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SERUM-FREE MEDIA

Serum-free media formulations are cell line–specific and require optimization for microcarrier culture

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Abstract

Background aims. Mesenchymal stromal cells (MSCs) are being investigated as potential cell therapies for many different indications. Current methods of production rely on traditional monolayer culture on tissue-culture plastic, usually with the use of serum-supplemented growth media. However, the monolayer culturing system has scale-up limitations and may not meet the projected hundreds of billions to trillions batches of cells needed for therapy. Furthermore, serum-free medium offers several advantages over serum-supplemented medium, which may have supply and contaminant issues, leading to many serum-free medium formulations being developed. *Methods.* We cultured seven MSC lines in six different serum-free media and compared their growth between monolayer and microcarrier culture. *Results.* We show that (i) expansion levels of MSCs in serum-free monolayer cultures may not correlate with expansion in serum-containing media; (ii) optimal culture conditions (serum-free media for monolayer or microcarrier culture) differ for each cell line; (iii) growth in static microcarrier culture does not correlate with growth in stirred spinner culture; (iv) and that early cell attachment and spreading onto microcarriers does not necessarily predict efficiency of cell expansion in agitated microcarrier culture. *Conclusions.* Current serum-free media developed for monolayer cultures of MSCs may not support MSC proliferation in microcarrier cultures. Further optimization in medium composition will be required for microcarrier suspension culture for each cell line.

Key Words: *mesenchymal stromal cell, microcarrier, serum-free medium*

Introduction

Mesenchymal stromal cells (MSCs; alternatively mesenchymal stem cells) are currently being investigated for many different therapies; there are 460 trials listed on Clinicaltrials.gov as of February 1, 2015 [1]. Cells expressing characteristics defined by the International Society for Cellular Therapy as MSC markers (surface markers CD73, CD90, CD105 and osteogenic, chondrogenic and adipogenic differentiation ability) [2] have been extracted from various sources of tissue, with varying growth properties [3–6]. Investigations into their clinical use include their potential to differentiate for tissue replacement (bone, cartilage) as well as their immunomodulatory properties *in vivo* and their expression of paracrine factors supporting tissue regeneration [7–15].

MSCs are predominantly grown in serum-supplemented media for use in clinical trials [16–19]; the first serum-free media (SFM) approved for use with MSC culture for clinical trials was only cleared by the US Food and Drug Administration in 2011 [20,21]. However, there is a growing recognition that SFM may be more desirable for several reasons, such as batch-to-batch variability of FBS, limited global supply of serum and potential xeno-contaminants [22–24]. These issues will only be magnified if MSC therapies become widespread, when production at industrial scales is necessary.

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Table I. Human MSC lines from various cell banks and their tissue sources used in this study.

MSC line	Tissue source	Cell bank	Catalog No.	Media used for initial expansion	Working cell bank passage
UC-MSC-one	Umbilical cord matrix	Promocell	C-12971	α10	9
UC-MSC-2	Umbilical cord matrix	ATCC	PCS-500−010	ATCC MSC basal media + supplement (PCS-500−040)	6
UCB-MSC	Umbilical cord blood	Medipost	G-12−363−2	α10	7
BM-MSC-1	Adult bone marrow	CTI Biotech	−	α10	8
BM-MSC-2	Adult bone marrow	RoosterBio	−	RoosterBio MSC growth medium	3
BM-MSC-3	Fetal bone marrow [47]	National University Hospital, Singapore	−	α10	8
Adipose MSC	Adipose tissue	ATCC	PCS-500−011	ATCC MSC basal media + supplement (PCS-500−040)	6

MSCs were obtained from a variety of tissues and cell banks. Cells were expanded according to the manufacturer's instructions, all which involved serum-containing media. Working cell banks were established and used for subsequent testing in SFM, with passage number of cells kept below 10 passages.

Hence, many different SFM formulations have been developed, published by academic labs [25−28] and reported as proprietary compositions by commercial companies [29−37]. Most serum-free formulations claim equivalent cell population doubling time compared with serum-containing media in monolayer culture and/or preservation or improvement in MSC characteristics such as expression of cell surface markers and differentiation capability [29,38−41]. Previous studies have compared different SFM formulations for MSC culture in monolayers, each study testing different MSC lines, showing that the media of choice can influence cell proliferation, differentiation and time of cellular senescence [33,42,43], albeit to different rates. Given the many different MSC lines and SFM formulations available, the question arises if there is one ideal formulation for all cell lines and the different methods of propagation or if each cell line and production method will require a different optimal media for expansion and maintenance.

In parallel, there is an anticipated need for large-scale production of MSCs that traditional monolayer culture might not meet [44]. Estimated amounts of MSCs required for one treatment dose vary between billions to hundreds of billions of cells, and a patient may require multiple doses [16,44,45]. This could result in a demand for production of trillions of cells per production batch. Whereas monolayer culture can only feasibly reach tens of billions even with automated multi-layer cell stacks, microcarrier cultures can potentially reach trillions of cells per batch [44].

Given the reported differences in cell phenotype through the use of different SFM formulations [38,46] as well as differences between monolayer vs microcarrier culture [33,47], we decided to carry out a comparative study of several MSC cell lines expanded in SFM in monolayer versus microcarrier culture. SFM developed in-house as well as several commercially available serum-free formulations were investigated. We found that.

(i) In monolayer culture, MSC cell density (cells per cm^2) achieved in SFM may not correlate with the density achieved in medium containing serum.
(ii) There is no single optimal SFM formulation that works for all cell lines. Each cell line achieved different levels of cell density (cells per cm^2) in the different SFM formulations. Hence, the optimal SFM conditions differ for each cell line.
(iii) With the use of several SFM, we found variable levels of correlation between efficiency of growth in monolayer cultures versus growth in microcarrier cultures. The optimal medium can differ between monolayer culture and microcarrier culture, even in the absence of shear stress induced by culture agitation.
(iv) Growth of MSCs in static microcarrier cultures does not correlate with MSC growth on microcarriers in stirred spinner culture.
(v) Levels of early cell attachment and spreading (1−2 h after seeding) on the microcarrier surface do not necessarily predict efficiency of cell expansion in agitated microcarrier culture.

Our results indicate that the use of SFM in MSC culture can give greater cell expansion than serum-containing medium, helping to meet the large numbers of cells projected for cell therapy. However, SFM optimized for monolayer culture of MSCs may not support the growth of MSCs in microcarrier culture. A number of SFM exist, and it is critical to select the right one in microcarrier culture during early investigative and preclinical trials for projected large-scale cell production in bioreactors. Furthermore,

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