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A preliminary early economic assessment of a novel BM-MSC secretome based therapy for Osteoarthritis patients

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Declaration

I declare that this document is an original work of my own authorship and that it fulfils all the requirements of the Code of Conduct and Good Practices of the Universidade de Lisboa.

Preface

The work presented in this thesis was performed at Stem Cell Engineering Research Group (SCERG) at iBB - Institute for Biosciences and Bioengineering of Instituto Superior Técnico, during the period February-December 2020, under the supervision of Prof. Frederico Ferreira (IST) and Prof. Mónica Oliveira (IST).

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Abstract

Mesenchymal Stromal Cells (MSC) derived secretome has been shown to have immunomodulatory and regenerative properties. Thus, and based on animal studies, MSC-derived secretome could become a potential biotherapeutic for managing Osteoarthritis (OA) of the knee. This work aims to contribute to the research and development of biotherapeutics. For this purpose, an early Health Technology Assessment (eHTA) is established, allowing to consider different cost and effectiveness scenarios, to support stakeholders informed decisions. The cost of goods (CoGs) per dose of biotherapeutic is calculated on the basis of biological, economic and manufacturing process parameters, through the implementation of Monte Carlo simulations. The biotherapeutic's CoGs are used in the Cost Utility Analysis (CUA). CoGs are combined with WOMAC (pain, function, and rigidity) scores, which are then converted to quality adjusted life years (QALY). A Multicriteria Decision Analysis (MCDA) has been initiated to complement the CUA, since the latter does not provide all the relevant information for decision making. The results suggest that for a scenario where the new therapy proves to be twice as effective (0.8 QALY per patient) as the current therapy - Autologous Protein Solution (APS) (0.4 QALY), and demonstrate a 75% reduction in CoGs per patient, it can be said that the biotherapeutic will dominate the existing therapy. This thesis will help to design scientific studies, indicating how to reduce the costs of the new biotherapeutic, and contribute to an improvement in the health care of OA patients.

Keywords: Osteoarthritis, Regenerative medicine, Mesenchymal stromal cells, MSC-derived secretome, Monte Carlo simulations, early Health Technology Assessment, Cost-Utility Analysis

Resumo

O secretoma derivado das Células Estromais Mesenquimais (MSC) demonstrou possuir propriedades de imunomodulação e regeneração. Deste modo, e baseado em estudos de animais, poderá vir a ser um potencial bioterapêutico para gerir a Osteoartrite (OA) do joelho. O presente trabalho visa contribuir para a investigação e desenvolvimento deste bioterapêutico. Para esse efeito, é estabelecida uma Avaliação de Tecnologia de Saúde Precoce (eHTA), que considera diferentes cenários de efetividade e custo. O custo dos bens (CoGs) por dose de bioterapêutico é calculado com base em parâmetros biológicos, económicos e de processo de fabrico, através da implementação de simulações Monte Carlo. Os CoGs do bioterapêutico são utilizados na Análise de Custo-Utilidade (CUA). Estes, são combinados com as pontuações das subescalas WOMAC dor, função, e rigidez, que depois são convertidos em anos de vida ajustados à qualidade (QALY). Uma Análise de Decisão Multicritério (MCDA) foi inicializada para complementar a CUA, uma vez que a última não fornece toda a informação relevante para uma tomada de decisão. Os resultados sugerem que para um cenário em que a nova terapia revelar ser duas vezes mais efetiva (0,8 QALY por paciente) do que a terapia atual - Solução de Proteína Autóloga (APS) (0,4 QALY), e demonstrar uma redução no custo de fabrico por paciente em 75%, poderá dizer-se que o bioterapêutico dominará a terapia existente. Este estudo ajudará a desenhar estudos científicos, indicando como reduzir os custos do novo bioterapêutico e contribuindo para uma melhoria nos cuidados de saúde de pacientes com OA.

Keywords: Osteoartrite, Medicina Regenerativa, Células estaminais mesenquimais, Secretoma, Simulação Monte Carlo, Avaliação preliminar de uma Tecnologia de Saúde, Análise custo-utilidade

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List of Abbreviations

ACI Autologous Chondrocyte Implantation.

APS Autologous Protein Solution.

AT Adipose Tissue.

ATMP Advanced Therapy Medicinal Product.

BM Bone Marrow.

CBA Cost-benefit analysis.

CEA Cost-effectiveness analysis.

CM Conditioned Medium.

CMA Cost-minimization analysis.

CoG Cost of Good.

CUA Cost-utility analysis.

DALY Disability-Adjusted Life-Year.

DSP Downstream Processing.

ECM Extracellular Matrix.

eHTA early Health Technology Assessment.

EMA European Medicines Agency.

ET Expansion Technology.

EU European Union.

EV Extracellular Vesicles.

FBS Fetal Bovine Serum.

FDA Food Drug Administration.

GAG Glycosaminoglycan.

GMP Good Manufacturing Practice.

HA Hyaluronic Acid.

HS Health Systems.

HT Health Technology.

HTA Health Technology Assessment.

IA Intraarticular.

IC Incremental Cost.

ICER Incremental Cost–Effectiveness Ratio.

KOOS Knee injury and Osteoarthritis Outcome.

LY Life-Year.

MCDA Multi-criteria Decision Analysis.

MSC Mesenchymal Stromal Cells.

NE North East.

NHS National Health Service.

NSAID Non-Steroidal Anti-Inflammatory Drug.

NW North West.

OA Osteoarthritis.

OAT Osteochondral Autograft Transplant.

OCA Osteochondral Allograft.

QALY Quality-Adjusted Life-Year.

SE South East.

SF-36 Short Form-36.

SW South West.

TESSEE Tool for Early Stem Cells Economic Evaluation.

UC Umbilical Cord.

UK United Kingdom.

US United States.

VAS Visual Analogue Scale.

WHO World Health Organisation.

WOMAC Western Ontario and McMaster Universities Osteoarthritis Index.

WTP Willingness To Pay.

Chapter 1

Introduction

In this section, an introduction to the present study is provided, defining the motivation, overview, objectives and outline of this thesis.

1.1 Motivation

The technological progress achieved by society in the last century resulted in many significant improvements in Health Systems (HS) around the globe. ”Innovation in medical technologies is critical to improvements in patient care, but it is also costly and uncertain” [3]. As such, the development of innovative biotherapeutics and novel cell therapies often reveal themselves as an alternative to conventional treatments of several diseases with the possibility of being tailored according to each individual health care needs. In the long term, they might become golden-standard approaches, getting us a tiny step closer to a cure.

Acknowledging the uniqueness of each individual, one of the main objectives of personalised medicine is answer to a particular disease according with each individual self, aiming to obtain a regenerative response as treatment [4]. Regenerative medicine is a multidisciplinary field which deals with engineering and medical science, endorsing the regeneration of diseased and injured tissues or even whole organs. It reveals as a promising alternative when the body does not respond to conventional methods, and the donated tissues or organs do not meet the transplantation demands of aged and diseased patients [4, 5].

As average life expectancy increases, the population is getting elderly, which is a driver to increase age-related diseases prevalence, such as knee Osteoarthritis (OA). This is not exclusively related to aging. It is also associated with a variety of risk factors such as obesity, gender, lack of exercise, genetic predisposition, bone density and injuries that have occurred [6]. According to World Health Organisation (WHO) [6], it is predicted that by 2050, there will be 2 billion people aged over 60 years old, in other words, more than 20% of the world’s population. Consequently, the global incidence of OA will increase. Of this 20%, it is estimated that three quarters will have OA symptoms and one third of those will be strongly incapacitated. These figures show that 130 million people worldwide will be affected by OA,

and almost a third of these, around 40 million will be severely crippled [6]. In addition, there is a higher prevalence among women than men. Globally, the ratio of people who develop OA symptoms are 10% male and 18% female, considering a sample older than 60 years old [6]. Nevertheless, the concern does not account only for OA patients themselves but also for the significant associated economic burden. In accordance to O'Brien et al. [7] in 2015, the annual costs were 23 billion EUR in the United Kingdom (UK), 5.3 billion EUR in Australia, and around 120 billion EUR in the United States (US). The ambulatory and inpatient care (including surgery), as well as work productivity losses, are considered to be the main cost sources [6].

Non-invasive treatments for knee OA have been proposed to avoid or delay the need of total joint replacement, which is the final procedure performed in these cases [6]. Despite those treatments having shown significant improvements, with varying success rates, they are strictly limited to repairing focal defects and they lack supporting evidence of reverting the disease [6]. In the OA course, a multitude of challenges are recognised [6], which in turn open up opportunities for stem cell-based therapies to assist in its treatment, since it is needed a more global approach towards managing this pathology.

This thesis evaluates the possible use of a Mesenchymal Stromal Cells (MSC) secretome based therapy for knee OA [8]. This suggestion is based on the collection of the following pre-clinical evidences: (i) After seven days, there was a reduction of pain and cartilage repair improvement for MSC secretome compared to the control [9]. The injection of MSC secretome leads not only to early pain mitigation but also to a supportive effect on the formation of cartilage in a murine OA model [9]. By employing the MSC secreted biofactors which contain regenerative properties, it will be possible to enhance the patterning and affordability of therapy and adjust it to clinical practice [9]. (ii) By twelve weeks, defects treated with exosomes have demonstrated an improved gross appearance and improved the histological score in comparison to the control group, as well as full repair of hyaline cartilage and subchondral bone with good surface regularity, entire connection to the adjacent cartilage, and Extracellular Matrix (ECM) deposition in a rat model [10].

The inflammatory environment of joints makes challenging to prevent the progression of the disease before cartilage loss. In the current study is envisaged a secretome-based therapy obtained from allogenic human MSC. This assumption is related with the following observations. Firstly, in diseased and ageing patients groups, in particular for inflammatory diseases, autologous MSC may be compromise and interfere with the therapy, meaning that patients own cells might not be the ones that best fit the therapy purpose [11]. Moreover, associated donor site morbidity imposes additional risks to the patient. Secondly, it is suggested the formulation of a cell free product. Here, it is explored the potential regulation of such inflammatory process using cell-free Intraarticular (IA) injections of factors present on the MSC driven secretome, resulting on a positive therapeutic outcome, i.e. stopping cartilage degeneration and potentiating its regeneration [11]. Finally, the search for a new modifying therapy for OA, exploring the therapeutic potential of the molecules presented on the MSC-derived secretome, offers further opportunities to identify novel biotherapeutics and drugs for OA therapies [11].

The process of financing medicines by the Government requires a detailed pharmaco-therapeutic and pharmaco-economic evaluation in order to ensure rationality in the co-funding and acquisition of

Health Technology (HT)s [3, 12]. New therapeutic approaches have higher uncertainty associated with the manufacturing process and production costs, requiring early Health Technology Assessment (eHTA) [3, 12]. eHTA use will contribute to provide insights on the possible cost-effectiveness relations for OA treatment. This information will allow better health management and healthcare system, which provides a more efficient use of limited resources, in order to maximise healthcare benefits at a reduced cost, and support decision on the value of implementing novel OA therapies [13]. Moreover, to establish the economic advantage, it is essential to compare the health gains arising from the novel MSC-derived secretome therapy with the additional costs that it can bring to the National Health Service (NHS) [3, 14]. Considering significant economic burden and increase on prevalence of OA, this thesis aims to establish a preliminary model to assess the potential use of a MSC-derived secretome therapy for OA management.

1.2 Topic Overview

The novel OA therapy discussed in this work is designated MSC-derived secretome. This name was chosen in order to provide a more general descriptive term, including as therapeutic agents, extracellular vesicles (cornerstones of this work), but also additional proteins (potentially present outside the extracellular vesicles), that have therapeutic potential, and are carried out through the bioprocess together with the extracellular vesicles to be included on the final medicine formulation.

Human Bone Marrow (BM) MSC-derived secretome, when formulate as a medicine, can be classified as an Advanced Therapy Medicinal Product (ATMP). ATMPs are based on biotherapeutics, cells, genes, or tissues [15]. MSC are able to regulate other cells behaviours through the extracellular biomolecules produced by them. Those molecules, when taken together, are called cell secretome [16]. Some of such molecules are released by the MSC inside vesicles called exosomes [16]. Therefore, the secretome is a substance that comprises a cocktail of proteins, including cytokines, growth factors and many others, and these are expressed by an organism and secreted into the extracellular space which comprises about 13 to 20% of all human proteins [16]. The two therapies compared throughout this thesis are MSC-derived secretome against Autologous Protein Solution (APS) - one of the advanced therapies for OA currently in use.

In order to study the potential value of using a new MSC-derived secretome therapy, an eHTA is performed. This evaluation is made from a societal perspective since it is the one that manages to capture the greatest number of points of view, for optimal allocation of resources maximising total society welfare [17]. Before a new therapy, such as the MSC-derived secretome therapy can be implemented, the decision-making bodies in healthcare need to guarantee that it is reliable, effective and cost-effective [18]. Regarding the later aspect, the sooner the eHTA is carried out (e.g. during the research process of the MSC-derived secretome), the quicker it is possible to rationalise investment in the development of the same therapy and decide on target therapeutic endpoints [18]. In this way, additional costs can be avoided, as it is already known in advance that a this therapy will not add sufficient value to society [3], maximising the return on investment and societal impact of the MSC-derived secretome therapy. The

assessment is also made to understand what clinical endpoints need to be reached to justify the use of such therapy.

