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Editorial
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The Future Need for Massive Expansion of Stem Cell Numbers for Stem Cell Therapies

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Editorial

When Stem Cell (SC) therapies ultimately become standard therapies, the estimated number of stem cells needed will be incredibly large. Even for clinical trials to establish FDA approval of stem cells in various disease therapies, the demand will be much greater than we can currently deliver. For stem cell therapies to be realized, we must find a way to increase the numbers of stem cells that do not differentiate prematurely, and meet the specific needs of the therapy in sufficient numbers while maintaining cell purity and stemness. The estimated cell dose is very high because of attrition and differentiation of stem cells [1-3]. To overcome these issues and maximize targeted delivery, the best approach is to inject large amounts of the SCs [4]. The estimates of needed SCs range between 5 to 10 million per kilogram of patient weight for a successful therapy [5-7]. This, multiplied by the up to 3,000 patients in a phase 3 clinical trial, would equal 2.1 trillion ($2.1x10^{12}$) SCs for transplantation [8]. SCs are difficult to scale up because they are anchorage dependent and very sensitive to mechanical and cell-cell contact [9,10]. With respect to human Mesenchymal Stem Cells (hMSCs), the gold standard of culture is T-flasks, which can yield approximately $2.5x10^6$ SCs per 75 cm^2 flask. There are several systems and bioreactors in use for growing hMSCs. They all aim to increase surface area and decrease shear to more efficiently culture SCs. There are two main classifications: Suspension reactors and Immobilized Matrix reactors.

Microcarrier Suspension Reactors

Stirred system has successfully been tested for hMSC expansion [11]. Since the cells are adherent, they must incorporate microcarriers. These porous spherical structures are suspended in the media and provide the cells an anchorage point for expansion. Some notable systems using microcarriers are spinner flasks, Paddle driven reactors, and wave bags. This type of system is much easier to sample, as the cells are suspended in the media.

Spinner Flasks (SF) and Stirred Tank Bioreactors (STBR): Spinner Flasks (SF) and Stirred Tank Bioreactors (STBR) are impeller driven systems which utilize microcarriers for hMSC Culture. The Impeller both properly mixes the media and keeps the microcarriers suspended in the liquid. Some commonly used microcarriers are Cytodex 1 and Cytodex 3 (GE), and Polystyrene and Collagen microcarriers (Pall). They are approximately 150 μm in diameter, range from 300 to 500 cm^2/g and boast very similar growth compared to static culture, but provide much larger overall cell harvest [12,13]. The advantage being that adherent cells are being cultured in a system mimicking a suspension culture, making it much more space efficient.

Mag3 bioreactor: From PBS Biotech also uses microcarriers, but utilizes a paddle wheel for mixing. This is performed though two gas inlets; one to drive the paddle wheel and the other to provide oxygenation to the system. With larger liquid handling arms, the paddle can be driven more slowly to decrease overall shear in the system [14]. The system is single use, and the paddle engages with the housing though magnets.

Wave bags: Wave bags can also be utilized for hMSC culture on microcarriers [11,15]. The advantage of wave bags over the other microcarrier based systems is that the mixing is done without an internal structure and eliminating this decreases shear. Little has been published on hMSC culture in these systems, but Computational Fluid Dynamics work by Jossen et al. [16] has shown areas of high shear at maximum deflection of the rocker. There is also some speculation that the force needed to suspend microcarriers can induce a breaking wave in the reactor. A fully formed wave would create enormous shear and could be detrimental to stem cell culture. Though microcarriers provide large amounts of Surface Area (SA), one disadvantage of their usage is that the carriers become an impurity during harvest. Under normal conditions the cells are lifted from

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Table 1: Specifications and reported culture values of hMSCs in both Microcarrier suspension and immobilized matrix systems.

Name	Type	Classification	Vendor	V (ml)	SA (cm^2)	SA:V	SCs/mL × 10^6	SCs/cm^2 × 10^4	Total SCs × 10^6	Source
-	PDMS Matrix	Immobilized	-	110	2,800	25.5	0.509	2	56	[19]
Quantum	Hollow Fiber	Immobilized	Terumo BCT	1,440	21,000	14.6	0.167	1.14	240	[20]
Mobius	STBR	Suspension	Milipore, Sigma	50,000	300,000	6	2	1.67	5,000	[23]
BIOSTAT RM	Wave Bag	Suspension	Sartorius	1,500	7,360	4.91	0.19	3.87	285	[16]
Mag 3	Paddle	Suspension	PBS	3,000	-	-	1.9	-	5,700	[14]

SA: Surface Area; V: Volume

the substrate, but since microcarriers are free-floating with the then lifted cells, there is an extra purification step that is needed [17].

Immobilized Matrix Reactors

Other systems increase SA by using fiber matrices or increased number of culture surfaces. These can be classified as immobilized matrix reactors, as the growth surface is stationary and media is perfused through the system.

Fixed bed reactors: Fixed bed reactors such as iCELL is by Pall are a static scaffold where the cells can adhere. Media is driven through the matrix, allowing gas and nutrient exchange [18]. Other researchers have created in house systems comprised of various materials, but operate in similar manner [18,19]. Cells expand on a static surface, and media is perfused through the matrix. Gas is either overlaid in the culture chamber or sparged in an auxiliary vessel and looped into the system.

Hollow fiber reactors: Hollow fiber reactors such as the Quantum system have also been tested as a culture matrix for hMSCs [20]. Cells can either grow in the lumen of the hollow fiber or on the exterior depending on the application. This system would allow very easy perfusion, however there is a concern of creating a media gradient since flow of media is unidirectional [21].

Parallel plate reactors: Parallel plate reactors provide a scale-out approach. This method is akin to cell stack systems, where multiple growth plates are placed together in a single vessel. This increases the overall usable cell growth surface and eases operator burden. The effect of hydrodynamic forces of unidirectional laminar flow across the SCs is not fully known, which is cause for some concern and future research (Table 1) [22,23]. One advantage of an immobilized culture surface is that during dissociation the cells lift away without bringing the matrix. If dissociation is performed enzymatically the enzyme must still be neutralized and buffer exchanged before injection. The caveat is that it is much harder to directly sample cells in these systems compared to microcarrier based reactors.

Conclusion

There is no consensus on how to best culture hMSCs. Current systems used for hMSC expansion are very different in size and features. Most agree that the cells are sensitive to media composition, cell-cell contact and dynamic forces such as shear. Future GMP production of SCs must weigh the pros and cons of each system to develop the needed harvests in the trillion cell range. There is a clear need for more robust, scalable, and automated bioreactors for expansion of stem cells.

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