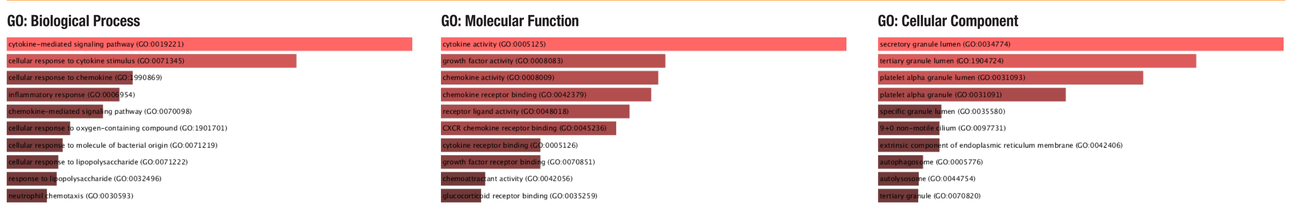
C) Gene Ontology (GO) Analysis

UPREGULATED:

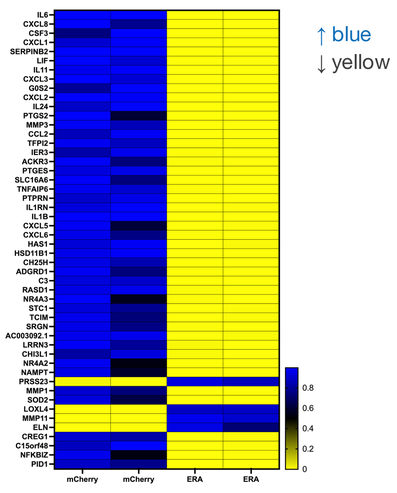
ECM = EXTRACELLULAR MATRIX



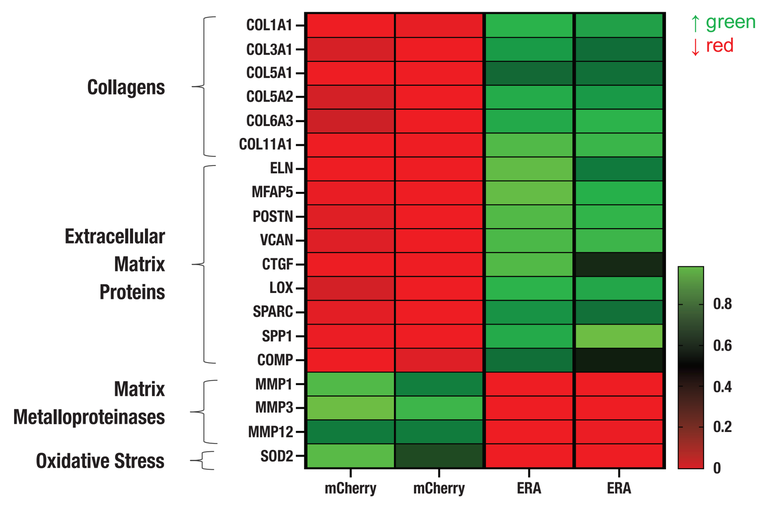
DOWNREGULATED:



D) Top 50 Differentially Expressed Genes



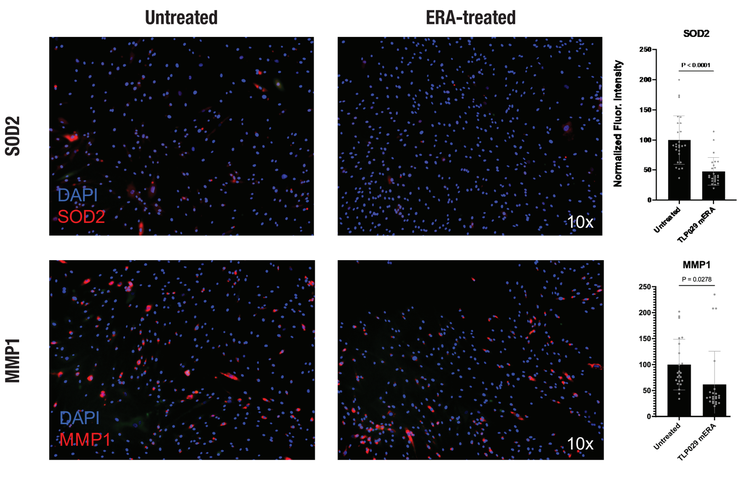
E) Genes of Interest



GENE	PROTEIN
COL1A1	Collagen Type I
COL3A1	Collagen Type III
COL5A1 COL5A2	Collagen Type V
COL6A3	Collagen Type VI
COL11A1	Collagen Type XI
ELN	Elastin
MFAP5	Microfibril-Assoc Protein 5
POSTN	Periostin
VCAN	Versican
CTGF	Connective Tissue Growth Factor
LOX	Lysyl Oxidase
SPARC	Secreted Protein Acidic and Cysteine Rich
SPP1	Secreted Phosphoprotein 1
COMP	Cartilage Oligomeric Matrix Protein
MMP1	Matrix Metallopeptidase 1
MMP3	Matrix Metallopeptidase 3
MMP12	Matrix Metallopeptidase 12
SOD2	Superoxide Dismutase 2

