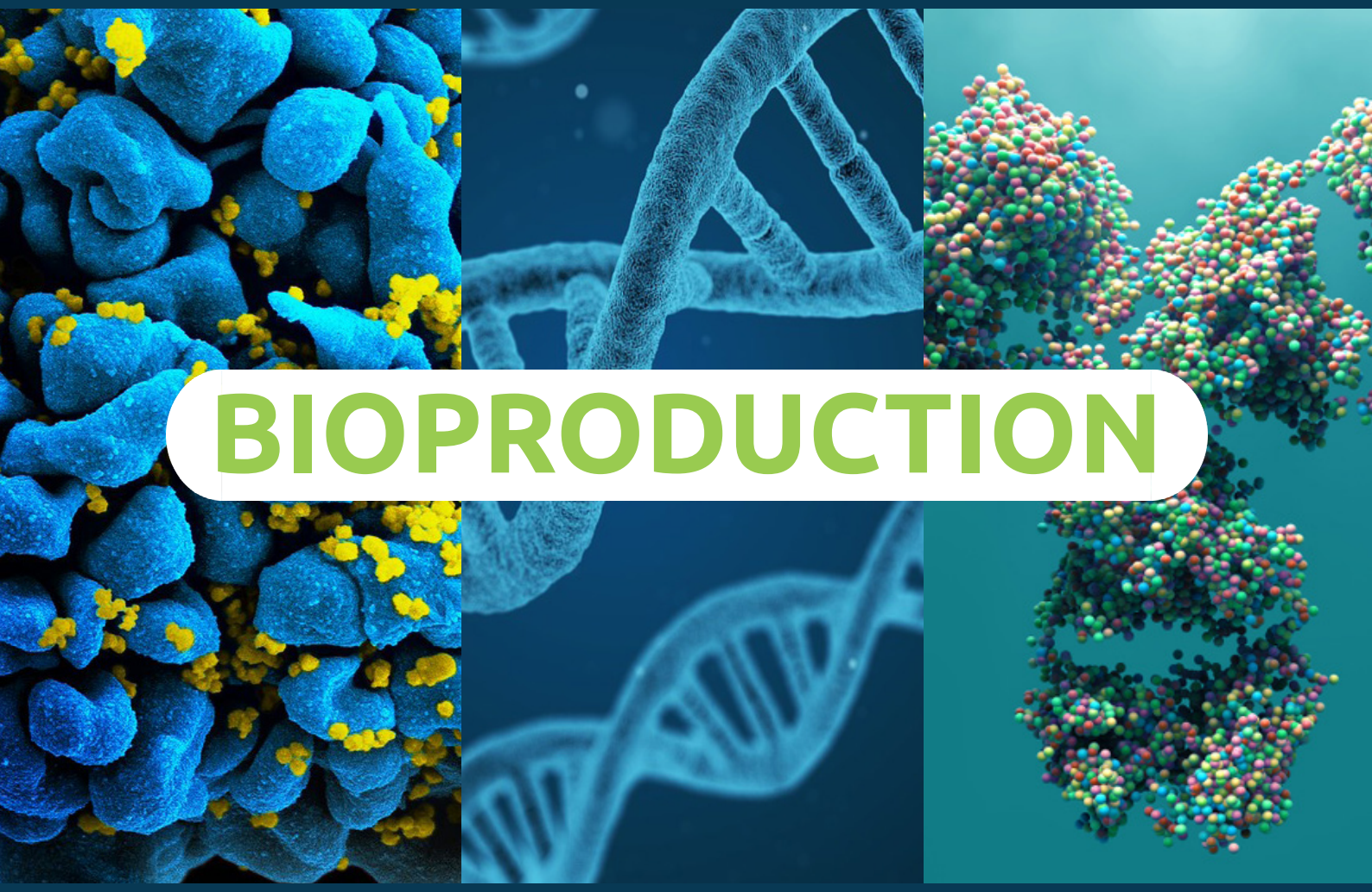
IMMUNOWATCH

EDITION N°3 - JULY 2021



BIOPRODUCTION

MABDESIGN
THE IMMUNOTHERAPY NETWORK

INTRODUCTION

MabDesign's Immunowatch is a one-of-a-kind information monitoring newsletter in the field of biologics. Its aim is to provide members of our association with the most recent and pertinent data gathered or generated through the key expertise of MabDesign and its collaborators in scientific research, business intelligence, market analysis and intellectual property.

It's general format includes a market study research, financial and economic data, invited contributions from scientists working in the industry or in academia and a section dedicated to intellectual property. The content of each edition is decided by an editorial composed of two field experts. While each edition usually focuses on one trending type of biologics, this current issue has been adapted to cover the bioprocessing aspects of these products and serve as a general introduction to subject. This editorial choice has been motivated by recent development, in terms of innovation and national strategies and by MabDesign's ongoing and/or upcoming actions and events in the bioprocessing field.

Immunowatch is done in collaboration with the MAbMapping Unit of the Ambition Recherche & Développement (ARD) Biomédicaments 2020 Phase II programme, funded by the Centre Val de Loire region.

BIOPHARMACEUTICALS
Innovation Dynamics in Health
IN REGION CENTRE-VAL DE LOIRE

MABDESIGN
THE IMMUNOTHERAPY NETWORK

Région Centre-Val de Loire

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EDITORIAL

Emmanuel Dequier

Emmanuel Dequier is Director at Grand Défi Bioproduction

The addition of drugs derived from biotechnologies i.e biologics (AKA biopharmaceuticals) to our arsenal of therapeutic and prophylactic solutions has been causing quite a revolution in the pharmaceutical industry for the past two decades. Indeed, biologics are now a major class of drugs with monoclonal antibodies being used to treat once incurable cancers and gene therapy intervening at the very heart of life's intricate machinery to repair defective genes or to boost the ability of our immune cells to fight off or circumscribe cancer cells. Promises of hope are now being converted into potent therapeutic successes with biotechnologies being the source of these innovation wonders.

Access to these new molecules remains however a challenge since the manufacturing of drugs produced or derived from living organisms is still highly complex. Innovation in the bioprocessing field is thus essential to ensure the large-scale production of these new therapies with guaranteed quality and controlled manufacturing cost. This will be sine qua non to ensure equity of access to treatment, the keystone to our healthcare system.

The panorama depicted in this edition of Immunowatch provides the current state-of-the-art with insights into the future innovations which will help us tackle these challenges in the bioprocessing field.

René Labatut

René Labatut is VP Head of Biologics Technology Innovation Strategy at Sanofi

The healthcare evolution is largely driven by bio-therapeutics for the next foreseen decades in conjonction with the Data revolution. Trends of moving towards prevention and root cause treatment are developing with the knowledge expansion speed increase. Multiple modalities are now developed to best answer patient needs. As exposed in this review, mAbs bio-production had reached a fairly high level of maturity and performance. Some others need significant performance increase to make them reasonably available to the patient community. All step of the bio-production value chain exposed here should contribute to this journey from the molecule design up to the final administration form in conjonction with patient monitoring. Therapeutics should be more and more adapted to the patient and disease detailed specificity. Agility in manufacturing should be largely increased to face the challenges of treatment customization. Product delivery area could be highlighted as critical to allow targeted action at lowest dose optimizing efficiency with better patient experience profile and also enabling some approaches like gene editing to deliver their full potential. New technological paradigm, combining multiple sciences and technologies should be considered in the process design to make accessible high performance from mass to personalized production. This could lead to significant industry landscape change favoring multiple actors network approaches versus single entities.

Nicolas Groux

Nicolas Groux is Chief Executive Director at MabDesign

Since 2015, our main objective at MabDesign has been to structure the French biopharmaceutical sector (therapeutic antibodies & recombinant proteins, vaccines, cell-based therapies, gene therapies, etc.). Building on our 200 association members, MabDesign is a front-row witness of the significant expansion of biopharmaceuticals both at the international and national level. Indeed, while this family of pharmaceutical products represented only for 4% of all marketed drugs worldwide at the beginning of 2021, it already accounted for 51% of the drug pipeline at the same time-point with a forecast increase in both percentages henceforth. We can further mention that out of the 8600 candidate-drug-developing companies, 56% are working on biopharmaceuticals.

With 530 pharmaceutical products currently under development by French entities, our nation currently ranks 3rd among European countries behind UK (860 products) and Germany (535 products). Importantly, therapeutic antibodies alone account for 169 products in this French biopharmaceutical pipeline. Several cell-based therapies (35 products) and gene therapies (78 products) have also made their way into this French pipeline. Our latest census shows that the French biopharmaceutical sector is composed of 705 companies: 181 of them involved in the development of these previously-mentioned 530 products with the support of 498 service-providers ensuring their development and 26 specialised training centres allowing for skill development of employees in this sector. The potency and specificity of this new class of drugs are providing numerous therapeutic hopes and has fuelled an extremely strong progression of the pipeline with an estimated average annual increase of drugs entering the preclinical phase of 33% for gene therapies, 17% for cell-based therapies and 11% for therapeutic antibodies over the next 5 years.

However, while the public health sector has high hopes on these novel therapeutic drugs to treat once incurable diseases, the cost of production of biopharmaceutical products is still greatly hindering their development. It is thus necessary to innovate both on the developability aspects and on the technologies supporting their development. This will require an important effort over time from all the various key-players of the health sector (product developers, CRO, equipment providers, training centres and support structures) who will have to work hand in hand with other related sectors which are now essential for the development of new technologies (robotics, cobotics, numerics, nano-electronics…).To face this major industrial and societal challenge, we at MabDesign, will be continuing our endeavour initiated 6 years ago to support you with strength and determination through our various events, training opportunities, expert consultancy services and tailored studies including intellectual property, business development, business intelligence and innovation funding.

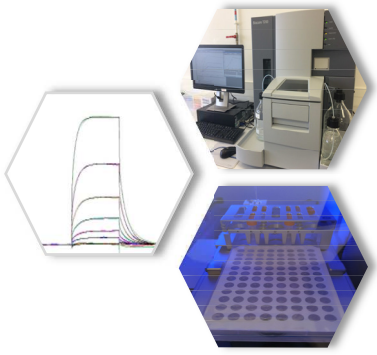
Fortuitously, the French government has recently renewed its commitment towards facing the biopharmaceutical challenge head-on with the announcement on June 29th of its 7-billion-euros investment plan of which 800 million will be dedicated solely to biotherapies. In this highly conducive context, MabDesign intends to pursue relentlessly its support to our 200 members and the 700 or so companies that make up the French Biopharmaceutical sector.

Agro-Bio

Monitoring solutions for your biologics production process

Critical Quality Attributes (CQA)

Biacore ™
SPR Technology



Fc receptors binding

- FcγRIII
- FcγRI
- FcRn
- FcγRII
- Fcγ
- C1q

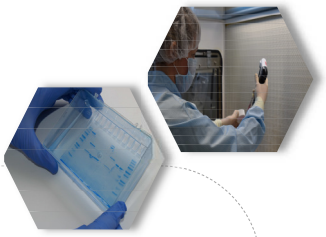
Affinity binding

- Ranking affinity
- K_D / k_a / k_d
- Similarity score

Calibration-Free Concentration Analysis (CFCA)

- Determine the active concentration of your drug

Host Cell Proteins (HCPs)



In-house HCP assay: *A global solution*

Antigen Selection → Immunization → Antiserum Purification → Antibody Validation → ELISA Development → ELISA qualification Validation to ICH guidelines

HCP Coverage 2-D:

% Determination

Optimization

Validation

Development of process specific HCP ELISA kit

Commercial HCP kit validation

Ph.: + 33 (0)2 38 64 83 50
https://agrobio.stago.com
techsupport@agro-bio.com

Agro-Bio

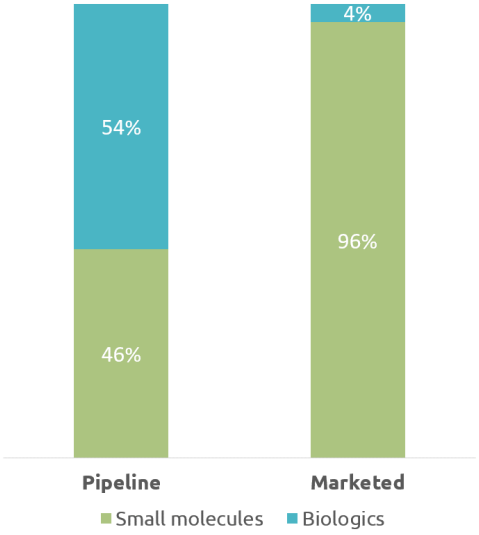
2 allée de la Chavannerie
45240 La Ferté Saint-Aubin
France

GLOBAL BIOPHARMACEUTICAL PRODUCTION MARKET

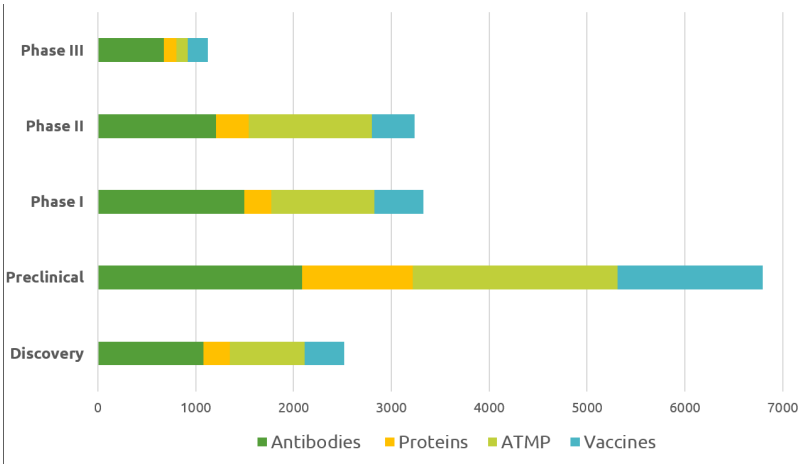
Discover the available products, pipeline candidates, major deals and biopharmaceutical companies

Biopharmaceuticals to produce in the future

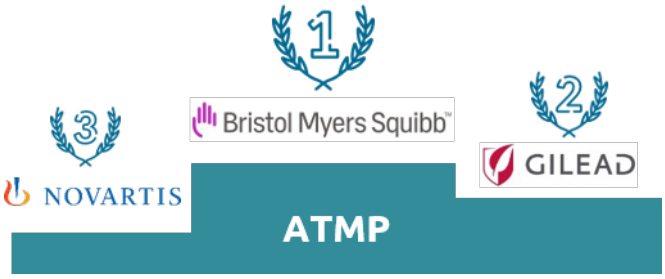
Small molecules vs biopharmaceuticals



Biopharmaceutical Pipeline



Top 3 companies developping biopharmaceutical products



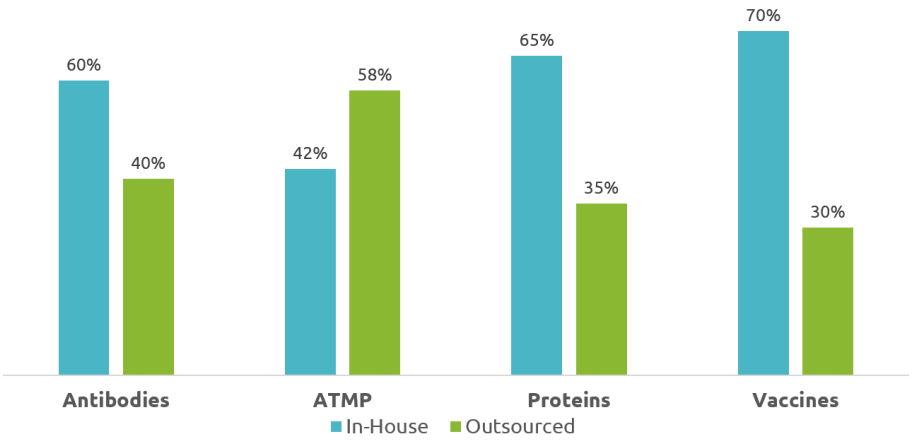
ATMPs: Advanced therapy medicinal products

