Core-shell Structured Fibrous Matrix Containing Two Different Growth Factors for wound repair

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Statement of Purpose: Coaxial electrospinning received much attention because water-soluble protein could be easily electrospun with polymers in organic phase. In coaxial electrospinning, two immiscible phases composed of organic phase and aqueous are electrospun through a dual nozzle. Thus, protein in aqueous phase can be electrospun to polymeric nanofibers (NFs) without dispersing protein particles in organic phase. Furthermore, encapsulated proteins can be protected from harsh environment such as organic solvent and initial burst of encapsulated proteins can be also controlled by coaxial electrospinning.[1, 2] A dual nozzle, composed of an inner outlet and an outer outlet, allows easy one-step fabrication of coaxial NF because different pairs of polymers participate in electrospinning process. In order to employ protein-loaded nanofibers for wound dressing devices, it is very crucial that the loaded proteins retain the original bioactivity drug for prolonged period because a half-life of released drug was shortened by attack of many hydrolytic enzymes. Thus, in order to prepare nanofibers for wound healing, we simultaneously encapsulated or immobilized two proteins in nanofibrous meshes by using co-axial electrospinning and chemical conjugation on the surface of nanofibers.[3]

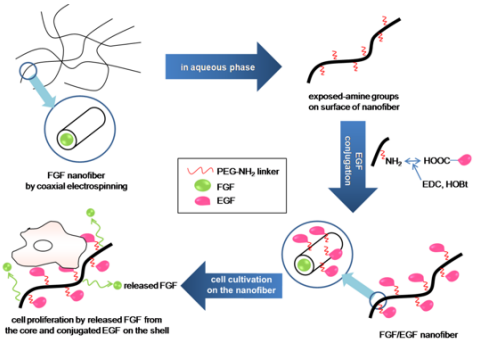

Figure 1. Schematic diagram of preparing bFGF/EGF NF.

Methods: For coaxial electrospinning, a dual nozzle was employed to simultaneously electrospin both the outer solution and the inner solution. The outer solution was a mixture of poly(ε-caprolactone)(PCL)-poly(ethylene-glycol)-NH_2 and PCL in a chloroform/MeOH mixture. For the inner solution, basic fibroblast growth factor (bFGF) was dissolved in 1% poly(vinyl alcohol) solution. Two immiscible phases were simultaneously electrospun to fibrous meshes through a dual nozzle. For surface modification of NF with epidermal growth factor (EGF), EGF was immobilized on the NF in the presence of HOBt, and EDC at room temperature with gentle stirring for 3 h. The bFGF-encapsulated and EGF-conugated nanofibers (bFGF/EGF NFs) were subjected to release test and followed by cell proliferation and differentiation study employing human keratinocytes and human fibroblasts. *In vivo* wound healing efficacy of the NFs was tested in female C57BL/6 mice with diabetic ulcers at dorsal area as described in the literature.[3] The NFs were applied to aseptically-treated wounds and wound healing rates were monitored for 7days. The recovered tissues were subjected to histological examination for confirming proliferation and differentiation of epidermal tissues.

Results: Coaxial electrospinning and surface-modification successfully generated NFs with a core-shell structure. As shown in Figure 2, because bFGF was encapsulated completely inside the NF, the NF with the conjugated EGF showed a significant peak of N_{1s} while the bFGF NF showed only C_{1s} and O_{1s} peaks. Further XPS result revealed that the atomic compositions of C, O, and N in the bFGF/EGF NF were 68.12%, 14.05%, and 4.27%, respectively, when the survey scan was performed for C_{1s}, O_{1s}, and N_{1s}.

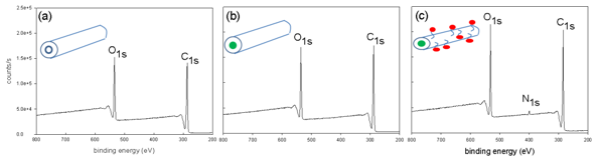

Figure 2. X-ray photoelectron scattering (XPS) spectroscopy of (a) NF, (b) bFGF NF and (c) bFGF/EGF NF.

When fibroblasts and keratinocytes were cultivated on bFGF/EGF NF, the degrees of cellular proliferation were 2.0 and 2.3 folds higher than control groups, respectively. bFGF/EGF NF dressed on diabetic ulcers effectively enhanced the expression levels of total collagen (blue) and cytokeratin (brown) after 7 days, which was confirmed by immunohistochemical staining (Figure 3).

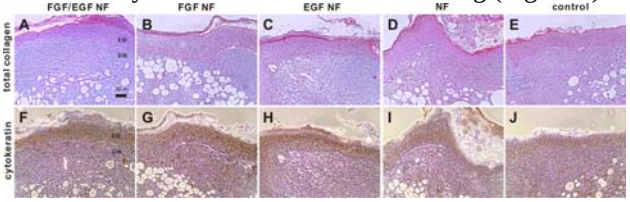

Figure 3. Histochemical staining of total collagen (blue; A to E) and cytokeratin (brown; F to J) in the re-epithelized tissues (7 days). Nucleus was counter-stained with hematoxylin (violet).

Conclusions: Two different growth factors were successfully encapsulated and immobilized in an electrospun NF. bFGF encapsulated in the NF showed cell proliferative activity toward fibroblasts and cultivated keratinocytes maintained the original phenotype by the immobilized EGF on the NF surface. *In vivo* study indicated that the repaired tissue was shown significantly superior prognosis in diabetic ulcers. Therefore, the multi-functional NF can be a promising candidate for drug delivery system and wound repair.

References:

1. Jiang H, et al. *J Control Rel* **2005**;108:237-243.
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3. Choi JS, et al. *Biomaterials* **2008**;29:587-596.