# RARE DISEASE CHALLENGE
# RaDiChal'21

## Preliminary Report

**The Spliceosome-mediated RNA trans-splicing gene editing approach for the phenotypic restoration of COL7A1 with sleeping beauty transposons via enriched fibroin-based nanoparticles**

**HEXAGENE**

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**Epidermolysis Bullosa**

## 1. Abstract

Functional impairment or complete lack of skin anchoring proteins, which results from numerous wide-ranged mutations in at least 18 genes, causes the fragile and continuously blistered epidermis disease known as Epidermolysis bullosa. In severe cases, mostly in recessive form, can be inside the body, such as the lining of the mouth, esophagus, and stomach. In addition, patients with rare dystrophic epidermolysis bullosa, which is the type our primary interest, might encounter aggressive metastasizing squamous cell carcinomas in cutaneous regions that may cause chronic ulcers. In such cases, RDEB jeopardizes patients' lives, mainly in the third to fourth decade. RDEB is caused by mutations in the COL7A1 gene that is responsible for encoding and secreting collagen type VII. These mutations lead to abnormal C7 synthesis or protein assembly defects in anchor fibers, resulting in poor epidermal adhesion and integrity. The two-third mutation counts are detected in clusters of exons between 47-118, proving consistent with published data. Spliceosome-mediated RNA trans-splicing offers a promising technique for developing novel gene therapy approaches for congenital and acquired disorders known as fatal and incurable so far. To do so, an exogenous DNA is injected into the fibroblast cell via fibroin-based nanoparticles, and a splice event in trans between the exogenous DNA and the endogenous target pre-mRNA is induced. The Trans-Splicing gene-editing technology will be used in our project to fix any mutations found between exons 47-118 and restore the COL7A1 gene's transcriptional activity via sleeping beauty transposons. Gene delivery vehicle decided as silk fibroin-based nanoparticle enriched with positively charged PEI and polyvinyl alcohol. The delivering agent will be administered with soluble microneedle tips via dermal patches to breach the stratum corneum and reach the dermis, the starting point of collagen secretion anomalies that lead to recessive dystrophic epidermolysis bullosa.

## 2. Problem

Recessive dystrophic Epidermolysis Bullosa disease occurs due to mutation of the collagen 7A1 gene, which has 118 coding exons. Any mutation in the exons or introns of the collagen 7a1 gene can cause the recessive dystrophic Epidermolysis Bullosa disease. Until this point in time, in excess of 800 detailed and unpublished grouping variations in COL7A1 have been distinguished in DEB patients (Wertheim-Tysarowska et al., 2011). It is challenging to produce a suitable genetic therapy for RDEB for many reasons. (i) This disease, which seems rare, does not have a single mutation that can be concentrated on (the prevalence of RDEB, respectively, is 1.35 per one million live births) (Eichstadt et al., 2019) *(figure 1)*. (ii) CRISPR method is less preferable in RDEB disease where a wide base interval needs to be corrected due to reasons such as off-target effect and low-efficiency rate. (iii) The use of viral vectors during genetic therapy transfer makes transfer planning difficult due to limited genome transfer, cytotoxic effects, the genome that is attempted to be transferred, and the integration of its hereditary material in a way that damages the genome and excessive immune response (Robbins & Ghivizzani, 1998). In addition, non-viral vectors currently in use have a low level of activity compared to viral vectors.

