Transmission electron microscopy

Utilising a powerful tool in biosafety as a novel approach to characterise the product quality of biologics such as vector-based vaccines and gene therapy products
Outline

1. Transmission electron microscopy: A short introduction
2. TEM in Biotechnology: Viral safety of biologics
3. From viral safety to product characterisation
4. Sample morphology, distribution & purity
5. Particle quantity: semi-quantification using TEM
6. Summary
1. Transmission electron microscopy: A short introduction

- Electron acceleration up to 300kV
- Electron interaction with stained sample as electrons transmit sample
- Image acquisition (analogue or digital)
- Resolving power below 1nm
- Deployed in medicine, bioscience and materials science for ultrastructural analysis

**Fig. 1** Transmission electron microscope
1.2 Transmission electron microscopy: A short introduction

Sample preparation in bioscience for TEM

I. Cellular diagnostics via positive staining (psTEM, sample is stained)
steps: chemical fixation ➔ resin embedding ➔ ultrathin sectioning ➔ staining

II. Particle diagnostics via negative staining (nsTEM, background is stained, particles appear bright)
steps: sample deposition and sedimentation on TEM grid ➔ staining

Staining agents
• Uranyl acetate (UA)
• Lead citrate
• Phosphotungstic acid (PTA)
2. TEM in Biotechnology: Viral safety of biologics

ICH Guidelines:

*ICH Q5A (R1) Quality of biotechnological products: viral safety evaluation of biotechnology products derived from cell lines of human or animal origin*

Where is TEM utilised?

1. Cell bank characterisation (psTEM)
2. Bulk harvest screening (nsTEM)

Quality Assurance?

➢ Qualified and validated assays following **GMP**
Fig. 2 Ultrathin section of mammalian cells with intracellular aggregation of virus particles (V). (scale bar = 1µm)
Fig. 3 nsTEM image of virus like particles (V) and latex beads (B). (scale bar = 200 nm)
3. From viral safety to product characterisation

- Morphology
- Distribution
- Purity
- Semi-quantity

- **Detection limit** for TEM analysis: $10^8 - 10^9$ particles per mL
4. Sample morphology, distribution & purity

What can TEM tell me about my sample?

1. Particle morphology:
   - Virus structure
   - Identity: enveloped/non-enveloped, capsid structure
   - Structural integrity: damaged or intact particles

2. Particle distribution:
   - Aggregation of particles (unsuitable process conditions in USP or DSP?)

3. Sample purity:
   - Background noise (cell debris, high protein contamination)
Fig. 4 nsTEM image of *Adeno-associated viruses* (V) & particle clusters/aggregations (C). (scale bar = 100 nm)
4. Sample morphology, distribution & purity

Percentage of counted particles

Average particle size in nm
Fig. 5 nsTEM image of damaged Adeno-associated viruses (V) & sample debris (D). (scale bar = 100 nm)
5. Particle quantity: semi-quantification using TEM

Procedure

- A suspension of beads with a known concentration and size are added to the test item
- Visualisation of test item and beads via nsTEM (negative staining)
- A defined amount of beads are counted on the TEM grid
- Test item particles are counted parallel to the beads
- Calculation of test item concentration in relation to the beads concentration
Fig. 6 nsTEM image of *Poxviridae* (V) & reference beads (B). (scale bar = 200 nm)
Fig. 7 nsTEM image of Poxviridae (V) & reference beads (B). (scale bar = 200 nm)
6. Summary

❖ TEM can deliver visual feedback on particle:
  ➢ morphology & structure/integrity
  ➢ distribution & aggregation
  ➢ purity
  ➢ semi-quantity

❖ TEM can visualise the ultrastructural properties of producer cells
  ➢ Information which can improve process conditions for vector-based vaccines and gene therapeutics in USP & DSP
neotem Bioanalytics

TEM expertise for powerful sample analysis

Contact: ashley.layland@neotembio.com

www.neotembio.com