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Overcoming Scalability Challenges to Increase Patient Reach in Cost-Effective Manners

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Ajinomoto Co., Inc. (Ajinomoto) is proud to support industrial customers to advance its capability of manufacturing induced pluripotent stem cells (iPSC) for cell therapy. There are some challenges to achieve scale-up iPSC manufacturing and Ajinomoto is developing technologies to address these issues in the industry.

This article is a summary of the presentation at iPSC manufacturing summit 2023 by Hiroki Ozawa at Ajinomoto. All information and titles are as of May 2023.

INTRODUCTION

As many of you may already be aware, there are currently over 100 clinical trials involving human pluripotent stem cells being conducted worldwide. When human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC) were first established, the cells were cultured on a feeder layer with fetal bovine serum (FBS). However, the use of feeder cells and FBS presents challenges including safety, consistency, and regulatory compliance. As a result, the culture system has been improved for clinical applications by transitioning to feeder-free and serum-free methods or by removing animal-derived materials altogether.

This table (Fig.1) shows the current clinical trials and their respective culture systems [1]. At Ajinomoto, we developed and provide animal-free culture medium for clinical and research applications. There are three key challenges associated with the manufacturing of human pluripotent stem cell (hPSC)-based products: safety, quality, and cost. At Ajinomoto, we have been working diligently to overcome these challenges.

CLINICAL TRIALS AND THEIR CULTURE SYSTEM

ID number	Year	Cell line	Derived Cell Type	Indication	Culture medium	Scaffold	Reference
NCT01217008	2010	ESC	Oligodendrocyte progenitor cells	Spinal Cord Injury	Serum-free	On-feeder	Carpenter et al.
NCT01345006	2011	ESC	Retinal Pigmented Epithelial Cells	Stargardt's Macular Dystrophy	Xeno-free	On-feeder	Schwartz et al.
UMIN000011929	2013	iPSC (auto)	Retinal Pigmented Epithelial Cell Sheet	Exudative AMD	Xeno-free	On-feeder	Kamao et al.
NCT02286089	2014	ESC	Retinal Pigmented Epithelial Cells	Advanced Dry AMD	Serum-free	On-feeder	Idelson et al.
NCT02239354	2014	ESC	Pancreatic beta-cell precursors	Type 1 Diabetes Mellitus	Xeno-free	Feeder-free	Schulz et al.
NCT02923375	2016	iPSC (allo)	Mesenchymal stem cells	Steroid-resistant Acute GVHD	Xeno-free	Feeder-free	Bloor et al.
NCT03119636	2017	ESC	Neural Precursor Cells	Parkinson's Disease	Xeno-free	Feeder-free	Wang et al.
UMIN000033564	2018	iPSC (allo)	Dopaminergic progenitors	Parkinson's Disease	Animal-free	Feeder-free	Doi et al.
NCT04106167	2019	iPSC (allo)	Natural killer cells	Cancer	Xeno-free	Feeder-free	Valamehr et al.
UMIN000036539	2019	iPSC (allo)	Corneal epithelial cell sheet	Limbal Stem-cell Deficiency	Animal-free	Feeder-free	Hayashi et al.
jRCTa031190228	2020	iPSC (allo)	Neural stem/progenitor cells	Spinal Cord Injury	Animal-free	Feeder-free	Itakura et al.
jRCTa050190104	2020	iPSC (allo)	Cartilage	articular cartilage damage	Animal-free	Feeder-free	Yamashita et al.
NCT04945018	2021	iPSC (allo)	Cardiomyocyte Spheroids	Heart Failure	Animal-free	Feeder-free	Tohyama et al.
NCT04696328	2021	iPSC (allo)	Cardiomyocytes Sheet	Ischemic Cardiomyopathy	Animal-free	Feeder-free	Miyagawa et al.

Fig.1 Clinical trials and their culture system

GUIDELINES TO BE CONSIDERED WHEN SELECT ANCILLARY MATERIALS.

