

# CELL & GENE THERAPY INSIGHTS

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MANUFACTURING PATHWAY

SPOTLIGHT

## FUTURE LEADER PERSPECTIVE

# Toward a scalable and consistent manufacturing process for the production of human mesenchymal stem cells

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The development of novel, affordable and efficacious therapeutics will be necessary to ensure the continued progression in the standard of global healthcare. With the potential to address previously unmet patient needs as well as tackling the social and economic effects of chronic and age-related conditions, cell therapies will lead the new generation of healthcare products set to improve health and wealth across the globe. However, if many of the small-to-medium enterprises (SMEs) engaged in much of the commercialization efforts are to successfully traverse the 'Valley of Death' as they progress through clinical trials, there are a number of challenges that must be overcome. No longer do the challenges remain biological but rather a series of engineering and manufacturing issues must also be considered and addressed.

Revenues for the cell therapy industry recently exceeded US\$1 billion [1], and with market approvals of stem cell therapy products, including Prochymal's (Osiris Therapeutics, Maryland, USA) human mesenchymal stem cell (hMSC) therapy, there is growing momentum and optimism that the cell therapy industry, learning from the failings of the tissue engineering

field, will come to fruition [2]. This is buoyed by government investment and industry commitment, as evidenced in the UK by the creation of the Cell and Gene Therapy Catapult and Manufacturing Centre [3], in Canada by the emergence of the Centre for Commercialization of Regenerative Medicine and centre for advanced therapeutic cell technologies

(with investment from FedDev Ontario and GE Healthcare) and a newly-introduced regulatory and reimbursement environment in Japan conducive for cell therapy manufacture [4]. Cell therapies are no longer solely a pursuit of scientific endeavor but a commercially viable industry in its own right – and a burgeoning one at that [1].

Human MSCs are a promising cell candidate for cell therapies due to their therapeutic efficacy, as determined by pre-clinical and clinical studies [5-8], their relative ease and multiple sources of isolation (Table 1), multi-lineage differentiation capacity and the ability to expand these cells *in vitro* [9]. With over 450 clinical trials involving the use of hMSCs by January 2015 [10], the interest in commercializing hMSC therapies is clear. However, in July 2011, the UK's Office for Life Science published a report which identified that "without the ability to manufacture, store, transport and distribute regenerative medicine products, the therapies would never become mainstream clinical practice" [11]. Bioprocess development and consistent manufacture is a key challenge that SMEs have faced, or will soon encounter, as they navigate through clinical trials; the ability to fulfil the increasing demand for cells at a quality and quantity required for therapeutic application.

### BIOPROCESSING CONSIDERATIONS FOR HMSC THERAPIES

There are a number of bioprocess challenges and considerations for the development of a hMSC therapy [36,37]; however this article will focus on those solely related to the expansion of hMSCs. Essentially, these challenges can be identified as follows:

- ▶ Cell quality – the cell forms the basis of the product
- ▶ Cell quantity – the number of cells required for therapeutic applications
- ▶ Cell nature – the anchorage-dependency of many cell therapy candidates
- ▶ Unprecedented and an undefined methodology

The large-scale *in vitro* expansion of cells, where the cell forms the basis of the product, is a paradigm shift for the biotechnology industry. Where the cell is the product, i.e., what is to be injected into the patient, there must be efficient harvest and the cell must retain its key quality attributes with respect to identity, potency, purity and safety [38] regardless of the intended application. In addition to cell quality, obtaining the numbers of cells required for therapeutic applications is another significant challenge. For the majority of applications, the expansion of hMSCs *in vitro* will be required to increase the number of functional cells to elicit a therapeutic benefit.

As illustrated in Table 2, the numbers of MSCs delivered to patients in clinical trials varies greatly but for a patient of 70 kg, 0.3 to 5 x 10<sup>8</sup> cells per treatment may be required. For allogeneic treatments this will therefore mean generating lot sizes of potentially trillions of cells [39].

▶ **TABLE 1** Sources of mesenchymal stem cells.

Source	Refs
Bone marrow	[7], [9], [12], [13]
Adipose tissue	[15]
Synovium	[16]
Trabecular bone	[17], [18], [19], [20]
Skin Tissue	[21], [22]
Adult peripheral blood	[23], [24]
Umbilical cord (Wharton's Jelly)	[25]
Cord blood	[26], [27]
Deciduous teeth	[28]
Fetal blood, bone marrow, liver & lung	[29], [30]
Muscle	[31], [32], [33]
Pericyte	[34]
Periosteum	[35]

CONSIDERATIONS FOR THE CULTURE OF hMSCS

Dissolved oxygen

In conventional mammalian cell culture (for the production of heterologous recombinant proteins), the level of dissolved oxygen in the growth medium is important and as such is always measured and often carefully controlled. Measurements in monolayer culture of hMSCs in T-flasks are usually limited to cell viability, confluency and those related to post culture functionality. However in order to inform the basis of the development of the larger scale production of hMSCs, the factors controlling the process need to be fully understood.

The general belief for hMSC expansion under controlled oxygen concentrations is that the concentration in the growth medium

should mimic the *in vivo* physiological conditions from which the hMSCs have been derived, in this case, bone marrow. However, there are conflicting results. Work has shown [52-54] that under “normoxic” conditions (i.e. 20 % O<sub>2</sub> / 75% N<sub>2</sub>, 5 % CO<sub>2</sub> v/v in the incubator, nominally ≅100 % dO<sub>2</sub>), the expansion of hMSCs is inferior compared to that obtained under “hypoxic” conditions (~ 2-5% O<sub>2</sub> v/v in the incubator, nominally ≅ 10-25 % dO<sub>2</sub>). Other studies, however, have demonstrated that, based on the concentration in the incubator, 10-25 % dO<sub>2</sub> (‘hypoxia’) can have an impact on either cell quality by attenuating cell differentiation [55] or cell quantity by reducing cell proliferation [56-58] in comparison to 100% dO<sub>2</sub> (‘normoxia’). Clearly the problem is not yet fully resolved and there are a number of possible

▶ **TABLE 2** — Examples of treatments and the number of cells transplanted or injected into patients during each trial.

