

PROFUSE-B8: A Spontaneously Immortalized Bovine Myoblast Cell Line for Cultivated Meat Production

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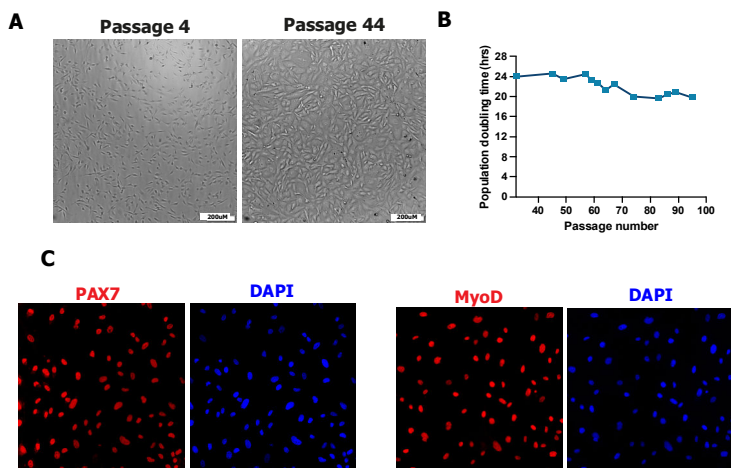


Introduction

The success of cultivated meat in the marketplace will depend on its ability to deliver a product that meets or exceeds the nutritional standards and sensory experience of conventional meat. This involves the production of a tissue consisting of fat, connective tissue, and importantly, muscle fibers. The muscle component is critical for the authentic mouthfeel, flavor, and and nutritional profile of cultivated meat. Undifferentiated cells, although abundant and relatively easy to culture, do not possess the specialized structures or functions that muscle tissue offers. Muscle tissue is inherently structured and organized, enabling it to provide the fibrous texture that consumers expect from meat. Undifferentiated cell biomass tends to be more gelatinous and lacks the fibrous quality that is crucial for the sensory experience of eating meat. This textural difference can significantly impact consumer acceptance, as texture is one of the primary factors influencing meat preferences. Muscle tissue contains myoglobin and other compounds that contribute to the characteristic taste of meat. Undifferentiated cells do not develop these flavor compounds, resulting in a product that may be nutritionally adequate but lacking in the sensory appeal that drives meat consumption. Cultivating muscle tissue allows for the preservation and enhancement of these flavor profiles, making it more likely to resonate with consumers.

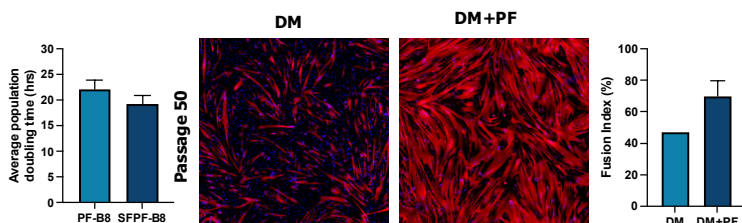
To successfully produce muscle-based cultivated meat products at scale, it's crucial to have a reliable and reproducible source of muscle progenitor cells with high proliferation and differentiation capabilities. Additionally, these cells must eliminate the need for repeated sampling from the animal source. Here we describe the generation of a spontaneously immortalized bovine myoblast cell line, PROFUSE-B8 with high muscle differentiation potential.. To address this, we have developed PROFUSE-B8 (PF-B8), a spontaneously immortalized genetic modification-free bovine myoblast. We have successfully grown the cell line in continuous 2D culture for over a year, achieving stable population doubling time, and exceeding >300 population doublings (100 passages). Additionally, this cell line has maintained its high capacity for myogenic differentiation and continues to respond effectively to the PROFUSE-S1 differentiation enhancement supplement, consistently achieving at least 95% fusion efficiency within 72 hours post-treatment. Additionally we successfully adapted a sub clone, PROFUSE-SFB8 (PF-SFB8), to grow in chemically defined serum free media. Moreover, we have demonstrated that the combination of PF-SFB8 cells treated with PROFUSE-S1 can successfully create significant muscle tissue when grown on 3D scaffolds. Thus, the PF-B8 cell line offers not only a valuable tool for advancing R&D, but has great potential for use in cultivated meat production.

