Topical delivery of synthetic mRNA expressing human contraception antibody (HCA) to the female reproductive tract for contraception

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Background

High rates of unintended pregnancies worldwide indicate the need for more acceptable contraceptive options. Human contraception antibody (HCA), an IgG1 monoclonal antibody that agglutinates and immobilizes human sperm, is a promising candidate for nonhormonal immunocontraception in women. Here, we explored an mRNA-based topical delivery method to induce expression of HCA in the female reproductive tract (FRT). The use of synthetic mRNA for contraception is novel and could provide several advantages including: efficiency, reversibility, safety, durability, and cost-effectiveness.

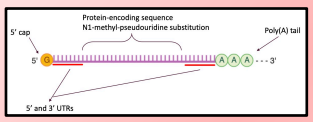
Objectives

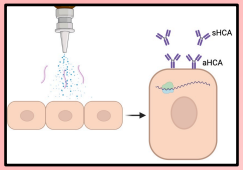
Optimize synthetic mRNA-mediated expression of HCA in the female reproductive tract (FRT):

- ✓ Achieve ≥ 10 µg/ml of HCA expression (minimum functional dose) in a vaginal epithelial cell line, a 3D vaginal tissue model, and a non-human primate model
- ✓ Maintain HCA levels in models by incorporating a GPI-linker
- ✓ Determine effects of prolonged mRNA exposure on cell viability
- ✓ Demonstrate bioactivity of mRNA-produced antibodies
- ✓ Study effects of variables within the vaginal environment such as temperature and low pH on mRNA transduction
- ✓ Express multimeric variants of HCA in cells using mRNA

Methods

mRNA Transfections: mRNA constructs were synthesized by the Santangelo lab using optimal modifications to enhance expression. Four constructs, GPI-anchored HCA (aHCA) and secreted HCA (sHCA) with or without Nanoluc (Nluc) incorporation, were used. Transfection experiments were conducted *in vitro* using a human vaginal epithelial cell line and a 3D vaginal tissue model and *in vivo* using rhesus macaques. Target cells were transfected with HCA heavy and light chain mRNA strands either by aerosol delivery or liquid droplets.





Expression Quantification: Levels of HCA expression post-transfection were measured by ELISA and Nluc luciferase detection.

Sperm Agglutination & Specificity: Bioactivity of mRNA-expressed HCA was assessed by kinetic sperm agglutination and sperm escape assays. Immunofluorescent staining was performed to confirm specificity.

Toxicity: Transepithelial electrical resistance (TEER) and MTT data were compared between mRNA-treated and control cells.

Results

Figure 1. Kinetics of mRNA-mediated HCA expression in models of the FRT. a) HCA expression in human vaginal epithelial (VK2) cells following a 1 µg mRNA transfection with a lipid-based vehicle was quantified by ELISA. **b)** Supernatants of 1 µg sHCA mRNA-transfected VK2 cells were collected and treated with furimazine for Nluc detection. **c)** Detection of aHCA in vaginal secretions of rhesus macaques over a 42-day period. Macaques received ~3 mg mRNA in 0.5 ml water using an atomizer.

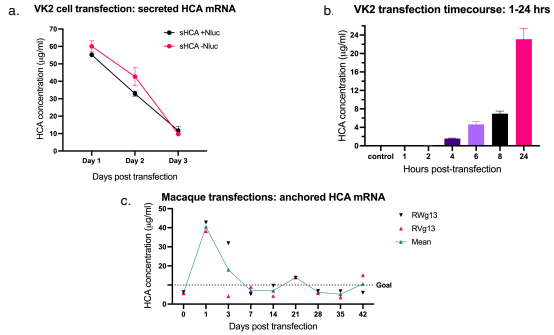


Figure 2. Bioactivity of mRNA-expressed HCA. a) Time elapsed to reach complete sperm agglutination were compared between commercial-grade HCA and mRNA-expressed HCA. **b)** Sperm escape assay using commercial-grade HCA and mRNA-expressed HCA. "Escaped" sperm are non-agglutinated progressive sperm cells after 5 min of antibody exposure. **c)** Agglutination potency of mRNA-expressed aHCA harvested on days 1 and 7 post-transfection. **d)** Immunostaining of sperm cells using cervicovaginal lavages (CVLs) collected from macaques on days 1 and 7 post-transfection.