In eHTA framework, a Cost-utility analysis (CUA) was initially performed. The price of a medicine is an important parameter when performing a CUA. Therefore, this thesis provides a preliminary estimation of the MSC-derived secretome manufacturing costs, more precisely, the estimated cost per dose of the new ATMP, implementing a stochastic simulations model to incorporate biological variability. Those manufacturing costs are then compared to the costs of the comparator therapy - APS, adapted from literature [19]. As there are no clinical studies for MSC-derived secretome therapy (evidence in humans), conclusions are achieved from various assumptions and comparisons with studies for APS therapy. Therefore, the conclusions hereby drawn are only valid in the context of the scenario that corresponds to the set of assumptions taken. When elaborating this preliminary eHTA, several assumptions are made: (i) One of the working initial assumptions taken is that the new ATMP and APS result on similar clinical outputs. However, APS is an emerging therapy that already has been applied in humans [19] and MSC secretome therapy does not. APS recurs to a patient's peripheral blood that contains high concentrations of anti-inflammatory and anabolic proteins and its application to treat OA results on positive outcomes concerning the patients' health status [19]. Nevertheless, bioactive factors secreted by MSC are not necessarily the same that the ones present in APS [16]. Still different studies point out the presence, for instance, of IL-10 and TGF-β, both on MSC secretome and APS, which have an anti-inflammatory therapeutic potential [16, 20, 21]. (ii) In addition, regarding the ATMP, animal model experiments results [9, 10] support the envisaged therapy, i.e. the outcomes of these studies can inform the role that therapy may play in individuals. (iii) Another aspect is that it is assumed that MSC immunomodulatory and hypoimmunogenic features allows the therapy in question to be allogeneic [22]. Therefore, it is hypothesised that the use of a MSC-derived secretome has higher probability of success when not to taken from the donor himself (OA individuals), but on a healthy donor with healthy MSC. Taken into account manufacturing costs and potential therapeutic impact, this thesis also informs about the potential therapeutic benefits in the form of Quality-Adjusted Life-Year (QALY), considering specific scenarios. CUA is a conventional approach used in some countries, still it is prone to much criticism. Because quantifying benefits of the therapy through QALY only takes into account health outcomes [3], regardless of the inherent social value of the MSC-derived secretome adoption process. It is considered by many that QALY metrics are unable to capture how patients, doctors or the general society perceive the value of a new ATMP [3]. So, it is essential to go further and explore a number of aspects that cannot be captured in CUA. Those include costs beyond manufacturing, benefits beyond patient health and risks, i.e. patient lost wages, treatment acquisition costs for patients, ease of administration for users, etc. Therefore, in this thesis the CUA is complemented with Multi-criteria Decision Analysis (MCDA) in order to support the decision to adopt or not the MSC-derived secretome therapy, which is still on pre-clinical trials. From this point on, one will conduct a preliminary economic evaluation of the new ATMP. This will be possible by making use of CUA, as well as MCDA model. Having the just described remarks, the stakeholders will have enough information to decide and take an informed and supported decision of whether or not to adopt the new ATMP. Summing up, this thesis is intended to contribute to

this decision-making process.

1.3 Objectives

The main goal of this thesis is to provide content suitable to answer the question of whether the new therapy - MSC-derived secretome should be adopted to knee OA management. In order to do so, an eHTA is carried out from a societal perspective to provide insights to those who might possibly adopt the new therapy - HS. The research developed in this thesis is highly relevant to inform whether the MSC-derived secretome product in the production conditions has the potential to be reimbursed or financed. In order to do so, the following objectives are required to be fulfilled:

- Establish a framework to estimate manufacturing costs of a dose of MSC secretome, which was investigated through a Monte Carlo simulations model;
- Estimate potential benefits of the MSC secretome, assuming different effectiveness levels of this therapy.

The research strategy consists of performing an eHTA to evaluate cost-effectiveness of the new treatment in comparison with APS therapy (the comparator). On a first approach, the level of effectiveness of the two therapies is assumed to be equal and cost-effectiveness is compared on the basis of cost. In other words, it is studied if the new therapy is more expensive than the comparator. Nonetheless, other scenarios are explored where the new therapy could present more encouraging clinical results. Therefore, to be worthwhile to pursue the ATMP treatment at higher costs than the ones of APS.

1.4 Thesis Outline

This thesis is organised in a total of six chapters which include Introduction, State-of-the-art, Methodology, Results, Discussion and Conclusion organised as follows:

- **Chapter 1** the current chapter introduces motivation, topic overview, objectives and thesis structuring.
- **Chapter 2** provides a State-of-the-Art which includes biological and economic context of OA; information on the therapeutic approach suggested and the comparator used - MSC-derived secretome and APS, respectively; topics related with stem cells, bio-processing, cells and extracellular vesicles manufacturing issues; and theoretical background on cost-effectiveness analyses.
- **Chapter 3** presents the methodology used on this thesis, namely set-up of the Monte Carlo model, the respective assumptions taken, description of the biological process for exosome production and economic processing models used. The most relevant equations used in the model simulations are also presented in this section, as well as information on the set-up of a sensitivity analysis, which was carried out in order to study the effect of selected inputs of the stochastic model and outputs forecasted.

- **Chapter 4** presents the results obtained, which include manufacturing costs of the MSC-derived secretome therapy, obtained through a stochastic simulation model, and the respective sensitivity analysis. This section presents the CUA which was applied to several levels of screening, and briefly discusses the results obtained in the light of the information previous reported in the literature. The MCDA was formulated to complement the results of previous analyses and it is also discussed on this chapter.
- **Chapter 5** provides a general discussion of the results in the light of previous reported studies and further discusses the assumptions taken and the model limitations.
- **Chapter 6** concludes the present study and presents opportunities for future work within this field of research.

Chapter 2

State of the Art

This chapter presents several concepts and results previously reported to support the current studies. Therefore the topics covered on this chapter include information on Cartilage, the tissue under study in the present work; OA and conventional treatments currently used; MSC and their secretome; evidence of BM-MSC-derived secretome therapy for OA; APS, an emergent OA therapy, used in this study as comparator; the manufacture process and its economic analysis; identified challenges for entry of new health technologies in the market; and methodologies on eHTA.

2.1 Cartilage

Cartilage is a connective tissue, which exhibits a reduced intrinsic capacity of self-repair, making challenging the treatment of damaged cartilage [23]. Cartilage is a tissue deprived of blood vessels, nerves and lymphatics. Cartilage is comprised of a rich ECM and of highly specialized cells, named chondrocytes, whose main function is to maintain a healthy cartilage. Those cells are sparsely distributed and are embedded within the ECM [23, 24]. The ECM is mainly composed of collagen fibres as type II collagen, a protein that provides structural support, proteoglycans and other constituents, present on lower amounts, such as non-collagenous proteins and glycoproteins that provide the tissue with sufficient mechanical properties for function *in vivo* [23, 24]. The presence of water is also crucial for the maintenance of this tissue, which accounts up to 80% of its wet weight [23, 24]. All these constituents provide elasticity to the cartilage as well as high tensile strength, which assist the weight-bearing joints to balance the weight in such a way that the underlying bone is capable of absorbing shock and weight [23, 24]. Some examples of these joints are the knees, hips, and lower lumbar spine. Cartilage can be divided in three types according to the relative amounts of its principal components: collagen fibres, ground substance (proteoglycans) and elastic fibres [23, 24]. Elastic cartilage provides strength and elasticity to organs and body structures[24]. Fibrocartilage has thick layers of strong collagen fibres and it is found in special pads (vital to reduce friction in joints) known as menisci and in the disks between spinal bones. Hyaline, also known as articular cartilage as it is found on the articulations, has many collagen fibres that provide strength to the tissue [24, 25, 26]. The articular cartilage is 2 to 4 mm thick

and it protects the joint surfaces of long bones in synovial joints [24, 25, 26]. This type of cartilage is a smooth and translucent tissue that acts as a cushion to absorb shock, enabling the movement between bones by providing a lubricated surface that reduces friction and facilitates the transmission of loads [24, 25, 26]. The collagen hereby present is mostly type II, and it is related to the tensile resistance of cartilage. Concerning the components of this cartilage 10% to 20% of the total mass is collagen, 5% to 10% are Glycosaminoglycan (GAG), the side chains on the proteoglycan core protein. GAGs are highly sulphated compounds with a global negative charge. They are able to hydrogen bonds with water, resulting in a highly hydrated mature tissue that can resist compressive loads [24, 25, 26]. When under mechanical load, proteoglycans endorse redistribution of water in cartilage, leading to an increase in osmotic pressure with water flow, which is fundamental to protect bones from loading [24, 25, 26]. Additionally, articular cartilage is divided into four major zones (superficial, middle, deep and calcified zone), which present chondrocytes with different shapes and ECMs with different composition and orientation of collagen according their distance related to articulating surface and the subchondral bone [24, 25, 26]. Besides these layers, the articular cartilage is separated by a tidemark from the subchondral bone [24]. The Superficial zone has a considerable quantity of flattened chondrocytes, sparse proteoglycans, and shortened collagen fibres, which are parallel aligned to the articulating surface in the joint [24, 25, 26]. This zone is the thinnest layer and makes up to 10-20% of the total cartilage thickness being responsible for enduring most of the tensile and shear stresses, protecting the deeper layers [24, 25, 26]. The Intermediate zone (middle) provides an anatomical bridge between the superficial and the remaining zones and provides bumper functions [26]. Here, chondrocytes content is lower and they present a spherical shape and are oriented in perpendicular or vertical columns parallel to the thicker collagen fibrils. This zone corresponds to 40-60% of the total cartilage thickness and, due to the larger osmotic water swelling, is more resistant to compressive forces [24, 25, 26]. Immediately below the intermediate zone is the Deep zone, where the chondrocytes are spherical. The largest diameter collagen fibres are perpendicular to the articular surface and radially oriented. This zone corresponds to around 30% of articular cartilage volume [24, 25, 26], contains the highest proteoglycans' amount and the lowest water content and, consequently, provides the greatest resistance to compressive forces. Finally, the tidemark that distinguishes uncalcified articular cartilage from the calcified tissue is a zone that participates in the process of endochondral ossification during longitudinal bone growth [26]. The calcified zone, with the role of attaching cartilage to bone, has a transitional structure with a very low number of cells, mineralized ECM and mostly type X collagen instead of type II [24, 26].

The Superficial zone has a considerable quantity of flattened chondrocytes, sparse proteoglycans, and shortened collagen fibres which are parallel aligned to the articulating surface in the joint [24, 25, 26]. It is the thinnest layer and makes up to 10-20% of the total cartilage thickness being responsible for enduring most of the tensile and shear stresses, protecting the deeper layers [24, 25, 26]. Going deeper, there is the Intermediate zone (middle) offering function and an anatomical bridge between the superficial and the remaining zones [26]. Here, the low content of chondrocytes appears closely spherical oriented in perpendicular or vertical columns parallel to the thicker collagen fibrils. This zone corresponds to 40-60% of the total cartilage thickness and due to the larger osmotic water swelling, it is more

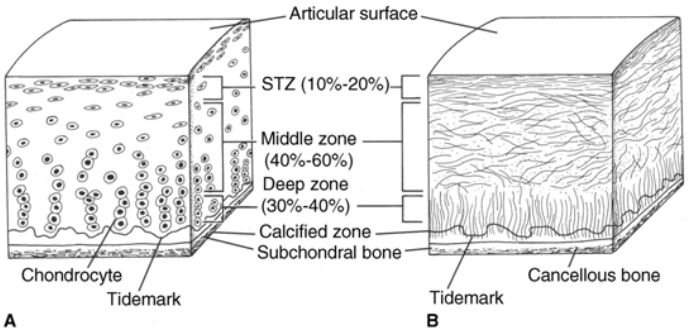

Figure 2.1: Schematic, cross-sectional diagram of healthy articular cartilage: A - cellular organization in the zones of articular cartilage; B - collagen fibre architecture. Adapted from [26].

resistant to compressive forces [24, 25, 26]. Immediately after to the intermediate zone is the Deep zone, where the chondrocytes are spherical. The largest diameter collagen fibres are perpendicular to the articular surface and radially oriented. This zone corresponds to around 30% of articular cartilage volume [24, 25, 26]. It contains the highest proteoglycans' amount and the lowest water content and, consequently, the greatest resistance to compressive forces. Also, the tidemark distinguishes uncalcified articular cartilage from the deeper calcified tissue that participated in the process of endochondral ossification during longitudinal bone growth [26]. Thus, the calcified zone has the role of attaching cartilage to bone. It has a transitional structure, a very low number of cells, mineralized ECM and mostly type X collagen instead of type II [24, 26].

2.1.1 Cartilage Lesions - Osteoarthritis (OA)

The word Osteoarthritis is composed of osteo-, -arth- and -itis referring, respectively, to bone, arthron (meaning joint), and inflammation. Therefore, OA is a disease of inflammation of bone and joint cartilage. At one time it was actually thought that inflammation was actually not the cause for OA development, and that it was mainly a degenerative disease resulting from simple wear and tear [24, 6]. However, the more recent data point out that in fact inflammation does play an important role in OA's evolution [24, 6]. In the articular join of a healthy individual, the two constituent bones slide easily, without friction, due to the layers of articular cartilage in each bone. Synovium is another important component of the synovial joints which is associated with articular cartilage, and together with the surface of the joint cartilage form the inner lining of the joint space [6, 24]. Furthermore, the synovium is not only composed of blood vessels, lymphatic vessels and loose connective tissue, but on its surface it has type A cells responsible for cleaning cellular debris and type B cells which help to lubricate both joint surfaces by producing synovial fluid components [6, 24, 27], which participate on the inflammation response.

