Introduction: Functional deficiencies after skeletal muscle trauma are a common problem in orthopaedic surgery. Therapeutic strategies to influence the outcome have been conserved for a long time. Representing a new approach the local injection of mesenchymal stem cells (MSC) has been shown to improve the regeneration process microstructurally.(1) In a previous study our group could show that the transplantation of 1 million MSC into a severely injured rat muscle enhanced the functional outcome four weeks after trauma.(2) Still unrevealed is the question if the effect of stem cell transplantation is "dose"-related or simply a switch-on – switch-off mechanism. No data exists yet about this issue, and if the first assumption was true, about the optimal number of cells, which have to be transplanted to obtain the maximum effect. The aim of the present study was to describe a dose-response relationship between the number of locally transplanted MSC and the resulting contraction force after skeletal muscle trauma. We hypothesized that the improvement of muscle function would increase with the number of applied cells.

Materials and Methods: Thirty-four female Sprague Dawley rats weighing 140 – 160 g (Charles River, Sulzbach, Germany) were used for this study (control group: n=10, treatment groups: n=6). The animals were housed at a constant temperature of 25 °C with free access to pellet food and water. All animal experiments were carried out according to the policies and principles established by the Animal Welfare Act, the NIH Guide for Care and Use of Laboratory Animals and the national animal welfare guidelines. The study was approved by the local legal representative (Landesamt für Arbeitsschutz, Gesundheitsschutz und technische Sicherheit, Berlin: Reg 0252/06).

Autologous MSC were harvested by tibial aspiration and cultured under standard conditions in monolayer. Hematopoetic cells were removed by medium changes. One week after an open crush injury of the left soleus muscle the animals were transplanted with increasing numbers of autologous MSC (0.1, 1, 2.5 and 10 x 10^6 cells) or saline in the control group. Muscle contraction forces were measured in vivo three weeks later. The sciatic nerve was indirectly stimulated in fast twitch (9 mA / 75 Hz, 0.1 s duration and 5 s intervals) and tetanic mode (9 mA / 75 Hz, 3 s duration and 5 s intervals). Maximum contraction forces were individually normalized to the uninjured right soleus muscle.

The arithmetic mean and standard deviation were determined for each measurement. Statistical significance analysis was performed using the non-parametric Wilcoxon test for dependent samples when individuals. The non-parametric Mann-Whitney-U test for independent samples was used for comparisons between the treatment and the control group. The level of significance was set to 0.05.

Results: The comparison of the results between the control and the treatment groups showed a significant logarithmic correlation between the number of the transplanted stem cells and the resulting contraction forces of the traumatized soleus muscles [coefficients of determination: 0.89 for fast twitch (p = 0.004, Fig. 2) and 0.87 for tetanic contraction (p = 0.002)]. Maximum forces did not differ between the control group and the group treated with 1 x 10^5 MSC. A significant improvement of twitch contraction force compared to the non-treated control group could be detected in animals three weeks after the transplantation of 10 million cells [68.5 ± 13.7 % versus 41.5 ± 17.9 %, (p = 0.019)]. Tetanic forces were significantly higher after the transplantation of 2.5 and 10 x 10^6 cells with 41 ± 5.1 % and 53.2 ± 6.6 % of the contraction forces generated by the internal control muscles than in muscles of the control group displaying values of 29.2 ± 12.4 % (p = 0.045 and 0.002).

![Graph A](image1.jpg)  
**Figure 1.** Correlation of normalized twitch (A) and tetanic (B) contraction forces 3 weeks after transplantation of different numbers of MSC.

Discussion: The presented study underlines the effectivity of mesenchymal stem cell transplantation after severe skeletal muscle trauma. For the first time a correlation of the number of injected stem cells and the resulting improvement of muscle contraction force could be demonstrated. Both, fast twitch and tetanic stimulation modes yielded maximum soleus contraction forces, which could be approximated to logarithmic functions when plotted against transplanted cell numbers. The resulting graphs describe a saturation of the achieved improvement confirming our hypothesis that there would be a biological limit for the functional benefit of the muscles. Despite of this saturation it seems to be prudent to transplant a high number of cells accepting a certain amount of apoptosis to gain the best functional result. A critical factor of MSC therapy of skeletal muscle trauma concerning a future clinical application is represented by the small window of opportunity between cell harvest and transplantation before the development of fibrosis.

References:  