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Supplemental Information

De Novo Synthesis of Elastin by Exogenous Delivery of Synthetic Modified mRNA into Skin and Elastin-Deficient Cells

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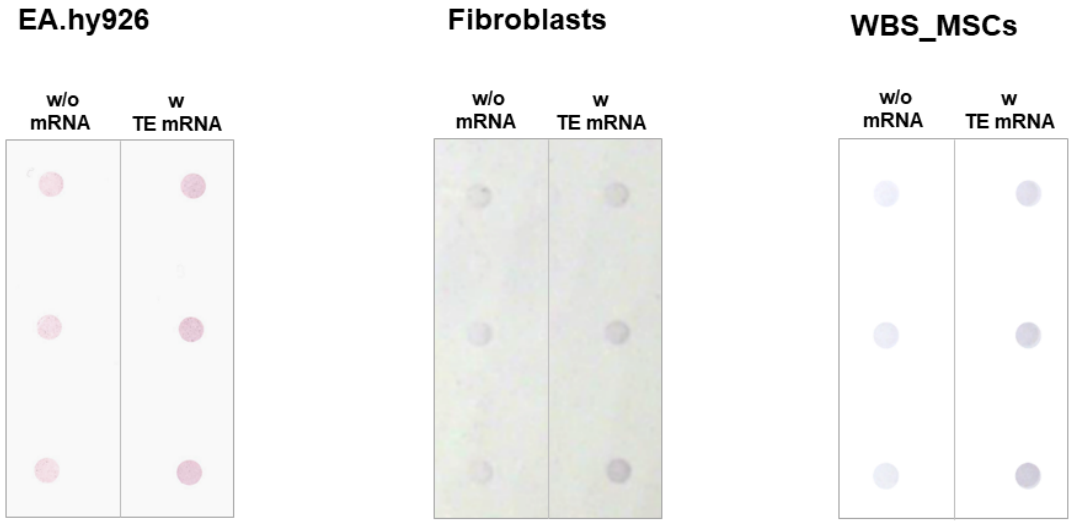
Supplementary Data:

Detection of elastin using dot blot assay

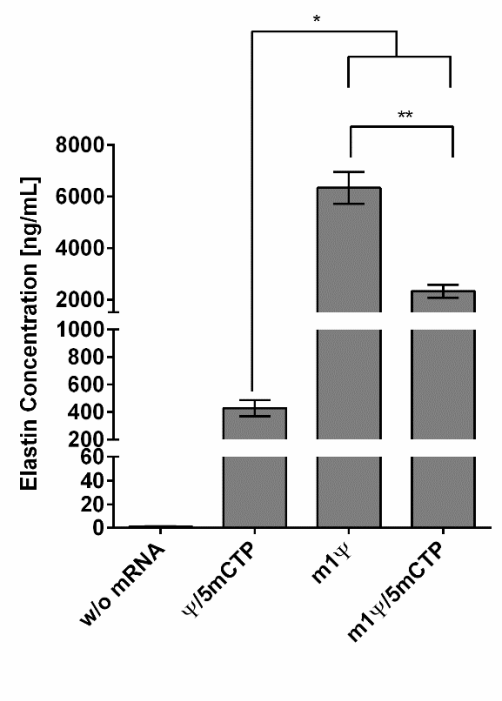


Supplementary Figure 1: Dot blot images with detected elastin after the transfection of EA.hy296 cells, human fibroblasts, or MSCs derived from WBS patient (WBS_MSCs) with synthetic modified TE mRNA. 3 x 10^5 EA.hy926 cells, human fibroblasts, or WBS_MSCs were transfected with 2.5 µg synthetic modified TE mRNA. As a control, cells were incubated with OptiMEM containing Lipofectamine 2000 without TE mRNA. After 24 h, 500 µl supernatant was blotted on nitrocellulose membrane and elastin was detected using polyclonal antibody to human aortic elastin and the subsequent detection of alkaline phosphatase conjugated secondary antibody.

Elastin ELISA

For the detection of translated elastin in the supernatant of mRNA transfected cells, 4 x 10^5 EAHy.926 cells were seeded, cultivated overnight, and transfected with 2.5 µg Ψ/5mCTP, m1Ψ or m1Ψ/5mCTP modified TE mRNA using Lipofectamine 2000. After 4 hr, transfection complexes were removed and 1 ml medium was added. The

