WHBA 2018 SUMMER SCHOOL
CELL THERAPY
BIOMANUFACTURING DESIGN
CHALLENGE

Challenge Statement

Production of Clinical-grade Cartilage Grafts: QbD Biomanufacturing

Overall Challenge Description

Quality by Design (QbD) of a commercial manufacturing facility for the production of a cell therapy product containing human mesenchymal stem cells (MSCs) for the treatment of joint cartilage defects. The facility should produce a clinical-grade product consisting of an injectable biomaterial (biocompatible, non-immunogenic, biodegradable) containing MSCs from one of the following two options (you choose): a) autologous (patient-derived) and b) allogeneic. The manufacturing facility should consider the entire healthcare product lifecycle (Fig. 1) by integrating upstream and downstream bioprocesses and accounting, through engineering design and regulatory requirements, for the various stages from cell banking for allogeneic grafts, collection and transport of autologous cells, to transport of the cellular product to the clinic, etc.

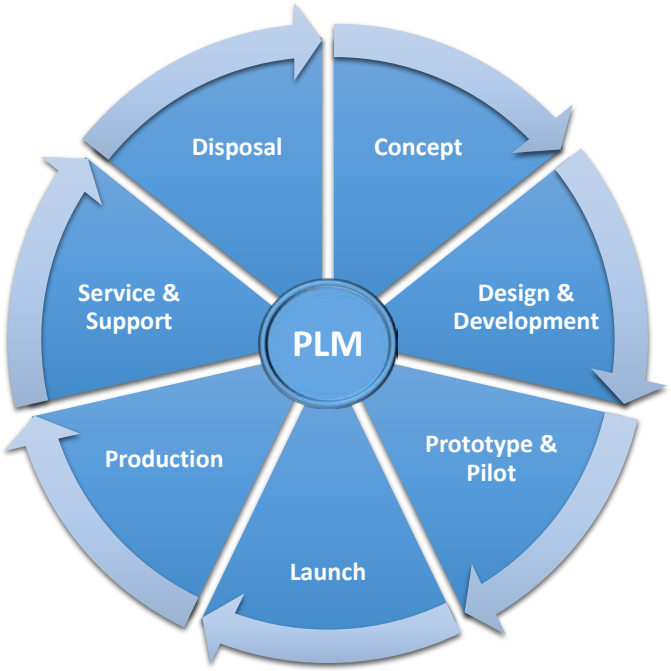

Figure 1. Diagrammatic representation of Product Lifecycle Management (PLM). PLM is an approach of integrated and cross-company administration and control of all product-related processes and data across the whole product lifecycle following the extended logistic chain – from design and production via sales through to disassembly and recycling.

1. **Challenge assumptions:**
   Human MSCs have been used for the treatment of a wide variety of clinical conditions such as cartilage defects, graft versus host disease, bone defects etc. The clinical-scale production of cellular therapy products containing either autologous or allogeneic mesenchymal stem cells is required for the commercialisation of such treatments. The products should satisfy FDA/EMA requirements and should be produced in cGMP facilities. You may assume that utilities such as process water, WFI, high pressure steam, low pressure steam and electricity as well as disposal options including waste water treatment, disposal via incineration and disposal via landfill are available on or convenient to your site and may be purchased at local costs. Single-use, disposable manufacturing practices should be employed. You can choose the location of your facility to be ANYWHERE IN THE US OR EU.

2. **Manufacturing targets:**
   Consider a 10-year manufacturing horizon. Please use the following production schedule in your design:
   - production year one: 25,000 items
   - production year two: 50,000 items
   - production year three: 75,000 items
   - production years four through ten: 100,000 items

3. **Cell therapy product:**
   You will produce the cartilage graft in 2 ml of biomaterial with 2.5 x 10^6 cells/500 µL/cm^2 in single use vials. Your response should include the analytics appropriate

for the demonstration of safety, efficacy and comparability. Table 1 provides representative production requirements of the desired cell therapy product by cell source.

