



The Effect of Adherent and Non-Adherent Culture Plates on the Detachment of PMA-Treated THP-1 Cell Line

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ABSTRACT

Introduction: Due to their role in regulating inflammation, monocytes, and macrophages are important immune system cells incorporated and evaluated in various *in vitro* and *in vivo* disease modeling experiments. Accordingly, investigating appropriate culture conditions to maintain the viability, phenotype, and functionality of these cells is considered in different studies. In this study, we tried to evaluate whether the type of culture plate affects the adhesion, survival, and morphology of PMA-treated monocytes.

Methods: The THP-1 cell line was cultured in adherent or non-adherent culture plates and cells were treated with PMA small molecule to be induced into macrophages. The morphology of treated cells and the viability of detached cells were assessed three days post-induction.

Results: Our results showed that the morphology and viability of PMA-treated THP-1 cells were the same in both types of plates.

Conclusion: We showed that the type of cell culture plate did not significantly affect PMA-treated THP-1 cells.

Keywords: THP-1 cell line, Macrophage, Adhesion, Culture plate, Morphology.

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INTRODUCTION

Macrophages, which are essential elements of the innate immune system, have key functions in defending against pathogens, causing inflammation, and maintaining tissue homeostasis (1, 2). Macrophages perform phagocytosis of pathogens, release cytokines and antimicrobial substances, and display antigens to activate adaptive immune cells. (1, 3, 4). It has been shown that macrophages display significant diversity, with distinct functional phenotypes that react to different environmental cues (3, 5). They can be traditionally activated (M1 phenotype) by pathogens and Th1 cytokines to generate proinflammatory substances. Alternatively, they can be activated (M2 phenotype) to contribute to the termination of inflammation and enhance the tissue repair (2,

4). Tumors, as abnormal tissues, are also infiltrated by tumor-associated macrophages (TAMs), which support angiogenesis, metastasis, and immunosuppression (1, 3). Therefore, macrophages and their cell of origin, monocytes, are cells of interest in various studies focusing on inflammation-affected conditions, including cancers.

Studies have shown that the type of substrate surface that macrophages are cultured on can affect their characteristics and functions. For example, when comparing a standard adherent (Ad) plate to a non-adherent (non-Ad) plate, researchers found that macrophages grown on non-Ad plates showed increased expression of pro-inflammatory genes and enhanced functional responses. This observation included the activation of signaling pathways attributed to the

inflammation, particularly when macrophages were exposed to inflammatory stimuli like lipopolysaccharide (LPS). These findings indicated that the surface of the culture dish can influence the behavior and immune responses of macrophages (6-8).

As another importance of the choice of appropriate culture plate, it should be noted that macrophages are very adhesive to Ad plates and their harvest is considered as a challenging step. Accordingly, the method used to detach macrophages from the culture plate is influential in maintaining the cell viability, the integrity of cell surface markers, and its functionality (7, 9, 10). There are two common ways to detach cells from culture plates: enzymatic methods (for example, by utilizing trypsin or accutase) and non-enzymatic methods (for example, by the usage of phosphate-buffered saline (PBS) and EDTA) (10).

Therefore, as the type of cell culture dish significantly affects the macrophage attachment, leading to variations in their phenotypic characteristics and functional capabilities, the choice of dish surface and the detachment method is crucial for accurately studying macrophage biology and its roles in immune responses. Understanding factors affecting the behavior of macrophages in the culture condition can help optimize their in vitro culture for further studies, ultimately enhancing the translation of findings to in vivo situations (7, 10, 11). In this regard, in this study, we tried to examine how the type of culture plate affected the adhesion, harvest, and survival of THP-1-induced macrophages.

MATERIALS AND METHODS

Monocytic Cell Line Culture

THP-1 monocytic cell line purchased from Pasteur Institute of Iran was cultured in RPMI1640 culture medium (Biosera, LM-R1638/500) supplemented with 10% FBS (Gibco, 20511), 1% L-glutamine (Gibco, 25030-024), 1% penicillin, 1% streptomycin (Gibco, BI-1203), and 1% NEAA (Gibco, 11140-035) in an incubator at 37°C and 5% CO₂. Cells were subcultured at a 1×10^6 cells/ml density.