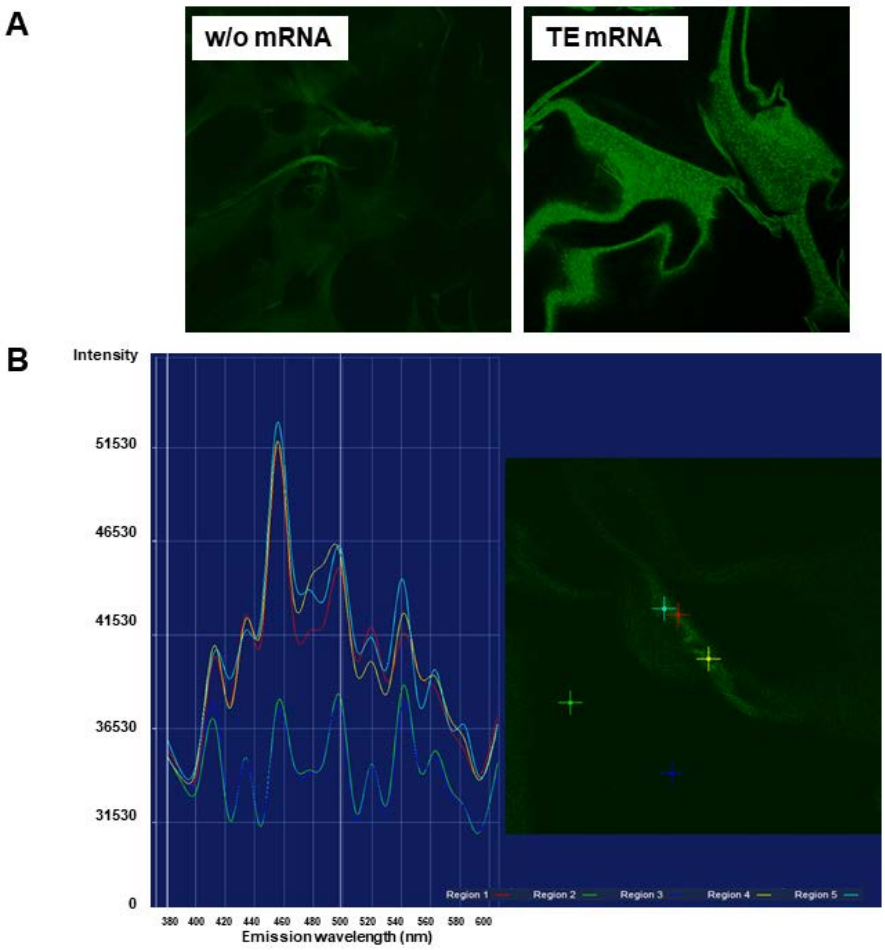
supernatant was collected after 24 hr and centrifuged 10 min at 3,000 x g to remove cell debris. Elastin concentration was measured using ELISA Kit for human elastin (Antibodies-online, Cloud-Clone Corp., Aachen, Germany) according to manufacturer's protocol. Cells incubated with OptiMEM containing Lipofectamine 2000 was used as control.



Supplementary Figure 2: Detection of elastin concentration after the transfection of EA.hy293 cells with Ψ/5mCTP, m1Ψ or m1Ψ/5mCTP modified TE mRNA using elastin ELISA. 4 x 10^5 EA.hy926 cells were transfected with 2.5 µg differently modified synthetic TE mRNA. After 24 hr, the elastin concentration was detected using elastin ELISA. As a control, cells were incubated with OptiMEM containing Lipofectamine 2000 without TE mRNA. Results are shown as mean ± SEM (n=4). Statistical differences were determined using one-way ANOVA with Bonferroni's multiple comparisons test. (*$p<0.05$, **$p<0.01$)

Detection of elastin by two-photon laser scanning microscopy (LSM)

To analyze the elastin fiber formation, 3 x10^5 fibroblasts were transfected with 2.5 µg Ψ/5mCTP modified TE mRNA and 30,000 cells were seeded on each 1 cm^3 gelatin scaffold (Gelfoam®, Pfizer, New York, NY, USA). After a cultivation for 21 days at 37°C and 5% CO_2, the elastin fiber formation was analyzed using two-photon LSM as described in the study by Fritze et al. [1]. Therefore, MiraTM 900 Ti:Sapphire Laser pumped by a Verdi V5 Diode-Pumped Laser (Coherent, Santa Clara, CA, USA) coupled to an upright Zeiss Axioskop 2 FS MOT motor-based confocal laser-scanning microscope was used. After excitation with 760 nm light, elastin autofluorescence was detected as a broad emission signal from 403 to 531 nm with a maximum at approximately 457 nm. At an excitation wavelength of 760 nm, the scaffolds seeded with TE mRNA transfected cells revealed an increased autofluorescence signal compared to the scaffolds seeded with cells without TE mRNA transfection.



Supplementary Figure 3: Analysis of elastin synthesis by using two-photon laser scanning microscopy (LSM). 30,000 human fibroblasts without or with 2.5 µg TE mRNA transfection were seeded on gelatin scaffolds, and cultivated for 21 days. **A)** Two-photon LSM images of scaffolds seeded with or without TE mRNA transfected fibroblasts at an excitation wavelength of 760 nm. **B)** Spectral distribution measured by setting the excitation wavelength to 760 nm. The crosses (+) show the analyzed region of interests (ROIs).

Literature

[1] O. Fritze, M. Schleicher, K. Konig, K. Schenke-Layland, U. Stock, C. Harasztosi, Facilitated noninvasive visualization of collagen and elastin in blood vessels, Tissue Eng Part C Methods 16(4) (2010) 705-10.