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Production of clinical-grade plasmid DNA for human Phase I clinical trials and large animal clinical studies

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Abstract

The use of plasmid DNA as vaccines for the treatment of cancer and infectious diseases is on the rise. In order to facilitate the manufacture of clinical-grade plasmid DNA for Phase I clinical trials, we developed a process whereby >200 mg plasmid could be produced in a single production run under Good Manufacturing Practices. A dedicated cleanroom (Class 10,000 with Class 100 biosafety cabinet) is utilized for production of the bacterial cell bank, fermentation, harvest/lysis of the biomass, and downstream purification. Fermentation requires three 16–18 h runs (∼12 L each) in shaker-flasks, yielding ∼60 g bacterial paste following batch centrifugation. The biomass is alkaline-lysed, pooled, and the resulting flocculent precipitate is separated by a novel vacuum step, followed by depth-filtration. Downstream processing includes anion-exchange chromatography, utilizing Qiagen silica-based resin, and precipitation with isopropanol. Following precipitation, the DNA is harvested by centrifugation, dried, formulated, and sterile-filtered using a Sartorius Sartobran 150 filter prior to Final-Filling. All processing steps utilize sterilized, single-use components. This process results in a product manufactured according to regulatory guidelines. The plasmid DNA is sterile with ≥95% supercoiled DNA, an A_{260}/A_{280} ratio ≥1.9, undetectable or extremely low residual endotoxin, RNA, genomic DNA, protein, and antibiotic. Residual solvent levels are negligible. The product yields the predicted profile upon restriction-enzyme digestion, is biologically active upon transfection and remains stable for several years at −20 °C. We have therefore developed a reproducible and cost effective process to manufacture clinical-grade plasmid DNA. This process can be adapted by other academic centers for human or large animal clinical trials.

Keywords: Clinical-grade plasmid DNA; cGMP; Gene therapy

1. Introduction

As of 2007, 18% of all gene transfer clinical trials, worldwide, utilized plasmid DNA as the vector of choice (http://www.wiley.co.uk/genetherapy/clinical). The percentage of gene transfer trials to treat cancer is 67.5%. To date, most of the plasmid vaccine trials target HIV, a variety of tumor antigens, malaria, and hepatitis B. They have uniformly been found to be safe and well tolerated.

Antibody and/or CTL responses were elicited after immunization in patients with HIV [1–3], while in HIV negative patients, CD4 but not CD8 responses have been observed [4]. Vigorous CTL responses were obtained utilizing a DNA-priming/vaccinia vector boost immunization schedule [5]. In patients with malaria, plasmid vaccines have been shown to induce CTL responses, but no antibody responses were detected [6–8]. DNA-priming followed by boosting with either a vaccinia vector or a recombinant antigen have shown improvement in CTL responses over DNA alone and limited

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protection against infection in one trial [9–12]. In patients with hepatitis B, vaccination with DNA-coated gold beads induced both antibodies and CTL responses in previously naive participants and antibody responses were observed in some individuals in another trial [3,14].

The manufacturing process for clinical-grade plasmid DNA vaccines encompasses a number of key steps [13–25]. Initially, a plasmid consisting of a plasmid backbone, including typical elements, such as the origin of replication, an antibiotic resistance gene other than the ampicillin resistance gene, a strong eukaryotic promoter and a polyadenylation signal sequence, and encoding the therapeutic gene(s) of interest, is generated. CpG motifs normally present in bacterial DNA may also increase the plasmid DNA potency by stimulating the innate immune system [23]. Subsequently, a bacterial cell bank containing the plasmid is established to create a uniform inoculum for further process development and large-scale fermentation. After fermentation, the lysis of the resulting biomass is generally performed using the well-established alkaline lysis method [24]. Further downstream processing essentially aims at eliminating impurities, such as host genomic DNA, RNA, proteins as well as endotoxin. There are a number of published procedures for downstream processing, however detailed information is scarce due to the proprietary nature of the manufacturing processes [14,16,18,19,21]. Chromatography is the most suitable downstream processing method for plasmid DNA. This technique is an established, scalable technology with a proven track record in pharmaceutical production. Because plasmid DNA is negatively charged, anion-exchange (AEX) chromatography is most useful in removing key impurities, such as RNA, chromosomal DNA, proteins, endotoxin and process additives. Ribonuclease A (RNase) is frequently used in downstream plasmid DNA processing. Although, this enzyme efficiently degrades RNA impurities, it is of bovine origin. Presently, manufacturers are developing purification processes that produce gram quantities of pure plasmid DNA without using RNase [15].

