CHICKEN FIBROBLAST-TO-ADIPOCYTE DIFFERENTIATION FOR CULTURED MEAT : A COMPARATIVE STUDY BY EMBRYONIC DAY

So Hee Kim [1], Chan Jin Kim[1], Eun-Yeong Lee[1], Yu-Min Son[1,]
Young Hwa Hwang[2] and Seon Tea Joo[12*]

[1]Division of Applied Life Science (BK21 Four), Gyeongsang National University, Jinju, Korea

[2]Institute of Agriculture & Life Science, Gyeongsang National University, Jinju, Korea

*Corresponding author email: stjoo@gnu.ac.kr

I. INTRODUCTION

As fat is one of the important factors that determine the taste and profile of meat, addition of fat is essential to manufacture cultured meat which is similar characteristics to traditional meat. In previous research, diverse ways to adding fat have been conducted to manufacture various types of cultured meat containing fat. Among them, fat addition by culturing adipocyte with trans-differentiation has recently been taken notice. The cultured adipocyte obtained by trans-differentiation from various cell types such as fibroblasts and osteoblasts can efficiently provide fat to cultured meat without wasting cells [1]. Most of all, fibroblasts, which occur throughout the body of an organism and can be easily observed in primary culture of satellite cell, is suitable for trans-differentiation into adipocytes in the cultured meat production process. This study aims to find out difference in trans-differentiation of primary fibroblasts into adipocyte-like-cell in different growth stage of chicken. In this study, it was expected to give efficiency to the establishment of cell lines and fat production for the production of cultured meat in the future.

II. MATERIALS AND METHODS

Fibroblasts were isolated from the skin tissue of chicken embryos between 10 and 20 days of incubation. The isolated cells were figured out as fibroblasts by staining for integrin beta 1, which are specific markers of fibroblasts. When the confluency exceeded 70%, the growth media was replaced with differentiation medium which elicit trans-differentiation into adipocyte. As each fibroblast was provoked to differentiate into a pre-adipocyte at 10, 15, and 20 days after the medium change. Changes in cell morphology were observed in bright field using CKX 53 (Olympus, Tokyo, Japan). After inducing trans-differentiation for 10 days, neutral lipids in adipocyte were stained with HCS LipidTOX green and the degree of differentiation was confirmed by flow cytometry. Harvested adipocytes were analysed using GC (gas chromatography) for fatty acid composition. All results of experiments were analyzed with one-way analysis of variance (ANOVA) using SAS 9.4 software (SAS Institute, Cary, NC, USA).

III. RESULTS AND DISCUSSION

Due to the morphological differences in the size and developmental stage of the embryos on days 10, 15, and 20, there were variations in the separation of fibroblasts. Fibroblast isolation was relatively easy on embryonic day 10 than other days as the skin tissue did not have many blood vessels and hairs. Homogeneity of each fibroblast isolated in three different days was confirmed by measuring the expression of integrin beta 1 (Fig. 1). Trans-differentiation degree of fibroblast was examined by staining with HCS LipidTOX green neutral lipid stain. Fibroblasts isolated from the 10 days embryos showed the highest accumulation of neutral lipids and images of stained cell showed neutral lipid accumulation decreased as the embryos approached hatching under the same conditions. In particular, 94% of the cells isolated on 10 days expressed LipidTOX in flow cytometry, which suggests that cells obtained from younger embryo such as those at 10 days can differentiate into adipocytes almost entirely. In the fatty acid composition analysis using gas chromatography, there was almost no significant among all of adipocytes which is derived from embryonic days 10, 15, and 20 (Tab 1). The cultured fat, obtained by harvesting differentiated adipocytes, had the highest concentration of oleic acid, followed by palmitic

acid. Fatty acid composition of cultured adipocyte is similar to real chicken, indicating that the cultured adipocytes are suitable for use in cultured meat manufacturing.

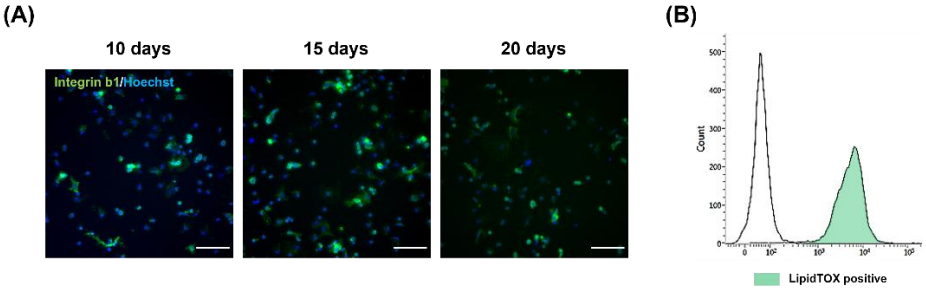

Figure 1. Representative immunifluorescence images of fibroblast isolated on 10, 15 and 20 days with Integrin beta 1 and Hoechst 33342

Table 1. Fatty acid composition (%) of neutral lipids derived from trans-differentiated fibroblasts isolated on different days

Fatty acid (%)	Embryonic day			SEM	P-value
	10 days	15 days	20 days		
C12:0	0.1[b]	0.1[a]	0.1 [a]	0.02	0.02
C14:0	0.6	0.5	0.6	0.02	0.70
C14:1	0.2	0.3	0.2	0.04	0.17
C16:0	22.6	21.8	22.3	0.25	0.65
C16:1	3.9	4.0	3.7	0.08	0.72
C18:0	18.7	19.0	18.2	0.23	0.50
C18:1	28.2	28.7	29.2	0.31	0.11
C18:2	16.2	15.4	15.3	0.30	0.37
C18:3	0.4	0.4	0.4	0.01	0.95
C20:0	0.5	0.5	0.5	0.02	0.72
C20:4	7.8	8.4	8.6	0.23	0.27
C20:5	0.2	0.2	0.3	0.03	0.09
C22:6	0.6	0.7	0.7	0.05	0.22
SFA	42.6	41.9	41.7	0.27	0.14
MUFA	32.3	33.0	33.2	0.29	0.26
PUFA	25.2	25.1	25.1	0.03	0.99

Experiment was perfomred in triplicate and indicated Means±SEM.
A P-value of < 0.05 was indicated significant.

IV. CONCLUSION

Isolating fibroblast on embryonic day 10 of the chicken embryo, depending on the development of the chicken embryo, enable efficient trans-differentiate into preadipocyte.

ACKNOWLEDGEMENTS

This research was supported by the Korean IPET through the Agri-Bioindustry Technology Development Program, funded by the Ministry of Agriculture, Food, and Rural Affairs (MAFRA) (Project No. 321028-5), Korea.

REFERENCES

1. Pasitka, L., Cohen, M., Ehrlich, A., Gildor, B., Reuveni, E., Ayyash, M., Wissotsky, G., Herscovici, A., Kaminker, R., Niv, A., Bitcover, R., Dadia, O., Rudik, A., Voloschin, A., Shimoni, M., Cinnamon, Y., & Nahmias, Y. (2022). Spontaneous immortalization of chicken fibroblasts generates stable, high-yield cell lines for serum-free production of cultured meat. Nature Food, 4(1), 35–50.