THP-1 Differentiation

THP-1 cells were differentiated towards M0 macrophages by treatment with 100 ng/ml of PMA (Gibco, P8139) in a medium, containing RPMI1640, 10% FBS, 1% L-glutamine, 1% penicillin/streptomycin, and 1% NEAA for 48 hours (12). Two types of Ad and non-Ad plates were used, and 1×10^6 cells/ml were seeded. After 48 hours of treatment with PMA, cells were rested for 24 hours and the medium without PMA was added.

Macrophage Harvest from Culture Plates

For the detachment of differentiated cells cultured on Ad or non-Ad plates, cells were washed with PBS (Biobench, 001) and incubated with EDTA (5mM) (Sigma, E5134-250G) in PBS (13) on ice for 40 minutes and after that, they were pipetted. Then, DMEM medium (Gibco, 121611) with 10% FBS was added. Detached cells were centrifuged at 1500 rpm for 5 min and washed with PBS containing 2% FBS.

Cell Viability Assessment

The total cell number and the percentage of viable cells were calculated by the cell counting method using a hemocytometer (Neubauer chamber) and cell staining with trypan blue dye (0.4%).

Statistical Analysis

Statistical analyses were performed using Prism version 9. Data were graphically represented as the mean $\pm$ standard deviation (SD). The statistical significance of differences between groups was determined by the unpaired student's t-test method. $P < 0.05$ was considered significant.

Ethical Considerations

The procedure performed in this study was approved by the Ethics Committee of Tarbiat Modares University (IR.MODARES.REC. 1402.042).

RESULTS

PMA treatment of THP-1 cells in different Ad or non-Ad plates

As presented in Figure 1, THP-1 cells had round and semi-adherent morphology before

differentiation induction; however, their morphology was changed into spindle-like and fried egg-like morphologies during PMA treatment in both culture plates. Moreover, 24 hours post-rest, the elongated adherent cells were obvious in both treated culture plates although untreated cells maintained their round and semi-adherent morphology (Figure 1).

Cell harvest-dependent factors in two different culture plates

The non-enzymatic method was employed to detach cells from both types of plates and two criteria were evaluated: the essential time for complete cell detachment and the cell viability. Our results showed that cells were detached from both culture plates 40 minutes post-incubation in a cold PBS/EDTA buffer. Moreover, as presented in Figure 2, more than 80% of cells were viable in both groups and there was no statistically significant difference between the two culture groups.

