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An Ultra-Thin Polymer Coating for the Tethering of Adenoviral Vector to the Surface of Coronary Stents

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An Ultra-Thin Polymer Coating for the Tethering of Adenoviral Vector to the Surface of Coronary Stents

Abstract
Our group has previously demonstrated stent-based gene delivery with either viral or plasmid vectors. However, these previous studies utilized bulky PLGA or collagen stent coatings, known to cause inflammatory reactions in stented arteries. In the present experiments we successfully attached adenoviruses either directly, or via anti-adenovirus antibodies to the steel surface of stents using chemical coordination with biphosphonates.

Disciplines
Biochemical and Biomolecular Engineering | Chemical Engineering | Engineering | Materials Science and Engineering | Polymer Science

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851. Suppression of Chemical Burn-Induced Neovascularization by Subconjunctival Injection of Adeno-Associated Virus Vector Expressing Angiostatin
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Ocular neovascularization (NV) is one of the leading causes of blindness. Many ocular surface diseases such as dry eye syndrome, ocular cicatricial pannus (OCP), limbal insufficiency and sequel of chemical burn frequently lead to cornea NV. The underlying mechanisms of these diseases include chronic inflammation, autoimmune response and stem cell loss. This major ocular complication can lead to corneal scarring, edema, lipid deposition, and inflammation that may not only significantly alter visual acuity, but also worsen the prognosis of the subsequent penetrating keratoplasty (PKP). Since ocular surface diseases usually have protracted courses, the control of their respective detrimental mechanisms usually depend on long term application of steroid or cytotoxic or immunosuppressive medications such as cyclosporine and cyclosporine A. Local and systemic side effects create dilemma for clinician. On the other hand, the recombinant AAV vector is currently considered as one of the most promising viral vectors for gene therapy. In this study, we investigate the efficacy of AAV vectors in a rat model of experimental corneal NV generated by silver nitrate (AgNO3) cauterization. The vectors containing either the mouse angiotatin gene (AAV-angiotatin) or the control green fluorescent protein gene (AAV-GFP). Two weeks after subconjunctival injection the vectors, gene transfer efficiency and therapeutic efficacy were evaluated under slit-lamp biomicroscopy every other day. Vessel length was measured by fluorescent isothiocyanate (FITC)-coupled concanavalin A (ConA) lectin angiography. AAV-angiotatin successfully suppresses the chemical-burn induced corneal NV as shown by Angiography analysis. The areas free from blood vessel invasion in the cornea correlated with the sites of virus injection. The therapeutic effect lasted for the entire 12-week duration of our experiments, while AAV-GFP expression persisted more than 6 months. This is the first report that subconjunctival injection of AAV-angiotatin can significantly inhibit the formation of corneal NV lesion. Importantly, subconjunctival injection can be used as a unique reversible gene therapy method. Therefore, the AAV vector represents a promising gene delivery system for efficient and long-term transduction in ocular tissue. Our study demonstrated the feasibility of gene therapy approaches for the treatment of corneal NV diseases. It may also become clinically useful for a variety of ocular diseases involving NV, such as wound- and inflammation-related corneal angiogenesis with limbal insufficiency, which is still pharmacologically untreatable.

852. Development of a Retroviral Vector Containing the Erythroid Band 3 Promoter Flanked by the Chicken β-globin 5′HS4 Insulator
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Expression of genes in retroviral vectors is subject to the epigenetic effects of surrounding heterochromatin after integration, resulting in transgene silencing or position effect variegation (PEV). It has been shown that an insulator element from the chicken β-globin locus (5′HS4) serves as a barrier against such position effects and thereby promotes long-term, uniform expression of a transgene but the mechanism by which an insulator suppresses PEV has not been elucidated. The erythroid anion exchanger 1 (AE1 or band 3) promoter is active only in erythroid cells. Using a transgenic mouse assay, we have analyzed human γ-globin gene expression using promoter elements from the erythroid form of the AE1 gene. Transgenic mice containing 2 different AE1 promoter sequences, -1738 to +22 (9 lines) or -357 to +22 (9 lines), fused to the γ-globin gene coding region 5 bases upstream of the initiation codon showed erythroid specific expression of the transgene with an average AE1/γ-globin mRNA level of 13.5±0.07% and 7.3±0.04% of total mouse α-globin mRNA per transgene copy, respectively. With both promoters, γ-globin expression was position independent and copy number dependent. However, γ-globin distribution in circulating erythrocytes was variegated in 14/18 lines. We hypothesized that flanking the 5′ and 3′ ends of the -1738 to +22 AE1/γ-globin gene construct with the chicken 5′HS4 would suppress PEV, resulting in more uniform expression of the transgene. In transgenic mice containing the insulated, long AE1/γ-globin construct, expression of the transgene was erythrocyte specific, position independent (6/6 lines express) and copy number dependent (p = 0.0001). Transgenic mice had an average AE1/γ-globin mRNA level of 19.8±0.03% of total mouse α-globin mRNA per transgene copy. AE1/γ-globin mRNA from the non-insulated construct initiated at cryptic sites upstream of the start site used by the endogenous gene. In contrast, the insulated AE1/γ-globin construct initiated transcription at the start site used by the endogenous gene. Distribution of γ-globin in all 6 lines was also uniform, with 100% of erythrocytes containing human γ-globin. We conclude that the erythroid AE1 promoter contains an insulator element upstream of position -1738. Furthermore, the chicken 5′HS4 insulator suppresses PEV by directing transcription initiation to the correct start site. We have constructed an oncoretroviral vector containing the -357 to +22 AE1/γ-globin gene with the chicken 5′HS4 insulator in the 3′LTR. Stabile transduction of the AE1/γ-globin gene and transfer of 5′HS4 to the 5′ LTR was demonstrated by Southern blot analysis of transduced K562 cell DNA. We hypothesize that an insulated AE1/γ-globin vector will express γ-globin at relatively high levels in primary erythroid cells.

