

PLGA-Chitosan nanoparticle-mediated gene delivery for oral cancer treatment: A brief review

L M Bakar^{1,3}, M Z Abdullah^{1,3}, A A Doolaanea², S J A Ichwan^{1,4*}

¹Department of Fundamental Dental and Medical Sciences, Kulliyyah of Dentistry,

²Advanced Drug Delivery Laboratory, Department of Pharmaceutical Technology, Kulliyyah of Pharmacy,

³Department of Biomedical Sciences, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, Kuantan, Pahang 25200, Malaysia

*E-mail: solachuddin@iiu.edu.my

Abstract. Cancer becomes a serious issue on society with increasing of their growth and proliferation, either in well economic developed countries or not. Recent years, oral cancer is one of the most threatening diseases impairing the quality of life of the patient. Scientists have emphasised on application of gene therapy for oral cancer by using nanoparticle as transportation vectors as a new alternative platform in order to overcome the limitations of conventional approaches. In modern medicine, nanotechnologies' application, such as nanoparticles-mediated gene delivery, is one of promising tool for therapeutic devices. The objective of this article is to present a brief review summarizes on the current progress of nanotechnology-based gene delivery treatment system targeted for oral cancer.

1. Introduction

Cancer is expected to grow worldwide and aggressively proliferate which can cause an increased-on mortality rate by year. The occurrence of cancer diseases had given huge burden on society and had caused 21.7% of mortality among chronic diseases globally in 2012 [1]. Cancers had caused enormous human potential lost and costs the nation billions of dollars. It is estimated that the costs of USD 87.8 billions for total medical care in treating the cancer for US in the year of 2014. Oral cancer is one of the 10 most frequent types of cancer worldwide, and an estimated 300,400 new cases occurred worldwide in 2012, with 145,400 deaths [2,3]. Smoking habit, alcohol consumption, smokeless tobacco product use and HPV infection are the associated factors found in oral cancer patients [2].

Most of oral cancers cells are derived from the stratified squamous cells which lining the mucosal epithelium. The malignant neoplasm has a tendency to proliferate more rapidly locally, invading adjacent local tissues including the bone of the mandible, and spreading to drain lymph nodes [4]. Conventional treatment strategies of oral cancer are either radiotherapy, chemotherapy and radical surgery, or combination of these treatments. However, these conventional therapies such as radiotherapy and chemotherapy, are non-selective and caused discomfort and inconvenience to the patients [5]. The traditional cancer treatment approaches are loathed for their repugnant side effects including the pain and discomfort linked with their administration.

2. Gene therapy in the management of oral cancer

Generally, cancer occurred via multiple genetic alterations, including deletions, point mutations, promoter methylation, oncogene amplification and tumor suppressor inactivation, due to extreme



exposure to carcinogen [6]. Specifically, oral cancer is a genetic disease commonly related to the genetic changes include proto-oncogenes activation and inactivation of tumor suppressor genes [7]. Early detection and recognition of oral cancer could decrease the mortality rate and improve the five-year survival rate. Proteomic and genomic technologies are the keys to overcome these obstacles. Hence, cancer research group has highlighted on the applications of gene therapy in oral cancer treatment. Gene therapy can potentially target cancerous cells while leaving normal tissue unharmed [8].

2.1. Gene therapy

Gene therapy can be described as the treatment of disease by introducing a piece of therapeutic genetic material into the targeted cells in order to either slowdown in the progression or to cure for the disease [9]. It has high potential as new treatment modality for genetic disease. Gene therapy research has started since 1980 with impressive achievement including the development of animal models, identifications of viral vectors, genes cloning, and the expression of these inserted gene [10]. Tumour suppressors, antigens, cytokines and suicide enzymes are among the common gene types, which are transferred in combating cancer. Mutations of p53 gene are present in half of oral squamous cell carcinoma compared to others gene such as BRCA-1, Fus-1 and endostatin [5,11]. Thus, p53 gene is the most frequent transferred tumour suppressor gene in order to restore the function of a tumor suppressor gene in cancer cells. This improvement resulted a novelty in oral cancer treatment via gene therapy which specifically attacks cancer cells including targeting the function of p53 tumor suppressor gene [5].