We have developed StemFit™ medium, stem cell culture media, and StemFit Purotein™, recombinant growth factors, for hPSC culture with several unique features. Since our products are designed for clinical applications, we have focused on using only animal-origin-free ingredients and optimizing performance with a single-cell expansion protocol. Culture medium and growth factors for cell and gene therapy products are considered ancillary materials. Since these materials have specific risks associated with their derivation, there are guidelines in place to ensure their safety and quality. ISO20399 outlines the requirements and recommendations for ensuring the safety and performance of these materials. The United States Pharmacopeia (USP) and European Pharmacopoeia (EP) focus mainly on qualification programs and risk assessment. Japan has established the Standards for Biological Ingredients (SBI), which apply to all animal-derived materials, including those derived from humans, to ensure their safety. Based on these guidelines, the use of animal-derived components in cell product manufacturing is generally not desirable.

GUIDELINES FOR AMS TO ENSURE THE SAFETY AND QUALITY

- Ancillary materials (AMs) have specific risk associated with their derivations.
- Some guidelines provide detailed instructions on how to ensure the safety and efficacy of the final cell and gene therapy products that are produced with the use of ancillary materials.

ISO

- ISO20399:2022 sets out the requirements and recommendations for both suppliers and users of ancillary materials (AMs) to ensure the safety and performance of the manufactured cell products.

USP/EP

- USP1043 provides guidance on the development of appropriate qualification programs for AMs used in cell, gene, and tissue-engineered products.
- EP5.2.12 focuses specifically on materials extracted from various biological sources or produced by recombinant DNA technology and addresses risk assessment, manufacturing, and quality control.

JP

- Standards for Biological Materials apply to all animal-derived materials, including those from humans.
- Several essential aspects need to be taken into account, including traceability, virus inactivation or removal processes during the production, and comprehensive quality testing along with the COA.

RISKS OF ANIMAL DERIVED INGREDIENTS

There are several risks associated with the use of animal-derived components in cell culture.
Firstly, there is a safety issue. Non-human animal components carry the risk of infection and immune reactions, while human plasma materials carry the risk of pathogen transmission.

Second, quality can be an issue with animal-derived components. There can be variations related to the donor, and it has been shown that bovine serum albumin (BSA) has significant lot-to-lot variability, resulting in inconsistencies in stem cell culture.

Lastly, supply can be a critical issue. Procuring fetal bovine serum (FBS) or human platelet lysate (hPL) can be challenging and is often a headache for procurement managers to secure a good lot of these materials.

THE RISKS WITH ANIMAL/HUMAN-DERIVED COMPONENTS

- The use of animal-derived materials presents potential safety and quality risks, as well as supply issues

Issues related to animal-derived materials

Safety

- Animal components risk zoonoses and immune reactions. (Astori et al., 2016)
- Risk of pathogen transmission from human plasma material (Gröner, 2008)

Quality

- Animal derived products has donor variations. (Price, 2017)
- Lot-to-lot variability in BSA results in inconsistencies. (Ieyasu et al., 2017)

Supply

- Procuring the materials such as FBS and hPL has become a challenging task due to their shortage, and securing a good lot is always difficult.

SINGLE CELL PASSAGING WITH StemFit™ MEDIA

. We've developed a medium called StemFit™ designed for single-cell culture for clinical and research use[2]. Typically, hPSCs are passaged as clumps; however, for reproducible protocols, it's important to dissociate them into single cells and seed a fixed number.

CGT Catapult performed a growth comparison test and found that cells cultured in StemFit™ with a single-cell protocol outperformed others commercially available media. Additionally, with lower seeding density, single-cell culture can achieve higher yields while reducing medium volume and change frequency.

Cloning experiments were also performed and validated that StemFit™ shows the best efficiency when combined with Laminin 511 E8 fragment.

SINGLE-CELL CULTURE

- StemFit was designed for single-cell culture to enhance the benefit of growth and cloning efficiency.

