Supporting Information: The Skin You're In: Design of Experiments Optimization of Lipid Nanoparticle Self-Amplifying RNA Formulations in Human Skin Explants

Anna K. Blakney[†], Paul F. McKay[†], Bárbara Ibarzo Yus[†], Judith E. Hunter[‡], Elizabeth A. Dex[‡], Robin J. Shattock[†,*]

[†] Department of Medicine, Imperial College London, London, W21PG, United Kingdom

[‡] Department of Plastic Surgery, Imperial NHS Trust, London, W68RF, United Kingdom
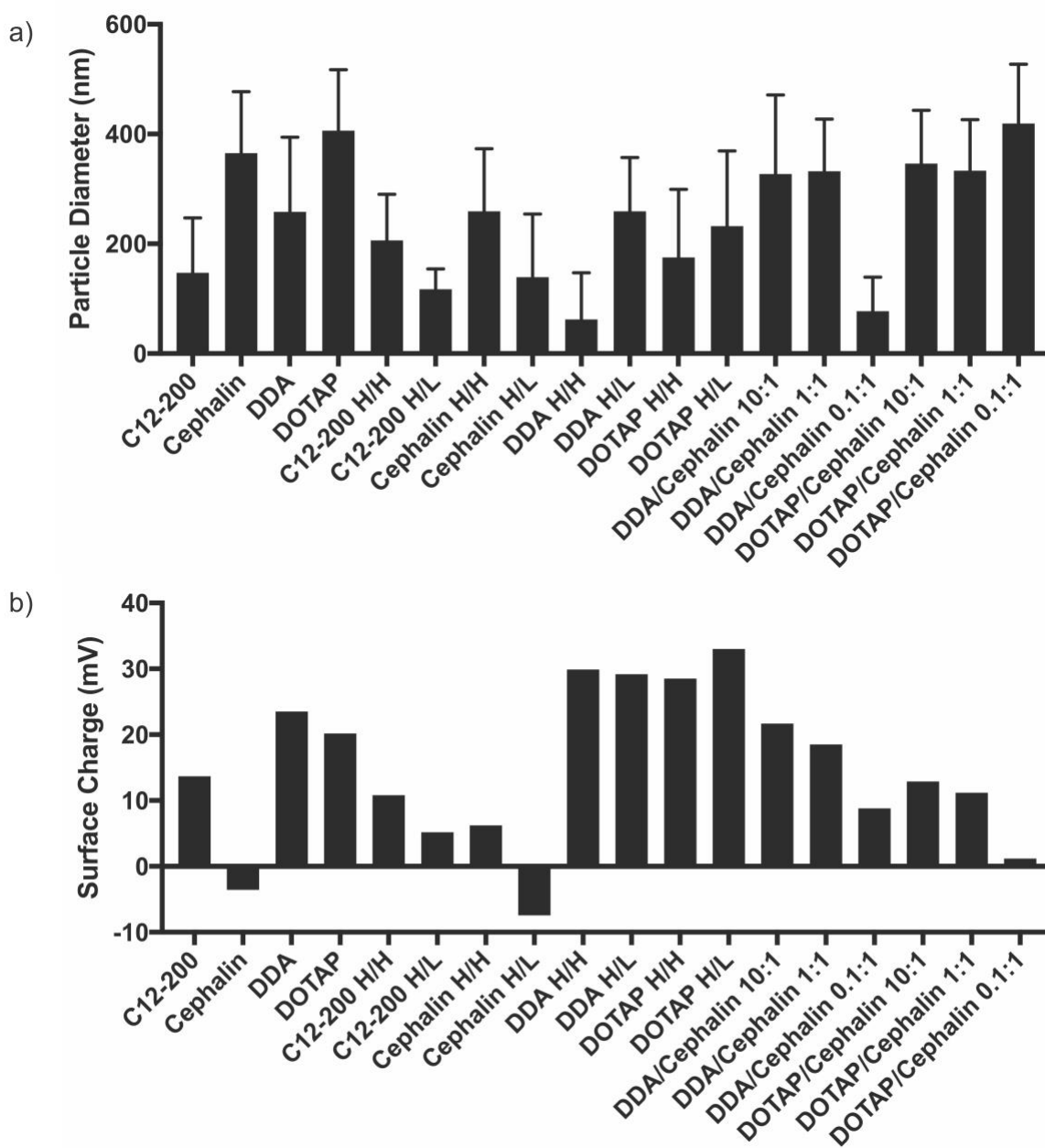
FIGURES

