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Controlled Release Systems for Non-Viral Vectors

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Adapting controlled release technologies to the delivery of non-viral vectors has the potential to overcome barriers that limit gene therapy. Controlled release systems can enhance gene delivery and increase the extent and duration of transgene expression relative to more traditional delivery methods. Delivery vehicles for controlled release are fabricated from natural and synthetic polymers, which function either by releasing the vector into the local tissue environment or by maintaining the vector at the polymer surface. Vector release or binding is regulated by the effective affinity of the vector for the polymer, which depends upon the strength of molecular interactions. These interactions occur through non-specific binding based on vector and polymer composition or through the incorporation of complementary binding sites (e.g., biotin-avidin). This review examines the delivery of non-viral vectors from natural and synthetic polymers, and presents opportunities for continuing developments to increase their applicability.

Introduction

Controlled release systems for low molecular weight drugs and proteins have become a multi-billion dollar industry, with products such as

Nutropin® Depot, Gliadel® wafer, Norplant, and CYPHER™ Stent. These systems illustrate the potential advantages of controlled release, which include: (1) maintained drug levels within a desirable range, (2) localized delivery to a target tissue or cell type to avoid adverse side effects, (3) decreased dose or number of dosages, and (4) facilitated delivery for fragile compounds (i.e., short half-lives). The adaptation of controlled release technologies to the delivery of non-viral vectors has the potential to overcome barriers that limit gene therapy. Controlled release can maintain elevated DNA concentrations in the cellular microenvironment, which improves gene delivery[1]. Additionally, non-viral vectors may have a relatively short half-life[2], and delivery vehicles can either prevent their degradation or provide a sustained release. This review examines gene delivery from biomaterials and discusses how continuing advances will increase their applicability.

Delivery mechanisms

Controlled release systems typically employ polymeric biomaterials that deliver vectors according to two general mechanisms: i) polymeric release in which the DNA is released from the polymer or ii) substrate-mediated in which DNA is retained at the surface. For polymeric release, DNA is entrapped within the material and released into the environment, with release typically occurring through a combination of diffusion and polymer degradation. Polymeric delivery may enhance gene transfer by first protecting DNA from degradation, and then maintaining the vector at effective concentrations, extending the opportunity for internalization. DNA release into the tissue can occur rapidly, as in bolus delivery, or extend over days to months[3-5]. Conversely, substrate-mediated delivery, also termed solid phase delivery, describes the immobilization of DNA to a biomaterial or extracellular matrix, which functions to support cell adhesion and places DNA directly in the cellular microenvironment. Cells cultured on the substrate can internalize the DNA either directly from the surface, or by degrading the linkage between the vector and the material[6].

Vehicle formulations

Vehicles for gene delivery can be fabricated from both natural and synthetic polymers and processed into a variety of forms, including nanospheres, microspheres, or scaffolds. Nanospheres are particles with diameters ranging from approximately 50 nm to 700 nm[7], consistent with the size of non-viral vectors. Nanoparticles are internalized and release DNA intracellularly. In contrast, microspheres with diameters ranging from 2 μm to 100 μm, are not readily internalized, but retained within the tissue to release DNA[8,9]. Released DNA can transfect cells at the delivery site, with the protein product acting locally or distributed systemically[9,10]. Alternatively, polymeric scaffolds function to define a three-dimensional space and can either be implanted or designed to solidify upon injection. These scaffolds can deliver DNA to cells within the surrounding tissue, or can target those infiltrating the scaffold[5,10].

A variety of natural and synthetic materials have been employed for DNA delivery, which can be categorized as either hydrophobic (e.g., poly(lactide-co-glycolide) (PLG), polyanhydrides) or hydrophilic polymers (e.g., hyaluronic acid (HA), collagen, poly(ethylene glycol) (PEG)). Synthetic polymers such as PLG and polyanhydrides have been widely used in drug delivery applications, as they are biocompatible and available in a range of copolymer ratios to control their degradation. Drug release from these polymers typically occurs through a combination of surface desorption, drug diffusion, and polymer degradation[11]. Alternatively, hydrogels, which are often more than 98% water and maintain the activity of encapsulated vectors, released DNA by diffusion from the polymer network[4], which can be controlled by crosslinking the polymer[12].