Condition		Number of cells delivered per treatment	Refs
<b>AUTOLOGOUS</b>			
BM-MSCs	Ischemic heart failure	20x10 <sup>6</sup> , 100x10 <sup>6</sup> or 200x10 <sup>6</sup> cells/patient	[40]
BM-MSCs	Amyotrophic lateral sclerosis	11-120x10 <sup>6</sup> cells/patient	[41]
BM-MSCs	Stroke	60-160x10 <sup>6</sup> cells/patient	[42]
BM-MNCs	Stroke	1 x 10 <sup>8</sup> cells/patient	[43]
BM-MSCs	Graft versus host disease	1-2x10 <sup>6</sup> cells/kg	[44]
BM-MSCs	Cartilage repair (osteoarthritic knee)	13x10 <sup>6</sup> cells/patient	[45]
BM-MSCs	Multiple sclerosis	32-52x10 <sup>6</sup> cells/patient	[46]
BM-MSCs	Multiple sclerosis	1-2x10 <sup>6</sup> cells/kg body weight	[47]
<b>ALLOGENEIC</b>			
Prochymal®	Graft versus host disease	2 or 8x10 <sup>6</sup> cells/kg body weight	[48]
BM-MSCs	Graft versus host disease	1.7-2.3x10 <sup>6</sup> cells/kg body weight	[49]
Prochymal®	Myocardial infarction	0.5x10 <sup>6</sup> , 1.6x10 <sup>6</sup> or 5x10 <sup>6</sup> cells/kg body weight	[50]
PD-MSCs	Diabetes	1.2-1.5x10 <sup>6</sup> cells/kg body weight	[51]

Prochymal® is an allogeneic bone marrow-derived MSC product from Osiris Therapeutics Inc (USA). Abbreviations: BM-MSCs: bone marrow-derived MSCs; BM-MNCs: bone marrow mononuclear cells; PD-MSCs: placenta-derived MSCs.

explanations for this difference, not least of which may be cell line specificity or culture conditions.

### Serum-free media

The culture of mammalian cells, be it for the production of proteins, vaccines or cell therapies, requires complex nutrients which have traditionally been provided in the form of growth-factor-rich media supplemented with Fetal Bovine Serum (FBS) [59]. It is widely acknowledged however that the addition of FBS is undesirable for a variety of reasons and efforts are being made to develop defined, serum-free processes [60]. The oft-cited reason for avoiding the use of serum is the risk of contamination through the introduction of adventitious, xenogeneic agents [60]. This is often the biggest driver for the switch from serum-based to serum-free processes given the perceived FDA aversion towards serum [61]; however this risk is mitigated in part by the rigorous screening and selection process required for GMP-grade serum.

Batch-to-batch variability of serum is another reason for the shift away from serum-based processes [62]. Serum is poorly defined and there can be significant variation between batches, resulting in a lack of reproducibility. For an industry where the “process is the product”, standardization is crucial and variation in culture conditions outside of pre-defined limits is unacceptable. Moving towards a well-defined medium will allow for the development of standardized, reproducible manufacturing methods and would avoid costly serum batch testing.

Moreover, Brindley *et al* posit that the biggest concern with employing serum-based processes is

not necessarily due to the perceived regulatory issue, as GMP-grade serum can be sourced, but rather a supply and availability issue [61].

In light of the concerns regarding the use of serum, there is now a growing body of literature investigating the use of serum-free media for monolayer culture [60,62–67] and microcarrier-based culture of hMSCs [68–70] with varying degrees of success. Table 3 provides an overview of various commercially available serum/xeno-free hMSC media.

## EXPANSION TECHNOLOGIES

To be able to obtain a sufficient number of cells for a cell therapy, be it autologous or allogeneic, *ex vivo* cell expansion is an essential step in the development process. There are numerous techniques which are currently employed for the scale-up or scale-out of adherent cells with their own respective advantages and disadvantages. This article will focus specifically on microcarriers. A more detailed comparison of the various expansion systems is provided by the author [71,72].

### Microcarriers

Microcarrier technology provides a significantly larger surface area per unit volume of bioreactor [73] compared to monolayer culture, and combines the potential ease of scalability, process monitoring and control capability associated with bioreactor cultures that makes bioreactor culture common place in the biopharmaceutical arena. Numerous types of microcarrier particles are commercially available with varying surfaces, charge, structures and other properties (Table 4).

