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Short Communication

Gastrimargus musicus muscle tissue culture

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Cells from legs of *Gastrimargus musicus*, a grey brown grasshopper were grown in simple media. The medium was tested for growth of muscle cells and with supplements. It was found that the medium containing calcium carbonate had round cells growing close to each other in clumps. The medium containing creatine had cells growing which were elongated and branched and had all the features of fully developed muscle tissue. The muscle tissues grew to a size, more than double that of the original grasshopper and were maintained in the medium supplemented with creatine for more than two months.

Key words: *Gastrimargus musicus*, cell culture medium, insect cell culture, muscle.

INTRODUCTION

Insect cells have been used in various fields of biotechnology as production factories of recombinant proteins, vaccines etc and even for basic research (Sudeep et al., 2005). Maintenance of cells in culture for long duration was a problem for earlier workers, till Grace in 1962 successfully established long-term cultures of insect cells (Lynn, 2002). Lery and Fediere (1990) have developed a serum free medium. Neurons and glial cells were obtained to facilitate the studies of development and membrane biophysics of the cells (Hayashi and Hildebrand, 1990). *Antheraea eucalypti*, a moth and *Aedes aegypti*, the mosquito cells were tissue cultured for the study of multiplication of yellow fiver virus (Converse and Nagle, 1967). The imaginal discs of fourth instar mosquito larvae (*A. aegypti*) and the male and female gonads of diapausing Cynthia pupae were cultivated in vitro by Fendrich and Trager (1963).

Akiyama et al. (2009) developed a bioactuator powered by insect dorsal vessel tissue which can work for a long time at room temperature without maintenance, showing insect dorsal vessel tissue to be a more promising material for bioactuators used at room temperature than other biological cell-based materials. The effect of molting hormone 20-hydroxyecdysone was investigated on hemocytes of *Galleria mellonella* (Izzetoglu and Karacali, 2003). Primary cultures of muscle tissue from 10-day-old embryos of *Locusta migratoria* and 11-day-old embryos of *Schistocerca gregaria*, were grown and maintained in two different media and showed different growth patterns (Duce and Usherwood, 1986). Intestinal smooth muscle cells and myenteric neurons were used for cell culture model development (Batista, 2007).

As it can be seen from the above discussion, insect cells have been developed for various types of cells and different types of media have been utilized for their growth. The work done by us highlights that, even with simple medium not only cell growth can be obtained but with simple addition of one compound, can cause growth of tissues like a network.

MATERIALS AND METHODS

The medium for culture of muscle tissue had the following constituents: ammonium molybdate 0.035 mM/L, cobalt chloride 0.210 mM/L, cupric sulphate 0.117 mM/L, zinc sulphate 0.294 mM/L, ferrous sulphate 1.980 mM/L, aspartic acid 2.675 mM/L, Tween 80 25 mg, cholesterol 4.5 mg, ethanol 1 ml, inositol 2.220 mM/L, nicotinic acid 1.300 mM/L and thiamine 0.237 mM/L. All the constituents were mixed and autoclaved. This basal medium was used for cell culture of muscle cells of *Gastrimargus musicus*. For the tissue to develop, some variations were tried with the basal medium. 150 mg creatine, 150 mg calcium chloride and 150 mg calcium carbonate was added to each 100 ml basal medium taken in separate flasks. The legs were excised from G. musicus and homogenized in phosphate buffered saline. 20 µl of the homogenized tissue sample was inoculated into separate flasks containing 100 ml of different media. The inoculated media were kept at 27 ± 1°C.

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Table 1. Growth of cells in different combination of basal medium.

S/No.	Medium	Type of cell
1	Basal medium	Single cells with profuse growth
2	Basal medium + calcium chloride	Single with good growth
3	Basal medium + calcium carbonate	Rounded cells which formed clumps
4	Basal medium + creatine	Elongated cells which formed elaborate network like muscle tissue

RESULTS AND DISCUSSION

The cultures were kept at 27 ± 1°C for 14 days. The growth of cells and development of tissues were visualized after 14 days. The cells were present in basal medium and in medium containing calcium chloride. The medium containing calcium carbonate had growth which was visualized from the flask. The growth looked liked clumps (Table 1). On visualization under microscope, cells were seen which were very compact. The cells were round and grew close to each other. The medium containing creatine had growth which on observation with naked eyes could be said as tissue. On visualization under microscope cells were found to be elongated, branched and deep orange in colour. They formed a compact matrix like structure (Table 1). The tissue was transferred into fresh medium where it continued to grow. Till writing the tissue was transferred the third time and the tissue was more than 2 inch long and 2.5 inch wide.

It can be said from this study that cells of G. musicus can be developed into tissues. The cells from the legs of G. musicus were cultured in different media and growth of diffused cells were found in basal medium as well as medium containing calcium chloride. It can be concluded that calcium chloride does not provide any advantage to the growing cells for them to bind to each other which is similar to that of the basal medium. The medium containing calcium carbonate had more compact growth and cells aggregated into clumps. Calcium carbonate in the medium helped the cells to grow close to each other and form clumps. Therefore, it can be concluded that calcium carbonate present in the medium somehow provided the cells with competence to form clumps. It has been reported that calcium plays an important role in cell adhesion (Kemler et al., 1989) but such effect is only shown by calcium carbonate and not by calcium chloride for the muscle cells of G. musicus. The medium containing creatine, developed tissue from the cells. Creatine helped muscle cells to grow and develop into tissue. The elongation of cells and branching seen in the tissue was clearly an indication of the matrix like muscle tissue. This aggregation of cells was of true tissue, unlike clumping of cells obtained in the medium containing calcium carbonate, where the cells grew close to each other but were round in shape and no connection between cells was observed.

The cells of muscle tissue grown in the medium containing creatine had an elaborate network like muscle tissue. The cells were branch and tips were tapered and pointed. The branching was observed as in the original muscle tissue from the legs of grasshopper. The cells which grew in the medium were different in one point from the original muscle tissue, in that they were not striated as in the original tissue from the grasshopper. It has been shown in other works that creatine is able to enhance differentiation in murine skeletal myoblast cell cultures by increasing the number of nuclei (Deldicque et al., 2007). Even though the work had been done on murine cells, it can be generally concluded that creatine promotes tissue differentiation.

Therefore from the above discussions and works of others, it can be concluded from the study that muscle cells from the legs of G. musicus can be grown in simple media and can be helped to develop into tissues, if the medium contains creatine.

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