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GENE DELIVERY

Polysaccharide-modified scaffolds for controlled lentivirus delivery *in vitro* and after spinal cord injury

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ABSTRACT

Gene delivering biomaterials have increasingly been employed to modulate the cellular microenvironment to promote tissue regeneration, yet low transduction efficiency has been a persistent challenge for *in vivo* applications. In this report, we investigated the surface modification of poly(lactide-co-glycolide) (PLG) scaffolds with polysaccharides, which have been implicated in binding lentivirus but have not been used for delivery. Chitosan was directly conjugated onto PLG scaffolds, whereas heparin and hyaluronan were indirectly conjugated onto PLG scaffolds with multi-amine crosslinkers. The addition of chitosan and heparin onto PLG promoted the association of lentivirus to these scaffolds and enhanced their transduction efficiency *in vitro* relative to hyaluronan-conjugated and control scaffolds that had limited lentivirus association and transduction. Transduction efficiency *in vitro* was increased partly due to an enhanced retention of virus on the scaffold as well as an extended half-life of viral activity. Transduction efficiency was also evaluated *in vivo* using porous, multiple channel PLG bridges that delivered lentivirus to the injured mouse spinal cord. Transgene expression persisted for weeks after implantation, and was able to enhance axon growth and myelination. These studies support gene-delivering PLG scaffolds for *in vivo* regenerative medicine applications.

1. Introduction

Biomaterial scaffolds are a versatile tool to create a permissive cellular microenvironment for regenerative medicine. Their tailorable architecture provides a structural niche for the engraftment of transplanted cells and the infiltration of endogenous progenitor cells. Functionalizing these scaffolds with viral vectors can expand their bioactivity, with the material providing a platform for maintaining vectors locally to obtain robust, prolonged expression of the transgene, which can either promote regeneration or counteract its inhibitors [1,2]. Strategies utilizing viral vectors permit a ready exchange of target genes, or delivery of multiple vectors, without altering the delivery system, as the vectors have similar physical properties independent of the gene sequence. Still, the transduction efficiency of viral vectors *in vivo* has been low historically, motivating the exploration of strategies that promote the retention or immobilization of viruses onto the biomaterial [3–10].

More recently, biomaterials have been designed to more efficiently incorporate and retain viral vectors [3–5]. In hydrogels, the physical properties such as mesh size, hydrophilicity, and degradation, dictate whether a vector is released or retained within the material [3,6,7]. In contrast, for macro-porous scaffolds, the fabrication procedures often diminish virus activity, which precludes the entrapment of vectors. Adding virus-affinity moieties to these biomaterial scaffolds further enhanced viral vector retention and led to localized transgene expression; however, the incorporation of these moieties can alter the biomaterial's properties [3,5], limiting their applications. As one approach, the envelope proteins of viral vectors were engineered to contain antibody-binding fusion proteins [8,9] or oligo-peptide tags and ligands [10] in order to control their association to materials. However, altering the virus coat can be time consuming and may reduce the virus transduction efficiency compared to the wild-type virus. Our strategy modifies the surfaces of biomaterials in order to promote interactions that can retain the vector while minimizing changes to the bulk properties.

Herein, we investigated the immobilization and controlled release of lentivirus on poly(lactide-co-glycolide) (PLG) scaffolds modified with polysaccharides post-fabrication. The polysaccharides employed in this study—heparin, hyaluronan, and chitosan—are naturally-derived polymers that have previously been incorporated onto PLG for regenerative medicine applications [11–19]. More recently, heparin has been

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utilized to immobilize and concentrate lentivirus for enhanced production efficiency [20], and this report extends this observation of binding to promote localized gene delivery. Heparin, hyaluronan and chitosan were immobilized onto the surface of porous PLG scaffolds using *N*-ethyl-*N*′-(3-(dimethylamino)propyl)carbodiimide/*N*-hydroxysuccinimide (EDC/NHS) chemistry that allows polysaccharides to be coupled to PLG without altering the bulk properties of the scaffold. Scaffolds modified with polysaccharides using this approach were then characterized for incorporation and retention of lentivirus on macro-porous structures, enhanced transgene expression both *in vitro* and *in vivo*, and evaluated for their ability to enhance tissue regeneration in a spinal cord injury model.