**TABLE 3**

**Comparative summary of different hMSC serum-free media and FBS-based medium.**

	Manufacturer	Glutamine supplement required?	Attachment substrate required?	Xeno-free available?	Dissociation reagent	Medium Exchange	Days until passage	Shelf-life of prepared medium	Notes
Mosaic	BD	✗	✓	✗	Accutase	Not required	3	28 days	Attachment substrate can be applied to as many flasks as necessary and stored for 3 weeks. No longer available for purchase.
DMEM + FBS	Multiple	✓	✗	✗	Trypsin-ED-TA	After 3 days	6	28 days	Simplest and most user-friendly protocol with no attachment substrate.
TheraPeak	Lonza	✗	✗	✓	Any non-animal derived dissociation reagent	After 3 days	6	28 days	Identical to DMEM passage protocol therefore most user-friendly. No attachment substrate needed
Mesen-Cult	Stem Cell Technologies	✓	✓	✓	Manufacturer's own dissociation and inhibition reagents	After 5 days and if medium appears "yellow"	5 or 6 days	5 days	Most involved protocol with prepared medium only available for 5 days (therefore needing many working aliquots). Attachment substrate needed 24 h before culture
StemPro	Life Technologies	✓	✓	✓	TryPLE Select	Every 2 days	6	14 days	Requires 2 medium exchanges during a routine passage. Attachment substrate required 1 h prior to culture
Prime-XV	Irvine Scientific	✗	✓	✓	TryPLE Express	Every 2 days	6	28 days	Requires 2 medium exchanges during a routine passage. Attachment substrate required a minimum of 1 h prior to culture
Xuri	GE Healthcare	✗	✓	✓	TryPLE Select	Every 2-3 days	6 days	30 days	Attachment substrate required. Coated vessels can be stored for 1 week when covered with Parafilm.

TABLE 4

Properties of commercially available microcarriers

Microcarrier	Manufacturer	Diameter (µm)	Matrix	Avg. density	Surface coating	Surface charge	Carrier porosity
<b>MAMMALIAN PROTEIN-COATED MICROCARRIERS</b>							
Collagen	Pall SoloHill®	125-212	Polystyrene	1.02	Type I porcine collagen	None	Non-porous
Cultispher-G®	PerCell-BioLytics	130-380	Type I porcine gelatin	1.04	None	None	Macroporous (porosity: 50 % pore size: 10 - 30 µm)
Cytodex 3™	GE Healthcare	141-211	Dextran	1.04	Type I porcine collagen	None	Non-porous
FACT III	Pall SoloHill®	125-212	Polystyrene	1.02	Cationic Type I porcine collagen	+	Non-porous
Global Euraryotic Microcarrier (GEM™)	Global Cell Solutions	75-150	Polysaccharide alginate	1.02	Porcine gelatin	Magnetically charged	Non-porous
SphereCol®	Advanced BioMatrix	125-212	Polystyrene	1.03	Type I human collagen (VitroCol®)	None	Non-porous
<b>RECOMBINANT PROTEIN-COATED MICROCARRIERS</b>							
Pro-Nectin® F	Pall SoloHill®	125-212	Polystyrene	1.02	Recombinant fibronectin	None	Non-porous
<b>XENO-FREE MICROCARRIERS</b>							
Cytodex 1™	GE Healthcare	147-248	Dextran	1.03	DEAE	+	Non-porous
Cytopore 1 and 2™	GE Healthcare	200-280	Cotton cellulose	1.03	DEAE	+	Micro/Macroporous (porosity: > 90 % pore size: 30 µm)
Enhanced Attachment	Corning	125-212	Polystyrene	1.02	CellBIND®	None	Non-porous
Glass	Pall SoloHill®	125-212	Polystyrene	1.02	High silica glass	None	Non-porous
Hillex® CT	Pall SoloHill®	90-212	Polystyrene	1.12	Cationic trimethyl ammonium	+	Non-porous
Hillex®	Pall SoloHill®	160-180	Dextran	1.11	Cationic trimethyl ammonium	+	Non-porous

TABLE 4 CONT.

Microcarrier	Manufacturer	Diameter (µm)	Matrix	Avg. density	Surface coating	Surface charge	Carrier porosity
MicroHex™	Nunc	Side-length: 125 µm Thickness: 25 µm	Polystyrene	1.05	Nunclon™ surface	Not specified	Non-porous
Plastic	Pall SoloHill®	125–212	Polystyrene	1.02	None	None	Non-porous
Plastic Plus	Pall SoloHill®	125–212	Polystyrene	1.02	None	+	Non-porous
PVA	Loughborough University	100–220	PVA	1.03	None	None	Non-porous
Star-Plus	Pall SoloHill®	125–212	Polystyrene	1.02	None	Charged	Non-porous
Synthemax II®	Corning	125–212	Polystyrene	1.02	Synthemax II®	None	Non-porous

## MSC MICROCARRIER STUDIES

### Microcarrier culture conditions

It has become increasingly clear that to develop an optimal microcarrier-based hMSC culture process, parameters for the culture must be identified and optimized. Table 5 provides a list of these various parameters for different stages of the process.

### Microcarrier selection

At present, there is no unified set of culture conditions for the expansion of hMSCs on microcarriers given the infancy of the research. Some groups have demonstrated successful growth on one particular microcarrier over another, for example Schop and colleagues found that when comparing nine different microcarriers, Cytodex-1 was selected after it demonstrated the highest seeding efficiency [74]. In contrast, Dos Santos and colleagues found that having previously used Cultispher-S [75] (a gelatin-based microcarrier), a xeno-free approach was required for the adoption of such a process for clinical-grade expansion, and therefore selected Plastic P102-L. Our group developed a systematic microcarrier screening process for hMSCs including 13 commercially available microcarriers and found that Collagen and Plastic P102-L microcarriers were optimal for hMSC growth for three different donor cell lines [76].

