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Short Communication

The Stagnant Adaptation of Defined and Xeno-Free Culture of iPSCs in Academia

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Abstract

Pluripotent Stem Cells were originally derived and cultured using a feeder layer of cells. Movements have been undertaken to transition from this method to one more defined, high-throughput, and without xenogenic factors. Tremendous research has been done in this area and many products have been developed, however, based on our analysis of recent publications in stem cell related journals many in academia are still using older methods like a feeder layer. In this short communication, we discuss the feasibility of transitioning to defined, xeno-free methods, how a standardized method could improve the field and industry, and that a study bringing together multiple institutions comparing culture methods could be done to evaluate the efficacy of these new methods.

ABBREVIATIONS

iPSC: Induced Pluripotent Stem Cells; **MEF:** Mouse Embryonic Fibroblasts; **ESC:** Embryonic Stem Cells; **FBS:** Fetal Bovine Serum; **BSA:** Bovine Serum Albumin

SHORT COMMUNICATION

Several revolutionary technologies [1,2] have been developed recently to improve the consistency and clinical potential of iPSC research, however, many academic labs have yet to transition to these culture systems. This brief review will discuss some of the issues that pertain to this topic, in particular the history, the current state of the field, the costs associated with the culture techniques, and what this means for the goals of the field as a whole.

Pluripotent stem cells were first derived and cultured using a MEF feeder layer [3,4]. This was never meant to be their permanent culture method and there was a movement to eliminate the feeder layer [5], xenogenic sources [6,7], and define all needed components of the culture early on [8,9]. The goal is to identify a defined, xeno-free culture system including the media, substrate, and dissociation reagent. Defined culture systems use chemically defined media whose every component and concentration is known while xeno-free culture systems for human (i.e., clinical) applications use by definition the biological components derived from human or are produced in culture recombinantly using human genetic sequences. Defined and xeno-free culture systems would minimize the inherent variability in biological components and standardize the experimental system in the research community and clinical products by providing the more stringent quality control necessary. While there are tremendous amounts of research and products available now for defined and xeno-free culture, this movement of applying these techniques has fallen flat so far. In 2016 (Jan – June), well over half of the articles published on iPSCs or ESCs in two highly regarded stem cell journals (*Cell Stem Cell* [10-27] and *Stem Cells* [28-55]) still use a feeder layer (27 of 46) [10-19,28-44], while no study utilized truly defined and xeno-free conditions. The remaining (20 of 46) did not use a feeder layer, but utilized undefined or xenogenic conditions in one way or another (e.g., Matrigel®, FBS, or BSA) [14,20-27,45-55]. One study utilized both in comparison so it was included in both lists [14]. This phenomenon of defined and xeno-free cultures not being published on is also seen when you look at all articles on PubMed. The share of "Stem Cell" articles with the phrases "defined culture" and "free" is not increasing over the last 20 years, see Figure (1). If these techniques were being implemented, it would be expected that these shares would be increasing. Scale-up and development of clinical grade products using human pluripotent cells need defined and xeno-free cultures, so why is the research community lagging behind in the adoption of these methods when many of the projects are translational research rather than basic science?

These methods may seem to be more expensive or prohibitive by the cost of materials or new reagents needed for the culture, as seen by the cost of materials (Table 1). However, when you look at the cost of implementation, defined and xeno-free methods are competitive in price and very close to that of the feeder system and undefined systems, see Figure (2). This may still be prohibitive for some labs with inherited feeder systems or cheaper ways to make media, but for the labs that can afford

Table 1: Cost of ESCs and iPSCs culture: Prices of making own media estimated using Thermo Fisher products. Substrate for Feeder condition could vary greatly based on the cells origin or if derived from animal in lab.

	Media (per 500mL)		Substrate (Units Vary)		Dissociation (per 100 mL)	
Feeder [3,4,17,65,66]	Make own	$126.33	Derive/Buy MEFS (7million cells)	$0/$66	0.25% Trypsin	$12.17
Xenogenic or undefined [67-70]	Make own/TeSR™-1	$140.6/$270	Matrigel® (10mLs)	$269.12	Accutase®	$17.00
Defined and xeno-free [1,2,58,59]	TeSR™-E8™	$206	Truncated Vitronectin (5mg)	$503.50	Versene	$10.31

Recipes and cost for making own medias are as follows:
Feeder Media (All Thermo Fisher products)
Knockout DMEM medium- $28.75 per 500mL
- Need 391mL - $22.48

supplemented with 20% KSR – $332.25 per 500mL
- Need 100mL – $66.5

1.1 mM nonessential amino acids - $17.96 per 100mL (10mM)
- Need 5mL - $0.90

0 mM L-glutamine - $24.50 per 100mL (200nM)
- Need 2.5mL - $0.98

1.1 mM β-mercaptoethanol - $7.46 per 20mL (50mM)
- Need 1mL - $0.37

penicillin-streptomycin - $20.05 per 100mL (1000x)
- Need 0.5mL - $0.10

4 ng/ml bFGF - $175 per 10ug
- Need 2ug – $35

Total: $126.33 per 500mL

Xenogenic Media (All Thermo Fisher products)
Knockout DMEM/F12 - $35.80 per 500mL
- Need 391mL - $28.00