In an OA patient, the most prevalent progressive joint disease, there is no longer much articular cartilage left separating the two bones, increasing significantly the friction degree between them, which then leads to inflammation [6, 24]. This fact will in turn sparks pain through the nerve endings in this joint space. OA disease affects the entire joint, which includes cartilage, subchondral bone, synovium, tendons and muscles [6, 24]. This clinical condition is caused by articular cartilage degeneration, synovitis

(inflammation of the synovial membrane), subchondral bone sclerosis and formation of the osteophytes. In OA situation, the particular joint referred to is the synovial joint [6, 24]. Regardless of the initial cause of joint cartilage damage, chondrocytes will initiate attempts to repair the cartilage [6, 24, 27]. They begin to produce less proteoglycans and more type II collagen at first, but then they produce a different type of collagen, type I collagen [6, 24, 27]. Unfortunately, this collagen type does not interplay with proteoglycans in the same manner as type II which in turn will decrease the elasticity of the cartilage matrix, allowing its decomposition [6, 24, 27]. However, over the years, the articulations of a patient suffering from OA become depleted in chondrocytes, which eventually follow programmed cell death [27]. In this way, the cartilage becomes softer, weaker, continues to lose elasticity, and starts to disintegrate in the synovial space [27].

As type A cells in the synovium attempt to remove the debris, immune cells like lymphocytes and macrophages are recruited into the synovial membrane, which produces pro-inflammatory cytokines that ultimately cause inflammation of the synovium as well, called synovitis [6, 24, 27]. Also, fibrillations are formed, essentially these crevices in what used to be a smooth joint surface. The cartilage continues to erode until the bone is exposed, allowing it to rub with the other bone, which causes the bone eburnation, making it resemble polished ivory [6, 24, 27]. Finally, at the extremities, the bone grows outwards, called osteophytes, which makes the joints appear wider, something that is more obvious when seen in the distal and proximal interphalangeal joints, or in the finger joints [6, 24, 27]. Consequently, OA induces joint pain, stiffness and loss of function (especially in the knees, hips, hands and other weight-bearing joints) [6, 24]. The cartilage modification, under an OA clinical condition, makes it more vulnerable to degradation. Changes on the ECM are characterized by the accumulation of advanced glycation end-products, reduced aggrecan size, reduced hydration, and increased collagen cleavage [27]. Those ECM changes endorse the decrease of cell density in the meniscus and ligaments, which will lead to joint infection [27]. The function of subchondral bone is impaired due to not only reduced osteocytes' amount, but also the different mineral composition. Mitochondrial dysfunction promotes catabolic processes and cell death over anabolic processes, since oxidative stress and reduced autophagy in chondrocytes varies their function [27]. The cartilage's fibrillation surface occurs in focal areas and it can be associated with a complete loss of staining for GAGs [27]. The density of chondrocytes in cartilage decreases with age. However, chondrocyte 'clusters' emerge during the development of OA near sites of damaged tissue and might indicate attempted repair or altered cellular signals [27]. Furthermore, OA chondrocytes become highly active with increases in both anabolic processes, i.e., matrix synthesis, and catabolic pathways, i.e., those induced by inflammatory cytokines [27].

Age seems to be the biggest risk element for OA, and cartilage often degrades over longer periods of time, which makes it really difficult to identify a single factor [27]. Inflammation also seems to be involved, and there are a number of proinflammatory cytokines like IL-1, IL-6, and TNF, among others, that seem to play an important role in the course of the disease [6, 24]. Some of these are more involved in breaking down cartilage through proteolysis, meaning increased catabolism, whereas others are more involved in blocking the formation of new cartilage (meaning decreased anabolism) [6, 24]. Also, joint injury, which brings with it a lot of inflammation, seems to be a major risk factor for OA, as well as

mechanical stress and obesity. Other risks factors include neurological disorders, genetic factors, and even certain medications, suggesting there are other mechanisms at play as well [6, 24].

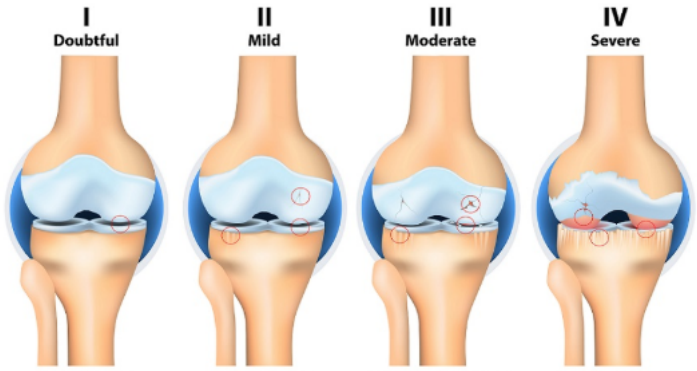

Figure 2.2: Schematic representation of knee OA stages: I - Minimum disruption, approximately 10% of cartilage loss; II - Joint-space narrowing, cartilage starts breaking down and there is the occurrence of osteophytes; III - Gaps in the cartilage can expand until they reach the bone; IV - Joint-space greatly reduced, 60% of the cartilage is already lost and there is a large osteophytes number. Adapted from [28].

2.1.2 Conventional Approaches to OA treatment

Several therapeutical strategies are being used to manage OA development, involving various interventions in the field of cartilage therapy [24]. Patients with OA are constantly dealing with persistent pain, rigidity and motion disability [6, 24]. Therefore, OA management includes as traditional treatments: physiotherapy, medication and lifestyle changes [24, 6]. At an early stage, OA patients are receive administration of Non-Steroidal Anti-Inflammatory Drug (NSAID)s, Hyaluronic Acid (HA) injections, simple analgesics and corticosteroid injections that will promote symptom relief rather than treat or reverse the OA process [6, 24]. Surgical procedures are recommended when considering a later stage of the disease and for larger cartilage defects. Cartilage can be treated through repair or regeneration [24, 6].

BM stimulation methods, that can contribute to cartilage healing, are Microfracture, Drilling and Abrasion Arthroplasty [29]. All three processes of BM stimulation are minimally invasive surgeries that share the same principles, breaching the subchondral bone by promoting its bleeding in order to create a blood clot at the lesion site and allowing pluripotent stem cells from the marrow to remodel the fibrin clot in the defect into fibrocartilage [29]. It has been shown excellent short-term clinical outcomes after marrow stimulation however, studies were also showed inconclusive durability and treatment failure beyond 5 years [29]. In addition to the methods mentioned above, cartilage repair regenerative and repair approaches includes Autologous Chondrocyte Implantation (ACI) and their advances Autologous Chondrocyte Implants Associated with the Matrix (MACI) and Autologous Matrix Chondrogenesis (AMIC), Osteochondral Autograft Transplant (OAT) and Osteochondral Allograft (OCA) which can be characterized as regeneration methods [29].

The ACI is performed in two phases, combining surgical treatment with cell cultured in-vitro and their implantation in the defect [29]. First, a section of cartilage is removed from a non-weight-bearing area of the affected joint and then transferred to a sterile nutrient solution. In cell culture, chondrocytes

are isolated from the cartilage tissue by enzymatic digestion with collagenase. The chondrocytes are then expanded in single-layer culture and cultivated for 3 to 5 weeks in order to amplify their amount [29]. Through a second surgical procedure - arthrotomy - the chondrocytes are injected into the defect. To protect the remaining chondrocytes on the implanted side and to prevent the mass from floating, a periosteal flap is still sewn over the defect and sealed with fibrin glue [29]. It is well documented that only the periosteal flap can have chondrogenic abilities and induce cartilage regeneration. After 11 years or more, follow-up studies show that 92% of patients were satisfied, with sustained improvement in clinical outcomes, showing that this technique results on long-term durable results [29]. Despite those promising results, there are still constraints such as the biological response of periosteal flap; the complexity and cost of two surgical procedures and the subsequent cartilage healing capacity loss due to dedifferentiation of the in vitro isolated and expanded chondrocytes [29].

OAT process consists in excising a piece of healthy cartilage from a less-demanding area of the body to cover a cartilage defect in a more important and affected area. So, before drilling the recipient site, it is important to proceed with donor harvesting [24, 30]. The site from where donor graft comes from should have ease of access and minimal morbidity associated with the harvest of the osteochondral plug(s) i.e. areas of lower contact pressure as femur (superolateral or the superomedial femoral trochlea) [24, 30]. The fact that defects could be filled immediately with mature, articular cartilage and that both chondral and osteochondral defects could be treated similarly, is seen as the major advantage of this technique [24, 30]. OCA uses harvested tissue as osteochondral plugs taken from a deceased donor in order to cover only the injured area [29]. The principle is nearly the same as OAT, however there is no restriction on the size or number of plugs that can be harvested from the donor bone, both of which are limited in OAT [24, 30]. The treatments described above are not disease modifiers and may not always be long-term effective [6]. They are quite expensive and invasive, and often do not meet patients' expectations [6]. In this way, regenerative medicine using stromal cells or derivative products of them could address several of these problems.

2.2 Mesenchymal Stromal Cells

MSC are multipotent adult stromal cells. As such, they are promising stem cells' candidates, being perceived as an attractive cell source to be used in repairing and rebuilding damaged or diseased connective tissues [24, 31]. This can be illustrated by the fact that MSC can migrate to the site of injury due to their capacity to suppress the inflammatory response to injuries and to promote wound repair and healing [24, 31]. Furthermore, they exhibit immunomodulatory and hypoimmunogenic features. MSC are hypoimmunogenic since there is barely any response in the presence of externally supplemented co-stimulatory molecules. Moreover, they are immunomodulators in the manner that they provide maintenance of peripheral tolerance, transplantation tolerance, autoimmunity, etc [24, 31]. They modulate several effector functions by releasing immunomodulatory factors that allow them to escape rejection mechanisms, enabling their use in allogeneic settings [22, 31]. MSC also demonstrate the ability to set-up a regenerative micro-environment being anti-apoptotic, anti-fibrotic, anti-inflammatory,

pro-angiogenic, which contributes to the vast amount of clinical trials based on their use [31].

MSC can be extracted from BM, muscle, Adipose Tissue (AT), synovium, and many other connective tissues. Therefore, they can be expanded in culture while maintaining their multipotency – Osteogenic, Chondrogenic, and Adipogenic cell lineages, due to their culture-dish adherence and high proliferative capacity *in vitro* [24, 32]. Nevertheless, the sustainability use of MSC, following the initial differentiation-based rationale, has been questioned by some researchers, since some studies report that MSC engraftment and subsequent differentiation into appropriate cell types can be sporadic [24, 32]. Adding to this, one must still face logistical and operational challenges associated with proper handling and cell storage to maintain the vitality and viability of the cells for transplantation [24, 32]. All those aspects point out that MSC not only show differentiation potential into multiple lineages, but also exert their therapeutic effects through the secretion of factors that decrease cellular injury and improve repair [16]. Consequently, MSC secrete a broad spectrum of trophic factors including growth factors, chemokines and cytokines, which facilitate the paracrine activity of these cells in tissue regeneration [16].

2.2.1 Existing therapies

Quite different production costs for MSC-based therapies were reported, according with the production technology and the target product type and quality [33]. For example, the cost of production of a MSC dose (70 million cells per dose), obtained through a total of five passages vary between 9,000 to 17,000 EUR according with the expansion method used (microcarriers in bioreactor vs planar technology) [33].

Table 2.1 summarizes examples of different products that are or have been submitted to approval or commercially available. The price of those cell therapies ranges from 400 to 720,000 EUR per dose [33].

Table 2.1: Examples of currently available human MSC-based medicinal products, both for allogeneic and autologous therapies related with bone and cartilage diseases.

Product	Company	Cell source	Therapy	Condition	Country
Allostem	AlloSource	ASC	Allogeneic	Bone Regeneration	USA
Cartistem	Medipost	UCB-MSC	Allogeneic	Osteoarthritis	South Korea
OsteoCel	NuVasive	BM-MSC	Allogeneic	Spinal cord Regeneration	USA
OvationOS	Osiris Therapeutics	BM-MSC	Allogeneic	Bone Regeneration	USA
Trinity Evolution	Orthofix	BM-MSC	Allogeneic	Bone Regeneration	USA
Trinity Elite	Orthofix	BM-MSC	Allogeneic	Bone Regeneration	USA
Ossron	RMS	BM-MSC	Autologous	Bone Regeneration	South Korea

2.2.2 MSC-derived secretome

MSC can be considered environmentally responsive cells since they secrete high levels of bioactive factors and signals as a result of their paracrine signalling. The MSC-derived secretome is comprised by bioactive factors (nucleic acids, soluble proteins, and lipids) produced by MSC that are secreted to

the extracellular space as soluble and/or as encapsulated in Extracellular Vesicles (EV)s [16, 34, 35]. The composition of the MSC secretome depends of the different microenvironment stimuli. Several studies have reported that secretome varies considerably when the cells were exposed to inflammatory situations, i.e., tissue injuries and diseases. Therefore, it has been hypothesised that MSC secretome factors can mediate the biological functions of MSC, in this particular case, cartilage repair [16, 34, 35]. Extracellular Vesicles (EV)s are membrane vesicles released by almost every cell type, including MSC, into the extracellular space to deliver biological signals. They form a variety of complex structures such as apoptotic bodies, microvesicles and exosomes. The apoptotic bodies are the biggest EVs ($>$1000 nm) which degenerate from the MSC during apoptosis; MSC-derived microvesicles (100–1000 nm) are shed from the plasma membrane; Exosomes are the smallest MSC-sourced EVs (30–200 nm) that are released through the fusion of multivesicular bodies with the plasma membranes [36].

