Visualization of the novel coronavirus (SARS-CoV-2) and identification of cytopathic effects in cell culture

Introduction

The novel coronavirus (SARS-CoV-2), which causes Covid-19 (coronavirus disease 2019), caused a global pandemic that claimed the lives of millions. Early identification and evaluation of this virus was a high priority in developing therapies aimed at reducing its effects on people and the global economy. In early 2020, the Oh lab, at Seoul National University College of Medicine in South Korea, succeeded in creating a methodology to isolate, culture and image the coronavirus, from a sample taken from the first positively identified Covid 19 patient in South Korea.

Figure 1. RMC Boeckeler PowerTome PCZ.

Instrumentation

The instrument used in this research application was the RMC Boeckeler MTXL model ultramicrotome, which precedes the new and improved PowerTome, the only fully upgradeable ultramicrotome in the market. Users have a choice of the PowerTome XL (PTXL) routine workhorse controlled via a digital tactile controller, eminently suitable for high-throughput work, to the PowerTome PCZ (PTPCZ) research level system with computer control and HD video options. The PowerTome is capable of accommodating glass knives up to 12mm wide, triangular tungsten carbide knives, and any commercial brand of diamond knife. The user can also choose from various add-on components for array tomography and cryo applications.

Procedure

A viral swab kit was used to collect an oropharyngeal sample from the patient with laboratory-confirmed Covid-19. This sample was inoculated into the Vero cell line, an immortalized kidney epithelial cell line (ATCC Cat # CCL-81). The cells were then cultured at 37° C in a humidified atmosphere of 5% carbon dioxide. The culture was observed using light microscopy after five days. Cultures were then fixed with primary fixative of 1% glutaraldehyde in phosphate buffer followed by secondary fixation with 2% osmium tetroxide. The culture samples were then embedded in a medium hardness resin (Embed 812, Electron Microscopy Sciences Cat # 14120), according to manufacturer instructions. The embedded fixed samples were then sectioned at 65nm using an RMC Ultramicrotome (Boeckeler Instruments Model MTXL). Sections were negatively stained with 4% uranyl acetate and positively stained with 1% lead citrate before viewing with a transmission electron microscope (JEOL USA Inc. Model JEM-1400) at 80kV.

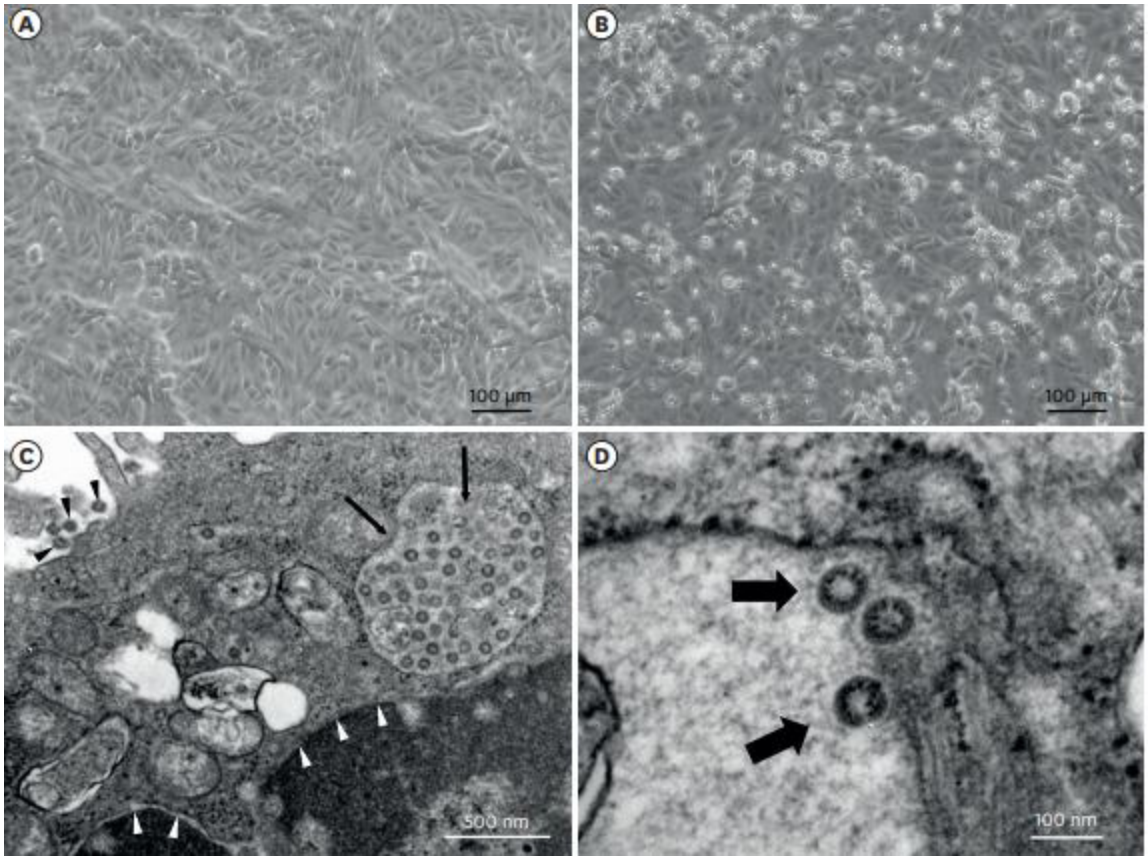

Figure 2. Cytopathic effects of SARS-CoV-2 in Vero cell cultures and electron microscopy image of SARS-CoV-2. Vero cells were inoculated with oropharyngeal swab sample. (A) Vero cell cultures in negative control. (B) Cytopathic effects consisting of rounding and detachment of cells in Vero cell cultures 3 days after the first blind passage. (C, D) Transmission electron microscopy image of Vero cells infected with SARS-CoV-2. White arrow head denotes nuclear membrane, black arrow head extracellular virus particles, and thin black arrow cytoplasmic vesicle including virus components (C). Thick black arrow denotes magnified virus particles with crown-like spikes (D).

Results

Cytopathic effects of SARS-CoV-2 on the cultured cells were evident 3 days after inoculation. Coronavirus was confirmed upon visualization of spherical particles with a characteristic ring of spikes, ranging 66 to 81 nm in diameter, reminiscent of a crown when viewed with by TEM. The virus was found to be present within cytoplasmic vesicles and the extracellular space adjacent to the plasma membrane. Subsequent whole genome sequencing and phylogenetic analysis indicated a strong clustering relationship with other SARS-CoV-2 samples collected in Wuhan.

Data source:
Journal of Korean Medical Science, 24 Feb, 2020, “Virus Isolation from the first Patient with SARS-CoV-2 in Korea”.

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