Targeted Inhibition of HIV-1 Utilizing a Bioengineered Nanofibrous Polyester Material

[1,2]Joshua R. Araya, [2]Lisa M. Fitzgerald, [2]Tarryn E. Phaneuf [2]Saif G. Pathan, [2]David W. Nelson,
[2,3]Derek J. Thoresen, [4]Martin J. Bide, [2]Tina M. Phaneuf, [2]Matthew D. Phaneuf
[1]Northeastern University, Boston, MA; [2]BioSurfaces Inc., Ashland, MA; [3]St Michael's College, Colchester, VT;
[4]University of Rhode Island, Kingston, RI

Background

Human Immunodeficiency Virus Type-1 (HIV-1) is a retrovirus that currently infects over 33 million people worldwide. Transmission of HIV-1 occurs when a viral envelope glycoprotein complex consisting of GP120 and GP41 binds to the CD4 receptor on the cell surface along with a co-receptor of the chemokine family, allowing the virus to enter the cells. GP120 has therefore been the target of numerous strategies in order to prevent HIV-1 infection. Galanthus Nivalis Lectin (GNL) is a glycoprotein which has been shown to have specificity for the carbohydrate portion GP120 of HIV-1. The goal of this study was to immobilize GNL onto a nanofibrous polyester (nPET) material and characterize GP120 affinity for the bioengineered surface. Our hypothesis is that immobilization of GNL onto a nanofibrous material will result in increased GP120 binding, thereby resulting in a surface with the ability to bind HIV-1.

Methods

nPET Electrospinning Methodology: Polyester chips (0.63dl/g IV) were dissolved in an organic solvent prior to electrospinning. Nanofibrous polyester flat sheets (nPET) were electrospun onto a rotating mandrel using proprietary parameters on a custom-designed computer-automated system (n = 4 sheets).

Surface Functionalization: nPET sheets (n=2) were then surface functionalized. nPET sheets were then reacted in a 0.5% (w:v) sodium hydroxide (NaOH) solution at 100°C for 30 minutes in order to create carboxylic acid groups. These materials were then rinsed in distilled water and air dried overnight (nPET-HYD).

Surface Characterization: nPET and nPET-HYD materials were then examined for functional group formation via Methylene blue (MB) dye uptake (87% purity). Segments ($1cm^2$; n = 2 segments/test group) were cut and weighed. Segments were then placed into 3ml of MB solution (5mg/L in Tris buffer, pH 8.0) for 24 hours followed by a wash in Tris buffer. Absorbance for the dyebath and wash solutions was then read. The number of carboxylic acid groups on each material was determined.

Physical Characterization: Tensile strength for nPET and nPET-HYD materials was evaluated. Materials (1.5cm length X 0.3cm width; n = 4 segments/test group) were cut, weighed and measured for thickness. Segments were placed into a Q-Test apparatus (0.5cm gauge length), with stretching occurring at a rate of 30.5cm/min.

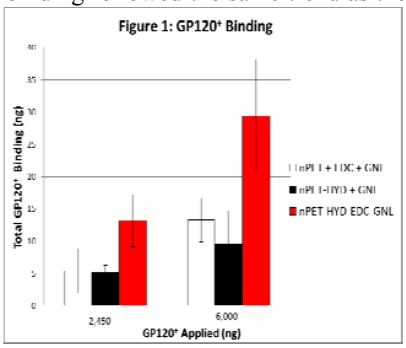
GNL Immobilization onto nPET-HYD Material: nPET (n = 4) and nPET-HYD (n = 8) segments were cut ($1cm^2$) and weighed. A carbodiimide cross-linker (EDC) solution was prepared (10mg/ml) and added to the nPET segments and one set of nPET-HYD segments. To the other set of nPET-HYD materials, 4ml of ethanol was added. All were mixed on an orbital shaker (100rpm) for 1 hour. A 1mg/mL solution of FITC-labelled GNL was prepared (GNL*). Segments were then placed into 2ml of GNL* and reacted for 18 hours. These materials were individually washed in PBS with 1% Tween 20 with sonication. Surface fluorescence was measured and the amount of GNL* bound determined. This GNL immobilization procedure was repeated two additional times with unlabeled GNL in order to assess $GP120^+$ binding.

Affinity of $GP120^+$ for GNL-Immobilized Material: The materials described above were then reacted overnight on an orbital shaker (100rpm) with sterile PBS containing 1% Albumin in order to block non-specific GP120 binding. GP120 was fluorescently tagged with DyLight 550 ($GP120^+$). Two sets of materials were exposed to two different $GP120^+$ concentrations (2,450 and 6,000ng/material) for 24 hours on an orbital shaker, followed by washing for 5 minutes in sterile PBS. $GP120^+$ binding was determined via surface fluorescence measurement.

Results/Discussion

Surface functionalization of nPET materials via NaOH hydrolysis was confirmed using MB uptake. Evaluating MB dyebath and wash solutions confirmed that nPET-HYD materials had the greatest uptake (0.433nmoles MB dye/mg weight material). Tensile strength for the nPET-HYD materials was not significantly different than nPET control materials (1.43 ± 0.26 kgf/mm^2 versus 1.51 ± 0.26 kgf/mm^2).

GNL* binding was 3.1 and 21.3 fold greater on nPET-HYD materials reacted with EDC (85 ± 5 µg/segment) as compared to nPET segments reacted with EDC and nPET-HYD segments alone, respectively. $GP120^+$ binding followed the same trend as the GNL binding with the nPET-HYD materials reacted with EDC having the greatest binding as compared to the controls for both applied $GP120^+$ concentrations (**Figure 1**) ($p < 0.03$).



Conclusions

This study demonstrates that GNL can be immobilized onto a surface-modified electrospun material and retain its affinity for GP120 binding. The next step is to evaluate this surface using the HIV-1 virus. This technology may have application as a filtration material for biologic fluids in order to remove free HIV virus.

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