2.3 Evidence of the Novel and Comparator therapies

2.3.1 Animal Studies with MSC-derived secretome

Evaluation of secretory products of MSC in two animal models is resumed in Table 2.2. These results suggest that MSC-derived secretome carry over therapeutic effects on OA disease models [37].

Table 2.2: Example of two *in vivo* (animal) studies in which the corresponding dosages for the therapy are presented.

Animal and OA Model	Sources	Secretome/Vesicle	Mode of delivery	Outcomes	References
Mouse OA model	Human BM MSC	Secretome	6 µL MSC-derived secretome	After 7 days, the injection of MSC secretome leads not only to early pain mitigation but also to a protective effect on the formation of cartilage	[9]
Rat defect model	Human MSC	Exo	100 mg exosomes/ 100 mL	By 12 weeks, defects treated with exosomes have demonstrated an improved gross appearance and improved the histological score in comparison to the control group	[10]

2.3.1.1 Usage Advantages

The use of the secretome, rather than MSC, as therapeutic agent in regenerative medicine [37, 38, 39], presents two main advantages. The first one is related with avoiding safety concerns associated with cellular contamination, potential presence of oncogenic cells and uncontrolled cell division [38]. Secondly, the effect of exosomes-based therapy is transitory, since the presence of exosomes is not permanent and they can be eliminated in case of adverse effects. The small size of exosomes can contribute to a lower immunogenicity or toxicity than when using artificial carriers [37, 38, 39]. Additionally, the manufacture of exosomes allows process optimization and clinical up-scaling, ensuring reproducibility and cost-effectiveness [37]. This allows a controlled selection of cell sources and the possibility of adopting cell lines with unlimited expansion potential resulting in a higher yield of the final therapeutic product [37, 39]. Contrarily to cells, when exosomes are used as therapeutic agents, safety, dosage, and ef-

fectiveness can be assessed using methods similar to the ones used for conventional pharmaceutical agents [37, 38]. This can significantly speed up the adoption of such novel therapies to clinical practice. Additionally, purified exosomes can be stored for longer periods without loss of their biological activity [37, 38].

Several research studies provide encouraging data to suggest the use of MSC-derived EVs to treat joint injury and OA. Firstly, assuming exosomes and multipotent MSC have similar properties immunogenic tolerance, a allogeneic therapy is possible without the risks of antigen presentation in cells driven from MSC differentiation [37, 38]. Secondly, signals carried in EVs are more stable than MSC, because they are 'locked' at an exact point in the original MSC, under specific controlled conditions and stimuli, consequently eliminating issues regarding the MSC long-term biological performance [37, 38]. Finally, it is valuable to use EVs in a pathological joint, since the biofactor profile is obtained in the absence of 'environmentally-responsive', i.e., the secretome will not dynamically modulate according with the evolution of the inflammatory profile of the resident joint tissues and cells [37, 39], resulting on a better characterized AMTP.

2.3.1.2 Effects in OA treatment

The effects of secretome, more specifically exosomes derived from stem cells in the OA context, can be classified into four different type of studies [37]: experimental OA models; experimental inflammation models; cartilage or osteochondral tissue regeneration; and osteoarthritis pathophysiology. In OA models exosomes have improved the cartilage anabolism and reduction of inflammation *in vitro*, and proved protective properties against cartilage deterioration and progression of osteoarthritis in rats after joint injury. In inflammation models, nanovesicles had an immunomodulatory and chondroprotective effect in joint inflammation *in vitro*, models, able to influence the performance of chondrocytes and multiple others cells; such results have been translated for outstanding anti-inflammatory effects *in vivo* (rats). In cartilage tissue regeneration, exosomes regenerative results have been evidenced in osteochondral defects produced in animal models, all of which result in those repairments with abundant hyaline cartilage formation in type II collagen. Those results have been assigned to the production of a regenerative cartilage immune phenotype and increased chondrocytes metabolic activity within the defects that were treated with exosomes [37].

2.3.2 Clinical Trials with Autologous Protein Solution

2.3.2.1 APS clarification

OA is considered a "wear and tear" disease, which progression leads to cartilage destruction, subchondral bone remodelling, and synovial membrane inflammation [40]. of cartilage. Joint cartilage depletion is not only a mechanic process. Inflammation is an important aspect of OA pathogenesis, triggering the production of proteolytic enzymes, that cause ECM degradation [41], along with secretion of inflammatory factors, such as pro-inflammatory cytokines, mediators that enhance catabolism of joint tissues affected by the disease. Some of such mediators are interleukins (IL), particularly IL-1 and IL-6; Tumour

necrosis factor-alpha (TNF-); and Matrix metalloproteinases (MMPs). For example, several MMPs proteases (aggrecanases), are involved in the articular cartilage destruction as they target aggrecan, the large proteoglycan responsible for cartilage resiliency [19, 40]. Moreover, animal experiments suggest that up regulation of IL-1-receptor antagonist (IL-1Ra), along with other antagonist factors, may decrease the progression of OA, suggesting approaches for further clinical trials [19].

APS is a therapy prepared from autologous peripheral blood, which is composed of white blood cells (WBCs) containing anti-inflammatory proteins, platelets containing anabolic growth factors, and concentrated plasma containing anti-inflammatory proteins [19]. The blood sample, after to be collected, is incubated with glass beads and centrifuged, which increases IL-1Ra production, along many other cytokines, and anabolic growth factors such as epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), IGF, TGF-β and anti- inflammatory cytokines of soluble tumor type II (sTNF-RII), IL-4 and IL-10 [20]. Thus, in APS therapy is obtained high concentrations of anti-inflammatory cytokines and growth factors from peripheral blood samples of compatible donors or the patient, exploring the interaction of the various components [20].

APS therapy contributes to the homeostasis of joints affected by OA adjusting the relative difference of IL-1Ra and IL-1b concentrations [20]. The administration of this therapy increases the joint cartilage metabolism towards stimulation of chondrocyte proliferation by growth factors. APS therapy mitigates the inflammatory cascade, although the pathways or mechanisms by which it acts are not fully described [19]. Several kits are commercially available to prepare APS from patient whole blood samples [19]. As aforementioned, this technique collects a volume of autologous peripheral blood that varies from 10 to 60 mL, depending on the kit of manufacturer [20]. After being processed, a 2.5 mL sample is injected on the patient, which contributes to improve the joint homeostasis, preventing degenerative changes in cartilage and bone [19]. The next section describes three studies where the effect of APS therapy on knee OA patients was investigated.

2.3.2.2 APS clinical evidence

Kon et al. [42] investigate, for a sample of 46 patients, whether the use of APS would reduce pain and could improve function in patients for 3 years period follow-up. An APS injection was administered to 31 of the patients, and a saline injection was administered to the remaining 15 patients - control group. Patient-reported outcomes and side effects were assessed by different scales at 12, 24 and 36 months through Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), Visual Analogue Scale (VAS), Short Form-36 (SF-36), Knee injury and Osteoarthritis Outcome (KOOS), among others parameters.

WOMAC includes a set of 24 standardised questions used by health professionals to evaluate the condition of patients with knee osteoarthritis, including pain (i), stiffness (ii) and joint function (iii) [43]. The WOMAC score can be a single score between 0-96, resulting from the sum of the scores for each question, or separate scores can be considered according to the respective pain, stiffness or function sub-scale. (i) The pain sub-scale consists of 5 questions that can assume a score between 0-20; (ii) The stiffness sub-scale comprises 2 questions, within a range of 0-8; (iii) The function's sub-scale is

composed of 17 questions where utility will vary in a range of values from 0-68. VAS is a surveying instrument that attempts to measure a feature or behaviour that is believed to vary through a continuous set of values and that cannot be directly measured. SF-36 is a survey of 36 items, reported by patients, concerning their health. The SF-36 is a measure of health condition and an abbreviated form of it, the SF-6D, is commonly used in health economics. KOOS assesses both the short- and long- term results of knee injuries. This instrument is an extension of WOMAC and consists of 42 items in 5 sub-scales scored separately; Pain, other Symptoms, Function in Daily Life, Function in Sport, and Quality of Life related to the knee.

The inclusion criteria applied include age between 40 and 75 years, willingness and readiness to be monitored during the study, knee OA grade 2 or 3 diagnosed according to the Kellgren-Lawrence scale, body mass index lower than 40, a total mean of 5 in pain score in sub scale WOMAC, average WOMAC index between 1.75 and 4 at screening and at baseline, failed at least 1 conservative OA therapy and signed an independent ethics committee-approved informed consent form. The study results report WOMAC pain score increases in a mean 65% and 41% improvement, respectively, for the APS treated group and control group over the same period. The VAS pain score improved 49% in the APS group versus 13% in the control group. However, a less significant benefit was reported in SF-36 sub-scales Bodily Pain. This trial allows to conclude that a single IA injection of APS is safe (like saline injection) and may bring to the knee OA patients an improvement in pain.

van Drumpt et al. [44] report a 11-patients study performed to establish the clinical efficacy of the APS adoption and possible adverse events. Patient-reported outcome was measured through WOMAC scale, on the first two weeks and 1st, on the 3rd, and 6th months. The inclusion criteria for this study included patients with at least 40 years of age, knee pain for at least 15 of the previous 30 days, diagnosis of knee OA stage 2 or 3 in Kellgren-Lawrence classification, body mass index lower than 40, a score higher or equal of 10 on the pain sub scale WOMAC, and agreeing to refrain from pain medication, other than acetaminophen, during the study period. There were no severe adverse events reported by the investigator and no events related that were connected to the device. However, there were specific events related to the injection procedure, including discomfort at the injection site (1 out of 11 patients), and procedural nausea (1 out of 11 patients), which were resolved and did not require any treatment. The average WOMAC pain score decreased from 11.8 ± 1.5 at the beginning to 4.2 ± 3.3 after a 18 months period, which corresponds to an improvement of 64.4% in knee pain. Stiffness and function WOMAC scores also made significant improvements of 58.3% and 61.0%, respectively. In resume, a single injection of APS to patients with early to moderate knee OA resulted in reducing symptoms throughout the duration of the study.

Hix et al. [45] report a study, where 10 patients were treated. The inclusion criteria used were similar to those previous mentioned for the two other studies. Side effects were perceived by seven subjects. One was severe (diverticulitis), which was not related to the system or treatment. One patient experienced side effects related to the procedure (arthralgia/musculoskeletal discomfort), and all other effects sensed by the remaining patients were not connected to the system or treatment. WOMAC index was used to assess patients' outcomes with report improvements on pain decrease of 72.5% after one

year. The pain score was reduced from 12.0±1.2 before APS injection to 3.3±2.9. This study also suggests that there is a significant correlation between increase in white blood cells concentration in APS and improvement in WOMAC pain rates.

2.3.3 Clinical Outcomes

An eHTA can contribute to design a clinical trial, namely on bringing to the decision on follow-up duration and therapeutic endpoints cost-effectiveness consideration. Survival, or years of quality life gained, are final endpoints, as they relate directly to the patient's state of health. Often, the therapeutic endpoints targeted in a clinical studies may not be the envisaged for after application of the therapy in the population at large [46, 2]. The need for such intermediate end-points result from challenges posed by the need to evaluate longer follow-up periods and larger patient sample size, many clinical trials evaluate intermediate endpoints [46, 2]. Translation of an envisaged MSC-derived secretome therapy into the clinical application requires to safety and efficacy, the latter by assessing as therapeutic endpoints, pain level and function (i.e. including mobility and daily activities), ideally for a 10-year follow-up, in a clinical study for a shorter period.

2.4 Process Economic Design

Chapter 2.3 described different evidences to the use of APS and the existing data to support the possible development of a MSC-derived secretome based therapy for OA disease; in both cases aiming to decrease pain and improve function. The current Chapter describes the main manufacture process drivers for estimation of the cost of MSC-derived secretome dose costs. However, the development of novel health therapies also includes additionally costly steps until they are approved and used on the health area.

2.4.1 Cost of Goods (CoGs)

2.4.1.1 Description

The set of total costs such as developing, marketing, manufacturing and delivering an ATMP to a patient is defined as CoGs. Moreover, their optimization will endorse the development and commercialization of more affordable ATMPs, which in turn are more likely to achieve reimbursement from payers and gain broader adoption for patient treatment. Careful process design may affect many of the existing costs. This can be accomplished at the time when the application of a new drug is being developed, underlining the importance of commercial strategy (beyond therapeutic strategy) when an investment in a new cell or biotherapeutic therapeutic product is involved. Furthermore, to analyse the manufacturing CoGs, Lipsitz et al. [47] designed a roadmap to plan key considerations and objectives for each step, in cell-based manufacturing; which is here, relevant for the production of MSC-derived secretome. These steps include: Tissue procurement, Material acquisition, Facility operation, Production (scaling up cell expansion), and Storage.

- **Tissue procurement** – includes screening, clinical acquisition, scheduling, variability in quality, transportation and regulatory compliance. According to this step and to build the model, one should decide whether to use allogeneic or autologous therapies [47].

- **Material acquisition** – comprises issues related to medium, supplements, cell cultures, commercial demand consistency and bioequivalence. One should adopt either xeno-free or serum-free media to construct the model [47].

- **Facility operation** – covers topics as forecast demand, required production scale/capacity, outsourcing or building a central or multicentre, and clean-room environment. Choosing between a "fresh" (non-cryopreserved) product or a cryopreserved product amenable to store longer will reduce the transportation times, influencing the facility costs. A choice between centralized or multicentre manufacturing would be made after establishing the demand forecast for the marketed product (considering targeted patients and geographical constrains), the scale/capacity of manufacturing needed, and consider the cost structure [47].