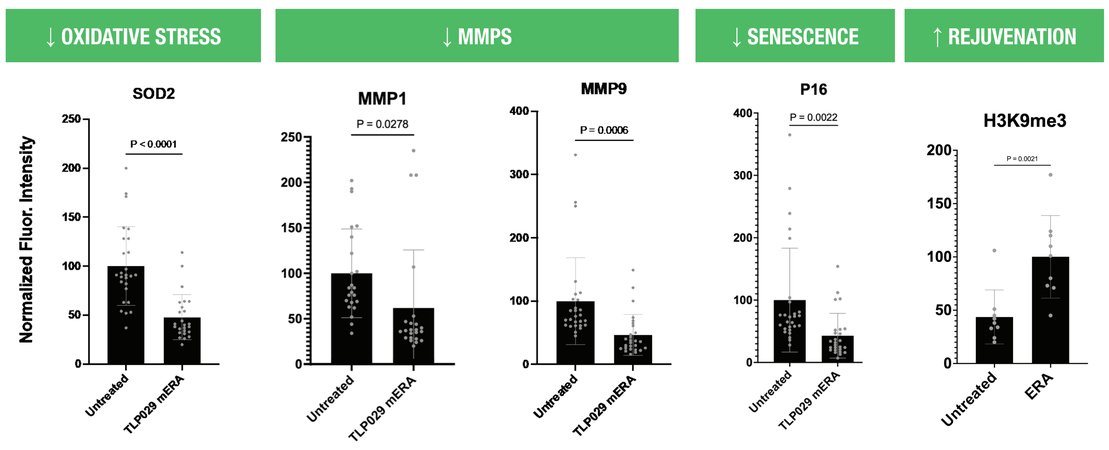
Transient reprogramming with ERA™ Has a Strong Global Transcriptional Effect on Adult Dermal Fibroblasts That Amounts to an Overall Enrichment of ECM Protein Deposition and Downregulation of Cytokine Signaling. Primary adult human dermal fibroblasts were transfected with ERA and harvested on Day 6 for bulk RNAseq analysis. A) MA plot showing the wide distribution of differentially expressed genes (in red). B) Volcano plot showing distribution of significantly differentially downregulated (blue) and upregulated (red) genes. C) Gene ontology (GO) analysis of ERA-treated fibroblasts (relative to control) showing the top 10 upregulated and downregulated GO categories. D) Hierarchical clustering heatmap of the top 50 most significantly differentially expressed genes between ERA-treated and Control groups. E) Selected genes of interest related to skin quality that were significantly up- or down-regulated

FIGURE 2



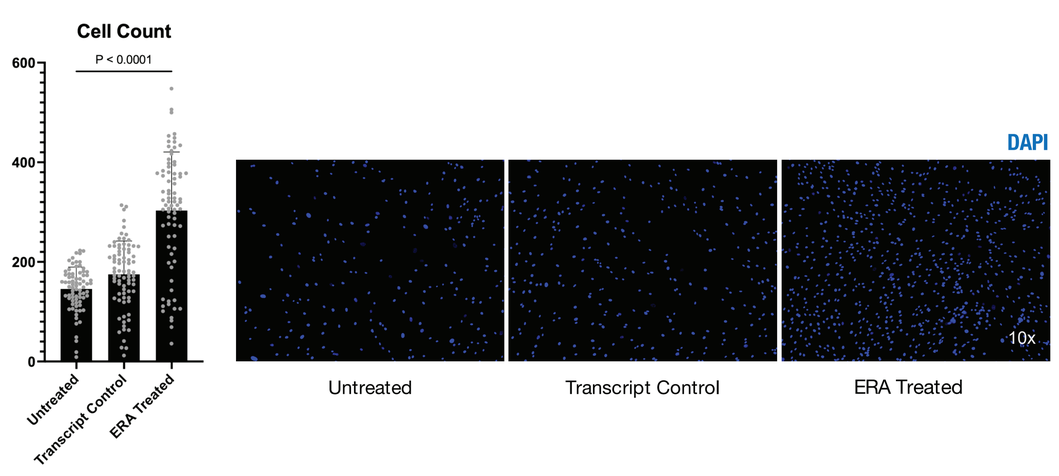
SOD2 and MMP1 Protein Expression is Decreased in Dermal Fibroblasts Treated with ERA™. Primary adult human dermal fibroblasts were transfected with ERA and fixed on Day 6 for analysis by quantitative immunofluorescence. Both SOD2 (oxidative stress marker) and MMP1 are known to be increased in aged skin.

