Journal of Controlled Release 167 (2013) 109–119

Contents lists available at SciVerse ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

GENE DELIVERY

Liposomal surface coatings of metal stents for efficient non-viral gene delivery to the injured vasculature

Sandra Ganly [a,b,1], Sean O. Hynes [a,1], Faisal Sharif [c], Ahmed Aied [d], Valerie Barron [a], Karl McCullagh [a,e], Jill McMahon [a], Peter McHugh [b], Jim Crowley [c], Wenxin Wang [d], Timothy O'Brien [a], Udo Greiser [a,d,*]

[a] Regenerative Medicine Institute (REMEDI), National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Ireland
[b] Department of Mechanical & Biomedical Engineering, National University of Ireland, Galway, Ireland
[c] Department of Cardiology, Galway University Hospital, Galway, Ireland
[d] Network of Excellence for Functional Biomaterials (NFB), National University of Ireland, Galway, IDA Business Park, Dangan, Galway, Ireland
[e] Discipline of Physiology, School of Medicine, National University of Ireland, Galway, Ireland

ARTICLE INFO

Article history:
Received 11 October 2012
Accepted 31 January 2013
Available online 10 February 2013

Keywords:
Stent
Non-viral gene therapy
Vasculature
Lipid
Lipoplexes

ABSTRACT

Despite the widespread use of drug eluting stents (DES), in-stent restenosis (ISR), delayed arterial healing and thrombosis remain important clinical complications. Gene-eluting stents (GES) represent a potential strategy for the prevention of ISR by delivering a therapeutic gene *via* a vector from the stent surface to the vessel wall. To this end, a model *in vitro* system was established to examine whether cationic liposomes could be used for gene delivery to human artery cells. Three different formulations were compared (DOTMA/DOPE, DDAB/DOPE or DDAB/POPC/Chol) to examine the effects of different cationic and neutral lipids on the transfection efficiency of lipoplex-coatings of metal surfaces. Upon completion of the characterization and optimization of the materials for gene delivery *in vitro*, these coatings were examined on a range of stents and deployed in a rabbit iliac artery injury model *in vivo*. Maximal transfection efficiencies for all coatings were observed on day 28, followed by declining, but persisting gene expression 42 days after stent placement, thereby, presenting liposomal coatings for gene eluting stents as treatment options for clinical complications associated with stenting procedures.

1. Introduction

Delivery of large biomolecules from drug eluting stents (DES) holds great promise for addressing the shortcomings of currently existing (small drug eluting) stenting platforms or catheter-based delivery systems such as inadequate concentrations of the pharmacotherapeutic agents at the lesion site, unwanted distal spread of the compounds to other organs and insufficient specific targeting of the cells involved in thrombosis, inflammation and neointimal hyperplasia after injury of the vasculature [1,2]. Several stable surface coatings for DES have been proposed [3–6] that withstand strain, are biodegradable, improve the biocompatibility of the devices or reduce thrombogenicity and may be further enhanced to meet specific delivery requirements in combination with unique pharmacologies of a specific therapeutic agent or to be used with re-designed stents to optimize their performance [7,8]. By contrast, catheter-based delivery [9] that may not allow sufficient time for a high enough therapeutic dose of agents to penetrate target cells and reach subcellular compartments such as the nucleus, halts the blood flow which may lead to hypoxia and necrosis.

Gene therapy utilizing viral or non-viral approaches has been heralded as a new era in the treatment of acquired or genetic diseases. Viral vectors are more efficient than non-viral vectors, however, non-viral vectors such as lipoplexes and polyplexes [10–12] are attractive for clinical applications because they may offer safety advantages [13–16], reduced host immune response and the ability to carry larger DNA constructs [17]. Steric stabilization of liposomes with polymer coatings such as polyethylene glycol (PEG) has overcome some of the challenges to successful drug delivery they encountered *in vivo*, mainly opsonization and clearance by the reticuloendothelial system [18,19] and facilitated longer lasting circulation in the vasculature with increased ability for localization in tumors [20–23] of these non-viral carrier systems. Especially the introduction of cationic lipids, methods for liposome and cationic lipoplex production and the characterization and optimization of their morphology [24–30] were major milestones on the path to development of efficient lipoplex-based transfection systems. However, several challenges for lipoplex-mediated gene delivery remain. These include the targeting of therapeutically sufficient doses at