### Medium selection

As mentioned previously, there is a growing body of literature focusing on hMSC monolayer culture with serum-free medium. Given

**TABLE 5**  
**Parameters for each hMSC microcarrier culture process step with a list of studies which have investigated this particular parameter.**

Stage of process	Parameter	Studies
Vessel configuration	Impeller selection	[79]
	Baffles	-
	Vessel geometry	-
Inoculation	Impeller delay	[82], [83]
	Intermittent/constant agitation	[80]
	Cell/microcarrier seeding density	[81], [80], [79]
Agitation	Agitation speed	-
	Intermittent/constant agitation	[68], [74]
Culture	Medium selection	[69], [70], [84]
	Microcarrier selection	[74], [85], [68], [86]
	pH	[87], [76]
	dO <sub>2</sub> / dCO <sub>2</sub>	[87], [85]
	Sparging	-
	Addition of extra microcarriers	[85]
Medium exchange	Level of medium exchange	[75]
	Frequency of medium exchanges	[75]
Harvest	Dissociation reagent	-
	Agitation speed	[88, 89]

A (-) indicates no studies were found to have investigated this parameter as of yet.

that work is still ongoing to determine the optimal monolayer hMSC culture conditions, it is likely that microcarrier culture conditions will lag behind, as is evident by fewer studies investigating serum/xeno-free hMSC microcarrier cultures. Dos Santos and colleagues have employed a completely xeno- and serum-free microcarrier culture process, where they used the StemPro<sup>®</sup> MSC xeno-free medium [68]. They found that they were able to effectively culture bone-marrow and adipose-derived hMSCs under such conditions, reaching a maximum cell density of 2.0 x 10<sup>5</sup> cells/mL for the bone marrow derived hMSCs in a working volume of 80 mL.

Using Prime-XV, we achieved a maximum cell density of > 3.0 x 10<sup>5</sup> cells/mL in a working volume of 100 mL on Plastic P102-L microcarriers [69].

### Seeding density

It has been suggested in the literature that seeding density has an effect on the proliferation of hMSCs grown as a monolayer, with lower seeding densities (100 cells/cm<sup>2</sup>) demonstrating increased proliferation compared to higher seeding densities (5000 cells/cm<sup>2</sup>) [77,78]. In animal and human MSC microcarrier culture, this is an area which has also received attention, with studies investigating the effect of different cell-to-bead ratios [79-81]. Frauenschuh and colleagues described the cell attachment process as following a Poisson distribution [81], and found that initial cell seeding densities ranging from 1-3 x 10<sup>6</sup> cells/100 cm<sup>2</sup> surface area had little effect on attachment. With respect to the cell-to-bead ratio, there appears to be consistency in the data presented with studies by Hewitt and colleagues [79] and Yuan and colleagues [80] suggesting that a ratio of 5 cells-to-bead may be optimal.

### Operating conditions

The combination of microcarrier culture with a bioreactor system provides all of the benefits associated with bioreactors such as a greater level of culture homogeneity achieved via agitation as well as process monitoring and control. This however means there is the need to consider and optimize the operating parameters of the bioreactor also.

Much of the research carried out thus far attempts to demonstrate the effect of some of the aforementioned parameters on hMSC yield and quality. Dos Santos and colleagues opted to employ an intermittent agitation



**TABLE 6**  
**Studies of MSC culture on microcarriers.**

Ref.	MSC source	Microcarrier	Working volume (mL)	Max. cell density (cells/mL)	Volume harvested (mL)	Notes
[84]	Porcine bone marrow MSCs	Cytodex-1	40	$\sim 1.0 \times 10^6$	40	
[74]	Human bone marrow MSCs	Cytodex-1	50	$\sim 1.5 \times 10^5$	2	Volume harvested was based on sample size
[83]	Porcine bone marrow MSCs	Cytodex-1	200	$\sim 4.0 \times 10^5$	-	No sample or harvest volume specified
[79]	Human placental MSCs	Cytodex-3	80-100	$\sim 3.8 \times 10^5$	-	Volume harvested was based on sample size
[90]	Human placental MSCs	Cytodex-3	-	$\sim 1.05 \times 10^6$	2	It is unclear what the working volume of the microcarrier culture was
[86]	Human bone marrow MSCs	Modified Cytodex-3	30	$\sim 5.0 \times 10^5$	30	
[75]	Human bone marrow MSCs	Cultispher-S		$\sim 4.2 \times 10^5$	50	
[91]	Rat ear MSCs	Cultispher-S	1000	$\sim 9.0 \times 10^5$	0.5	Harvest volume was based on sample size
[80]	Human bone marrow MSCs	Cultispher-S	125	-	125	
[92]	Human bone marrow MSCs	Cultispher-G	200	$\sim 3.4 \times 10^5$	1	Harvest volume was based on sample size
[68]	Human bone marrow and adipose tissue MSCs	Plastic P102-L	80	$2.0 \times 10^5$ (bone marrow MSCs) $1.4 \times 10^5$ (adipose tissue MSCs)	0.5	Carried out in xeno-free medium. Harvest volume was based on sample size
[93]	Human bone marrow MSCs	Collagen	2000	$9.0 \times 10^4$	-	Volume harvested was based on sample size
[69]	Human bone marrow MSCs	Plastic P102-L	100	$3.2 \times 10^5$	100	Serum-free medium culture. Full volume harvested

strategy whereby during the first 24 h, the culture was agitated for 15 min at 25 rpm after which followed a period of non-agitation for 2 h [68]. After this, the culture was agitated constantly at 40 rpm for the duration of the culture. Schop and colleagues instead employed an agitation strategy of constant agitation at 30 rpm for 18 h, after which the culture was constantly agitated at 40 rpm [74].

Medium exchange regime is another key consideration; the regularity and amount of medium exchange has to be controlled carefully. With respect to hMSC microcarrier culture, the effect of medium exchange regime was demonstrated by Eibes and colleagues who compared two medium exchange regimes; (i) a 25% medium exchange every 48 h and (ii) a 25% medium exchange every 24 h starting after day 3 [75]. They found that the first medium exchange regime resulted in a significant depletion of glucose during the exponential phase of cell growth, with an associated increase in ammonium concentration which reached inhibitory values. The second medium exchange regime did not result in such adverse metabolite concentrations, with glucose present throughout the exponential phase and the level of ammonium not reaching inhibitory values [75].

