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Review

DNA molecules and human therapeutics

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Nucleic acid molecules are championing a new generation of reverse engineered biopharmaceuticals. In terms of potential application in gene medicine, plasmid DNA (pDNA) vectors have exceptional therapeutic and immunological profiles as they are free from safety concerns associated with viral vectors, display non-toxicity and are simpler to develop. This review addresses the potential applications of pDNA molecules in vaccine design/development and gene therapy via recombinant DNA technology as well as a staged delivery mechanism for the introduction of plasmid-borne gene to target cells via the nasal route.

Key words: Plasmid DNA, vaccine development, gene therapy, staged delivery nasal route.

INTRODUCTION

Recombinant DNA technology and the sequencing of the human genome have led to pertinent discoveries especially in the fields of gene therapy and nucleic acid vaccines. The DNA molecule is one of the most vital sources for the development of a novel group of therapeutics modelled on its endogenous structure (Wolf et al., 1990; Prather et al., 2003; Patil et al., 2005). DNA-based therapeutics includes covalently closed circular plasmids containing transgenes for gene therapy, ribozymes, DNAzymes and oligonucleotides for antisense and antigene applications (Patil et al., 2005; Shroff et al., 1999). Gene therapy processes involve the introduction of one or more functional and specific genes into a human recipient to repair certain genetic defects and aberrations. Plasmid DNA vaccine is developed from a pathogen's gene to provide immunity against diseases by allowing the foreign genes to be expressed transiently in transfected cells, mimicking intracellular pathogenic infection and inducing both humoral and cellular immune responses (Gurunathan et al., 2000; Durland and Eastman, 1998). Plasmid DNA vaccines offer a platform to immunize with gene-based materials that are expressed by the cells of the recipient, and this provides a greater control over the immunisation process because the investigator determines which antigens and co-stimulants to use, where to elicit the response, which cytokines to be co-expressed, amongst others (Prather et al., 2003; Durland and Eastman, 1998). Even though most of the plasmid-based therapeutic products are in the clinical trial stage, they have materialized lately to yield extremely promising candidates for drug therapy for many diseases such as influenza, cancer, AIDS, neurological and cardiovascular disorders (Patil et al., 2005). One of the major merits of plasmid-based therapeutics over conventional low molecular weight pharmaceuticals is their specific selective identification of molecular targets and pathways leading to high specificity of action (Leitner et al., 2000; Mountain, 2000). Plasmid DNA uptake and metabolism considerably enhances the stability and minimizes the degradation of pDNA-based therapeutics. High molecular weight pDNA can effectively be delivered into cells using both synthetic and natural delivery systems (Donnelly et al., 1997; Davis, 2001). Besides enhancement in pharmacodynamics, pDNA delivery methodologies have achieved efficient targeted introduction of gene-based molecules into desired tissues. This article reviews the design and development of plasmid-based therapeutic products and their applications in gene therapy and genetic vaccination. A controlled nasal delivery mechanism for the introduction of plasmid-borne therapeutic genes to target cells, their uptake and improved pharmacokinetics/ pharmacodynamics.