- **Production: scaling up cell expansion** – all the personnel, aseptic processing, automation, and quality control selection are subjects considered in this step. A typical cell therapy dose is estimated to include 10^7-10^8 cells per dose, although full dose ranges varying from 10^4-10^{12} cells per Kg. So, one should identify and evaluate the combinations of upstream (cell expansion) and downstream (MSC-derived secretome harvesting) technologies that minimize CoGs [47]. In the current project, decisions on MSC exosomes' number per dose will also be discussed.

- **Storage** – packaging cryopreservation agents, storage temperature and storage time. MSC-derived secretome can be stored for long periods without loss of biological activity ($-20°$C for 6 months or $-80°$C for up to 2 years). This avoids common storage and transportation issues - need to be transported frozen, enabling the use of EV as 'off-the-shelf' instead of 'made-to-order' ATMP that will incur less manufacturing and storage costs [47, 37].

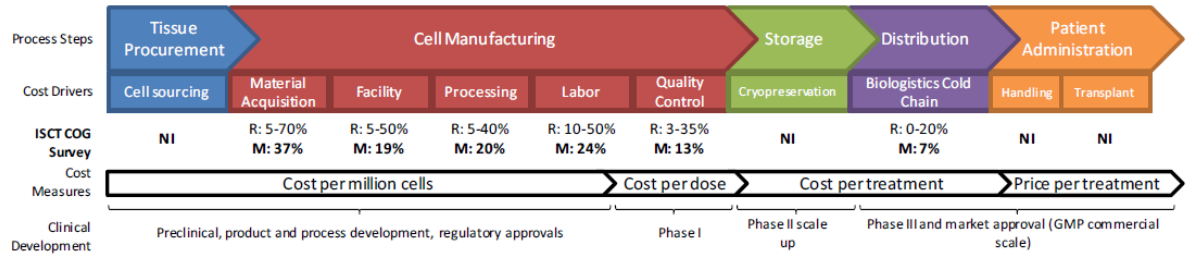

Figure 2.3: CoG survey respondents indicated the expected cost of each stage of the production process, identifying key cost drivers. Each stage and associated cost drivers can be aligned with a guiding cost measure and a stage of clinical development. M, mean response; NI, not included in survey questions; R, range of responses. Derived from [47].

2.4.1.2 Economic Aspect

A cell therapy economic evaluation cannot be fully understood without taking into consideration every relevant aspect of a product life cycle and how it can influence product cost. Figure 2.4 identifies the different costs associated with production of a cell derived product. Additionally, it is worth to mention that the identification of the costs often incur fixed or overhead costs that are overall manufacturing expenses of a product, including the cost of operating a Good Manufacturing Practice (GMP) facility (such as heating, ventilation and air conditioning (HVAC) light, rent, or capital costs) and variable costs characterized as production costs varying according to the production (such as the time of health professionals or supplies) [47, 2].

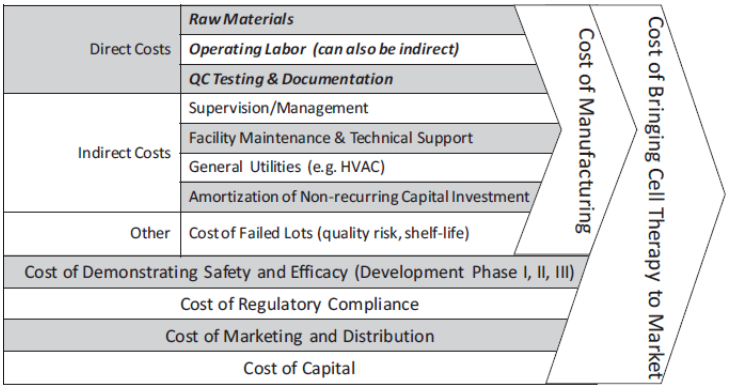

Figure 2.4: Costs related to cellular therapy business model. Derived from [47].

2.4.2 Modelling background - TESSEE

There are several manufacturing and reimbursement challenges currently impairing the widespread adoption of cell-based therapies to support regenerative medicine practices in healthcare systems. eHTAs are performed in order to provide knowledge on manufacturing innovations, reducing the CoGs and improving the long-term value that future stem cell based therapies should yield to ensure reimbursement.

The model for this thesis is an adaptation of the open source tool, Tool for Early Stem Cells Economic Evaluation (TESSEE) [33], now adopted to MSC exosomes ATMP rather than a cell therapy. TESSEE takes into account the key attributes desired for bioprocess modelling (discrete event simulation) and health economics of stem cell therapies under considerable values of uncertainty. TESSEE provides a platform to simulate the operation of a stem cell bioprocessing facility, allowing the calculation of the cost of goods. It also mimics biological variability, allowing the implementation of probabilistic distributions and random sampling of input values from these distributions to result for example in the number of cells per batch, the cost of the goods per dose, etc. This tool estimates CoGs to inform on potential prices, for scenarios of simulation of particular disease progression. TESSEE allows to handle and process large volumes of data, generated by a considerable number of individual simulations. For the present work, the model for MSC-derived secretome was developed from scratch, based on TESSEE bioprocess modelling, while for health economics aspects, a different approach than TESSEE's was

chosen (Chapter 3).

2.5 Health Technologies' development and market entry challenges

The industry of HTs is one of the most rigorously supervised industries, facing many challenges, as it is under intense scrutiny and regulation. Manufacturers must fulfil regulatory issues relating to medical technologies safety while demonstrating its cost-effectiveness, and the possible benefits to healthcare payers and purchasers. Usually, the organisations in charge of the regulatory cycle for technologies are the competent authorities, manufacturers, and certification bodies [48].

2.5.1 Regulatory background

The HS of a given country is influenced by the regulatory framework which obviously have an impact on manufacturing, on the quality system, labelling, necessary clinical data, and fees during the approval process.

According to the European Medicines Agency (EMA), therapeutic products derived from stem cells are included in ATMPs. The number of ATMP currently manufactured are considerably lower than the small pharmaceutical molecules. The same quality control requirements are imposed for all the medical products and manufacturers need to demonstrate that the product is safe and performs according to its intended use, including compliance with Good Manufacturing Practice GMP. For approximately 10 to 15 years, both ATMPs and other therapies are subjected to clinical trials in order to be approved.

When approved, ATMPs are authorized for marketing in the European Union (EU) due to the centralized approval procedure by the EMA [48]. In contrast, Food Drug Administration (FDA) in the US is an agency responsible for ensuring the safety, quality, benefits that outweigh the risks of use, efficacy, and safety of drugs, vaccines, and other biological products. Integrating FDA, the Centre for Biologics Evaluation and Research (CBER) or the Centre for Drug Evaluation and Research (CDER) guarantee the access to safe and effective drugs, including generic drugs and biological therapies. Stem cells therapies- derived therapeutic products are categorized as biological therapies, in order to enhance the health of the US population [48]. European and American approaches have some differences, one may expect that those will be mirrored in the number or type of devices approved and the speed at which the devices are introduced in each market [48].

2.5.2 Reimbursement and evidence development

Many of the benefits brought to patients in terms of healthcare are the result of innovation. Nevertheless, to achieve success in healthcare sector it is not enough to have a new product or technology, but is also needed to ensure the successful application of such products, reimbursement and acceptance by the different stakeholders. Manufacturers need to be able to provide such new products as competitive costs, contributing making significantly to the high value for all healthcare stakeholders [48].

The medical products industry is slowly being shifting from dominant cost-plus pricing approach to value-based reimbursement models, where it is followed the value-based pricing approach [48].Therefore,

standardised methods used to assess economic benefits, including standard quality of life metrics (e.g. QALYs) and maximum Willingness To Pay (WTP) metric, which is the maximum cost at or below a consumer will buy a unit of a product.

The introduction of a new therapy may have wider economic implications for the healthcare organisation (e.g. improved workflow in hospitals) and for healthcare financing, (i.e. the need for new reimbursement schemes). The funding of schemes where the service and not the production itself represents the benefit of healthcare should be investigated, both in terms of procurement and reimbursement. Public procurement focuses on setting the price of the scheme between producers and providers of healthcare services (e.g. setting and negotiating prices). Reimbursement, on the other hand, is composed of coverage, that drives subsequent coding and payment procedures, i.e. the medical product that is not covered by insurance plans will never be reimbursed [48]. Jurisdictions are different from country to country and the differences between America and Europe are significant. They should, therefore, be studied so that it is possible to discuss pricing and reimbursement [48]. In the US, manufacturers and producers have more room to establish prices and determine the value of a new therapy for reimbursement. Nonetheless, private insurance companies and government agencies, such as Medicare and Medicaid, set monetary standards for specific procedures and therapies. Although interventions below the WTP thresholds of $50,000 to $150,000/QALY are perceived by academic assessments and public pricing committees as being cost-effective, there is no explicit cost-effectiveness threshold in the form of $/QALY [49, 50]. For several European countries, stricter price and reimbursement controls are in place since public healthcare is the norm with NHSs and market access depends on positive recommendations from health technology assessment agencies [49, 50]. The National Institute for Health and Care Excellence (NICE), in UK, defines explicit cost-effectiveness thresholds in the form of cost per QALY, which is between £20,000 and £30,000 [51]. On the other hand, in Germany, the main economic analysis of health is the analysis of the budget impact, which is used for price negotiation, combined with international price potential. In this country cost-effectiveness analysis has a limited role [52, 53].

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2.6 Economic Evaluation

In order to determine how much a HS should pay depends on the resulting health gain as well as social equity, the quality of patient experience, impacts on the wider economy and the quality of evidence on which to base a decision, factors which are taken into account by governments or insurers who finance HS. Thus, there are many criteria to be used by decision-makers [54].

2.6.1 early Health Technology Assessment

Health Technology Assessment (HTA) plays a major role in the decision-making process to approve applications of the novel medical product, either an ATMP, a service or a small pharmaceutical drug in the healthcare sector, as well as assessing the cost-effectiveness of such technologies[55]. Therefore, HTA, also referred to as health economic evaluation, balances the costs of the health technology with the benefit it brings to patient car; informing on a systematic, transparent, unbiased and robust manner. At the end of this process, the aim is to identify which health technologies are the most valued in society and which should be invested in [55]. HTA has common principles with evidence-based medicine (EBM) and clinical practice guidelines (CPG), such as gathering and examining evidence from research in a reproducible and organised way, making it accessible and operational for decision-making purposes. As HTA aims at policy decision making, assessments are done to fulfil the need for reliable information to support a decision and knowledge, produced in scientific research, is then transmitted to support the decision- making process [55]. The major challenge on eHTA is related with uncertainty associated with lack of the technology, economic and health outcomes. Still, such eHTA can inform on design of clinical trials before information on clinical effectiveness being available, supporting the decisions on targets to be established to health economic evidence straight at initial stages of clinical research. In addition, an early assessment may facilitate patients' timely access to the more beneficial technologies, as well as respond to policymakers and insurers on the issue of new funding arrangements [55, 48]. In a realistic situation, the developing process of biotherapeutics does not demonstrate linear behaviour, yet it can be presented in this form for the sake of simplicity. According to Figure 2.5, the process starts with ”idea generation” and then ends with ”post-market surveillance” [48].

The development can be pursued or dropped depending on the decision points termed gates. At each decision point, new evidence that has become accessible since the previous decision point can be taken into consideration and, in this way, management flexibility can be incorporated. Additionally, to help manufacturers develop safe, effective and efficient medical products, it is of paramount importance to know the consecutive and connected development phases from raw material procurement to final disposition [48]. Undoubtedly, eHTA would have a crucial role taking go/no-go ATMP decisions. Guidelines for pre-and clinical trial design are created in order to avoid manufacturing and scalability plans, resulting in incompatible costs with a potential reimbursement price. Therefore, it has been adopted to appraise the management and the strategy of the novel therapeutic product, in this particular case, by determining the commercial viability of MSC-derived secretome in OA release. Moreover, if a no-go decision has been taken, the development will be stopped, and the associated costs as well as the market access and

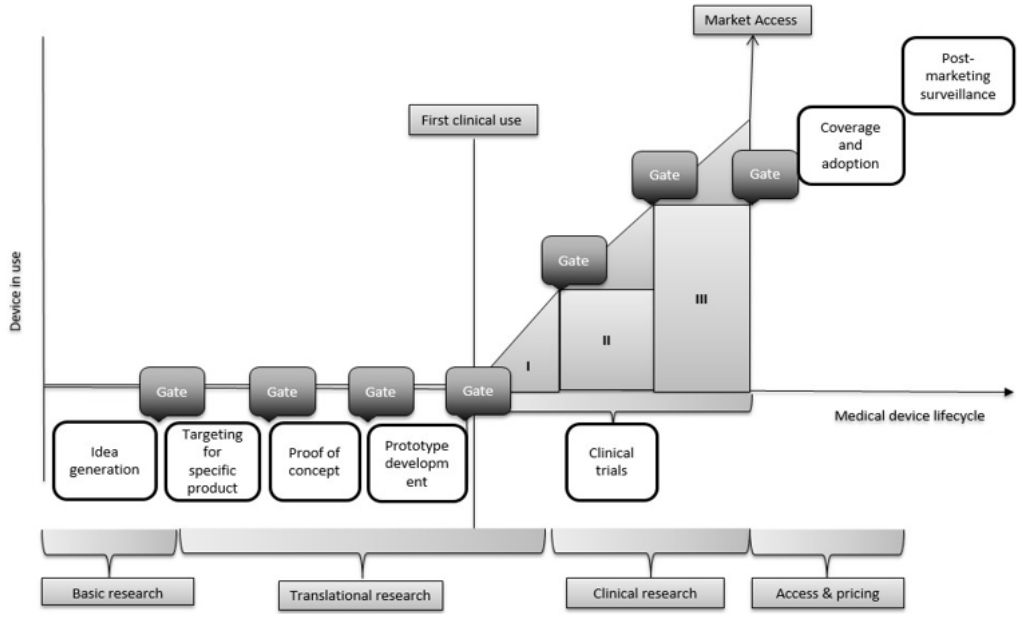

Figure 2.5: A simplified flowchart of stages in biotherapeutics' development. Adapted from [1].

reimbursement risks will be alleviated [56, 1]. The study perspective to be adopted is one of the initial decisions to be taken when conducting an eHTA, as it determines which costs are deemed relevant, affecting the remaining assessment. Secondly, a comparison of two or more health alternatives must be made. Additionally, to perform the assessment it is necessary to have a comparator i.e. a cost-effective alternative usually used therapy, to compare the inputs (costs) and outputs (consequences) associated with each of the alternatives. Thirdly, the evaluation also requires an adequate length of follow-up to fully evaluate the impact of the ATMP [2, 46]. Some study perspectives are on the table, according to the point of view from which costs and outcomes are seen in economic analysis.

