Screening of food ingredients with proliferative activity for skeletal muscle cells

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Objectives: As global population is projected to reach 10 billion by 2050, shortage of protein-rich foods is expected as early as 2030. While livestock industry is required to be more productive to meet the demand, concerns on environmental impact, such as land use and greenhouse gas emission, has been raised in recent years. As those concerns grow, demand on alternative proteins increases all over the world. Cultured meat, made of animal cells cultured in vitro, is considered one of the most promising sources of animal protein in future, as it has less environmental impacts compared to conventional meat. In vitro animal cell cultures are generally performed in a medium supplemented with fetal bovine serum (FBS) which contains proteins, vitamins, carbohydrates, lipids, hormones, growth factors, minerals and trace elements. Though FBS has been widely used to grow most types of animal cells for the research purpose, use of it in cultured meat production isnotconsidered viable due to problems such as high cost, safety concern, low production capacity and animal welfare. As replacing FBS with food ingredients leads to cost reduction and safer products, it makes cultured meat more affordable and acceptable to consumers. Therefore, we screened several food ingredients to identify ones which can be used as FBS substitute.

Materials and Methods: Bovine primary myoblasts were enzymatically isolated from cheek meat of cattleslaughtered in commercial slaughterhouse. The purity of the isolated myoblasts was tested by immunostaining, and myotube formation capability was con- firmed by culturing under differentiating condition. Expansion of myoblasts was performed on collagen type 1 coated plate with medium containing FBS for three subsequent passages. The cells were harvested and used for comparison of medium components. In order to examine applicability of the developed medium to other species, chicken myoblasts were also used. Chicken primary myoblasts were isolated from E10 chick embryos. Cells were expanded under the same condition as bovine myoblasts.

To compare the effectsof food ingredients on cell proliferation, myoblasts were seeded in medium supplemented with food ingredients or FBS and cultured for 4 days. Culture with serum free medium was conducted for control. Cells were counted on day 4 of culture. Expression of Myogenin, Desmin andMyosin heavy chain was analyzed by RT-qPCR at days 2, 4 and 6 of culture. After 4 days of proliferation, cells were induced to form myotubes by changing medium to induction medium supplemented with 2% horse serum.Food ingredients used in this study were commercially available dry powders.

Results and Discussion: At passage 0, most isolated bovine cells (95 %) were Pax7 positive, demonstrating low level of contamina- tion of fibroblasts in myoblasts. The myoblasts formed large multinucleated myofiber when cultured in differentiation medium. As these results showed high purity and muscle differentiation capacity of isolated myoblasts, thebovine myoblastswere used in further analysis to compare the effects of medium components on proliferation and differentiation.

Under serum free condition, the number of muscle cells remained almost unchanged for 4 days. The cells showed higher expression of the muscle differentiation markers compared to those grown in medium containing FBS. Less myotubes were formed under eventual differentiating condition. As these changes caused by omitting FBS from medium, cell proliferation and myotube differentiation of cells grown in medium supplemented with various food ingredients were compared to evaluate the capability to replace FBS. Among more than ten ingredients we tested, only ingredient X supported cell proliferation under serum free condition. No in- crease in muscle differentiation marker expression was observed compared to those in cells cultured with FBS-containing medium. By optimizing ingredient X concentration, growth factors and attachment factors, proliferation and myotube formation comparable to those of cells grown in the FBS-containing mediumwereachieved.On the other hand, the effects ofingredientX on chicken myoblasts proliferation were not as remarkable as those on bovine myoblasts.

The results of this research showed that FBS can be replaced with ingredient X, and eventual development of affordable food-grade medium for cultured meat production is considered achievable.Since the effects of ingredients on bovine and chicken cells were confirmed to be different, our finding implies that Food grade FBS substitutes are needed to be optimized for each cell.

Key words: Medium for cultured meat production