**Cell Therapy CE Applications**

Facilitator: *Jane Luo, SCIEX, USA*

Scribe: *Andrew McClain, BMS, USA*

**Abstract:**

Cell therapy uses living cells as a drug to treat a disease. Many cell therapies are being developed to halt and reverse disease, restore damaged organs, and ultimately, cure many life-threatening conditions. For example, CAR T cell therapy is a type of cancer immunotherapy treatment that uses immune cells called T cells that are genetically altered in a lab to enable them to locate and destroy cancer cells more effectively. Although cell therapies bring a tremendous opportunity to patients, they also pose significant challenges to analytical quality control. Capillary Electrophoresis (CE) is a valuable technology for characterizing novel pharmaceuticals such as fusion protein drugs, bsmAbs, ADCs, and AAVs. At this roundtable, we'll discuss current challenges, approaches, and opportunities offered by CE for cell therapy.

**Questions for Discussion:**

1. Which CE technologies are currently available for characterizing lentiviral vectors (LVV) for VSV-G, p24, and the nucleic acid payload?
2. Which orthogonal technologies are currently being used to characterize LVVs?
3. What are the advantages and disadvantages of using CE vs. other orthogonal technologies for LVV characterization?
4. How important is Full, Partial, and Empty analysis for Lentiviral Vectors?

**Discussion:**

1. CE is used to analyze the lentivirus vector, not the cell itself.
    a. Intermediates or "raw materials" for Cell Therapy
2. Brainstorm: What can CE do for the Cell itself?
    a. Experience: Conference where someone mentioned using CZE to analyze sperm. David Michels and Norman Dovichi published some papers previously using CE for single-cell analysis
    b. No CE publications on CART cells yet
    c. Idea: Probe to bind to a receptor and do affinity CE
        i. Is concentration too low?
        ii. Multiple cells vs. single cell
        iii. Isolate Genomic DNA and RNA, amplify with PCR, and do analysis.
    d. Kit: Qiagen for Nucleic Acid assay
        i. Reliable, long-lasting!
        ii. Elutes in small volume, which concentrates the Nucleic acids after isolation from the cells.
    e. Idea: Cell-based applications for CE should be coming soon (keeps on getting larger and larger)
        i. Mini-benchtop CE could be put into a clinical setting for cell testing.

f. FACS analysis (Fluorescence-Activated Cell Sorting): fluorescence detection with flow cytometry.
    i. Around 1 million cells are required.
g. If we can extract proteins from cells, and separate them by CE on the Simple Western, and then use antibody binding with the cell, it could analyze a cell therapy product.
h. Is there a pI difference between transfected vs. un-transfected cells?
    i. should be detected in CE-SDS (make the assumption we have the chimeric antigen receptor cells)
        1. May be available on the market in the future.
    ii. Standard curve will allow quantification.
i. Amy Herr- Nature Communications Paper: "3D projection electrophoresis for single-cell immunoblotting" Grist et al. (2020)
    i. Tiny PDMS devices to sort cells.
    ii. Can this be applied to CAR-T cells?
    iii. Why can't this technology be transferred to T cells?
        1. Likely hasn't been shown due to the rarity of samples.
j. If a cell has been transfected, it expresses an antibiotic resistance gene (defense mechanism)
k. Companies have been doing genetic engineering within their own companies, but reference standards may become commercially available soon.
    i. Barrier to entry for the field and for analytical development groups
    ii. AAV reference standard available through Charles River
    iii. Other companies have pre-made AAVs for sample analysis. (expensive)
    iv. Creative Biolabs have CAR-T cells available (expensive and specific)
    v. SignaGen: sells lentiviral vectors w/ different genes in them (non-harmful ex. GFP, of varying sizes)
        1. expensive as well ($500 for 30 µL)
l. If MW of chimeric antigen receptor is known, can be detected by CE-SDS
m. Idea: a small ligand that could bind to the cell (w/ a fluorescence probe) can be tracked
    i. Cell alone vs. Cell w/ Ligand and Receptor
n. Idea: Cells may contain impurities when re-injected into a patient
    i. ex. viral, cell waste, residuals after purification
    ii. What is the quality control post-transfection?
        1. Purity, integrity, etc.
        2. Process monitoring?
    iii. CE can be used to detect these!

3. Sciex Tech Notes:
    a. Lentivirus (Genome Integrity)
        i. Lower titer compared to AAV:
            1. 10^9 transducing unit/mL vs. 10^13 GC (genome copy)/mL
        ii. A large portion of products are empty, need to separate empty from full
        iii. Lentiviruses known to cause diseases in humans (risk)
        iv. AAV cap in genome size provides challenges, which is why a lentivirus may be used. Lentivirus has an 8kb carrying capacity

v. Challenges with expressing bigger genes.
    1. % of full-length genome may be as low as ~40% due to how the lentivirus will package the larger genes (UCLA paper)
    2. Higher license needed as well (safety)
vi. BSL-2 Hood for sample preparation and virus handling.
    1. Replication deficient AAV can be done with BSL-1 (open bench with lab coat, gloves, and goggles)
    2. EHS approval needed

b. Lentivirus Protein Analysis
    i. LVVs have ~20 different proteins
    ii. CE-SDS to profile these proteins and compare across samples (process monitoring, batch to batch comparison)
        1. Biotechne poster- Maurice ciEF and CE-SDS analysis of LVV capsid proteins (and FLEX for fraction collection for AAV capsid proteins)
    iii. Quantification of p24 can be used for titer determination