- **Healthcare provider perspective** – includes all the costs associated with providing the healthcare service.
- **Patient/family perspective** – the costs directly affecting the patient, such as out-of-pocket costs, a co-payment of health utilization, time away from work.
- **Societal perspective** – costs that affect society (including those of patient and healthcare provider) in delivering health service, all medical and non-medical costs (hospitalisation, long-term care, home care, social welfare services) [2, 17].

Different studies, such as randomized controlled trials, observational studies, uncontrolled experiments, and descriptive series can evaluate the efficacy of a new intervention, assess how the new therapy could, for example, prevent further complications associated with a disease [57]. For eHTA, as clinical trials have not yet been performed, the analysis is based on hypotheses derived from analogous approaches. Thus, other units come into play when the value of an intervention cannot be translated into monetary benefits. It is extremely important that the health metric used allows comparison across clinical areas, which reflects the benefits but also the possible uncertainties about the application of

the new intervention such as additional costs, changes in survival and/or quality of life, susceptibility to changes in quality of life and reflect trade-offs between different aspects of health [50, 58]. The number of Life-Years (LY) gained is the simplest way to measure the effectiveness of a therapeutic intervention. However, this measure does not consider the patient's quality of life during the additional number of years of life [58, 57]. For this reason, QALYs have been introduced, integrating in this metric the notion of quality of life. In order to classify health, utilities can be explained as a person health states ranging from 0 (death) to 1 (perfect health) [58, 57].

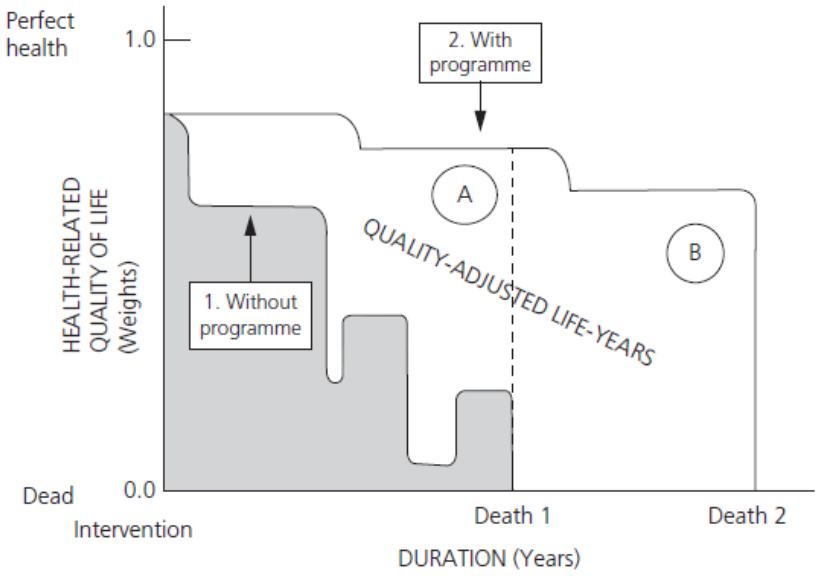

Figure 2.6: QALYs gained from an intervention. Adapted from [2].

Figure 2.6 shows the quality of life associated with patient's health o following a sharper decline without intervention (lower curve - Death 1) or if the patient receive the treatment with a longer individual live (high curve - Death 2). The area between the two curves corresponds to the increase in number of QALYs resulting from the intervention.

The values of health status preferences also called utilities are used to represent the strength of individuals' preferences for different health states. Utility measurement usually calls for a quality of life based on generic health-related preferences (HRQoL) instruments such as EQ-5D and the Health Services Index (HUI). As discussed in section 2.3.2.2, OA-specific tools (e.g., WOMAC) are often preferred to generic ones in clinical studies, particularly due to their higher sensitivity in detecting a minimal variation in OA condition. Nevertheless, WOMAC (as well as most other OA-specific instruments) covers three dimensions - pain, stiffness and function and therefore cannot directly produce utilities, not being used in economic evaluations [59].

Comparing QALY to Life-Year (LY), the first is a more comprehensive measure that captures morbidity and mortality. For an intervention, each life year gained is multiplied by the corresponding health utility. This analysis is widely applied in academic settings and in various public HS in order to assess the value of the new therapies to be introduced in the market, and guide their approval and reimbursement. Nevertheless, this measure of effectiveness is associated with several obstacles. The first is a

streamlined health model and further research would be needed to understand the impact of the different challenges that alter the quality of life. Secondly, it is regarded as a deficient indicator of quality of life (e.g. 2 years with a health utility of 0.5 years is not the same as 5 years with a health utility of 0.2 in qualitative terms, but both produce the same number a QALYs with a value of 1), possibly leading to mistaken decisions of reimbursement. Furthermore, the fact that the determination of utility is based on measures communicated by patients, there may be a certain degree of bias in the response, which leads to a negative impact on the results. One of the other main obstacles is related to ethical matters, because while QALYs shows a positive ethical standpoint in the evaluation of people at different stages of life with the same perception of quality of health, there is the reverse of considering a disabled person less valuable in terms of quality of life than a non-disabled person [50].

In addition to QALY, other metrics combining quality of life and survival may be enumerated:

- Disability-Adjusted Life-Year (DALY)
- Healthy-Years Equivalent (HYE): conjectured number of years in perfect health lived that would be equivalent to the precise number of years spent in imperfect health
- Saved Young Life Equivalents (SAVE): equivalence between health gains by the program to be equivalent to save and restore one young life to full health

2.6.2 Traditional Economic Analyses

In an early stage of drug development, it is important to economically evaluate and determine the value of such therapy or drug. To assess such interventions, one may use one of the following frameworks: Cost-effectiveness analysis (CEA), Cost-benefit analysis (CBA), CUA and Cost-minimization analysis (CMA) [60, 61].

CEA tries to identify which intervention leads to more health benefits at the same cost or the intervention where the same health benefit can be reached for a lower cost. This approach measures the benefits in natural units, i.e. deaths or illnesses. Even so, this framework illustrates the inability to compare efficiency assessments across interventions which produce different outcomes, as well as the need to focus on a single outcome of an intervention, even when an intervention generates several distinct benefits [60, 61].

CBA presents a comparison of benefits against costs of a given intervention. In here, benefits are expressed in monetary units. The aim is to directly compare monetary effect outcomes and monetary costs of an intervention against benefits and costs of alternative interventions or absence of intervention. By doing this, one should obtain the number of society's resources to be allocated to attain a specific goal. CBA main presumption is the existence of social welfare, and it can be expressed and maximized by moving additional resources to aspects of production where there is a greater social benefit. A reservation regarding this perspective is that despite being able to incorporate the broadest range of effects across an extensive range of interventions, it requires evaluating benefits of death and disease in monetary terms. [60, 61].

CUA is a form of CEA and measures the cost per unit of utility, i.e. in terms of 'healthy years' which are characterized by a multidimensional utility-based measure, combining life-years gained with some judgement on the quality of those life years. CUA includes the cost in monetary units and utility in units (such as QALYs) and it compares the cost-utility of an intervention with other interventions, with different objectives, deciding which describes the best way of spending a given treatment budget or healthcare budget as a whole. The outcome is a ratio that represents the number of QALYs or Disability-Adjusted Life-Year (DALY)s gained as a result of the intervention and the cost in monetary units of this intervention. A CUA's caveat is the significant increase in the complexity of assessing outcomes [60, 61].

CMA is performed when in the presence of known or assumed equal health effects of alternatives. In this case, the decision only depends on the costs, assuming that there is no difference in effectiveness, so the least costly alternative is the most efficient. However, few interventions are equally effective. This analysis is incomplete since it can only compare alternatives with the same outcomes and the adoption of the same, thus producing biased results as it ignores the correlation between the magnitude of effect and cost [60, 61].

CEA and CUA are the most frequent approaches used in resource allocation regarding the healthcare sector by reporting costs related to a particular health outcome [60, 61]. According to the aforementioned description and, in this particular case, CUA should be the more suitable framework, however explicit rationalisation of healthcare on CUA basis is only available in the UK. This is due to the fact that there are two standard features such as NHS ("Beveridge") and social security ("Bismarck") based HS, which also mean that there are different concepts of illness, health perspectives and medical practices depending on the basis of the system [62].

Systems that focus on values such as universality and equity are known as Beveridge systems. On the other hand, systems based on universality and plurality, freedom and solidarity are called the Bismarck type. Thus, the Beveridge-type system argues that the national health service of several countries such as the UK, Italy and some Scandinavian countries are placed on social equity and a universal system of coverage, yet there is a reduced consumer choice in the offer of services. In contrast, a health policy relying on a Bismarck-type system is reflected in countries such as Germany, where there is both a plurality of providers and an abundance of choice, with the result that individuals can be treated differently from one another. As an example of a more different case, the US, which makes use of private insurance systems, is supported by a strong concept of patient dominance [62].

So, in relation to Beveridge situation that encourages the use of CUA, once the parameters' cost, effectiveness and utility have been established, analyses are done by calculating the amount of money spent per unit of effectiveness or utility, respectively. Those analyses are not only dependent on the market, but there are also regulatory issues that can affect costs heavily [63].

2.6.2.1 Comparing Cost and Effect

To exactly reproduce the opportunity cost of the new ATMP, a comparison should be made against the next best alternative - the comparator, as mentioned previously. The incremental costs and incremental effectiveness of this health intervention can be graphed on a cost effectiveness plane where each point

represents how different the new ATMP is to the comparator therapy [60].

Therefore, the cost-effectiveness plane consists of four quadrants in which the incremental costs (vertical axis) and incremental effectiveness (horizontal axis) of an intervention can be displayed. There are four quadrants through which an analysis can be made.

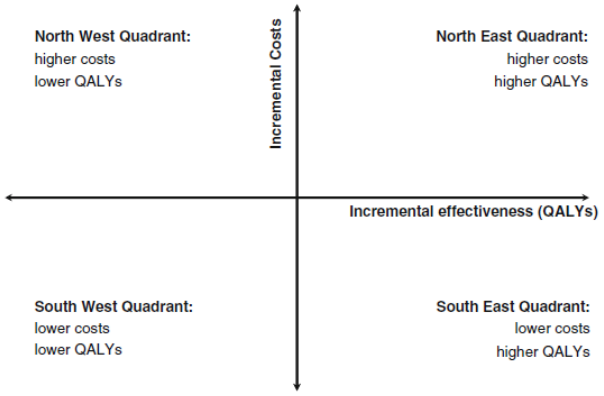

Figure 2.7: Cost-effectiveness plane. Derived from [60].

The North West (NW) quadrant contemplates higher costs and is less effective, against the South West (SW) quadrant which despite having lower costs is less effective. On the one hand, the North East (NE) quadrant has higher costs and is more effective; on the other hand, the South East (SE) quadrant has lower costs and is more effective. Thus, of the four quadrants, the latter would be the ideal scenario in which the intervention should be inserted in. The economic evaluation outcome is named as Incremental Cost–Effectiveness Ratio (ICER). It measures the incremental cost of an activity relative to incremental next best alternative, divided by the incremental effectiveness between those same two alternatives expressed in units of health (e.g., life years gained) or QALY, see Equation 2.1 [60, 56, 61].

$$ICER = \frac{C_{new} - C_{usualhealthcare}}{E_{new} - E_{usualhealthcare}} \tag{2.1}$$

Estimating costs as well as health effects of the alternative intervention is essential to properly inform decisions on its use. However, it is not sufficient to communicate the appropriate ICERs and therefore the cost-effectiveness threshold should be used. Moreover, when an alternative is cost-effective but not affordable, it means that an inaccurate threshold is being used to assess the cost-effectiveness and therefore does not reflect the scale and value of what should be given up in order to implement the alternative [2].

For a health intervention to be profitable, the ICER must be below a cost-effectiveness threshold, which is the expression of the maximum WTP for the new ATMP. If a fixed budget is established and the objective of decision-makers is to maximise the health of a given population, the threshold represents the loss of opportunities that will arise after resources are moved from different sectors of HS [2, 56]. Using dominant WTP thresholds, the headroom analysis (amount of additional cost for which treatment is still cost-effective given its effectiveness) can inform the maximum reimbursable price of a product [2, 56].