2. Materials and methods

2.1. Surface-modified PLG scaffolds and bridges

To form a 5 mm disk-shaped scaffold, a 1:30 ratio of PLG (75:25 ratio of D,L-lactide to L-glycolide, inherent viscosity: 0.76 dL/g; Lakeshore Biomaterials, Birmingham, AL) and salt (250–425 μm) was mixed using wet granulation, mechanically pressed at 1000 psi, equilibrated under 800 psi of carbon dioxide for 16 h and then released at 60 psi/min to foam into the final structure as previously described [5]. The scaffolds were leached overnight in distilled water. To form a 90% porous bridge for a mouse spinal cord hemisection lesion, a 1:1 PLG and salt (63–106 μm) mixture coated cylindrical sugar fibers drawn from 103 °C caramelized sucrose, was packed into a mold, was equilibrated under 800 psi of carbon dioxide for 16 h and was then released at 60 psi/min, as established previously [21]. The bridges were leached 30 min in distilled water. All materials were from Fisher Scientific unless otherwise indicated.

Scaffolds and bridges were modified by first incorporating the crosslinkers (1,6-diamino hexane (HDA, 1.5 mg in 10 μL isopropanol, which represents the solubility limit for HDA and is comparable to previous reports [11]), or chitosan (2.5–250 μg in 10 μL 2% acetic acid, 8183 mol. wt.)) onto the surface and then immersing the scaffold for 4 h into 1 mL of 1 M MES buffer in the presence of 9 mg EDC (CreoSalus Inc., Louisville, KY) and 6 mg NHS (Research Organics, Cleveland, OH). Heparin (180 USP/mg, ~16,000 Da [22]) and hyaluronan (Genzyme, 1330 Da, Cambridge, MA) were attached onto the crosslinker modified PLG by incorporating 2.5–250 μg of these polysaccharides in 10 μL MES buffer onto the crosslinker-modified scaffolds and then immersing the scaffold into the above EDC/NHS in MES solution overnight. The modified scaffolds and bridges were washed with distilled water and then dried until use.

The surface of the scaffolds was imaged using the Leo Gemini 1525 (Zentrum für Werkstoffanalytik Lauf, Pegnitz, Germany) at 10 kV after coating with osmium tetroxide. Conjugation of polysaccharides onto PLG was quantified by immersing the modified scaffolds for 3 h in dyes known to associate with them and then measuring the absorbance extinction of the solution. Chitosan was quantified using the extinction of Orange II at 480 nm, heparin using toluidine blue (Fisher Scientific) at 610 nm, and hyaluronan using alcian blue at 620 nm. Dye solutions were made according to previously established methods [23–25]. All materials are from Sigma-Aldrich unless otherwise indicated.

2.2. Lentivirus production and association to PLG scaffolds

Human embryonic kidney cells (HEK 293T) cells were cultured in Dulbecco's modified eagle medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin. All cell culture materials were purchased from Invitrogen. Lentivirus was prepared using a previously established technique [3–7]. These cells were co-transfected with plenti-CMV-luciferase, plenti-CMV-sonic-hedgehog or plenti-UbC-beta-galactoside encoding vectors and the packaging vectors: pMDL-GagPol, pRSV-Rev, pIVSVSV-G using Lipofectamine 2000 (Roche Biosciences, Palo Alto, CA, USA). Supernatant was collected after 48 h, concentrated in PEG-it (SystemBiosciences, Mountain View, CA, USA) for 24 h, precipitated using ultracentrifugation and resuspended in PBS. Titer was determined using a qPCR lentivirus titer kit (Applied Biological Materials, Inc., Richmond, BC, Canada).

