臨床用間葉系幹細胞の製造法開発

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Background

Human mesenchymal stem cells (hMSCs) demonstrate regenerative properties and multipotentiality, and have been proposed as a potential candidate for cell therapies. It is well known that adipose derived stromal cells (ADSCs) can be easily harvested with less discomfort, low donor-site morbidity and high amount compared to bone marrow-derived stem cells. Classical media used for generating hADSCs are typically supplemented with ill-defined supplements such as fetal bovine serum (FBS). Ideally, culture media are desired to have well-defined serum-free formulations that support the efficient production of hADSCs while maintaining their therapeutic and differentiation capacity. Towards this objective, we aim to compare the use of several commercial serum-free media combination with or without RetroNectin® (TaKaRa Bio) treated flask. The hADSCs from subcutaneous adipose tissue with the collagenase based isolation method. The primary end point for this study is a comparison of hADSCs expanded in combination the serum free media and RetroNectin with classical media using FBS, and the secondary is the function of these cells.

Human Mesenchymal Stem Cell (hMSC)

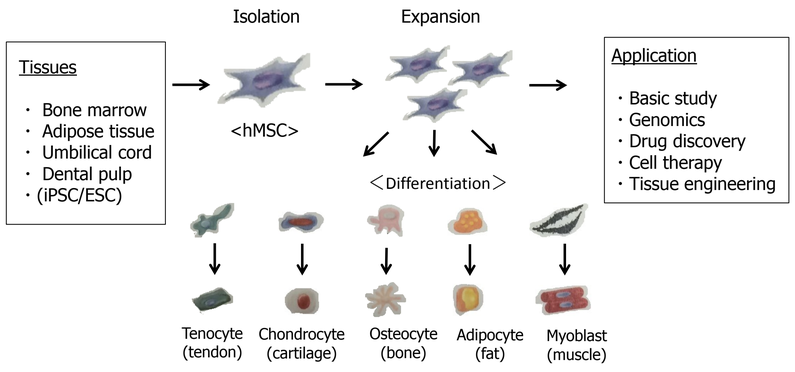

Figure 1 Schematic representation of multipotent differentiation of hMSC from several tissues.

Advantage of hMSC from Adipose tissues for clinical application.

◆Potency
・Differentiation potential to mesodermal cells
・Immunosuppression
・Cytokine production
・Homing

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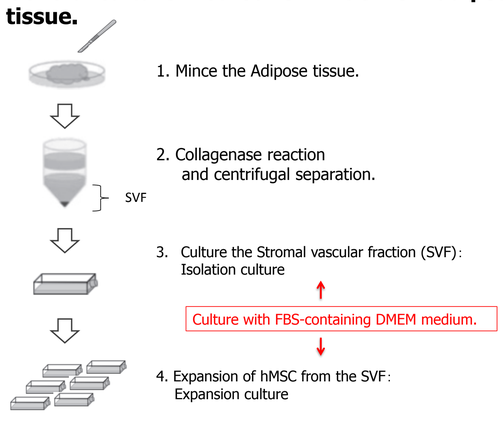
◆Disease
・GvHD
・Cardiac failure
・Crohn's disease
・Rheumatism
・COPD exacerbation
etc.

◆Advantage of ADSC (Adipose derived stem cells)

Bone marrow derived	Adipose derived
Small amount from bone marrow stroma.	Abundant from subcutaneous adipose tissue.
Highly invasive	Less invasive
Decreases with ageing.	Less influence with aging.

General culture method for ADSC from adipose tissue.



Objective

Culture media are desired to have well-defined serum-free formulations that support the efficient production of hADSCs while maintaining their therapeutic and differentiation capacity.

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We aim to compare the use of several commercial serum-free media combination with or without RetroNectin® (TaKaRa Bio) treated flask.
The hADSCs from subcutaneous adipose tissue with the collagenase based isolation method.

・Primary：Comparison of expansions
・Secondary： The function of these cells

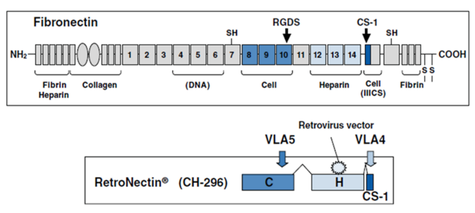
Results

The hADSCs from subcutaneous adipose tissue with the collagenase based isolation methods.