FIGURE 3



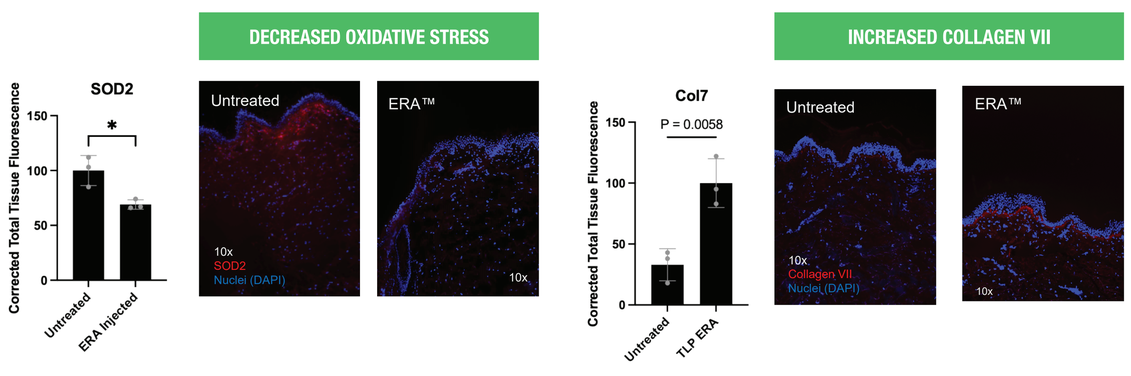
Treatment with ERA™ Results in a Broad Panel of Changes Related to Aging and Skin Quality. Primary adult human dermal fibroblasts were transfected with ERA and fixed on Day 6 for analysis by quantitative immunofluorescence. SOD2, MMP1, MMP9, and p16 (senescence marker) are known to be increased in aged skin, while H3K9me3 (rejuvenation marker) is known to be decreased.

FIGURE 4



Cell Proliferation is Increased in Fibroblasts Treated with ERA™ Primary adult human dermal fibroblasts were transfected with ERA and fixed/counted on Day 6.

FIGURE 5



ERA™ Rejuvenates Ex Vivo Human Skin. Freshly excised abdominal skin was obtained from a 52yo female undergoing elective surgery. 12mm punch biopsies were put into culture and intradermally injected with ERA. On Day 6, skin explant cultures were processed for immunofluorescence. Collagen VII is a key ECM protein that maintains the integrity of the dermal-epidermal junction and is known to decrease with age.

Conclusion

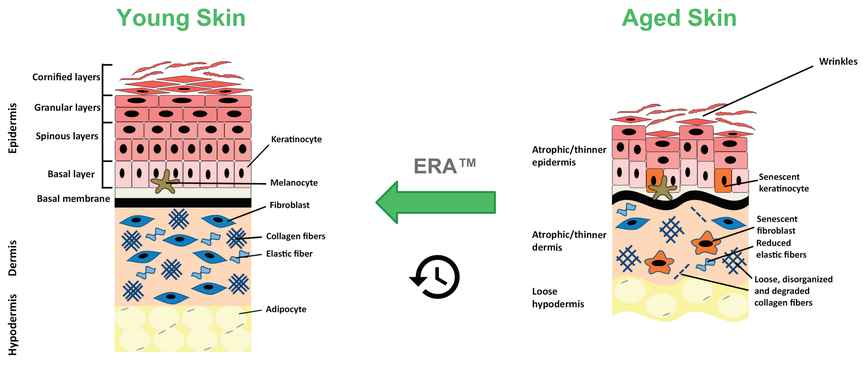
Compared to controls, human adult fibroblasts treated with ERA displayed:

- A global change in the transcriptome that amounted to an overall deposition of ECM proteins and decreased cytokine signaling
- Increased gene expression of various collagens and other ECM proteins
- Decreased gene and protein expression of MMPs and oxidative stress markers
- Decreased protein expression of the senescence marker p16
- Increased protein expression of the rejuvenation marker H3K9me3
- Increased cell proliferation

Compared to controls, human skin explants treated with ERA displayed:

- Decreased protein expression of the oxidative stress marker, SOD2
- Increased protein expression of collagen VII

Discussion



ERA™ increases fibroblast proliferation and ECM protein deposition while simultaneously reversing the intrinsic hallmarks of aging. These molecular changes suggest that ERA™ has the potential to reverse the clinical signs of aging skin.

SCAN TO LEARN MORE

REFERENCES: 1) Sarkar et al. Nat Comm. 11:1545 (2020); 2) Langton et al. Mechanisms of Ageing and Development 156:14–16 (2016); 3) Treiber et al. Dermato-Endocrinology 4:232–235 (2012); 4) Qin et al. Br J Dermatol. 177:1337–1348 (2017)

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