Turning the body into a biofactory: in situ generation of immunostimulatory virus-like nanoparticles

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BACKGROUND

- Glioblastoma multiforme is a lethal brain tumor characterized by a highly immunosuppressive microenvironment.
- Fibrin gene-activated matrices (GAMs) serve as an *in situ* biofactory for the sustained and localized delivery of therapeutic pDNA.
- Virus-like particles (VLPs) formed by self-assembling viral proteins that mimic viral structures are safe and powerful immunostimulators.

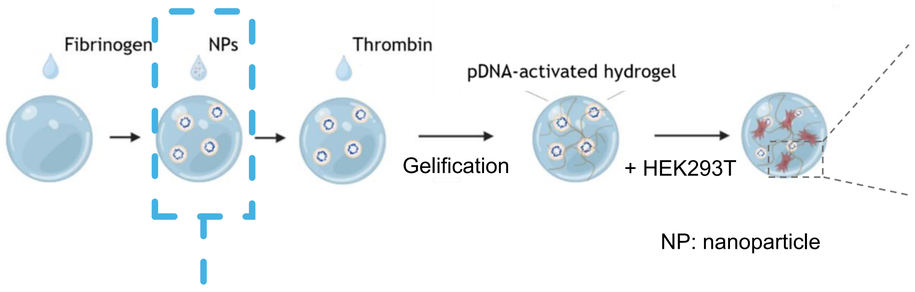
OBJECTIVES

- To evaluate fibrin GAMs as platforms for sustained in situ production of immunogenic VLPs
- To assess their ability to activate dendritic cells and overcome glioblastoma-associated immunosuppression.

METHODS

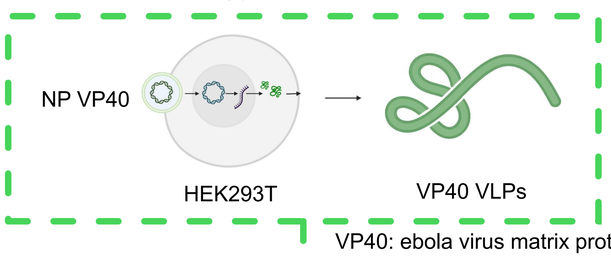
The biofactory scaffold: gene-activated matrices (GAMs)

Production:

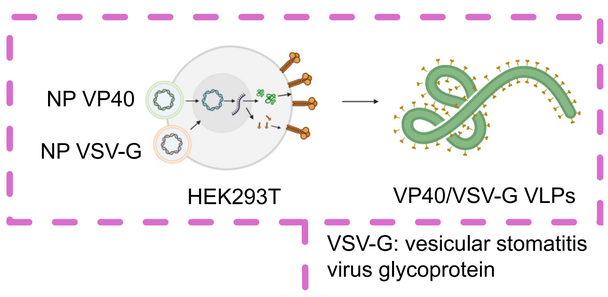


The biofactory machinery: in situ assembly of virus-like particles (VLPs)

