

Abstract of the doctoral dissertation of Karolina Zygmunt, M.Sc., entitled:

Assessment of the paracrine effect of fibroblasts on the myogenesis process towards obtaining meat by *in vitro* techniques.

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Cultured meat is a promising supplement to traditional meat husbandry, utilizing the ability of muscle cells to form advanced three-dimensional structures *in vitro*, which closely resemble muscle tissue. Using the potential of muscle satellite cells – precursors of muscle cells – for self-renewal, proliferation, and differentiation, combined with advanced cell culture techniques, has enabled successful attempts to obtain structures reflecting muscle tissue *in vitro*. However, reducing the production costs of the final product and further improving bioengineering techniques requires further research from scientists in many fields.

The aim of this study was to evaluate the paracrine effect of fibroblasts – connective tissue cells – on the myogenesis of bovine muscle satellite cells in a two-dimensional culture system (Transwell inserts) and a three-dimensional culture system (spinner flask bioreactors). At the initial stage, using DOE (Design of Experiments) statistical analysis, a culture medium composition was developed that supported muscle cell proliferation while maintaining a low rate of spontaneous differentiation.

The results indicate that the most important factor determining the rate of cell division was the type of serum used, followed by the addition of the mitogenic factor – basic fibroblast growth factor (bFGF). Glucose concentration did not significantly affect the proliferation rate; however, under conditions of reduced glucose (1 g/L), the self-renewing cell phenotype was maintained.

Assessment of the paracrine effect of fibroblasts on the myogenesis process in two-dimensional cultures using transcriptome analysis (RNA-Seq) demonstrated that fibroblasts promote early myogenic differentiation through paracrine signaling. Under proliferation-promoting conditions (in growth medium), increased expression of early differentiation genes such as *EGR1*, *IL6*, *NR4A1*, *SOCS3*, and *EYA2* was observed, while the expression of late differentiation genes was downregulated. After induction of differentiation by modifying cell culture conditions, this effect was enhanced, indicating that fibroblast paracrine signaling may

regulate the extent of myogenic differentiation. The ratio of paracrine signals also proved significant, as it changed during culture due to the faster rate of fibroblast proliferation, which could have an inhibitory effect on terminal differentiation, including through increased TGF- β signaling.

Assessment of the paracrine influence of fibroblasts on the course of myogenesis under three-dimensional conditions, using gelatin microcarriers and spinner flask bioreactors, revealed significantly higher expression of genes involved in proliferation (such as *PAX7*, *MYF5*, *MYOD*) and early differentiation (*MYOG*, *EGR1*). Furthermore, skeletal myosin – a differentiation marker – protein expression levels were significantly higher in the co-culture with fibroblasts.

In summary, fibroblasts stimulate early myogenic differentiation of muscle cells through paracrine signaling, as confirmed by analyses conducted using two-dimensional and three-dimensional cell culture models. By modulating the ratio of paracrine signals from fibroblasts, it is possible to induce myogenic differentiation. Furthermore, the obtained results indicate the potential of using paracrine signaling elements, such as extracellular vesicles (EVs), as an effective substitute for expensive biological and chemical factors added to the culture medium.