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Ying Gao Balch*

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Research Article

Subculture human skeletal muscle cells to produce the cells with different Culture medium compositions

Ying Gao Balch
The University of Arkansas at Pine Bluff, Department of Human Sciences
Corresponding Author: Ying Gao Balch, The University of Arkansas at Pine Bluff, Department of Human Sciences
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Abstract

This study aimed to subculture human skeletal muscle cells (HSkMC) using a culture medium with different compositions to determine the most efficient medium for the growth of the human skeletal muscle cells. The culture media was divided into three groups: Group1. An HSkMC growth medium. Group 2. An HSkMC growth medium + with 10% high glucose (GH). Group 3. An HSkMC growth medium + 10% fetal bovine serum (FBS). HSkMC from groups 1 to 3 gradually became round in shape and gathered in clusters. These changes differed between the groups. In group 3, the HSkMC clusters were more in numbers and gathered as significantly more prominent than in the other groups under the EVOS-Microscope shown. We concluded that by manipulating the composition of the culture medium, it is possible to induce HSkMC to promote the best growth.

Keywords: human skeletal muscle cells (hskmc); EVOS-Microscope

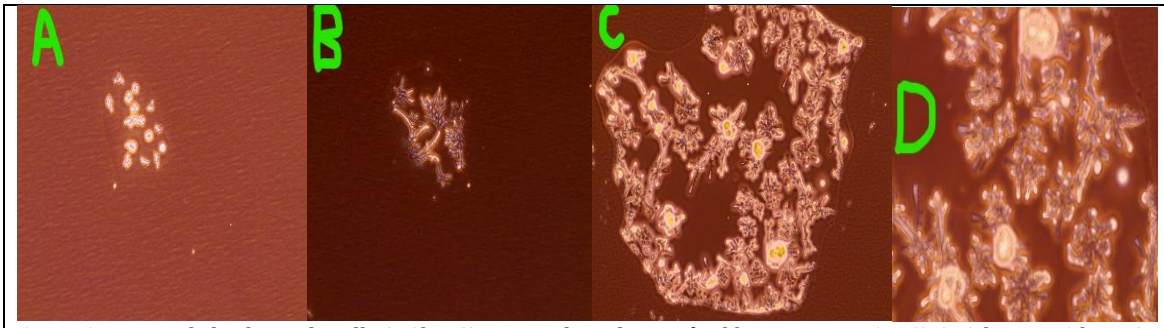
Introduction:

Evidence suggests that inflammation and apoptosis play a role in a skeletal muscle cell associated with diminished skeletal muscle cell function with healthy aging [1, 2-5]. Besides, Apoptotic signaling pathways can activate cysteine-dependent, aspartate-specific proteases (caspases-3), which are the end of proteases and integral to the final produced cell death [6-7]. Caspases-3 typically exists in an inactivated state in the cytoplasm, but it can be activated by proteolytic cleavage, and then hetero-dimerization and activates pro-caspase-3 start the caspase cascade [8, 9]. Once the proteolytic cascade is switched on, it eventually cleaves and enables pro-caspase-3 to induce the inflammation to the loss of skeletal muscle [9]. This study aimed to subculture human skeletal muscle cells (HSkMC) using culture media with different compositions to determine the most efficient medium for the growth of the human skeletal muscle cells to ensure that the research experiment more efficient.

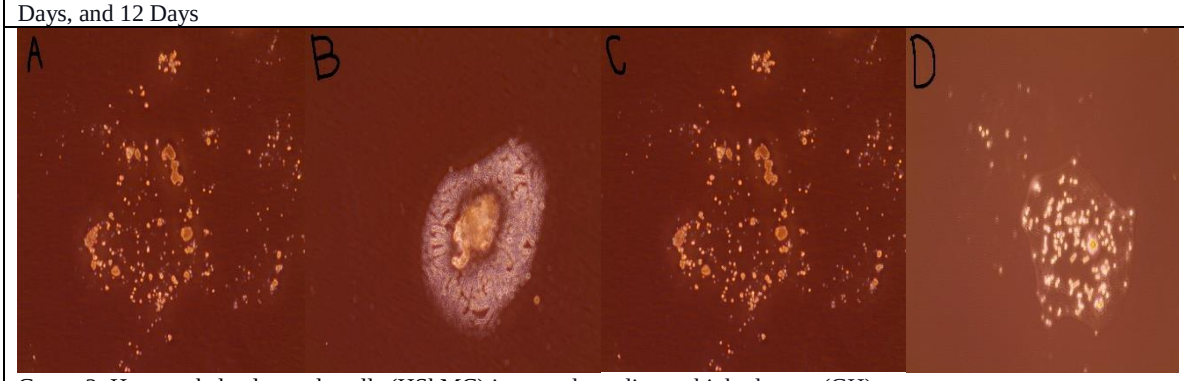
Materials and methods:

Human skeletal muscle cells source: normal human skeletal muscle. Single donor: 52 years old black female and in the formulation of cryopreserved at the second passage in basal medium containing 10% FBS and 10% DMSO (Cell Application, INC.).The Growth Medium at 4°C in the dark immediately upon arrival. Cryopreserved Vials have stored cry vials in a liquid nitrogen storage tank immediately upon arrival. To examine under a microscope to check if all the cells were attached to the bottom of the flask. Decontaminated the exterior of the cell culture flask with 70% alcohol. Placed the sealed flask in a 37°C, 5% CO_2 incubator for 2 hours as shipped. In a sterile Biological Safety Cabinet, opened the flask's cap very slowly and carefully, removed the transport medium by aspiration, and added fresh growth medium: 15 ml for a T-75 flask. Placed the flask in a 37°C, 5% CO_2 humidified incubator with the loosened cap to allow gas exchange. To change the medium every other day.