** All data has been generated by MabDesign unless stated otherwise*
Source: Globaldata

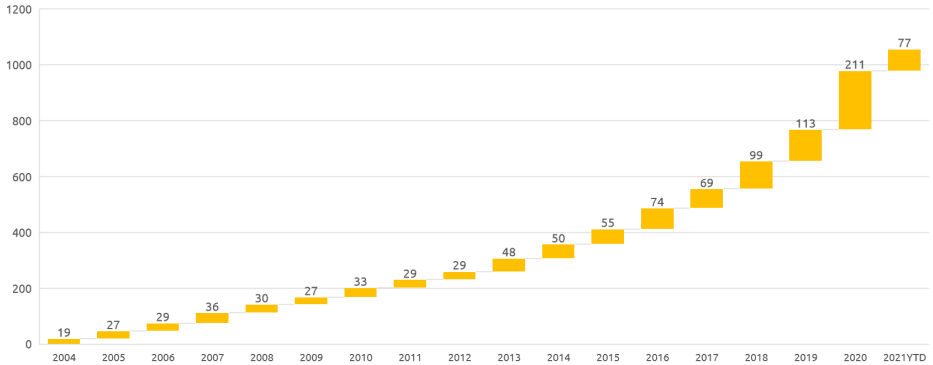
BIOPROCESSING

Companies manufacturing most marketed biologics in-house & Outsourced

API's manufacturing of marketed biologics (%)



Deals between product developpers and bioprocessing companies (Number of deals by year)

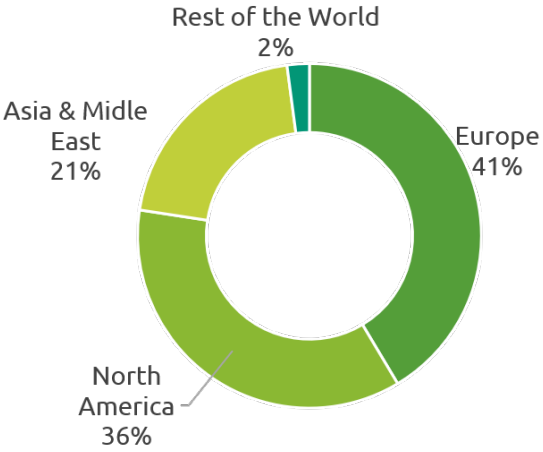


Service companies with the highest number of deals for bioprocess

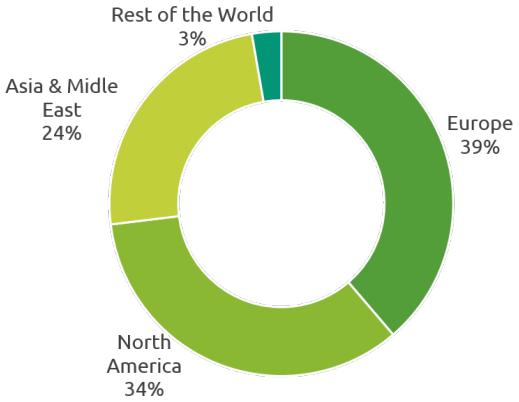
1. Lonza
2. Catalent BIOLOGICS
3. AGC Biologics
4. patheon by Thermo Fisher Scientific
5. SAMSUNG BIOLOGICS
6. FUJIFILM Diosynth biotechnologies
7. Boehringer Ingelheim
8. WuXi Biologics Global Solution Provider
9. Rentschler Biopharma
10. emergent biosolutions

Biopharmaceuticals CDMO Market

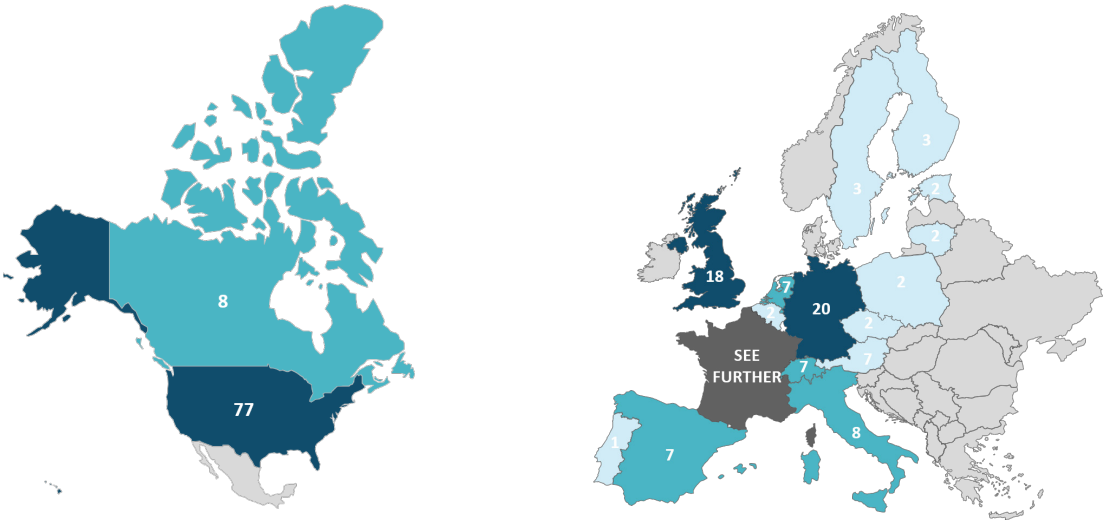
Biopharmaceuticals CDMO : Distribution by location of Headquarters



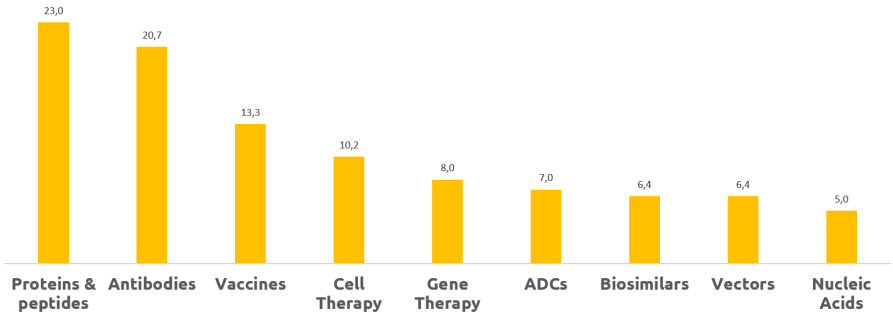
Biopharmaceuticals CDMO : Distribution by location of Manufacturing Facility



Biopharmaceuticals CDMO : Distribution by location of Headquarters



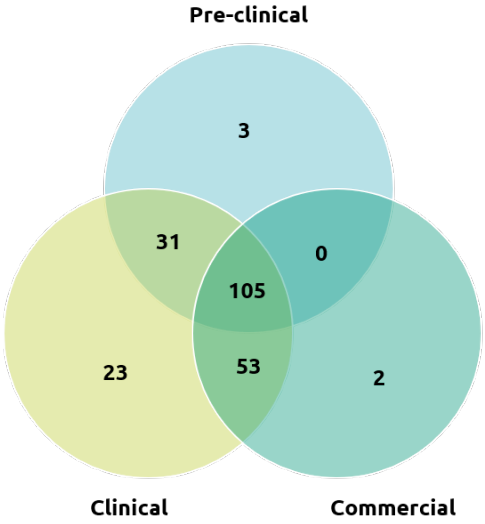
BioPharmaceutical CDMOs : Distribution by Type of Biologics (%)



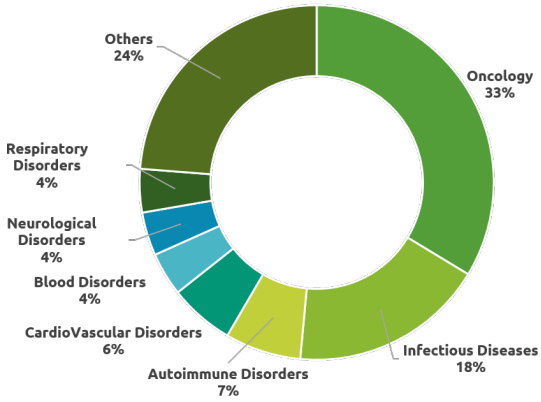
** All data has been generated by MabDesign unless stated otherwise*
Source: Globaldata & Roots Analysis : Biopharma Contract ManufacturingMarket (3rd Edition), 2019-2030

Biopharmaceuticals CDMO Market

BioPharmaceutical CDMOs : Distribution by Scale Of Operation



Demand analysis : Distribution of annual demand for Biopharmaceutical Manufacturing



Biopharmaceuticals CDMO Market, 2021

6,9 USD billion
CAGR 8,1%
(2019-2030)

SWOT

S — Strenghts

- Companies are seeking help from CDMOs
- Wide range of services and a long-term relationship with them
- Availability of specialized facilities
- Offering flexible manufacturing solutions
- Offering lower cost

W — Weaknesses

- Concerns associated with service/product quality
- Facility constraints
- Inability to cope with analytical testing and drug product release requirements
- Lack of physical capacity of downstream purification equipment

O — Opportunities

- Strong biologics pipeline
- Biologics have already demonstrated their strengths
- Several biosimilars are expected to enter the market.
- Several niche and evolving therapeutics are anticipated to sustain growth in the CDMO

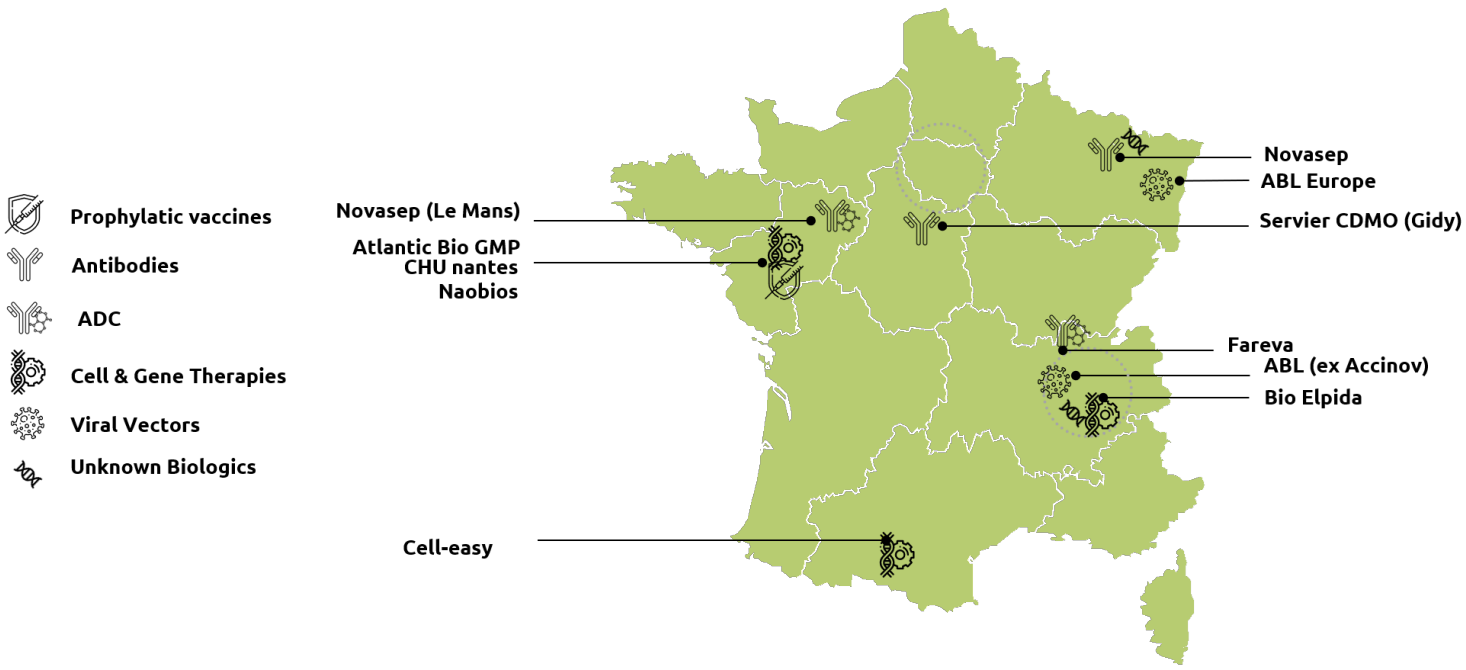
T — Threats

- Complex nature of such therapeutic subject to stringent regulatory surveillance.
- Reproducibility is one of the primary concerns related to the manufacturing of biologics.
- Therapeutics success could motivate developpers to build their own facilities

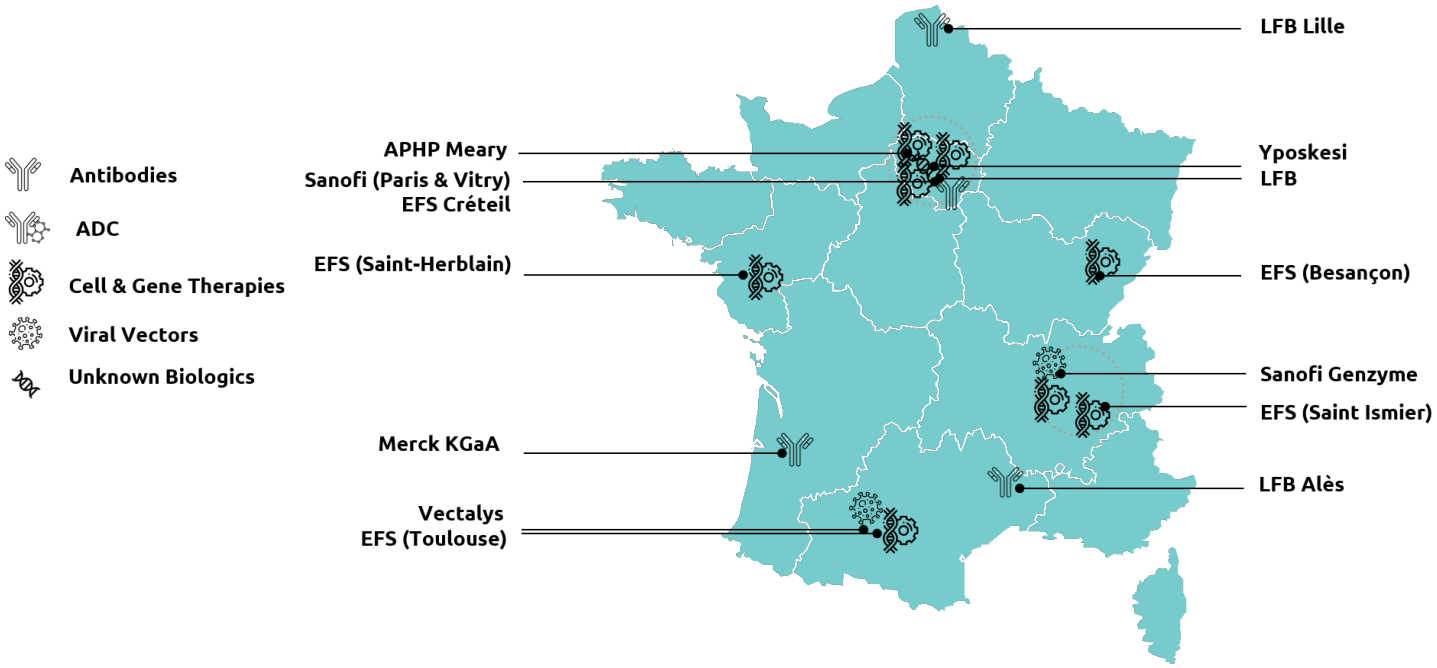
** All data has been generated by MabDesign unless stated otherwise*
Source: Globaldata & Roots Analysis : Biopharma Contract ManufacturingMarket (3rd Edition), 2019-2030

GMP Bioprocessing companies in France

Outsourced production



Mixed production



** All data has been generated by MabDesign unless stated otherwise*
Source: Globaldata

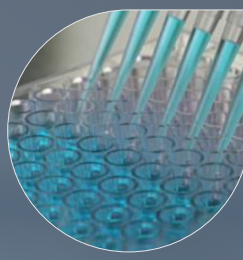
IDBiotech
Immuno Diffusion Biotechnologies

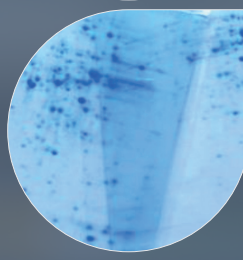
Your Partner for the management of HCP risks of your bioprocess

Host Cell Proteins (HCP) fit the definition of the Critical Quality Attribute (CQA) due to their potential to affect product safety and efficacy. Biopharma companies are therefore advised to manage this risk right from the early phases of the candidate biopharmaceuticals lifecycle development and to generate a sufficient knowledge to demonstrate control before submitting regulatory filing.