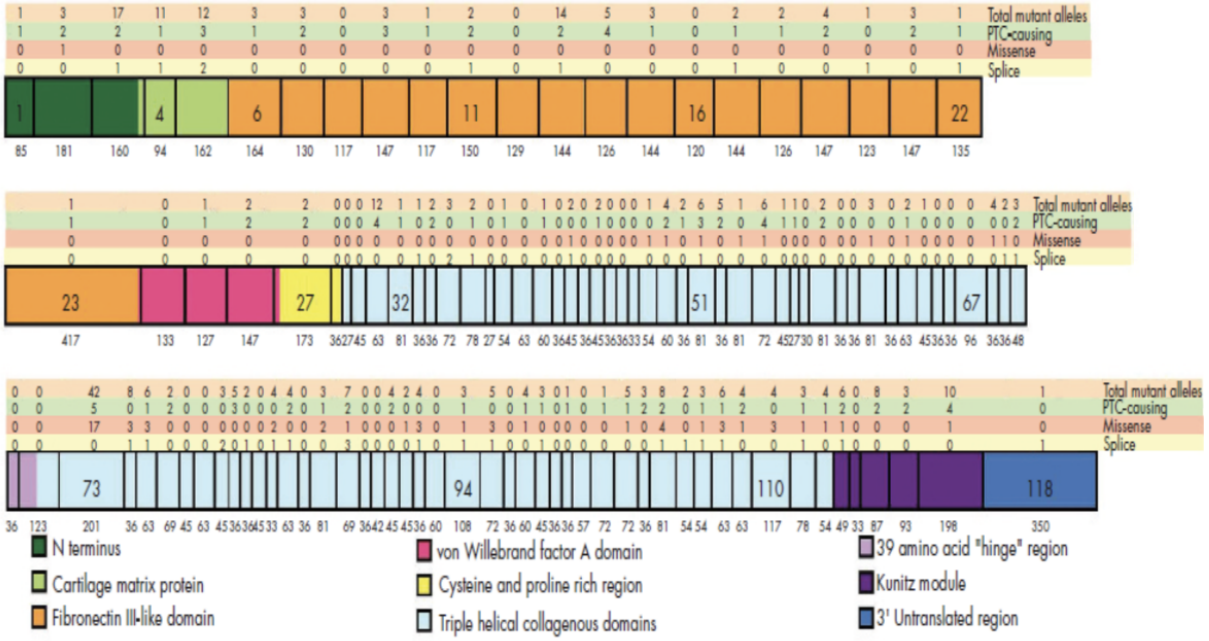


Figure1

## 3. Solution

We searched for the most effective and safe method for our disease, considering the above limitations. As a result of our research, we believe that transferring the Splicesome-Mediated RNA trans-splicing (SMaRT) method with the Sleeping Beauty transposon within the fibroin nanoparticle will be the most effective method.

The trans-splicing method is similar to the way pre-MRA, which occurs naturally in eukaryotic cells, gets rid of its introns via splicesosome and turns into mRNA. In this project, we found that targeting more exons and maximizing the number of mutations and efficiency would be 3' exon replacement for RDEB disease. According to the results we obtained from the article written on this subject, we developed a 3' exon replacement model that targets more than 4 kb of the gene and can target 2/3 of the mutations in the gene (Tockner et al., 2016).

We decided to use the Sleeping Beauty Transposon system to maintain the effectiveness of the material we will transfer reliably and effectively. Transposons can move or reposition themselves within and between chromosomes to insert themselves into different places within the genome.

The Spliceosome-mediated RNA trans-splicing therapeutic approach for the phenotypic restoration of COL7A1 with sleeping beauty transposons via enriched fibroin-based nanoparticles project aims to reconstruct wide-ranged exons between 47-118 cover two-thirds of the patient with recessive epidermolysis bullosa. Integration of RNA Trans-splicing Molecule (RTM) gene transported between two inverted terminal repeat (ITR)

will be ensured through Sleeping Beauty transposase. The delivery of transposons will be conducted with fibroin protein-based nanoparticles that have high biocompatibility and non-immune response to fibroblast cells via clathrin-mediated endocytosis. To increase cellular uptake, positively charged PEI and as an inner core PVA that enable high therapeutic entrapment and tunable particle sizes will be components of enriched fibroin nanoparticles. The coaxial electro spraying method, which can inject materials with electrostatic forces, will be used to embed transposon and polyvinyl alcohol into fibroin nanoparticles. Eventually, the project aims to use soluble microneedles as dermal patches to pass the stratum corneum layer, deliver the obtained transposon-loaded fibroin nanoparticles to the fibroblast cells, and effectively release them in a controlled ,and a sustainable manner.

