Nanofibrous EGF gene delivery system employing MMPs-responsives linkers

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Statement of Purpose: In our previous study, nanofibrous scaffold was fabricated to control DNA release according to digestion of MMP-cleavable linker in the presence of metalloprotease (MMP). The nanofibrous mesh was prepared by electropinning employing amine functionalized diblock copolymers and a linear PEI was subsequently conjugated to the surface-exposed amine groups by a MMP-cleavable linker. Plasmid DNA encoding human epidermal growth factor was incorporated to MMPs-responsive nanofibrous mesh by an ionic interaction between DNA and surface-immobilized PEI of the fibrous meshes. MMP cleaved PEI moiety from the matrix and released PEI and DNA spontaneously formed an electrostatic complex in the presence of MMP.

Methods: PCL-PEG block copolymer was completely dissolved in an organic solvent at 10% (w/v). The polymer solution was injected through 27G needles at injection speed of 1ml/h. The primary amine group of the electrospun nanofibers was hydrated in PBS. A peptide composed of 7 amino acids (DGPLGVC) was reacted with surface-exposed amine groups with EDC and NHS. N-Succinimidyl 3-(2-pyridyldithio)-propionate (SPDP)-activated linear polyethylenimine (LPEI) was subsequently reacted with the peptide-conjugated nanofibrous meshes. Plasmid DNA encoding human epidermal growth factor (phEGF) was loaded according to various N/P ratios of LPEI and DNA. Released LPEI/DNA complexs were quantified at 72h with MMP-2 or without MMP-2. Transfection efficiency of the release fraction (72h) was measured in human dermal fibroblast cells. LPEI/DNA particle (N/P ratio=16) was employed as a control.

Results: Fluorescamine assay showed that the amount of the exposed amine groups on the fibrous mesh was 0.434nmol/mg. The amount of conjugated LPEI to the nanofibers was confirmed by XPS. Survey scans spectra of C_{1s}, O_{1s}, and N_{1s} confirmed that LPEI density on the LPEI-nanofiber was 0.173nmol/mg, suggesting that 4.3μg/mg LPEI was conjugated to exposed primary amines.

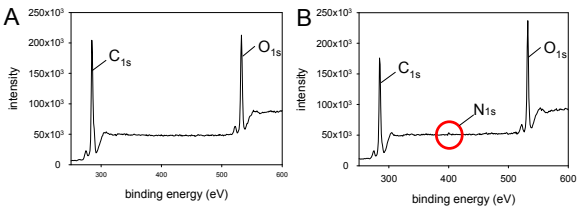

Figure 1. X-ray photoelectron scattering (XPS) spectroscopy of (A) nanofiber without LPEI and (B) LPEI-immobilized nanofiber.

DNA incorporation efficiency and DNA release were examined by Picogreen assay. DNA incorporation efficiency was increased by increasing N/P ratios because cationic charge densities on the surface of the nanofibrous meshes were also increased. The released LPEI was quantified by advanced protein assay system. MMP significantly increased release rates of DNA and LPEI from NF because of digestion of a MMP-reactive peptide. However, in the absence of MMP, DNA and LPEI releases were further attenuated compared to the MMP-treated group.

Table 1. DNA incorporation efficiency at various N/P ratios of LPEI-conjugated nanofibrous matrix (NF).

NF	the initial amount of DNA (μg/NF)	the incorporated amount of DNA (μg/NF)	DNA incorporation efficiency (%)
2	16.5	1.6 ± 0.3	9.7 ± 1.8
4	8.3	1.6 ± 0.1	19.2 ± 1.5
8	4.1	1.2 ± 0.3	28.0 ± 7.3
16	2.1	1.4 ± 0.1	68.1 ±2.8

Table 2. The amount of released DNA and LPEI from nanofibrous matrix with MMP-2 or without MMP-2 at 72h.

NF 16	The amount of released DNA		the amount of released LPEI	
	μg/device	%	μg/device	%
MMP (+)	0.67 ± 0.01	46.98 ± 0.36	1.55 ± 0.05	36.04 ± 1.16
MMP (-)	0.22±0.01	15.2 ± 0.27	0.10 ± 0.05	2.32 ± 0.95

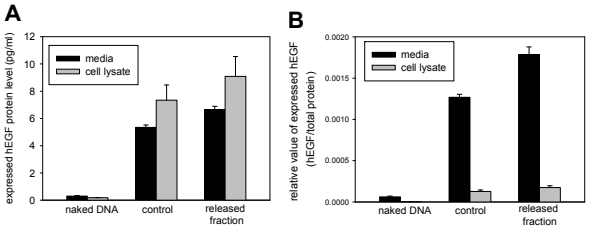

Figure 2. hEGF expression levels in human dermal fibroblasts. (A) The amount of expressed hEGF and (B) relative value of expressed hEGF comparing the total amount of expressed protein in fibroblast.

In vitro transfection efficiency of the released fractions (72h) from LPEI-conjugated nanofibers was similar to control. The total amount of expressed hEGF was 16pg/ml in released fraction group.

Conclusions: With increasing N/P ratios, the DNA binding efficiency to the nanofibers was also increased. Release rates of DNA and LPEI from NF increased in the presence of MMP. In vitro transfection efficiency showed no difference between the released fraction and the control group.

References: H.S. Kim, H.S. Yoo J. control. Release 2010; 145: 264-271