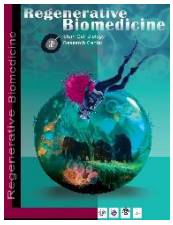

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Workshop Report

Human Embryonic and Mesenchymal Stem Cells: Report of a 1-day workshop held at Stem Cell Biology Research Center, Yazd, on 14th May 2025

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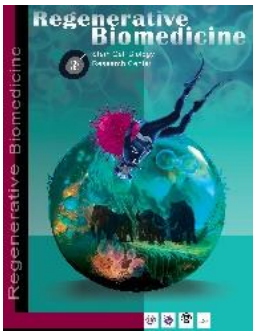
Abstract

This report provides a brief summary of the proceedings from one-day workshop entitled “Human Embryonic and Mesenchymal Stem Cells” held at the Stem Cell Biology Research Center based in Yazd Reproductive Sciences Institute at Shahid Sadoughi University of Medical Sciences, Yazd, Iran on 14th May 2025. This workshop introduced participants to the biological characteristics, culture methods and laboratory handling of human embryonic stem cells (hESCs) and mesenchymal stem cells (MSCs). ESCs are derived from the inner cell mass during the blastocyst stage of the developing embryo *in vitro*. They are pluripotent and can theoretically give rise to any type of cells and also provide an ideal screening tool for potential drugs in the pharmaceutical industry and allow the study of early human development and infant cancers. Co-culture with a feeder layer of inactivated human or mouse embryonic fibroblasts is the conventional method for the hESCs culture and expansion that providing a facile stem cell expansion system with continuous medium conditioning while effectively preventing the interaction between hESCs and feeder cells. MSCs as a representative of adult stem cells can be isolated from almost all tissues and effectively expanded *in vitro*. These *in vitro* expanded cells have been shown to possess the potential to differentiate into specific cell lineages.

Keywords: Human Embryonic Stem Cells, Mesenchymal Stem Cells, Human Foreskin Fibroblast (HFFs), Co-culture, Workshop



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Introduction

One of the most available sources of human fibroblasts is neonatal foreskin that can be isolated from both neonatal and adult foreskin tissues. Fibroblasts are spindle-shaped, elongated, cells known for their strong ability to migrate and proliferate. They are the major cellular parts of connective tissues with multiple biological functions. They communicate with neighboring cells and tissues through by secreting extracellular matrix molecules, cytokines, growth factors and chemokines. Not only do these cells have wound-healing applications but they are also the most popular source for pluripotent stem cell biotechnology (1). Following the first derivation of human embryonic stem cells in 1998 using mouse embryonic fibroblasts (2), hFFs have been used as a human source feeder layer (3, 4, 5) to prevent the risk of animal pathogen transmission to hESCs and their derivatives for future cell-based therapeutic applications (6). Here, we have used Yazd human foreskin fibroblast (YhFF#8), produced in our center (1), as a human feeder source.

In this regard, one-day workshop titled "Human Embryonic and Mesenchymal Stem Cells" has recently been held by the Stem Cell Biology Research Center based in Yazd Reproductive Sciences Institute at Shahid Sadoughi University of Medical Sciences. This workshop was divided in to two sessions. During the workshop two types of cells (YhFF#8 and Yazd 4) were used in practical sessions.

In addition to the practical sessions, Dr. Behrouz Aflatoonian, the head of the Stem Cell Biology Research Center explained about the Stem Cells and their classification (Fig. 1).

Figure 1. Theoretical session to introduce stem cells by Dr. Behrouz Aflatoonian.

The first practical session was about human mesenchymal cells.

In this part of the practical session, passage of YhFF#8, P14 as a feeder layer for Yazd4 hESCs (Yazd4, P5+6) was taught. Practical session began with the passage and culture of the YhFF#8, first by observing, and then each of the attendee passage one flask individually. For this method, culture medium was removed from a tissue culture flask of YhFF#8 and the cells were washed with phosphate buffered saline (PBS, without calcium and magnesium) then Trypsin-EDTA 0.25% was added and flasks incubated at 37°C at 5% CO2 for 3 min. Flasks were tapped to detach of the cells from the bottom of the flasks. Trypsin-EDTA was neutralized by adding Dulbecco´s Modified Eagle Medium (DMEM, high glucose) enhanced with 10% fetal bovine serum (FBS). The mixture was drawn up and transported to the conical tube to centrifugation at 1000 rpm for 5 min. The pellet was seeded in to a tissue culture flask containing DMEM+10% FBS medium and incubated in the humidified atmosphere at 37°C at 5% CO2. The second practical session

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focused on the passage and vitrification of hESCs (Fig. 2) (3, 5, 6).

Figure 2. Practical session to train attendees on working in Human Embryonic Stem Cells by Mahdieh Karimi.

For this session YhFF#8 were treated with mitomycin C for 4 hours to induce mitotic inactivation. After washing three times with PBS, the cells were detached using trypsin-EDTA. The cells centrifuged at 1000 rpm for 5 minutes, and then plated into 50µl microdrops at a concentration of 3500 cells per drop and covered with mineral oil on dishes (Each dish was plated with seven microdrops of feeder layers). The next day, the microdrop medium containing the feeders was substituted with human ES medium (HES medium) (containing KnockOut DMEM (K.O.DMEM), KnockOut Serum Replacement (K.O.SR), L-Glutamine/B-mercaptoethanol solution, Non-Essential Amino Acids (NEAA), bFGF, Gentamicin). The hESCs (Yazd4; (5)) were mechanically passaged using a mouth pipette and pulled Pasteur pipette. For vitrification, small pieces (larger than pieces usually used for passaging) of hESC colonies were scraped off and transferred in to a small drop of HES medium. Then, colonies were transferred in to homemade vitrification solution 1 and 2 (VS1 and VS2) containing DMEM+20%FBS, NEAA, Dimethyl Sulfoxide (DMSO), Ethylene Glycol and Sucrose. Small colonies were drawn on to the cryotech and cryowing tools (3), plunged in to liquid nitrogen and then transferred to long cryovials which were sealed with cap located in the cryocane and placed in a liquid nitrogen storage container.

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Conflict of interest

The authors have no conflict of interest to declare.

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