Determining drug activity against SARS-CoV-2 using lab-generated lentiviral vectors pseudo-typed with the SARS-CoV-2 spike glycoprotein

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Introduction

The SARS-CoV-2 spike glycoprotein mediates virus entry into target cells by binding to angiotensin converting enzyme 2 (ACE-2) receptor protein [(1)]. By understanding and inhibiting the interaction between SARS-CoV-2 spike glycoprotein and ACE-2 receptor, SARS-CoV-2 could be prevented from entering host cells, thus averting the spread and infection within the population. As a safer, reproduceable and practical alternative for studying SARS-CoV-2 activity outside of biosafety level 3 containment, this study used variants of SARS-CoV-2 spike glycoprotein, pseudo-typed onto replication incompetent lentiviral vectors [(2)] as a tool for the development of a high through-put drug screening assay .

Aims

- To generate a plasmid expressing the SARS-CoV-2 spike glycoprotein.
- To generate lentiviral vectors pseudo-typed with the SARS-CoV-2 spike glycoprotein.
- To assess the effects of drugs on the infectivity of SARS-CoV-2 pseudo-typed lentiviral vectors in a drug treatment assay.

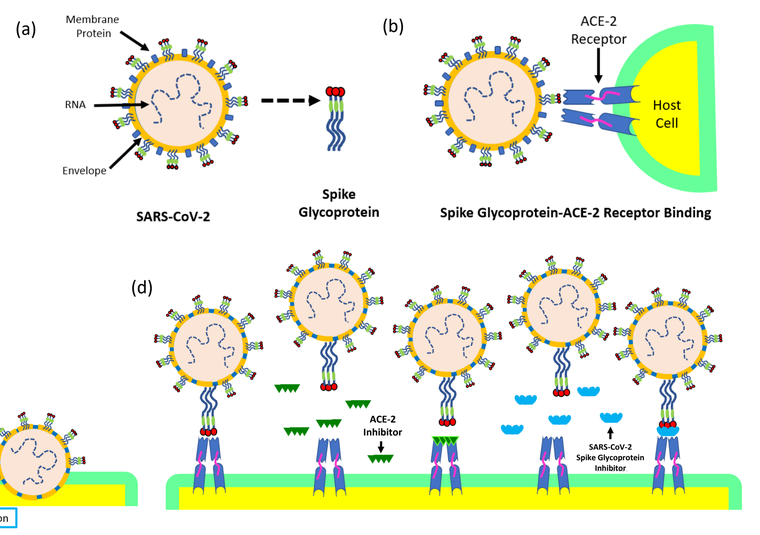

Figure 1: *SARS-CoV-2 structure and binding. (a) SARS-CoV-2 virus and spike glycoprotein structure. (b) Spike protein binding to ACE-2 receptor. (c) Virus cell fusion process mediated by the spike glycoprotein. (d) Competitive inhibitor binding of both SARS-CoV-2 and ACE-2 receptor preventing binding and viral cell fusion process. Images adapted from Huang et al. (2020) [(1)].*

Methods

- A plasmid expressing the SARS-CoV-2 spike glycoprotein (pCMV14-3xFlag-SARS-CoV-2) was generated by placing a FLAG-tagged SARS-CoV-2 gene under the control of a CMV promoter.
- Generated lentiviral particles were analysed for reporter activity by undertaking luminescence-based and viability assays to optimise methods for pseudo-virion production.
- Dexamethasone and heparin were assessed for their ability to impede potential viral entry through interruption of the SARS-CoV-2 spike glycoprotein mediated viral entry process.