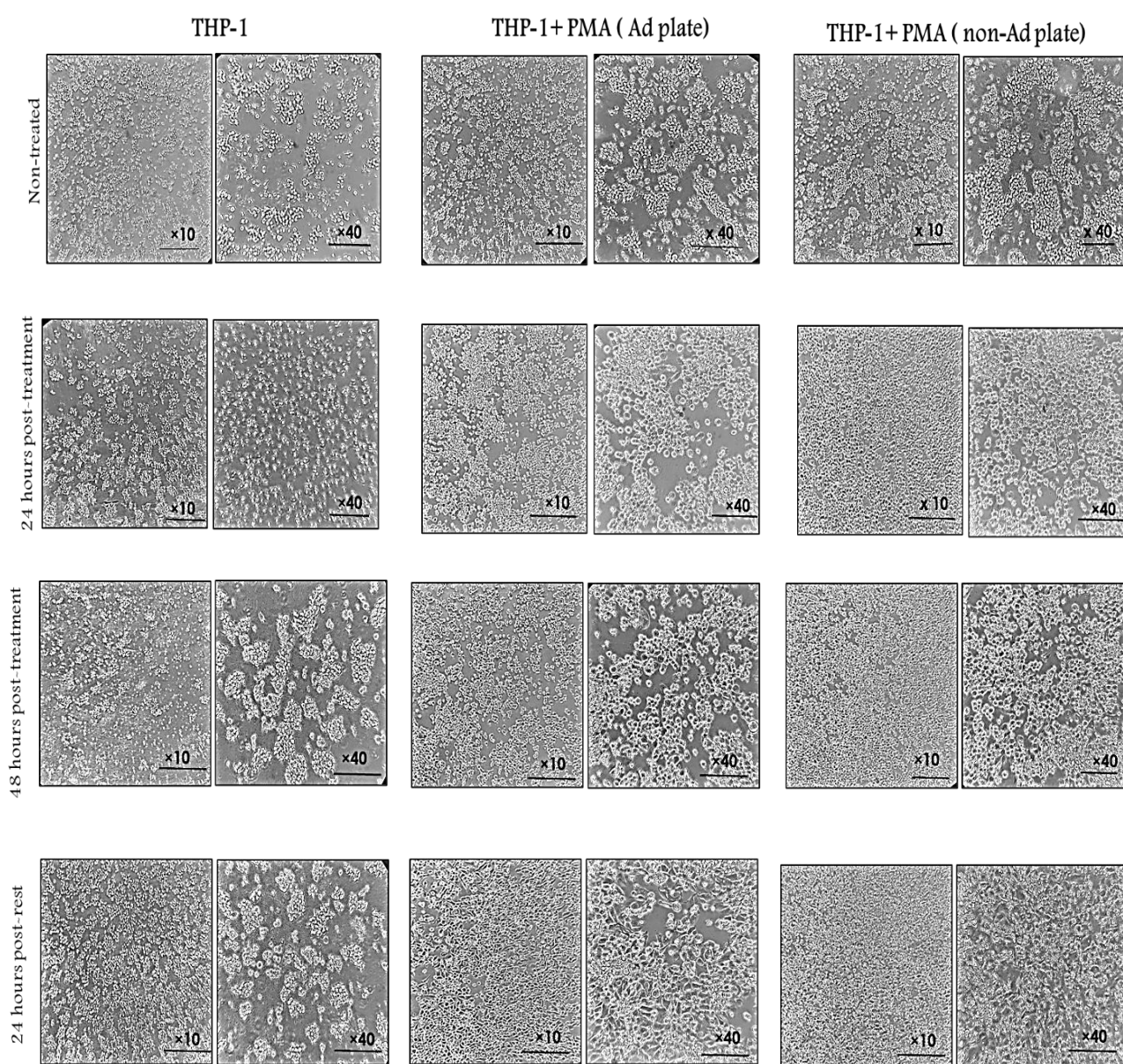


Figure 1. Morphology of THP-1 cells during treatment with PMA. Phase contrast images of THP-1 cells before and after treatment with PMA (100 ng/ml) in two different adhesives (Ad plate) and non-adhesive (non-Ad plate) culture plates.

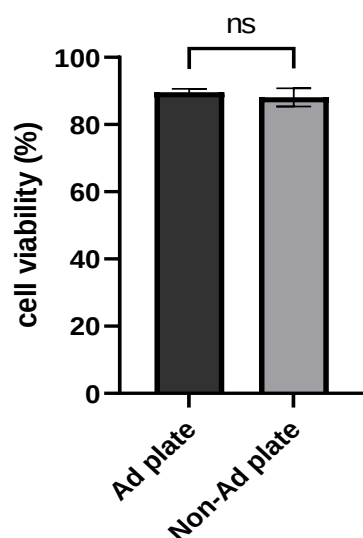


Figure 2. Cell viability percentages after cell harvest from two different culture plates. The “ns” indicates no significant difference between the groups.

DISCUSSION

Since monocytes and their derivatives (macrophages and dendritic cells) play important roles in regulating inflammation and innate immunity, researchers have studied their function and application in immunological research (14-16). In this regard, different types of monocytes from distinct sources, including cell lines or primary cells, have been used to be differentiated into macrophages although the source of origin may impact their functional characteristics and their applications in different experiments (17). The usage of THP-1 cell line has some key advantages over primary human monocytes, including providing a homogeneous cell population, minimizing donor-associated variability, being readily available, and the ease of culture and maintenance in *in vitro* culture conditions without the need for more specialized culture conditions (17). Genetically engineered THP-1 cell lines can be generated to overexpress or to be knocked down for specific genes of interest, making them suitable for mechanistic studies (18, 19).