General Process Description

Transplantation of live cells as therapeutic agents is poised to offer new treatment options for a wide range of acute and chronic diseases. However, the biological complexity of cells has hampered the translation of laboratory-scale experiments into industrial processes for reliable, cost-effective manufacturing of cell-based therapies. Unquestionably, the development of bioprocess technologies for the transfer of the current laboratory-based practice of stem cell tissue culture to the clinic as therapeutics necessitates the application of engineering principles and practices to achieve control, reproducibility, automation, validation and safety of the process and the product. The successful translation will require contributions from fundamental research (from develop- mental biology to the 'omics' technologies and advances in immunology) and from existing industrial practice (biologics), especially on automation, quality assurance and regulation. The timely development, integration and execution of various components will be critical—failures of the past (such as in the commercialization of skin equivalents) on marketing, pricing, production and advertising should not be repeated. Designing cell manufacturing processes according to quality-by-design (QbD) principles will become essential. QbD integrates scientific knowledge and risk analysis into manufacturing process development and is already being adopted by the biopharmaceutical industry. Linking measurable molecular and cellular characteristics of a cell population to final product quality through QbD is a crucial step in realizing the potential for cell therapies to transform healthcare.

Manufacturing of cell therapy products (CTPs) for clinical application typically requires several of steps, both upstream & downstream: acquisition or generation of the starting cell type; cultivation; modification; harvest; concentration; purification; formulation, fill and finish (preparing the CTP at the correct concentration and composition, dispensing into the final product 'container', and any post-fill processing); storage; and shipping of the product.

Table 1 – Production requirements for the production of the desired cell therapy product by cell source

Indication	**Autologous-based treatment**	**Allogeneic-based treatment**
Therapeutic cell type	Autologous MSC-derived cartilage graft	Allogeneic MSC-derived cartilage graft
Cell source	Autologous MSC from bone marrow	Allogeneic MSC isolated from several adult and fetal sources
Patients per year	15,000 (USA)	100,000 (worldwide)
Cells per dose	2.5×10^6 cells/500 µL/cm^2	2.5×10^6 cells/500 µL/cm^2
Critical cartilage defect size (cm^2)*	1	1
Delivery attributes	Fresh	Frozen

* For grade 4 lesions higher than 1 cm^2, surgical intervention is required.

The biomanufacturing facility to be designed should produce a clinical-grade product consisting of an injectable biomaterial (biocompatible, non-immunogenic, biodegradable) containing MSCs from either: a) autologous (patient-derived) or b) allogeneic. The upstream consists of 4 modules, as shown in Fig. 2: a) cell growth module, b) bioreactor module, c) scale module and d) control module. Decisions will have to be made in terms of the bioreactor design selected, cell growth format (choose either **single cell** or **hydrogel** cultures), scale of culture – all of which will inform the selections in the control model (oxygen, pH, temperature, etc).