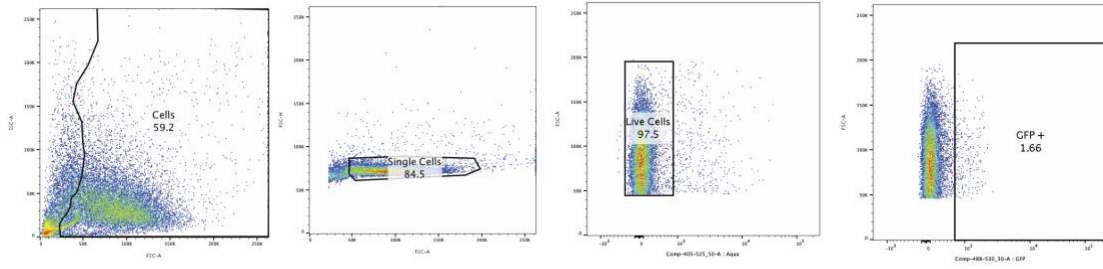**Figure S1**. Characterization of lipid nanoparticle a) diameter and b) surface charge.

Figure S2. Gating strategy for eGFP expressing cells from human skin explants as determined by flow cytometry after three days of culture.
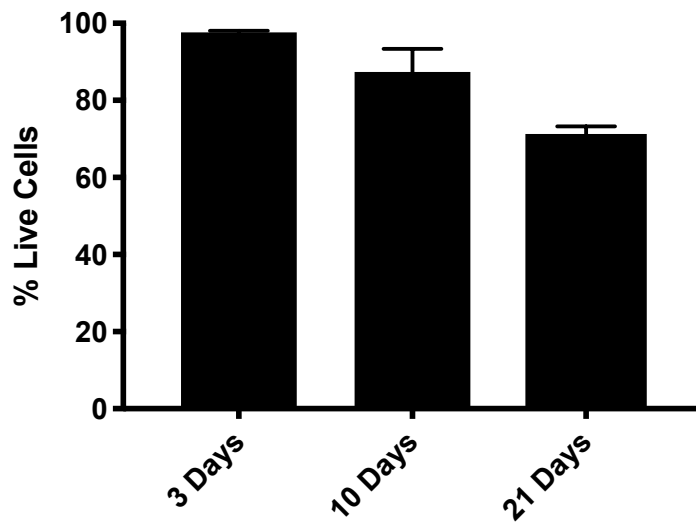
Figure S3. Explant viability over the course of 21 days as determined by flow cytometry.
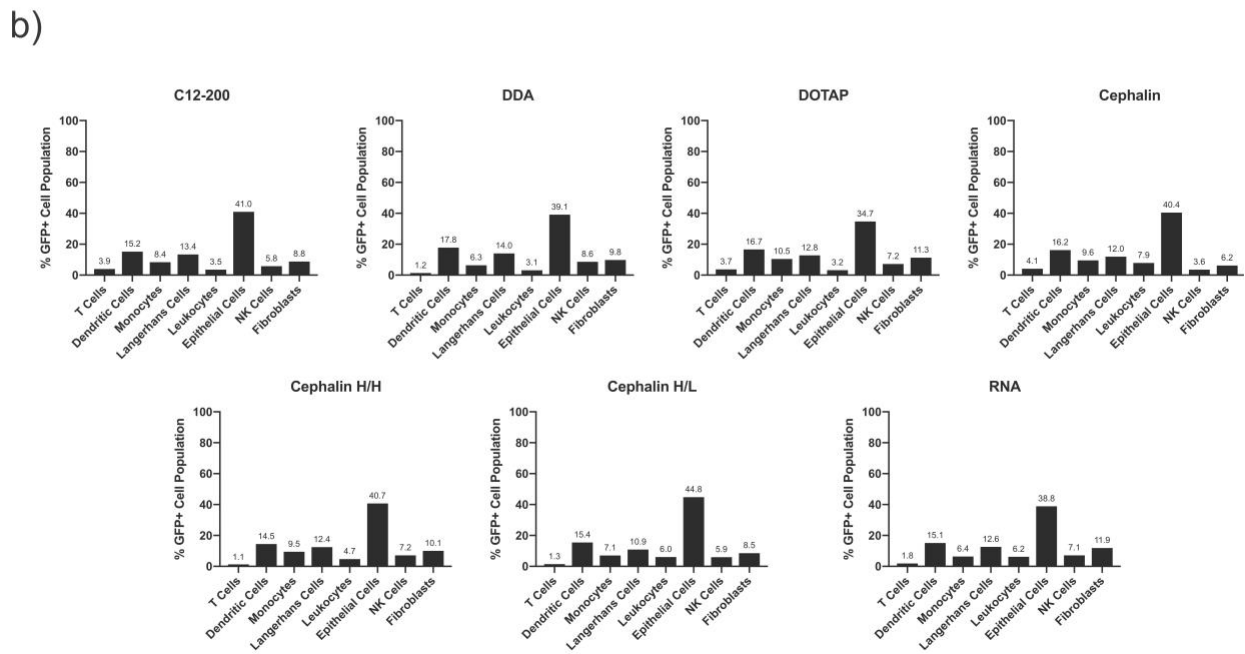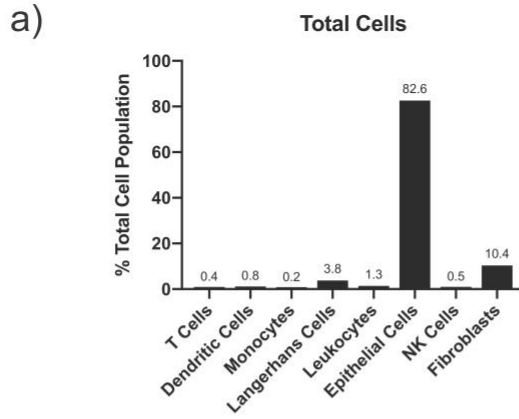
Figure S4. Identity of cells present in human skin explants and GFP+ cells after ID injection of LNP formulations as determined by flow cytometry after three days of culture. a) Identity of cells present in human skin explants. b) Identity of GFP-expressing