The demand for plasmid DNA has resulted in a growing industry for the contract manufacture of both research-grade material for animal studies and cGMP-grade product for human clinical trials. Several trials have shown that DNA vaccines are safe in patients with cancer although so far, the efficacy appears to be limited [17,26–30]. Recently, Bergman et al. have demonstrated that xenogeneic DNA vaccination in dogs with malignant melanoma is safe, leads to the development of anti-tyrosinase antibodies and is potentially therapeutic [26,31].

In order to fulfill the need for plasmid DNA to pursue these canine studies and to also provide clinical-grade plasmid DNA for human clinical trials aimed at treating patients with melanoma at MSKCC, we have developed in-house an efficient, cost effective, large-scale purification process to produce (>200 mg) clinical-grade plasmid DNA for Phase I clinical trials. Regulatory requirements stipulate that the production of any agent intended for use in human clinical trials must be performed under current Good Manufacturing Practices (cGMP) [32]. This implies that the manufacturing process is fully in-control, performs as intended and includes the use of methodologies that ensure the identity, safety, purity, and potency of the manufactured product. Regulatory agencies, such as the US Food and Drug Administration (FDA) and the European Agency for the Evaluation of Medicinal Products (EMEA), provide guidance regarding the specifications, quality testing, and manufacturing standards of plasmid DNA products [33–35].

We have established in the Gene Transfer and Somatic Cell Engineering Facility (GTF) at MSKCC a simple process that allows the manufacture of pilot- to Phase I-scale (200 mg) quantities of clinical-grade plasmid DNA. This process, described herein, utilizes a novel clarification step for the rapid preparation of multiple liters of bacterial lysate, a single chromatographic purification step, and disposable/single-use components throughout.

2. Materials and methods

2.1. Manufacturing facility: description, sanitization and environmental monitoring

The plasmid DNA production facility in the GTF consists of a 72 ft^2 Class 10,000 modular cleanroom suite and an adjacent 24 ft^2 gowning room. The facility contains a dedicated Class 100 double HEPA filtered biosafety cabinet for open aseptic manipulations, and all necessary equipment required for the fermentation, harvesting, and purification of clinical-grade plasmid DNA. A schematic diagram of the production suite and of the flow of materials and personnel is shown in Fig. 1.

The layout and design of the production rooms and equipment permit effective cleaning and decontamination. The external and internal surfaces and auxiliary parts of the equipment are cleaned prior to each batch process as per our area clearance program and standard operating procedures (SOPs). Removable parts are autoclaved. Procedures and cleaning schedules for equipment and areas used in the production are documented. Validation studies were performed to demonstrate elimination of microbial contaminations using established cleaning procedures. Approved cleaning agents are used for cleaning all equipment, countertops and biosafety cabinets. Procedures exist for the proper disposal of waste materials.

The GTF's environmental monitoring program stipulates that after cleaning and prior to every production, the inner and outer surfaces of equipment, as well as all work surfaces are monitored for viable particles using RODAC plates (BD Diagnostics, Sparks, MD, USA). Additionally, prior to and during the process, the air in the production rooms and biosafety cabinets are routinely monitored for viable particles, using a Millipore (Billerica, MA, USA) MAirT air sampler, and for non-viable particles using a MetOne par-