Title:	A combinatorial approach for robust transgene delivery and targeted expression in mammary gland for generating biotherapeutics in milk, bypassing germline gene integration
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Abstract:	Protein expression in the milk of transgenic farmed animals offers a cost-effective system for producing therapeutics. However, transgenesis in farmed animals is not only cumbersome but also involves risk of potential hazard by germline gene integration, due to interruptions caused by the transgene in the native genome. Avoiding germline gene integration, we have delivered buffalo β-casein promoter-driven transgene construct entrapped in virosomes directly in the milk gland through intraductal perfusion delivery. Virosomes were generated from purified Sendai viral membrane, containing hemagglutinin-neuraminidase (HN) and fusion factor (F) proteins on surface (HNF-Virosomes) which initiate membrane fusion, devoid of any viral nucleic acids. Intraductal delivery of HNF-Virosomes predominantly transfected luminal epithelial cells lining the milk duct and buffalo β-casein promoter of the construct ensured mammary luminal epithelial cell specific expression of the transgene. Mammary epithelial cells expressed EGFP at lactation when egfp was used as a transgene. Similarly, human interferon-γ (hIFN-γ) was expressed in the mammary gland as well as in the milk when hIFN-γ was used as a transgene. This combinatorial approach of using Sendai viral membrane-derived virosomes for entrapment and delivery of the transgene and using buffalo β-casein promoter for mammary gland specific gene expression provided a better option for generating therapeutic proteins in milk, bypassing germline gene integration avoiding risks associated with animal bioreactor generated through germline gene integration.
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