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Effect of Time Transport on Mesenchymal Stem Cell Surface Markers: Unveiling the Influence of Cellular Translocation on Cellular Phenotype

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ABSTRACT

Background: The transportation of mesenchymal stem cells (MSCs) is a critical step in their clinical application for regenerative medicine. However, the impact of prolonged transportation time on the surface markers of MSCs remains poorly understood. **Objective:** This study aims to reveal the effects of prolonged transportation time on MSC surface marker expression and its implications for therapeutic efficacy. **Methods:** Human MSCs were isolated from umbilical cord tissue and divided into control and experimental groups, with the experimental group subjected to simulated transportation conditions. **Results:** Flow cytometry analysis revealed a significant decrease in the expression of surface markers CD73, CD90, and CD105 in the experimental group compared to the control group. **Conclusion:** This decrease in surface marker expression may compromise the differentiation potential and immunomodulatory capacity of MSCs. Further studies are needed to elucidate the underlying mechanisms and develop optimization strategies to preserve MSC surface markers during transportation, ensuring the delivery of high-quality MSCs for regenerative therapies.

Keywords: Mesenchymal Stem Cells, Surface Marker, Transportation Time

INTRODUCTION

Mesenchymal stem cells (MSCs) have emerged as a remarkable tool in regenerative medicine due to their ability to differentiate into multiple lineages and their potential to promote tissue repair and regeneration.[11,12] However, the successful clinical application of MSCs relies not only on their intrinsic properties but also on the preservation of their surface during transportation from the donor to the recipient site.[13] Transportation time, encompassing the period from harvest to transplantation, can subject MSCs to various environmental and physical stresses that may potentially compromise their therapeutic efficacy.[14]

While numerous studies have focused on optimizing isolation, culture conditions, and differentiation protocols, the influence of transportation time on the surface markers of MSCs remains an understudied area. Surface markers might be measured using several parameters, including MSC surface markers such as CD73, CD90, and CD105, play a crucial role in maintaining the self-renewal

capacity and multilineage differentiation potential of MSCs.[1,2,3] Any alterations in the expression or functionality of these markers during transportation could have significant implications for the clinical outcomes of MSC-based therapies.

Understanding the effect of time transport on MSC surface markers is of paramount importance to understanding how transportation time affects the quality of MSC. Thus, investigating the impact of transportation time on MSC surface markers is essential for enhancing the quality control measures in cellular therapy protocols.

METHODS

Isolation and Culture of UC-MSCs

MSCs were obtained from human umbilical cords. A Phosphate buffer solution (PBS) with 5% Penstrep antibiotic from Gibco TM Invitrogen, NY, USA, was used as the transport medium[4]. The Wharton's jelly, separated from the umbilical cord, was minced and placed into a 75 cm^2 flask containing Dulbecco's Modified Eagle's Medium (DMEM) from Sigma-Aldrich, Louis St., MO. The DMEM was mixed with 100 IU/cc penicillin/streptomycin from Sigma-Aldrich and 10% fetal bovine serum (FBS) from Gibco TM Invitrogen, NY, USA. The cu ltured Wharton's jelly was then incubated at 37°C with 5% O2. MSCs typically emerged within 7-10 days, and the medium was changed every 3 days. Once reaching 80% confluence, the MSCs were passaged using trypsin, and the 4th passage was used for the experiment

MSC Surface Marker Characteristic

Flow cytometry analysis was used to verify the MSC phenotype of isolated cells. Briefly, the cells were trypsinized, centrifuged, and then incubated with specific antibodies including anti-rat CD70, CD90, CD105, and Hematopoietic Lineage antibodies conjugated with fluorescein allophycocyanin (APC), isothiocyanate (FITC), phycoerythrin (PE), and peridinin-chlorophyll-protein (perCP)-Cy5.5.1 (BD Bioscience, San Jose, CA, USA). As a negative control, an isotype-specific conjugated anti-IgG from BD Bioscience was used. Cells were analyzed using a BD Accuri C6 Plus flow cytometer.

Sample Preparation, Temperature Control, and Surface Marker Analysis

MSCs were harvested at the 6^{th} passage and 80% confluency for transportation simulation. Cells were detached using free animal TrypLE and resuspended in a 0,9% Natrium Chloride solution[3,4]. Transportation containers were prepared using sterile, sealed, and temperature-controlled packaging. Temperature control was achieved using gel packs and insulated materials to maintain a 2-8ºC temperature range throughout transportation. Transportation time point was used in this study starting from 0, 2, 4, 10, 14, and 24 H. MSCs from each transportation time point and the control group was analyzed using flow cytometry to evaluate the expression of MSC surface markers including CD70, CD90, CD105, and Hematopoietic Lineage.