Naked DNA

Naked DNA delivery by traditional mechanisms generally results in low but sustained expression *in vivo,* which is limited by poor uptake due to factors such as degradation and clearance. Physical (e.g., ultrasound, hydrodynamic injection) and chemical (e.g., cationic lipids/polymers) methods are continually being improved to enhance cellular uptake of naked DNA by altering cell permeability or enhancing cellular interactions[13]. Nevertheless, polymeric delivery represents an alternative approach that can increase residence time within the tissue and protect against degradation.

Naked DNA interacts weakly with many polymers, leading to release from the vehicle with rates modulated by the polymer properties. Collagen based materials released naked DNA *in vitro* for times ranging from hours to days[4,14], yet intramuscular implantation of collagen pellets maintained the DNA locally for 60 days[15]. HA-based hydrogels also release the DNA; however, the rate of release can be controlled by the extent of crosslinking[12,16]. For synthetic polymers such as PLG, the integrity of the DNA can be affected by degradation of the polymer to lactic acid and glycolic acid[5]. PLG polymers can provide release rates ranging from a few days to more than 60 days (Figure 1), with the fabrication method and the polymer composition regulating release[15,17,18]. Ethylene vinyl-co-acetate (EVAc) polymers can similarly provide a sustained release of DNA on the time scale of weeks[19].

DNA releasing polymers administered to multiple sites *in vivo* have demonstrated the capacity to transfect cells locally and promote sustained protein production. An injectable PLG formulation delivered subcutaneously led to 28 days of expression with 50 µg of DNA[17]. An implantable PLG scaffold delivering 500 µg of DNA was able to transfect cells within and adjacent to the scaffold, and promote physiological responses[5]. Collagen minipellets containing 50 µg of DNA

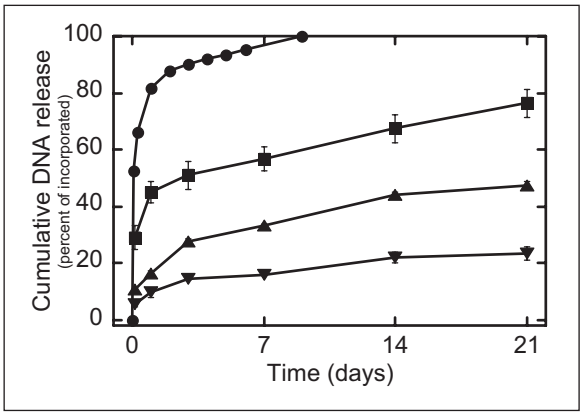

Figure 1. *Range of release rates of DNA from PLG scaffolds achieved through variations in the fabrication process.*

administered intramuscularly elicited systemic effects for at least 60 days, which was significantly longer than direct DNA injection[9].

DNA Complexes

Although naked DNA provides transfection *in vivo,* packaging DNA with cationic lipids or polymers can enhance *in vivo* transfection. Complexes of naked DNA with cationic polymers or lipids facilitate cellular internalization, by creating a less negative surface charge and providing stability against degradation[20]. The presence of complexation agents can also maintain the stability of DNA complexes during polymer processing[21], and in some cases increase encapsulation efficiency[22]. Porous PLG or collagen scaffolds with encapsulated polyplexes or lipoplexes achieved substantial transfection *in vitro*[4,23] and *in vivo*[4], but with significantly altered release profiles compared to naked DNA, due to interactions of the complexation reagents with the biomaterial or with adsorbed serum components[24].

Interactions between complexation agents and the polymer have been adapted to specifically immobilize DNA complexes to a substrate. Poly(L-lysine) (PLL) and PEI were modified with biotin residues for subsequent complexation with DNA and binding to a neutravidin substrate[6,25]. Complexes were formed with

mixtures of biotinylated and non-biotinylated cationic polymer at a constant N/P ratio. For complexes formed with PLL, the number of biotin groups and their distribution among the cationic polymer were critical determinants of both binding and transfection (Figure 2). Increasing the number of biotin groups per complex led to increased binding[6]. However, transfection was maximal when complexes

