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Expansion of embryonic stem cells in suspension and fibrous bed bioreactors

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ABSTRACT

Applications of embryonic stem (ES) cells in cellular transplantation and tissue engineering require scalable processes for mass production of these cells with controlled qualities. The main objective of this work was to evaluate two cell culture processes for long-term expansion of murine embryonic stem (mES) cells. With serial passaging, suspension cultures in spinner flasks were able to expand mES cells as aggregates for 12.5-fold in each passage of 4 days. However, extending the culturing time to 6 days in each passage caused significant loss in cell viability and induced differentiation as indicated by the reduced expression levels of SSEA-1 and Oct-4. Long-term expansion of mES cells in a fibrous bed bioreactor (FBB) was also studied for 30 days in 2 passages, 15 days in each passage. With periodically refreshing the culture medium, a high expansion fold of 60–77 was achieved in each passage. Flow cytometry and RT-PCR were used to analyze key pluripotency and differentiation markers. The results showed that the expanded cells in both suspension and FBB cultures remained in a highly pluripotent state, which was also confirmed with the embryoid body (EB) forming efficiency test. It is concluded that both the suspension and FBB cultures are suitable to support long-term expansion of undifferentiated mES cells. However, the FBB culture can sustain cell growth for a longer period without frequent passaging, requires less media and labor, and is thus more economical to use for mass production of ES cells.

1. Introduction

Pluripotent stem cells (PSCs), including embryonic stem (ES) cells and induced pluripotent stem cells (iPSCs), have unlimited capacities to self-renew and differentiate into all cell types, and thus have a great potential for applications ranging from cell therapies, drug discoveries, disease modeling to tissue engineering (Engle and Puppala, 2013; Rajamohan et al., 2013; Wu and Hochedlinger, 2011). However, for these applications, a scalable and efficient culture system for economical mass production of ES cells with consistent properties is required and must be developed (Abbasalizadeh and Baharvand, 2013; Sharma et al., 2011). The expansion of ES cells based on common laboratory procedures is carried out in two-dimensional (2-D) static cell culture systems such as T-flasks, which are limited by the available surface area and difficult to scale up (Oh et al., 2005; Want et al., 2012). It also requires frequent subculturing or cell passages in order to maintain the undifferentiated state of ES cells, which is labor intensive, time consuming, and expensive, and cannot meet the projected market demand for ES cells (Ouyang and Yang, 2008; Subranmanian et al., 2010).

More recently, suspension cultures of PSCs and adult stem cells as cell aggregates (Alfred et al., 2010; Cormier et al., 2006; Gilbertson et al., 2006; Kehoe et al., 2008; Sen et al., 2001; zur Nieden et al., 2007) or microcarrier cultures (Alfred et al., 2011; Chen et al., 2013; Lock and Tzanakakis, 2009) have been extensively studied in stirred-tank bioreactors (see Table 1). Stirred-tank bioreactors with pH, dissolved oxygen and temperature controls can provide relatively homogenous and well-defined environments for stem cell growth and expansion (Chaudhry et al., 2009; King and Miller, 2007; Serra et al., 2010). ES cells cultured on microcarriers in stirred tanks could reach a high expansion fold of 30–70 and final cell density of $\sim 3.5 \times 10^7$ cells/mL (Abranches et al., 2007; Fernandes et al., 2007; Fok and Zandstra, 2005), and the culturing period for each passage could be extended to 8 days (Fernandes et al., 2007). A high expansion fold of 439 were

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Table 1
Comparison of PSC expansion cultures in suspension bioreactors.

