Optimization of the production and purification of non-viral vectors for gene therapy applications

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ABSTRACT

Cancer ranks the second leading cause of death globally and has significant societal and economic costs. To address this issue, the production of biopharmaceuticals is standing out. Nucleic acids are gaining popularity in preventing infections and as therapeutic agents in gene therapy, aiming to fix the target dysfunction by transfecting eukaryotic cells with gene-based products. Non-viral vectors, including plasmid DNA and minicircle DNA (mcDNA), attracted increased importance among these. Despite their clinical relevance, current manufacturing strategies are still complex and involve multi-step purification processes, ultimately increasing their cost. To overcome this obstacle, this work investigates applying ionic-liquid-based aqueous biphasic systems (IL-ABS) as a primary capture strategy of p53-mcDNA biopharmaceuticals. Considering the medium complexity in which the p53-mcDNA is produced, a clarification and concentration step with IL-ABS is critical before moving to high-resolution chromatographic purification. p53-mcDNA was produced resorting to recombinant *Escherichia coli* cells under optimized conditions to promote cell growth and parental plasmid (PP) bioproduction. Afterward, recombination was induced using L-arabinose, yielding the p53-mcDNA. The fraction containing the PP and p53-mcDNA was subsequently isolated using a commercial kit, and their partitioning behavior in ABS comprising bromide-based ILs and citrate potassium salt was investigated. Ongoing studies are focused on optimizing the separation of PP and p53-mcDNA using the designed IL-ABS, after which a sample of increased complexity will be applied.

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