Following the aforesaid, the adoption of CEA has become the method of choice for several HTA

entities. Nonetheless, not all the concerns of value for decision-makers can be adequately outlined in this method. In particular, the use of CUA metric and the incremental cost per QALY have become the way forward for several HTA bodies within the Beveridge system, since these two are in line with the social values defended by the referred system. Yet, concerning value assessment, only the length of life and health-related quality of life are measured, not existing an adequate insight into social value, innovation and socio-economic burden [64].

Among countries dealing with a Beveridge system, there is exclusively in the UK evidence of an open reasoning of healthcare based on CUA. This country exhibits some restrictions namely on the budget and, consequently, a concern about the opportunity cost, particularly with regard to the reallocation of existing services and the process of embracing a new therapy. Using CUA with a clear decision-making threshold would be more suitable in order to consider the opportunity cost since the threshold is designed to reflect the value of the services that would be displaced. Having said this and in accordance with the saved principle of equity, all QALYs are valued in the same way inattentive of who receives them (with the exception of 'end-of-life' treatments) as well as the values in the instrument preferred to estimate QALYs come from a survey of the general population [62].

2.6.3 Literature review of health technologies in CUA context

Table 2.3 portrays several studies (not solely about OA) reviewed which served as a starting point to elaborate the MSC-derived secretome CUA.

Table 2.3: Cost-effectiveness studies on which this work was based.

Title	Year	References
Cost-Utility Analysis of Telemedicine and Ophthalmoscopy for Retinopathy of Prematurity Management	2008	[65]
Cost Effectiveness of Intra-Articular Hyaluronic Acid and Disease-Modifying Drugs in Knee Osteoarthritis	2018	[66]
Cost-utility analysis for the treatment of knee OA in three European: Platelet-Rich-Plasma dedicated kit versus Hyaluronic acid	2019	[67]
The Cost-Effectiveness of Platelet-Rich Plasma Compared with Hyaluronic Acid Injections for the Treatment of Knee Osteoarthritis	2020	[68]
The Value of a New Diagnostic Test for Prostate Cancer: A Cost-Utility Analysis in Early Stage of Development	2020	[69]
Cost-Utility Analysis of Prophylactic Dextrose Gel vs Standard Care for Neonatal Hypoglycemia in At-Risk Infants	2020	[70]

2.6.4 Multi-criteria Decision Analysis

Traditional approaches measuring the value of a health technology are typically based on considerations (benefits and costs), focusing on health outcomes and utilities.

As the value of health technology has many dimensions and it is not strictly confined to its benefit or clinical effect, more comprehensive alternatives, and broader manner of measuring value in health have been explored to support the decision-making process, namely the approval of new health technologies.

Consequently, HTA entities generally regard other criteria in addition to cost-per-QALY ratios such as equity and fairness and interventions' prioritization for vulnerable populations, in order to perform their decision-making processes i.e. in technology adoption, reimbursement, and pricing [71].

Golan et al. [72], conducted a review of which criteria should be used in eHTA. This study covered several countries and the US state of Oregon, and it was proposed that the criteria in question should be grouped into three distinct parcels:

- Need, appropriateness and clinical benefits;
- Efficiency, including cost effectiveness;
- Quality, solidarity and other ethical or social values.

The application of the MCDA in HTA has been explored to establish priorities and inform government [64]. In addition, the uncertainties associated with the process are assessed, taking into account an explicit set of criteria and their relative importance in a fully transparent procedure, in order to support the decision-making process [64]. At the same time, a spectrum of stakeholders' views is incorporated to convey a more societal perspective, such as the debate between patients and doctors in order to assess and select the therapies and technologies that are best suited and contribute most to a progressive improvement of the health status of the general population. Although MCDA methods identifying and including the personal preferences of the patient, they also bring with them some disadvantages among which the complexity of MCDA models and the time required to perform the model [64, 71, 54].

MCDA is a systematic process of formal approaches aimed at supporting individuals or groups to explore certain important decisions. Consequently, it clarifies the criteria by which the decision-makers would compile all the imperative aspects for a complete value appraisal of a new medicine or healthcare intervention [64, 71, 54]. MCDA specifies how each of those criteria should be measured, and the importance given to each of them, acting as descriptors of performance for measuring the extent of satisfying those [64, 71, 54]. Also, the whole process is finalised with a decision, in response to a multi-criteria problem. Therefore, the main steps to adopt in a decision analysis method are:

1. Structure the problem by establishing the decision context;
2. List the set of options to be appraised;
3. Identify the objectives and criteria that manifest the value in relation to the consequences of each option;

4. Describe the performance value of each option against the criteria by determining the scores of the options - Scoring;
5. Select weights for each of the criteria to measure their relative significance in a decision problem - Weighting;
6. Incorporate the weights and scores for each of the options to derive and overall value;
7. Scan the results;
8. Perform a sensitivity analysis.

Thus, MCDA is used to clarify what value the new therapy could add through a more global point of view (societal perspective) and not only considering the manufacturing costs of the MSC-derived secretome therapy and the benefits exclusively in the health of the patient analysed in CUA. In this way, conclusions can be drawn about whether or not to embrace the novel therapy in order to inform the bodies involved in the decision-making process.

2.6.5 Summary

There are no clinical trials on the therapy evaluated in this study - MSC-derived secretome, so the suggestion of this work is supported by evidence of animal studies [9, 10]. The entire work is of an exploratory nature. Due to the lack of clinical information, i.e. lack of patients' outcomes, it is chosen a therapy already used in patients with OA - APS, which will serve as a comparator throughout the study. Measured utilities of APS therapy, i.e. values that represent an individual's preference for a given health condition, are assessed on WOMAC scales. The benefits of APS therapy are assessed using three endpoints: pain, function and stiffness. WOMAC results should be converted into QALYs, in order to proceed with the study. It is assumed that the benefits of the biotherapeutic will be the same as those of the APS comparator therapy. Therefore, the effectiveness of the new biotherapeutic (measured in QALYs) is based on assumptions that will need to be validated in the future. The eHTA of this new biotherapeutic is a multidisciplinary process that relates economic, medical and social aspects in the context of its adoption. It will first resort to a CUA that will serve as a starting point for comparing costs and effectiveness between the two therapies. Next, a multi-criteria model will be initiated where the aspects present in it are based on potential biotherapeutic advantages found in literature [37, 38].

Chapter 3

Methodology

In this chapter the Model conception and implementation are presented. This Chapter intends to develop a two-phase model: Biological Process modelling and Health Economic Process modelling in order to be able to generate information which allows health bodies involved to make a decision on the uptake of MSC-derived secretome therapy.

3.1 Overview

This works aims to investigate the health-economic value of introducing an envisaged MSC-derived secretome therapy for OA relief. The eHTA targets HTs that is still in an early research and development stage, that information and knowledge about manufacture is also scarce, implying to make a variety of decisions and assumptions throughout the study [1]. Therefore, the process of manufacturing the biological therapeutic is described and designed according to a given set of literature-based assumptions [1].

The idea of the present work is to transpose the information on animal studies [10, 9] which already have scientific evidence to individuals. The estimation of manufacturing costs is obtained through Monte Carlo simulations to incorporate biological uncertainty and considering that the manufacture process has not yet been developed. The estimated manufacturing costs of MSC-secretome therapy are used into a CUA. APS is used as comparator therapy. The current study is focused on informing on a clinical trial phase II, designed to include 200 patients administered with the envisaged therapeutic agent. In a first approach, as central scenario, the CUA will be performed to evaluate whether there are (or not) incremental benefits assuming health outcomes to these therapy similar to the ones reported for patients administered with APS therapy [42, 45, 44]. Based on such model, a cost-effectiveness comparison will then be formulated (MSC-derived secretome against APS). After that, a MCDA model was formulated To considering aspects not previously captured in the CUA model. Figure 3.1 portrays a general insight of the timeline of the various models that comprise the methodology of this thesis. The sub-model I is implemented in sub-model II - Monte Carlo simulations. The sub-model III uses the estimated CoGs from sub-model II to perform the CUA. The sub-model IV outlines aspects that were not captured throughout

sub-model III.

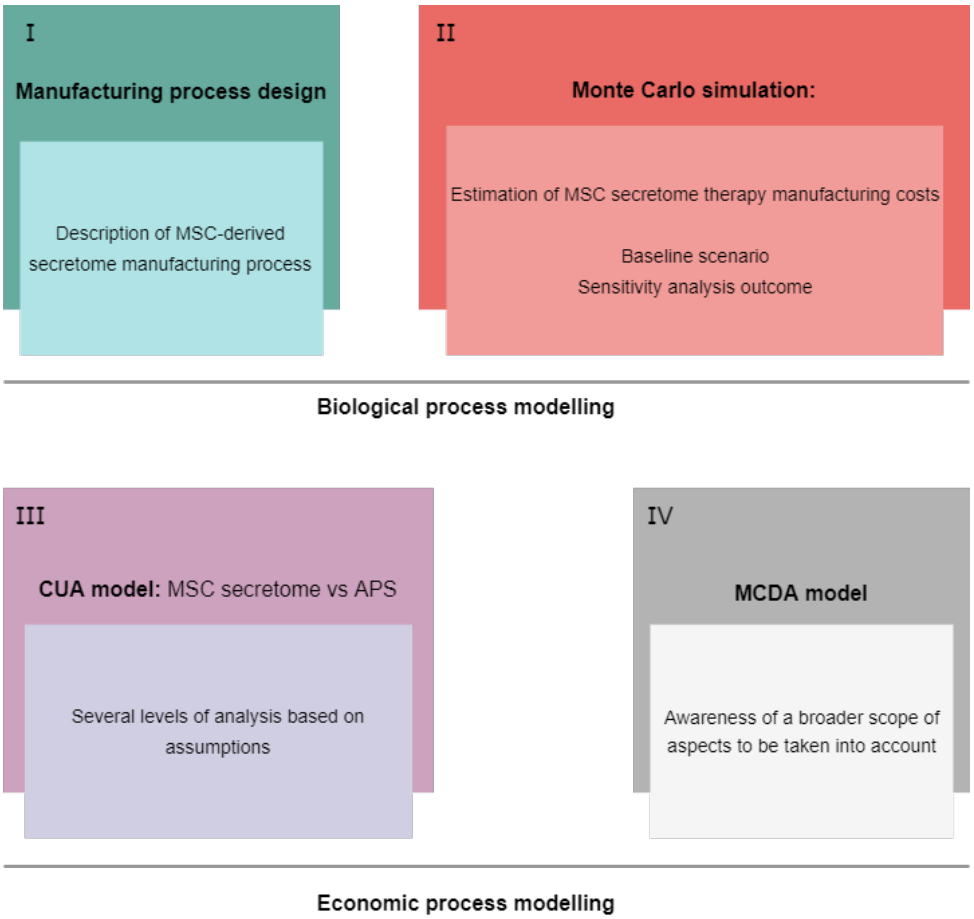

Figure 3.1: Overview of all sub-models sequence present in this chapter. I-Description of manufacturing process, II-Monte Carlo simulation, III-CUA model, IV-MCDA model.

3.2 Biological Process modelling

Several decisions have to be considered concerning the design of the manufacture process of MSC-derived secretome. Those include selection of the type of stem cells to be used, the culture medium, type of technology for cells expansion, production of exosomes, isolation of exosomes and packing of the final product [33].

Stem cells selection

As discussed in Section 2.2, MSC isolated from BM are the ones more widely used in cartilage repair, however sourcing this cells implies an invasive procedure which may cause donor site morbidity and it is limited on collected volume, implying a low yield of cells obtained. As MSC have immunomodulatory and hypoimmunogenic features, they allow the therapy in question to be allogeneic [22]. Allogeneic MSC based therapies since they are less donor availability dependent and can rely on healthy donors with no compromised MSC. For frozen biological therapeutic products, allogenic products also allow to optimize logistics and have the potential to optimize process stages to reduce COGs, as product obtained from

cells driven from one donor can be administered to several patients, benefiting from economies of scale through a scale-up of manufacturing approach [33].

Culture media selection

The culture medium selected influences the biological process performance as it provides basic nourishment, growth enhancing agents, salts, co-factors and a liquid matrix for stabilization of the cells and gas exchange [15]. The culture media is designed to include a basic formulation supplemented in order to meet cell's needs [15]. For MSC the most common supplement is animal serum, typically Fetal Bovine Serum (FBS). However, in accordance to GMP the use of FBS for MSC expansion is an issue when such cells are to be used as an ATMP. Both EMA and FDA recommend to avoid the use of raw material driven from mammals (including serum) on the preparation of medicines; this recommendation aims to decrease the risk of contamination (e.g. prions) [15]. Xeno-free medium is a formulation that does not contain any components of animal origin, but may contain components of human origin, such as human serum or insulin. Following regulatory agencies recommendations, increasingly cases have been reported in which xeno-free medium is transported from research laboratories to clinical applications, complying with GMP standards [73]. It is expected that cell expansion in xeno-free medium prepared under GMP conditions, with defined and safe components for human use, becomes the norm in future FDA authorised clinical studies [73].

Expansion platforms selection

The selection of the type of MSC expansion technology is important to define the therapeutic manufacturing process [33]. Cell expansion technologies can categorize as planar or bioreactors, being also classified, respectively, 2D and 3D [33]. In the present work 2D planar technologies were selected for cell expansion. Cell based therapies usually require higher stem cells quantities, ranging from 10^6 to 10^9 cells per dose per patient [74]. The supply of "off-the-shelf" stem cell products is greatly limited by areas and volumes of expansion [33]. In addition, 2D culture flasks not always allow monitoring and control of nutrients that may impair cell yield [33]. A better cell performance can be achieved using 3D platform expansion such as spinner bottles and bioreactors. However, being this work based on a cell-free therapy, i.e. based only on the substances that the cells secrete, the number of cells needed per dose per patient is much lower than would be expected in conventional cell therapy [33]. For these aforementioned reasons, the use of 2D technology such as T-flasks is a sufficient condition to conduct the study.

