Production and characterization of pseudotyped particles to develop an immunosensor for SARS CoV 2

Maria-Camila Lopez[1]
Viviana Vasquez[2]
Javier A Jaimes[3]
Mauricio Rojas[4]
Jahir Orozco[2]
Maria-Cristina Navas[1,*]

[1]*Grupo de Gastrohepatologia, Facultad de Medicina, Universidad de Antioquia, Lab 434 SIU, UdeA, Medellin, Colombia.*
[2]*Max Planck Tandem Group in Nanobioengineering, University of Antioquia, Complejo Ruta N, Torre A, Laboratorio 4-166, Calle 67, Nº 52-20, Medellin, 050010, Colombia.*
[3]*Department of Microbiology & immunology, College of Veterinary Medicine, Cornell University, 930 Campus Rd., VMC Box 5, Ithaca, NY 14853-640, United States of America*
[4]*Grupo de Inmunología Celular e Inmunogenetica, Facultad de Medicina, Unidad de Citometria de Flujo, Universidad de Antioquia, Medellin, Colombia.*
*maria.navas@udea.edu.co

Studying SARS-CoV-2 requires biosafety level 3 (BSL-3) facilities due to its virulence and transmission risk by aerosols. We have produced viral-like particles that express the spike glycoprotein of this emergent coronavirus by taking advantage of the pseudotyped particles tools to circumvent this issue. The resultant pseudovirions display the spike protein of SARS-CoV-2 expressed on the surface of the particle and therefore could mimic the interaction between the virus and the angiotensin-converting enzyme 2 (ACE2), which has been described as the primary host cell target. This simple, but highly beneficial tool, significantly reduces biological risk in the first steps of the immunosensor development requiring only a BSL-2 facility.

The pseudovirions were obtained by co-transfection of three plasmids (*gag-pol* of Murine Leukemia Virus (MLV), firefly luciferase gene/MLV RNA packaging signal/ 5'/3' flanking MLV long terminal repeat and SARS-CoV2 S) in HEK-293 cells and harvested 72 hours post-transfection [1]. The product was characterized by flow cytometry and indirect immunofluorescence. The spike protein was detected on the surface of the pseudovirions using an anti-SARS-CoV-2 Spike polyclonal antibody and an anti-Rabbit IgG secondary antibody cross-linked allophycocyanin, with pseudotyped particles in the presence of the secondary antibody as fluorescence control. The infectivity quantification of the pseudovirions was assessed by transduction of VERO-E6 cell using 10-fold serial dilutions; the Luciferase activity was detected 72 horas post transduction and the title was calculated based on the endpoint dilution and relative luciferase expression.

Current experiments are directed to detect the pseudovirions by the interaction with a thiolated ACE2-reactive peptide linked to maleimide-coated magnetic particles, which electrochemical reaction will be followed by chronoamperometry by a biotinylated anti-ACE2 antibody conjugated with Streptavidin-Horseradish peroxidase (HRP).

REFERENCES

[1] Millet JK, Tang T, Nathan L, Jaimes JA, Hsu H-L., Daniel S, Whittaker GR. Journal of Visualized Experiments, 145 (2019) 3-9