Results and Discussion

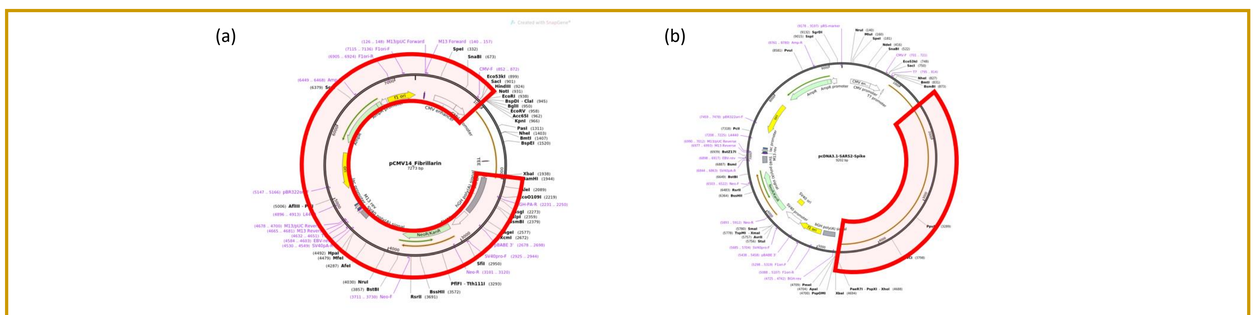

Figure 2: *Generating pseudo-SARS-CoV-2 plasmid (a) Schematic of pCMV14-3XFlag-Fibrillarin (Addgene), with cut CMV14-3XFLAG backbone highlighted in red (6273bp). (b) Schematic of pcDNA3.1-C9-SARS-CoV-2-Spike (Addgene) with cut, amplified and digested SARS-CoV-2-Spike DNA insert highlighted in red (4000bp). Both highlighted plasmid fragments were ligated to form the pCMV14-3XFLAG-SARS-CoV-2 (SC2) (~10,300bp).*

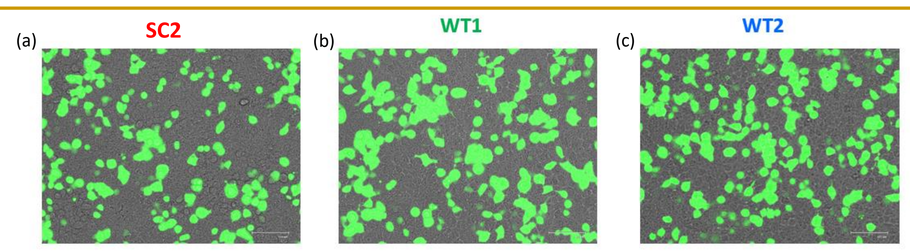

Figure 3: *Lentiviral particle production. Fluorescent images of plates seeded with HEK293T cells, 48 hours following transfection with the three plasmids for lentiviral vector generation. Pseudo-typing using (a) pCMV14-3xFlag-SARS-CoV-2 (b) pcDNA3.1-C9-SARS-CoV-2-Spike and (c) pcDNA3.1-SARS-CoV-2-Spike(wt) using Method 2 of lentiviral vector generation.*

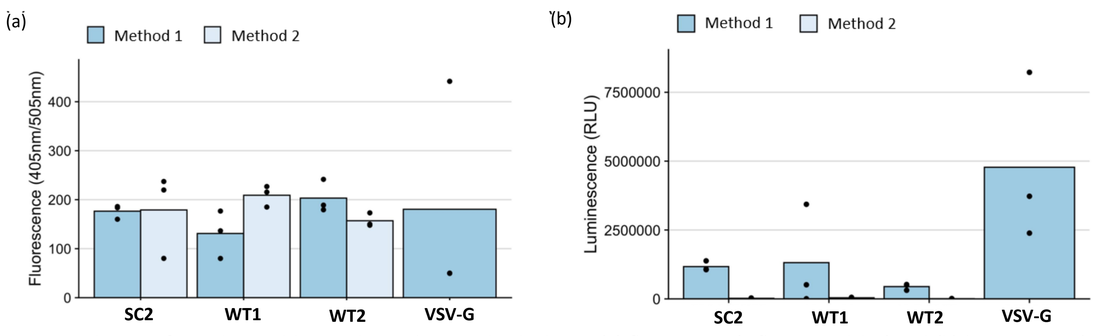

Figure 4: *Transduction of pseudo-typed lentiviral particles. Graphs displaying (a) fluorescence (reflecting viability) and (b) luminescence (reflecting transduction) readings following viability and luciferase-based proliferation assays on pseudo-typed lentiviral particles generated using Method 1 and Method 2 of lentiviral vector generation. pCMV14-3xFlag-SARS-CoV-2 = SC2, pcDNA3.1-C9-SARS-CoV-2-Spike = WT1, pcDNA3.1-SARS-CoV-2-Spike (wt) = WT2, Vesicular Stomatitis Virus glycoprotein = VSV-G.*