## 4. Method

The primary purpose of drug delivery systems covers administration and the delivery of particles designed as a pharmaceutical agent to the target region of the body safely and correctly to avoid immunogenicity and toxicity responses. Delivery systems should protect our therapeutic from degradation, increase its permeability, managing releasing patterns, prevent changes its pharmacokinetics, and enhance cellular uptake. The convenient structural features of protein-based nanoparticles allow corporations with biodegradable polymers to maintain a sphere formation to secure controlled and continued deliverance. Our therapeutics should be administered above a proper level to boost its efficiency, and curative effect expect a successful treatment(Davidov-Pardo et al., 2015). Besides fibroin's versatile, high binding property and highly tunable character, as a proven encapsulating material, the protein can be used as an advanced carrier for an extensive type of size molecules, enzymes, and genetic materials(Pham et al., 2018). More than 10 types of preparation methods for fibroin nanoparticles were evaluated. The electrospray manufacturing method is decided to prepare fibroin nanoparticles because of its high drug entrapment and adjustable particle size properties(Qu et al., 2014). The fundamental logic behind the technique is the ejection of target polymers into the nanodroplets by applying electrostatic forces. . Polyvinyl alcohol (PVA) is a semicrystalline polymer that can be used when preparing stable distributions to advance therapeutic solubility. It has been widely used in the biomaterial field as a synthetic and water-soluble material (FDA approved)(Cao et al., 2017). At the beginning of establishing the coaxial system, the dual-capillary electrospray head will be set up to form two segregate channels. The next step will be the injection of polyvinyl alcohol/transposable element and silk fibroin into the internal and external sections, respectively.

Transfer methods for gene restoration treatments consist of two different methods, vector-based, including viral and non-viral, and physical approaches (Chen et al., 2016). Our project aims to deliver fibroin enriched nanoparticles to the dermis layer of the skin because our target disease Recessive Dystrophic Epidermolysis Bullosa stems from dysfunctional Collagen VII protein produced by fibroblasts in the dermis layer of the skin. In this aspect, the lower layer of skin, which has fibroblast density, is a potential target to administrate treatment. As a promising approach, microneedles can reach exactly to dermis layer passing

through the stratum corneum (Chen et al., 2016). The fact that microneedles have pain-free and less immunogenicity and tumorigenicity rate also creates an advantage over viral methods such as adenovirus, retroviral, etc. (Jiao et al., 2020). Different types of MNs such as metal MNs, coated MNs, dissolving MNs are used to overcome various challenges in gene delivery (Chen et al., 2016). Dissolving microneedles are superior to other types in dose capacity, harmlessness, and feasibility (Chen et al., 2016). Also, dissolving microneedles utilizes soluble material not to harm the skin.

## 5. Feasibility

All proof of concept stages are specified in the method section, and the existence of research based on the biocompatibility of fibroin protein with fibroblasts and research implying epidermolysis bullosa for the use of sleeping beauty transposon makes our project possible at the theoretical stage.

## 6. Risks

Digestion of fibroin, an organic polymer, by macrophages and reduced particle efficiency is a potential risk. In order to test these risk factors, it should be determined how macrophages break down nanoparticles with mammalian experiments. If this rate is at a level that will affect transposon delivery, macrophage activity can be reduced with immunosuppressive drugs for the period of therapy. In addition, the patients with EB must experience the microneedles to be used. At the theoretical stage, the efficiency analysis of our project, which is designed for areas where wounds have not yet occurred, should be done for areas where injuries have occurred to elucidate whether our dermal patches are appropriate material for injured regions or not. If it is not suitable for areas with wounds, application methods such as wound dressing, which reduces the advantage of direct injection under the skin and works with a less effective release mechanism, should be evaluated.

## 7. References


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