* Corresponding author at: Regenerative Medicine Institute, National Centre for Biomedical Engineering Science & Department of Medicine, National University of Ireland, Galway, Ireland. Tel.: +353 91495166, +353 91495833; fax: +353 91495547, +353 91495585.
E-mail address: udo.greiser@nuigalway.ie (U. Greiser).
[1] The two first authors contributed equally to the paper.

0168-3659/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jconrel.2013.01.036

the precise location of the disease or tissue damage, extended, stable encapsulation, extended release of the gene and minimization of drug distribution to distal organs.

Gene eluting stents (GES) offer an ideal platform for local delivery of a smaller, more efficient dose of therapeutic genes, protect the cargo load from ubiquitous nuclease digestion because of the layer-by-layer DNA deposition technique and facilitate better penetration of transgenes due to their direct contact with the vascular bed and persistence at the site of implantation while serving as reservoirs for vectors allowing prolonged release with improved kinetics and better control over adverse systemic side effects [1]. Increased specificity and limited side effects of coated, gene eluting stents will increase their efficacy, however, the large size and physical chemistry of the transgenes as well as stent loading restrictions associated with smaller surfaces of stent struts mean limitations in drug loading. These limitations prompted us to develop new protocols and designs for the formulation of the vector system and for testing the stability of the coatings and the release kinetics of the large pharmacological agents.

Early pioneering work on the release of genes from coated stents by R.J. Levy's group [31–35] demonstrated feasibility for plasmid DNA- or adenoviral-mediated gene delivery. At present, strategies for delivering therapeutic genes *via* non-viral and viral gene transfers to the vessel wall include introducing them directly through catheters at the time of angioplasty [36–39] or *via* gel-coated surfaces of the balloon [40,41]. However, catheter-based delivery has not resulted in significant improvement of in-stent restenosis [42,43]. Recently, lipo(poly)plex-based gene delivery from PLGA/gelatine-coated meshes has been reported in a rabbit iliac artery restenosis model [44]. Substantial GFP expression was detected only at early time points while encountering limitations of these coatings due to rapid dissolution *in vivo*. However, clinical applications may require efficient, localized and long term delivery of a therapeutic gene of up to several weeks, which points to the necessity of developing alternative delivery systems.

Thorough safety and efficacy testing of gene eluting stent technologies in pre-clinical animal models is paramount for successful clinical translation. However, there is no single animal model which is optimal for testing stents. Although recognized as the gold standard in preclinical testing of vascular devices due to similarities to the human cardiovascular anatomy in terms of size and physiology [45], the porcine model has a number of shortcomings. It is not cost effective for high throughput testing and there are significant logistical concerns with the porcine model. Such model is most appropriate for immediate preclinical testing for therapeutic benefit. There are relevant smaller rodent models such as the Zucker (diabetic model) rat [46] and a number of inbred and genetically modified mouse models [47–50]. However, for rodents even as large as rats, specially modified equipment including specifically manufactured stents must be used which does not reflect the dimensions used in humans. In addition, reproducibly injuring the vessel wall is a significant technical challenge in such small caliber vessels. As a compromise we used a cost effective rabbit model in the current study to test efficacy of reporter gene delivery from stents. Furthermore, the size of the rabbit common iliac arteries allowed for use of human sized equipment, albeit in a more muscular vascular environment than seen in the human coronary bed.

Our present study examines the use of three different liposomal formulations (DOTMA/DOPE, DDAB/DOPE or DDAB/POPC/Chol), to a reporter transgene. We initially established a model *in vitro* system (based on gene delivery from metal coupons) for the optimization of lipoplex-mediated transfection efficiencies of VERO cells and of human smooth muscle cells (hCASMCs). We then assessed whether liposome-based surface coatings would result in efficient and prolonged gene delivery from cobalt chromium stents or phosphorylcholine-modified stainless steel stents in the common iliac arteries of normocholesterolemic rabbits.