skin cells from explants treated with LNP-formulation RNA. The bar represents the mean for n=3, and the mean is detailed on top of each bar for each cell type.
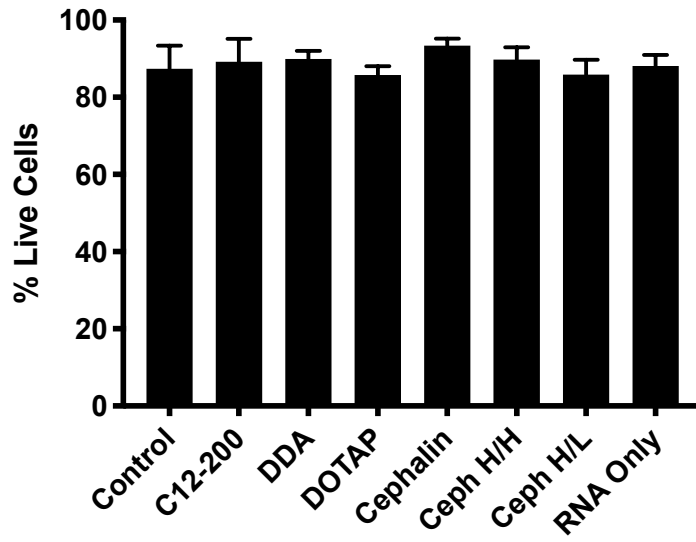
Figure S5. Skin explant cell viability in response to LNP-formulation saRNA, as determined by live/dead staining and flow cytometry after three days of culture.

TABLES

Table S1. Panel of antibodies used to identify GFP expressing cells in human skin explants.

Antibody	Labelling	Clone	Vendor	Test Volume (μL)
CD1a	PerCP-eFluor 710	HI149	Biolegend	5
CD3	eFluor 450	OKT3	Biolegend	5
CD14	Qdot 605	Tük4	Biolegend	1
CD11c	PE	3.9	Biolegend	5
CD45	Alexafluor 700	2D1	Biolegend	2
CD56	PE-Cy5	CMSSB	Biolegend	5
CD90	PE-Cy7	5E10	Biolegend	5