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Review Article
Manufacturing Processes

A comprehensive review of quantum bioreactor cell manufacture: Research and clinical applications

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ABSTRACT

The Quantum cell expansion system manufactured by Terumo-BCT is perhaps the most widely reported Good Manufacturing Practice–compliant bioreactor used for the expansion of adherent cell populations, both for research purposes and clinical cell–based therapies/trials. Although the system was originally designed for adherent cell expansion, more recently suspension cultures and extracellular vesicle manufacturing protocols have been published using the Quantum system. Cell therapy research and regenerative medicine in general is a rapidly expanding field and as such it is likely that the use of this system will become even more widespread and perhaps mandatory, for both research and development and in the clinic. The purpose of this review is to describe, compare and discuss the diverse range of research and clinical applications currently using the Quantum system, which to our knowledge has not previously been reviewed. In addition, current and future challenges will also be discussed.

Introduction

The Quantum (Terumo-BCT, Lakewood, CO) is perhaps one of the most widely used bioreactor systems, to date, that is compliant for Good Manufacturing Practice (GMP). Here, we review the current status of the Quantum cell expansion system and give a flavor of its applications for research and clinical use. Further, we suggest strengths and weaknesses of the system from a user outlook and highlight future perspectives. Throughout, it should be noted that there may be other bioreactor options available for each of the applications that were outside the scope of this review.

The Quantum Hollow-Fiber GMP Bioreactor

The Quantum cell expansion system is a functionally closed, automated, scalable, hollow-fiber bioreactor [1,2]. The bioreactor component comprises up to 11 500 hollow fibers, which create an internal surface area of 2.1 m^2, equivalent to 120 T175 tissue culture flasks [1,3]. Temperature/gas controls and critical processes such as feeding, removing waste products and harvesting cells are automated (Figure 1). The bioreactor has proven effective for the expansion of a variety of adherent cells, suspension cells, extracellular vesicles (EVs) and viral vectors (as in Table 1 [4–34]; discussed later).

Why is Up-Scale Advanced Therapy Medicinal Products (ATMPs) Manufacture Needed?

Therapeutic doses of cell-based therapies can be very high depending on the application, for example, intravenous therapies often use $>10^9$ cells, requiring a scaled-up manufacture to produce adequate cell numbers and to ultimately reduce costs [4,35-37]. Many ATMP developers and manufacturers are moving away from autologous approaches to treatment and bespoke manufacture, in favor of allogeneic "off-the-shelf" approaches [38]. Up-scale manufacturing lends itself well to the need for large allogeneic banks of cells. Traditional biopharma facilities often use automated, closed technologies, which are designed to repeatedly manufacture identical products at high volume while balancing operation efficiency, cell doubling considerations, process stability and the risk of batch loses [5]. Flexibility is required as more ATMP products move toward gaining regulatory approval; however, cell therapies must be produced

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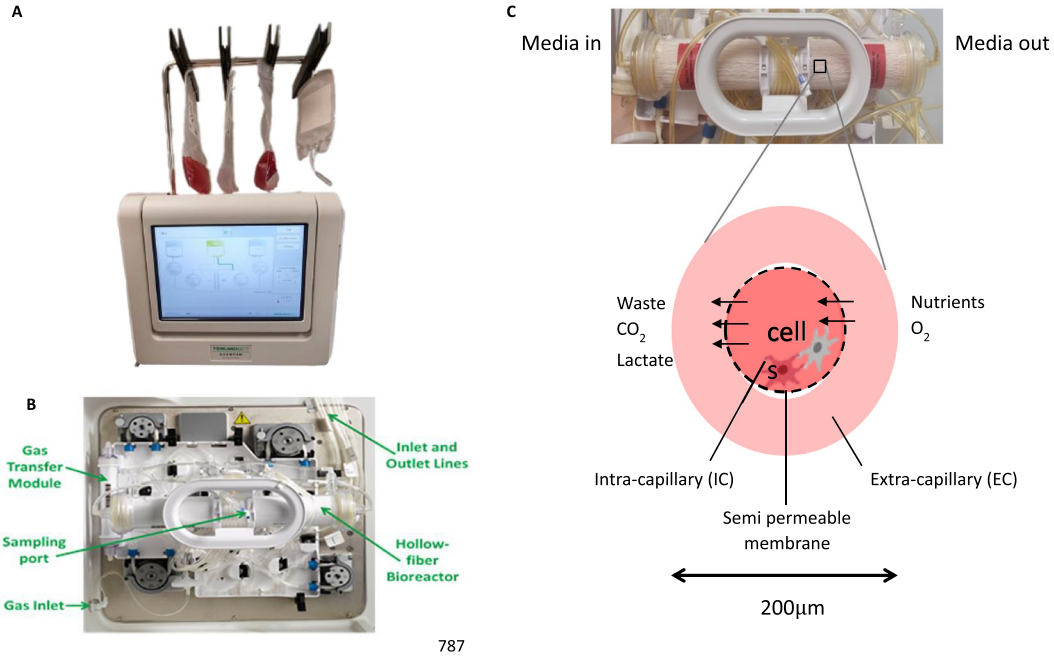

Figure 1. (A) The Quantum Cell Expansion System. (B) A single-use cassette, including the hollow-fiber bioreactor is inserted inside the Quantum Cell Expansion System, with the reactor connected to a gas supply and tubing inserted into the peristaltic pump system to move culture media through the system. (C) Schematic depicting the hollow fiber perfusion system inside the bioreactor, indicating the flow of nutrients, waste, CO_2, O_2 and lactate across the semi-permeable membrane of the fibers.

Table 1
Overview of Quantum-expanded cultures and substrates.

