**Application of micro/nano scale substrates for skeletal muscle tissue regeneration**

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**Introduction**

Skeletal muscles have a high capacity of self-repair but are not able to regenerate when a significant loss of tissue occurs, resulting in severe loss of function. Although transplantation of cells has been tested by direct injection, limited regeneration were observed due to the low viability of the intramuscular injected cells and restricted cell integration into the host tissue (Salehi et al. 2017). To address these issues, we developed various substrates such as electrically conductive nanofibrous scaffolds (Ostrovidov et al. 2017), microfabricated fibers (Ebrahimi et al. 2018), and injectable cell carriers in different scale from micro to nano with different topographies to deliver the cells and mimic the native structure of skeletal muscle tissue.

**Materials and methods**

We have fabricated different type of substrates in micro and nano scale: 1) sub-micron ribbons as an injectable cell carriers were microfabricated using Poly (lactic-co-glycolic acid) (PLGA) and the feasibility of their application to deliver the mouse myoblasts cells (C2C12) with high efficiency was tested (Fig.1 A-B), 2) Conductive fibers made of Gelatin-PaNi were prepared using electrospinning technique and the effect of electrical conductivity and electrical stimulation of cells were studied on inner organization of cell structures and differentiated myotubes (Fig.1 C-D) 3) Microfabricated fibers with two various topographies ”patterned and unpatterned” were prepared using Gelatin methacrylated hydrogel (GelMA) and the synergistic effect of topography and Agrin stimulation was studied on myogenesis (Fig.1 E)

**Fig.1** Different substrates (A-B) injectable cell carriers(Salehi et al. 2017) (C-D) electroconductive spun fibers (Ostrovidov et al. 2017) (E) microfabricated fibers (Ebrahimi et al. 2018)

**Results and discussion**

Injectable cell-carriers, 50µm width and 500µm length, could provide the proper contact guidance for generating aligned structures before injection and mechanical support during injection of cells. Moreover, microfabricated fibers with diameter of 500µm with a well-defined grooved surface could show the possibility of growing larger scale of muscle tissue. The micropatterned fibers efficiently could induce myoblast alignment and enhanced myotube formation. Treatment of myoblasts with agrin during differentiation also enhanced myogenesis in addition to enhancing AChR clustering (Ebrahimi et al. 2018). The enhanced myotube formation and myogenesis as well as improvement of intracellular organization and the functionality was confirmed as an effect of nanofibrous scaffolds made from electrically conductive polymers. Higher calcium transients and contractions with higher amplitude and regularity were observed when myotubes were electrically stimulated on electrically conductive nanofibrous scaffolds.

**Conclusion**

We anticipated that the studied substrates promote the formation of organized muscle microtissue and could be used for local delivery of cells while maintaining the cellular organization, viability and functionality.

**References**

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