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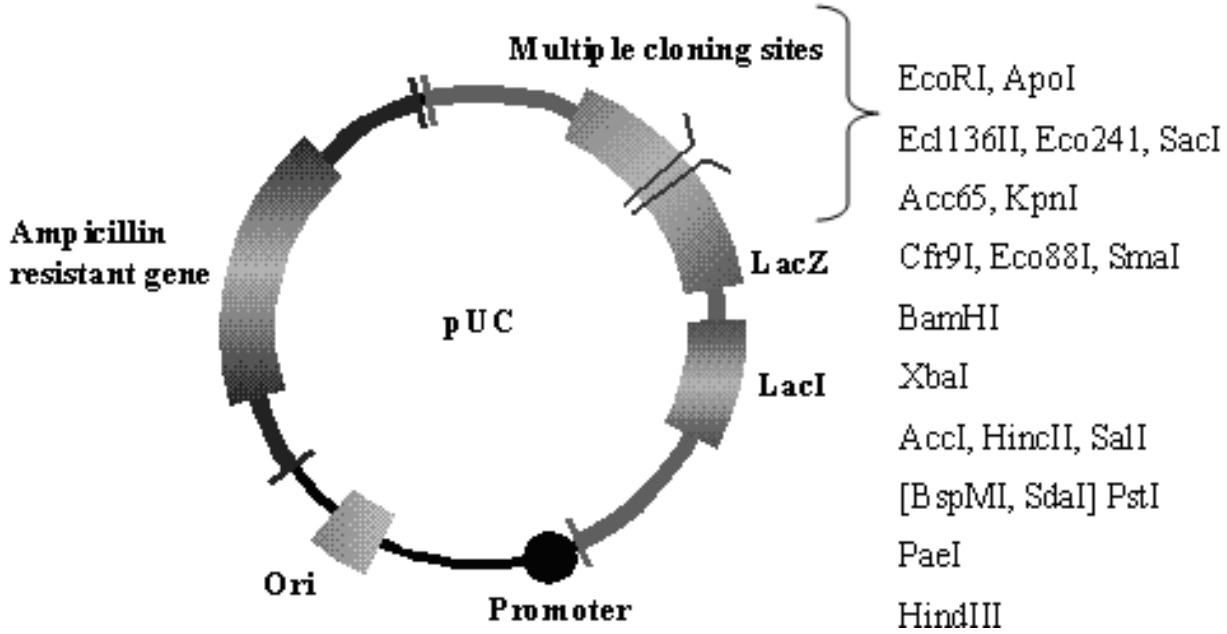

Figure 1. A plasmid vector construct from the pUC family containing an ampicllin resistant gene, origin of replication, promoter, LacI repressor gene, LacZ gene for α-peptide of β-galactosidase and a multiple cloning region showing different restriction endocleases for sequence specific cleavages.

PLASMID-BASED THERAPEUTIC PRODUCT DESIGN AND DEVELOPMENT

The formulation and engineering of plasmids to obtain maximum transfection during therapeutic application is conceptually straightforward. Figure 1 shows a typical pUC-based pDNA construct. The pDNA should contain rudiments for maintenance and propagation in the bacterial host and for expression of the transgene in the human host. The elements required for bacterial propagation include the origin of replication and any plasmid-encoded functions such as RNAI and RNAII sequences required for replication (Ferber, 2001; Prather et al., 2003). Besides the transgene of interest, the pDNA molecules should contain regulatory indicators such as promoter and enhancer sequences for regulating gene expression. Promoters available for recombinant gene expressions in mammalian cells include those from human cytomegalovirus, simian virus, human elongation factor and human ubiquitin. Human promoters have greater resiliency against immune response activation than viral promoters. Enhancers are mainly tissue specific regions in the pDNA that enhance the production of the gene of interest and transcription efficiency (Patil et al., 2005; Gurunathan et al., 2000). An antibiotic resistant gene typically called selection marker should be included for the assortment of successful transformants upon initial introduction of the plasmid into the bacterial host and to create selective pressure against a prevalence of plasmid-free segregants arising in the population (Wolf et al., 1990; Patil et al., 2005). The choices that exist for the selectable marker can be categorised into two groups; those that require both plasmid and host-based genetic manipulations and those that require only plasmid-encoded sequences (Patil et al., 2005; Prather et al., 2003). During design of a plasmid vector suitable for therapeutic application, the regulatory environment must be considered. A key regulatory concern which is the possibility of pDNA integration should be addressed during the design of the plasmid. Promoters and terminator regions should also be carefully selected to restrict their biological activities on the sequences inserted on the plasmid. Possible adverse effects resulting from selection markers such as ototoxicity and nephrotoxicity must be considered in the choice of a selection marker. Kanamycin which is an amino glycoside antibiotic is known to be free from such concerns associated with β-lactams (Wolf et al., 1990; Doolan and Hoffman, 2001; Patil et al., 2005).