Strategy 1: ebola VLPs



Strategy 2: pseudotyped ebola VLPs



RESULTS

1. Nanoparticle optimization within GAMs

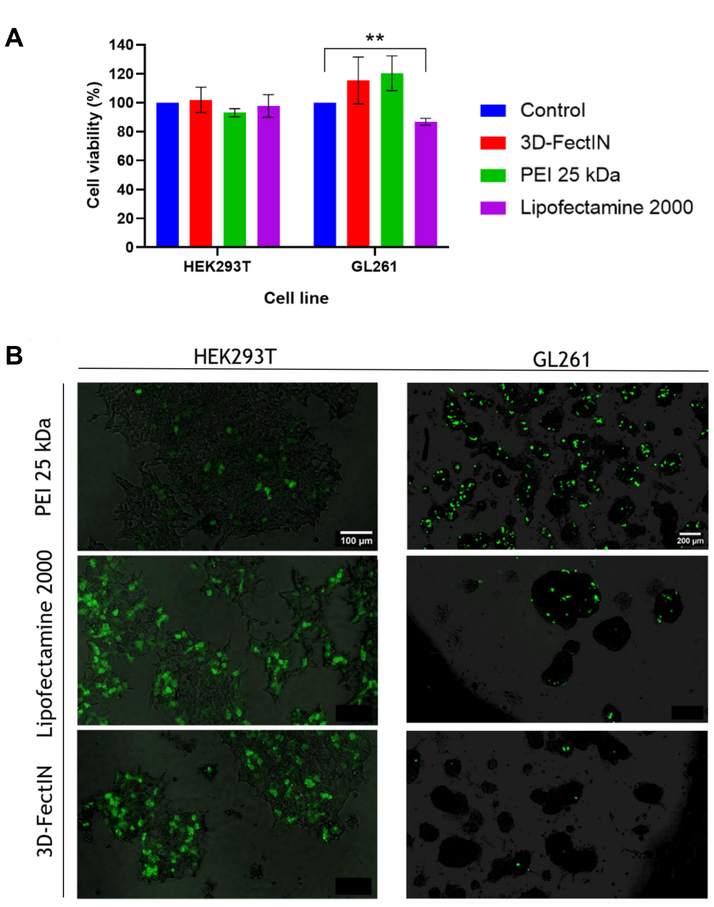


Figure 1. (**A**) Cell viability (24 h) by Alamar Blue assay (mean ± SD; n=3; **p < 0.01). (**B**) GFP expression (48 h) within GAMs (300 ng/cm² pDNA dose) (scale bars = 100 and 200 µm).

2. Characterization of GAM-derived VP40 VLPs and release kinetics

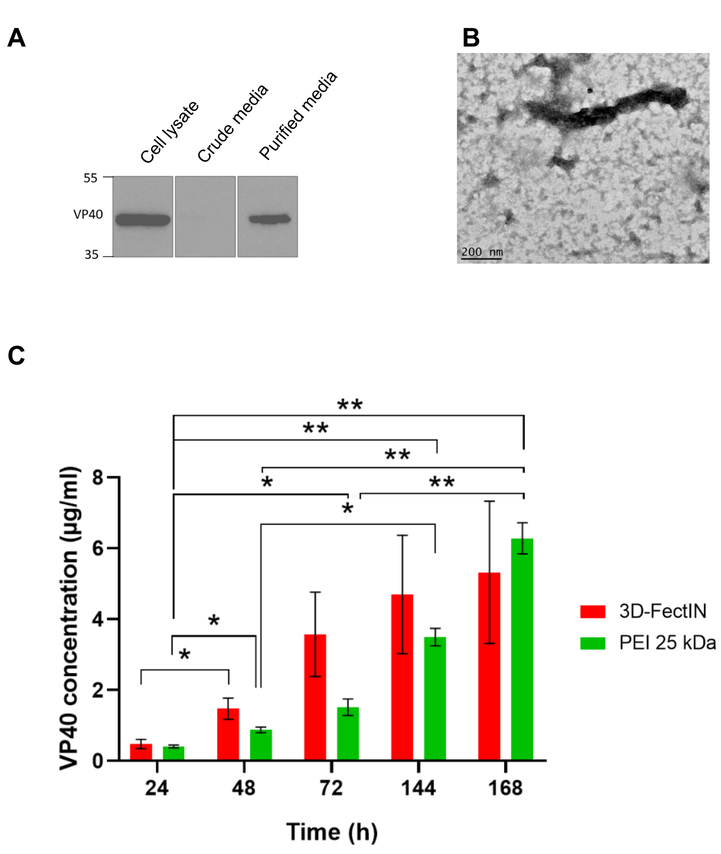


Figure 2. (**A**) VP40 expression in HEK293T cells and conditioned media by Western blot. (**B**) Filamentous morphology of purified VLPs by TEM (scale bar = 200 nm). (**C**) Time-course kinetics of VP40 production in hydrogel supernatants (indirect ELISA; mean ± SD; n=3; *p < 0.05 and **p < 0.01).