ELISA using anti-HCP polyclonal antibodies is the gold standard method for the quantification of HCP impurities in bio-therapeutics. Commercial ELISAs are usually used during preclinical and clinical phase I & II. To secure the bio-project, health regulatory authorities strongly advise to develop and validate a process-specific ELISA as soon as possible and anyway starting from phase III.

IDBiotech offers custom immuno-solutions for HCP risk management in accordance with regulatory guidelines





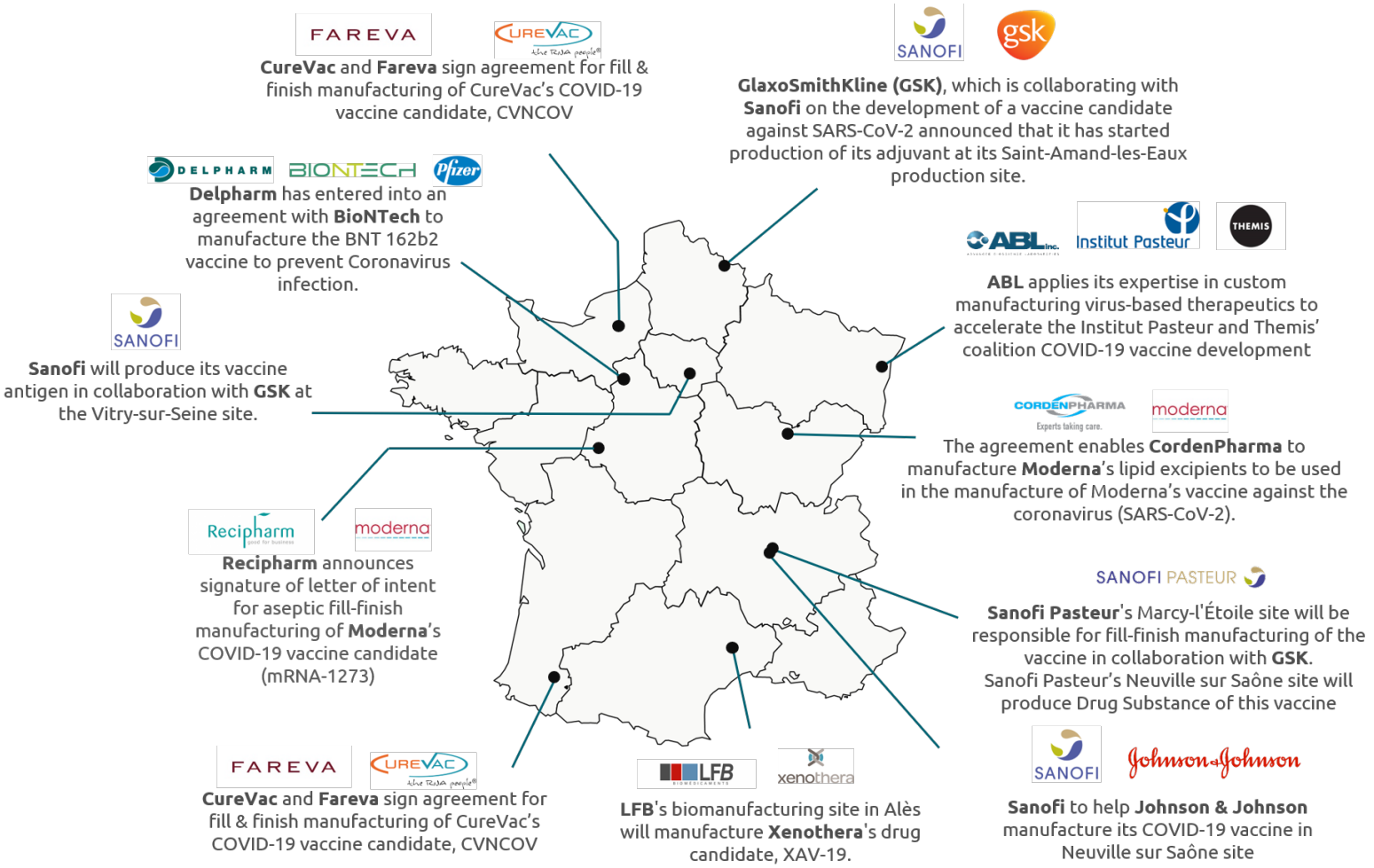
- Selection of the commercial HCP ELISA kit that best suits to your bioprocess
- HCP proteome pattern using 2D-DIGE electrophoresis
- Coverage assessment of anti-HCP antibodies by 2D-DIBE western-blotting
- Custom production and qualification of anti-HCP antibodies
- Custom development and validation of process specific HCP ELISA
- Manufacturing of ready-to-use process specific HCP ELISA kits
- Sample testing (HCCF, IPS, and DS)

By choosing IDBiotech as your partner you ensure high added value support for your project

- De-risking entry into the clinical phase (drug substance characterized, safety controlled)
- Helping valorization of drug candidate at various steps (IP, fundraising, licensing)
- Ensuring compliance of your CMC documents with health authorities requirements (FDA, EMA)
- Accelerating your pharmaceutical development stages by supporting the optimization of your bioprocess

IDBiotech - 1, Rue Marie Curie 63 500 Issoire France - Tel : +33 (0)4 73 54 95 01 - contact@idbiotech.com - www.idbiotech.com

BIOPROCESSING COMPANIES IN FRANCE: FOCUS COVID-19



For more information, see COVID-19 Special Edition: https://www.mabdesign.fr/immunowatch/

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Source: Globaldata

Promega

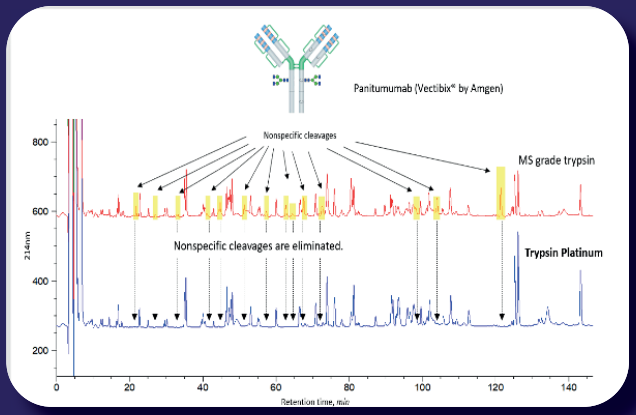
cGMP Promega Expertise for Bioproduction and QC Release

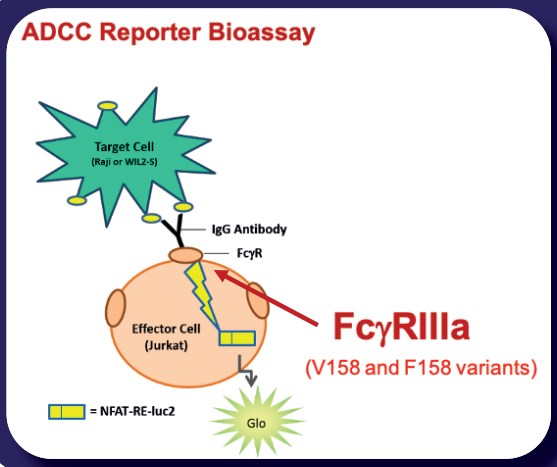
cGMP Manufacturing

Using Promega technologies ensure reliable quality control for batch release and let you benefit unique and high quality reagents for your biologics development strategy.

Mass Spectrometry for Sample analysis:

- New Trypsin Platinum NEW for High cleavage specificity
- ProAlanase NEW
- SoluMax surfactant NEW
- Ide-S and Ide-Z proteases
- Glycosidases





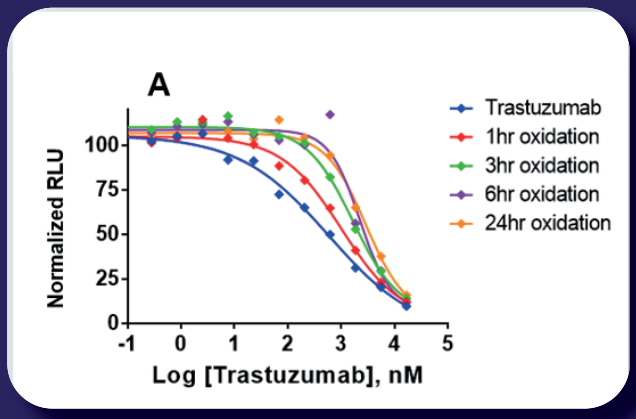
Accurate reporter Bioassays for:

- MOA validation
- Stability monitoring
- Batch to batch reliability
- Lots release controls

+ others assays available: T Cell Activation, VEGF, PD-1…

New Lumit™ FcRn Binding Immunoassay: NEW

- Allows checking for stability and potential modifications like oxydation of your antibodie's production
- Fast, HTS compatible and easy protocol



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BIOLOGICS AND THEIR CHEMICAL COUNTERPARTS

Laure DELHON & Gavin VUDDAMALAY[1]

1. MabDesign, Lyon, France

Chemical molecules, or small molecules, were historically the first type of pharmaceutical drugs to be developed and marketed. It started with Salicin, an active ingredient derived from willow bark and used for pain relief during centuries. It was first manufactured in pill form by Joseph Buchner in 1828 and afterwards marketed as a small molecule, known by its common name, Aspirin. The small molecule pharmaceutical has been thriving since then fuelled by our growing knowledge and expertise in R&D and in the industrial production of these drugs. This acquired proficiency is reflected in today's pharmaceutical landscape whereby small molecules represent 96% of all marketed drugs. The first biopharmaceutical emerged nearly 150 years later with the market approval of Orthoclone OKT3® in 1986. The latter is used as an immunosuppressant drug to reduce acute allografts rejection in transplanted patients. Orthoclone OKT3® is a therapeutic monoclonal antibody targeting CD3 molecules on T cells. Its development has been made possible through the hybridoma technology developed by Nobel-Prize-winners G. Köhler et C. Milstein in 1975.

A biopharmaceutical (AKA biologics) is derived from living organisms such as humans, animals, plants, microorganisms and/or by biotechnology methods (recombinant DNA techniques/ cell culture). Biopharmaceuticals include modified human proteins, monoclonal antibodies, growth factors, vaccines, enzymes, living entities such as cells and tissues, etc. As such, biologics can be composed of sugars, proteins, or nucleic acids or complex combinations of these substances. In comparison, small molecules are chemically synthetized organic compounds of known structure. Table 1 summarizes the other main differences between biologics and small molecules. Biologics' intrinsic characteristics inevitably led to the creation of a whole new drug-production industry for this new type of drug known as bioprocessing.

Production of drugs involves a highly-controlled environment constrained by strict regulations and standards combined with numerous quality control (QC) procedures to ensure that the end-product has retained the desired properties and potency with no contaminants. However as compared to the chemical synthesis of small molecules, bioprocessing of therapeutic or preventive products involves additional steps and QC procedures due to the complexity and on the biological origin of these drugs. As such, Cost of Goods (CoG) is significantly higher for biologics than from small molecules. It is however expected that the emergence of biosimilars will help to lower these costs. A biosimilar is a biologic medical product highly similar to another already approved biopharmaceutical with no significative clinical differences. It can be considered as the equivalent concept of generic drugs for small molecules. The first biosimilar to be approved in the EU was the infliximab biosimilar in 2013.

Although biologics appear to be a real challenge to produce, it represents a growing class of therapeutic drugs or therapies, characterized by high specificity, better efficacy and improved side effects profiles, as compared to small molecule drugs . Indeed, our latest data depicts a biologics-pipeline of 16780 drug candidates, spanning from R&D to clinical phases, being developed by a total of 4900 companies worldwide. Importantly, the global biologics market was valued at 248 billion € in 2020 with a compound annual growth rate (CAGR) forecast at approximately 12% over the next five years.

	Chemical molecules	Biologic molecules
Molecular weight	<1000 Da	> 1000 Da (mAb~ 150kDa)
Structure	Simple and small structures, that can be entirely characterize	Producted exclusively from alive cells, complex structures, hard to be entirely characterize
Production	Chemically synthesized	Cell culture, recombinant DNA technology
Reproducibility	Identical copies (generics, bioequivalence)	Impossibility to have identical copies
ADME tools	Available/extensive ADME understanding	Understanding of ADME still evolving
Dosing route	Oral often possible	Usually parenteral (IV, SC, IM)
Dosing interval	Daily (typically)	Intermittent dosing
Half life (t1/2)	Short (typically several to 24 hrs)	Long (typically days or weeks)
Distribution (Vd)	High, distribution to organs/tissues	Lower, usually limited to plasma and/or extracellular fluids
Metabolism	Generally liver and kidney, pathway well characte-rized	Catabolized and degraded into amino acids, biotransformation not occuring
Excretion	Mainly biliary and renal	Mostly recycled by body
Clearance (CL)	Mostly linear PK (nonlinearity due to saturation)	Slow clearance
Potency and selectivity	Generally less selective	High selectivity (affinity/potency)
PK analytes	Drug and metabolites	Antibody and ADA
PK bioanalysis	LC-MS/MS methods	Mostly ELISA
PD	Short acting	Long acting
PK/PD	PK usually not driven by PD due to dominance on non-target mediated binding	PK and PD mechanistically connected
hERG	Yes	No
Formulation	Complex and diverse	Simple formulation
Stability	Generally stable	Very sensitive to environment changes Require very strict conditions for transport and stockage
Immunogenenicity	Low immunogenic potential, execption hapten protein compound	High immunogenic potential
Toxicity	On- and off-target related toxicity	Typically exaggerated pharmacology
Food effects	Potential concern	Generally no
Drug/drug interaction	Potential concern (PK and/or PD related)	Generally no (few examples, mostly PD related)
Prices	Low prices, therefore, severe competition from chemical generics after patent expiration	High prices with very less competition

** adapted from LEEM document and Roots Analysis : Biopharma Contract ManufacturingMarket (3rd Edition), 2019-2030*

HUMAN AND ANIMAL CELLS AS FACTORIES FOR THERAPEUTIC MOLECULES

Pr. Eric Olmos[1] and Pr Danièle Bensoussan[2]

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2. Unité de Thérapie Cellulaire et banque de Tissus, CHRU de Nancy, Allée du Morvan, 54511 Vandoeuvre les Nancy

1. INTRODUCTION

Animal and human cells have progressively become a versatile platform to produce various therapeutic biomolecules. This category of expression systems mainly includes mammalian (essentially hamster, mouse and human cells) and insect cells. From a general point of view, non-human mammalian cells are still the most used at the industrial scale (production of glycosylated recombinant proteins and of adenoviral vectors among others) but some continuous human cell lines can be used in vitro in a bioreactor to obtain a supernatant enriched in a specific protein or in extracellular vesicles after selective isolation. Moreover, human stem cells can be used in vitro as a source of differentiated cells, as with induced pluripotent stem cells (iPS).

