Skeletal muscle in a dish: towards making skeletal muscle in vitro

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Appendix

English Summary

Skeletal muscle is the most abundant tissue in the human body, playing a critical role in numerous physiological functions. Over the past few decades, our understanding of skeletal muscle function and its associated diseases has significantly progressed, largely due to the development of advanced in vitro models. These sophisticated systems have become vital for studying stem cell-based regenerative medicine, testing new therapeutic strategies, and investigating the mechanisms underlying both healthy and diseased muscle states.

In this thesis, we explored various facets of recreating human skeletal muscle in vitro. Our research ranged from describing the presence of non-coding RNAs (ncRNAs) in the differentiation of induced pluripotent stem cells (iPSCs) to pioneering innovative materials for in vitro applications. Additionally, we evaluated advanced 3D models within the context of skeletal muscle disease, advancing our understanding of this complex tissue.

Chapter 1 provides a general introduction into stemcell biology. It delves into the early embryonic germlayer development mainly focusing on mesoderm formation and myogenesis (skeletal muscle development). It also discusses the different subtypes of ncRNAs and their reported role in the context of skeletal muscle development. Last, it provided an overview of engineering techniques used sofar to establish in vitro models for the modeling of skeletal muscle.

Chapter 2 investigated the role of ncRNAs and epigenetic memory during early germ layer differentiation, revealing a subset of ncRNA biotypes specific to mesoderm, endoderm, or ectoderm differentiation using multi-omics sequencing. Our study emphasized the importance of ncRNAs in regulating differentiation and identified potential targets for refining human iPSC-based differentiation protocols. Notably, transfer RNA (tRNA) fragments emerged in our analysis as an unexpected biotype, opening questions about their regulatory role in early germ layer differentiations. This research provides a great source of potential ncRNAs, for future functional studies aiming at improving the differentiation of iPSC-derived cell types. This could particularly relevant for to improve differentiation from IPS towards muscle progenitors. Furthermore, the findings can deepen in the understanding of skeletal muscle differentiation.

Chapter 3 focused on developing a novel magneto-active biocompatible material, demonstrating significant improvements in myoblast differentiation in three-dimensional (3D) models. This magneto-conductive material for melt electrowriting (MEW) enhances muscle fiber alignment and maturation, mimicking the mechanical properties of in vivo skeletal muscle tissue. Current models mostly rely on electrical stimulation for actuation so the magnetic property of this new model open new possibilities.

Chapter 4 evaluated various 3D skeletal muscle models, highlighting the effectiveness of cantilever-based models in testing restored functionality. Microtissues proved optimal for gene correction testing. Preliminary results from a vascularized skeletal muscle-on-a-chip model showed promising differentiation efficiencies. By consistent conditions across different models, we pioneered a systematic approach for selecting the appropriate 3D model tailored to specific experiments needs, a necessity as more models are developed.

Chapter 5 established reliable RNA staining techniques for skeletal muscle tissues and microtissues. The detailed staining protocol developed for RNA and protein en-

hances the ability to analyze gene and protein expression accurately. This method is a valuable tool that can be applied to both in vivo skeletal muscle and 3D in vitro skeletal muscle models.