Species	Cell type	Number of studies	References
Human	BM-MSCs	14	[1,3,15,16,23–31,35]
	AD-MSCs	6	[36–41]
	UC-MSCs	2	[25,37]
	Neural MSCs	2	[7,38]
	Periosteum MSCs	1	[39]
	CD3+ T cells	2	[40,41]
	CD4+ T cells	1	[42]
	CD25+ T cells	1	[42]
	Dendritic cells	1	[43]
	Fibroblasts	1	[44]
	Skeletal muscle fibroblasts	1	[44]
	HEK 293T	1	[45]
	Embryonic stem cells	1	[2]
	IPSC endothelial cells	1	[18]
	IPSC	1	[17]
Porcine	BM-MSCs	1	[46]
Murine	Embryonic fibroblasts	1	[2]
Substrates			
	Fibronectin	22	[1–16,18,24,27–31,47–50,37,39–41,46]
	Cryoprecipitate	3	[16,25,36]
	Vitronectin	2	[16,17]
	Laminin and vitronectin	1	[17]
Media			
	Alpha MEM	10	[1,16,24,26,28,29,36,37,46,49]
	DMEM	11	[2–5,25,27,30,31,39,45,48,50]
	Other custom XSFM	2	[17,44]
	PRIME-XV T-cell Expansion XSFM	2	[40,42]
	RPMI	1	[43]

AD-MSCs, adipose-derived mesenchymal stromal cells; BM-MSCs, bone marrow–derived mesenchymal stromal cells; DMEM, Dulbecco's modified Eagle's medium; HEK 293T, human embryonic kidney 293 SV40 T-antigen cells; IPSC, induced pluripotent stem cell; MEM, modified Eagle's medium; RPMI, Roswell Park Memorial Institute; UC-MSC, umbilical cord-mesenchymal stromal cells, XSFM, xeno- and serum free media.

using standardized, reproducible, GMP-compliant processes that can be tracked, documented and audited [39].

Controlling and Monitoring Manufacture in the Quantum System

Hypoxia and normoxia

The physiologic oxygen concentration experienced by cells in the tissues of the body is markedly lower than the 21% oxygen tension commonly used for cell expansion *in vitro*. Previous research in our group and by others has demonstrated that the culture of cells in hypoxic conditions can be therapeutically beneficial in terms of retention of a desirable *in vivo* tissue-forming phenotype and a slowing of plastic adherence de-differentiation [10–14]. The Quantum bioreactor has a direct gas connection, which allows users to connect any blend of gas. Terumo-BCT states that an internal investigation demonstrated that a comparison of hypoxic and normoxic conditions resulted in no significant difference in cell yield, viability or phenotypic, morphological or differentiation potential, but the cell types grown were not clearly defined (personal communication, Terumo-BCT, October 2022). Hanley *et al.* [15] showed that the yield of bone marrow–derived mesenchymal stromal cells (BM-MSCs) was 75% greater in 5% oxygen cf. 21% oxygen in sister populations grown on tissue culture plastic. Further work needs to be undertaken to establish the use of maintaining a more "physiologic" oxygen tension during cell manufacture in the Quantum system.

Coating the hollow fibers

Cell adhesion is critical for the proliferation and differentiation of anchorage-dependent cells and relies on the surface chemistry of the tissue culture substrate. The hollow fibers of the Quantum are non-adherent and require coating with a substrate that is permissive to cell attachment and allows the cells to retain an *in vivo* phenotype. As summarized in Table 1, fibronectin, vitronectin and human donor pooled cryoprecipitate (CPPT) have most commonly been used as substrate coatings for the Quantum for the expansion of MSCs, whereas laminin or fibronectin have been used for the expansion of human-induced pluripotent stem cells [16–18]. Gelatin, human serum albumin, collagen I, poly-l-lysine and poly-d-lysine were also tested for MSCs but produced lower yields compared with the more commonly used substrates [16].

Fibronectin is a high molecular weight (440 kDa) glycoprotein found in the blood and as a solid component linking proteins in the extracellular matrix [19]. Plasma-derived fibronectin is widely used in cell culture, although recombinant and cellular fibronectin is also commercially available. Fibronectin benefits from being a fully defined protein that is suitable for multiple cell types. Vitronectin is a low molecular weight (75 kDa) serum protein that similarly interacts with cells via integrin-mediated adhesion to RGD motifs [20]. Currently, recombinant vitronectin and fibronectin products and other similar synthetic surface-coating reagents are commercially available that adhere to GMP manufacturing guidelines. However, when considering the use of fibronectin, the choice of commercial supplier requires careful consideration, with several vendors no longer being recommended for ATMP manufacture by the US Food and Drug Agency or requiring extensive, costly adventitious viral testing.

Cryoprecipitate is derived from human blood by freezing fresh plasma, then thawing and centrifuging to collect a precipitate. It is rich in clotting factors such as fibrinogen, factor VIII, factor XIII and von Willebrand factor, and it also contains fibronectin, platelet microparticles and immunoglobulins. The fibrinogen concentration in CPPT varies from 10 g/L to 25 g/L, depending on the donor and the processing [21]. When CPPT is used as a Quantum substrate, it is usually prepared as a pooled product from four to six donors [21]. In summary, the efficiency of CPPT in coating the Quantum bioreactor differs between batches, and the product is also likely to be more difficult to obtain than the other commercial bioreactor substrates described. Frank *et al.* [16] compared BM-MSCs that were Quantum expanded on fibronectin, recombinant vitronectin and CPPT. All substrates performed well for BM-MSC manufacture, with similar cell yield and no differences in growth kinetics, morphology, adherence to the International Society for Cell and Gene Therapy (ISCT) flow profile and capacity to trilineage differentiate. Therefore, the choice of coating reagent should be determined by the user dependent of their specific cell type, ideally tested on plastic before Quantum expansion and further influenced by whether the cell product needs to be manufactured in accordance with GMP.

