Nanofibrous Bicomponent Scaffolds for the Dual Delivery of NGF and GDNF: Controlled Release of Growth Factors and their Biological Effects

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Introduction: Repairing severely injured peripheral nerves requires suitable devices for bridging the gap and guiding axon growth. Due to limited availability and donor site function loss for autografts, tissue engineering-based therapy using nerve guide conduits is a promising alternative and hence scaffolds with desired physical, chemical and biological properties are needed. Electrospun nanofibrous scaffolds offer distinctive advantages in facilitating Schwann cell migration and neurite outgrowth [Wang HB, et al., *Acta Biomaterialia*, 2010, 6:2970-78]. Nerve tissue regeneration can be enhanced by the local delivery of biological cues including nerve growth factor (NGF) and glial cell line-derived growth factor (GDNF). Therefore, nanofibrous bicomponent scaffolds were electrospun for the controlled release of NGF and GDNF [Liu C, Wang M, *MRS Fall Meeting*, Boston, MA, 2013]. In this study, the dual delivery of NGF and GDNF from novel bicomponent scaffolds and their biological effects were investigated.

Methods: Using dual-source dual-powder electrospinning (DSDP-ES) (Wang C, Wang M, *J. Mater.Sci.-Mater.Med.*, 2012, 23:2381-97) and emulsion electrospinning (E-ES), novel bicomponent scaffolds were made, encapsulating NGF in poly(D,L-lactic acid) (PDLLA) fibers and GDNF in poly(lactic-co-glycolic acid) (PLGA) fibers. PLGA emulsions (12 or 15% w/v) and PDLLA emulsions (15 or 20% w/v) were used for fibers. The ratio of NGF/PDLLA fibers to GDNF/PLGA fibers in scaffolds were varied and the scaffolds made were designated as: (1) S1, S2, S3, S4 and S5 for GDNF/PLGA (12%w/v) and NGF/PDLLA (15%w/v) scaffolds at the fiber ratio of 1:0, 0:1, 1:1, 1:2 and 2:1; (2) S6, S7, S8, S9 and S10 for GDNF/PLGA (15%w/v) and NGF/PDLLA (20%w/v) scaffolds at the fiber ratio of 1:0, 0:1, 1:1, 1:2 and 2:1; (3) S11 for PLGA and S12 for PDLLA monocomponent scaffolds without growth factors (GFs). The morphological and structural properties of scaffolds were assessed using SEM and TEM. *In vitro* release behaviors of NGF and GDNF were studied using ELISA kit assay. For evaluating bioactivity of GFs, PC12 cells were cultured in DMEM at 37°C with 5% CO_2. After 1 day, culture medium was replaced with medium containing intact GFs or GFs released from monocomponent scaffolds and replenished every 2 days. For biological investigations, PC12 cells were seeded on scaffolds and cultured for up to 7 days. MTT assay was used for cell proliferation study.F-actin and neurofilament were stained at day 4 and 7 for investigating cell differentiation under confocal laser scanning microscopy (CLSM). Neurite length was measured using Image J.

Results: Smooth GDNF/PLGA and NGF/PDLLA fibers with core-shell structures were produced via E-ES. Through DSDP-ES, both types of nanofibers were evenly distributed in bicomponent scaffolds and desirable fiber ratios (and hence GF ratios) could be achieved. NGF and GDNF could be successfully incorporated in fibers with encapsulation efficiency above 80%. In a 42-day test period, GFs released from mono- or bicomponent scaffolds showed a burst release withi 24 h, followed by a sustained release. Both NGF and GDNF from scaffolds with various fiber ratios exhibited similar release profiles but different release amounts(Fig.1). MTT results showed bicomponent scaffolds had significant enhancement in cell proliferation compared with the control (Fig.2). GF bioactivity evaluation showed that the differentiation percentage of PC12 cells in the release group (providing released GFs) were only slightly lower than that of the control (the intact GFs), indicating bioactivity of released NGF and GDNF were preserved at maximum. The biological investigations revealed that GF-containing scaffolds significantly enhanced cell differentiation in terms of neurite outgrowth and branching of PC12 cells. High GF release amounts led to higher differentiation levels (Fig.2). Synergetic effect of NGF and GDNF on cell differentiation occurred at the 2:1 ratio of NGF/PDLLA to GDNF/PLGA in bicomponent scaffolds.

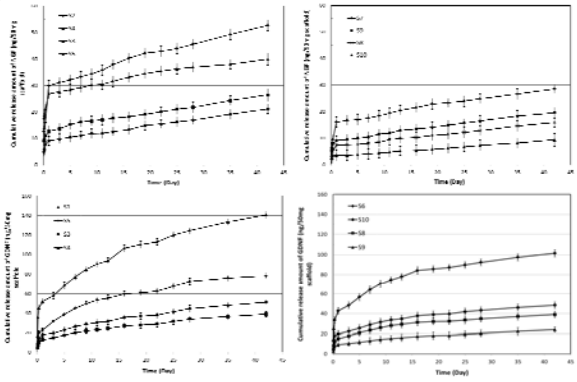

Fig.1. *In vitro* release profiles for bicomponent scaffolds (Above: NGF release; below: GDNF release).

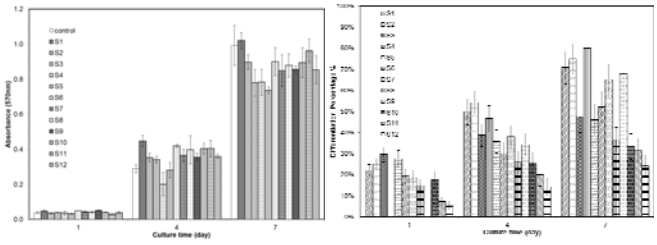

Fig.2. PC12 cell proliferation (left) and differentiation (right) on mono- and bicomponent scaffolds.

Conclusions: Bicomponent scaffolds containing designed amounts of NGF and GDNF with good post-release bioactivity could be made via DSDP-ES with E-ES. Sustained release of both GFs could be achieved. Compared with the control, mono- and bicomponent scaffolds containing GF or GFs induced much enhanced PC12 cell differentiation. Furthermore, NGF and GDNF released from bicomponent scaffolds exhibited synergetic effect on cell differentiation at certain fiber ratio(s).