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Abstract

New Combined Approach to Simplify the Minicircular DNA Lysate and Directly Apply in Ion Exchange Membrane Chromatography †

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Cervical cancer (CC) is one of the most prevalent cancers in women worldwide [1]. This type of cancer is caused by the human papillomavirus (HPV), namely by its most virulent high-risk genotypes, HPV-16 and HPV-18. After a persistent infection, E6 and E7 oncoproteins expressed by this virus are responsible for the inhibition and degradation of p53 and pRB tumor suppressor proteins, respectively [2].

Gene therapy has been explored in recent years due to its potential in the treatment of acquired and incurable diseases. This therapeutic approach is based on delivering a strange DNA sequence to a host cell to change the function of a particular molecule that may play an important role in the development of a disease [2,3]. Minicircular DNA (mcDNA) has become a promising non-viral DNA vector, not only because it lacks prokaryotic sequences considered harmful (such as antibiotic resistance genes, replication origin, and CpG motifs that are generally associated with safety concerns), but also because it is a smaller vector when compared to conventional plasmid DNA (pDNA), thus presenting better transfection efficiency, cell permanence and gene expression [4]. Micro RNAs have been identified as gene silencers that degrade or inactivate a targeted messenger RNA. In particular, microRNA-375 (miR-375) has been proven to silence the expression of E6 and E7 oncoproteins in high-risk HPV-infected cells [5].

Thus, the present work aims at the production and purification of an mcDNA vector that encodes the pri-miR-375 gene, which can be used for gene therapy against CC in the future. However, the biotechnological process of the obtained mcDNA involves several steps (namely centrifugations; concentration with organic solvents; clarification with high salt concentrations; purification methods consisting on size exclusion or affinity chromatography that are time-consuming or are not available commercially [5], respectively) that make the upstream process quite expensive from the pharmaceutical industry's point of view, and the development of alternative strategies is essential.

Concerning the above mentioned, this research work suggests a new recovery approach, based on the crude lysate treatment with diatomaceous earth to direct filtration and removal of all cell debris and precipitated impurities, combined with a second step using a Sartobind membrane to eliminate the RNA. The main advantage of using the new filtration product associated with the lysate treatment with diatomaceous earth is related to its highly porous and permeable structure that enable the lysate to easily flow, preventing clogging and blocking of the filter when filtration is carried out. This approach allows

the substitution of several steps pre-established for the lysate preparation, namely the cell debris centrifugation in the end of alkaline lysis, the isophopanol concentration step and respective centrifugation and the ammonium sulfate clarification step and respective centrifugation [6], saving significant time, resources and environmental impact. Following this step, the simplified lysate was directly loaded in a Sartobind® Q75 membrane (with a pore size > 3 µm, allowing the internalization of larger molecules in the microporous structure and a high connection capacity) to explore anion exchange chromatography (due to the presence of quaternary ammonium ligand) [7]. After some preliminary studies of equilibrium and elution conditions, the elimination of a large amount of RNA was optimized in the flowthrough during the lysate loading step, and the rest of the RNA contamination was eluted by NaCl increasing step gradient. The efficiency of both steps of this combined process was proven through the quantification of resultant samples recovered in the end of each step, using a well studied and applied CIMac™ pDNA analytical column [8].

Overall, some studies continue to be carried out to improve the isolation and the maximum yield of the mcDNA vector recovered. Thus, it is expected that this combined strategy can be scaled-up and successfully applied in the biopharmaceutical industry to quickly and economically purify DNA vectors.

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