Considering that stem cells can be the source of more differentiated cells, their use in vivo make them cell factories. The use of human cells as an in vivo factory has been performed for decades with hematopoietic stem cells (HSC) for the reconstitution of hematopoietic blood cells and immune cells. On the same model, specific T cells like Viral specific T cells (VST) can proliferate in vivo and differentiate into cytotoxic anti-viral T cells. Mesenchymal stromal cells (MSC) when used in regenerative medicine, can also be considered as cell factory able to produce differentiated cells in vivo.

From a process engineering point of view, the success of the development and strengthening of biotherapies using animal and human cells should be based on an advanced knowledge of the cell behavior in their culture system, namely the deciphering of the couplings between cells and their culture environment.

In view of the above, we will first present hereafter the operative and expected applications of animal and human cells as in vitro factories but also as in vivo factories, focusing on mesenchymal stromal cells. Then, the current and future strategies of optimization of the interactions between the cells and their biochemical and hydromechanical environments will be discussed.

2. HUMAN AND ANIMAL CELL CULTURE FOR THE PRODUCTION OF THERAPEUTIC BIOMOLECULES: MAIN APPLICATIONS

2.1. Industrial applications of continuous cell lines

Compared to primary cells, which are derived from extraction and/or digestion processes of biological tissues, continuous cell lines theoretically have an unlimited capacity to divide, thus allowing for a major expansion of the use of animal cells in industrial biotechnology processes. These continuous lines are either cells that have undergone a mutation of genes involved in the cell cycle, or cells

transformed by an oncogene, or cancer cells taken from an organism. Thus, as early as 1975, the fusion of rodent lymphocytes and myeloma cells allowed the creation of hybridomas and the production of monoclonal antibodies, which are mainly used today in diagnosis.

Gradually, in the 1980s, the use of these cells to produce therapeutic biomolecules was accepted by the regulatory authorities; the scaling up of culture processes to ensure a significant reduction in production costs was facilitated by obtaining (i) cell lines that were robust to agitated conditions and (ii) optimized culture media. The technology of cloning a gene coding for a protein of interest into an expression vector introduced into the cell has opened broad horizons for the production of customized recombinant proteins, making mammalian cells sophisticated platforms for the industrial production of these biomolecules. For example, in 1987, tissue plasminogen activator (tPA) was the first therapeutic molecule produced by Chinese Hamster Ovary (CHO) cells to be approved for marketing. Since these breakthrough innovations, the development and marketing authorizations of molecules produced by animal cells and in particular by mammalian cells have continued to rapidly increase (Figure 1). The market for recombinant proteins dedicated to therapeutic use, and especially that of monoclonal antibodies, is the major application sector driving the industrial use of animal cells. Monoclonal antibodies are used in the treatment of cancer, cardiovascular diseases, respiratory diseases, infections, ophthalmic or inflammatory diseases. They are currently finding new uses in the treatment of severe infections such as SARS-CoV2 (antibodies bamlanivimab and etesevimab).

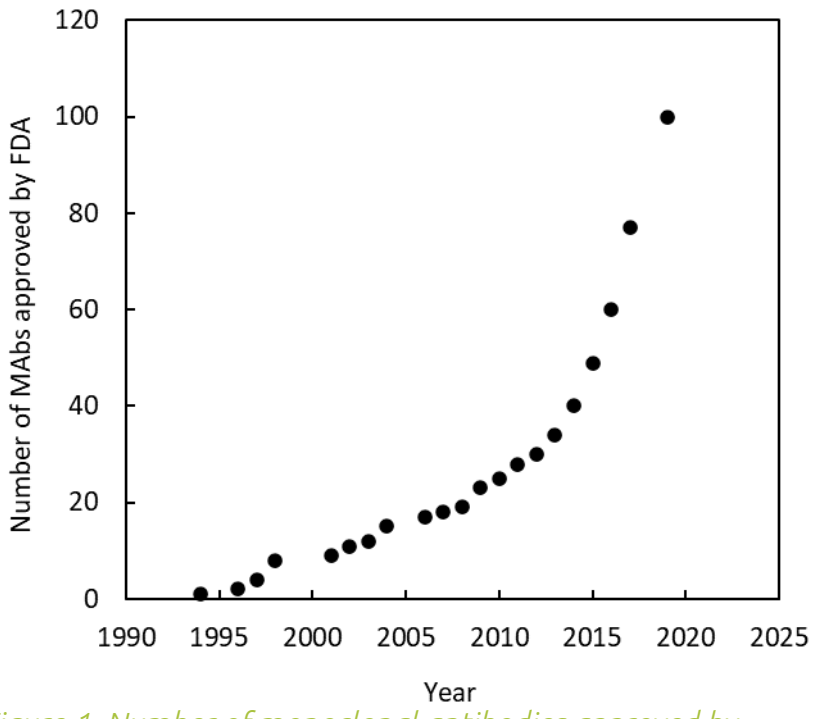

Figure 1. Number of monoclonal antibodies approved by FDA.

The most common cell line is the CHO cell, which alone seems to account for approximately 75 % of recombinant protein production. In addition to mammalian cells, insect cells, infected with a baculovirus carrying the gene coding for the protein of interest, are also attractive tools for the transient production of proteins on a smaller scale. A list of the main mammalian cell lines and their recognized applications is given in Table 1.

More recently, new lines of human origin are emerging for the production of adenoviral vectors or recombinant proteins (HEK293, PER. C6)[1]. They present a higher advantage as they allow the production of a non-immunogenic glycan. The main drawback is that they are susceptible to human virus contaminations requiring viral inactivations. HEK293 (Human Embryo Kidney 293) and its different variants, transfected with viral DNA, is the most prominent human cell line used for protein expression. Other cell-lines, including HT-1080 (human fribrosarcoma), CAP (from human amniocytes), PER.C6 (from human retinoblast), and HuH-7 (from human hepatoma) are used for the generation of factor VII, adenoviral vectors, immunoglobulins, or factor IX, respectively[2].

Cell	Biological source	Some common uses
CHO	Chinese Hamster Ovary cells	Recombinant proteins
VERO	Kidney epithelial cells extracted from an African green monkey	Human and veterinary viral vaccines (rotavirus, rabies, poliovirus)
BHK	Baby Hamster Kidney fibroblasts	Factor VIII, veterinary viral vaccines
HEK 293	Human embryonic kidney	Adenoviral vectors (SARS-CoV2)
Hybridomas	Murine hybrid cell line	Monoclonal antibodies
PER.C6	Derived from human embryonic retinal cells	Recombinant proteins, adenoviral vectors (SARS-CoV2)
NS0	Derived from the non-secreting murine myeloma	Recombinant proteins
MDCK	Madin-Darby canine kidney cells	Viral vaccines (flu)
MRC5	Human fetal lung fibroblast cells	Human viral vaccines (flu)

Table 1. Most common mammalian cell lines and related applications.

2.2. Human cells for the production of extracellular vesicles

Extracellular vesicles (EV), including exosomes, micro-vesicles, and apoptotic bodies display different sizes (from 30 nm for exosomes to 5 µm for apoptotic bodies), derived from most of cell types, and implicated in intercellular communication participating in physiological but also pathological processes like initiation and progression of tumor[3]. Among EV, exosomes are generated secondary to the invagination of the endosomal membrane, harboring a unique composition, and are further released from the cell by membrane fusion[4]. Exosomes have been described as micro-carriers releasing proteins and lipids, non-coding and coding RNAs, micro-RNAs (miRNA) for the regulation of biological processes, genomic DNA and transference of antigens or immunogenic molecules for the activation of T-cells during immune responses[5]. They exhibit many characteristics from the originated cells with which they share properties, making them relevant (i) as biomarkers for the diagnosis of a wide variety of health disorders and (ii) for cell-free therapeutics as they could present a better safety profile than the original cell therapy[6]. For example, they can be generated from cells interfering with immune response like dendritic cells, macrophages and MSC inducing either immunostimulating or immunomodulatory effects. Monocyte-derived-exosomes appear to be able to escape immune phagocytosis, allowing their increased circulation and efficacy compared to other exosome types[7]. Interestingly, they can also be derived from tumor cells, and they can elicit an anti-tumor cellular immune response and easily target the tumor due to their membrane homology with tumor cells. However, there are controversial reports showing that tumor cell-EV promote cancer progression through different mechanisms like induction of angiogenesis, escape to apoptosis, and intensification of tumor cell proliferation[3]….

Thereby, due to their high biocompatibility and ability to penetrate through biological barriers, their enhanced stability and limited immunogenicity, exosomes are very promising tools for targeted drug-delivery going beyond the limits of liposomes[3]. Many kinds of chemotherapeutics drugs have been loaded into exosomes like paclitaxel or doxorubicin, allowing an increased uptake by tumor cells compared to the liposomal form of the drug and even overcoming drug resistance mechanisms. The cargo can also be therapeutic RNA or proteins.

Despite the advantages and promising interests of EV, many challenges remain for bringing exosomes into the clinic[8]. First, cell source selection is a critical step depending on the targeted tissue and on the elucidation of potential deleterious effects, as mentioned previously for tumor cell-EV. Exosome generation from standardized cell lines would be of great interest. The scale-up of the culture systems of cells must take into account serum-free medium, stirred tank bioreactors. Second, the definition of a purification process for achieving high yield and purity is a limiting milestone. Ultracentrifugation is the well-known method for exosome isolation. Improvements are needed to reach a large-scale manufacturing of exosomes and new methods are currently emerging like size-exclusion chromatography, ciliated micropillars nano-traps, acoustic wave separation and flow field-flow fraction[9]. Third, specific quality controls (QC), including potency assays, have to be sought and developed. New technologies are necessary to allow an on-line fast QC. Finally, while overcoming those production concerns, a key issue will be to determine the optimal dose, the maximum tolerated dose, the minimum efficacy dose and the route of administration.

Different early phase clinical trials have been initiated and, for some of them, completed in different indications like tissue repair, cancer, Graft versus Host Disease (GVHD) or chemotherapy delivery, confirming the great interest for this promising therapy and that there is still a long way to move on and bring them to the bed side[9].

2.3. Induced Pluripotent stem cells: a cell factory for the generation of more mature derived cells

Induced pluripotent stem cells (iPSCs) were first obtained by Yamanaka and colleagues while reprogramming adult cells, transferring 4 limiting transcription factors (Oct3/4, Sox2, c-Myc, and Klf4)[10]. Compared to embryonic stem cells (ESC), iPSC could present molecular anomalies (epigenic traces of their somatic origin, genomic instability possibly due to the reprogramming process, or alteration of mitochondrial DNA) but also different advantages, mainly the capacity to be generated in an autologous setting avoiding immunological problem. Those pluripotent stem cells can then be differentiated in many types of cells (neurons, MSC, …). However, although the autologous setting is attractive to overcome immune conflicts, a large-scale production of stable and controlled iPSC lines is only possible in an allogeneic setting. Some iPSC banks for research or clinical trials are currently developed in different countries but many challenges remain to meet the medical needs [11]: (i) overcoming immune rejection is an issue by developing large HLA typed banks to allow HLA matching with the recipient or by gene editing, (ii), implementing QC to ensure safety and efficiency of cells (especially genomic sequencing). Finally, although iPSCs are considered devoid of ethical problems compared to embryonic stem cells, there are currently raising ethics proceedings in France considering iPSC could be potentially differentiated into gametes (Ethic law in project).

2.4. MSC: a cell factory producing soluble factors depending to the inflammatory environment

Discovered in 1970s in bone marrow (BM), Mesenchymal Stromal/Stem Cells (MSC) have since been found in multiple tissues like adipose tissue, dental pulp, menstrual blood or extra-embryonic source such as Wharton's Jelly (WJ) or placenta[12]. In 2006, the International Society for Cellular Therapy (ISCT) defined Mesenchymal stem/stromal cells as (1) adherent to the cell culture plastic; (2) able to differentiate into osteocytes, chondrocytes, and adipocytes; (3) with a phenotype that is positive for the CD73 CD90 CD105 mesenchymal markers and negative for the CD34 CD45 HLA-

DR hematopoietic markers (MSC can modulate both innate and adaptive immunity by cell contacts, secretion of cytokines, chemokines, growth factors, and release of extracellular vesicles[13]. Briefly, immune properties of MSC rely on two main mechanisms: a direct interaction of MSC with immune cells and a paracrine effect of MSC. The main factors expressed by MSC in response to immune cell interactions are: microRNAs, PD-L1, HLA-G, Prostaglandin E2 (PGE2), cytokines (transforming growth factor-β (TGFβ), Interleukin (IL)6, IL10, HGF, VEGF…), and one enzyme (Indoleamine 2,3–dioxygenase: IDO). Immune properties of adult MSC have been widely reported[14]. Two MSC phenotypes -immunomodulatory or immunostimulatory- depending on the inflammatory context and the stimulation of their TLR were described[15]. In a cell anergy context, they could exhibit a pro-inflammatory phenotype, MSC1, making it possible to reduce apoptosis and promote T-cell survival. On the other hand, in case of inflammation, they could adopt an immunosuppressive and anti-inflammatory phenotype, called MSC2. This dual phenotype according to environment brings new opportunities for therapeutic uses. However, these immunomodulatory cells are slightly impacted by the host immune system (lack of expression of MHC class II antigens and CD80/CD86 costimulatory molecules, weak expression of MHC class I antigens) allowing there use in an allogeneic setting without considering HLA compatibility. Current data suggest also that MSC exert strong antimicrobial effects both indirectly, across their role in the host immune response against pathogens and directly, by the secretion of antimicrobial peptides and proteins (AMPs), but also by the expression of molecules such as IDO and IL17[16].

Up to now, more than 800 clinical trials have been performed with MSC from different sources in various indications, highlighting their safety and, in some of high phase clinical trials, their efficacy. Market authorization has been obtained in USA and Europe in GVHD treatment or in Crohn's disease fistulas.

In an allogeneic setting, high numbers of MSC have to be generated. In academic laboratories, MSC production is mainly performed in 2-dimension (2D) culture flasks which is time consuming and yields not compatible with the medical need. Currently, industrialization of 2D models begins with the marketing of bioreactors. Bioreactor increases cell culture surface and limits steric hindrance. A more versatile and scalable technology of culture has also arisen last recent years: the 3-dimensional model, consisting in using microcarriers as adherence support, in stirred tank bioreactors, while reducing the operating cost of the process. Regardless of differences due to MSC source, different challenges remain: (i) the recovery of MSC from microcarriers to achieve a good yield, (ii) the maximum volume of culture while keeping stable MSC characteristics (most of the cultures in stirred bioreactors operate today at a scale of few liters [17,18], (iii) and on-line monitoring of culture parameters.

As previously mentioned, exosomes are generated from MSC as they exhibit anti-inflammatory and immunomodulatory capacities and would allow a cell-free therapy. However, although the production of standardized homogeneous batches would be an improvement compared to whole MSC, the main advantage of the whole cells would be lost. The strength of MSC relies on their main drawback: to be a "living drug". The use of EV instead of cells induces the loss of adaptability capacities (dual phenotype MSC1 and MSC2). In multifactorial immune pathologies with concomitant inflammation and lymphopenia as described in sepsis, could it be possible to do without this main benefit?

