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Standardization of Primary Hepatic Cell Culture in Chicken

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A B S T R A C T

Liver is one of the important organs in the body that regulates various metabolisms in the body and having multiple functions. It is difficult to study all the functions of the liver *in vivo*, because of other organs interventions. Hence, the liver parenchymal cells (hepatocytes) were cultured *in vitro* in order to study the different activities of the liver. In the present study, we standardized culturing of chicken embryo hepatocytes from 12 to 14 days old embryos. Here we have used 0.125% trypsin EDTA to disaggregate the parenchymal cells of the liver. The cells were cultured using growth media added with 10% Fetal Bovine Serum and Tryptone Phosphate Broth.

Introduction

The liver is the largest internal organ in the body performs a wide range of physiologically important metabolic functions including synthesis of plasma proteins, glycogen storage, fatty acid biosynthesis, bile secretion, detoxification (xenobiotic metabolism) and blood glucose generation etc. (Hewitt *et al.*, 2007; Puviani *et al.*, 1998; Gebhardt, 1996). It also involves in metabolic homeostasis by regulating carbohydrate, lipid, and amino acids levels and the major site of gluconeogenesis. Unlike in other animals, in Poultry, liver is the major site of *de novo* fatty acid biosynthesis (Leveille, 1966, 1969; Pearce, 1977). However, it is difficult to study specific liver activities *in vivo* owing to interfering influences from other organs like kidney, gut, and lungs, which metabolize drugs and the muscle involvement in glucose homeostasis (Lee *et al.*, 2014).

Hence, culturing the liver parenchymal cells *in vitro* is helpful for us to study the various activities of hepatocytes.

Materials for cell culture

Standard cell culture equipment

Laminar flow hood, humidified chamber (CO_2 (5%) incubator), Standard inverted microscope, Centrifuge, Water bath.

Materials required

Pipettes, Gloves, Scissors and forceps, 0.22µm filters, Muslin cloth, Magnetic stirrer, Beakers, 25-cm^2 flask, micro pipette tips.

Tissue required

12-14 days old chicken embryo

Media and the reagents required

Phosphate Buffer Saline (PBS), Trypsin EDTA (Gibco by Life Technologies, USA), Growth medium – M-199 medium (Sigma Aldrich, USA) and Fetal Bovine Serum (Gibco by Life Technologies, USA).

Protocol

In previous studies, various methods have been exercised in attempts to isolate liver parenchymal cells. These include chemical, mechanical and enzymatic protocols as well as in combination of these methods. However, it's difficult to isolate hepatocytes with adequate viability by following mechanical and chemical protocols (Wang *et al.,* 1985).

Hepatocytes have a unique epithelial cell polarity and intercellular structures including bile canaliculi, tight and gap junctions and the cells also possess high regeneration capability. But they lose these specific properties very fast when isolated and cultured *in vitro* (Reid and Jefferson, 1984). 12 to 14 days old embryonated eggs were utilized and at this stage we will get a visible and appreciable amount of liver samples for the culture of hepatocytes. The whole egg was disinfected by swabbing with 70% alcohol and placed in a small beaker with their blunt end facing up. The egg shell was cracked at broad end by using sterile forceps, and white shell membrane was peeled off to expose the chorioallantoic membrane (CAM) with its blood vessels. Further, membranes were pierced with sterile curved forceps and the embryo was raised out by grasping it gently and placed in a 9-cm Petri dish containing autoclaved sterile Phosphate Buffer Saline (PBS) and cleaned thoroughly. The head, limbs and wings of the embryo were detached and the ventral side of the embryo was cut opened by means of sterile scissors. Further, the abdomen portion was separated to locate the liver and the liver lobes were collected aseptically using sterile forceps into another petri dish contained sterile PBS.

The liver lobes were separated and the part of hepatic vessels and unnecessary tissue were detached. The gall bladder was removed carefully without piercing. The liver lobes were minced into pieces using sterile scissors and the unwanted tissue, such as membranes, fat or other attached tissues were dissected. After that, the liver pieces were transferred to another petri dish having PBS to clean several times to wash off all the all debris and blood tinge. Finally, the tissue pieces were cleaned once again by re-suspending in a beaker containing PBS and allowed the pieces to settle down and the supernatant was discarded (the same was repeated two more times).

These tissue pieces were shifted to a sterile beaker containing sterile magnetic bar and approximately 10-15 mL (maintained at room temperature) of 0.125% trypsin-EDTA (Gibco by Life Technologies, USA) was added for disaggregation of cells. Further, the beaker was placed on magnetic stirrer in an incubator at room temperature for stirring at around 100 RPM for less than 10 minutes. The beaker was taken out from the stirrer and allowed the left over pieces to settle at the bottom. The supernatant was filtered through sterile double layered muslin cloth into a fresh, sterile beaker. The filtrate was taken into a 50 mL tube and centrifuged at 3000 RPM for 5 minutes. The resultant pellet was re-suspended in 5 mL growth medium, M-199 (Sigma-Aldrich, USA) with Fetal Bovine Serum (FBS; Gibco by Life Technologies, USA) to stop the action of trypsin.

The cells with medium were centrifuged at 3000 RPM for 3 minutes. The ensuing pellet was re-suspended in 5 mL of growth medium

containing 10% FBS, 1% Tryptone Phosphate Broth (Sigma-Aldrich, USA) and Antibiotic Antimycotic solution (100000 units Penicillin, 10mg Streptomycin and 25µg Amphotericin B; HiMedia).

The cell suspension was to diluted $1x10^6$ cells /mL in growth medium with the help of hemocytometer or electronic cell counter, and seeded approximately $2x10^5$ cells /cm^2 in each 25 cm^2 tissue culture flask. The culture flasks were incubated in humidified incubator at 37^0C temperature with 5% CO_2 and 95% air. Further, the medium was changed at regular intervals (36-48 hours) as dictated by the depression of pH by changing the colour of medium to pink to yellowish.

The optimal conditions for hepatocyte proliferation require a balance of necessary components for cellular proliferation. Further, these cells grow in patches and extend from all the sides. Though the cells with the above said protocol can proliferate and survive for 8 to 10 days from the day of culture, further studies are required to maintain the cellular growth and multiplication. Thus, it is critical to optimize cell culture for each experiment type and to use as long as possible identical batch reagents and media.

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