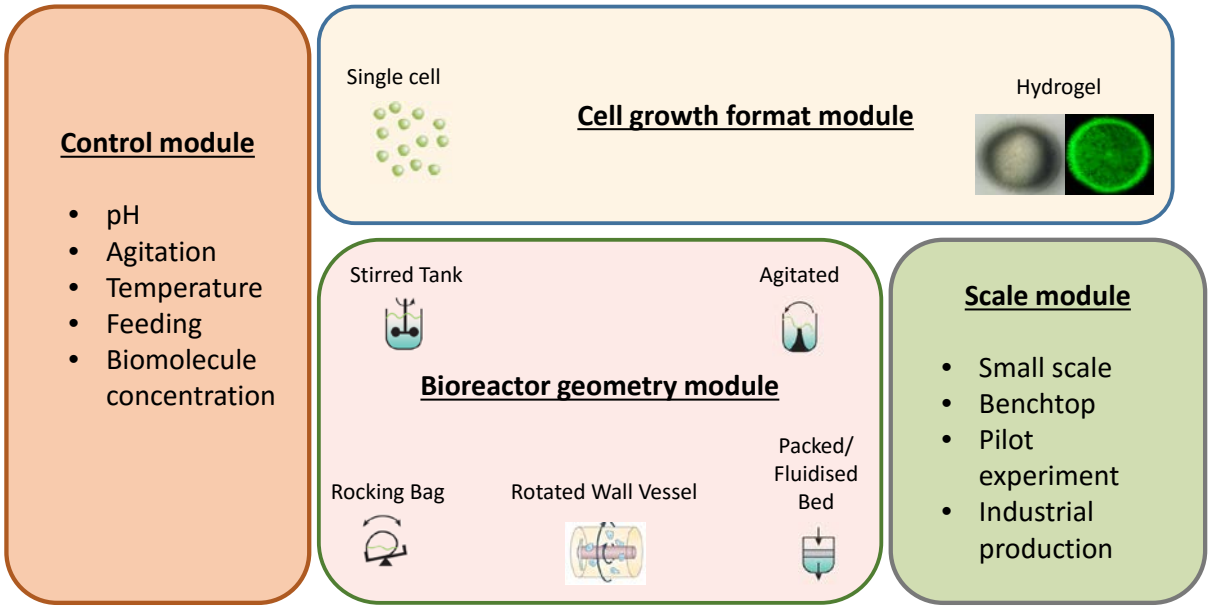

Figure 2. QbD biomanufacturing combining standardized, well-characterized modules, which reduces the process development required for each new cell type, although not all modules are compatible with each other. Cells can be grown in various formats. Bioreactor size ranges from small-scale screening devices, to benchtop, pilot, and industrial-scale devices. Larger stirred tanks have not yet been used for stem cell applications. Small-scale bioreactors are critical for manufacturing patient-specific products. Ideally, these systems are disposable, integrate isolation and selection with cell growth and formulation operations, and minimize cell manipulations. They also provide a basis for developing strategies to manufacture allogeneic cells in small lots, which is useful for early and mid-stage clinical programs or for rare indications. Bioreactors use agitated or perfused geometries. Parameters currently controllable in bioreactors include agitation, pH, dissolved oxygen, temperature, feeding, and biomolecule concentration (adapted from Lipsitz YY, Timmins NE, Zandstra PW, Nat Biotechnol 2016: 34(4): 393-400).

QbD encompasses product and process description, characterization, design, monitoring and continuous improvement, as illustrated in Fig. 3. It is guided by a thorough understanding of the fundamental biology and engineering underlying a product and its production. QbD begins by describing the desired product quality characteristics (quality target product profile; QTPP), identifying attributes that directly influence the safety and efficacy of the product (i.e. critical quality attributes), identifying the parameters that influence these attributes (i.e. critical process parameters) and developing a design space that quantifies how parameter variability affects the quality attributes.

For the selected cell therapy products, the QbD parameters need to be identified and applied in the biomanufacturing process.