Media, monitoring cell growth and harvesting

A plethora of culture media types have been used in the Quantum and have been established for different applications (summarized in Table 1). For example, BM-MSCs have been cultured in alpha-minimum essential media, Dulbecco's minimum essential media and GlutaMax (Gibco supplement, used as an alternative to L-glutamine), adipose-derived MSCs (AD-MSCs) in alpha-minimum essential media and GlutaMax, T cells in RPMI and a chemically defined xeno-free media (PRIME-XV T cell expansion XSFM media). Most types of media are compatible with the Quantum and are chosen to suit the cell type being cultured rather than the system itself. Certain cell types require additional supplements in addition to basal media to support growth in the Quantum and to induce or retain a desirable phenotype. These include fetal bovine serum (FBS), human platelet lysate (hPL), insulin–transferrin–selenium, glucose, vitamins (e.g., vitamin C [ascorbic acid] for chondrocytes) and amino acids (such as L-glutamine) [22]. The Quantum is designed to allow for the addition of cytokines, growth factors and supplements before and during the culture process enabling bespoke manufacturing processes.

Monitoring of cell growth kinetics in the system is commonly undertaken using lactate and/or glucose readings, sampled in the conditioned media acquired during a manufacturing growth phase. Sampling conditioned media via the sampling port for this purpose allows for analysis of the media currently circulating in the EC circulation loop. There are established protocols for using these metabolic measures (glucose and lactate) and that consider these alongside the perfusion rate through the bioreactor, in order to establish the cellular production rate per day [3]. This allows for prediction of local doubling time and, once worked up with matched tissue culture plastic (TCP) populations on the user's specific cells, can be used to predict the number of cells in the bioreactor system at any specific time. Plotting the calculated lactate production rate over time can allow the user to determine when the cells are in an expansion phase and, once this begins to plateau, can inform on an appropriate time to harvest. An additional/alternative method of assessing cell growth, indirectly, is to establish and monitor a "sister" population seeded onto traditional tissue culture plastic vessels (flasks/plates). Although not directly comparable with the conditions in the Quantum system (particularly in terms of substrate and media perfusion), such parallel cultures allow the operator to evaluate how well a particular culture is growing at the low densities most commonly used in the bioreactor and can inform critical decisions such as need to alter perfusion rates and time to harvest. Harvesting cultures from the Quantum is usually undertaken via standard trypsinization for adherent cell populations with the use of animal derived trypsin or recombinant TrypLE (Thermo Fisher Scientific, Waltham, MA, USA). A range of concentrations have been used previously; however, 0.25% solutions tend to be preferred for adherent bioreactor cell cultures cf. the 0.05% concentration more commonly used in traditional tissue culture plastics.

Cell and EV Manufacture

Applications of Quantum cell manufacture for research

The Quantum system has been widely used in the up-scale expansion of many different cell types for research purposes. Some have been aimed at yielding maximal numbers of cells from a single passage, whereas others have looked to the Quantum as a method of automating processes in developing protocols that will be transferable for GMP cell manufacture. Most of the research studies reviewed in this manuscript have focused on the derivation and expansion of BM-MSCs or from subcutaneous fat/adipose (AD-MSCs), isolated and/or expanded on fibronectin (Table 1). For BM-MSC isolation, BM aspirate can be loaded directly into the Quantum and large numbers of BM-MSCs (in the region to 10–35 million cells) yielded from the first passage [1,15,23–31]. Once isolated, these BM-MSCs have been reseeded into the Quantum and further culture expanded, with cell yields typically being greater than 100 million MSCs [1,15,19,25,31].

Several studies have demonstrated that Quantum-derived BM-MSCs adhere to the ISCT guidelines in terms of cell-surface marker profile and trilineage differentiation potential [1,15,23,25,28–30,32,33]. Functionality of Quantum-manufactured BM-MSCs has been demonstrated using *in vitro* activated T-cell suppression assays [15,26,29,30]. Further, the efficacy of Quantum-derived BM-MSCs have been demonstrated *in vivo* in rodent models for the treatment of ischemic stroke and cartilage joint surface injury [15,34].

Important considerations for GMP manufacturing process development and safety are whether Quantum expansion is successful using cryopreserved banks and determining whether expansion in the system induces any chromosomal aberrations. Rojewski *et al.* [1] demonstrated that BM-MSCs which had previously been cryopreserved could be expanded in the Quantum, however their doubling time was 1.4-fold higher in comparison to seeding freshly isolated cells. In addition, Jones *et al.* [24] and Mennan *et al.* [25] demonstrated no detectible changes in Quantum-manufactured cultures cf. BM-MSCs expanded in parallel on standard TCP in terms of telomere length and spectral karyotype, micronucleus formation and tumorigenicity.

Process optimization in the Quantum is continually being sought in terms of limiting the frequency of “open” procedures, improved automation and reducing costs. Hanley *et al.* [15] calculated that for a theoretical clinical trial using BM-MSCs to treat 60 patients, the use of the Quantum required 0.02% of the number of “open” procedures compared with TCP, thus significantly reducing the likelihood of product contamination. This study also indicated that, at the time of publication, the cost of manufacturing 200 million BM-MSCs was comparable in either the Quantum or TCP; however, the labor costs were much lower, primarily due to the reduction in time to harvesting the cells from the bioreactor (approx. 45 min), in comparison with TCP (approx. 6 hours) [15]. Further, Russell *et al.* [29] indicated that the manufacture of 100 doses of 100 million BM-MSCs would be manufactured a month earlier using Quantum cf. TCP and that the reagents and consumables costs would be nearly halved by using Quantum, equating to a saving of $976.41 per dose.

