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Negative Staining and Immunocytochemistry of HIV-1 Vectors

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HIV-1 vectors intended for gene therapy applications are primarily produced by transient transfection methods and may contain factors that affect the transduction potential of these vectors[**1,2**]. To characterize these factors, we have developed negative and immunocytochemical staining methods to directly visualize HIV-1 vector particles using Transmission Electron Microscopy (TEM).

The HIV-1 vector, CS-CGW, pseudotyped with Vesicular Stomatitis Virus G-protein (VSVG), Ross River Virus (RRV) envelope protein, or RD114 envelope was examined by TEM after negative staining with either 1% aqueous Uranyl Acetate (UA) or NanoVan (Nanoprobes, Yaphank, NY). Vector samples were mixed with equal proportions of latex beads for quantitation of vector particles. For negative staining, the vector supernatant was adsorbed directly onto formvar/carbon coated grids or spun (80,000 rpm, for six minutes in a TLA 100.1 ultracentrifuge) onto coated grids. Following negative staining, vector particles were identified by size and morphology. UA was absorbed by the vector particles, resulting in dark spherical structures with indistinguishable structure. NanoVan staining resulted in lighter staining of the vector particles allowing more definition of core components. A higher proportion of vector particles were seen in samples prepared by ultracentrifugation. The concentration of vector particles in samples prepared by direct adsorption was lower, but more uniform allowing for quantitation of particles. We also characterized vector particles using immunocytochemistry with antibodies directed against viral envelope proteins (VSVG/RRV/RD114) or HIV-1 core protein (p24). Immunostaining was conducted on vector samples adsorbed onto grids or on thin sections taken from embedded (Unicryl, Electron Microscopy Sciences, Hatfield, PA) samples. Better staining was obtained with anti-RRV with both types of specimens. Minimal staining was found with anti-p24 or VSVG antibodies. However, quantitation of immunoreactive particles was easier on samples adsorbed onto grids versus sectioned material.

In conclusion, we used negative and immunostaining methods to evaluate HIV-1 vectors intended for use in gene therapy. Our data indicate that NanoVan is superior to UA for negative staining in visualizing the structure of HIV-1 by TEM. The immunostaining of HIV-1 vector particles was better using sectioned material rather than adsorbed samples, but for quantitation of intact or disrupted particles, the latter was more useful. The methods described here can be used for characterization of a variety of retroviral vectors and viruses.

References

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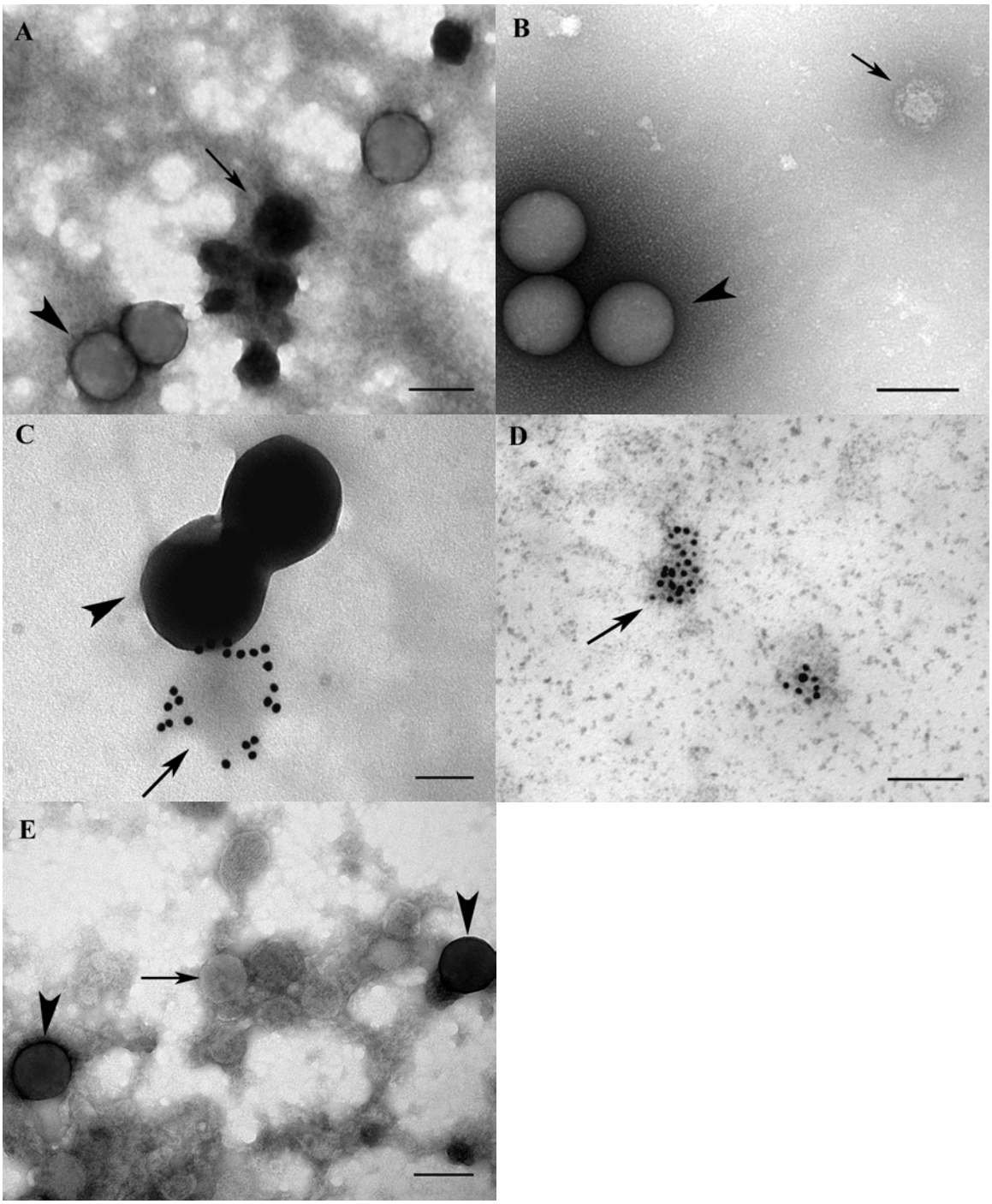

Negative and Immunogold staining on HIV-1 vectors: HIV-1 vector, CS-CGW, mixed with latex beads was stained with A) uranyl acetate (bar =50 nm) or B) NanoVan (bar =100 nm). CS-CGW pseudotyped with RRV and mixed with latex beads was stained with anti-RRV antibodies and a secondary antibody conjugated to 10 nm gold particles following C) direct adsorption onto grids or D) after embedding and sectioning (bar =25 nm). E) CS-CGW mixed with beads was centrifuged directly onto grids and stained with NanoVan (bar = 100 nm). The beads in all samples are indicated by arrow heads; vector particles are shown by arrows.

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