Muscle Force

Introduction:
Muscle healing following severe injury is slow and often incomplete, resulting in the formation of dense scar tissue (1,2), leading to permanent damage and hyperfunctioning recovery. Growth factors of GFs have the ability to enhance muscle growth and therefore muscle healing. Insulin growth factor type 1 (IGF-1), basic fibroblast growth factor (b-FGF), and nerve growth factor (NGF) were used in a previous study to investigate the capacity of enhance myoblast proliferation and fusion in vitro (3). The aims of this study are to investigate the effect of these specific GFs on muscle regeneration and muscle healing in vivo.

Material and methods:
Both gastrocnemius muscles of 42 mice (C57 BL/10Fr+/+) were lacerated and repaired. Serial injections of GF (100 ng/ml) were performed in one leg at D1, 3, and 5 post-injury. The same volume of physiologic solution was injected in the contralateral leg. Each GF was injected in 14 mice. Seven days after the surgery, two animals per group were sacrificed and muscle regeneration was evaluated using hematoxilin-eosin staining and quantitative histology. The regenerating myofibers in the laceration site were counted and the diameter of 200 regenerating myofibers was measured in 10 different randomized areas on the slide using a micrometer ruler. At 1 month post-injury, muscle healing was assessed in the 3 groups using regular histology (h-e) and muscle contractility testing (n=9). Specifically, fast twitch strength (FT) and tetanus strength (TS) were measured by electrical field stimulation. Both gastrocnemius muscles were removed and mounted in a double jacketed organ bath of 5 ml at 36°C in Krebs solution and constantly bubbled with a mixture of 95% O2 and 5% CO2. The initial tension was set to 20 mN, and isometric contractions were measured with strain-gauge transducers coupled with a TBM4 strain gauge amplifier and recorded on computer using a data acquisition program. The muscles were stimulated with square wave pulses of 0.25 msec duration at maximal voltage (50V). First, 1 Hz stimulation was applied and the muscle twitches recorded, then 6 tetanic stimulations were applied with 0.5 sec train duration at 100 Hz in every 10 seconds. Finally, the muscle was weighted using a microbalance. For the regeneration study, the distribution of diameter sizes among the four groups was compared using non-parametric statistics (Kruskall-Wallis and Kolmogorov-Smirnov tests). Differences with p<0.01 were considered statistically significant. Regarding the physiological evaluation of contractile properties, based on the data obtained in our preliminary experiments, 80% of power with a type 1 error rate of 0.05 was achieved with 6 subjects per samples. The repeated measures ANOVA test was used to compare groups. Differences with p<0.05 were considered statistically significant.

Results:
The treated muscles showed numerous regenerating myofibers throughout the injured region. Regenerating myofibers were increased 3.5 fold with both b-FGF and IGF-1, and 1.5 fold with NGF compared to control (Figure 1: A,B). The mean diameter of the regenerating myofibers was 32 +/- 9 µm for NGF (Kolmogorov-Smirnov p=0.02), 36 +/- 10 µm for b-FGF (Kolmogorov-Smirnov, p=0.0001), and 38 +/- 10 µm for IGF (Kolmogorov-Smirnov, p=0.0001). One month after injury, muscle healing was histologically evaluated by counting the area of small and non-activated regenerating myofibers. In the treated muscle. In the non-treated muscle, muscle regeneration was less advanced and fibrotic scar tissue covered the superficial area of the injured site(Figure 1: C,D). The FT strength was increased 2 and 1.6 fold compared to the control in b-FGF and IGF-1 groups respectively (ANOVA, p=0.0001). Similarly, TS was increased by 1.8 with b-FGF and by 1.4 with IGF-1. In this study, NGF showed no significant effect on muscle healing (Figure 2).

Discussion:
The development of an approach based on the use of growth factors aimed at a faster and more complete recovery may revolutionize the significant “down-time” following a muscle injury. Our study demonstrates that the direct injection of specific growth factors into a muscle injury can significantly improve healing. Specific growth factors were not only able to improve muscle regeneration but also showed more complete muscle healing. The discrepancy between in vitro and in vivo results for NGF may be due to a maintained increase of NGF in the inflammatory injured muscle (4) and to the fact that an exogenous application of NGF may induce a hyperalgesic state, which results in disease of the injected limb. At one month the muscles treated with IGF-1 and b-FGF showed accelerated healing over the control as well as a higher functional recovery. These muscles contained only a few areas of fibrosis compared to the control. These results open new horizons for the treatment of all types of muscle injuries.