In addition to BM-MSCs, MSCs derived from alternative tissues have been widely expanded in the Quantum system (Table 1). Following BM, the most frequently used source of MSCs that have been expanded in the bioreactor are AD-MSCs [36–51]. These studies have included loading raw liposuction aspirate into the Quantum, isolating and expanding the AD-MSCs in the system [36–49] as well as reseeding Quantum-isolated AD-MSCs [36,49] and seeding AD-MSCs prior isolated and expanded on TCP [48,50]. MSCs in the order of hundreds of millions are typically harvested following Quantum isolation, with reported cell viabilities of >95% [36,50]. Adherence to the ISCT MSC profile was maintained following AD-MSC Quantum expansion [36–48]. Other cell types successfully expanded in the Quantum system include MSCs isolated from umbilical cord digests [25], Wharton’s jelly [37], neural tissues [38] and periosteum [39], human iPSCs [18], HEK239T cells [45], fibroblasts and skeletal myoblasts [44] monocyte-derived dendritic cells [43], as well as animal-derived cells such as mouse embryonic fibroblasts [2] and porcine MSCs [46].

Besides MSCs, there are also several reports of T-cell expansion protocols in the Quantum [40–42]. We realize that the expansion of T-cell populations is far more established in other bioreactor systems, for example, the Xuri Cell Expansion system (Cytiva, Marlborough, MA, USA) [52], the CliniMacs Prodigy (Miltenyi Biotec, Bergisch Gladbach, Germany) [53,54] and the Cocoon cell therapy manufacturing platform (Lonza, Basel, Switzerland) [55] and the use of the Quantum for this purpose is still in early process development. However, we thought it worthy to include a few examples of this alternate use of the system in our review. In brief, Nankervis *et al.* [41] optimized the Quantum expansion process for CD3+ T-cells, demonstrating that perfusion feeding allowed efficient gas exchange and continued feeding of cells which in turn increased yields. Coeshott *et al.* [40] further demonstrated that CD3+ T cells could be expanded from the Quantum when seeded at low (30×10^6 cells) or high (85×10^6 cells) densities, yielding 1.48×10^{10} and 2.51×10^{10} cells, respectively. The 8–9 days required to culture expand these large yields has the potential to reduce the production time for CAR-T cell therapies and indicates that lower seeding densities could be used for individuals with low numbers of circulating CD3+ T cells [40]. It has also recently been shown that the Quantum cells can more efficiently support the up-scale expansion of CD4+ and CD25+ regulatory T cells in comparison with standard TCP [42].

Applications of Quantum for EV manufacture

So far, we have reviewed the multiple and diverse cell expansion protocols using the Quantum system, but an emerging additional purpose for the Quantum is to enhance the production of cell-derived products such as EVs. EVs are small cell-derived particles ($<1\ \mu$m) that contain mRNA, miRNA and protein cargo that is encapsulated by a lipid membrane [56]. Protocols have been developed to enrich EVs (particularly those <200 nm in size) for use as an alternative to cell-based therapies [57]. However, there are still hurdles to overcome in the manufacture of clinically applicable EVs.

The Quantum platform represents an opportunity to overcome current limitations of the field, mainly poor vesicle yields, of which the resulting EVs are highly heterogeneous, labor-intensive and vary between batches. Already, some studies have begun to establish this reality, being able to scale-up the production of EVs, typically of MSC origin. These studies differ by many factors that have yet to be standardized, including different harvest medias (either EV-depleted FBS/hPL or serum free to minimize contaminating EVs) and isolation protocols (Table 2) [57]. Although this is a common caveat in EV research, the Quantum requires further research to devise an optimal protocol to harvest EVs. Such factors for exploration should include the optimal time-point to harvest EVs, informed by glucose and lactate readings, and the chosen strategy for collection, which can be done continuously, in batches, or at the end of the run. As more studies emerge, full disclosure of the used materials, protocol and experimental outputs (metabolic measures, predicted cell number and cell number at harvest) in consideration to EV outputs will be integral.

What remains to be established is whether Quantum-derived EVs retain their therapeutic attributes and how this changes the final product, since culture conditions can greatly affect EV characteristics [62]. Already, Quantum MSC EVs have been shown to significantly improve neurological recovery in swine or monkey models of traumatic brain injury [58,60,61]. However, such studies based this conclusion in comparison with vehicle controls, and no direct comparison has been made to tissue culture plastic, for example.

Table 2
Extracellular vesicle manufacture in the Quantum.

Cells loaded for expansion	Expansion media	EV harvest media	EV harvest frequency	EV isolation protocol	EV characterization	EV characteristics	Reference
3×10^6 human BM-MSC	N/D		Start on day 3, followed by every 48 h for 6 d and every 24 h for the final 4 d	250*g* for 5 min → 3000*g* for 30 min → 0.22-μm filtration → 100 000*g* for 120 min	qNano (IZON)	N/D	[58]
20×10^6 human BM-MSC	αMEM + 1% Lglutamine + 5% HPL	αMEM + 1% L-glutamine	Every 48 h for 12 d once peak expansion was achieved	1000*g* for 15 min → 0.2-μm filtration → 100 000*g* for 180 min (Ti45 rotor)	NanoSight; Protein assay; Flow cytometry	$9.8–44.9 \times 10^{11}$ per harvest (0.9–6.2 mg protein); $9.8–15.6 \times 10^{13}$ total particle yield, CD9+, CD63+, CD81+ and CD47+	[59]
10×10^6 monkey BM-MSC	αMEM + 10% EV-depleted FBS		Every 48 h for 4 d, then every 24 h for 2 d	250*g* for 5 min → 3000*g* for 30 min → 0.22-μm filtration → 100 000*g* for 120 min	qNano (IZON)	111-nm mode size	[60]
10×10^6 monkey BM-MSC	αMEM + 10% EV-depleted FBS		Start on day 3, followed by every 48 h for 4 days and every 24 h for the final 2 d	250*g* for 5 min → 3000*g* for 30 min → 0.22-μm filtration → 100 000*g* for 120 min	qNano (IZON)	N/D	[61]
30×10^6 human WJ-MSC	αMEM + [20% FBS OR 5% hPL]	αMEM	Every 24 h for 3 d once peak expansion was achieved	2000*g* for 30 min → 20 000*g* for 30 min →100 000*g* for 70 min (Ti70 rotor)	NanoSight; Flow cytometry	$1.13 \pm 0.5 \times 10^{13}$, [FBS] $1.64 \pm 0.2 \times 10^{13}$ [hPL] total particle yield, 113 ± 6.2 nm [FBS], 125 ± 4.4 nm [hPL], mode size, CD63+ and CD81+	[37]
10×10^6 monkey BM-MSC	αMEM + 10% EV-depleted FBS		Start on day 3, followed by every 48 h for 4 d and every 24 h for the final 2 d	250*g* for 5 min → 3000*g* for 30 min → 0.22-μm filtration →100 000*g* for 120 min (JS-24.38 swing bucket rotor)	qNano (IZON); TEM; Western blot	30- to 150-nm size range, peaking at 100 nm, CD63+ and Alix+	[26]