#### Microcarrier harvest

A key factor in the choice of microcarrier is the ability to efficiently harvest the cells after hMSC expansion, a decision which will impact downstream processing. In the majority of the studies listed in Table 6, the working volume is below 200 mL and there is little focus on the harvesting procedure, or the ability to effectively harvest should the process increase in scale. This would appear to suggest

that most, if not all of the work involving the expansion of hMSCs on microcarriers, has focussed solely on the attachment, expansion and culture conditions of hMSCs. Detachment of cells from the microcarrier surface and subsequent retention of cell quality is equally as important as cell attachment and proliferation given that the product of interest for cell therapies is the cell itself. This problem will only be exacerbated as expansion scale increases and therefore it is crucial to consider cell harvesting strategies from the outset so as to ensure a viable, holistic bioprocess.

#### TRANSLATIONAL INSIGHT

The successful development of clinically-relevant, reimbursable cell therapy products and other advanced therapeutics will require a shift in mindset toward one that values, understands and applies translational research – the type of research that takes science from the bench and addresses technological, manufacturing, commercial, regulatory and clinical challenges, thereby enabling the delivery of healthcare and economic benefits. It is my contention that early-career researchers (ECRs), from both industry and academia, with a strong grounding in translation research, are therefore critical to accelerate the development process [82]. Although the translational pathway can be resource intensive, application-specific and fraught with obstacles, there are fundamental principles which can provide guidance along the prickly path [82]. Further understanding of how a product's CQAs correlate with clinical efficacy and better characterisation techniques will result in more consistent, standardized manufacturing processes, and as with biologics

production, there will be a continued increase in the cell densities that are obtained. But perhaps most importantly, with ever-increasing numbers of highly-trained, multidisciplinary ECRs emerging from world class training centres (the Catapult, CCRM and UK EPSRC Centres for Doctoral Training to name but a few), there is great reason to be optimistic and I fully believe that this generation of ECRs will harvest (pun intended) the crops planted and nurtured by the

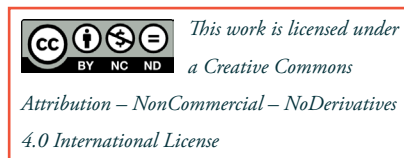
previous generation of translational scientists, engineers and clinicians who have pioneered the field.

### FINANCIAL & COMPETING INTERESTS DISCLOSURE

*The author has no relevant financial involvement with an organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This*

*includes employment, consultancies, honoraria, stock options or ownership, expert testimony, grants or patents received or pending, or royalties.*

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### REFERENCES

- Mason C, Brindley DA, Culme-Seymour EJ, Davie NL. Cell therapy industry: billion dollar global business with unlimited potential. *Regen. Med.* 2011; 6(3), 265–72.
- Cyranoski D. Cell-therapy conference feels (cautious) momentum. 2012; <http://blogs.nature.com/news/2012/05/cell-therapy-conference-feels-cautious-momentum.html>
- Technology Strategy Board: New centre to play vital role in future of health care. 2012.
- Azuma K. Regulatory Landscape of Regenerative Medicine in Japan. *Curr. Stem Cell Rep.* 2015; 1(2), 1–11.
- Caplan AI, Correa D. The MSC: an injury drugstore. *Cell Stem Cell* 2011; 9(1), 11–5.
- Caplan AI. Why are MSCs therapeutic? New data: new insight. *J. Pathol.* 2009; 217(2), 318–24.
- Pittenger MF, Martin BJ. Mesenchymal stem cells and their potential as cardiac therapeutics. *Circ. Res.* 2004; 95(1), 9–20.
- Barry FP, Murphy JM. Mesenchymal stem cells: clinical applications and biological characterization. *Int. J. Biochem. Cell Biol.* 2004; 36(4), 568–84.
- Pittenger MF, Mackay AM, Beck SC *et al.* Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; 284(5411), 143–7.
- Heathman TRJ, Nienow AW, McCall MJ, Coopman K, Kara B, Hewitt CJ. The translation of cell-based therapies: clinical landscape and manufacturing challenges. *Regen. Med.* 2015; 10(1), 49–64.
- Office for Life Sciences. Taking stock of regenerative medicine in the United Kingdom. 2011.
- Friedenstein AJ, Piatetzky S, Ii, Petrakova KV. Osteogenesis in transplants of bone marrow cells. *J. Embryol. Exp. Morphol.* 1966; 16(3), 381–90.
- Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. *Cell Tissue Kinet.* 1970; 3(4), 393–403.
- Risbud MV, Shapiro IM, Guttapalli A *et al.* Osteogenic potential of adult human stem cells of the lumbar vertebral body and the iliac crest. *Spine.* 2006; 31(1), 83–9.
- Zuk PA, Zhu M, Mizuno H *et al.* Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng.* 2001, 7(2), 211–28.
- De Bari C, Dell’accio F, Tylzanowski P, Luyten FP. Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis Rheum.* 2001; 44(8), 1928–42.
- Noth U, Tuli R, Osyczka AM, Danielson KG, Tuan RS. *In vitro* engineered cartilage constructs produced by press-coating biodegradable polymer with human mesenchymal stem cells. *Tissue Eng.* 2002; 8(1), 131–44.
- Osyczka AM, Noth U, Danielson KG, Tuan RS. Different osteochondral potential of clonal cell lines derived from adult human trabecular bone. *Ann. N. Y. Acad. Sci.* 2002; 961, 73–7.
- Sottile V, Halleux C, Bassilana F, Keller H, Seuwen K. Stem cell characteristics of human trabecular bone-derived cells. *Bone* 2002; 30(5), 699–704.
- Tuli R, Tuli S, Nandi S *et al.* Transforming growth factor-beta-mediated chondrogenesis of human mesenchymal progenitor cells involves N-cadherin and mitogen-activated protein kinase and Wnt signaling cross-talk. *J. Biol. Chem.* 2003; 278(42), 41227–36.

