The influence of culture medium on the procoagulant nature of MSCs via Tissue Factor (TF) expression

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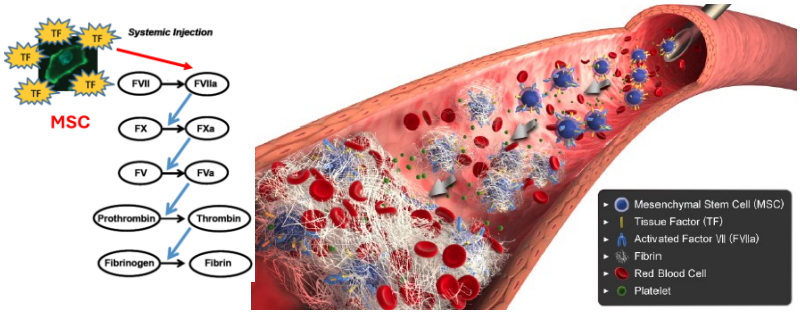
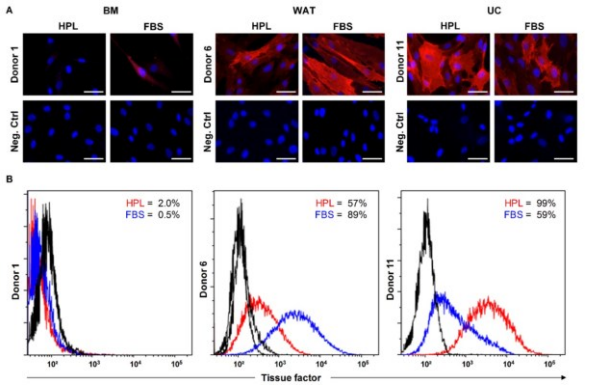
Background

In 2023, the Ministry of Health, Labour and Welfare (MHLW) expressed concern over cases of vascular occlusion, such as pulmonary embolism, following the intravenous administration of Mesenchymal Stem Cells (MSCs), and issued a recommendation emphasizing the need for safety measures. Tissue factor (TF; CD142), a factor of the extrinsic coagulation pathway, is expressed on the surface of Mesenchymal Stem Cells (MSCs) and is involved in thrombosis when MSCs are administered intravenously 1). When MSCs with high TF expression are administered into the bloodstream, a thrombus is formed, initiated by the MSCs via the reaction between cell-surface TF and blood components. While MSCs in vivo express little to no TF, culturing MSCs in media containing additives such as Fetal Bovine Serum (FBS) or Human Platelet Lysate (HPL) strongly induces TF expression. This induction shortens the MSCs' blood coagulation time and increases their procoagulant activity (prothrombogenicity) 2). Myoridge Co. Ltd. has previously offered various customized culture media for MSCs tailored to user needs (media performance and cost) and has launched a catalog product, Xeno Free MSC Medium (Ex-MSC XF). This document presents the results of a study investigating the influence of culture medium on MSC TF expression and procoagulant activity.



1)Biochem. Biophys. Res. Commun. 431(2): 203-209, 2013

The influence of culture medium on TF expression in human MSCs (Bone Marrow (BM), Adipose Tissue (WAT), and Umbilical Cord (UC))



