Understanding the delivery of an ASO therapy for Epidermolysis Bullosa

Authors

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EPIDERMOLYSIS BULLOSA (EB)

- rare genetic disorder
- causes painful blistering of the skin with little abrasion
- 10.3 cases per million people in Australia

RECESSIVE DYSTROPHIC EB (RDEB)

- tissue separation in the sublamina densa
- most severe form of EB
- extensive scarring caused by blistering from birth
- chronic wounds
- pseudosyndactyly
- chronic inflammation
- increased risk of developing squamous cell carcinoma

GENETIC CAUSE OF RDEB

- caused by mutations in *COL7A1*
- large reduction or complete loss of type VII collagen
- reduced anchoring between skin layers
- revertant mosaicism can cause spontaneous correction of *COL7A1* in some cell popluations

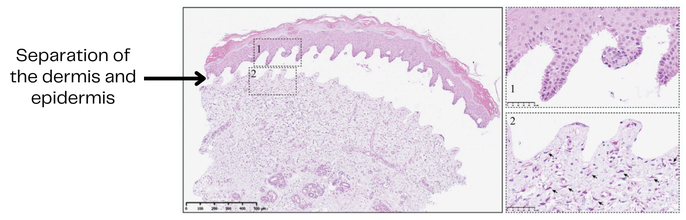


Figure 1. Skin section from a patient with RDEB. Formalin fixed and paraffin-embedded, with HRP-Magenta (Dako) chromogen staining. Imaged sourced from Condorelli et al. J Invest Dermatol. 2024.

RDEB CURRENT TREATMENT OPTIONS

- symptomatic therapies
- Beremagene geperpavec by Krystal Biotech
  - viral delivery of COL7A1 transgene
- possibility of antisense oligonucleotide (ASO) therapies

OBJECTIVE

Understand the efficacy, stability, and delivery pathway of a chemical delivery agent carrying an ASO in different skin cell models for the possible treatment of RDEB.

Hypothesis: Delivery of the ASO wil occur in under 30 minutes in ReCell comparable to plated cells

Aims:

1. Measure the ASO delivery efficiency in ReCell
2. Track the delivery of the ASO in plated cells over time
3. Measure the ASO activity in plated cells over time

METHODOLOGY

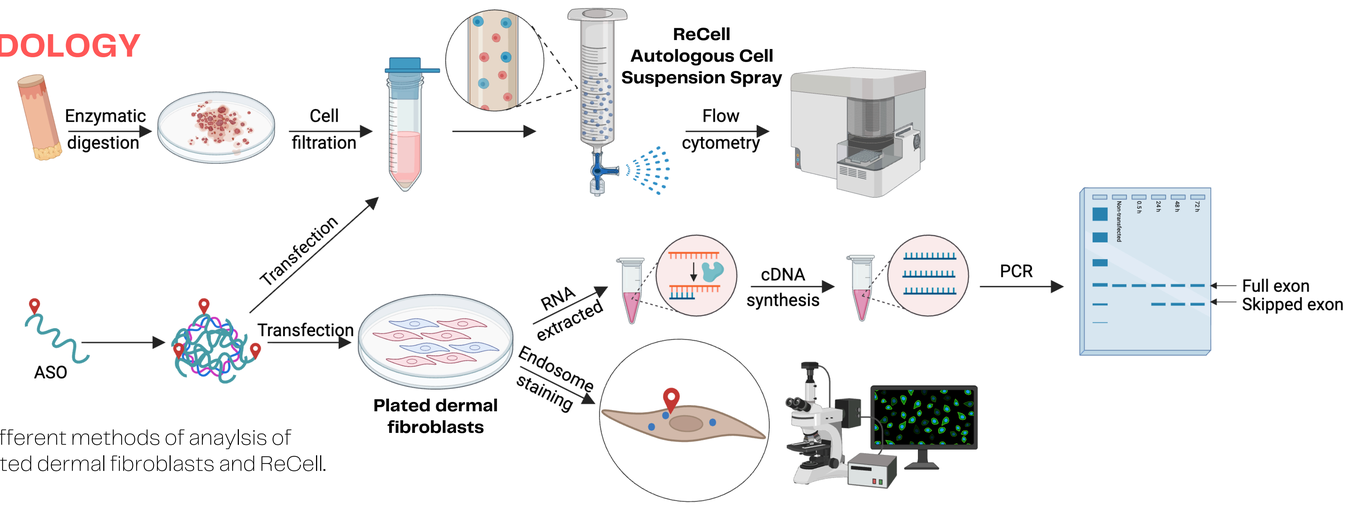


Figure 2. Defferent methods of anaylsis of delivery in plated dermal fibroblasts and ReCell.

RECELL TRANSFECTION IN 5 MINUTES

ReCell (Spray on Skin) is a method used for burn surgery, taking a small biopsy of healthy skin which undergoes enzymatic digestion before the cells are sprayed back onto to the burn. Has translational power for skin related genetic disorders.

ASO transfection is seen within 5 minutes in ReCell based on priliminary results.

