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Cell therapy for denervated tissue

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Abstract
Background: Peripheral nerve injury results in denervation of tendons and muscles. The biology of denervated muscle has been well studied but little is known about the associated tendons. Denervation of muscle leads to atrophy which includes muscle fiber shrinkage and cell death, a process that is influenced by the lack of acetylcholine (ACh) signaling to the muscle cells. Recovery of long-term denervated muscle function is often poor. This thesis describes how a cell therapy approach using adipose tissue-derived stromal vascular fraction (SVF) may be used to protect and regenerate denervated muscle. Previous studies have shown how adipose tissue-dervied stem cells (ASCs), commonly expanded from the SVF, have pro-regenerative effects on the injured peripheral nervous system, and how ASCs differentiated towards a "Schwann cell-like phenotype" (dASCs) reduce muscle atrophy. In this thesis work, we studied the possible mechanisms underlying the regenerative potential of both SVF and culture expanded dASCs.

Hypotheses: We hypothesized that: 1) denervated tendon displays morphological and biochemical properties that resemble the chronic degenerative tendon condition known as tendinosis; 2) denervated muscle upregulates expression of muscarinic acetylcholine (ACh) receptors and apoptosis-associated signaling mechanisms; 3) dASCs enhance the proliferation of myoblasts *in vitro* through secretion of ACh; 4) SVF influences the proliferation, differentiation, and survival of myoblasts *in vitro* via secretion of growth factors; and 5) SVF can preserve denervated muscle tissue. To test our hypotheses, two model systems were used: an *in vitro* model based on indirect co-culture, and an *in vivo* rat sciatic nerve transection model.

Results: Denervated tendon displayed morphological changes similar to tendinosis, including hypercellularity, disfigurement of cells, and disorganized collagen architecture, along with an increased expression of type I and type III collagen. In addition, levels of neurokinin 1 receptor (NK-1R) were upregulated in the tendon cells. In denervated muscle, there was an increased expression of muscarinic ACh receptors, as well as of genes associated with apoptosis, such as caspases, cytokines (e.g., tumor necrosis factor-alpha; TNF-α), and death domain receptors. We subsequently used TNF-α as an inducer of apoptosis in an *in vitro* rat primary myoblast culture model. TNF-α activated/cleaved caspase 7 and increased poly ADP-ribose polymerase (PARP) levels. Moreover, Annexin V and TUNEL were increased after TNF-α treatment. Indirect co-culture with SVF significantly reduced all these measures of apoptosis. Proliferation studies showed that both dASCs and SVF enhanced growth of myoblasts *in vitro*. With dASCs, the effect was partially explained by secretion of ACh, and for SVF by released growth factors, such as hepatocyte growth factor (HGF). In both cases, the signal was mediated via phosphorylation of ERK1/2 (MAPK). HGF also had an inhibitory effect on the differentiation of myoblasts into myotubes. Finally, the protective effects of SVF were confirmed *in vivo*: injections of SVF into denervated muscle significantly increased the mean fiber area and diameter, as well as reduced the expression of apoptotic genes and TUNEL reactivity.

Conclusions: Denervated tendons undergo severe degenerative changes similar to tendinosis. Furthermore, SVF has the ability to reduce muscle atrophy *in vivo*. Using *in vitro* systems, we showed that this might occur through secretion of growth factors which activate MAPK signaling and anti-apoptotic pathways. In conclusion, SVF offers a promising approach for future clinical application in the treatment of denervated muscle.

Keywords
adipose tissue-derived stem cells, apoptosis, co-culture, denervation, myoblasts, peripheral nerve injury, proliferation

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