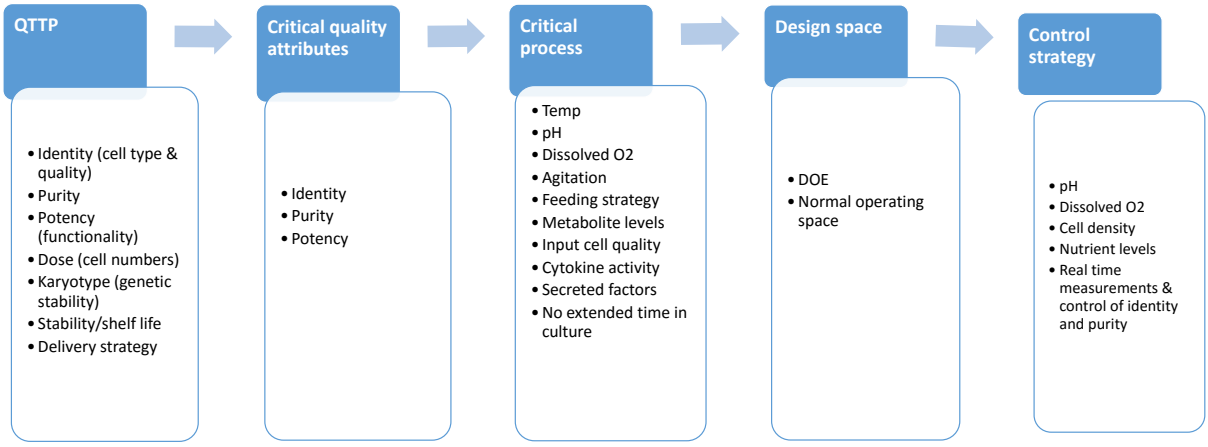

Figure 3. The QbD process. The first step in applying QbD is to define the quality target product profile—the characteristics of the CTP that assure its quality, safety, and efficacy. Second, the quality attributes that are critical for meeting the Quality Target Product Profile are determined by a risk assessment. Third, the critical process parameters and materials attributes that affect critical quality attributes are identified, and their effects on critical quality attributes are quantified in a design space. Fourth, a control strategy is developed to ensure that critical process parameters remain within the 'normal operating range' that ensures the production of quality product. Finally, the process is validated in the manufacturing facility at scale, and is continually monitored during manufacturing runs and improved as knowledge about the process increases (adapted from Lipsitz YY, Timmins NE, Zandstra PW, Nat Biotechnol 2016: 34(4): 393-400).

In designing the biomanufacturing processes for the cellular therapy product, you should consider (not inclusive) the following operations:

1. **Upstream Processes:** You will have to design a media preparation area that may use a proprietary media formulation specific to your company or off-the-shelf,

commercially available media provided by companies such as BD™, Life Technologies™ or Lonza™. The media should be suitable for the cell type used. Please decide on the type of vessels to use (steam-in-place, disposable or both) and ensure there is space for storing both the raw materials and the media after it is prepared.

2. **Cell Banking & Vial Thaw (Allogeneic)**: Design the on-site cell banking facilities, which are normally composed of liquid nitrogen (LN2) tanks, with room for roughly 500 vials/tank. The tank needs to be refilled with LN2 every two weeks and has a working volume of 10 dm^3. You will use one vial of Mesenchymal Stem Cells that are undifferentiated for each batch of product that will be produced in your facility. For this exercise, please assume the starting cell number to be 250,000 MSC/vial.

3. **Cell Isolation (Autologous):** Autologous applications will require the design of an on-site cell isolation facility, that must include bone marrow aspiration and MSC isolation from the aspirate. After successful isolation, cells will be expanded immediately. Assume that you can isolate around 400,000 MSC per bone marrow aspirate.

4. **Cell Expansion & Differentiation:** After vial thawing and/or cell isolation, cells are expanded in culture. It is not unusual for the doubling time of MSCs to be 60 hours. The cells will need to be expanded by passaging into larger and larger volumes and after the final scale up the culture will need to be differentiated into chondrocytes. You will need to decide, design and create the type of bioreactor you will use for the expansion and differentiation steps of the process. Note that one may select to leverage the same type of bioreactor for different steps. Please note that MSCs grow adherently, so the use of solid supports is mandatory.

5. **Purification:** After differentiation into chondrocytes, the cells need to be collected and sorted as some may have not fully differentiated. Design a process to collect/harvest the stem cells and also sort them, since you are only interested in the fully-differentiated chondrocytes.

6. **Inactivation:** Virus Filtration/Inactivation is a safety step in the manufacturing process. You will need to decide where in the process this step will take place and using what method. There are a number of methods that one may utilize such as filtering, solvent/detergent treatment, low pH inactivation, heat treatment, and chromatography, to name a few. Remember you do not want to destroy the product in this step so select appropriately.

7. **Stabilizing Material for Shipping:** The material will be provided to the end consumer as a ready-to-use, biocompatible graft, which needs to be stabilized prior to shipping. Please design the appropriate stabilization unit, packaging and shipment areas.

8. **Testing of Raw Materials and Product:** Please account for quality control of the materials used and the final product (release assay). Choose and describe the analytical equipment and Bioassays used to demonstrate that the product meets release specifications (especially with regards to the levels of desired cell phenotypes).

9. **Storage and Shipment:** Please include the facility to package and prepare the graft for distribution taking into consideration GMP regulations and the sensitivity of the material.

10. **Supply Chain and Storage of Raw Materials and Intermediates:** Please consider the supply chain and storage space and the material stock that should exist at any given time.

11. **Production Waste:** Please design the pretreatment, "kill tanks" that will feed into the county/city sewage facility. You have rented a pre-existing space that includes a sewage system that you can utilise.

12. **Cost Data:** Please consider the appropriate costs (based on location) for electricity, sewer, water, WFI, etc.

References/Additional information

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9. Hambor, John E. "Manufacturing Stem Cells at Scale." Bioprocessing International, Vol 10, No 6, June 2012, pp 22-23.