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Development of effective vaccines against emerging and re-emerging pathogens is a worldwide public health priority. In response to the current pandemic, global vaccine manufacturers have developed several types of SARS-CoV-2 vaccines, including adenovirus (AdV)-vector based vaccines. Production of viral vector vaccines requires optimization of several cell culture parameters. Using wildtype AdV type 5 infection with suspension HEK293 cells as a model system, we evaluated various conditions (including time of harvest, multiplicity of infection (MOI), cell density, insulin supplementation, and temperature shift) to optimize adenovirus production. In addition, we evaluated cell growth and AdV5 production in different chemically defined media and feeds. Viral titers were quantified by qPCR, TCID50 and Focus Forming Assay (FFA). While Gibco™ CD 293™ Medium supports comparable cell density to other commercially available media, cell density attained in Gibco™ Dynamis™ Medium was >2-fold higher. Predictably, upon AdV5 infection, cell density and cell viability decreased for all infected cultures from 2 to 7 days post infection (dpi). Addition of feed on 0 and 2dpi (with temperature downshift on 3dpi), resulted in peak AdV titers on 4 or 5 dpi for Dynamis™ Medium and CD 293™ Medium, respectively. The AdV5 titers in both Dynamis™ Medium and CD 293™ Medium showed comparable or better peak titers than other commercially available media. Furthermore, peak virus titers were not dependent on insulin in either Dynamis™ Medium or CD 293™ Medium. Finally, infectivity assays (TCID50 assays and FFA) confirmed the AdV5 titers obtained with qPCR. Overall, these results demonstrate Dynamis™ Medium and CD 293™ Medium can effectively support HEK293 cell growth and high adenovirus production.