853. An Ultra-Thin Polymer Coating for the Tethering of Adenoviral Vector to the Surface of Coronary Stents
Ilia Fishbein,1 Ivan S. Alferiev,1 Gordon S. Wong,2 John M. Vohs,2 Jeanne M. Connolly,1 Robert L. Wilensky,3 Robert J. Levy.1
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Our group has previously demonstrated stent-based gene delivery with either viral or plasmid vectors. However, these previous studies utilized bulky PLGA or collagen stent coatings, known to cause inflammatory reactions in stented arteries. In the present experiments we successfully attached adenoviruses either directly, or via anti-adenovirus antibodies to the steel surface of stents using chemical coordination with bisphosphonates. Bisphosphonate derivatives of polyallylamine possessing primary amines (PAA-BP), or both primary amines and latent thiols (PrSSPA-BP) were custom synthesized. The dynamics of PrSSPA-BP adsorption to the...
specific tethering of a clinically significant amount of the recombinant
for AdV immobilization on the stent surface, which allow site-
arteries was determined by immunohistochemistry 1, 3 and 7 days
arteries (n=8). The extent of transgene expression in the stented
Stents bearing AkAb-tethered AdV-GFP were deployed in rat carotid
retained their transducing potential up to a fourth round of relocation.
does not result in measurable cell transduction. Moreover, meshes
the mesh borders, while the same amount of free virus (MOI=1)
polymer coating does not exceed 5 nm. Reaction corresponds to monolayer deposition of the polymer. Additionally,
anti-adenoviral antibodies (anti-hexon, anti-fiber and anti-knob) was
pyridyldithio (PDT) groups on the stent surface. A panel of reduced
was achieved by SPDP modification of AdV with subsequent
AkAb-primed surface was profoundly inhibited (87%) by a 1000-
fold excess of knob protein. Alternatively, vector immobilization
was achieved by SPDP modification of AdV with subsequent
cross-linker, SPDP, was employed to introduce thiol-reactive,
corresponds to monolayer deposition of the polymer. Additionally,
corneal endothelial cells. The influence of the polymer shell
fast (less than 1 hour) saturation of the steel surface with PrSSPAA-
backbone through a cleavable bond. Fluorometric studies showed
surface of 316L stainless steel was studied using a compound
comprised of a fluorescent marker, dansyl, attached to the polymer
blood flow in ischemic and non-ischemic limbs (contra-lateral limb)
and mortality. Therapeutic angiogenesis seeks to treat PAD via the
vascular endothelial growth factor (VEGF)-activating transcription
employed. The purpose of this study was to investigate the role of
growth and proliferation of new blood vessels. Unfortunately this
expressed. Physiological angiogenesis occurs in the context of multiple
isoforms of which three, VEGF121, VEGF165 and VEGF189, represent >99% of the VEGF
pre-mRNA is spliced to give seven isoforms of which three,
vein with a single dose (500mg) (n=13) and the other (control) group (n=10), included b-
nek arteries 10 days post-operatively. One group received a single unilateral
femoral artery ligation and excision and were divided into two groups
METHODS: In total, 23 New Zealand White rabbits underwent
femoral artery ligation and excision and were divided into two groups
at 10 days post-operatively. One group received a single unilateral
(ischemic limb TA muscle) intra-muscular injection of ZFP-VEGF
(500mg) (n=13) and the other (control) group (n=10), included b-
galactosidase injection (500mg) (n=7) and sham treatment (n=3).
The ischemic and contra-lateral TA muscles of all animals were
harvested 21 days post-operatively. In a subgroup of these rabbits,
neuropathy and cell transduction. The thickness of polymer coating
does not exceed 5 nm. Reaction corresponds to monolayer deposition of the polymer. Additionally,
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