Benefits and necessity of single-cell culture

SOP

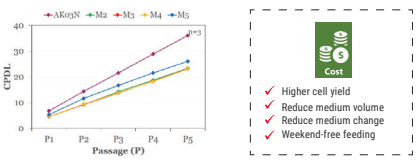
- Reproducible protocol: A fixed number of cells spread evenly
- Efficient differentiation: Optimal cell number and density are key to differentiation efficiency

CRISPR

- Need to handle and select the edited cells during CRISPR
- The cloning efficiency is directly related to CRISPR performance

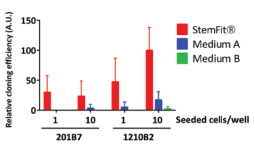
StemFit for single-cell culture

Growth



CGT Catapult 2016

Cloning



GENETIC STABILITY OF HPSCS IN CULTURE

Another important factor to consider in cell culture is the genetic stability. Ensuring the safety and quality of hPSC products is crucial because of the de novo mutations, and mutated cells that can form and take over the population. So, how can we reduce the chance of mutations and prevent culture selection or adaptation?

In culture, stressors such as reactive oxygen species (ROS) and acid can damage DNA and cause genetic alterations. Oxidative glycolysis increases ROS levels, but reducing oxygen in the culture can lower this risk. Lactate accumulation is a main cause of culture acidification, and low pH primarily causes DNA damage.

Cells cultured in StemFit™ with single-cell protocols showed less lactate accumulation. We believe that lower cell numbers and density contribute to reduced acidification in the culture.

Certain genetic mutations, such as BCL2L1 and p53, can provide cells with a culture advantage. Cells with these mutations can be selected and adapted in culture, eventually taking over the population. In unstable culture conditions, these cells are more rapidly selected as normal cells tend to die, increasing the chance of detecting mutations. Recent reports suggest that culture medium and conditions can improve genetic stability. Culturing with StemFit™ may result in fewer genetic mutations. Several studies have also shown improved genetic stability with StemFit™, contributing to stable cell culture for clinical applications[3].

ADVANCING GENETIC STABILITY IN CULTURE

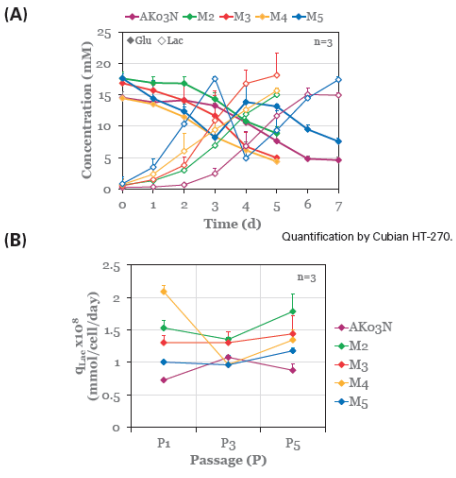
- Stressors such as ROS and acid cause damage to DNA and genetic alterations.

Acquisition of genetic alterations

- Oxidative glycolysis increases the potential for leakage of ROS into the iPSC cytoplasm. (Turinetto et al., 2017)
- Less oxygen lowers mutation rate by reducing oxidative damage-induced base-pair changes. (Thompson et al., 2020)

- Culture density-related medium acidification primarily causes DNA damage. (Jacobs et al., 2016)
- Low pH inhibits glucose consumption, arrests cell cycle, and leads to cell death. (Wilmes et al., 2017; Liu et al., 2018)

Single-cell culture with StemFit™ results in low lactate accumulation



METABOLIC APPROACH TO CONTROL CELL MANUFACTURING PROCESSES

Our unique approach for hPSC derived product manufacturing - metabolic approach provides a way to overcome undifferentiated cells. One of the biggest concerns for hPSC-based products is residual undifferentiated cells, as they can form teratomas. There are several approaches to eliminate undifferentiated hPSCs. For example, it has been reported that using CD30 antibodies and sorting against Chorin antigens can successfully enrich target cells. However, cell sorting is costly and time-consuming. Therefore, we propose a different approach. Cell metabolism is determined by cell lineage and the surrounding microenvironment to sustain cellular function. However, this relationship also works in reverse.