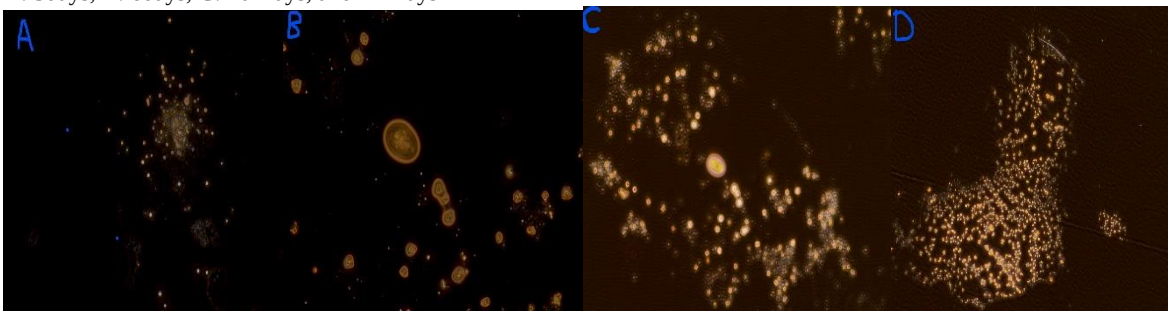
Preparing cell culture flasks for culturing HSkMC: To ensure the Biological Safety Cabinet, with HEPA filtered laminar airflow, was in proper working condition. Clean the Biological Safety Cabinet with 70% alcohol to ensure it was sterile. It was turned the Biological Safety Cabinet blower on for 10 min before cell culture work. To make sure all serological pipettes, pipette tips, and culture medium were sterile. All of them follow the standard sterilization technique and safety rules. It should always be wearing gloves and safety glasses when working with human cells even though all the strains have been tested negative for HIV, Hepatitis B, and Hepatitis C. Handle all cell culture work in a sterile hood.



Group 3 Human skeletal muscle cells (HSkMC) in growth medium + fetal bovine serum (FBS) A: 3days, B: 6days, C: 10 Days, and 12 Days



Group 2: Human skeletal muscle cells (HSkMC) in growth medium + high glucose (GH)
A: 3days, B: 6days, C: 10 Days, and 12 Days



Group 1 Human skeletal muscle cells (HSkMC) Growth medium
A: 3days, B: 6days, C: 10 Days, and 12 Days

Preparing subculture mediums:

Took the Skeletal Muscle Cell Growth Medium from the refrigerator and decontaminated the bottle with 70% alcohol in a sterile hood. Pipetted 15 ml of Skeletal Muscle Cell Growth Medium into 20 ml for each T-75 flask with different culture medium: Group 1. HSkMC growth medium. Group 2. HSkMC growth medium + 10% high glucose (GH). Group 3. HSkMC growth medium + 10% fetal bovine serum (FBS). Placed the flask in a 37°C, 5% CO_2 humidified incubator with the loosened cap to allow gas exchange. To change the medium every other day. Water treated in a Milli-Q water purification system (TGI Pure Water Systems, USA). HSkMC growth medium, high glucose medium, and FBS of all acquired from (USA).

Results:

The three groups' culture medium, 1 to 3, gradually became round in shape and gathered in clusters under the EVOS-Microscope shown. These changes differed between the groups. In group 3, the HSkMC clusters were more in numbers and gathered as bigger aggregates under EVOS-Microscopy shown. That groups 1 and 2 were similar in terms of the mean area of each aggregate; however, only in group 3, the number of aggregates and the total size of aggregates clusters were significantly more than in the other groups. We concluded that by manipulating the composition of the culture medium, it is possible to induce HSkMC to promote the best growth.

Discussion

HSkMC was capable of growing by manipulating the culture medium composition. In groups 1 to 3, HSkMC got rounder increasingly during culture. Under the EVOS-Microscope shown, HSkMC was kept in culture media with a high glucose concentration without FBS; however, this characteristic led to a decreased cellular density, smaller formation, and aggregation of oval-round cellular clusters comparison with group 3. In group 3 exhibited the most significant results for aggregates clusters with observation under the EVOS-Microscope.

In all three groups in which essential medium was used as primary culture medium, it was observed the formation of cellular clusters; Group 2 was exposed to a stimulus with high glucose in the absence of FBS. FBS provides essential nutrients and growth factors to human skeletal muscle cells in culture and sustaining their proliferation [10, 11, 13]. Besides, FBS also has antioxidant properties, thus being critical for cellular survival. Therefore, groups 1, 2 of this study have observed the apoptotic effect caused without FBS [15]. High glucose concentration has been

considered to be an effective inductor of the human cells. Still, some problems have been associated with its use throughout the culture. Some researchers have stated that a high concentration of glucose in culture media for a long time causes oxidative stress, cellular damage, early senescence, and apoptosis [15, 16]. Thus, it is likely that the absence of antioxidant extrinsic factors, such as β-mercaptoethanol, might have contributed to a more significant cellular loss by oxidative stress in group 1. 2. This oxidative stress can be caused by a high glucose concentration for an extended period and the absence of FBS [12].

EVSO-Microscope analysis shown that in groups 1 and 2 in the oval-round cell clusters were less numerous and more isolated from each other than those in group 3 and 12 final days of group 3. Therefore, it was believed that the addition of extending days had promoted more efficiency by the effect of supplements like FBS during 12 days. Fetal bovine serum is composed of a mixture of hormones, growth factors, antibodies, and protein components responsible for maintaining cellularity in many cell cultures [14]. Moreover, Researchers believe that adding specific substances to the medium can promote human skeletal muscle cells' best growth at the proper culture mediums, like adding extrinsic factors, supplements, and a high glucose concentration.

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