2)Theranostics. 8(5): 1421–1434, 2018

Methods and Results

Human Adipose-Derived MSCs

Thaw and seed
DMEM/10%FBS
2 days

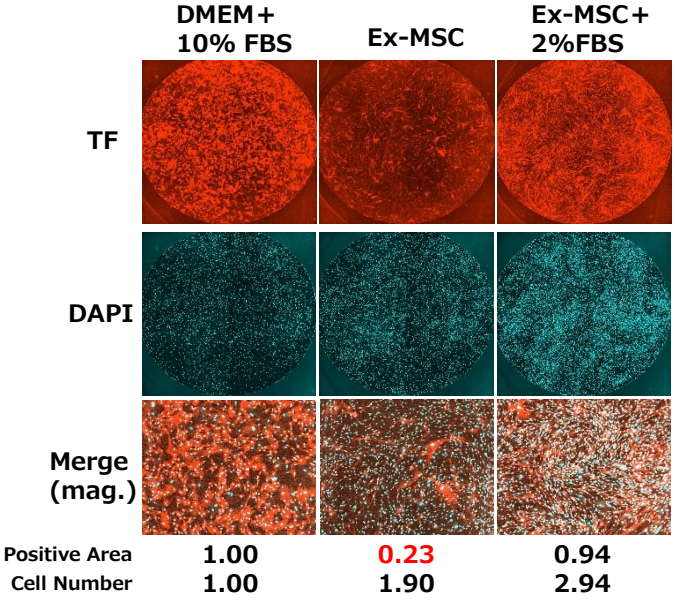
Increase of TF expression

Seeding: 1500 cells/well into 96-well plates (in DMEM/10% FBS)
Overnight culture

Cells were cultured in several types of media
3 days

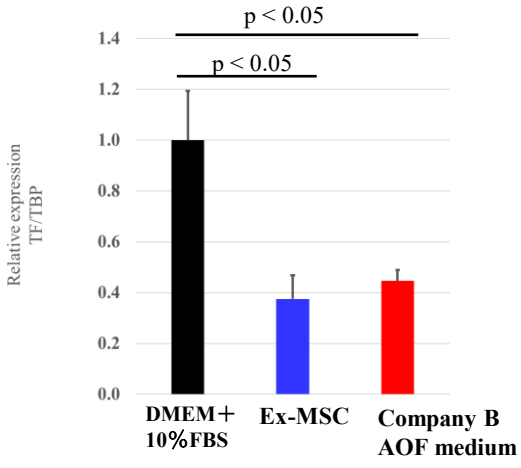
- Fluorescence Microscopy (DAPI, TFantibody)
- TF Expression Quantification by PCR
- Analysis of TF expression on the cell surface by FCM
- Coagulation analysis after mixing MSCs with human blood plasma

TF Expression Analysis by Fluorescence Microscopy



	DMEM+ 10% FBS	Ex-MSC	Ex-MSC+ 2%FBS
Positive Area	1.00	0.23	0.94
Cell Number	1.00	1.90	2.94

Quantification of TF Expression by Real-time PCR



・ADSC TF expression was suppressed in the Xeno-Free medium (Ex-MSC) compared to serum-containing media.
・The addition of FBS to Ex-MSC increased TF expression (dependent on FBS concentration)

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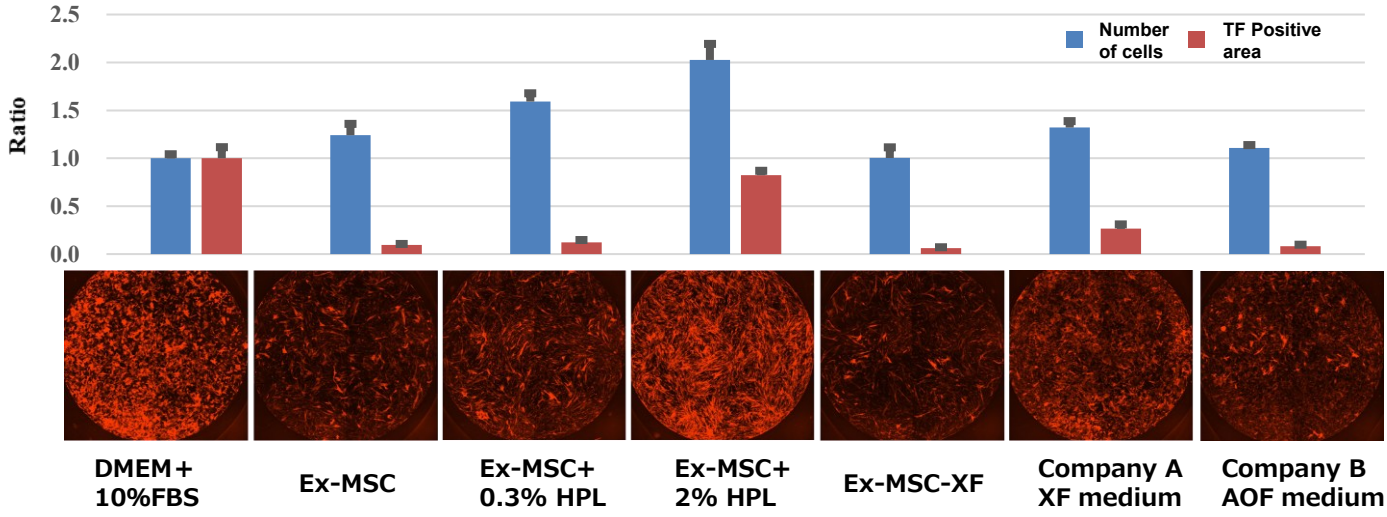
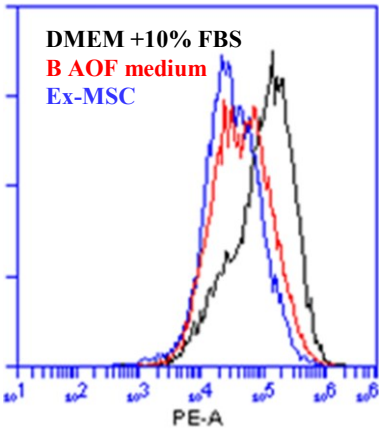
Immunofluorescence images of TF in ADSCs cultured in seven media