2.2. Viral vector

Adenovirus (Ad) is currently considered as a promising carrier for cancer gene therapy due to its unique features such as efficient infection, high loading capacity, high levels of gene expression (though transient), lack of insertional mutagenesis and to infect non-dividing cells. In 2008, Bennett and Macguire had showed remarkable vision improvement for Leber's congenital blindness after the injection of modified virus carrying the normal healthy genetic materials [10]. However, the clinical application of viral vector is restricted by short vector's half-life, hepatocytotoxicity, low accumulation and the host's immune response [12]. Moreover, adenoviruses will be allowing for prolonged survival outside of the body due to unstable condition towards exposure of chemical or physical agents and adverse pH conditions. This fact is significant in any environmental risk assessments that are undertaken. Besides, even though viral vector has gene transfer efficiency, it has many other drawbacks that will limit its application *in vivo*. As a result, new research focuses increasingly on the development of synthetic vectors not based on viral systems.

2.3. Non-viral vector

Even though non-viral carriers are less efficient in gene transfer as compared to viral carrier, they possess the advantages of safety, simplicity of preparation and production, high gene encapsulation capability, and less of specific immune response [13,14]. The non-viral vectors consist of naked nucleic acids delivered by injection, liposomes, cationic polymer-based carriers, cationic peptides, nanoparticles, and other means. These non-viral vectors were designed to be able to penetrate the physical barrier of the cell and the nuclear membranes then exert sustained and high-level of gene expression.

3. Significance of nanotechnology in gene therapy

Although gene therapy can offer better safety profiles as it is more cancer-selective as compared to conventional medicine, its application is hindered by limited plasmid DNA penetration of cell membrane barrier, vulnerability to enzymatic degradation and nucleases in biological matrices, safe choice of carrier system material and low transfection efficiency. Application of nanotechnology has drawn a significant impact including the medical therapeutics. In Greek, the word "Nano" means "dwarf" [15]. Nanomedicine, which were referred to usage in diagnose, treat, and prevent diseases at the basic cellular and molecular level, is one of the application development of nanotechnology in the

field of medicine [16]. Presently, there are various avenues by means of nanotechnology have been evolved in research and development as to improve the quality of life of the patient. Moreover, the cancer research group took the advantages of nanotechnology application in designing, characterising, producing, and delivering process for an alternative to the conventional cancer therapy. Particularly, nanotechnology system been applied either in drug delivery system or gene delivery system with the usage of nanoparticles.

3.1. Nanoparticles

Nanoparticle can be defined as any produced particle that has a characteristic structures dimension from 10 to 1,000 nm in size [17]. This sub-micron size range is an advantage in delivery system for the movement of the nanoparticles within the cells and tissues. Due to this minute size, nanoparticles can easily get through fine capillaries and cross the physiological barrier before being taken up by the cells efficiently [18]. They have been developed in different structures based on their proposed properties and functions. The nanoparticles that are frequently utilised for the cancer treatment include polymeric nanoparticles, liposomes, dendrimers, carbon nanotubes, nanoshells, magnetic nanoparticles, and gold nanoparticles [16]. Basically, nanoparticles can be used to provide targeted delivery of therapeutic material, enhance oral bioavailability, sustain therapeutic effect in target tissue and increase stability of therapeutic agent against enzymatic degradation, especially of protein, peptide and nucleic acids drugs. These targeting capabilities of nanoparticles will be depending on its particle size, surface charge, hydrophobicity, and surface modification [19].

