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Technical procedure in vitro evaluation of human stem cell growth using the MTT assay

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Abstract: The aim of this study is the practical determination of the proliferation rate of human stem cells in a biological environment using laboratory methods. Stem cells are unique in their ability to self-renew and differentiate, which distinguishes them from most differentiated cells, such as cardiac muscle cells, which cannot regenerate after injury. This makes stem cells highly significant in the fields of regenerative medicine and experimental pharmacology. The study utilized the MTT assay, a colorimetric technique that enables the visualization of cell viability through mitochondrial activity. Viable cells reduce MTT into a formazan dye, measurable by a spectrophotometer. The experiments were conducted using an inverted microscope and spectrophotometer in laboratory conditions. Based on data evaluation, we assessed the proliferation activity and formulated conclusions about the mitotic behavior of stem cells under controlled in vitro conditions. This method offers a useful approach for monitoring cellular responses to pharmacological treatments and for supporting tissue regeneration research.

1 Introduction

This article focuses on the practical assessment of human stem cell proliferation in a biological environment using the colorimetric MTT assay and standard laboratory equipment. The regeneration of tissues and cells is a key topic in modern biomedical research, especially for conditions involving irreversible cell damage, such as heart attacks where lost cardiomyocytes cannot regenerate naturally [1].

Stem cells are unique due to their ability to self-renew and differentiate into various cell types—a property known as pluripotency—making them valuable tools in regenerative medicine and drug testing.

Proliferation, the process of increasing cell numbers through mitosis, must be tightly regulated to maintain tissue balance and prevent disease. This article includes theoretical background on cell metabolism and proliferation, specifics of stem cell growth, and detailed steps for conducting the MTT assay.

Authors Niu at al. state that the MTT assay is based on the conversion of yellow MTT to purple formazan by mitochondrial dehydrogenases in living cells, providing a reliable method for quantifying cell viability. In the theoretical background, they emphasize that the amount of formazan produced is directly proportional to the number of viable cells, making the MTT assay an effective tool for assessing cell proliferation and cytotoxicity [2]. The MTT test is a simple and effective method for quantifying cell viability and proliferation. It measures the conversion of yellow tetrazolium to purple formazan in the mitochondria of living cells. The resulting product is dissolved and analyzed by spectrophotometry, providing an indirect measure of live cell count and growth over time.

The practical component was carried out under laboratory conditions using accessible equipment, demonstrating the MTT assay as a reliable tool in biomedical research [1,3,4].

2 Stem cells and cell proliferation

Stem cells are undifferentiated cells with the ability to self-renew and differentiate into various cell types. They are essential for embryonic development and tissue regeneration in adults. Mesenchymal stem cells (MSCs) can replicate continuously and specialize into multiple cell types—a property called pluripotency.

Stem cells vary in their potency:

- Totipotent: capable of generating all cell types including embryonic and extraembryonic tissues.
- Multipotent: can produce several related cell types.
- Unipotent: limited to one specific cell type.

Sources include:

- Placental and amniotic fluid: high potential and fewer ethical concerns.
- Umbilical cord blood: rich in hematopoietic and mesenchymal stem cells.
- Adult tissues: like bone marrow and fat, offering accessible research material.

Cell proliferation—the increase in cell number—is crucial for tissue growth and healing but must be tightly regulated. Disruptions in this process are linked to diseases like cancer.

The MTT assay is a common method to assess cell viability and proliferation in vitro. It measures the reduction of the yellow MTT compound into purple formazan by metabolically active cells. Absorbance is read spectrophotometrically, indicating the number of viable cells. Though simple and cost-effective, the method depends on mitochondrial activity and may not reflect actual proliferation under all conditions [1,3,5,6].

3 Results and discussion

3.1 Stem cell proliferation testing

MTT assay is a colorimetric technique widely used to assess cell viability by measuring mitochondrial activity, which reflects the number of living cells. The assay relies on the ability of active mitochondria in viable cells to reduce the yellow tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) into dark purple formazan crystals. Dead or damaged cells lack this capability, making the method a sensitive indicator of cell health and proliferation [7,8].

