Ultrastructural study of the effect of cultural media on the cultured hepatocytes.

Thesis

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By

Sarah Hassan Tawfik

M.B.B.Ch

Under Supervision of

Prof. **Ali Ahmed Fouad El Hendawi**

Professor of pathology

Faculty of Medicine

Cairo University.

Prof. **Dr. Soheir saied Mansy**

Professor of pathology

Electron microscopy department

Theodor Bilharz Research Institute.

Faculty of medicine

Cairo University

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Equipments and Consumables: Automatic pipettes (Eppendorf, USA), stainless steel mesh, metal comb, surgical set, gloves and sutures, sterile syringes, pipette aid and pipettes (Falcon, USA), tips, vials with caps, conical tubes, plastic cups and petri dishes (Corning, USA).

Apparatus: Hemocytometer (Paulmarine, Germany), Perfusion pump (Unipan, type 371, Poland), Incubator (Labtech, Korea), Shaker (rocking type MR-1, BOECO, Germany), Light microscope (Olympus, USA), Cooling centrifuge (Heraeus, Germany), Refrigerator and Deep freezer (Brunswick Scientific, USA).

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Abstract

Background: In vitro culture of isolated rat hepatocytes faces so many challenges in order to maintain hepatocytes' functional and morphological characteristics without deterioration for a prolonged period of time. Numerous literature reports documented these challenges and difficulties and proposed solutions for them in order to meet the requirements of hepatocytes-culture applications such as bioartificial liver support systems that are used as a bridge to transplantation for patients with intractable liver diseases.

Aim of the work: The aim of this work is reviewing previously published literature concerning hepatocytes culture media with a practical ultrastructural study of hepatocytes in basic culture media as a first step for a forthcoming more specialized work.

Materials and methods: This study was conducted on isolated hepatocytes from Wister rats weighing 200-250gms. Isolated hepatocytes were cultured in RPMI 1640 growth media formulation supplemented with FBS under four different culture conditions. Condition A; hepatocytes were cultured in 10% FBS supplemented growth media formulation, on non coated culture plates. Condition B; hepatocytes were cultured in 7% FBS supplemented growth media formulation, on non coated culture plates. Condition C; hepatocytes were cultured in 10% FBS supplemented growth media formulation, on collagen coated culture plates. Condition D; hepatocytes were cultured in 10% FBS supplemented growth media formulation, on polylysine coated culture plates. Freshly isolated and cultured hepatocytes under different conditions (at fixed time intervals, i.e. 24 and 48 hours after primary culture) were processed for light and electron microscopic examination using Zeiss light microscope and Philips TEM 208 S electron microscope respectively.

Results: By light microscopic examination, freshly isolated hepatocytes were mainly arranged in the form of groups and clusters formed of mononucleated and binucleated hepatocytes with well defined cell borders. Only few hepatocytes were found separate with ill defined cellular membranes. Cultured hepatocytes under all tested conditions showed reduction in clustering and grouping 24 hours after their primary seeding except for condition B under which hepatocytes retained their grouping ability. Extensive necrosis was detected 48 hours after hepatocytes primary culture under all culture conditions except for condition D under which viable hepatocytes groups were detected. Electron microscopic examination of freshly isolated hepatocytes showed groups of rounded cells with well defined intact cell membranes and surface microvilli, few hepatocytes with damaged cell membranes were detected. No intercellular tight junctions or bile canalliculi were found. Ultrastructural study of hepatocytes' clusters formed under condition B revealed clusters of hepatocytes with ill defined cellular membranes, with no bile canaliculli or intercellular junctional connections. Signs of cellular

degeneration were detected 48 hours after hepatocytes culture under conditions C and D, with mitochondrial relative resistance for degradation when compared with other cellular organelles.

Conclusion: This work emphasized on the need of in vitro hepatocytes culture models to extra supplements such as hormones and growth factors beside FBS and substrate coating for prolonged maintenance of hepatocytes specialized phenotype. This study also declared the importance of electron microscope for the follow up of cultured cells as it may confirm or contradict the results of light microscopic examination.

Key words: Transmission electron microscope – light microscope – cell culture media – cultured hepatocytes.

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- BAL: Bioartificial liver devices.
- BSC: Biological safety cabinets.
- CDC: Centre for disease control and prevention.
- C NOS: Calcium dependant nitric oxide synthase.
- DMEM: Dulbeco Modified Eagle's Minimal Essential medium.
- EC: Endothelial cells.
- ECM: Extracellular matrix.
- EGF: Epidermal growth factor.
- FBS: Fetal bovine serum
- FCS: Fetal calf serum.
- FGF: Fibroblast growth factor.
- GFAB: Glial fibroblast acidic protein.
- GM-CSF: Granulocyte-Macrophage colony stimulating factor.
- HEPES: Hydroxyethyl piperazin e-n-2-ethane sulfonic acid.
- HGF: Hepatocyte growth factor.
- HSPGS: Heparan sulphate proteoglycans.
- IL: Inetrleukins.
- KGF/FGF 7: Keratinocyte growth factor/fibroblast growth factor.
- MCSF: Macrophage colony stimulating factor.
- MEM: Minimal essential medium.
- NPC: Non parenchymal cells.
- OLT: Orthostatic liver transplants.