Culture Condition（Medium and FBS or alternative）

Culture Medium	FBS or alternative	RetroNectin
Dulbecco's Modified Eagle's Medium (DMEM: As control)	10%FBS	−
	5% auto-plasma	−
		+
Cellartis® DEF-CS™ 500 Xeno-Free Culture Medium (DEF-XF)	−	+
	5% auto-plasma	
Serum free Medium-X (SFM-X)	−	+
	5% auto-plasma	

RetroNectin® ; A recombinant human fibronectin fragment



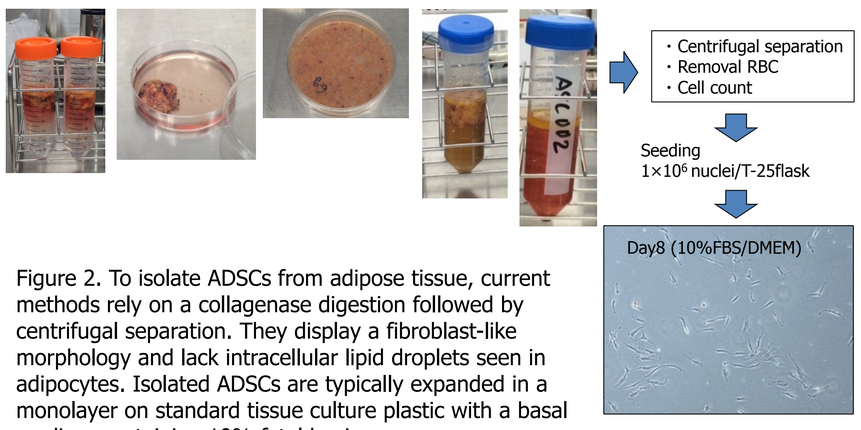


Figure 2. To isolate ADSCs from adipose tissue, current methods rely on a collagenase digestion followed by centrifugal separation. They display a fibroblast-like morphology and lack intracellular lipid droplets seen in adipocytes. Isolated ADSCs are typically expanded in a monolayer on standard tissue culture plastic with a basal medium containing 10% fetal bovine serum.

For "the Isolation" culture (passage 0), ADSCs were well expanded in DEF-XF medium containing 5% auto-plasma with RetroNectin

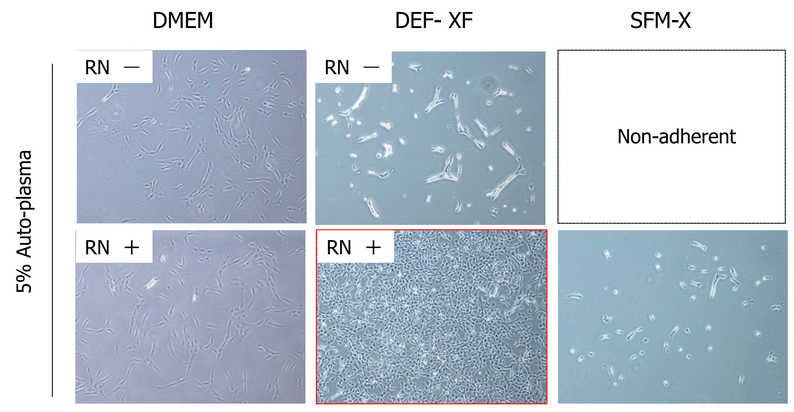


Figure 3. Cultivation of primary hMSC (Passage 0) from adipose tissue using different two commercial serum free media. Cells were inoculate at 1,000,000 nuclei from SVF into RetroNectin-coated or non-coated T-25 flasks, each containing 10 mL of DEF-XF, SFM-X and a classical DMEM medium with or without 5% auto-plasma. After 24 hrs, nonadherent cells in each medium were removed. The adherent cells were allowed to grow for additional 7 days.

For "the Expansion" culture (passage 1~), ADSC were well expanded Serum free medium-X medium with RetroNectin® (without auto-plasma)