αMEM, alpha minimum essential media; BM-MSC, bone marrow–derived mesenchymal stromal cells; EV, extracellular vesicle; FBS, fetal bovine serum; hPL, human platelet lysate; WJ-MSC, Wharton's jelly–mesenchymal stromal cells.

These studies also lack adequate EV characterization in accordance with the International Society of Extracellular Vesicles minimal reporting criteria [63,64]. Such criteria are necessary since, to date, no existing protocol can isolate pure EVs, and it is important to establish what co-isolates may contribute to findings. Zhang *et al.* [26] have better accommodated this, associating CD63+ and Alix+ Quantum-derived particles of 30–150 nm with improved cognitive function in the same traumatic brain injury model, but further characterization is necessary to fully define a Quantum EV preparation. Here, following International Society of Extracellular Vesicles recommendations should dictate the minimum information on EVs, but future research should focus on a wider-view by using 'omic techniques. This will be crucial in moving towards the use of the Quantum system to manufacture EVs for clinical application.

In this regard, several aspects of EV manufacture will need to be adjusted for GMP compliance, and the Quantum system represents an opportunity to move the field further toward a clinically translational product. Bellio *et al.* [37] have begun some of the work necessary, exploring the difference between Wharton's Jelly–MSCs expanded in 20% FBS or 5% hPL before EV harvest in serum-free media, demonstrating no significant differences in particle yield (1.13×10^{13} particles/mL, FBS versus 1.64×10^{13} particles/mL, hPL) or mode size (113 nm FBS versus 125 nm hPL), and both preparations were shown to express EV-specific markers, CD63 and CD81, with few differences being found in their miRNA cargo. Furthermore, both EV enrichments improved cardiac function post-myocardial infarction. Mendt *et al.* [59] have taken this work a step further, using the Quantum to achieve GMP-grade EVs, by transfer of conditioned media from the bioreactor in a closed system to conduct isolation, in accompaniment to sterility testing. This generated a total of $9.8\text{-}15.6 \times 10^{12}$ particles per run, with $9.8\text{-}44.9 \times 10^{11}$ particles being collected every 48 hours depending on the day of harvest. Here, EVs were generated for the purpose of making "iExosomes," engineered EVs with the ability to target oncogenic Kras, used to treat patients with pancreatic cancer [59]. Here, both the reporting of Quantum outputs (matching particle counts to lactate readings [37] and characterization of individual batches [59]) and EV characterization is much improved. However, insights into protocol development, the effect on EVs due to their Quantum origin and consideration to potential non-EV particulate is still necessary to address. As such, whilst GMP grade EVs are achievable, these avenues would need to be explored to make the Quantum an established source of clinically applicable EVs.

Clinical applications of Quantum-derived cells

We have independently searched for publications and trials in which the Quantum system is used clinically and have also requested all available information from Terumo-BCT. Clinical trial information available on the NCT database was not always detailed enough to divulge the cell manufacture methodology, and therefore this summary is unlikely to be exhaustive. Here we present a flavor of the range of clinical applications currently undertaken in the system (Table 3) [4,9,23,34,65-74], but it is beyond the scope of this review to fully analyze the findings from each study. Clinical trials using the Quantum explore a range of cell types to evaluate their efficacy for varying indications. The use of a bioreactor such as the Quantum can enable more centers to treat patients with cell-based therapies, where an on-site GMP compliant laboratory is not available. One such example is a case report of a 24-year-old man with grade III graft-versus-host disease (GVHD) following an allogeneic stem cell

Table 3
Quantum-manufactured cells in the clinic.

