**Introduction:** Previous studies suggest that normal innervation contributes to wound healing. Our laboratory has shown that neuronal sprouting and infiltration in the developing scar are observed during healing of medial collateral ligament (MCL) [1]. Denervation leads to impaired mechanical properties of healing MCL, decreased blood flow and diminished angiogenesis [2]. Altered levels of mRNA for angiogenic molecules are found in denervated MCL healing scar, including a striking increase in mRNA levels for the angiogenesis inhibitor thrombospondin-1 (TSP1) [3]. Nerve growth factor (NGF) is a neurotrophic factor that plays an important role in promotion of neuronal growth and sprouting. Recently NGF has also been found to positively influence angiogenesis and skin wound healing. For this study, we hypothesized that the addition of exogenous NGF would improve healing of ligament, promoting re-innervation, angiogenesis and in vivo blood flow.

**Materials and Methods:** Two groups of 30 male Sprague-Dawley rats (BW 342±32g) underwent unilateral transection of the MCL. One group was given NGF (total 10μg) and the other was given phosphate buffered saline (PBS) (as control) by osmotic pump, for 7 days after injury. After 7, 14 and 42 days, MCL was assessed for in vivo blood flow using laser speckle perfusion imaging (n=10 at each time point). Reinnervation, vascular density and production of the angiogenesis inhibitor were assessed by immunohistochemical staining for the pan-neuronal marker PGP 9.5, the endothelial marker vWF and the angiogenesis inhibitor thrombospondin-2 (TSP2) (n=3). Using computer-aided image analysis, neuronal density was assessed as the fractional area occupied by PGP 9.5 stained axonal profiles on a random sample of histologic sections. Vascularity was similarly estimated by counting the number of blood vessels and by measuring the area fraction surrounded with vWF positive vascular profiles. In addition, angiogenesis inhibitor expression was estimated by the fractional area occupied by TSP2 immunoreactivity in relation to the total area.

Mechanical properties were assessed on an EnduraTec mechanical testing unit (Bose) (n=7). The limbs were potted with polymethylmethacrylate in custom-built clamps at 60 degrees of knee flexion. Specimens were cycled twice from -2N to 1N and then held at 0mm. The remaining structures, except MCL, were carefully removed. The specimen was cycled again from -2N to 1N twice and ended at 0.01N to establish ligament zero. The ligament was held at 0.1N and then digital images were taken to measure the width and thickness. The cross sectional area (CSA) was calculated assuming rectangular geometry. The creep protocol was defined as 30 cycles from 0.01N to 1.5N at 1Hz, and then held at 1.5N for 20 min. At the end of the static protocol, the ligament was returned to 0.01N at a ramp rate of 0.03Hz, and then distracted at a rate of 8mm/min to failure. The failure load was recorded. The ultimate tensile strength and the stiffness (linear slope of the load-deformation curve between 25 and 75% deformation) were calculated. Mann-Whitney U test (histology) or Student’s t-test (mechanics) was used for statistics.

**Results:** PGP 9.5 labeling was significantly increased in the NGF group compared to control at both 14 and 42 days (NGF: 0.52±0.09%, mean ± SEM vs control: 0.28±0.05%, p<0.05 and 0.93±0.07% vs 0.71±0.07%, p<0.01 for 14 and 42 days, respectively). In vivo blood flow in the NGF group was not different from that in the control group at any time point. The number of vessels was significantly increased in the NGF group by 14 and 42 days (NGF: 1.79±0.09 vs control: 1.52±0.18, p<0.05 at 14 days, and NGF: 2.38±0.18 vs control: 1.66±0.11, p<0.05 at 42 days). The fractional area of vWF surrounded vessels was significantly increased in the NGF group at 7 days; NGF: 1.54±0.14% vs control: 1.24±0.13%, (p=0.05). By 14 and 42 days, no significant differences were found. TSP2 expression was seen surrounding vessels at the site of injury in both groups. The fractional area of TSP2 expression in the NGF group was decreased compared to control at 14 days post-injury; NGF: 0.27±0.05% vs control: 0.52±0.09%, (p<0.01).

The ligament CSA was significantly increased in the NGF group compared to control at 7 days (NGF: 3.41±0.37mm² vs control: 2.46±0.17mm², p<0.05). No significant differences in the CSA were found by 14 and 42 days. NGF treatment increased the failure load of the MCL by 40% at 42 days (35.37±4.07N vs 24.88±1.98N, p<0.05) (Graph A). The ultimate tensile strength of the NGF treated ligaments was increased by 55% at 42 days (26.39±2.97MPa vs 16.89±2.18MPa, p<0.05) (Graph B). NGF increased the stiffness by 30% at 42 days (19.62±0.98N/mm vs 14.93±0.97N/mm, p<0.01) (Graph C). No differences in mechanical properties between NGF and control group were noted at 7 and 14 days post-injury. There were no detectable differences in creep properties at any time points after injury.

**Discussion:** NGF promoted re-innervation, angiogenesis, and decreased angiogenesis inhibitor expression in healing ligament. Previous studies have shown that denervation negatively affects vascular responses [2,3]. NGF has been demonstrated to stimulate angiogenesis by exerting proliferative and mitogenic effects on endothelial cells. An increase in both re-innervation and angiogenesis promoted by NGF might contribute to the healing of ligament. NGF also up-regulates the neuronal synthesis of SP and CGRP in sensory neurons. These neuropeptides may also play an important role in wound healing, by exerting effects on fibroblasts and endothelial cells.

Local application of NGF accelerates skin wound healing and increases the breaking strength of healing skin wound in normal and diabetic mice. Clinically, topical administration of NGF promotes healing of neurotropic and vasculitic ulcer in human. NGF improved the failure load, ultimate tensile strength and stiffness of healing MCL. NGF may have a potential to enhance ligament healing.


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