BIOPROCESS ENGINEERING OF PLASMID-BASED THERAPEUTIC PRODUCT

Since Wolf and co-workers (1990) reported the uptake and extended *in vivo* expression of transgenes via injection of naked DNA into the leg muscle of mice, the use of pDNA molecules as vectors for gene therapy or vaccination has gained significant interest. Consequently, well in excess of hundred pDNA-based gene therapies, cancer vaccine and prophylactic vaccine clinical trials have been initiated (Shroff et al., 1999; Gurunathan et al.,

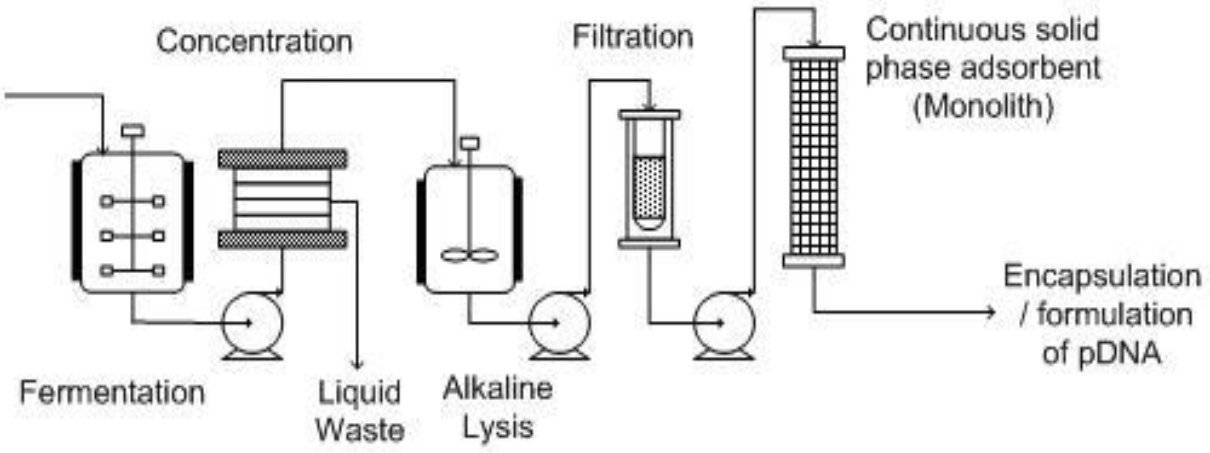

Figure 2. Proposed process flow sheet for single-stage purification of plasmid-based therapeutic products (Danquah et al., 2008b).

2000). This mass evolvement has yielded some early but encouraging results in the fight against some serious infectious diseases such as malaria (Doolan and Hoffman, 2001) and AIDS (Mascola and Nabel, 2001). However, although this novel vaccine technology appears very promising, it exhibits some limitations in the production of large quantities of pDNA to satisfy the growing market demands. At the laboratory scale, production and purification of plasmids are generally viewed as relatively easy and simple procedures. However, plasmid production under laboratory conditions generally leads to volumetric titers ranging from 5-40 mg/L (Prazeres et al., 1999; Prather et al., 2003). In the context of milligram range doses and large patient populations, these processes and their associated productivity levels are inadequate for economically viable plasmid production. Although a few publications mention large-scale plasmid production processes achieving productivity levels of several hundreds of milligrams per litre (Lahijani et al., 1996; Chen et al., 1997; Schmidt et al., 2001; Carnes et al., 2006; Danquah and Forde, 2008a), which represents a significant improvement over standard laboratory processes, it is likely that considerable room still exists for significant process improvements to be realized. The achievement of high-volume pDNA manufacturing hinges on fermentation optimisation and a high throughput continuous purification methodology (Danquah and Forde, 2008a; Prather et al., 2003; Durland and Eastman, 1998). The process flow sheet in Figure 2 illustrates process development in the creation of an optimised fermentation scheme and a single-stage continuous purification step for pDNA product manufacturing with product purity and integrity meeting relevant regulatory standards. We have published elsewhere the performance of this technology in the creation of a rapid and economically-viable production platform for pDNA (Danquah and Forde, 2007; Danquah et al., 2008b). Table 1 shows data on current pDNA production levels.