Cell type	Indication	Cell yield/dose	Period in Quantum	Trial ID	Reference
BM-MSCs	Graft-versus-host disease (GVHD)	Two doses of 1.5×10^6 cells/kg	23 d (2 passages)	N/A (hospital exemption)	[23]
	Immune modulation in patients receiving full-thickness corneal transplants	$2\times 8.0 \times 10^7$or $2\times 1.6 \times 10^8$	6–8 d	Not registered	Ongoing trial, no results published to date (VISICORT)
	Type 2 diabetes and DKD	8×10^7, 1.6×10^8 or 2.4×10^8 fixed doses of ORBCELL-M	N/D	NCT02585622	Ongoing trial, no results published to date (NEPHSTROM)
	Idiopathic Parkinson disease	1×10^6 cells/kg, 3×10^6 cells/kg, 6×10^6 cells/kg or 1×10^7 cells/kg	3 passages	NCT02611167	[35]
GM-neural MSCs with cytosine deaminase (CD-NSCs)	Phase 1 trial for recurrent high-grade gliomas	1×10^7 or 5×10^7	7–10 days +9 days	NCT01172964	[7,65]
	Phase 1 trial for recurrent high-grade gliomas	5×10^7 to 1.5×10^8	7–10 days +9 days	NCT02015819	[66]
GM-neural MSCs with carboxylesterase (CE-NSCs)	Phase 1 trial for recurrent high-grade gliomas	N/D	N/D	NCT02192359	Ongoing trial, no results published to date
AD-MSCs	Allogeneic adipose-derived stromal cells for patients with ischemic heart disease and ischemic heart failure (phase 1)	1×10^8 cells	2 passages	NCT02387723	[67]
	Allogeneic adipose-derived stromal cells for patients with ischemic heart disease and ischemic heart failure (phase 2)	1×10^8 cells	2 passages	NCT03092284	[68]
	Stem Cell Therapy in IschEmic Non-treatable Cardiac Disease (SCIENCE)	1×10^8 cells	2 passages	NCT02673164	[69]
	Primary graft dysfunction after lung transplantation	1×10^8 cells	2 passages	NCT04714801	Ongoing trial, no results published to date
	Aqueous deficient dry eye disease	22×10^6 MSCs/mL	N/D	NCT03878628	[51]
MultiStem (BM-derived Multipotent Adult Progenitor Cells, or MAPC)	MultiStem in Ischemic Stroke (MASTERS)	4×10^8 or 1.2×10^9 cells	16 d (2 passages)	NCT01436487	[70,71]
	MultiStem Administration for Stroke Treatment and Enhanced Recovery Study (MASTERS-2)	1.2×10^9 cells	16 d (2 passages)	NCT03545607	Ongoing trial, no results published to date
	TReatment Evaluation of Acute Stroke for Using in Regenerative Cell Elements (TREASURE)	1.2×10^9 cells	16 d (2 passages)	NCT02961504	Rationale in [72] Ongoing trial, no results published to date
	Efficacy and Safety Study of HLCM051 (MultiStem) for Pneumonic Acute Respiratory Distress Syndrome (ONE-BRIDGE)	9×10^8 cells	16 d (2 passages)	NCT03807804	[73]
	Phase 1/2 Study to Assess MultiStem Therapy in Acute Respiratory Distress Syndrome (MUST-ARDS)	9×10^8 cells	16 d (2 passages)	NCT02611609	[74]
Fibroblasts and keratinocytes	Autologous dermo-epidermal skin substitute for the treatment of burns	N/D	N/D	NCT03227146, NCT03229564,	Ongoing trial, no results published to date
	Autologous dermo-epidermal skin substitute for the treatment of full-thickness skin defects	N/D	N/D	NCT03394612	Ongoing trial, no results published to date

BM-MSC, bone marrow–derived mesenchymal stromal cells; DKD, diabetic kidney disease; N/A, not available; N/D, not determined.

transplant [23]. Because of the severity of the condition and the speed at which treatment was required, BM-MSCs were grown in the Quantum bioreactor. A 25-mL sample of BM aspirated from the original donor yielded 6×10^6 cells following 23 days in culture (2 passages), and two infusions of 1.5×10^6 cells/kg was found to reduce the severity of GVHD to grade I in 3 months.

VISICORT is evaluating the potential for Quantum allogeneic BM-MSCs to modify the immune system's response for patients receiving a full-thickness corneal transplant. In this phase 1b trial, healthy BM-MSCs are expanded in the Quantum for 6–8 days, cryogenically preserved in individual dose infusion bags. This study reports on eight patients, in whom the safety and tolerability of two intravenous

infusions of cryopreserved BM-MSCs at 7 days and 1 day before the corneal transplant procedure are tested. Group 1 (n = 4) will receive two infusions of 8×10^7 cells and group 2 (n = 4) will receive 1.6×10^8 cells. Subject to satisfactory results, a larger phase 2 trial is planned.

The Novel Stromal Cell Therapy for Diabetic Kidney Disease (NEPHSTROM) study is a double-blind, placebo-controlled phase 1/2 trial investigating the safety, feasibility, tolerability and preliminary efficacy of an intravenous infusion of ORBCELL-M (Orbsen Therapeutics, Galway, Ireland) Quantum manufactured BM-MSCs (http://clinicaltrials.gov: NCT02585622). Up to 48 patients with type 2 diabetes and progressive kidney disease will be exposed to either placebo, 8×10^7, 1.6×10^8 or 2.4×10^8 BM-MSCs (n = 12 each group), and markers of diseases assessed for up to 18 months' post-infusion. Another phase 1 study assessed the safety and tolerability of intravenous allogeneic BM-MSCs in patients with idiopathic Parkinson disease (http://clinicaltrials.gov: NCT02611167) [35]. Here, 20 patients were sequentially enrolled and received a single infusion of either 1×10^6 cells/kg, 3×10^6 cells/kg, 6×10^6 cells/kg or 1×10^7 cells/kg. The study concluded infusions at these concentrations were safe, well tolerated and not adversely immunogenic [35].

Allogeneic neural stem cells (NSCs) have been expanded in the Quantum to produce clinical-grade genetically modified NSCs stably expressing the prodrug-activating enzyme cytosine deaminase (CD-NSCs), and subsequent adeno-viral transduction to express a modified human carboxylesterase (CE-NSCs) [7]. A single intracerebral administration of either 1×10^7 or 5×10^7 CD-NSCs in 15 patients with recurrent high-grade gliomas in a phase 1 trial has successfully demonstrated safety, migratory ability of the cells to tumor sites and successful conversion of a subsequent orally administered prodrug 5-fluorocytosine into an active chemotherapy agent (http://clinicaltrials.gov: NCT01172964) [65]. Consequently, a further phase 1 trial investigated the feasibility of multiple doses of CD-NSCs; 15 patients received a median of 4 (range 2–10) intracerebral CD-NSC doses, ranging from 5×10^7 to 1.5×10^8 CD-NSCs in combination with 5-fluorocytosine ± leucovorin, with no clinical signs of immunogenicity (http://clinicaltrials.gov: NCT02015819) [66]. The authors recommended a dose of 1.5×10^8 CD-NSCs to be used in phase 2 trials. A further, ongoing phase 1 trial due for completion mid-2023, is investigating the safety and therapeutic dose of intracranially administered CE-NSCs (http://clinicaltrials.gov: NCT02192359).