2.5. Adoptive T cell immunotherapy: T cell factories allowing in vivo cell expansion and differentiation into cytotoxic T cells

The recovery of virus-specific T-cell immunity is crucial for patients after hematopoietic stem cell transplantation (HSCT) to avoid viral infections or reactivations. Based on donor lymphocyte infusions experiments, ex vivo virus-specific T cells (VST) were generated for patients with viral infection, refractory to anti-viral drugs[19]. VST can be expanded ex-vivo but also isolated in vitro from a donor leukapheresis using an immunomagnetic separation system (Miltenyi Biotec). In this last situation, a very small number of isolated specific T cell is obtained and infused into the patient, requiring an in vivo expansion when T cells interact with antigen-presenting-cell presenting viral peptides. As mentioned before, such VST are generated for a dedicated patient. Thus, large scale manufacturing is not an issue. The Prodigy platform, a closed, automated system, compliant with GMP guidelines developed by Miltenyi Biotec, allows bringing this medicine to the bed side. On a clinical point of view, randomized controlled studies are still missing to conclude on efficacy and safety of these allogeneic VST. Such a phase III clinical trial is currently including patients on a European scale (sponsor: Munich university).

2.6. Comparison with other expression systems

Today, most of recombinant glycoproteins produced by biotechnology are produced in mammalian cells. Other expression systems can be used, such as bacteria (Escherichia coli), yeast (Pichia pastoris, Saccharomyces cerevisiae), insect cells (Sf9, Sf21, BTI 5B1-4) or plants. These systems have clear advantages as they allow higher product concentrations; their production processes are considered as more easily scalable with reduced production costs (particularly in terms of culture media costs) compared to mammalian cell production processes. However, they lack the ability to implement complete post-translational modifications of the protein, notably glycosylation. Thus, their use is generally restricted to the production of small proteins or to the vaccine industry. Notable examples are the production of human insulin by the yeast Pichia Pastoris, the production of pseudo-viral particles for the design of the vaccine against the Papilloma virus (GARDASIL9) by Saccharomyces cerevisiae or viral vectors by Sf9 insect cells. The main advantages and disadvantages of mammalian and non-mammalian cells as expression systems are reported in Table 2.

Cell source	Advantages	Drawbacks
Mammalian (human or animal)	Excretion of proteins; growth in suspension or adhered on microcarriers in mixed bioreactors; scale-up robustness	Risk of viral contaminations; high culture costs; moderate yields and cell concentrations (except in perfused or optimized fed-batch modes of culture)
Non-mammalian (yeasts, bacteria, plant, insect)	Higher yields; fast growth; less risk of viral contaminations; lower production costs; scale-up robustness	Partial post-translational modifications of therapeutic proteins; risk of protein aggregation and inclusion body formation

Table 2. Advantages and drawbacks of mammalian and non-mammalian expression systems adapted from O'Flaherty et al. (2020)[20].

3. INDUSTRIAL PROCESSES AT THE SERVICE OF CELL FACTORIES

A production facility needs to interact with its socio-economic environment, with infrastructures allowing the routing of raw materials and people, and the management of flows leaving the factory (waste, products). The optimization of internal flows (communication, goods, etc.) is also one of the keys to the efficient running of the production means. Like an industrial plant, the animal cell can be described as a «factory» not so much because of the production it generates but essentially because of the complexity of the extra- and intracellular exchanges and interactions observed. To describe this complexity of flows, modelling tools are used, such as metabolic modelling, which represents the cell factory in the form of a «road map» and quantifies the distribution of flows within this mesh. For instance, a recent study modelled a CHO cell with 2101 genes and 4527 metabolites interconnected by 7436 reactions[21] ! Even if, in the near future, we can hope that this type of model will be used to control the production process, the approach favored in recent years by the engineering sciences to size and optimize culture processes is a systemic approach, which incorporates the intracellular machinery without precisely describing it. Thus, the determination of optimal culture conditions is based on the empirical or semi-empirical control of extracellular environments (that we will call the inlets of the cell factory) promoting the targeted performances of the cell (that we will call the outflows). By extension, the successful scale-up of animal cell culture processes to an industrial scale implies tacitly, but resolutely, that the maintenance of bioproduction performance from one scale to another can be guaranteed by the conservation of the input flows into the cell factory (the same causes promoting the same effects), provided that the intrinsic biological characteristics of the cell are also maintained (Figure 2). With the progress of knowledge and modelling tools, this systemic approach is progressively moving towards a multi-scale modelling that integrates all the length and time scales of the production process: metabolites/reactions, cell/physiological state, cell populations, bioreactor/hydrodynamics, process/pilot (see paragraph 4).

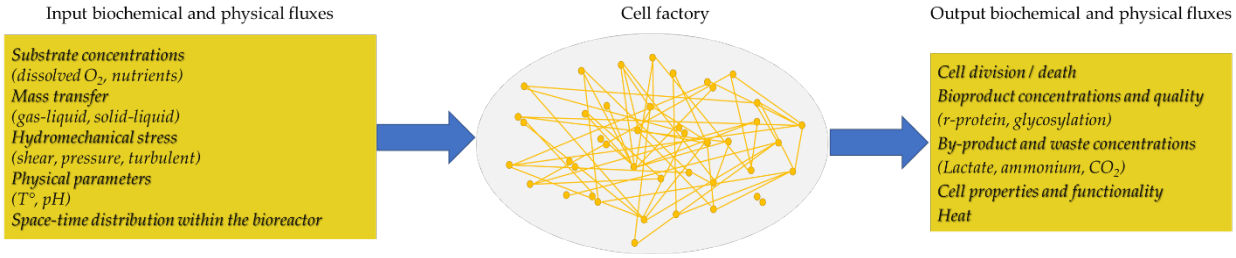

Figure 2. The concept of cell factory: the key integrated scale for process optimization.

Controlling the microenvironments of the cell factory relies on the deployment of experimental and numerical methodologies that will aim to (i) strengthen the understanding of cell/environment couplings and (ii) predict, in a sufficiently anticipated manner, the temporal evolutions of cellular responses, knowing the dynamics of these at a given time. Among the challenges taken up or to be taken up, let us mention in particular:

Control and optimization of substrate and nutrient concentrations. To achieve this goal, the culture processes are or should be based on the use of culture media, if possible, chemically defined, the composition of which will have been optimized during preliminary screening phases; this strategy makes it possible, in particular, to eliminate possible batch-to-batch variability in the culture medium. While the use of such media is gradually standardized for the industrial production of recombinant proteins (for these applications the use of compounds of plant origin or recombinant compounds in place of animal or human sources is also very frequent), the

addition of compounds of animal or human origin (fetal calf serum, human platelet lysate, growth factors) remains classic for the culture of primary human cells (MSC). However, as the kinetics of nutrient consumption or generation of metabolic products modifies this initial biochemical environment, it is also necessary to have methods for measuring, as far as possible «on-line», the concentrations of substrates and metabolites. Thus, the 2010s have seen the emergence and concretization of spectroscopic sensor technologies implemented in bioreactors (dielectric, RAMAN, near infrared, etc.) including on industrial production lines. Once combined, these sensors can not only measure in real time the concentrations of glucose, lactate, and glutamine but also the concentration and quality of the monoclonal antibody produced[18]. They are also required to improve the performance of fed-batch or continuous-perfused modes of culture.

Knowledge of hydromechanical environments. Most of the time, the use of agitated bioreactors imposes a turbulent, multiphase (gas-liquid, liquid-solid or gas-liquid-solid) hydrodynamics, notably characterized by its temporal variability and spatial heterogeneity. The description of the local hydrodynamics imposed on the cells during their culture makes it possible to anticipate the appearance of cellular damage in case of too strong agitation, to predict modifications in the size of cellular aggregates, or to generate mechanical environments suitable for the differentiation of stem cells, for example[18]. It can also contribute to a better understanding of the phenomena of mixing and mass transfer (oxygenation, desorption of CO2) or even to optimize bioreactor designs for a targeted application[22]. However, it requires the deployment of sophisticated experimental and numerical methodologies (numerical fluid mechanics, particle image velocimetry) which still have limitations in the case of complex multiphase flows. To overcome these limitations, the use of macroscopic scale-up criteria (mechanical power, mean Kolmogorov scale, volumetric oxygen transfer coefficient, etc.) makes it possible to maintain the hydromechanical stresses within acceptable limits or to dissolve the oxygen at a controlled rate.

4. STRENGTHEN (FURTHER!) THE INTERACTIONS BETWEEN THE CELL FACTORY AND THE CULTURE PROCESSES

The versatility and industrial operability of continuous cell lines or primary cells will be all the more strengthened that the constraints of cell factory operation are integrated early into the culture process optimization strategies and/or by using advanced modelling approaches. Thus, the prediction of the evolution of the physical cell environments during the transition to an industrial production scale (mechanical constraints, concentration heterogeneity) must be carried out during the process development phases, using mechanistic simulation approaches (CFD, compartments) and, if necessary, dedicated scale-down methodologies (two-stage systems, bioreactor screening).

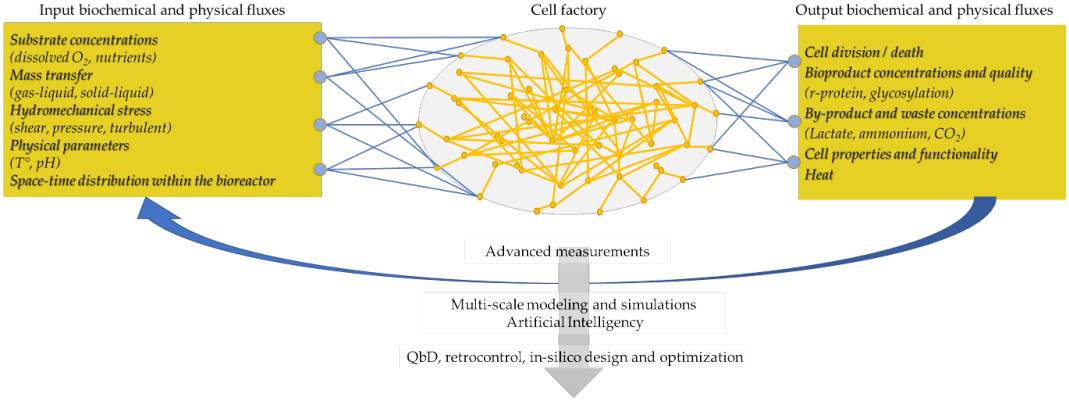

Figure 3. Towards in-silico design and optimization of the cell factory microenvironments.

The construction of predictive multiscale models, helping not only to predict cell performance under given culture conditions but also to optimize them, is currently underway and should be strengthened in the coming years (Figure 3). It requires the use of online measurement technologies to characterize cell dynamics and predict the actions needed to maintain cell productivity through implementation of optimal control strategies. On-line monitoring of cell functionality is also an important challenge to be met in the coming years to allow the application of these advanced understanding approaches. In this general framework of Quality By Design applied to animal and human cell cultures[23], the contribution of Artificial Intelligence and in particular Deep Learning by Neural Networks, numerical simulation of flows coupled to cell kinetics and new generations of on-line sensors will be essential to «close the loop».

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Production processes for biotherapeutics

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Bioproduction processes harness the ability of cells to manufacture proteins and orient this towards clinical and commercial purposes. From early roots in vaccine manufacturing in the 1900's, today these processes are largely focused on production of therapeutic proteins (principally monoclonal antibodies), and more recently viral vectors for gene therapy.

Early commercial processes for mammalian cell culture-based production of therapeutic proteins were described from the late 1980s / early 1990s onwards (1). As compared to current bioproduction processes, these were typically low yielding (e.g., titres ≤0.5 g/L for monoclonal antibodies at harvest, (1)), and complex (as regards the number of unit operations). Since then the overall technology platforms have reached something approaching maturity. Todays' processes for monoclonal antibody (MAbs) manufacturing (as one example) are largely based on a fed-batch approach, with two to three chromatography steps in the downstream process (DSP). Titres are typically in the order of 2 – 5g/L at harvest for more recently developed processes (last 5 – 10 years) (1). Biosimilar production processes may still mimic these early formats (lower titres, more unit operations) in order to remain as similar as possible to the originator product.

In general, mammalian cell culture-based bioproduction processes start with the thawing of a small volume of concentrated cells (see Figure 1 for a typical example). This is usually a vial from a working cell bank. The cells are then serially expanded in a seed train, prior to inoculating a Production Bioreactor (2). When the cells have reached a critical mass in the production bioreactor, some form of "switch" is activated, to focus the cells on producing the protein of interest (and focus less on replicating themselves). This is usually effected by a shift in temperature or pH, addition of a chemical compound (such as sodium butyrate) or a combination of these elements.

Box 1. Key terms

Harvest: The cells, media and intermediate protein product at the end of the Production Bioreactor.

Master cell bank: An aliquot from a single pool of cells, which generally has been prepared from a selected cell clone under defined conditions, and then dispensed into multiple containers and stored under defined conditions. The MCB is used to derive all working cell banks. (ICH Q5A, ICH Q5D).

Production Bioreactor: Bioreactor vessel where the final step of bioproduction occurs (after cell expansion in the seed train) to produce the desired protein/product of interest.

Seed train: Serial expansion of producer cells, starting from a vial (usually from a working cell bank; volume of ~1mL) through a series of flasks and then into to small bioreactors, up to a volume that is sufficient to inoculate the final bioreactor (called the Production Bioreactor). Typically, the seed train cell mass is about 20% of the initial volume of the Production Bioreactor.

Titre: the quantity of product present in the bioreactor, usually expressed as g / litre (or genomes/L for AAV and LV).

Working cell bank: The Working Cell Bank is prepared from aliquots of a homogeneous suspension of cells obtained from culturing the MCB under defined culture conditions. (ICH Q5A, ICH Q5D).

Working volume: the amount (volume, or weight) of liquid (cells, media, and feeds) that is used in the Bioreactor. This is typically less than the geometric vessel volume, as a certain amount of free space is required at the top of the bioreactor (the "headspace") to enable gas exchange and also to ensure the filters, etc, situated at the top of the bioreactor, do not come in to direct contact with the vessel contents.

For production of gene therapy viral vectors such as Adeno-associated viruses (AAV) or Lentiviral vectors (LVV), the main approach currently involves transient transfection of plasmids encoding the

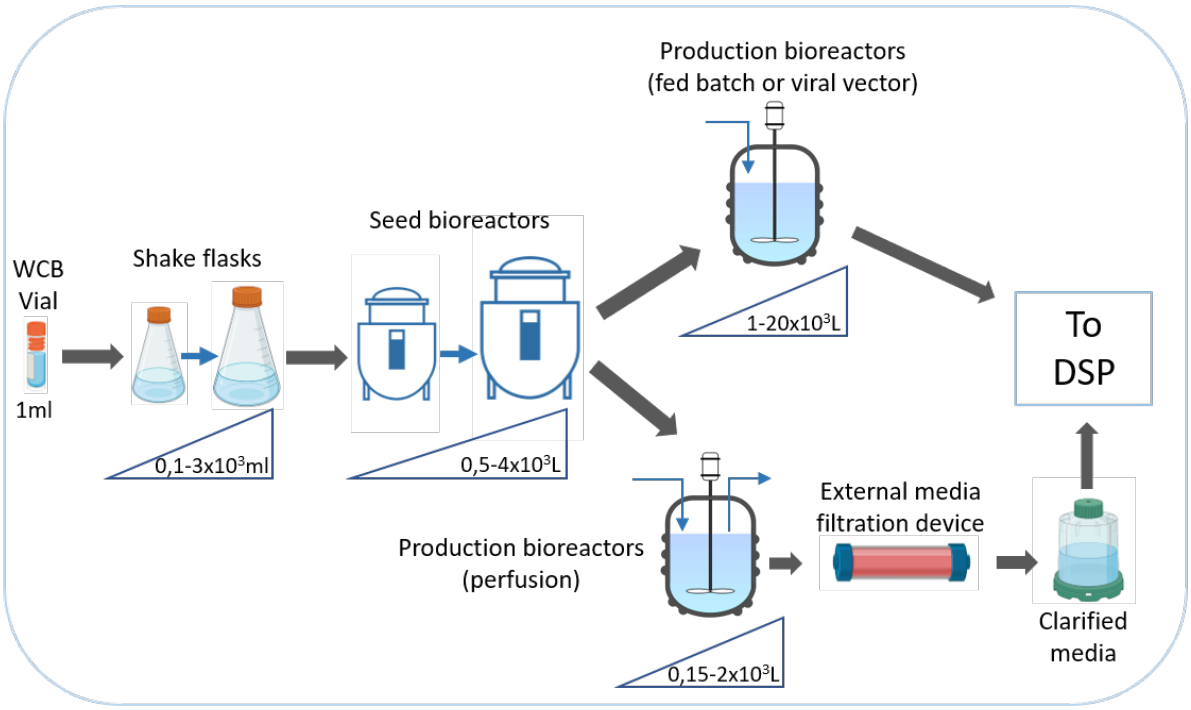

Figure 1a. Generic schema for a mammalian cell culture bioproduction process (USP). Cells are progressively expanded in containers of increasing size, until enough culture mass and viability is obtained to inoculate a Production Bioreactor (Fed Batch or Perfusion mode). Approximate volumes are provided.