We have confirmed that certain amino acids and trace metals can manipulate hPSC culture. This is a metabolic approach for hPSC-derived cardiomyocyte production. As cardiomyocyte transplantation requires large cell quantities, we developed a tryptophane supplemented customized StemFit™ medium to enhance the cell proliferation rate. The undifferentiated hPSCs utilize glycolysis and glutamine oxidation to generate the energy, on the other hand the mitochondria matured in cardiomyocytes, and they rely on lactate oxidation. In other words, we took a simple strategy, used selection medium AS501 after cardiomyocyte differentiation, which we deprived glucose and glutamine from, and added lactate. The residual iPSC die and only the cardiomyocytes get selected. The cardiomyocytes adapt metabolically in this process, and the cells become matured. This strategy successfully eliminates the residual hPSCs very efficiently and improves the cell quality[4]. Additionally, the cost is economically affordable.

3D SUSPENSION CULTURE

With growing interest in 3D suspension culture, we have carefully formulated our media to allow for an optimized protocol in 3D.

The required number of cells for transplantation varies among cell types. Pancreas, heart, liver, and blood cells require a large quantity of cells for transplantation.

One of the major issues in this field is the cost. This study shows the results of an autologous cost calculation[5]. The key takeaway is that automation significantly reduces COGS. However, the cost of reagents still remains. For large-scale production, a scalable culture system is required. Conventionally, a 2D culture system is used, but a 3D culture system is promising for scalable culture.

To obtain 10^{10} cells using a 2D culture system, 60L of medium would be needed with a multilayer flask. On the other hand, 3D culture can achieve more efficient culture and save on medium volume. However, cell proliferation is usually slower in 3D culture, so it can take an extra week.

3D suspension culture is promising, but there are challenges such as low expansion rate, shear stress, passage issues, aggregate control, and cell line stability. We support overcoming these challenges with an optimized formulation and technical consultation for an improved protocol.

DEVELOPMENT SUPPORT FOR CELL MANUFACTURING PROCESS

- Although 3D suspension culture is promising for scaling up cell culture, there are still challenges that need to be addressed.
- Ajinomoto has been working on efficient 3D suspension culture development.

Challenges with 3D culture

Low expansion rates or overall low cell yield

Shear stress

Passage

The size and shape of aggregates

Culture protocol need to be optimized for each cell lines

How Ajinomoto can support

- Optimized formulation for suspension culture.
- The protocol is key - sharing the data of Ajinomoto's suspension culture technology with orbital plate shaker and spinner flask.
- Troubleshooting and proposing for improving culture conditions

References.

[1] Expert Opin Biol Ther. 2023 Jan-Jun;23(6):479-489
[2] Sci Rep. 2014 Jan 8;4:3594
[3] bioRxiv [Preprint]. 2023 Mar 1:2023.02.28.529447
[4] Circ Res. 2017 May 12;120(10):1558-1560
[5] Cytotherapy. 2017 Dec;19(12):1383-1391

The Ajinomoto Group: Strengths for iPS Cell Culture Medium Development

Ingredient manufacturing technologies

As the world's leading supplier of high-quality amino acids, which are mainly used for pharmaceuticals, Ajinomoto Co. can supply amino acids free of animal-derived components and with full traceability.

Composition and formula design

With our heritage of research into amino acid nutrition and metabolism, Ajinomoto Co. possesses the technologies and "know-how" to quickly determine the optimal composition of the dozens of components that comprise a culture medium.

Analysis technologies

Ajinomoto Co.'s analysis technologies for amino acids and trace ingredients are highly sensitive and highly accurate. This permits us to formulate a high-performance culture medium with exacting quality control.

StemFit™: The Perfect Fit for Stem Cell Research

Conventionally, iPS cells were cultivated in a culture medium that included mouse cells and other animaland human-derived components. StemFit™ is highly safe because the risk of accidental biological contamination is minimized. To confirm this point, Ajinomoto Co. consulted with the Pharmaceutical and Medical Devices Agency (PMDA) of the Government of Japan, which determined that StemFit™ does not contain any animal- or human-derived components after an intensive review process.

StemFit™ is a high-performance, high-quality culture medium. Cells proliferate in the StemFit™ culture medium at a high growth rate, making research not only more efficient, but also more cost effective.

Ajinomoto is committed to the advancement of regenerative medicine with StemFit™ Media and Growth Factors. Chemically defined and Animal-Origin-Free, our products enable consistent experimental results and support the development of cell therapy products.

For more information, please visit https://www.ajitrade.com/stemfit/ and chat with us today Or contact us:

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