3. Production optimization of VP40/VSV-G VLPs

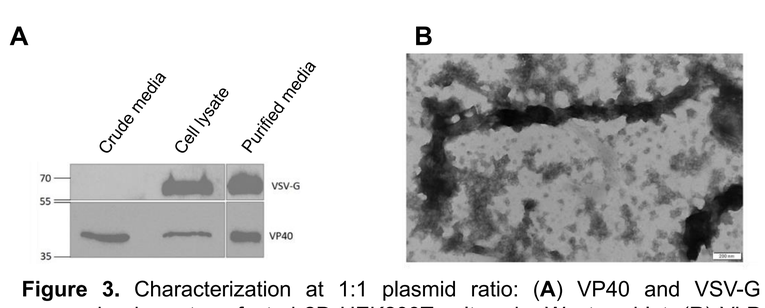


Figure 3. Characterization at 1:1 plasmid ratio: (**A**) VP40 and VSV-G expression in co-transfected 2D HEK293T culture by Western blot. (**B**) VLP morphology in purified media by TEM (scale bar = 200 nm).

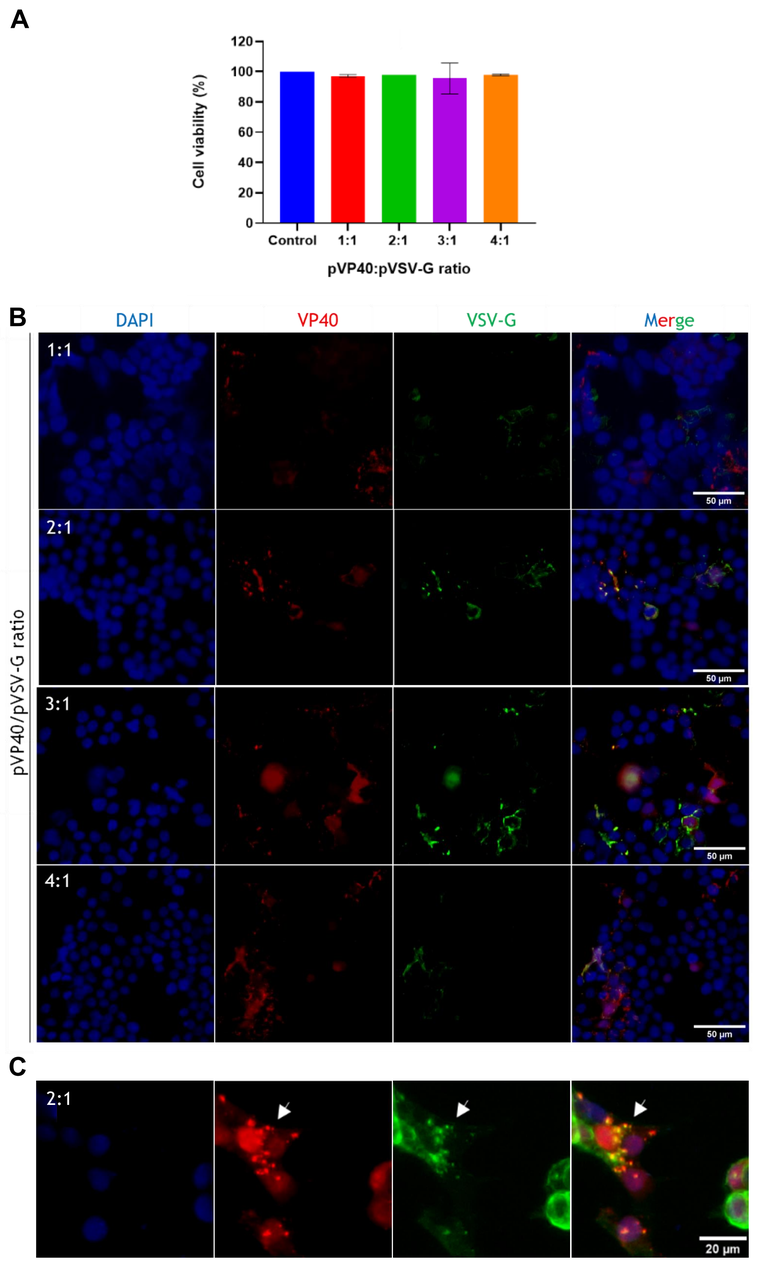


Figure 4. Screening of plasmid ratios in 2D HEK293T culture. (**A**) Cytotoxicity by Alamar Blue (24 h; mean ± SD; n=3). (**B**) VP40/VSV-G immunofluorescence at 200 ng/cm² pDNA (scale bar = 50 µm). (**C**) VP40/VSV-G expression at 250 ng/cm²; arrows indicate punctate cytoplasmic colocalization, suggesting particle assembly (scale bar = 20 µm).

4. High-resolution molecular characterization of single VP40/VSV-G VLPs

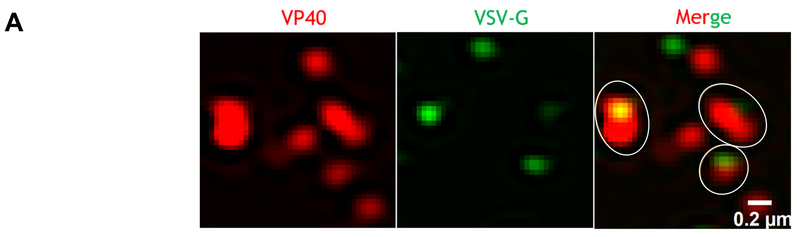


Figure 5. (**A**) Airyscan confocal microscopy of purified VLPs; circles indicate pseudotyped particles (scale bar = 200 nm). (**B**) Quantitative VP40/VSV-G colocalization analysis (Fiji ComDet plugin; mean ± SD; n=6)