In the experiment, the MTT substrate was introduced into 96-well microplates containing cultured cells. The conversion of MTT to formazan was carried out overnight, with the formazan product being insoluble and settling at the bottom of the wells. To enable accurate spectrophotometric measurement, a solvent such as DMSO (dimethyl sulfoxide) was added the next day to dissolve the crystals into a homogenous purple solution. The absorbance of this solution, directly proportional to cell number, was read using a spectrophotometer [7,9,10].

3.2 Cell culture procedure

Proper cell culture techniques are essential to ensure consistent and reliable results in any biological assay. In this study, MSCs were maintained under sterile in vitro conditions using essential laboratory equipment such as laminar flow cabinets, CO_2 incubators, centrifuges, inverted microscopes with imaging systems, and dry heat sterilizers. The culture environment was carefully controlled to replicate physiological conditions, including temperature (37°C), humidity, and CO_2 levels (5%) to maintain pH via a bicarbonate buffering system.

Cells were grown in a nutrient-rich culture medium composed of DMEM/F-12 mixed 1:1 with Alpha MEM, supplemented with 5 mL of fetal bovine serum (FBS). This medium combination provided essential amino acids, glucose, vitamins, and trace elements necessary for optimal growth and differentiation. Sterility was maintained at every step, and all materials were pre-sterilized or handled under aseptic conditions. The medium was changed every 2–3 days to replenish nutrients and remove waste products.

Additional steps involved preparing the working environment in the laminar box with UV sterilization before initiating any cell handling. All instruments, such as pipettes, Petri dishes, and media containers, were introduced into the sterile workspace without direct contact. Before and after use, the workspace was cleaned thoroughly. The cells were regularly observed under an inverted microscope to monitor morphology, density, and contamination [11,12].

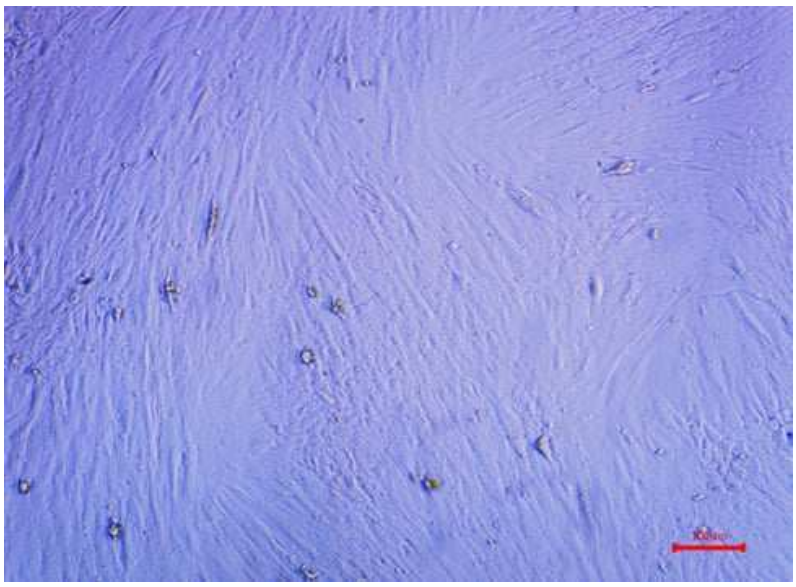

Figure 1 MSCs on day 20 of cultivation

3.3 Execution of the MTT assay

Figure 1 shows MSCs on the 20th day of cultivation at each point, the yellow MTT reagent was added to the wells, and the cells were incubated for approximately 3 hours at 37°C to allow sufficient conversion to purple formazan by viable cells. After incubation, DMSO was added to dissolve the formazan crystals, resulting in a uniform-colored solution suitable for quantification. On figure 2 there is the conversion of MTT to formazan.