3.1.1. Particle size

The size and size distributions of nanoparticle will affect their capability to enter the cells [20]. Tumor vasculatures are abnormal and have large pores with diameter size of 100 nm to 700 nm, whereas normal vessel junctions of 5-10 nm [21]. These abnormal pores allowing nanoparticle to pass into the tumours. Formulating nanoparticles will have distinct advantages as they have better intracellular uptake and resulted with greater efficiency of uptake [19]. The size of nanoparticles to across different barriers is reliant on the target site, tissue and circulation [20].

3.1.2. Surface charge

Surface charge is crucial for the nanoparticles delivery into the cells or tissues. It will determine the nanoparticle either will accumulate in blood vessel or bind to oppositely charged cells membrane [22]. Positively surface charge is needed to interact with negatively charge of cells membrane and nucleus membrane in order to increase the rate of nanoparticles internalization [20]. In addition, positively charge nanoparticles were reported that able to escape from endosomal/lysosomal vesicle during cellular internalization, whereas negatively charged nanoparticle remained in the vesicle [19].

3.1.3. Hydrophobicity and Hydrophilicity

In targeted delivery, the persistency of nanoparticles is essential in systemic circulation of the body. Commonly, nanoparticles with hydrophobic surface are easily marked and extensively eliminated by the fixed macrophages of the mononuclear phagocytic system organs [21]. Therefore, surface of conventional nanoparticles requires modification with different molecules, so that the circulation time and persistence in the blood will increase too. At the meantime, a cloud of chains created by coating hydrophilic polymers to nanoparticle are expected to repel plasma proteins during the nanodelivery process [22]. Hydrophilic polymers help in stabilization of the nanoparticles and in better targeting of the site because they aid in forming a stealth layer that tend to reduce the nonspecific uptake by the cells [19].

3.1.4. Surface modification

Performance of nanoparticles *in vivo* is closely related of changes in morphological characteristics, surface chemistry, and molecular weight [19]. Modification of nanoparticles creates anti-adhesive properties on the particle surface, which acts as steric barrier in reducing the massive clearance by circulating macrophages of the liver and promoting the possibility of undergoing improved permeation

process [22]. Molecular weight of the polymer used will influenced the release profile, as it is inversely proportional to the *in vitro* release mechanism system. The higher molecular weight of the polymer used will slower the release of drugs [23]. Advance design through modification may circumvent some of the difficulties faced by active molecules.

3.2. Biodegradable nanoparticles

Among different types of nanoparticles, polymer-based nanoparticles are widely used for vector delivery system [22]. Biodegradable nanoparticles can be fabricated from poly (D,L-lactide-co-glycolide); PLGA and chitosan. Biodegradable nanoparticles have been favourably chosen as non-viral vectors due to its capability of good encapsulation, control release, grand bioavailability and less toxic properties [19].

3.2.1. PLGA (*polylactic-co-glycolic acid*)

PLGA is a composition of two types of monomers; lactic acid and glycolic acid bonded by ester linkages and it has been given approval by the US Food and Drug Administration (FDA) and European Medicine as a carrier for parenteral administration [19,20,24]. The biodegradable polymers have shown to possess more efficacy and versatile nanoparticles due to their characteristics like prevention of rapid clearance by the reticuloendothelial system (RES) and enhanced plasma half-life leading to decreased dosage of the therapeutic agents. By modulating polymer characteristics, release of therapeutic agent from nanoparticles can be control. Nanoparticles containing encapsulated plasmid DNA could serve as an efficient sustained release gene delivery system due to their rapid escape from the degradative endo-lysosomal compartment to the cytoplasmic compartment and their sustained intracellular retention [19,25]. In order to achieve greater level of gene expression, the intracellular delivery is also being crucial part to look in gene therapy approaches.

It is proposed that the gene transfer efficiency of non-viral plasmid vector on oral cancer cells can be optimized via encapsulation with chitosan and PLGA. The PLGA-chitosan matrix will be employed to encapsulate plasmid DNA by the most commonly used fabrication method for nanoencapsulation, such as solvent-evaporation. This method has proven to be non-destructive to the integrity of the DNA [26].