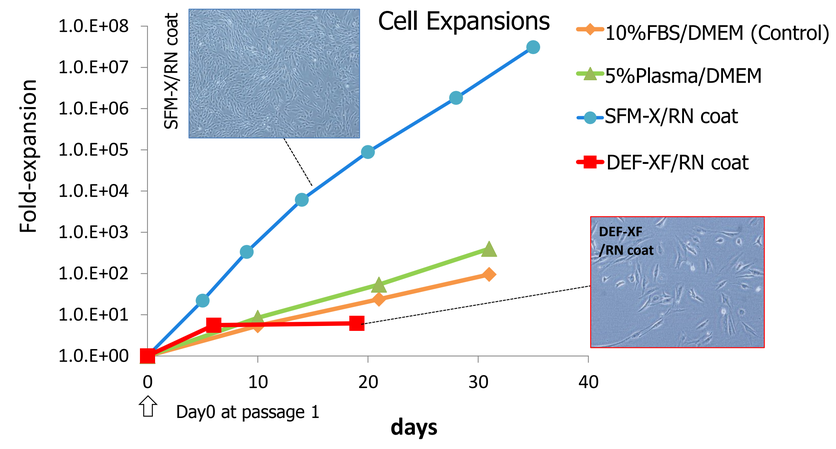


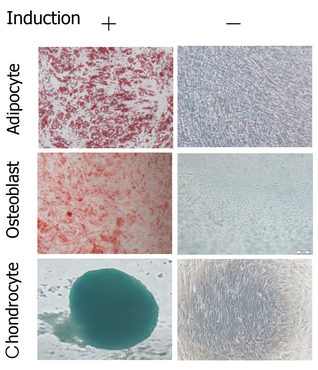
Figure 4. ADSCs expansion in the two Serum free media without auto-plasma. ADSCs were expaned for 5 passages (SFM-X media) or two passages (DMEM) or one passage (DEF-XF).

Function of the hADSC, expanded in serum free method with RetroNectin®

(A) Immophenotype

Culture Condition		SFM Method with RetroNectin
passage		P5
Positive marker (≧95%+)	CD73	99.6
	CD90	96.2
	CD105	100
Negative marker (≦2%+)	CD34	0
	CD45	0
	HLA-DR	0
	CD11b	0
	CD14	0

(B) Differentiation potential



(C) Growth factor production

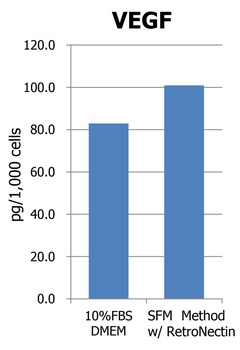


Figure 5. Functional analysis of hADSCs expanded in the serum free media with RetroNectin ®. (A) Phenotype and (B)differentiation potentials were examined with the criteria from the International Society for Cellular Therapy (ISCT).(C) Comparison of VEGF secretion determined by ELISA for classical media (10%FBS/DMEM) and serum free with RetroNectin® condition.

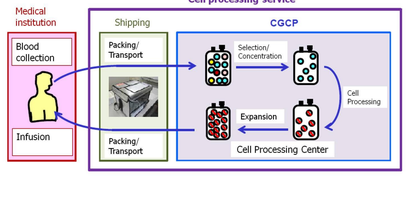
Discussion

In our experiments, we isolated the hADSCs from subcutaneous adipose tissue with the collagenase based isolation methods. Firstly, the initial step of the isolation culture, we confirmed that hADSCs were well expanded in combination DEF-XF serum free medium containing auto-plasma with RetroNectin coated flask compared with 10% FBS conditioned DMEM medium. And the following expansion culture, we confirmed that hADSC were greatly expanded in Serum free media-X with RetroNectin (without auto-plasma).
Furthermore, the combination method with RetroNectin and serum free medium expanded ADSC met typical MSC characteristics according to the ISCT position paper (Dominic M 2006) in terms of morphology, differentiation and phenotype. In conclusion, the combination of using RetroNectin and serum free medium, depending on culture phase (isolation or expansion), would achieve higher cell numbers more rapidly than those expanded in classical media such as DMEM medium containing FBS. Therefore, the expansion method could provide clinical dose of hMSC from subcutaneous adipose tissue.

Cell manufacturing service at TaKaRa bio Inc.

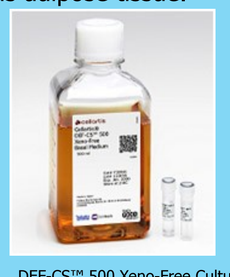
CGCP
Center for Gene and Cell Processing

- Licensed for Cell manufacturing by MHLW. Facility No. FA5150002
- Multiple Cell processing room
- Using single-use materials.



In this study, the hADSCs manufacturing combination with the serum-free media, DEF-CS™ Xeno Free (Cellartis®) and RetroNectin® allowed to rapidly establish primary cultured cells compared with classical media, and continuously expand with high efficiency in serum free medium-X under the RetroNectin® treated condition. Furthermore, we demonstrate that the expanded hADSCs could meet the criteria for MSC that proposed by ISCT, such as adherent to plastic, specific surface antigen expression and multipotent differentiation potential (adiopgenic, oseogenic and chondrogenic) *in vitro*. Therefore, the expansion method could provide clinical dose of hMSC from subcutaneous adipose tissue.

RetroNectin® (TaKaRa Bio)



DEF-CS™ 500 Xeno-Free Culture Medium (Cellartis®)

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演題発表に関連し、
開示すべきCOI関係にある企業等はありません。