21. Toma JG, Akhavan M, Fernandes KJ *et al.* Isolation of multipotent adult stem cells from the dermis of mammalian skin. *Nat. Cell Biol.* 2001; 3(9), 778–84.
22. Chunmeng S, Tianmin C. Effects of plastic-adherent dermal multipotent cells on peripheral blood leukocytes and CFU-GM in rats. *Transplant. Proc.* 2004; 36(5), 1578–81.
23. Zvaifler NJ, Marinova-Mutafchieva L, Adams G *et al.* Mesenchymal precursor cells in the blood of normal individuals. *Arthritis Res.* 2000; 2(6), 477–88.
24. Kuznetsov SA, Riminucci M, Ziran N *et al.* The interplay of osteogenesis and hematopoiesis: expression of a constitutively active PTH/PTHrP receptor in osteogenic cells perturbs the establishment of hematopoiesis in bone and of skeletal stem cells in the bone marrow. *J. Cell Biol.* 2004; 167(6), 1113–22.
25. Sarugaser R, Lickorish D, Baksh D, Hosseini MM, Davies JE. Human umbilical cord perivascular (HUCPV) cells: a source of mesenchymal progenitors. *Stem Cells* 2005; 23(2), 220–229.
26. Hong SH, Gang EJ, Jeong JA *et al.* In vitro differentiation of human umbilical cord blood-derived mesenchymal stem cells into hepatocyte-like cells. *Biochem. Biophys. Res. Commun.* 2005; 330(4), 1153–61.
27. Moon YJ, Lee MW, Yang MS *et al.* Expression profile of genes representing varied spectra of cell lineages in human umbilical cord blood-derived mesenchymal stem cells. *Acta Haematol.* 2005; 114(2), 117–20.
28. Miura M, Gronthos S, Zhao M *et al.* SHED: stem cells from human exfoliated deciduous teeth. *Proc. Natl Acad. Sci. USA* 2003; 100(10), 5807–12.
29. Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. *Blood* 2001; 98(8), 2396–402.
30. In 'T Anker PS, Noort WA, Scherjon SA *et al.* Mesenchymal stem cells in human second-trimester bone marrow, liver, lung, and spleen exhibit a similar immunophenotype but a heterogeneous multilineage differentiation potential. *Haematologica* 2003; 88(8), 845–52.
31. Bosch P, Musgrave DS, Lee JY *et al.* Osteoprogenitor cells within skeletal muscle. *J. Orthop. Res.* 2000; 18(6), 933–44.
32. Wada MR, Inagawa-Ogashiwa M, Shimizu S, Yasumoto S, Hashimoto N. Generation of different fates from multipotent muscle stem cells. *Development* 2002; 129(12), 2987–95.
33. Qu-Petersen Z, Deasy B, Jankowski R *et al.* Identification of a novel population of muscle stem cells in mice: potential for muscle regeneration. *J. Cell Biol.* 2002; 157(5), 851–64.
34. Diefenderfer DL, Brighton CT. Microvascular pericytes express aggrecan message which is regulated by BMP-2. *Biochem. Biophys. Res. Commun.* 2000; 269(1), 172–78.
35. Sakaguchi Y, Sekiya I, Yagishita K, Muneta T. Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. *Arthritis Rheum.* 2005; 52(8), 2521–29.
36. Brandenberger R, Burger S, Campbell A, Fong T, Lapinskas E, Rowley JA. Cell therapy bioprocessing. *Bioprocess Int.* 2011; 9, 30–7.
37. Rowley JA. Developing cell therapy biomanufacturing processes. *Chem. Eng. Prog.* 2010; 106, 50–5.
38. Carmen J, Burger SR, Mccaman M, Rowley JA. Developing assays to address identity, potency, purity and safety: cell characterization in cell therapy process development. *Regen. Med.* 2012; 7(1), 85–100.
39. Rowley J, Abraham E, Campbell A, Brandwein H, Oh S. Meeting lot-size challenges of manufacturing adherent cells for therapy. *Bioprocess Int.* 2012; 10, 16–22.
40. Hare JM, Fishman JE, Gerstenblith G *et al.* Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial. *JAMA* 2012; 308(22), 2369–79.
41. Mazzini L, Ferrero I, Luparello V *et al.* Mesenchymal stem cell transplantation in amyotrophic lateral sclerosis: A Phase I clinical trial. *Exp. Neurol.* 2010; 223(1), 229–37.
42. Honmou O, Houkin K, Matsunaga T *et al.* Intravenous administration of auto serum-expanded autologous mesenchymal stem cells in stroke. *Brain* 2011; 134(6), 1790–1807.
43. Bang OY, Lee JS, Lee PH, Lee G. Autologous mesenchymal stem cell transplantation in stroke patients. *Ann. Neurol.* 2005; 57(6), 874–82.
44. Perez-Simon JA, Lopez-Villar O, Andreu EJ *et al.* Mesenchymal stem cells expanded *in vitro* with human serum for the treatment of acute and chronic graft-versus-host disease: results of a phase I/II clinical trial. *Haematologica* 2011; 96(7), 1072–6.
45. Wakitani S, Imoto K, Yamamoto T, Saito M, Murata N, Yoneda M. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. *Osteoarthritis Cartilage* 2002; 10(3), 199–206.
46. Yamout B, Hourani R, Salti H *et al.* Bone marrow mesenchymal stem cell transplantation in patients with multiple sclerosis: a pilot study. *J. Neuroimmunol.* 2010; 227(1–2), 185–9.