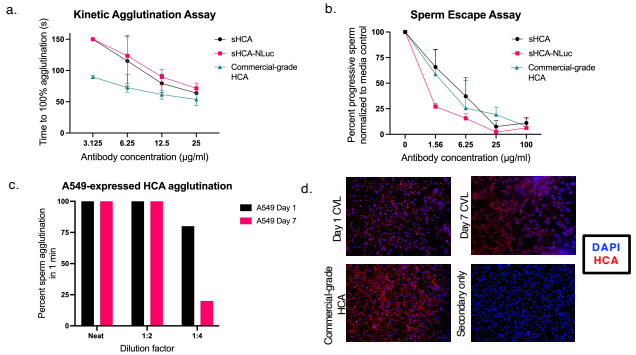


Figure 3. Cell viability following prolonged exposure to mRNA. a) No significant difference between MTT viability results of untreated cells and cells exposed to 1 µg mRNA after 7 days ($p > 0.05$). **b)** TEER measurements of vaginal tissue following mRNA aerosol treatment compared to untreated control tissue.

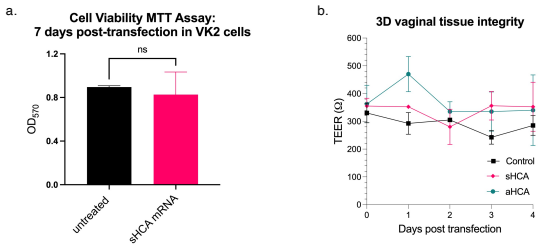


Figure 4. mRNA transfection efficiency. a) Percent cell uptake of mRNA was quantified by counting the number of cells expressing aHCA on cell membranes. **b)** Immunofluorescent staining of aHCA-associated Nluc throughout a 3D vaginal tissue model four days post aerosol transfection. **c)** mRNA was exposed to 0.5% lactic acid and 37 °C prior to cell transfection. No significant differences in Nluc expression among experimental conditions ($p > 0.05$).

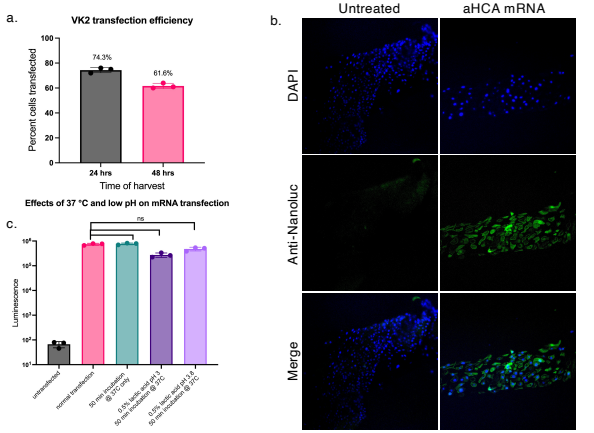
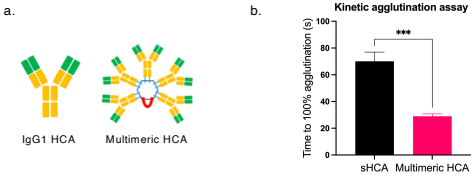


Figure 5. mRNA-expressed multimeric variant of HCA. a) Depiction of IgG1 HCA and multimeric HCA variant assembled with IgM tailpiece and J-chain (red). **b)** Sperm agglutination time was significantly improved using multimeric HCA compared to IgG HCA ($p < 0.001$).



Conclusions

Vaginal cells were conducive to aerosol mRNA transfection and subsequently produced antibody in all models. HCA expression was reversible and did not disrupt cell viability or tissue permeability. Greater than 10 µg/ml of agglutinating antibody was produced over consecutive days. The GPI-anchored HCA demonstrated enhanced retention compared to secreted HCA and may be especially suitable for longer-lasting contraception. A multivalent variant of HCA assembled with IgM tailpiece was expressed in cells from mRNA and had increased potency compared to IgG1 HCA. These data support the further exploration of topically delivered mRNA for the expression of HCA in the FRT as nonhormonal female contraception.

Funding

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