3.2.2. Chitosan

Natural based products are attractive candidates for therapeutic agents due to their safety and fewer side-effects [27]. Chitosan is one of the multipurpose polysaccharide of biological origin and naturally occurring by the repeating units of linear polysaccharide and amino polysaccharide. Chitosan is derived from chitin by partial deacetylation under a strong alkaline condition [28]. Chitin become the second most abundant polymer found in nature after cellulose whereby this polysaccharide is widely existed like crustaceans, insects and certain fungi [29].

Chitosan is a favourable nanocarrier, due to its biocompatible, biodegradable, nontoxic, cheap and has low immunogenicity as compared to other cationic polymers [30]. Chitosan has also been reported to carry an antimicrobial, antifungal, and wound-healing properties, whereby it is approved for dietary applications in Japan, Italy and Finland and by the FDA as well for wound dressings [31-33]. Moreover, chitosan and its derivatives have a significant impact in design of drug delivery systems and biomedical engineering area [34].

Chitosan is intensively utilized as assisting in gene delivery as it exhibits numerous of attractive properties [35]. The amine groups in its structure make it special as it is hydrosoluble and positively charged. These unique criteria will response with negatively charged molecules like DNA, upon interaction in an aqueous solution. Hence, chitosan can successfully bind DNA and protect it from being degraded by the nucleases. The positively charged chitosan will bind to targeted cell membranes and is reported to decrease the trans-epithelial electrical resistance of cell monolayers as well as to increase paracellular permeability [36]. Besides, chitosan solutions have been reported to increase trans and para-cellular permeability in a reversible, dose-dependent manner that also depends on the molecular weight and degree of deacetylation of the chitosan [37].

Chitosan-based gene delivery systems also seemed capable to overcome several issues raised such as difficulty of targeting and transporting through cell membrane, degradation occurring in endolysosomes and intracellular trafficking of DNA to the nucleus [38,39]. Application of chitosan-based gene delivery has been reported towards numerous cancer cells including A549 (lung adenocarcinoma), HeLa (cervical carcinoma), HepG2 (hepatocellular carcinoma), and OVCAR-3 (Human ovarian adenocarcinoma) [40].

3.2.3 PLGA-Chitosan nanoparticles

PLGA and chitosan alone is a promising carrier for gene therapy applications. Structure modification in terms of their *in vivo* stability, target specificity and desirable intracellular release of gene therapeutics have enhanced the potential of PLGA and chitosan [40,41]. Incorporation of PLGA and chitosan nanoparticles possesses an attractive material choice and offers a flexible technology platform for gene delivery system [42]. The advantage of combining PLGA nanoparticles with chitosan has recently materialized their biocompatibility, their tuneable size and surface properties, possessing an overall positive charge that promotes complex formation with negatively charged nucleic acids [43]. They also showed no electrophoretic mobility, indicating the successful uptake of all pDNA into the complex. Besides, they are able to offer protection of nucleic acids from enzymatic degradation in both the extracellular and intracellular spaces [41]. Application of PLGA-chitosan nanoparticles has never been tried for oral cancer gene therapy. Our group is currently conducting a research project in the utilization of PLGA-Chitosan nanoparticles to deliver tumor suppressor genes in oral squamous carcinoma cells.

4. Conclusion

Nanotechnology is predicted to alter health care in dentistry. Nanoparticle-based approach has unlimited potential with novel applications continuously being developed for use in cancer diagnosis, detection, imaging, and treatment. Hence, application of nanoparticles-mediated gene delivery will be useful for genetic manipulation techniques. The PLGA-Chitosan Nanoparticle-Mediated Gene seems to be a promising approach for oral cancer treatment. Nanotechnology perhaps holds its promise as an innovative avenue for oral cancer therapy.

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