- pCMV14-3xFlag-SARS-CoV-2 was generated for use outside of biosafety level 3 containment (Figure 2).
- Lentiviral vectors pseudo-typed with pCMV14-3xFlag-SARS-CoV-2 were generated and the production method optimised (Figure 3 and 4).
- A SARS-CoV-2 spike glycoprotein lentiviral pseudo-typing system was developed and used to assess the inhibition of heparin and dexamethasone on viral entry (Figure 5).
- Drug assays on pCMV14-3xFlag-SARS-CoV-2 pseudo-typed lentiviral particles were repeated with heparin and dexamethasone to confirm consistency of results (Figure 6).
- The use of gemcitabine as a positive control indicated the decrease in cell proliferation and decrease in viable cells due to drug treatment and not poor transfection efficiency of pseudo-typed lentiviral particles (Figure 6).
- In the future, the system could be used as a high through-put drug screen to measure inhibition and interaction of SARS-Cov-2 spike glycoprotein and variants (N429K, Y453F and N439K del 69-70) with its receptor.

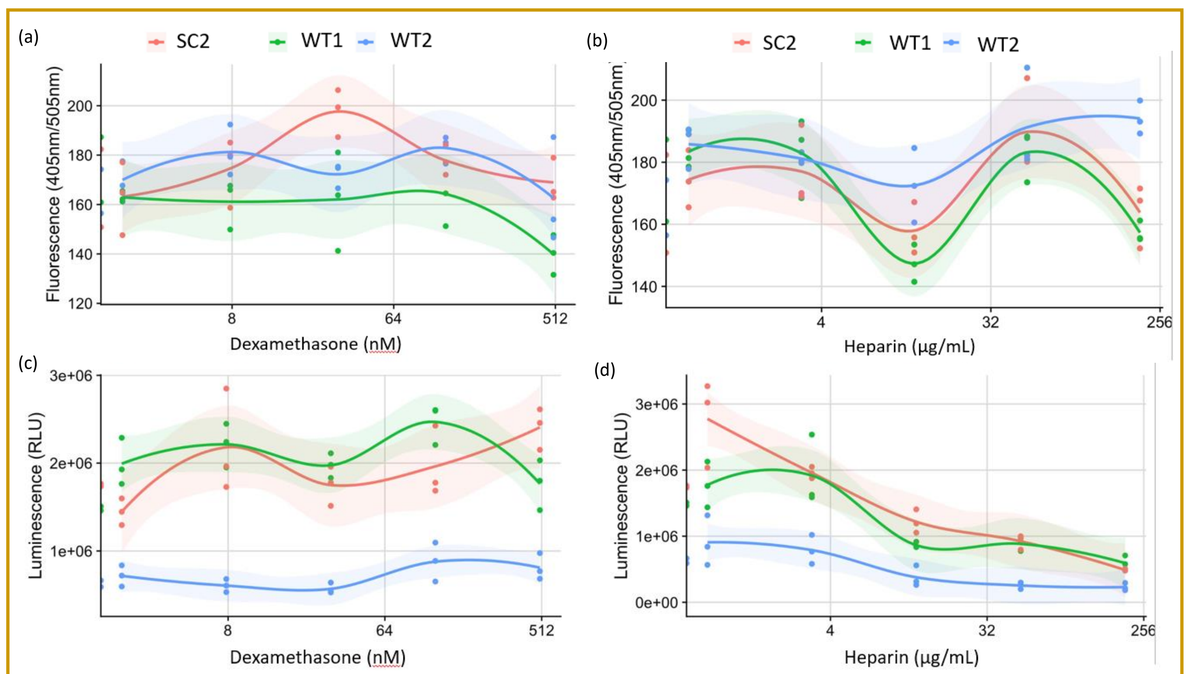

Figure 5: *Pseudo-viral particle drug assay. Graphs showing effects of heparin and dexamethasone on pCMV14-3xFlag-SARS-CoV-2 (SC2), pcDNA3.1-C9-SARS-CoV-2-Spike (WT1) and pcDNA3.1-SARS-CoV-2-Spike(wt) (WT2) pseudo-typed lentiviral particles incubated with HEKACE2 cells. (a) and (b): Viability assays. (c) and (d): Luciferase-based proliferation assays.*