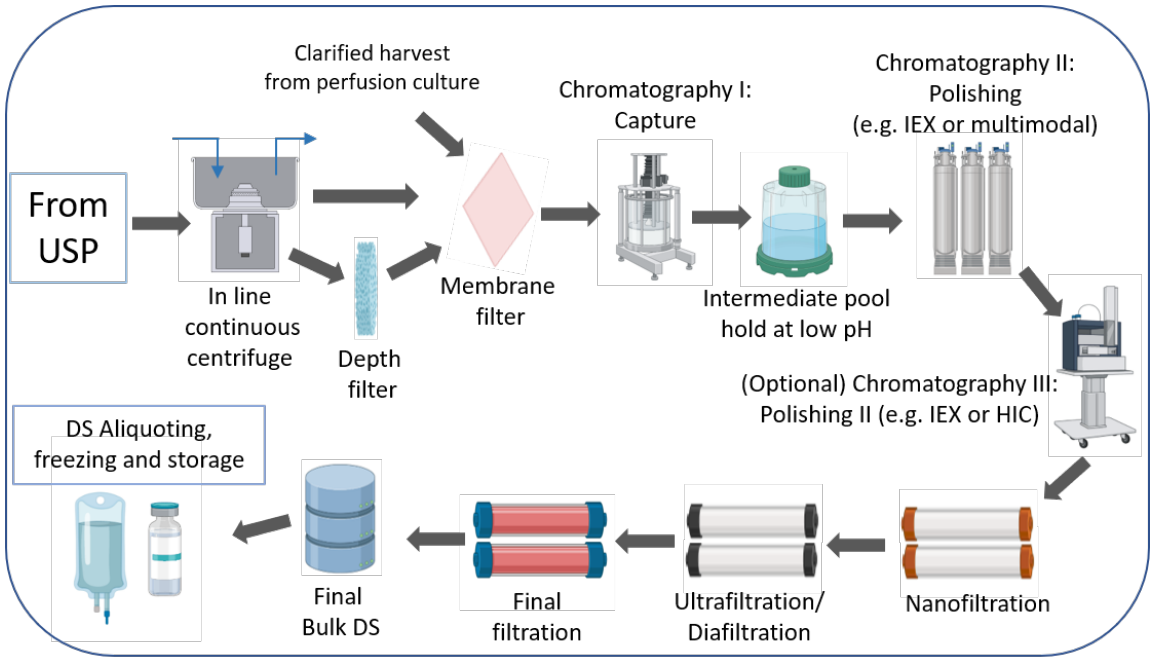

Figure 1b. Generic schema for a mammalian cell culture bioproduction process (DSP). Cells and cell debris are removed from harvest via centrifugation and/or filtration, or by perfusion. The clarified harvest is then processed through a series of chromatography and filtration steps to separate the product of interest from impurities. A 0.2um filtration step (not shown here) is usually included between each unit operation. For viral vector production processes, fewer chromatography steps are used, and nanofiltration is not common.

viral genes and the therapeutic gene of interest in to mammalian cells (usually HEK 293 (3)) directly in the production bioreactor (4), when the cells have reached a certain mass and viability. The use of HeLa cells and baculoviruses have also been described (5), and stable cell lines for AAV and LVV production are becoming more widespread (6, 7). For adenoviruses (e.g., some COVID vaccines are adenovirus based), the virus is capable of actively reproducing in HEK 293 cells, so a "seed stock" of virus can be used to initiate the viral vector production process in the Production bioreactor.

When the Production Bioreactor step has completed, the first step of the downstream process (DSP) is primary recovery or clarification (8). Cells and cell debris are removed via in-line centrifugation, depth and/or membrane filtration, or via a combination of these approaches. Further purification generally comprises two different chromatography steps (capture and polishing), with one or two columns being used for polishing. Nanofiltration and ultrafiltration/diafiltration round off the purification steps. Fully formulated drug substances (where there is no need for compounding and additional formulation in drug product manufacturing) are becoming more common. This is possible, except where multiple DP formulations exist for the same product. In these cases, partially formulated DS is more common.

There are various bioproduction formats to choose from (Box 2), and these principally depend on the needs of product development or market supply (e.g., single use vs multiple use processes), or are decided early on in the development programme for specific reasons (e.g., the choice of perfusion[1] or continuous processing (9) vs fed batch production bioreactors). Single use systems provide flexibility and segregation for multi-product facilities, but can become expensive (and environmentally impactful in terms of plastic waste) as the number of required batches increases. At a certain point, fixed/multiple use (e.g., stainless steel bioreactors) makes more operational and economic sense, and an economy of scope and/or scale develops for aspects like cleaning and steaming, along with their validation. Hybrid approaches (e.g., stainless steel bioreactors, with single use bags for media, buffer and DS intermediate storage) are quite common also.

As regards the cell lines used for bioproduction, Chinese Hamster Ovary (CHO) cells are the cell line of choice for MAb manufacture (10). Significant efforts have been invested in to CHO cell engineering to create high yielding clones (11), with removal of undesirable characteristics (e.g., enzymatic modification of the protein product). Most biopharmaceutical producers today have their own proprietary CHO clones, which they use for bioproduction of multiple different products. There is a large body of evidence and experience with CHO-produced biopharmaceuticals (12) as regards the final protein profile, post-translational modifications, the type and quantity of process impurities (such as proteins and DNA from CHO cells) and CHO susceptibility to contamination by putatively present viruses (also known as adventitious agents). Some complications may arise with newer biopharmaceutical formats, such as bi-specifics, where it may be challenging to tune the process to the quality requirements of two different protein moieties on the same molecule. This consideration may also drive the choice between different production formats (e.g., fed batch vs perfusion). Other murine cell lines, such as Sp2/0 and NS0 were used in the past, but are less commonly employed for newer processes.

For MAbs purification technologies, there is a wide and mature offering from multiple suppliers. Capture resins using Protein A as a ligand remain the standard workhorse for MAbs manufacturing processes (as the Protein A ligand recognises the Fc region of the MAbs (13)). For other protein therapeutics, the capture mode can be less specific, with potentially fewer offerings available on the market. Holding the eluted antibody intermediate at the low elution pH is a standard step

1 https://www.meticulousresearch.com/product/continuous-bioprocessing-market-5079

to inactivate putatively present adventitious viruses. This step is typically followed by one or two polishing steps, using ion exchange or hydrophobic interaction resins. Newer resins based on multi-modal exchanges (multiple different binding chemistries in the same resin) have led to the removal of one column in some cases, leading to two column processes. For the subsequent steps of nanofiltration (viral removal filtration) and ultrafiltration/diafiltration (buffer exchange and product concentration), there are numerous offerings on the market. Shield filters are also available to install before the nanofilter, which can reduce the presence of aggregates and increase the performance of the nanofiltration step.

Box 2. Principal types of mammalian cell culture bioproduction processes

Batch mode: Cells and media are placed in a Production bioreactor, and cultured without addition of further media and feeds until the culture is deemed to be complete (product has been produced to desired quantity and quality), or the culture is exhausted (loss of cell viability or viable cell number).

Continuous Process: The bioreactor is operated in a perfusion mode, and the collected perfused media is then continuously processed, either for the initial steps of the DSP (e.g., initial capture of the intermediate product from the bioreactor harvest), or straight through the DSP until the freezing of the final bulk drug substance.

Fed-Batch mode: Cells and media are placed in a Production bioreactor, and the culture is initiated. After a specified period of time, additional media and/or feeds are added sequentially to the bioreactor until the culture is deemed complete or the culture is exhausted. This is the most common mode for biologics production. Culture durations are typically 10 – 14 days, with feeding commencing around Day 4 – 6. Starting volumes are typically around 70% of the working volume of the Bioreactor, and final volumes are usually close to the maximal bioreactor working volume.

Fixed installation / Multiple use: Production containers and tubing, piping, etc, designed to be cleaned, steamed and re-used many times. Vessels are typically made of stainless steel, whereas chromatography columns can be made from stainless steel or a mix of stainless steel and hard durable polymers (e.g., acrylic). Tubing piping, connectors, can also be made from steel, or durable polymers (or a mix of both).

Perfusion mode: Cells and media are placed in a Production bioreactor, and culture is initiated in a batch mode for a number of days. When the culture has reached a certain point (defined by quantity of cells present and/or their viability), the culture is switched to perfusion mode. Perfusion involves the removal of (used) cell culture media, while the cells are retained in the bioreactor. This is accomplished using such technologies as spin-filters (located inside the bioreactor, or externally) or alternating tangential flow (ATF) devices. The principle is that a force is applied to the media and cells, which pushes the media through the filter, while retaining the cells in the culture. The recovered media is collected and stored, and the product purified from this during DSP. The removed volume of media is replaced by an equal volume of fresh media (typically equivalent to one working volume of the bioreactor per day). Sometimes it may be necessary to remove ("bleed") biomass from the culture to maintain the perfusion equilibrium. A perfusion bioreactor can operate from 30 – 60 days (or even longer), until the culture is intentionally terminated.

Single Use: Production containers and tubing, piping, etc, made from polymeric materials (plastics) that are designed to be used once for a bioproduction process. It is generally not possible to clean and re-use them (they are not designed for this). Single use systems include cell culture flasks, bioreactors, chromatography resins and membranes, filters, and tubing, connectors, etc, and also storage vessels for buffers, media and production intermediates. They are generally supplied sterile (gamma-irradiated).

2 https://bioprocessintl.com/upstream-processing/expression-platforms/30-years-upstream-productivity-improvements/
3 https://www.antibodysociety.org/resources/approved-antibodies/

Future perspectives: MAbs manufacture

After 30+ years of continuous technological development and numerous commercial launches of biopharmaceutical products[2,3] (1) bioproduction technologies have reached a point of maturity, certainly for MAbs manufacture. Average yields attained with commercial- scale mammalian manufacturing processes are ~70 - 80% (1).

As described above (and in Box 2), there is a wide scope of choice in terms of platforms, equipment and technologies. Each approach (e.g., continuous processing, single use systems) has its proponents, but the choice ultimately comes down to the needs of the product and the market, and the fit to your operational model. As cell culture yields have improved, and downstream processing technologies become more efficient (e.g., higher resin binding capacities), the use of smaller overall volumes and single-use approaches is now possible in a wider range of circumstances than was the case 10 years ago. For certain "older" technologies, e.g., stainless steel that needs to be cleaned and steamed, this can make sense where larger quantities/more batches are required, and/or where approaches for cleaning and steaming development and validation are well established. Fixed/ multiple use systems have the benefit of being less susceptible to supply chain disruption for single use items, which is becoming more prevalent during the COVID pandemic (e.g., sequestering of certain filters, etc, by national governments to prioritise vaccine manufacturing). This can reduce reliance on external supply chains.

Despite the maturity of the industry, technology offerings continue to evolve. Different formats for working cell banks (e.g., WCB as high density cell preparations in bags, which can reduce the overall production time by "jump starting" the cell culture processes), are starting to be used. Continuous processing is also gaining momentum, but remains a choice (as described above), and the different platforms can be used in mixed modalities (e.g., Production Bioreactor in Fed-Batch mode, but one or more seed expansion steps in Perfusion mode).

One main area of focus currently is data analysis and insights. While the fundamental approaches were established in different industries many years ago (14), there is a growing interest in collecting and mining bioproduction process data. More advanced approaches are based on data historian systems (e.g., Pi system from OSIsoft) that can collect data in real-time, which is then processed by (commercially available) data analytics platforms such as Simca and Discoverant[4]. Algorithms can be developed which enable prediction of key aspects of bioproduction operations, e.g., harvest timing and yield, and can also act as alert systems for process performance (e.g., loss of pH control linked to problems with process inputs). Even for early stage process development, there is an interest in aggregation of datasets to enable multivariate data analytics, to identify (previously unknown) relationships between different process parameters, and product quality and/or process performance. Often such analyses can highlight "non-obvious" linkages (e.g., shifts in product quality linked to early stages of cell culture, or linkages between the DSP and USP parts of the process). Significant effort has also been invested in Process Analytical Technology (PAT), principally driven by data acquired from probes (e.g., probes for metabolites, cell viability and density, or "multi-use" probes such as RAMAN (15). These enable high degree of process monitoring, with options for process control, and their data can be used to drive predictive process models or "digital twins", virtual versions of physical systems that may be used to optimize processing. On-line protein analytics is not widely implemented, and will most likely need improvement in the robustness of on-line auto-samplers to gain further usage.

4 https://www.hallam-ics.com/blog/introduction-and-optimization-of-data-historians

Future perspectives: gene therapies – viral vector manufacturing

The situation as regards bioproduction for cell and gene therapy viral vector manufacturing (e.g., AAV, LV) is quite different from that described above for MAbs. The overall state of bioproduction technology and understanding is ~20 years behind that of MAbs, and the industry will need to close this gap within the next five years to realise the promise of these therapies. Some specific challenges include low yields, limited offering of DSP technologies, poor stability of LV vectors, and viral and impurity clearance[5].

Yields: Overall titres at harvest for the two main types of vectors – AAV and LV – average 10^9 and 10^7 viral genomes per mL of harvest, respectively. Overall process yields range from 30 – 50% for AAV, and 15 – 40% for LV (Unpublished Data from Yposkesi). These quantities are manageable today given the sector's focus on rarer diseases and smaller patient populations. However, with the foreseen growth of therapeutic indications requiring higher doses and larger patient populations (for less rare indications), the situation is not sustainable. An increase in titres of 10 – 100 fold per batch will be required. The current predominant approach to producing LV and AAV vectors is based on transient transfection of the gene of interest and the associated viral genes, encoded across three or four plasmids, which takes place directly in the production bioreactor. This choice is largely driven by a need for flexibility (the ability to combine different transgenes with different serotypes, and also capsid variants). Even though large quantities of plasmids are used (which adds significantly to the cost of the produced batches), the incidence of interaction between the plasmid transfection complexes and the target cells is not sufficient, largely explaining the low titres. In the case of AAV, where not all plasmids may be transfected into all individual cells, this can increase the proportion of "empty" vectors in the final product (16). There is an increasing focus on the use of stable cell lines, but existing controllable stable expression systems are prone to "leaking" (and the viral proteins can be toxic to the cells), which necessitate intensive efforts to select a well-controlled clone. Development of new approaches are urgently required here, e.g., better controlled expression systems, faster methods for stable cell line development and cloning, methods for increasing the interaction between transfection complexes and process cells.