Virus (4×10^7 particles) was pipetted onto the scaffold to assess lentivirus incorporation and retention on the PLG scaffold, and these quantities are similar to those previously reported [4,5]. After a 10-minute incubation, the scaffolds were placed into a 96-well plate and washed with PBS (Sigma-Aldrich) to remove unassociated virus. The supernatant was titered using the qPCR kit. The scaffolds were placed into another 96-well plate, incubated in PBS at 20 °C, and removed after 1–3 days. The supernatant was again titered using the qPCR kit.

2.3. Lentivirus transduction in vitro

Transduction efficiency and proliferation were assessed qualitatively. Virus (4×10^7 particles) encoding for beta-galactosidase was pipetted onto the scaffold and washed with PBS after a 10-minute incubation, consistent with previous reports [4,5]. HEK 293T cells (1×10^6) were seeded onto the scaffold in a 6-well plate, similar to previous reports [4,5]. After 4 h, the scaffold was transferred into another 6-well plate and cultured for 3 days. To view transduction, scaffolds were fixed in 4% paraformaldehyde, incubated with 5-bromo-4-chloro-indolyl-β-D-galactopyranoside (X-gal) (Inalco SP, Milano, Italy) for 16 h and imaged using light microscopy (Leica Microsystems, Wetzlar, Germany). To assess non-transduced cells, scaffolds were counterstained with Nuclear Fast Red (Trevigan, Gaithersburg, MD).

To quantitatively assess transduction efficiency, virus (4×10^7 particles) encoding for luciferase was pipetted and dried onto the scaffold and washed with PBS. The scaffolds were incubated in PBS for 0 to 2 days at 20 °C, with 5×10^4 or 1×10^5 HEK 293 T cells subsequently seeded onto a scaffold in a 96-well plate (BD Falcon), consistent with previous reports [3,6,7]. After 4 h, the scaffold was transferred into a black 96-well plate and cultured for 3 days. Bioluminescence was assessed using the IVIS imaging system (Caliper, Hopkinton, MA, USA) as described previously [26]. The scaffolds were incubated with 50 mM of D-luciferin (Molecular Therapeutics, Inc., MI) for 4 h and imaged with two 10-second intervals.

2.4. Cell infiltration, gene expression in vivo

Gene expression was assessed following lentivirus delivery in the injured spinal cord. To assess transduction, virus (2.8×10^7 particles) was pipetted and dried onto a porous, 7-channel PLG bridge and stored at −80 °C until use. Animals were treated according the Animal Care and use Committee guidelines at Northwestern University. Surgery was performed as previously described ($n = 4$ per scaffold and time-point) [2,5,26]. C57Bl6 female mice (20 g, Charles River) were anesthetized using isoflurane (2%). A laminectomy was performed at T9–T10 for bridge implantation into a hemisection lesion that is 2.25 mm in length. The injury site was stabilized by suturing the muscles together and stapling the skin after covering the site with Gelfoam. Baytril (enrofloxacin 2.5 mg/kg SC, once a day for 2 weeks), buprenorphine (0.01 mg/kg SC, twice a day for 3 days), and lactate ringer solution (5 mL/100 g, once a day for 5 days) were administered post-operatively. Bladders were expressed twice daily until function recovered.

To quantify luciferase expression, mice were injected intraperitoneally with 150 mg/kg body weight of D-Luciferin (Molecular Therapeutics, Inc., MI). Bioluminescence was immediately assessed using the IVIS imaging system (Caliper, Hopkinton, MA, USA) as described previously [3–7]. The animals were imaged at 5 minute intervals until peak expression was confirmed. The ability of lentivirus delivering bridges to promote regeneration of axons after spinal cord injury was investigated by loading 7.0×10^6 particles of sonic hedgehog- or luciferase-