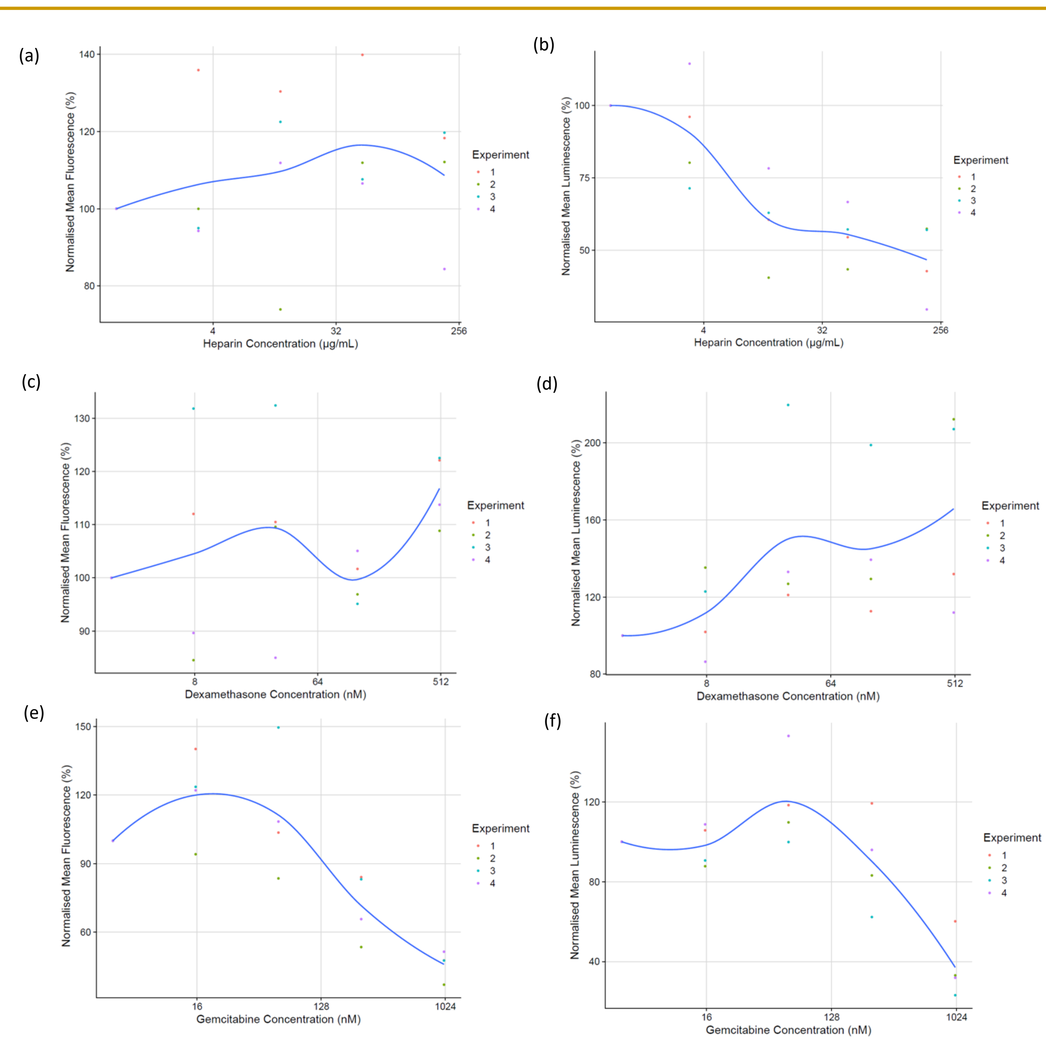

Figure 6: *Evaluation of pseudo-viral particle drug assay with gemcitabine (control). Graphs showing effects of heparin, dexamethasone and gemcitabine (control) on pCMV14-3xFlag-SARS-CoV-2 (SC2) pseudo-typed lentiviral particles incubated with HEKACE2 cells. Graphs indicate cell death due to presence of gemcitabine. (a), (c) and (e): Viability assays. (b), (d) and (f): Luciferase-based proliferation assays. n= 4.*

References

(1). Huang Y, Yang C, Xu XF, Xu W, Liu SW. Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. Acta Pharmacol Sin. 2020;41(9):1141-9.
(2). Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun. 2020;11(1):1620.