Figure 2 Conversion of MTT to formazan

The absorbance was measured at a specific wavelength using a spectrophotometer. Positive control wells containing only live cells and blank wells with only media were included to ensure measurement accuracy. The absorbance readings from blank wells were subtracted from sample readings to eliminate background noise and isolate the true signal of cellular metabolic activity.

Cell detachment was performed using trypsin, a proteolytic enzyme that facilitates the removal of adherent cells from culture vessel surfaces. The trypsinized cells were centrifuged to form a pellet and resuspended in fresh medium before being distributed evenly into wells. Care was taken to ensure uniform volume across wells (typically 100 µL) to prevent errors in optical readings. The figure 3 shows the 96 – well plate after the addition of DMSO. On figure 4 is shown the operation of trypsin.

Following the assay, the spectrophotometric data was analyzed to assess cell growth dynamics. Higher absorbance indicated increased proliferation. Table 1 shows the measured values, and it is clearly visible that the viability in sample 1 was 87.78%, while in sample 2 it was 84.07%. This means that a higher number of live cells was present in sample 1 [2,13-15].

Table 1 Measured averaged spectrophotometer values with resulting concentrations

	1	2	3	4	5	6	7	8	9	10	11	12	
A			sample 2	sample 2C							B	Control	490
B			0.252	0.233							0.159	0.282	490
C			0.238	0.233							0.153	0.291	490
D			0.228	0.215							0.154	0.251	490
E			0.232	0.227							0,153	0.259	490
F		average	0,237	0,227								0,270	490
G		%	87,78	84,07								100%	490

The concentration was calculated by direct proportion, according to the formula:

$$c = \frac{absorbance\ of\ sample - absorbance\ BLK}{absorbance\ of\ control - absorbance\ BLK} \cdot 100\%$$

After placing the cells into a 96-well plate, tetrazolium is added to each well, and the plate is then incubated for three hours at 37°C. During this time, the mitochondria of viable cells convert the MTT substrate into purple formazan. As a result of this activity and conversion, dark purple crystals are formed. These dark purple crystals settle at the bottom of the wells. Figure 5 shows straining with tetrazolium.