47. Connick P, Kolappan M, Patani R *et al.* The mesenchymal stem cells in multiple sclerosis (MSCIMS) trial protocol and baseline cohort characteristics: an open-label pre-test: post-test study with blinded outcome assessments. *Trials* 2011; 12, 62.
48. Kebriai P, Isola L, Bahceci E *et al.* Adult human mesenchymal stem cells added to corticosteroid therapy for the treatment of acute graft-versus-host disease. *Biol. Blood Marrow Transplant.* 2009; 15(7), 804–11.
49. Herrmann R, Sturm M, Shaw K *et al.* Mesenchymal stromal cell therapy for steroid-refractory acute and chronic graft versus host disease: a phase 1 study. *Int. J. Hematol.* 2012; 95(2), 182–8.
50. Hare JM, Traverse JH, Henry TD *et al.* A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. *J. Am. Coll. Cardiol.* 2009; 54(24), 2277–86.
51. Jiang R, Han Z, Zhuo G *et al.* Transplantation of placenta-derived mesenchymal stem cells in type 2 diabetes: a pilot study. *Front Med* 2011; 5(1), 94–100.
52. Grayson WL, Zhao F, Bunnell B, Ma T. Hypoxia enhances proliferation and tissue formation of human mesenchymal stem cells. *Biochem. Biophys. Res. Commun.* 2007; 358(3), 948–53.
53. Hung SP, Ho JH, Shih YV, Lo T, Lee OK. Hypoxia promotes proliferation and osteogenic differentiation potentials of human mesenchymal stem cells. *J. Orthop. Res.* 2012; 30, 260–66.
54. Estrada JC, Albo C, Benguría A *et al.* Culture of human mesenchymal stem cells at low oxygen tension improves growth and genetic stability by activating glycolysis. *Cell Death Differ.* 2011; 19, 743–55.
55. Fehrer C, Brunauer R, Laschober G *et al.* Reduced oxygen tension attenuates differentiation capacity of human mesenchymal stem cells and prolongs their lifespan. *Aging Cell* 2007; 6(6), 745–57.
56. Holzwarth C, Vaegler M, Gieseke F *et al.* Low physiologic oxygen tensions reduce proliferation and differentiation of human multipotent mesenchymal stromal cells. *BMC Cell Biol.* 2010; 11, 11.
57. Zeng H-L, Zhong Q, Qin Y-L *et al.* Hypoxia-mimetic agents inhibit proliferation and alter the morphology of human umbilical cord-derived mesenchymal stem cells. *BMC Cell Biol.* 2011; 12(1), 32.
58. Wang DW, Fermor B, Gimble JM, Awad HA, Guilak F. Influence of oxygen on the proliferation and metabolism of adipose derived adult stem cells. *J. Cell. Physiol.* 2005; 204(1), 184–91.
59. Jayme D. Development and optimization of serum-free and protein-free media. In: *Medicines from animal cell culture*, Stacey G, Davis J (Eds). John Wiley & Sons, Chichester, UK 2007; 29–44.
60. Felka T, Schafer R, De Zwart P, Aicher WK. Animal serum-free expansion and differentiation of human mesenchymal stromal cells. *Cytotherapy* 2010; 12(2), 143–53.
61. Brindley DA, Davie NL, Culme-Seymour EJ, Mason C, Smith DW, Rowley JA. Peak serum: implications of serum supply for cell therapy manufacturing. *Regen. Med.* 2012; 7(1), 7–13.
62. Jung S, Sen A, Rosenberg L, Behie LA. Human mesenchymal stem cell culture: rapid and efficient isolation and expansion in a defined serum-free medium. *J. Tissue Eng. Regen. Med.* 2012; 6(5), 391–403.
63. Bernardo ME, Avanzini MA, Perotti C *et al.* Optimization of *in vitro* expansion of human multipotent mesenchymal stromal cells for cell-therapy approaches: further insights in the search for a fetal calf serum substitute. *J. Cell. Physiol.* 2007; 211(1), 121–30.
64. Lindroos B, Boucher S, Chase L *et al.* Serum-free, xeno-free culture media maintain the proliferation rate and multipotentiality of adipose stem cells *in vitro*. *Cytotherapy* 2009; 11(7), 958–72.
65. Agata H, Watanabe N, Ishii Y *et al.* Feasibility and efficacy of bone tissue engineering using human bone marrow stromal cells cultivated in serum-free conditions. *Biochem. Biophys. Res. Commun.* 2009; 382(2), 353–58.
66. Hartmann I, Hollweck T, Haffner S *et al.* Umbilical cord tissue-derived mesenchymal stem cells grow best under GMP-compliant culture conditions and maintain their phenotypic and functional properties. *J. Immunol. Methods* 2010; 363(1), 80–9.
67. Heathman TRJ, Coopman K, Nienow AW, Kara B, Hewitt CJ. Process development to drive the consistency of mesenchymal stem cell production from multiple donors. In: *Scale-up and manufacturing of cell based therapies IV*. San Diego, California 2015.
68. Dos Santos F, Andrade PZ, Abecasis MM *et al.* Toward a clinical-grade expansion of mesenchymal stem cells from human sources: a microcarrier-based culture system under xeno-free conditions. *Tissue engineering. Part C, Methods* 2011; 17(12), 1201–10.
69. Heathman TRJ, Glyn VaM, Picken A *et al.* Expansion, harvest and cryopreservation of human mesenchymal stem cells in a serum-free microcarrier process. *Biotechnol. Bioeng* 2015; DOI: 10.1002/bit.25582.
70. Tan KY, Teo KL, Lim JFY, Chen AKL, Reuveny S, Oh SKW. Serum-free media formulations are cell