The Cardiology Stem Cell Centre in Rigshospitalet, University Hospital Copenhagen, Denmark, holds a Danish manufacturing authorization (no. 23909) and a tissue establishment authorization (no. 32298) and produce GMP-grade allogeneic Quantum expanded AD-MSCs (certificate of GMP compliance no. DK IMP 92217). These AD-MSCs have recently been used in both phase 1 and 2 trials in patients with ischemic heart disease and ischemic heart failure, (http://clinicaltrials.gov: NCT02387723, NCT03092284 and NCT02673164). In each case, 1×10^8 cryopreserved allogeneic AD-MSCs were intramyocardially injected into the peri-ischemic area, in a series of small injections (12-20) with the aim of regenerating the damaged myocardium; initial safety and feasibility studies demonstrated no complications or serious adverse events [67]. Results from two randomized double-blind placebo-controlled phase 2 trials, one single-center (81 patients) and one Horizon 2020-funded multi-center European trial (138 patients) for the same clinical indication, are yet to be published (for trial design, see [68,69]). Manufactured AD-MSCs from the Cardiology Stem Cell Centre have been used at the same dose (1×10^8 cells) in a phase 1/2 double-blind placebo trial in 30 patients undergoing lung transplantation, with the aim of reducing post-transplantation immunological reactions and ischemic reperfusion injury (http://clinicaltrials.gov: NCT04714801) and at 2.2×10^7 ASCs/mL in a phase 1 trial in 7 patients with aqueous deficient dry eye disease (http://clinicaltrials.gov: NCT03878628) [51].

MultiStem (Athersys, Inc., Cleveland, OH, USA) is a proprietary commercial pharmaceutical-grade composition of Multipotent Adult Progenitor Cells (MAPCs) specially formulated for clinical use by the clonal expansion of BM-MSCs in the Quantum. A successful randomized, double-blind, placebo-controlled phase 2 trial for patients suffering from ischemic stroke (MultiStem Administration for Stroke Treatment and Enhanced Recovery Study [MASTERS]; http://clinicaltrials.gov: NCT01436487 [70,71]) demonstrated a safe intravenous administration of up to 1.2×10^9 cells, 24–48 hours post-symptom onset. This has led to two further phase 3 trials (http://clinicaltrials.gov: NCT03545607 and NCT02961504) to fully evaluate the efficacy of MAPC in the treatment of ischemic stroke [72]. MultiStem has also been used in a series of clinical trials for acute respiratory distress syndrome. Phase 1 and 2 trials across the United States and Japan for pneumonia- and coronavirus disease 2019–induced acute respiratory distress syndrome (http://clinicaltrials.gov: NCT03807804 [73] and NCT02611609 [74]) have demonstrated a tolerance for the intravenous administration of up to 9×10^8 MAPCs and have led to a phase 2/3 trial with 400 patients, due for completion in December 2023 (http://clinicaltrials.gov: NCT04367077). denovoSkin is an autologous bio-engineered skin graft developed for use in severe burns and skin reconstructive surgery, whereby autologous fibroblasts and keratinocytes are expanded in the Quantum prior to being seeding onto a hydrogel scaffold (CUTISS AG, Schlieren, Switzerland) [75]. Phase 2 trials, which were due for completion by December 2022, are currently underway in adults and children for severe skin burns (http://clinicaltrials.gov: NCT03227146 and NCT03229564) and full-thickness skin defects (http://clinicaltrials.gov: NCT03394612).

It is clear that the use of the Quantum bioreactor is gaining traction in providing adequate cell numbers for application in clinical trials. The safety and efficacy of the cells produced by the system has been demonstrated many times, indicating the system is suitable for producing large numbers of cells while maintaining phenotypic characteristics.

Discussion

The up-scale expansion of MSCs using traditional TCP techniques remains an ongoing challenge. Large-scale cell manufacture requires a level of automation while retaining high cell quantity and quality. The surface area provided for cell attachment by the hollow fiber design of the Quantum is estimated to support a theoretical cell yield of 1000×10^6 MSCs; however, cell yields achieved in the literature vary significantly from 12×10^6 to 605×10^6 MSCs [31,36]. Harvests for non-adherent cells in the Quantum are even greater, with reports of up to 25 100×10^6 T cells [40]. Side-by-side comparisons of growth rates in sister populations grown on TCP have shown a faster growth rate in the Quantum [1,15,25,31,39,49]. Further, analyses of cells grown in TCP and the Quantum have demonstrated comparable characteristics in terms of phenotype and functionality [1,15,25,31,39,49]. The higher growth rate observed in the Quantum, however, could be influenced by the continuous perfusion of nutrients in the bioreactor cf. intermittent media changes in TCP.

Most studies using the Quantum have the end goal of developing a product that will be compliant with GMP manufacture. These studies have included manufacture for allogeneic and autologous applications, both of which have utility, such as when rapid, up-scale expansion of autologous cells is required, e.g., for GVHD and for manufacturing large numbers of cell doses for "off-the-shelf" allogeneic cell applications. Some current GMP manufacture includes the use of xeno- and blood products, for example, FBS. However, to future-proof these protocols, additional scrutiny and more rigorous testing may be needed and ideally protocols developed which move away from the use of xeno- and blood products. In order to coat the Quantum fibers, there needs to be a move away from human

cryoprecipitate. Therefore, further studies similar to those performed by Frank *et al.* [16] are required to compare the efficiency of GMP substrates. Moreover, identification of serum-free media that can support cells within the hollow fiber system is required. The use of commercially available serum-free media will have significant cost implications for the manufacture of cells within the Quantum system, due to the large media volumes required. Therefore, in-house development of serum free media may be beneficial.