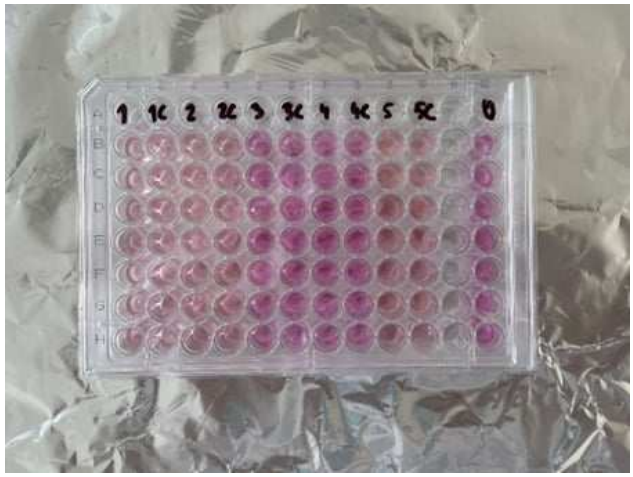

Figure 3 96-well plate after the addition of DMSO solvent

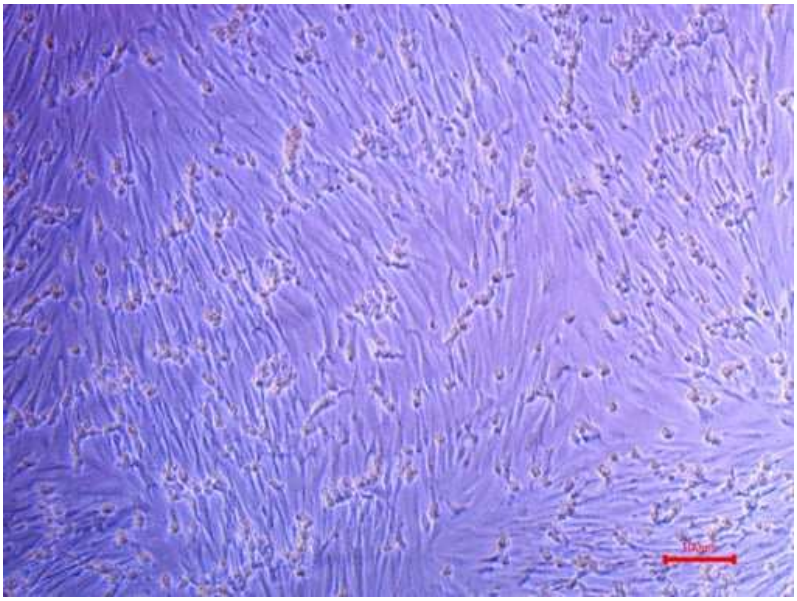

Figure 4 Action of trypsin immediately after application to cells

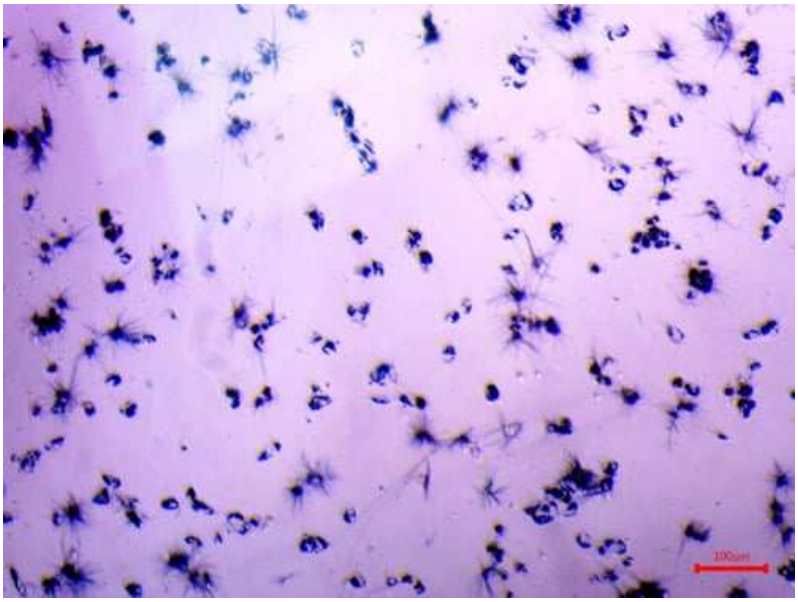

Figure 5 Formed crystals of purple formazan

4 Conclusion

This article focuses on evaluating the proliferation of human mesenchymal stem cells using the colorimetric MTT assay. This method quantifies cell viability based on metabolic activity and serves as an indirect indicator of proliferation under laboratory conditions.

In the practical part, cells were cultured over several days, and their activity was assessed by measuring absorbance via spectrophotometry. The results confirm that the MTT assay is suitable for monitoring cell growth in vitro. This experimental approach also allows observation of environmental effects on proliferation and can be applied to assess the cytotoxicity of tested substances.

The main contribution of this work lies in the practical validation of the methodology using accessible lab equipment and its successful application for tracking cell growth. Limitations of the method were identified—primarily its dependence on mitochondrial activity, which may not always reflect true proliferation. Therefore, complementary techniques such as BrdU incorporation, Ki-67 staining, or flow cytometry are recommended for a more complete view of the cell cycle.

Future research could explore other stem cell types, their behavior under different culture conditions, and their response to growth factors or drugs. These studies could help optimize culture protocols and support the development of effective regenerative medicine strategies.

The MTT assay, despite being simple and cost-effective, has limitations, such as dependence on mitochondrial activity, which may not always represent true proliferation. Therefore, it is recommended to combine it with complementary assays like BrdU incorporation, Ki-67 staining, or flow cytometry for a comprehensive understanding of the cell cycle and viability.

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