STAGED DELIVERY MECHANISM VIA THE NASAL ROUTE

Currently, pDNA immunisation can be achieved using intramuscular/intradermal injection, gold particle bombardment and needleless injection (Donnelly et al., 1997). These delivery methods are associated with some shortcomings thereby damping their effectiveness and immunological performance. Briefly, naked pDNA is unstable in biological fluid due to degradation by endonuclease and low uptake by cells leading to reduced expression of the encoded gene. Therefore, the development of a new and effective carrier system will be the key element to improve the potency of pDNA vaccines.

The nasal route is an important arm of the mucosal and systemic immune system, since both humoral and cellular immune responses can occur (Davis, 2001). This revolutionary approach will overcome several of the problems of existing vaccine delivery including the need for medical personnel to administer needles, the cost and logistics of storing and transporting vaccines and hygienic needle use and disposal. Intranasal vaccination offer non-invasive administration and easily accessible for a larger population, thus, will greatly assist the development of global health vaccination particularly in third world countries with the potential to reach greater numbers of people. Nasal delivery system can be achieved via different pharmaceutical forms such as dry powders, solutions or suspensions generated as different sized particles or droplets. The schematic pathways (Figure 3) in the nasal mucosa have been described by Kuper et al. (Kuper et al., 1992) where the particulate is taken up by the microfold epithelial cell, which is located in between the epithelial cells in lymphoid associated epithelium, and can elicit a local mucosal response or lead to a tolerance. The short residence time and low permeability of the formulations within the nasal cavity have contributed in poor bioavailability. The attention has therefore shifted to the evaluation of mucoadhesive

Table 1. Comparison of up-to-date top plasmid yields and productivity of various pilot-scale and industrial fermentation processes (Danquah and Forde, 2008a).

Fermentation process	Plasmid	Biomass conc. (g/L)	Yield (mg/L)	Productivity (mg/Lh)	Specific yield (mg/g)	Reference
Fed-batch (Pilot-scale)	pUK21CMVβ	70.0	200	5.6	2.6	Schmidt et al., 2003
Fed-batch (Pilot-scale)	pSVβ	8.3	20	0.6	2.4	O' Kennedy et al., 2003
Fed-batch (Pilot-scale)	VCL1005G/A	38.8	218.6	11	5.6	Lahijani et al., 1996
Fed batch NTC3019, 37°C (industrial-scale)	pBR322 derived	60.5	438	13	7.2	Carnes et al., 2006
Fed-batch (Pilot-scale) 37 - 42°C	pUC19	10.8	529	22	49	Danquah and Forde, 2008
Fed-batch NTC3019, 30 - 42°C (industrial scale)	gWiz GFP	48.5	1070	26	22.1	Carnes et al., 2006
Fed-batch NTC3019, 30 - 42°C (industrial-scale)	pNTC7264-hmPA-EGFP	42.9	1497	36	34.9	Carnes et al., 2006

Dry biomass concentration is estimated based on: 1.0 OD600 ≈ 0.5 g/L.