1) PF-B8 is a stable spontaneously immortalized bovine myoblast cell line



Following enzymatic digestion and FACS sorting, purified myoblasts population was maintained by serial passaging. A spontaneous immortalization event occurred around passages 13-17 A) Representative images of the pre-immortalization (passage 4) and post-immortalization (passage 44) cells. The line was deemed immortalized upon reaching passage 25 (~60 population doublings). B) Population doubling time was assessed up to passage 95 (>300 PDs). C) PF-B8 shows 100% positivity for MyoD and Pax7, validating its identification as a myoblast line.

3) PROFUSE-B8 is easily adapted to grow under serum free conditions

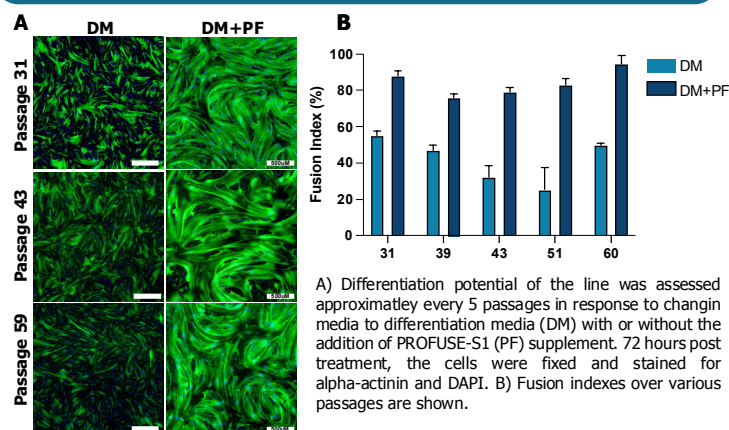


PF-B8 cells were thawed from the working cell bank. Upon the first passage after thaw the media was changed over 4 passages the media was serially diluted from 20% serum to 0% serum in a defined growth media formulation. A master cell bank of the adapted serum free cells or PROFUSE-SFB8 was made and the line was then passaged continuously and monitored for population doubling and differentiation potential

Conclusions

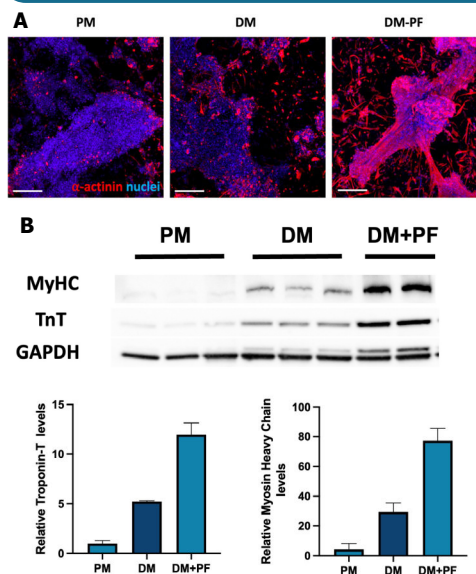
We demonstrate the successful generation of the first spontaneously immortalized bovine myoblast cell line which retains an extremely high differentiation potential. The PROFUSE-B8 line is capable of continuous adherent growth for at least 300 population doubling times retaining its differentiation potential. Moreover the line is responsive to PROFUSE-S1 differentiation enhancing media supplement. The line is easily adapted to grow in serum free media, while retaining its growth and differentiation properties. Moreover PROFUSE-SFB8, can adhere and grow muscle tissue on 3D scaffolds. Altogether, the PROFUSE-B8 line is an ideal line for the pro

2) PF-B8 maintains high differentiation potential which is enhanced upon treatment with PROFUSE-S1



A) Differentiation potential of the line was assessed approximately every 5 passages in response to changing media to differentiation media (DM) with or without the addition of PROFUSE-S1 (PF) supplement. 72 hours post treatment, the cells were fixed and stained for alpha-actinin and DAPI. B) Fusion indexes over various passages are shown.

4) PROFUSE-SFB8 successfully attaches to and differentiates on 3D scaffolds



A) Briefly, 1.5×10^6 PROFUSE-SFB8 cells were seeded in a hydrogel onto PGLA/PLLA scaffolds. Culture medium was then added to the scaffolds and replaced every 2 days. The constructs were cultured for 7 days in serum free proliferation medium and 3 days in serum free differentiation medium. Fixed samples were immunostained and imaged using the Andor BC43 confocal imager. Representative images of muscle fiber production in 3D are shown. The production of maturation associated muscle markers are significantly elevated in PROFUSE-S1 (PF) treated 3D muscle construct, as demonstrated by western blot analysis (B).

Acknowledgments



Funded by the European Union

רשות החדשנות Israel Innovation Authority