# Induction of intrafusal muscle fibers from human satellite cells and their innervations by human stem cell-derived sensory neurons

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**Statement of Purpose:**

While extrafusal muscle fibers generate muscle contraction and cause skeletal movement, intrafusal fibers serve as the sensory organs to detect the amount and rate change of muscle length (proprioceptors). Impairment of the sensory circuit will cause motor deficits, especially in movement coordination and fine motor activity, and has been involved in a wide range of diseases, such as autism and Parkinson's disease. Intrafusal muscle fibers have been induced in vitro from rat embryonic muscle cells (Rumsey, 2008) and the establishment of its connection with rat sensory neurons from DRG in a defined in vitro system has been demonstrated (Rumsey, 2010). The goal of this study is to develop a human-based in vitro muscle sensory circuit by utilizing human stem cells as the source. The aim is to generate intrafusal fibers from human satellite cells and establish its innervation by the stem cell-derived human proprioceptive sensory neurons.

**Methods:**

Human satellite cells from muscle biopsies were utilized as the source for the induction of intrafusal muscle fibers, while human neural progenitor cells were used as the source for the proprioceptive neurons. Serum-free medium was developed for the induction of intrafusal fibers as well as for their co-culture with proprioceptive sensory neurons. The generated intrafusal fibers and their innervation by sensory neurons were analyzed by phase microscopy, immunocytochemistry and electro-physiology.

**Results:**

According to the phase microscopy, both bag and chain intrafusal fibers were induced from human satellite cells with the novel induction protocol. Quantification of bag fibers in the culture indicated a consistent enhancement of bag fiber induction by Neuregulin. Co-immunostaining of Phalloidin and Egr3 (a transcription factor essential for intrafusal fiber differentiation) confirmed the intrafusal fiber identity of the induced bag fibers. The human muscle culture enriched with intrafusal fibers was then co-cultured with human sensory neurons (Guo et al 2013) in a new serum-free medium, and was then analyzed by immunocytochemistry. Sensory axonal terminals (stained by Peripherin) bifurcated when approaching a bag fiber (stained with BA-G5), suggesting an innervation of the bag fiber. Electrophysiological properties of induced intrafusal fibers were also analyzed by patch-clamp.

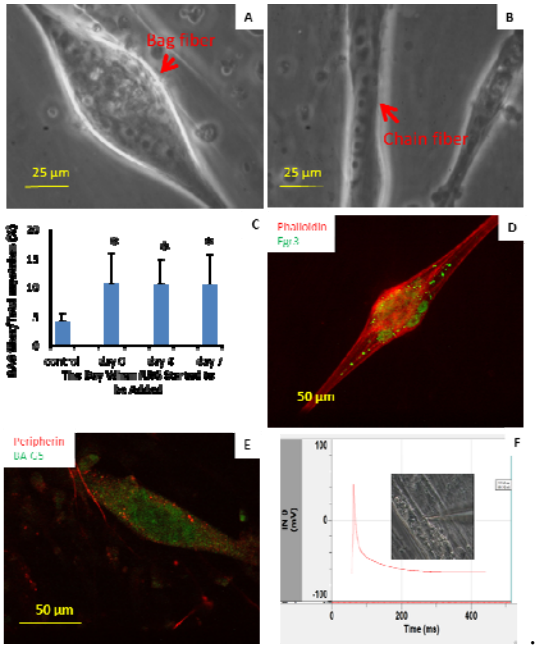


Figure 1. Induction of intrafusal fibers from human skeletal muscle stem cells and their co-culture with human sensory neurons. A&B) Phase microscope pictures of both bag (A) and chain (B) intrafusal fibers in the induced culture. C) Quantification of bag fibers in the induced culture when Neuregulin was added at different times. D) Co-immunostaining of Phalloidin and Egr3 confirmed the intrafusal fiber identity of the induced bag fibers. E) Co-culture of human intrafusal fibers with human sensory neurons was analyzed with immunocytochemistry. F) A sample trace of an Action Potential recorded from intrafusal fibers by patch-clamp.

**Conclusions:**

This study presented the first induction of human intrafusal muscle fibers from human muscle stem cells, and their innervation by human sensory neurons. This human-based in vitro model of the proprioceptive circuit of muscle movement will provide a valuable tool to study this circuit as well as its related diseases.

**References:**

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