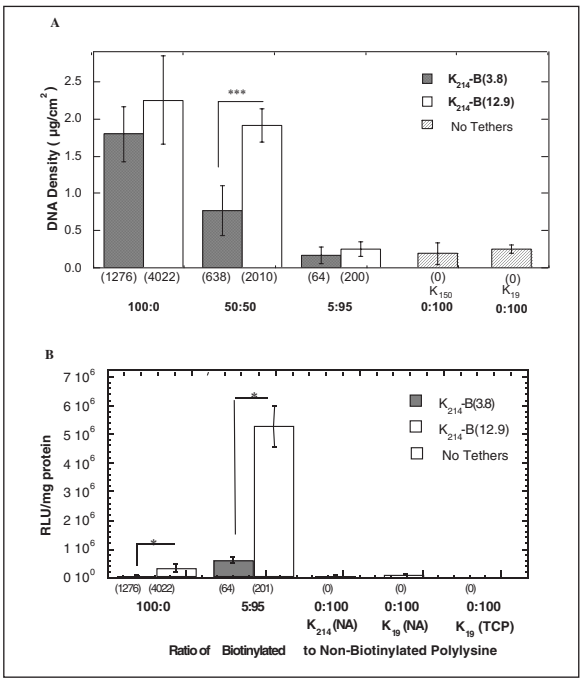

Figure 2. *Density and transfection of substrate associated DNA/polylysine complexes formed with varying biotin distribution. (A) DNA density and (B) transgene expression for complexes formed at a charge ratio of 5.5:1. The notation K_{214}-B indicates that polylysine (K) has 214 monomers on average and is biotinylated (B), with the number of biotin residues per polylysine following. The numbers in parentheses below each bar represent the average moles of biotin per mole of DNA. The data is presented as the average ± the standard deviation and the symbol * indicates statistical significance at a level of p <0.05 for the comparisons indicated. p values were obtained using the student t test with the single comparisons. The label NA and TCP indicates the substrate was neutravidin and tissue culture polystyrene respectively.*

contained biotin residues attached to a small fraction of the cationic polymers[25]. At this condition, less than 100 ng of immobilized DNA mediated transfection, which was increased 100 fold relative to bolus delivery of similar complexes[6]. For complexes formed with PEI, substantial transfection was observed, but was independent of the number of biotin groups present on the complex, which suggests that complex binding occurred by non-specific interactions with the substrate[25]. Other systems have used non-specific binding to mediate delivery. PLGA and collagen membranes were coated with phosphatidyl glycerol (1-5%) to support binding of complexes formed with polyamidoamine (PAMAM) dendrimers[26]. *In vivo* studies demonstrated a six to eight-fold enhancement in transfection relative to naked DNA delivery.

Applications

Gene Therapy: Numerous clinical trials have been completed or are pending for a multitude of pathologies including malignancy (e.g., colo-rectal, bladder, and brain). Most trials have not shown significant therapeutic efficacy or clinically useful responses, likely due in part to inefficient gene transfer[27,28]. Polymeric-based gene delivery systems may enhance delivery of the vector and extend the duration of transgene expression to achieve sufficient protein quantities that act locally or systemically. For example, IL-2, IL-12, and TNF-α expression induced by a DNA releasing gelatin sponge inhibited tumor growth in heterotopic nodules of tumor bearing mice[29].

Functional Genomics: Transfected cell arrays represent a high throughput approach to correlate gene expression with functional cell responses, based on gene delivery from a surface[30]. In principle, this system can be employed for numerous studies, such as screening large collections of cDNAs[30] or targets for therapeutic intervention. Transfected cell arrays were formed using a substrate-mediated approach in which plasmids or adenoviruses were mixed with collagen and spotted onto glass slides or into wells[30,31]. Plated cells were transfected and could be analyzed for cellular responses using a variety of imaging or biochemical techniques.

Conclusions

In comparison to traditional gene delivery systems, controlled release can enhance gene delivery by increasing the extent and duration of transgene expression, while reducing the need for multiple interventions. These polymer-based gene delivery systems capitalize on both specific and non-specific interactions between the biomaterial and vector, to achieve either release into the extracellular space or immobilization at the surface. While the potential to use these polymeric systems has been established, the design parameters by which to optimize or control gene transfer are not well understood. Vector and biomaterial development, combined with studies that correlate system properties (e.g., dose, release rate) with the extent of transgene expression (i.e., quantity and duration of protein produced, location of transgene expression) will lead to molecular scale design of delivery systems. The development of these systems may increase the efficacy within current gene therapy trials, and may also extend the applicability of gene delivery to other areas such as functional genomics.

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