Culture type	Cell types	Culture volume (mL)	Seeding density (10^6 mL^{-1})	Final density (10^6 mL^{-1})	Culture time (day)	Expansion fold	Doubling time (h)[a]	Production rate (10^6/day/mL)	$Y_{lact/gluc}$ (g/g)	References
Aggregates	mESC	50	0.05	–	15	53.4	23.5	–	–	Fok and Zandstra (2005)
		100	0.0375	1.1	5	29.3	24.6	0. 22	0.5–0.6	Cormier et al. (2006)
		100	0.0375	0.5–1.2	4	13.3–32	19.2–25.7	0.12–0.29	–	zur Nieden et al. (2007)
		100	0.025	1.0	4	40	18	0.24	–	Kehoe et al. (2008)
		100	0.0375	1.1–1.8	6	29–48	25.8–29.6	0.17–0.29	0.4–0.5	Alfred et al. (2010)
		50	**0.08**	**1.10 ± 0.01**	**4**	**13.75**	**25.4**	**0.28 ± 0.01**	**0.48 ± 0.08**	**This study**
	miPSC	30–50	0.05	0.8–1.2	3	16–24	15.7–18	0.25–0.38	–	Fluri et al. (2012)
		100	0.05	0.75–3.5	4	15–70	15.7 – 24.6	0.18–0.86	–	Shafa et al. (2012)
	hESC	100	0.018	0.45	6	25	31	0.072	–	Krawetz et al. (2010)
		50	1.0	2–2.4	7	2–2.4	133–168	0.14–0.2	–	Singh et al. (2010)
		–	0.06	0.32	7	5.3	70	0.037	–	Kehoe et al. (2010)
		50	0.106	1.89	6	17.7	34.7	0.30	–	Amit et al. (2011)
	hPSC	50	1.0	2–3	7	2–3	106–168	0.14–0.29	–	Zweigerdt et al. (2011)
		100	0.3	2.4	7–10	8	56–80	0.2–0.3	0.5–0.9	Abbasalizadeh et al. (2012)
		100	0.4–0.5	2.0–2.4	7	4–5	72–84	0.23–0.28	0.5–0.8	Olmer et al. (2012)
	hiPSC	100	0.4–0.5	1.0–2.0	3–4	2–4	72	0.33–0.5	–	Wang et al. (2013)
Micro-carriers	mESC	50	0.05	–	15	192	47.5	–	–	Fok and Zandstra (2005)
		80	0.01–0.1	2.5–3.9	8	39–250	24.1–36.3	0.31–0.475	–	Abranches et al. (2007)
		30–80	0.05	1.9–3.5	8	38–70	31.3–36.6	0.23–0.43	0.7–0.85	Fernandes et al. (2007)
		50	0.06	0.25–0.9	3	4.2–15	18.4–34.8	0.06–0.28	0.5–0.8	Storm et al. (2010)
		100	0.008–0.03	1.5–3.56	5–6	68–439	16.4–23.7	0.29–0.59	–	Alfred et al. (2011)
		30	0.05	2.8–4.2	8	85	30	0.34–0.52	0.8 ± 0.1	Fernandes-Platzgummer et al. (2011)
		700	0.05	4.3	11	85	41	0.39	0.85 ± 0.15	
	hESC	80	0.0625	0.15	5	2.3	100	0.02	–	Phillips et al. (2008)
		50	0.05–0.2	0.5–1.8	8	~10	~57.8	0.06–0.2	–	Lock and Parikh (2008)
		50	0.1–0.2	3.5	5–7	35	23.4–32.8	0.5–0.68	–	Oh et al. (2009)
		30	0.1	0.5–1.0	7	5–10	50.5–72.4	0.06–0.13	0.5–0.8	Storm et al. (2010)
		60	0.1	2.8	6	28	25.3–30.3	0.47	0.3–0.9	Marinho et al. (2013)
	hiPSC	–	0.025–0.1	0.18–0.37	8	3.3–7.4	66.5–83.6	0.02–0.04	–	Kehoe et al. (2010)
		50	0.08	1.4–1.9	6	18–23	31	0.23–0.32	–	Fan et al. (2013)
FBB	mESC	**10**	**0.08**	**5.46 ± 0.95**	**15**	**68.3**	**59.1 ± 0.2**	**0.36 ± 0.06**	**0.54 ± 0.13**	**This study**

Abbreviations: h, human; i, induced; m, mouse; ESC, embryonic stem cell; PSC, pluripotent stem cell (including both ESC and iPSC).
Bold highlights the results in this study which is indicated in the last column.
[a] Apparent doubling time calculated from the expansion fold and the total culture time, including the lag phase. The doubling or generation time during the exponential growth phase would be shorter.