DSP technology: As compared to the options available for MAbs manufacturing, the choice of resins, etc, for viral vector production is much more limited, and less understood (due to less experience in production). For AAV, DSP was initially based on a one-column approach, using a capture resin. These resins were initially serotype specific (necessitating different resins for different serotypes), but now newer resins are available which can capture a broad range of AAV serotypes with good affinity (17). Separation modes that require transit of the virus through the resin beads require large resin beads, for which there are limited offerings. The quantity of target material (the viral vectors) present as a proportion of the overall mass of the batch is also much lower for AAV and LV production processes than for MAbs, which creates additional challenges. In general, suppliers have started to address these challenges, but there is a lot of ground to still to cover to arrive at well-understood offerings.

Impurity and viral clearance: The initial approach for DSP steps in viral vector manufacturing that "one resin would do everything" is not generally supported by the levels of process impurities observed in the final bulk product (Unpublished Data from Yposkesi). There is a growing use of (at least) a second (polishing) chromatography step to reduce these down to levels that are expected from Regulatory Authorities. However, this additional step further reduces the overall process yield, and the overall level of understanding (e.g., choice of resins, performance) is at a very early stage. For clearance

5 https://themedicinemaker.com/manufacture/advanced-medicine-replicating-the-mab-success-story

of putatively present contaminating viruses (a.k.a., adventitious agents), the situation is much less understood than for MAbs. This is principally due to the lack of choice in resins, the use of human cell lines (versus murine for MAbs), which implies a different panel of viruses and risks to be evaluated (e.g., COVID can replicate in human cell lines), and the fact the product (the gene therapy vector) is a virus itself. This whole area will require an intensive focus in the coming years

LV stability: LV vectors are used today predominantly in *ex vivo* mode for therapies such as CAR-T, but the use of LV as a therapy in their own right (*in vivo*, as is the case currently for AAV) is a growing sector. LV are fragile viruses, and yields can be significantly affected by the bioproduction process, especially due to exposure to ambient temperatures. Large losses can occur during non-automated DP filling steps. Steps to reduce processing time and better ensure the stability of the LV drug substance or drug product (e.g., via different buffer formulations) are required (18).

Concluding remarks

As compared to early commercial protein production processes in the 1990's, today's bioproduction platforms and technologies are well developed. Yields are now quite high and it is relatively quick to establish stable cell lines and cell banks. There is a choice of different production formats (single use, fixed, hybrid), and PAT is quite well established with probes, although less so with online protein analytics (autosamplers). For DSP, yields of 80% are quite common, with stable technology offering for resins, filters, etc. Known weak points in processing (e.g., filter integrity failures) have established regulatory mechanisms for correction (e.g., reprocessing) resulting in minimal batch losses. For MAbs, process understanding as regards product and process impurity clearance, and viral removal, is well established. The current situation for MAbs can be described as mature, with a current focus on data analysis and insights, and continuing technological evolution. The situation for bioproduction for cell and gene therapies is more rudimentary and will require intense efforts over the coming 3 – 5 years to close the gap. The safety profiles of the viral vectors being used are better understood and combined with standardised production workflows, improved quality and accuracy of the analytics, will help make gene therapy products available to many more patients.

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FILL AND FINISH PROCESS

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1. Groupe IMT

INTRODUCTION

The culture and purification steps detailed above highlight the complexity and singular costs leading to the harvest of a molecule of interest.

The Fill & Finish steps of this molecule of interest, i.e. the manufacturing of the biomedicine, do not differ fundamentally from those of traditional injectable/sterile chemical drugs, but the extreme industrial risks and the very nature of the product (its biological nature, the size and the complexity of the molecule of interest) bring a very particular sensitivity to the steps that will follow: formulation and filling. This results in some specificities.

FORMULATION

The vast majority of current biomedicines are formulated as parenteral products in unit dose or infusion packaging; they are generally poorly absorbed and therefore not very bioavailable.

However, prospects are developing for pulmonary or even topical applications.

Generally speaking, the formulation excipients must not impact the stability, efficiency and safety of the proteins, antibodies, peptides or nucleic acids produced, or even improve them.

In consequence the objective of the formulator is multiple; indeed, the main risks lie in the loss of biological activity, the appearance of immunogenic risks or the formation of aggregates.

To date, the question of stabilization generally involves a freeze-drying step. The excipients/adjuvants must consequently also be adapted to the implementation of this step.

The main families of excipients used are sugars, polyols, surfactants, polymers, amino acids and salts.

Let us recall the definition of excipients as proposed by the International Pharmaceutical Excipients Council (IPEC): *substances other than the API (active pharmaceutical ingredient) which have been appropriately evaluated for safety and are intentionally included in a drug delivery system with a view to:*

- *Aiding in the manufacturing process of the said drug delivery system;*
- *Protecting, supporting or enhancing the stability or bioavailability of the active ingredient;*
- *Improving patient acceptability;*
- *Providing any other characteristics guaranteeing the safety and efficacy of the pharmaceutical product during its storage or use.*

Furthermore, in industrial coherence and in view of the innovative nature of these «new» medicines and subsequently of potential innovative adjuvants, particular attention must be paid to secure the supply chain.

FILLING TECHNOLOGIES

Let's consider the majority of biomedicines and in consequence the need of packaging for the parenteral route.

As with any drug of this type, the process must guarantee the characteristics of sterility, apyrogenity and absence of particles.

Generally speaking, in view of the sensitivity of biological molecules, filling processes in «aseptic manufacturing» mode are selected.

On account of that, environmental control is the key.

The specifications associated with this type of production are largely defined in the GMP.

By systematically considering the regulatory guidelines, environmental control will be mastered

in different ways: via a process in a controlled atmosphere area (class A + B / ISO 5), via the implementation of RABS and isolators, via the use of disposable devices.

Whatever the solutions chosen, the control of sources of contamination (chemical, biological, particulate, cross-contamination) always follows the same logic, in synthesis:

- Control of raw materials;
- Quality and qualification/validation of equipment;
- Implementation of robust methods;
- Staff training;
- Control of the working environment.

Caution: In the case of mAbs, for example, it may be difficult to identify cross-contamination if the mAbs produced have similar characteristics. In this case, specific identification controls must be implemented.

In the pharmaceutical industry, aseptic filling processes present, in the end, the highest risks for patients, and therefore for manufacturers. Taking this into account, the steps that constitute them are singularly controlled and supervised.

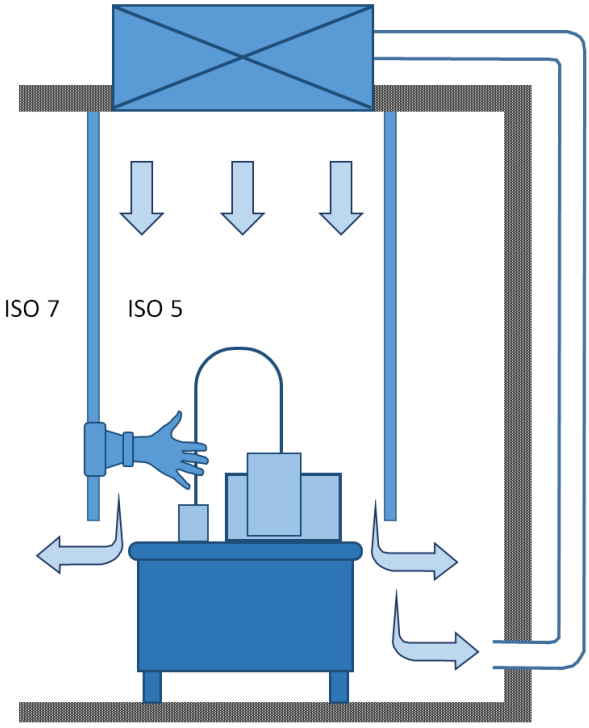
Hence, different options are possible:

Production in Clean Areas, (BPF class A in B / ISO 5). This scheme pushes to the maximum the commitment of the site, due to the size of the surfaces to be treated – the whole area – and the amount of potential risk (human in particular). The literature (GMP, FDA, ISO, WHO, PDA, ISPE) on the subject is dense. The means of control must concern materials, heating- ventilation and air-conditioning systems (HVAC), pressure cascades, procedures, monitoring, qualification...

Restricted Access Barrier System. An alternative strategy consists in concentrating the control zone within an open system (RABS), it is induced by the constant increase of the regulatory requirements for the control of the production environment and by the increase of the toxicity of the implemented products, and thus the protection of the operator.

This equipment puts a «semi» physical barrier between the operator and the product. They are suitable for products for which personal and environmental risks are not sensitive.

Installed in ISO7 classrooms, they work according to the following principle:

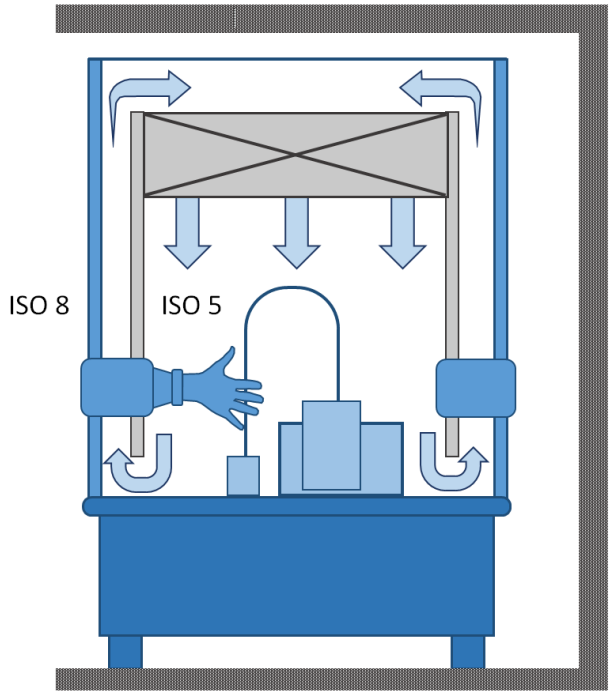


RABS DIAGRAM :

Two types of RABS are available, passive or active, depending on whether they use the HVAC system of the room where they are installed or whether they use a stand-alone system.

Isolators implement tight barrier techniques to achieve total physical separation between an internal and the external environment. This equipment is therefore closed, sterilizable and equipped with transfer devices to guarantee the sterility of incoming and outgoing elements.

An isolator works according to the following principle:



ISOLATOR DIAGRAM :

According to FDA Guidance - Sterile Drug Products Produced by Aseptic Processing / APPENDIX 1: ASEPTIC PROCESSING ISOLATORS:

The interior of the isolator should meet Class 100 (ISO 5) standards. The classification of the environment surrounding the isolator should be based on the design of its interfaces (e.g., transfer ports), as well as the number of transfers into and out of the isolator. A Class 100,000 (ISO 8) background is commonly used based on consideration of isolator design and manufacturing situations. An aseptic processing isolator should not be located in an unclassified room.

The regulatory framework is evolving and new guidelines pay particular attention to the control of transfers. Indeed, the transfer steps are the most risky phases in an isolator. They take place in continuous or discontinuous mode.

Suppliers offer different transfer and integrity test systems for these installations.

In general, barrier systems improve the level of sterility assurance; an isolator can be considered to provide a higher level of sterility assurance than a RABS, which in turn is higher than a traditional ISO 5 laminar flow area.

The isolator allows a complete and controlled decontamination of the machine and its environment.

For all these reasons, the current trend for new installations is clearly leaning towards isolator technology. The development of filling technologies with more and more automated equipment reinforces this orientation.

To go further and in particular to override cleaning, sterilization and associated validation steps, the use of **single-use** (SU) filling devices is shifting towards development.

Single-use technologies are the standard in USP and DSP, and are now available at filling stations.

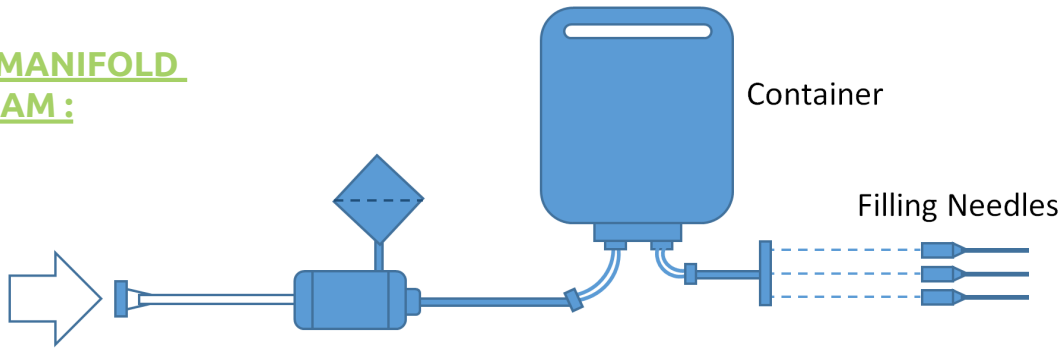
In addition to saving on cleaning and sterilization steps, the use of pre-sterilized and ready-to-use single-use systems offers more flexibility than traditional stainless steel systems.

We are not talking about a complete single-use filling line! The matter consists of the use of tubing and devices - or manifolds - that can be integrated into a «classic» line.

The accurate definition of User Requirement Specifications (URS) with suppliers is the key to successful implementation of this kind of technology.

Single-Use systems are equipped with connectors for aseptic connection and disconnection of manifolds and upstream containers. Special attention must be paid to the study of container/ content interactions. Here again the accuracy of the URS is important.

SINGLE-USE MANIFOLD DIAGRAM :



The use of Clean Rooms, RABS, isolators and Single-Use devices provides constantly improved performance and safety guarantees and represents the majority of current installations. They implement glass packaging items requiring washing, sterilization and depyrogenation upstream of the process. However, there are also technical alternatives, such as «closed vial» or «blow fill seal» technologies, of which the principles are based on the aseptic use of plastic polymers for filling and sealing.

These latter solutions are adapted to large-volume packaging but do not all allow the freeze-drying stage.

LYOPHILIZATION

Lyophilization, or freeze-drying, is a drying process designed to maintain product stability while ensuring optimal rehydration properties when applied to the patient.

This technology is widely used in traditional chemical pharmaceuticals and is well suited to the preservation of biological products.