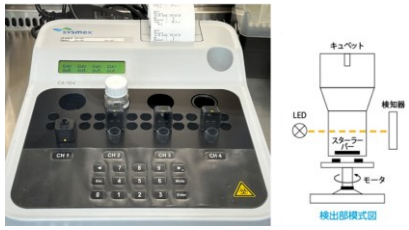


Analysis of Cell Surface TF Expression using FCM

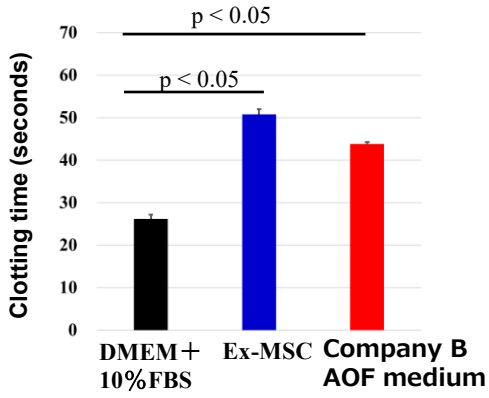


Clotting Time Measurement of ADSCs and Human Plasma (Clotting Assay)

CA-104, Sysmex



ADSC cell suspension (1.5 x 10e5 cells/mL, containing citrate) was mixed with an equal volume of human blood plasma. The mixture was set in an automatic coagulation measuring device (CA-104, Sysmex), and the clotting time (seconds) after the addition of calcium chloride (recalcification) was measured.



・TF expression in ADSCs was reduced by culturing them in Ex-MSC.
・ADSCs cultured in Ex-MSC exhibited lower TF expression and a prolonged blood clotting time compared to ADSCs cultured in FBS-containing media, resulting in low procoagulant activity.

Summary

・Ex-MSC allowed for large-scale expansion culture while maintaining low Tissue Factor (TF) expression in human adipose-derived MSCs, compared to FBS-containing media.

・Low TF expressing MSCs cultured in Ex-MSC showed a significantly prolonged blood clotting time in the clotting assay, suggesting they possess low procoagulant activity and a low propensity for thrombosis (low thrombogenicity).

・It was reconfirmed that Fetal Bovine Serum (FBS) and Human Platelet Lysate (HPL), which are commonly used in MSC culture media, induce TF expression in MSCs.

・Recent academic literature suggests that TF should be an evaluation item for cell products intended for intravenous administration (Stem Cells Transl. Med, 11, 2-13 2022). Therefore, when considering the intravenous administration of MSCs in the future, it is deemed important to select an appropriate culture medium, taking into account the TF expression level and procoagulant activity of the MSCs.

Data Contributors

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