5. Activation of peripheral blood-derived moDCs by GAM-released VLPs

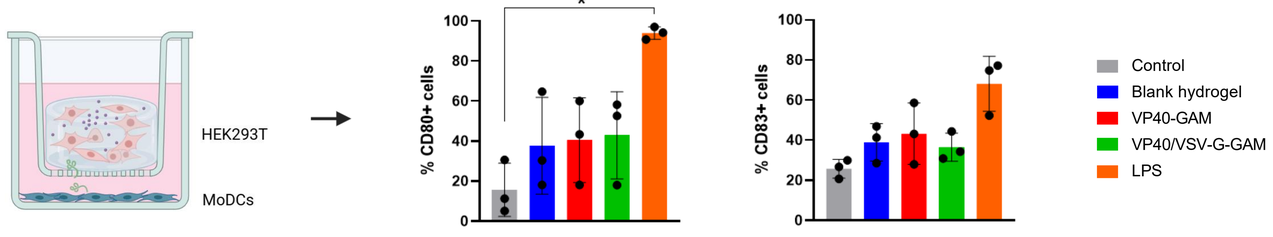


Figure 6. (**A**) Evaluation of monocyte-derived dendritic cells (moDCs) activation via VLP diffusion (48 h) from Transwell inserts by flow cytometry (mean ± SD; n=3).

CONCLUSIONS

- **Non-toxic fibrin-based gene-activated hydrogels** were successfully developed for the controlled production and release of immunostimulatory VLPs.
- *In vitro* release studies demonstrated sustained **VLP production for at least one week**.
- **VLPs** were successfully **pseudotyped with VSV-G**, with **more than 20%** of particles co-expressing VP40 and VSV-G proteins in 2D cultures.
- An **increase in moDC activation** was observed at 48 h, including in scaffold-only conditions. This early response suggests the onset of sustained VLP expression, which peaks between 48–72 h.

Acknowledgments

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