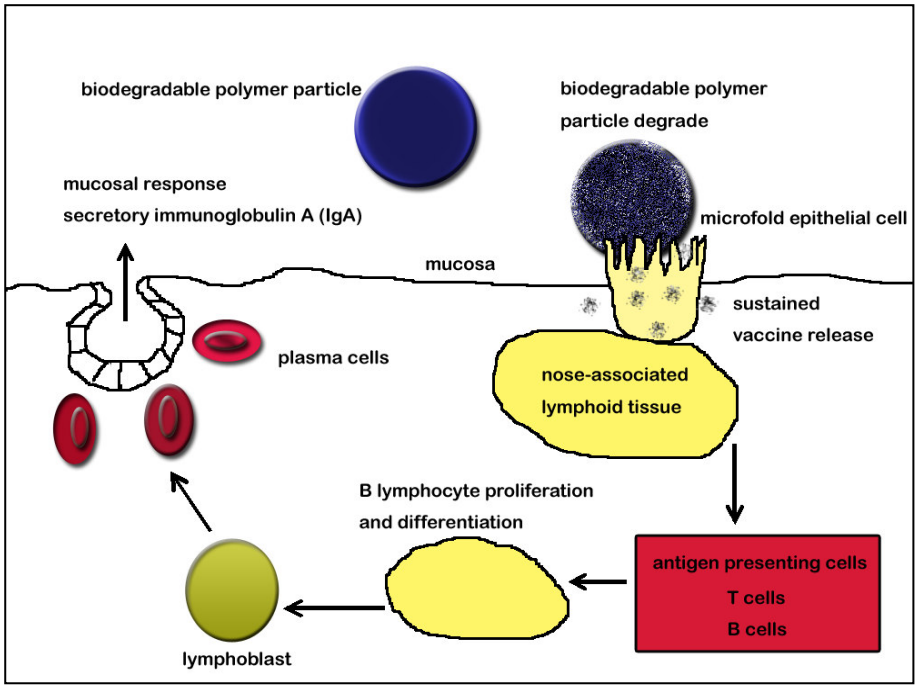

Figure 3. Hypothetical pathway to elicit a local mucosa response via nasal mucosa. After the intranasal immunization, the biodegradable polymer particle is sampled by the microfold epithelial cell and the vaccine released in sustainable fashion. It is then passed on to underlying lymphoid cells in the submucosa for processing and presentation. This results in the activation of T-cells that help B-cells to develop into IgA plasma cells.

polymers that demonstrate additional permeation-enhancing capabilities in nasal delivery (Ugwoke et al., 2005). This is achieved by encapsulating the plasmid-based therapeutic product into a mucoadhesive and biodegradable polymer system which is able to maintain sustained vaccine release over a period of time to specific sites. The biodegradable polymers can degrade in physiological environment, yielding naturally occurring metabolic products. Their degradation rate can be controlled to meet target usage such as staged delivery of vaccine. The method of staged delivery of plasmid–borne gene vectors can help to optimise the pharmacokinetics and pharmacodynamics of the release mechanism.

The design and use of biodegradable polymers for the controlled delivery of entrapped vaccines is an approach that holds promise for improving the duration and effectiveness of vaccine delivered locally or systematically via nasal route. Toxicity due to spikes in vaccine concentration can also be reduced. On the other hand, the immune response associated with pDNA vaccine can be augmented using a protein booster in a prime-boost immunization system (Schultz et al., 2000). For instance, stronger humoral and cellular immune responses were elicited when immunised with a combined vaccine regime consisting of DNA prime encoding the HCV E2 and subsequently boosted with the respective recombinant protein (Song et al., 2000). Encapsulation of DNA-based prime and protein booster in different segments of biodegradable particulate system enable improved and homogenized overall immune response.

CONCLUSION

Plasmid DNA presents a novel alternative option for immunological disease control and prevention. With the recombinant DNA technology, one can re-design obsolete production and storage methods of conventional therapeutic techniques that have been ineffective in controlling certain diseases. By using pDNA, it is possible to address several diseases or disease strains in one vaccine, which will meet growing need for multivalent combination vaccines leading to fewer injections. Delivery of pDNA vectors harbouring therapeutic sequences via the nasal route also creates a good platform for staged/controlled delivery of transgenes to specific target cells. However, a greater body of clinical data and further enhancements in bioprocess engineering and delivery are required before pDNA products herald the new generation of therapeutics.

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