- line&#x2013;specific and require optimization for microcarrier culture. *Cytotherapy* 2015; 17(8), 1152–65.
71. Rafiq QA, Coopman K, Hewitt CJ. Scale-up of human mesenchymal stem cell culture: current technologies and future challenges. *Curr. Opin. Chemical Eng.* 2013; 2(1), 8–16.
  72. Rafiq QA. Developing a standardised manufacturing process for the clinical-scale production of human mesenchymal stem cells. *Department of Chemical Engineering PhD*, 2013.
  73. Nienow AW. Reactor engineering in large scale animal cell culture. *Cytotechnology* 2006; 50(1–3), 9–33.
  74. Schop D, Van Dijkhuizen-Radersma R, Borgart E *et al.* Expansion of human mesenchymal stromal cells on microcarriers: growth and metabolism. *J. Tissue Eng. Regen. Med.* 2010; 4(2), 131–140.
  75. Eibes G, Dos Santos F, Andrade PZ *et al.* Maximizing the *ex vivo* expansion of human mesenchymal stem cells using a microcarrier-based stirred culture system. *J. Biotechnol.* 2010; 146(4), 194–197.
  76. Rafiq QA, Coopman K, Nienow AW, Hewitt CJ. Systematic microcarrier screening and agitated culture conditions improves human mesenchymal stem cell yield in bioreactors. *Biotechnol. J.* 2016.
  77. Both SK, Van Der Muijsenberg AJ, Van Blitterswijk CA, De Boer J, De Bruijn JD. A rapid and efficient method for expansion of human mesenchymal stem cells. *Tissue Eng.* 2007; 13(1), 3–9.
  78. Fossett E, Khan WS. Optimising human mesenchymal stem cell numbers for clinical application: A literature review. *Stem Cells International* 2012; doi: 10.1155/2012/465259
  79. Hewitt CJ, Lee K, Nienow AW, Thomas RJ, Smith M, Thomas CR. Expansion of human mesenchymal stem cells on microcarriers. *Biotechnol. Lett.* 2011; 33(11), 2325–35.
  80. Yuan Y, Kallos MS, Hunter C, Sen A. Improved expansion of human bone marrow-derived mesenchymal stem cells in microcarrier-based suspension culture. *J. Tissue Eng. Regen. Med.* 2012; DOI:10.1002/term.1515
  81. Frauenschuh S, Reichmann E, Ibold Y, Goetz PM, Sittinger M, Ringe J. A microcarrier-based cultivation system for expansion of primary mesenchymal stem cells. *Biotechnol. Prog.* 2007; 23(1), 187–93.
  82. Rafiq QA, Ortega I, Jenkins SI *et al.* The early career researcher’s toolkit: translating tissue engineering, regenerative medicine and cell therapy products. *Regen. Med.* 2015; 10(8), 989–1003.
  83. Yang Y, Rossi FM, Putnins EE. *Ex vivo* expansion of rat bone marrow mesenchymal stromal cells on microcarrier beads in spin culture. *Biomaterials* 2007; 28(20), 3110–20.
  84. Ferrari C, Balandras F, Guedon E, Olmos E, Chevalot I, Marc A. Limiting cell aggregation during mesenchymal stem cell expansion on microcarriers. *Biotechnol. Prog.* 2012; 28(3), 780–7.
  85. Jung S, Panchalingam KM, Rosenberg L, Behie LA. *Ex vivo* expansion of human mesenchymal stem cells in defined serum-free media. *Stem Cells Int.* 2012; 123030.
  86. Kehoe D, Schnitzler A, Simler J, Dileo A, Ball A. Scale-up of human mesenchymal stem cells on microcarriers in suspension in a single-use bioreactor. *Bioprocess Int.* 2012; 24(3), 28–38.
  87. Yang HS, Jeon O, Bhang SH, Lee SH, Kim BS. Suspension culture of mammalian cells using thermosensitive microcarrier that allows cell detachment without proteolytic enzyme treatment. *Cell Transplant.* 2010; 19(9), 1123–32.
  88. Schop D. Growth and metabolism of mesenchymal stem cells cultivated on microcarriers. Ph.D. 2010.
  89. Nienow AW, Rafiq QA, Coopman K, Hewitt CJ. A potentially scalable method for the harvesting of hMSCs from microcarriers. *Biochem. Eng. J.* 2014; 85(0), 79–88.
  90. Nienow AW, Hewitt CJ, Heathman TRJ *et al.* Agitation Conditions for the Culture and Detachment of hMSCs from Microcarriers in Multiple Bioreactor Platforms. *Biochem. Eng. J.* In Press, 2015.
  91. Yu Y, Li K, Bao C *et al.* *Ex vitro* expansion of human placenta-derived mesenchymal stem cells in stirred bioreactor. *Appl. Biochem. Biotechnol.* 2009; 159(1), 110–118.
  92. Sart S, Schneider Y-J, Agathos SN. Ear mesenchymal stem cells: An efficient adult multipotent cell population fit for rapid and scalable expansion. *J. Biotechnol.* 2009; 139(4), 291–299.
  93. Sun LY, Hsieh DK, Syu WS, Li YS, Chiu HT, Chiou TW. Cell proliferation of human bone marrow mesenchymal stem cells on biodegradable microcarriers enhances *in vitro* differentiation potential. *Cell Prolif.* 2010; 43(5), 445–456.
  94. Kehoe DE, Jing D, Lock LT, Tzanakakis ES. Scalable stirred-suspension bioreactor culture of human pluripotent stem cells. *Tissue Eng. Part A* 2010; 16(2), 405–421

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