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A Field Emission Scanning Electron Microscopy Method to Assess Recombinant Adenovirus Stability.

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A field emission scanning electron microscopy (FESEM) method was developed to assess recombinant adenovirus (rAd/p53) stability. This method was designed to simultaneously sort, count, and size the total number of rAd/p53 objects observed in an image. To test the method we treated a rAd/p53 preparation [1,5] with thermal incubation at 37°C for 0, 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 hours, and then monitored the effect on stability by assessing the anatomy of the virions using FESEM, with automated image-analysis (Image-Pro Plus v.4.1, Media Cybernetics, Silver Spring, MD), and transmission electron microscopy (TEM). In addition to electron microscopy (EM), the infectious activity of the thermally stressed rAd/p53 samples was quantitated using an established flow cytometry method. [2,3]

Unconjugated gold particles were mixed with each sample post-incubation to facilitate focusing and to provide an internal control for sizing of the virus particles. Viral specimens were subsequently fixed and stained on carbon coated copper grids, and then prepared for EM image-analysis. The T=0 time point showed that virions were evenly distributed over the grid, and the icosahedral geometry of the virus was evident. [4] As shown in Figure 1, FESEM image-analysis revealed a decrease in the total number of detectable single rAd/p53 particles and an increase in apparent micro-aggregates composed of multiple viral particles (multiplets) as early as 2 hours. In addition there was an observed decrease in the size of the single rAd/p53 particles and an increase in multiplet size with time at 37°C. The described changes predominated after 4 hours of incubation. The changes noted in virus morphology were concomitant with the observed loss in viral infectivity (Figure 2). The FESEM results were reproduced with TEM.

In conclusion, the results reported in this study suggest a novel method of assessing the stability of recombinant adenovirus products using FESEM image-analysis. The decrease in single rAd/p53 particles and the increase in higher order multiplets suggest that the method may be useful for monitoring or assessing stability.

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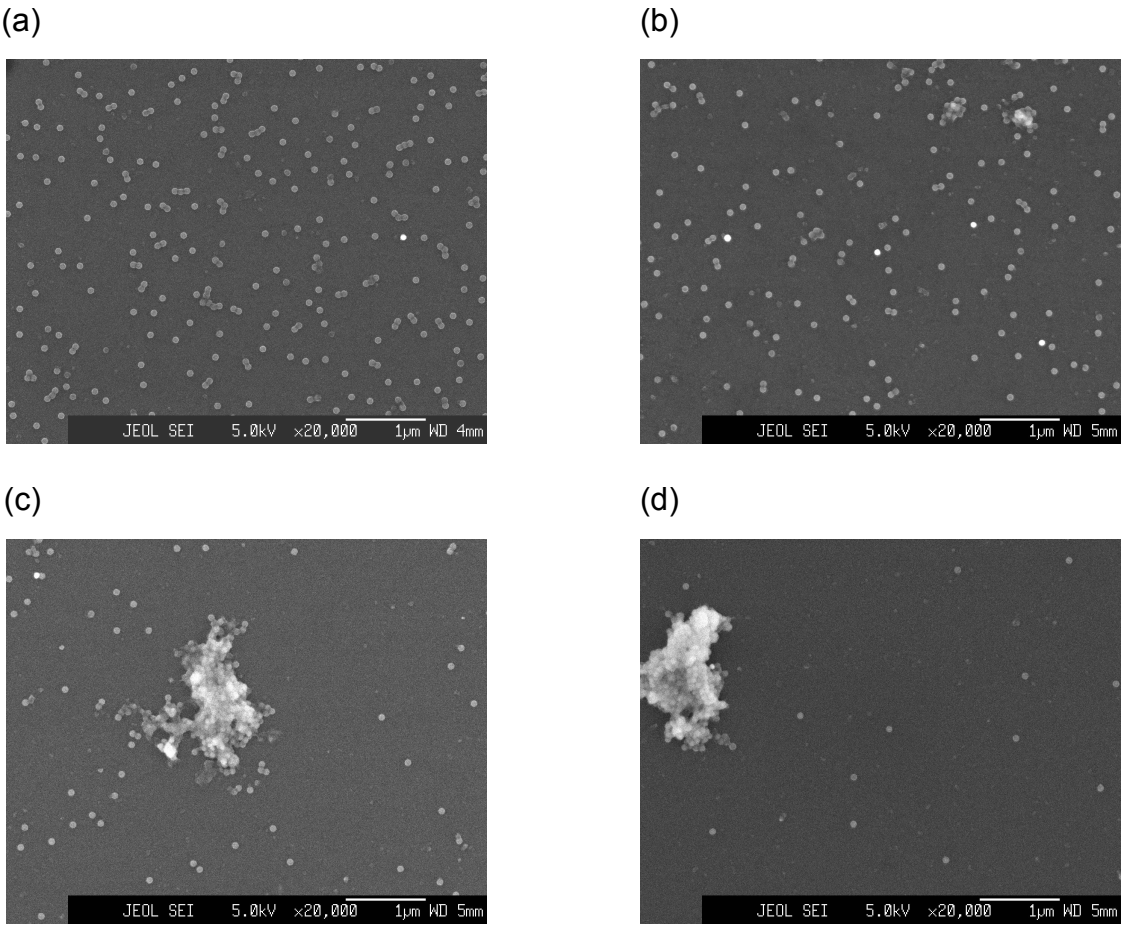

FIG.1. Representative FESEM images of rAd/p53 in PBS incubated at 37°C at the various times indicated. (a) T=0 (b) T=0.5 hours (c) T=2.0 hours (d) T=4.0 hours

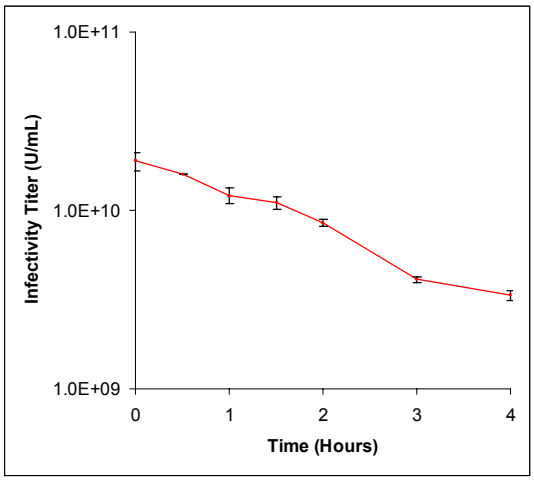

FIG. 2. Representative plot showing kinetics of rAd/p53 infectivity during thermal stress at 37°C.

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