MMP-responsive release of DNA from electrospun nanofibrous matrix in diabetic ulcers

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Statement of Purpose: A nano-scaled scaffold for gene therapy was prepared by electrospinning. The nanofibrous scaffold was designed to control DNA release rates according to a concentration of metalloprotease (MMP) using a MMP-cleavable linker. Electrospun nanofiber was fabricated by amine functionalized diblock copolymers and a linear PEI was subsequently conjugated to the surface-exposed amine groups by a peptide linker. DNA was loaded in the scaffolds 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.

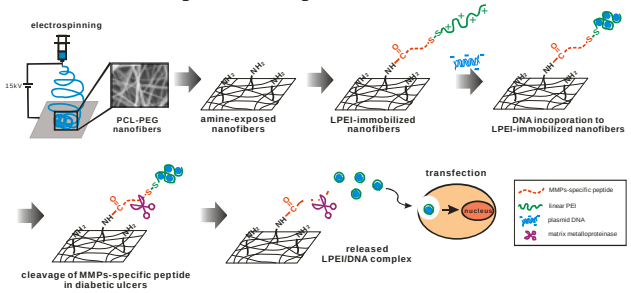

Figure 1. Schematic diagram of enzymetically responsive DNA-conjugated electrospun nanofibers.

Methods: PCL-PEG block copolymer was completely dissolved in an organic solvent to prepare 10% (w/v) polymer solution. 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 0.1M phosphate buffer. 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. Enhanced green fluorescence plasmid DNA (pEGFP) was loaded according to various N/P ratios of PEI and DNA. LPEI/DNA complex release profiles were examined for 72h with MMP-2 or without MMP-2. Transfection efficiency of the release medium was measured in NIH3T3 cells. In vivo GFP expression was evaluated in diabetic animals.

Results: Fluorescamine assay showed that the amount of exposed amine groups on the fibrous meshes was 0.387nmol/mg, suggesting that 3~4% amine group of total amount of primary amine groups was exposed. The conjugated amount of 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 0.384nmol/mg, suggesting that 99.2% of the exposed amines participated in the reaction. With increasing N/P ratios, DNA binding efficiency to the nanofibers was also increased. However, the amount of incorporated DNA was decreased when N/P ratios increased from 2 to 16. MMP significantly increased release rates of DNA and LPEI from NF because of digestion of a MMP-reactive peptide between LPEI and NF. However, in the absence of MMP, DNA and LPEI releases were attenuated compared to the MMP-treated group.

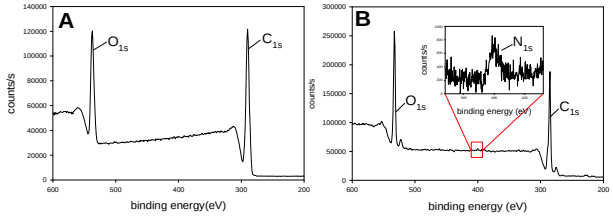

Figure 2. X-ray photoelectron scattering (XPS) spectroscopy of (A) nanofiber without LPEI and (B) LPEI-immobilized nanofiber. The inset is an enlargement of the nitrogen peak.

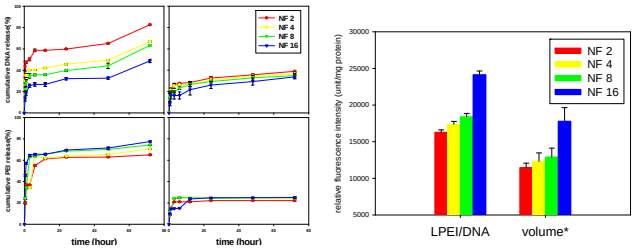

Figure 3 (left). Release profiles of DNA (A, B) and LPEI (C, D) from nanofibers with MMP-2 (A, C) or without (B, D) for 72h. Figure 4 (right). In vitro transfection efficiency of the released fractions (72h) from LPEI-conjugated nanofibers. *The same volume of the concentrated released fractions (0.1ml) was employed for the transfection study.

In vitro transfection efficiency of the released fractions (72h) from LPEI-conjugated nanofibers was increased when N/P ratios increased because of their condensation density. Higher expression level of GFP was observed when a NF with MMP-cleavable linker was administered to diabetic wounds.

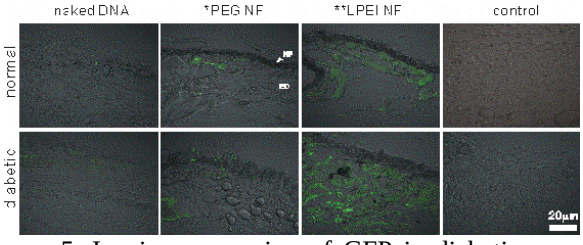

Figure 5. In vivo expression of GFP in diabetic wound and normal wound. *Nanofiber without LPEI; **LPEI-immobilized nanofiber via a MMP-cleavable linker.

Conclusions: Sustained release of LPEI/DNA complex was obtained by employing a MMP-cleavable linker between electrospun nanofibers and LPEI. 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.

References: Choi, J.S. Biomaterials. 2008;29;587-596.Lee, J.I. Acta biomaterialia, 2008;4;791-798.