2. Materials and methods

2.1. Preparation of liposomes and lipoplexes

The DDAB-based lipoplexes were prepared as described by Hayes et al. [51] with modifications: chlorofom solutions containing 2 μmol dimethyldioctadecylammonium bromide salt (DDAB; Avanti Lipids), 5 μmol 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC; Avanti Lipids) and 6.5 μmol cholesterol (Chol; Roche) or 5 μmol DDAB (Avanti Lipids) and 5 μmol DOPE (dioleoylphosphatidylethanolamine; Avanti Lipids) were mixed in glass tubes. The organic solvent was removed at 85 °C, 100–200 mbar for 45 min in a rotary evaporator (Büchi). Lipid films were resuspended in 400 μl of a 50% EtOH solution, sonicated in a waterbath for 15 min and mixed with 200 μl plasmid DNA (pDNA) solution containing 50% EtOH for 45 min before removing excess EtOH in a rotary evaporator at 85 °C, 100–200 mbar. To examine the effects of different cationic lipids, DOTMA (N [1 (2,3 dioleyloxy)propyl] n,n,n trimethyl-ammonium chloride)/DOPE lipoplexes (lipofectin; Invitrogen) were prepared as previously described [28]. Plasmid DNA (pDNA) encoding either a LacZ, EGFP or luciferase reporter gene was isolated from *E. coli* bacteria and prepared with the Endofree Plasmid Giga Kit (Qiagen). All plasmids were obtained from Clontech, Palo Alto, USA. Isolated pDNA (1 mg/ml) was stored in TE buffer (100 mM NaCl, 10 mM Tris–HCl).

2.2. Preparation of lipoplex-coated coupons

SS316L stainless steel coupons (Abbott Vascular, Galway, Ireland) were washed several times with distilled water, rinsed with ethanol, dried with medical wipes (Kimberley Clarke, USA) and placed for 30 min under the laminar flow box prior to coating with lipoplexes. Liposomal formulations, containing 100 μg of plasmid DNA, were prepared as described above, and applied to stainless steel coupons (SS316L), by directly applying the 300 μl lipoplex solution in successive small doses of 30 μl droplets onto the surface utilizing a micropipette (Gilson). The metal surfaces were air dried for several hours under a sterile, laminar flow box. In most cases, coupons were used in transfection studies a few hours later. However, we also examined the potential for efficient gene delivery after long term storage of the liposome-coated metal surfaces. Each coupon (10 mm Ø) was placed in an individual well of a 24-well plate. The plates were sealed with parafilm, placed in a −20 °C freezer for 24 h and transferred to −80 °C for long term storage (12 months).

2.3. Lipoplex release studies from metal coupons

Flourescence spectroscopy of eluted lipoplexes: Lipoplex-coated coupons were incubated with 100 μl of DMEM, 10% FBS and penicillin/streptomycin (P/S) on the surface at 4 °C for 0, 1, 7, 10, 14, 21 or 28 days. The collected supernatant at each time point was mixed with 100 μl Quant-iT™ picoGreen® solution (PG) (Invitrogen, Ireland). Five minutes after mixing the supernatant with the PG solution, the fluorescence intensity was measured using Varioskan Flash multi-reader (Thermo Scientific) with an excitation of 480 nm and an emission of 520 nm. Metal coupons coated with 100 μg of Gaussia luciferase plasmid DNA were used as positive controls.

Microscope images of fluorescently labelled surfaces: 100 μl of DMEM, 10% FBS and (P/S) PG were added to the lipoplex coated coupon surfaces and incubated for one day at 4 °C. The medium was then replaced with 100 μl of PG, incubated for 5 min at room temperature and removed by pipetting before visualization. Images were taken with Fluorescein isothiocyanate filter functionalized Olympus Ix81 inverted microscope.