Penicillin-streptomycin - $20.05 per 100mL (1000x)
- Need 0.5mL - $0.10

0 mM L-glutamine - $24.50 per 100mL (200nM)
- Need 2.5mL - $0.98

1% nonessential amino acids - $17.96 per 100mL (10mM)
- Need 5mL - $0.90

1.1 mM 2-mercaptoethanol - $7.46 per 20mL (50mM)
- Need 1mL - $0.37

20% (v/v) knockout serum replacement – $332.25 per 500mL
- Need 100mL – $66.5

5 ng/ml recombinant human FGF2 - $175 per 10ug
- Need 2.5ug – $43.75

Total: $140.60 per 500mL

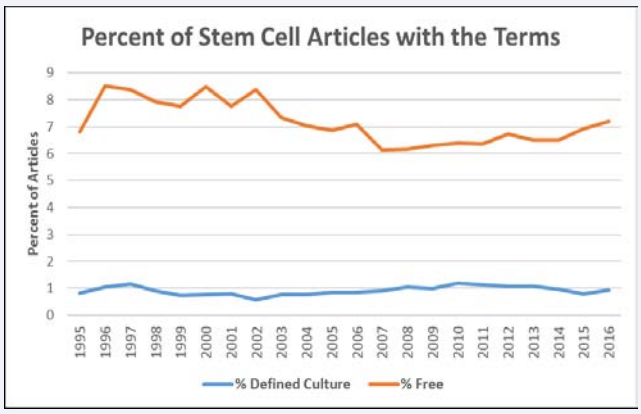

Figure 1 Graph showing the trend of stem cell articles relating to the topics of "defined" or "free" cultures: For this analysis, searches were conducted on PubMed and the "results by year" was analyzed. The searches "stem cell", "stem cell AND defined culture", and "stem cell AND free" were conducted. The graph shows the percent of stem cell articles that contain the terms "defined culture" and "free" over the past 20 years [56].

this minor increase what else needs to be done to promote the adoption of these changes? The companies supplying these alternatives have provided methodology for transitioning to defined, xeno-free systems and have shown validation of the products [1,2,57-60]. While the end goal of some of these group's studies may not be a therapy or large scale production, it will undoubtedly help the field if researchers are united in utilizing the same, consistent experimental system instead of those with tremendous inherent variability [61] or those that garner large patient by patient inconsistency [62]. So what is the next step for those in this industry, does the field need to validate the technologies more or on a larger scale? There are some claims of lower efficiency of the new methods [63,64], so perhaps it is that research groups are attempting to adopt the new techniques and are obtaining poor results?

Defined, xeno-free cultures of pluripotent cells are imperative for their industrial scale production and clinical application. In 2010 the International Stem Cell Forum funded a project comparing the performance of different media for culturing human ESCs [74]. Emerging stem cell products [72] including those in clinical trials [75] will benefit from a similar organized

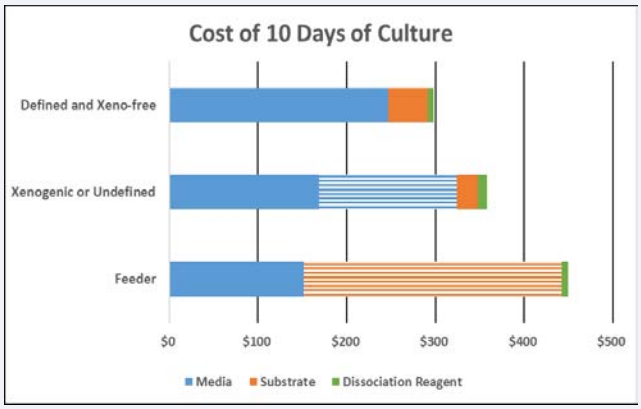

Figure 2 Cost estimate of pluripotent cell culture using the materials described in Table 1: We assume 5 6-well plates are used to culture cells for 10 days with amount of materials recommend by the supplier [1,2,4,58,65,66,68-71]. For note, a well is 9.8cm^2, therefore for the 5 6 well plates the total area is 294cm^2.

standardization project for evaluating the advanced culture technologies. Our cost analysis results shown in Figure (2) and Table (1) indicate that the difference in cost between undefined/xenogenic and defined/xeno-free culture systems is becoming negligible. Therefore, it is an ideal time to validate and adopt those technologies by the laboratories not only in industry but also in academia. Benefits and limitations of the new technologies should be evaluated objectively to establish standards. These standardizing efforts streamline the materialization of revolutionary technology discovered in academic and start-ups laboratories.

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