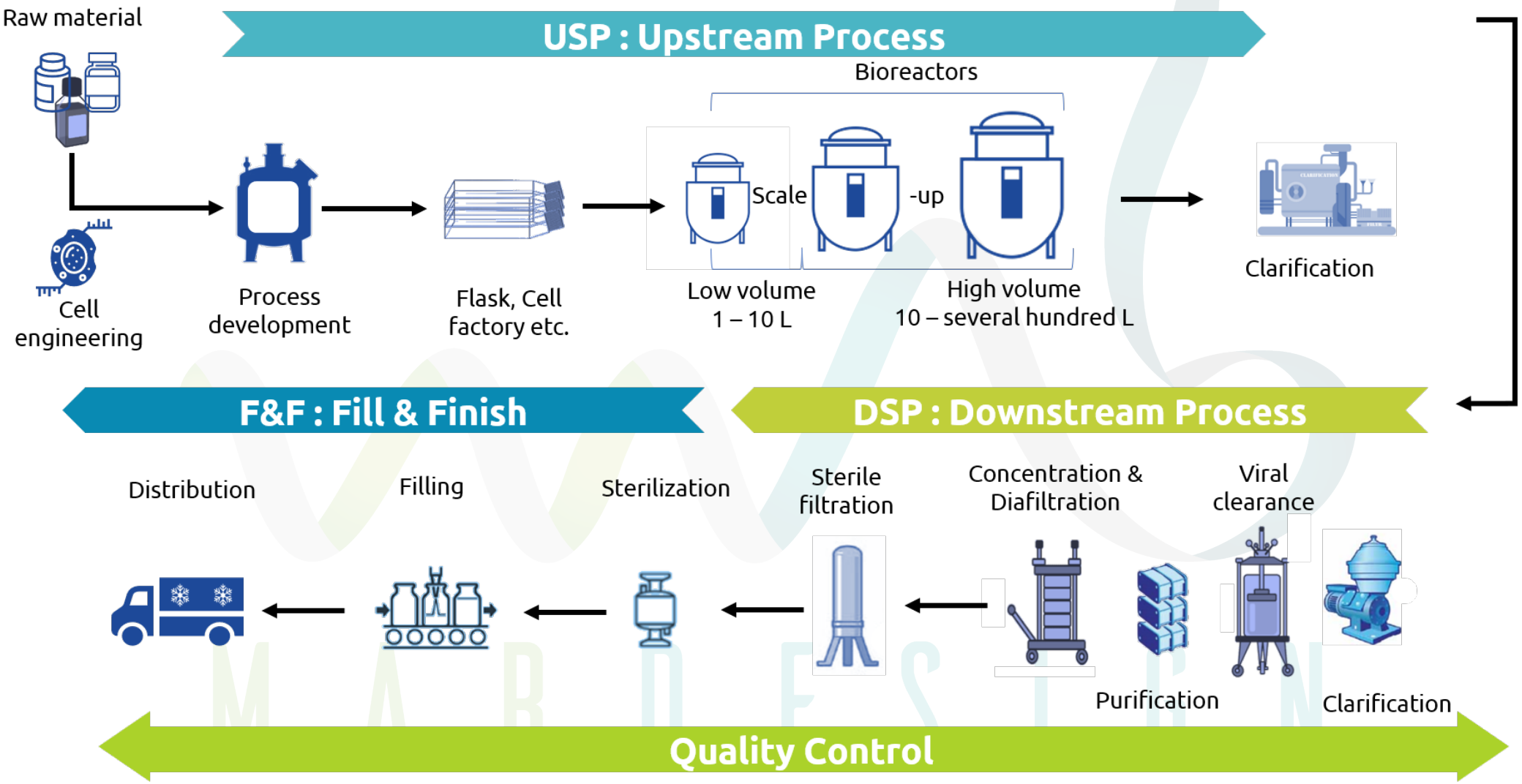
The parameters for lyophilization are not specific and accordingly remain traditional.

Attention must be paid to the choice of buffers to ensure reconstitution without altering – in particular – the secondary and tertiary structures of proteins.

In the race for cost control and competitiveness, pharmaceutical companies have greatly improved the performance and flexibility of the upstream and downstream stages of biological drugs preparation, while reducing dead times and footprints. The same tends to be true today for the Fill & Filling stages, in certain respects, via the different steps presented in this paragraph.

This approach is also fully in line with the logic of batch size reduction, flexibility and response to the issues of personalized medication and patient centric design.

Main stages of bioproduction (by MabDesign)



THE IMMUNOTHERAPY NETWORK

Launching of the BioprocessWatch

We hope that this edition of Immunowatch has served its purpose of being a general introduction to the field of bioprocessing. Our editors-in-chief and our internal editorial team have indeed reflected upon the content and have hand-picked the different contributors to depict the fundamentals, terminology, key expertise and complexity of this field.

As of going to press, there was a total of 529 biopharmaceutical drug candidates being developed by French companies. Importantly, these candidates include therapeutic antibodies, recombinant proteins, vaccines, cellular therapies, gene therapies and advanced therapy medicinal products. With this significant pipeline, France currently ranks third behind the United Kingdom and Germany in Europe as biologic developer. As such, France can no longer be confined solely a land of prophylactic vaccines. Obviously, this explosion of biologics R&D needs to be met with adequate bioprocessing capabilities for both clinical and commercial batches. The French bioprocessing industry will thus have to live up to the challenge of providing for this increasing demand in biologics manufacturing at competitive rates to establishing itself as a key player in Europe and at the global level and at the same reclaim its health sovereignty. This can only be made possible through government support and endorsement, funding schemes, involvement of academia and the private sector, and the development of innovative technologies.

For several years now, our association has been actively participating in national and regional programmes and organising scientific events and gatherings focusing on bioprocessing. In parallel, we have also been providing strategic consultancy services together with various training opportunities to key actors of this field, including academia, public bodies, SMEs and biotech and pharmaceutical companies, that are involved in the shaping of the bioprocessing industry in France through their R&D, innovation, technologies, services and products. In line with these past and current actions and to furthers our commitment and support to the French bioprocessing industry, MabDesign is pleased to announce the launching of its new information-monitoring letter, BioprocessWatch, dedicated entirely to the field of bioprocessing. Each edition of BioprocessWatch will focus on a current challenge, a critical step or a recent innovation linked to the manufacturing of a specific biopharmaceutical or affecting the whole field. BioprocessWatch will feature invited scientific contributions from academia and/or the industry, the most recent pipeline, economic and financial data (where applicable), insights into the intellectual property related to the theme and opinion articles from one or two experts working in the field.

On a final note, we would like to acknowledge the tremendous support from the Centre-Val-de-Loire (CVL) region, through their new Ambition Recherche et Developpement (ARD) CVL Biomédicaments programme, in making the launching of BioprocessWatch possible and for its continued trust in our association's missions and actions.

See you in a few months.
The BioprocessWatch editorial team at Mabdesign

SKILL DEVELOPMENT

In the bioproduction field

A new training alliance between Campus Biotech Digital, schools and training organizations to support skills development of the French bioproduction sector

By MabDesign

The **Campus Biotech Digital** is the winner of the Engineering Action for professional training and innovative support of Programme d'Investissement d'Avenir. The Campus is a new training center which aims to be positioned at the crossroads of biotechnology and digital skills, by offering spaces for the design of innovative educational content focused on industrial needs. It is driven by a leading industrial consortium (bioMérieux, Novasep, Sanofi, Servier), allied with a panel of key players in biotechnology training: EASE, ENSTBB-Bordeaux INP, ESTBB, IFIS, le Groupe IMT, MabDesign and Sup'Biotech. These schools and training organizations will support the Campus in developing the curricula including digital training modules. This alliance materializes the desire of all the players to be united to structure and support the development of skills in biotechnology.

This digitized Campus will offer training courses with innovative and immersive learning to optimize the acquisition of knowledge by learners. New digital tools will be exploited to improve the learning experience and raise the challenge of transforming training to place it in the world of Factory 4.0: big data, digital twins, serious games, immersive reality, virtual reality, augmented reality, and artificial intelligence to support cognitive approaches and promote optimal memory anchor.

At the launch of the Campus, 13 pedagogic curricula have been identified, covering the entire bioproduction chain and the major skills needed for the future industrial processes. The Schools and Training Organizations will support the Campus in the development of its courses by sharing the complementary pedagogic approaches of each actor. The final ambition of this alliance is to support France in becoming a European leader in bioproduction.

Read the Press Releases:

https://www.mabdesign.fr/wp-content/uploads/2021/06/CP_Alliance-nume%CC%81rique_VF.pdf

https://www.mabdesign.fr/wp-content/uploads/2021/03/CP_Alliance_Pedagogique_FINAL.pdf

How the Bio³ Institute supports the regional biopharmaceuticals programme aimed at developing digital learning

By Groupe IMT

The proportion of biopharmaceuticals in the drug market has been increasing over the past twenty years. The region Centre Val de Loire, which is at the cutting edge in the pharmaceutical sector, aims at developing research and structuring this promising field through its Biopharmaceuticals programme, piloted by the University of Tours. Its mission involves several cross-disciplinary actions combining science, innovation and education, with the aim of increasing the "biopharmaceutical" culture and ecosystem of the territory. A training program is conducted to meet the needs of academic and industrial players in the sector with regard to these new challenges. It involves the development of digital tools and the creation of innovative educational resources on relevant themes, such as biopharmaceuticals and biomanufacturing. These new resources will respond to the regional socio-economic challenges and will be part of a larger dynamic consistent with the national context, such as the Grand Défi Biomédicaments carried out by the Conseil National de l'Innovation and the Alliance France-Bioproduction.

The Bio3 Institute, through its position in the national strategy and its expertise in the field of biomanufacturing professions, is working on the development of these new educational resources by mobilising the professional skills of other actors in the programme (LabEx MAbImprove, Polepharma, MabDesign, Universities of Tours and Orleans, CHRU Tours). It has a 2,500 m2 technological platform designed as a mini-biomanufacturing plant that respects flows (materials, products and personnel) and constraints (procedures, controlled atmosphere area). In addition to providing training, the Bio3 Institute supports research teams (start-ups, industrialists, academics) by making industrial equipment available in a GMP-like environment to perform production trials.

Through this program, the Groupe IMT, the Bio3 Institute and the University of Tours are mobilised to create several e-learning training modules that will focus on biotechnologies and the tools of biopharmaceuticals production (molecular engineering, cell banks), technical and regulatory prerequisites related to biomanufacturing, as well as in situ analytical aspects of bioprocesses. In this national context and this territorial movement, the Bio3 Institute is also launching a survey in order to identify more specifically the training needs of today's and tomorrow's biomanufacturing sector. Click on this link to participate: https://forms.office.com/r/CkXYfKYK9w

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Implementing virtual reality as a « learning by doing » pedagogic modality

By ENSTBB

Upstream process (USP) at bioreactor level are classical steps in production of therapeutic proteins. Proficiency of these culture processes is crucial for the product quality. Indeed, several Critical Process Parameters (CPP) can modify Critical Quality Attributes (CQA) of the therapeutic protein, which may tune its potency or, conversely affecting patient safety. Therefore, one can understand the challenges of acquiring robust skills in control of cultures in bioreactor. Depending of the learner profile, skill acquisition is a process that takes place at different paces. Moreover, a type of learning element can more or less be adapted to a given learner. Thus, multiplication of media allows to smartly adapt to learner skills.

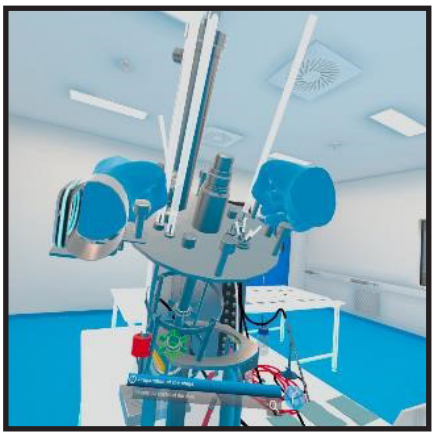
For this purpose, ENSTBB school has developed, with the company OUAT!, learning modules of virtual reality dedicated to microbial and mammalian cell culture bioreactor set up. These modules are adapted to apprehend the bioreactor components (vessel, probes, control unit, etc…), to understand the steps preliminary to culture running (calibration, set up of elements, sterilization, etc…). With the support of Bordeaux INP (through "Pedagogic Innovation" projects), these modules are completed by short demonstration videos to apprehend the bioreactor set up in the whole process.

As bioproduction requirements are increasing in France and learning methods have been disrupted by the pandemic, this type of pedagogic tools can address to the various audience met at ENSTBB:

- Engineering students in initial formation or apprenticeship, who are acquiring their basic skills
- Employees of companies in continuous training

"Learning by doing" (even virtually!)

These digital tools make it possible to enhance the real situation in front of the device. Thus, during the assembly of the bioreactor, the situations have already been visualized during videos and the sequences integrated by the learner in virtual reality. The learning process if usually completed by real protein bioproduction through practical works on stainless steel or in single use bioreactors. Individuals are then more available and attentive to more complex phases such as management of the process.



Bordeaux INP ENSTBB

MAC-BIORÉ, the new bioproduction project that brings together the strengths of Sup'Biotech and IPSA

By Sup'Biotech

Paris, May 18th 2021

Sup'Biotech, a school of biotechnology engineers and IPSA, a school of aeronautics and space engineers, have signed a framework cooperation agreement for a collaboration of expertise in Bioproduction between their laboratories. This is the first time that two engineering schools of the IONIS group plan to work together on a joint research project. This collaboration is now taking shape thanks to a project in Bioproduction, MAC-BIORÉ (Modeling, Analysis and growth Control in BIOREactors), whose objective is to facilitate the management of bioreactors by the design of instrumentation, like hardware and software equipment, capable of ensuring rigorous self-regulation of the process.

Bio-production is the production of molecules of interest from living cells to whom products (sugar, vitamins, etc.) are administered to ensure their growth. Cell culture requires reconstitution of the original conditions of the cell medium. This requires controlling temperature, pressure, humidity, pH and other parameters, hence the use of bioreactors. The bioreactor is the reservoir in which the cells evolve.

There are several applications for cell culture, in 2020, 80% of new drugs came from biotechnology.

For proper conduct of the bio-production process, the culture medium is controlled through the acquisition of measurements. Two types of measurements are made: real-time measurements using probes and offline measurements obtained following a sampling step.

Samplings regularly carried out on the bioreactor make it possible to:

- Ensure the proper functioning of the bioreactor
- Check for possible contamination of the environment by other organisms
- Quantify the production of molecules of interest

These sampling steps are constraining:

- They expose the operator to potentially pathogenic biological agents and chemical risks
- They disrupt the culture (increased risk of contamination, variation in volume)
- They require the presence of an operator

In order to facilitate piloting, it is necessary to design instrumentation capable of ensuring self-regulation of the process, by reducing or eliminating sampling.

Scheduled for the 2021-2025 period, this project will involve 5 teacher-researchers from the group and will allow the two schools to prepare their students for the challenges of the 4.0 factory. Also called the «factory of the future», it is the programmed evolution of production sites through the use of new high-performance technological trends. It relies on intelligent equipment that adapts and learns on its own. An approach logically called to profoundly transform many process industries in the years to come, including biotechnology.

MacBioré requires multidisciplinary expertise, particularly in Automation, Control Theory and Fermentation Engineering. These needs are at the origin of the merger of the Sup'Biotech and IPSA teams.

IPSA will bring its skills in Control Theory, a discipline which studies the possibility of acting on a dynamic system. It is taught from the 3rd year and throughout the engineering cycle. Its applications are very numerous in aeronautics when it comes to stabilizing any system in the face of external disturbances.

Sup'Biotech for its part will share its expertise in Fermentation Engineering, as engineers in Bioproduction are trained every year. That major attracts 34% of students in 2021. Sup'Biotech also opens in September a Bachelor with a bio-production component.

In order to work on these subjects and meet this challenge, a laboratory equipped and shared between the two schools is being built at Sup'Biotech, which can accommodate around ten students and researchers.

The joint research work of the two schools as well as the pooling of the same experimental space will allow the sharing of experiences and the creation of new educational activities. Engineering students will be the first to benefit from this Bioprocess - Systems Control synergy. They will develop transversal skills and consequently be more competitive on the job market. A pedagogical collaboration program piloted by the two research teams and integrating the existing training structure was built. This program will be rolled out from the 2021/2022 academic year and is based on two stages: 1) Initiation of IPSA and Sup'Biotech students to Bioprocesses and Control Theory respectively, then 2) Consolidation by an end-of-year project common for the students of the two schools.

«We are very proud to have started this collaboration and hope that this project can one day be developed on an industrial scale knowing how important control is for bioreactor manufacturers»

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MABDESIGN is a French membership organization in the field of biomedicaments. Created in 2014 MABDESIGN is managed by ABL Europe, Biomérieux, DBV Technologies, Institut Pasteur, Lyonbiopôle, Pierre Fabre, Sanofi, Thermo Fischer, TreeFrog therapeutics and 3 qualified people.

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Operational since September 2015, MABDESIGN has over 200 members, including pharmaceutical and biotechnology companies, service providers, training organizations, and equipment suppliers at the cutting edge of technology.

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