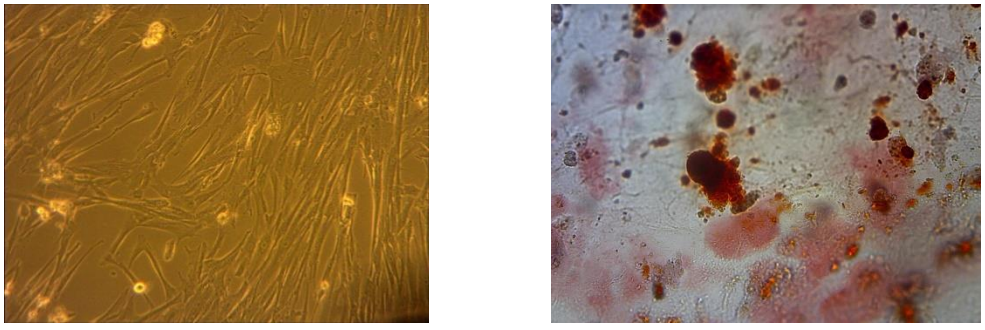
Statistical Analysis

Statistical analysis was performed to compare the expression levels of surface markers between different transportation time points and the control group. Descriptive statistics, such as mean and standard deviation, were calculated. Statistical significance was determined using appropriate tests, such as one-way analysis of variance (ANOVA), with p-values below 0.05 considered significant.

RESULT

MSC Characterization

In this study, we examined the properties of MSC including plastic adherent, morphology, and surface marker. The MSCs at the 6th passage had a typical appearance of fibroblast-like cells that formed single layers on the plastic flask (Figure 1). To determine their surface markers, flow cytometry analysis was performed and showed MSC had high levels of CD73 (97.9±0.32%), CD90 (98.4±0.87%), and CD105 (97,9±0.23%) but low expression of hematopoietic lineage (0,1±0.38%).



(c)

Figure 1. Isolated cells performed fibroblast like morphology and plastic adherent capacity

Prolonged Transportation Time Reduce MSC Surface Marker Expression

The analysis of surface marker expression revealed significant differences between the control and experimental groups. Prolonged transportation time led to a decrease in the expression levels of several surface markers commonly associated with MSCs, including CD73, CD90, and CD105 ($P<0,05$). On the other hand, the prolonged transportation time increases hematopoietic lineage markers [Figure 2].

DISCUSSION

The observed decrease in surface marker expression of MSCs after prolonged transportation time raises concerns regarding their therapeutic potential. Surface markers, such as CD73, CD90, and CD105, play crucial roles in defining the identity and functional characteristics of MSCs.[2,5,6] Their decreased expression may compromise the ability of MSCs to differentiate into specific lineages and modulate the immune response effectively.[7–9]

The underlying mechanisms responsible for the decrease in surface marker expression during prolonged transportation time require further investigation. The cellular stress may induce by transportation conditions, such as temperature fluctuations and mechanical disturbances, which triggers

signaling pathways that modulate surface marker expression.[10] Additionally, interactions between MSCs and the components of the transportation environment, such as packaging materials or media supplements, could contribute to these changes.

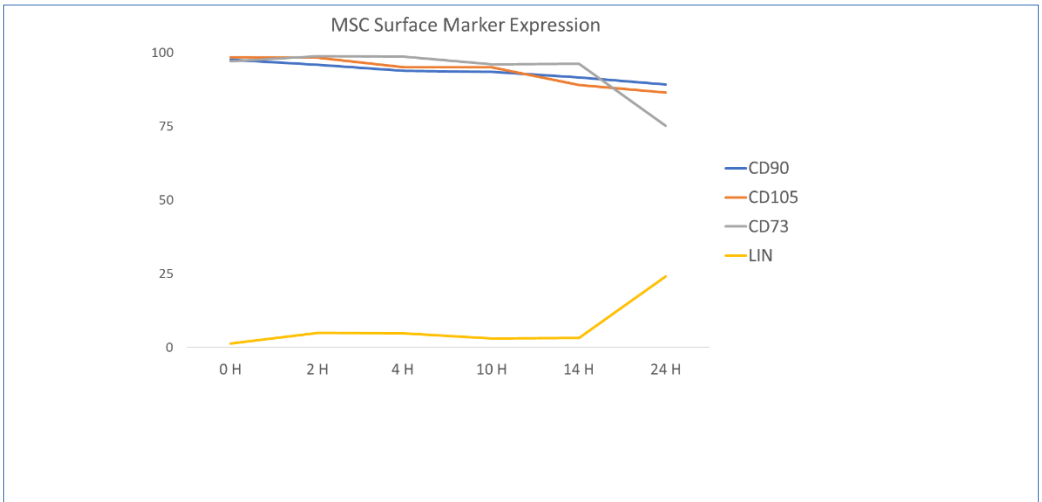


Figure 2. Prolonged Transportation Time leads to a decrease in the MSC marker and an increase in the Hematopoietic Lineage Marker

To mitigate the negative effects of prolonged transportation time, optimization strategies should be considered. These may include the development of specialized transport containers with better temperature control, the use of protective agents to minimize cellular stress, and the implementation of shorter transportation routes or expedited delivery methods. Moreover, further studies should assess the functional consequences of decreased surface marker expression on the therapeutic efficacy of MSCs after transplantation.

CONCLUSION

The findings of this study indicate that prolonged transportation time can lead to a decrease in surface marker expression of MSCs, which may have implications for their therapeutic potential. Surface markers, such as CD73, CD90, and CD105, are important for maintaining the identity and functional characteristics of MSCs, including their differentiation capacity and immunomodulatory properties. The observed decrease in surface marker expression raises concerns about the effectiveness of MSC-based therapies after transportation.

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AUTHORS' CONTRIBUTIONS

HW and AP concept and design the study and wrote the manuscript, CRSI prepared and analyzed the data, and NAA contributed to revisions of the manuscript and approved it for publication.

COMPETING INTERESTS

The authors declare no competing interests.

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