THP-1 cells can be differentiated into macrophage-like cells, allowing for the study of various inflammatory responses and macrophage polarization (M1 and M2 phenotypes) under controlled conditions (20). While blood-derived monocytes are essential for studying human-specific immune responses, THP-1 cells can provide a practical and versatile alternative for simulating inflammation studies, particularly when consistency, ease of use, and genetic manipulation are critical required factors (20, 21).

In our study, the induction of THP-1 differentiation into macrophages was conducted according to Phuangbubpha et al.'s protocol (12), using a concentration of 100 ng/ml PMA for 48 hours. Although different studies have employed different concentrations and time points for the differentiation induction, Phuangbubpha et al.'s study suggests that concentrations lower than 100 ng/ml result in insufficient differentiation into macrophages (22), conversely, concentrations higher than 100 ng/ml lead to an increased number of dead cells and a higher rate of cell detachment (12, 23, 24). Additionally, in our study, although the expression of monocyte and macrophage surface markers was not investigated in the control and treated groups, we observed the cell morphology changes throughout PMA induction, as described in the Phuangbubpha et al.'s study, which might be attributed to the differentiation into macrophages.

In vitro culture conditions of monocytes and macrophages face several challenges that can affect experimental outcomes and the reliability of results. Macrophages tend to adhere tightly to culture surfaces, making them challenging to detach without influencing cell viability [23]. This issue can hinder the ability to perform routine passaging or experimental manipulations, necessitating the use of specific detachment techniques (7, 25). Addressing these challenges requires careful optimization of culture protocols, including selecting appropriate culture plates, culture media, harvest techniques, and environmental conditions to ensure that the cultured macrophages can accurately reflect their *in vivo* behavior. Accordingly, our results showed that the morphology and the detachment

process were the same for cultured cells in both Ad and non-Ad culture plates; these findings were consistent with those of Song et al. (7).

On the other hand, various techniques have been used to isolate *in vitro* cultured adherent cells for functional and phenotypic analyses. However, the cell harvest methods might influence the surface proteins and characteristics of the cultured cells. The culture of certain cells, such as macrophages, which might adhere firmly to culture dishes, requires optimum cell detachment strategies to achieve viable and functional cells (7, 8). As suggested in Song et al. study, the detachment of tightly adherent macrophages from culture dishes poses significant challenges (7). For instance, cell incubation with accutase (as an enzymatic detachment approach) has been shown to yield better recovery of viable cells compared to the incubation with other enzymes, particularly for macrophages cultured on tissue/cell culture plates (7). Although enzymatic digestion is a commonly used method for isolating cells, it often leads to the degradation of cell surface proteins and extracellular matrix components. Therefore, prolonged exposure to enzymes can lead to the loss of cell surface proteins, affecting surface marker-dependent cell analysis and diminishing cell survival (7, 10). Conversely, it has been shown that the usage of a non-enzymatic method (e.g. PBS/EDTA on ice) can maintain both cell viability and cell surface markers, and therefore, we used this approach in our study (13). Although we did not analyze the expression of surface markers and the function of PMA-induced cells, according to Chen et al.'s study (13), the function and the phenotype of macrophages are maintained in this non-enzymatic method compared to the enzyme method. Based on our results, the cell culture plate did not influence the detachment process and after utilizing the cell harvest method, cells derived from both culture plates maintained their viability. Notably, the attachment of macrophages to culture dishes is mediated by a variety of proteins that are adsorbed into the dish surfaces (26, 27). Understanding these interactions is essential for optimizing culture conditions for the field of macrophage research and for accurately modeling their responses *in*

vitro and therefore, it is suggested to be considered in future studies.

CONCLUSION

In conclusion, we showed that the type of cell culture plate (Ad or non-Ad) did not significantly affect PMA-induced THP-1 cell morphology and viability after harvest. Moreover, our non-enzymatic detachment approach maintained the cell viability cultured in both culture plates. Evaluating cell surface markers and cell functionality is suggested to be considered in future studies.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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