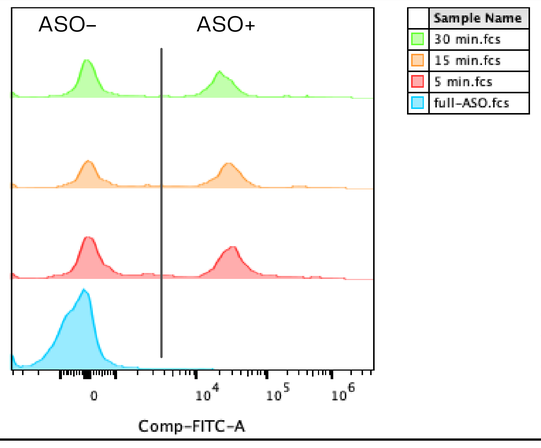


Figure 3. Histogram of ASO transfected ReCell at different time points, 5 min, 15 min and 30 min, will a full stain without ASO transfection as control.
Cells were gated for live cells via viability dye 780, then displayed on the FITC channel. ASO is tagged with fluorescein which shows as positive on the FITC channel.

Further analysis required to get the total percentage efficiency in the cell types of interest, fibroblasts and kerainocytes.

ASO DELIVERY TO CELLS IN UNDER 30 MINUTES

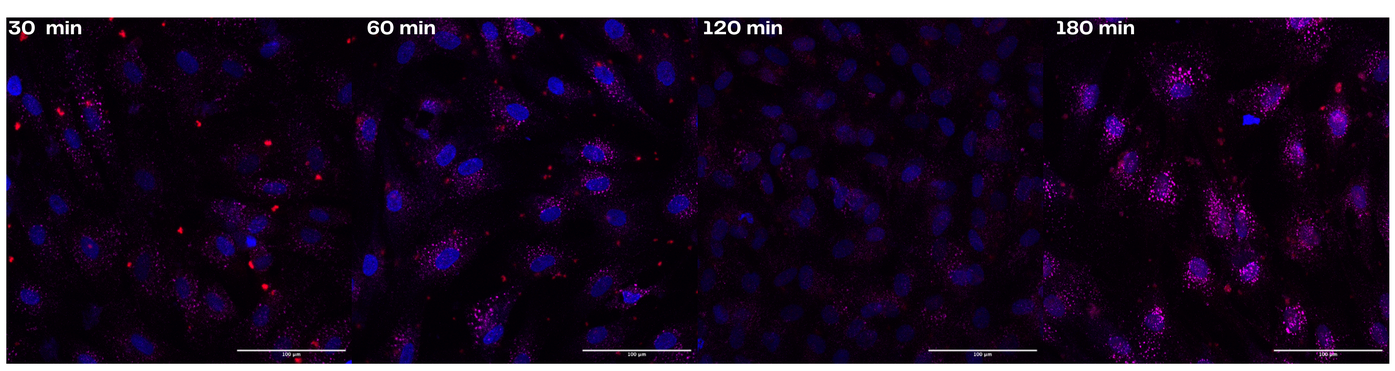
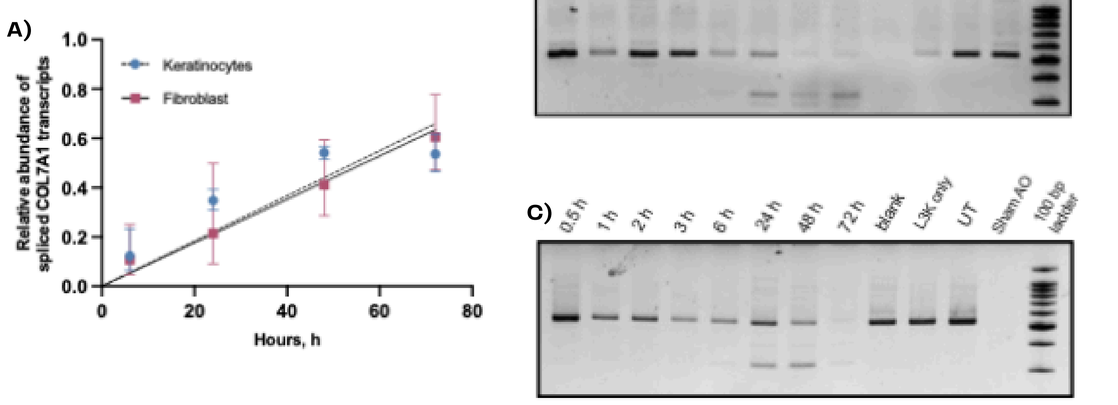


Figure 4. Images of patient dermal fibroblasts transfected with an antisense oligonucleotide tagged with hexachloro-fluorescein (Hex', red) at different timepoints (30, 60, 120, and 180 min). Stained for endosomes (purple) and Hoechst (blue). Imaged at 40 x magnification, scale bares are 100 µm.

SPLICED COL7A1 AT 6 HOURS

Figure 5. Splice COL7A1 expression in dermal primary fibroblasts and keratinocytes over time after ASO transfection. A) Relative abundance of spliced COL7A1 graphed over time. B) ASO transfected dermal fibroblasts COL7A1 RT-PCR. C) ASO transfected keratinocytes COL7A1 RT-PCR.



CONCLUSION

- transfection in 30 minutes in plated primary dermal fibroblasts
- spliced COL7A1 in plated primary dermal fibroblasts at 6 hours
- spliced COL7A1 in plated primary keratinocytes at 6 hours
- 5 minutes for delivery in ReCell
- new method of delivery for RNA thereapeutics
- future work
  - further analysis on delivery at different time points
  - optimise ReCell delivery for higher efficiencies

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