When optimizing protocols within the Quantum, the cost of the systems consumables and reagents can pose a significant challenge. There may be a reluctance to test multiple different optimization steps and technical replicates, due to the prohibitive cost of the bioreactors, their ancillary products (e.g., media bags) and the affiliated reagent costs of the large volumes of media, sera and coating reagents required. There are several ancillary products required for manufacture in the system, which include specialized bags for holding media/phosphate-buffered saline, waste conditioned media and for loading the cells/reagents. These ancillary products must be purchased through Terumo-BCT, thus maintaining non-competitive pricing and potentially limiting how "future-proofed" novel products may be should the company cease selling these products. At each step where media, reagents or phosphate-buffered saline must be added to the specialized ancillary bags, an "open" procedure must take place in which these reagents are "drawn into" the bags using a specialized peristaltic pump; potentially opening the system to contamination. These loaded bags are then "sterile welded" onto the disposable bioreactor cassettes. However, as the popularity of the system gains momentum for clinical applications, other biological companies (e.g., Thermo Fisher Scientific pre-culture filled cell media bags) are starting to develop products that could be directly loaded onto the Quantum in the future, including specialized GMP-compliant media pre-loaded into bags. Moreover, the starting minimum cell seeding density recommended for Quantum expansion can also represent a limiting factor, particularly when working with cells derived from primary tissues or rare specimens. Although hybrid processes can be used, in which the cells are isolated and culture expanded on tissue culture plastic before seeding within the bioreactor, it is not always desirable for cells to have undergone large numbers of preceding population doublings. A hybrid approach also increases the number of "open" procedures and moves away from optimal GMP manufacturing protocols. To address these issues, Terumo-BCT have now developed a new smaller bioreactor, called the Quantum Flex. The Quantum Flex system offers the opportunity to use fewer cells ($<$30 million T cells or $<$5 million MSCs), allowing for easier process development before switching to the larger sized bioreactors required for up-scale manufacturing. The utility of this system will become apparent as more studies using this product are published.

The Quantum bioreactor is popular and widely used across academia, in health care settings and industry. It is proposed that one of the major advantages of the Quantum system is that it can significantly reduce costs associated with the required numbers of expert GMP manufacturing staff who would be required to maintain the large numbers of tissue plastic cultures comparable with the surface area of the Quantum bioreactor. In our experience as users, it is possible that a single user could manage the oversight of multiple Quantum systems simultaneously. However, a period of expert training is required to fully develop a working knowledge of the technology and how to troubleshoot both hardware and software errors in order to maintain optimal performance of the instrument. Additionally, we are aware that the use of multiple bioreactors reflects a "scale out," rather than a "scale up" of manufacturing processes.

Although there are many advantages of this system, as discussed within this review, improvements could be made to further benefit the user and to ensure a more consistent manufactured product, as illustrated in Table 4 Currently, the Quantum does not have any monitoring system or alarm for the gas perfusion system. Hence, the user must ensure that the gas supply can be accessed via the input regulator and by monitoring the cells and media. As phenol red is not typically desirable for GMP manufacture, this may pose a difficulty (personal communication with ATMP manufacturing facilities, September 2022). Moreover, glucose and lactate measurements to determine cellular metabolic activity of the cells expanded in the Quantum are undertaken via daily conditioned media sampling from the outlet port and testing outside of the system using ancillary equipment. It would be beneficial for the Quantum to have an internalized means of testing and recording these parameters, such that the user could be automatically alerted to change the media flow rate in response to metabolic activity changes, or even better, that the system could automatically "self-monitor" the cells growth to ensure the cells are maintained in an active growth phase.

Conclusions

This review of existing literature has highlighted the wide-scale use of the Quantum bioreactor in research and translational settings. We have highlighted a need to develop standardized protocols for each cell (or cell-derived) Quantum product, allowing for direct comparisons across studies/trials, and the need to ensure consistent, well-characterized endpoints, essential for the clinical application of Quantum-derived products.

Author Contributions

Conception and design of the study: CH, CM, HSM, RD, TL, LR, JP and KW. Acquisition of data: CH, CM, HSM, RD, TL, LR, JP and KW. Analysis and interpretation of data: CH, CM, HSM, RD, TL, LR, JP and KW. Drafting or revising the manuscript: CH, CM, HSM, RD, TL, LR, JP and KW. All authors have approved the final article.

Table 4
Advantages and disadvantages of the Quantum bioreactor system.

	Advantages	Disadvantages
Cost	Reduced labor costs compared with traditional TCP expansion	High start-up costs and ancillary equipment/consumables
Single-use bioreactor	Time efficient	Expensive
	No risk of cross-contamination	Plastic waste/disposal
	Process flexibility	Not fully customizable
Hollow fiber system	Large surface area with continuous perfusion	Cannot monitor cell morphology or visualize cell growth
	Single-user can operate multiple bioreactors	No online control to allow assessment offsite
		Requires relatively high starting cell yield
		Not fully optimized for Extracellular vesicles or suspension cultures
		Requires large media volumes held at room temperature
	Reduced chance of contamination due to the closed system	Difficult to detect a contamination early
Regulatory	GMP-compliant; CE marked and FDA, MHRA compliant	
	Reduces need for costly clean rooms	

CE, Conformite Europeenne; FDA, Food and Drug Administration; GMP, Good Manufacturing Practice; MHRA, Medicines and Healthcare products Regulatory Agency; TCP, tissue culture plastic.

Declaration of Competing Interest

The authors have no commercial, proprietary or financial interest in the products or companies described in this article.

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