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In situ gene therapy via non-viral delivery of CRISPR-Cas9 to the skin aiming for recovery of autosomal recessive congenital ichthyosis (ARCI)

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Autosomal recessive congenital ichthyosis (ARCI) is a rare, but severe keratinisation disorder characterized with regions of dry scaled skin, impaired skin barrier function, higher transepidermal water loss and a meaningful susceptibility to infection. Gene editing tools like CRISPR-Cas9 are the ideal for correcting rare monogenic skin diseases like ARCI. We aimed to efficiently deliver CRISPR-Cas9 components (Cas9 protein (RNP) or Cas9-mRNA) to primary human keratinocytes (KCs) and skin stem cells (HPEKPs) using lipid-based nanoparticles (LNPs) for homology directed repair of TGM1 gene. Fluorescently labelled LNPs composed with different helper lipids including DSPC, DOPC, DOPE, DSPG and ES, were optimized systematically as high-performance gene delivery vectors via cellomics quantitative cell analysis. Concurrently, ApoE addition and 0.5%, 1.5% & 5% concentrations of polyethylene glycol (PEG) in LNPs were also assessed. Among the 5 helper lipids; DOPE showed the highest levels of cellular uptake, the inclusion of ApoE exhibited up to a 12.7-fold increase in LNPs uptake. Increasing PEG up to 5% resulted in a decrease in cellular uptake likely due to a physical steric barrier effect. Furthermore, 80-90% of CRISPR-RNAs were encapsulated with LNPs whereas RNPs did not show any significant encapsulation. Through RNPs/LNPs formulations, 12-17% cutting of genomic DNA was achieved while with CRISPR-RNAs it increased up to 30% with no significant cytotoxicity. To observe the effect of addition of permanently charged cationic lipids on LNPs, DOTMA (1,2-di-O-octadecenyl-3-trimethylammonium propane), was added to the LNPs composed of DOPE. Increased cutting efficiencies were seen with the addition of ≤ 10% DOTMA, which however, was also associated with cytotoxicity. Further increase of DOTMA up to 30% and 50% did not further improve the cutting efficiencies, due to strong cytotoxic effects. Conclusively, for efficient delivery of CRISPR-Cas9 components to skin cells comprehensive screening of individual components of nanoparticles libraries is required.

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