3.2.1 Sub-model I: Planning of the MSC-derived secretome manufacturing

To establish manufacture process model it was defined different stages needed to the production of the new desired therapeutic product, composed of a protein cocktail. A Monte Carlo approach was used to compute the model variables to integrate on the model the variability associated with the biological variables (namely cell growth behaviour). An outline of the manufacturing process can be seen below

- Figure 3.2, starting with the upstream phase, following Downstream Processing (DSP), and ending at the final phase of ATMP, ready for administration into the individuals.

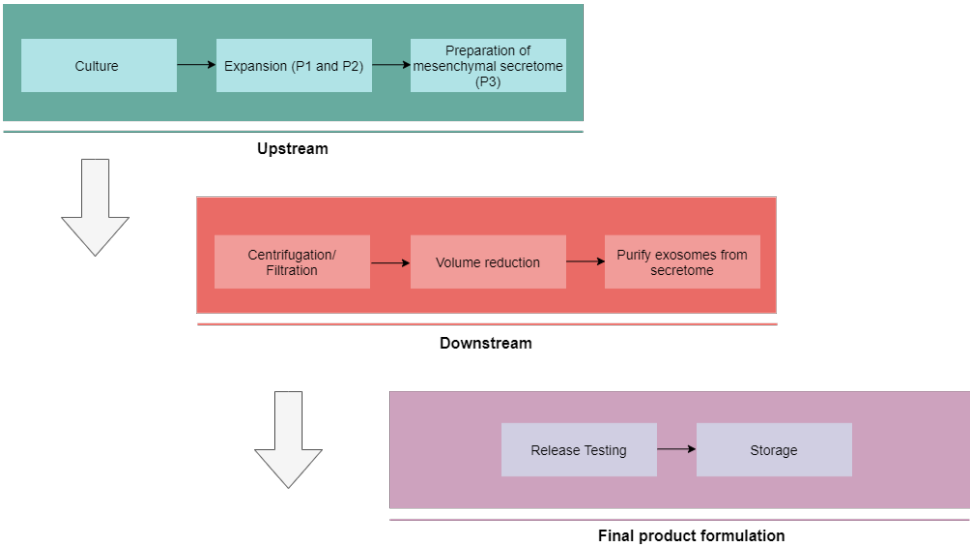

Figure 3.2: Process diagram with the main stages of MSC-derived secretome therapy manufacturing.

3.2.1.1 Upstream

The upstream process includes the stages from the isolation and early culture of the MSC, up to the cell bank, cell expansion, exosome production and final exosome harvesting [75]. While on processes where cells are the final product, it is required a cell isolation stage, such step will not be here considered as the final goal is not collect the cells but the secreted substances released by them – the secretome [9]. Still, it is considered all the steps to be implemented after cells thawing, including cell viability and counting tests [75]. In resume, there will considering three successive single stages within the upstream process: Cell culture, Cell expansion (passages 1 and 2), and Secretome preparation (passage 3) [9]. The duration of the latter passage will be only 24 hours and does not aim at cell expansion phase, but to stimulate the cells to produce the secretome in a simpler cell culture that facilitates downstream [9].

Expansion

The expansion phase is composed by three passages: P1, P2 and P3. P1 and P2 last 7 days each and P3 lasts 24 hours [9]. The growth of cells in culture can be modelled according to the cell growth curve, which comprises four phases: the latent phase before the start of growth, the exponential growth phase, the stationary phase with rapid cell number increase, stationary phase when the cell population number is maintained after cells growths had slows down and the death phase where cells dead occurs due to lack of nutrients. However, the later phase is not pertinent for this study [33]. Thus, cells must undergo at least one passage during the registration phase where the culture medium must be changed, for medium renewal purposes [33]. Cell expansion has been modelled according to Equation 3.1 and the growth curve is represented in Figure 3.3, where lag phase and exponential phase (label not shown) of passage 1 takes place on the first 7 days of culture. At day 7 with cells occupying between 70 and

90% of the T-flask surface area, cells are harvested upon enzymes action and seeded on new T-flasks, providing higher areas to follow up cell expansion. Therefore, Passage 2 takes place from day 7 to 14, with a 2 day lag phase followed by another exponential phase until day 14. Finally passage 3 lasts one day, from day 14 to 15.

- X_0 - represents the initial number of cells.
- μ - represents the growth rate which can vary depending on the passage (see Table 3.1)
- t-t_0 - time difference between the day when the number of cells is being analysed and the initial instant.

$$\frac{dX}{dt} = \mu X \Leftrightarrow \frac{dX}{X} = \mu t \Leftrightarrow ln(\frac{X}{X_0}) = \mu(t - t_0) \Leftrightarrow X = X_0 e^{(\mu(t-t_0))} \tag{3.1}$$

Table 3.1: Growth rate mean per passage.

No. of Passage	$\boldsymbol{\mu}$
1	0.52 (0.32-0.61)
2	0.48 (0.29-0.71)
3	0.48 (0.27-0.49)

The growth rate, $\boldsymbol{\mu}$, can assume both deterministic or stochastic values according to triangular distribution, as sustained above.

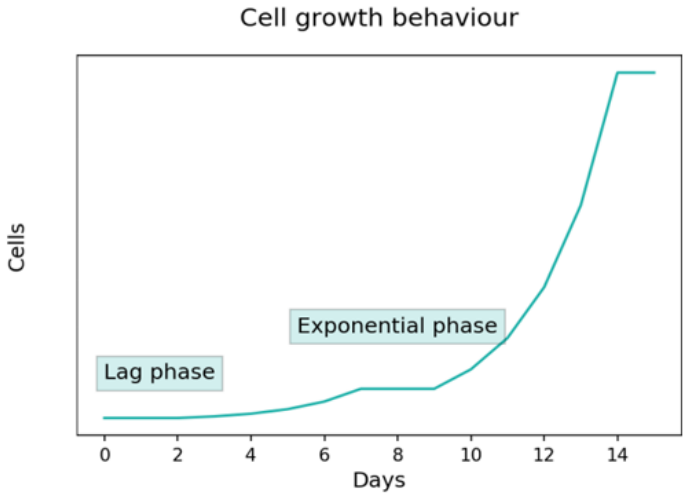

Figure 3.3: Representative graph based on the cell growth over 15 days according to the exponential growth rate formula, resulting from simulation, under the conditions described.

Once the cells are harvested, it is assumed that they will expand according to the draft present in Figure 3.3. In order to more accurately describe the diverse biological behaviour and to take into account uncertainty (to a certain degree) the present work portrays a stochastic cell growth model. To achieve this, in the absence of a distribution of values for growth rates, but only having access to

key parameters, such as range and mean values, a triangular distribution was used - Table 3.1 [33]. Triangular distributions are used when the shape of a given distribution of a variable is unknown. To apply it, one only needs to assess the minimum, maximum and modal values of the observed data [76]. Because one only had access to high, low and mean values of cell growth rates distribution, Equation 3.2 was used to estimate the mode of the triangular distribution from the mean value [76].

$$mean = \frac{l + mode + h}{3} \tag{3.2}$$

A scenario in which we only used the mean values is from now on designated as “fixed”. On the other hand, the scenario (or simulation type) in which a triangular distribution for cell growth rates [33] was used is designated as “random” - because it takes into account stochasticity.

Preparation of secretome

This stage corresponds to the passage 3 which lasts 24h. It corresponds to the stage in which cells are subject to a stimulating medium so as to obtain secretome [9]. Importantly, with the aim to facilitate downstream, the culture medium of a simpler composition (lower proteins and factors) than the ones used for the expansion phase is used.

3.2.1.2 Downstream

DSP concerns the unit operations between the final cell culture stage and final therapeutic product formulation. To ensure the final product functional properties, high purity and yield in a reliable manner, it is required to define critical quality attributes (CQAs) to the cells and secretome and manufacturing process has to be adapted accordingly [77]. Therefore, the DSP technologies will be selected considering the CQAs for the cells and final secretome formulation which are identity, purity, potency and safety, to be maintained even in scale-up and scale-out situations in the processes manufacturing [78].

Given that the biological process of producing secretome involves large volumes it is mandatory to perform a volume reduction and concentration of the final product steps [79]. Here some assumptions are also made. Following each of the first three centrifugations, the pellets (cells, dead cells, cell debris) are disposed of, and the supernatant is kept for the next step [80]. By way of contrast, following the two 100,000×g centrifugations, pellets (exosomes + contaminating proteins), exosomes are preserved as pellet and supernatants are discarded [80]. At the end of the process, the exosomes and proteins are again centrifugated and washed in order to be isolated and prepared for the final stage [80].

3.2.1.3 Final Product Formulation

The washing and purification steps aims to remove undesired substances present in the cell medium, such as proteins and culture media supplements that should not be present or only present at residual amounts on the final formulation. The obtained secretome, rich in exosomes , should comply with a final target dosage and then cryopreserved [33].

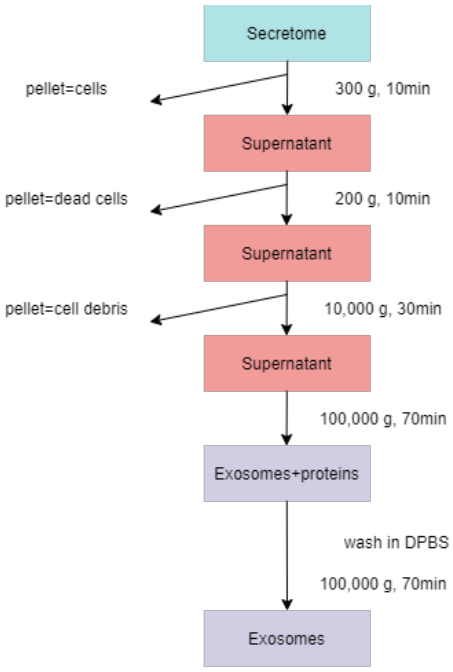

Figure 3.4: Flowchart for the exosome purification procedure based on ultracentrifugation differential. Speed and duration of each centrifugation are stated on the right of the arrows. Adapted from [80].

To establish a target dosage for the secretome/exosome based therapy, different pre-clinical studies and clinical trials (*with completed, recruiting or yet enrolling by invitation status*) were considered (Table 3.2). The studies on animal models (rats), presented in Table 2.2, used dosages [9, 10] that are obviously much lower than the intended dosage for humans (whom have higher body weights). However, the compilation of such studies is important, as there is still no clinical data obtained for humans patients for knee OA therapy based on either BM-MSC-derived exosomes or secretome. The duration of the final formulation phase will depend proportionally on the batch size and number of exosomes per dose (considering a 2 mL cryovial) of final product.

Table 3.2: Some clinical trials use either secretome or vesicles to treat the conditions proposed. The respective dosages of the therapies and the method of their administration are also presented.

Phase study	Condition	Secretome/Vesicle Types	Mode of delivery	References
Phase 1 Phase 2	Cerebrovascular Disorders	Allogenic MSC-derived exosome enriched by miR-124	200 μg total protein of allogenic MSC-generated exosome.	[81]
Phase 1	Coronavirus	MSC-derived exosomes	Five times aerosol inhalation of MSCs-derived exosomes (2.0 x 10^8 exosomes/3 mL).	[82]
Phase 1	Healthy	BM-MSC-derived exosomes	Once aerosol inhalation of MSC-derived exosomes: 1X corresponds to 2.0 x 10^8 exosomes/3 mL; 2X correspond to 4.0 x 10^8 exosomes/3 mL [...] 10X correspond to 20.0 x 10^8 exosomes/3 mL.	[83]
Phase 1 Phase 2	Alzheimer	AT-MSCs-derived exosomes	5 (Low) μg, 10 (Mid) μg or 20 (High) μg MSC-Exos/1 mL; Twice a week during 12 weeks.	[84]
Phase 2	COVID-19 SARS-CoV-2 Pneumonia	MSC-derived exosomes (EXO1 and EXO2)	Twice a day during 10 days inhalation of 3 mL solution contained 0.5-2.0 x 10^{10} exosomes of the first or second type, respectively [...].	[85]
Phase 1 Phase 2	OA	Umbilical Cord (UC)-MSCs derived Conditioned Medium (CM) (Secretome)	[...] 5 x 10^6 allogeneic UC MSCs in NaCL 5 mL, two weeks later 2 mL/knee of UC MSCs secretome, four weeks later 2 mL/knee CM. All via IA injection.	[86]

Table 3.2 reports exosome/secretome based therapies for other conditions than not OA. Moreover, Table 3.3 resumes information concerning the comparator therapy. Together, the information on both tables support the assumptions done on suggested doses to be applied in clinical applications of this novel exosome/secretome based therapy for OA.

Table 3.3: APS relevant studies.

Therapy	Patients	Mode of delivery	References
APS	n=46	2.5 mL once.	[21]
APS	n=10	2.9±0.2 mL once.	[45]
APS	n=10	2.5 mL once.	[44]

3.2.2 Design of sub-model II

The main process components and key considerations to be taken into account in the sub-model II are outlined in Figure A.1. In this section, a diagram with key parameters highlighted is presented in order to proceed with the analysis - see Figure 3.5.

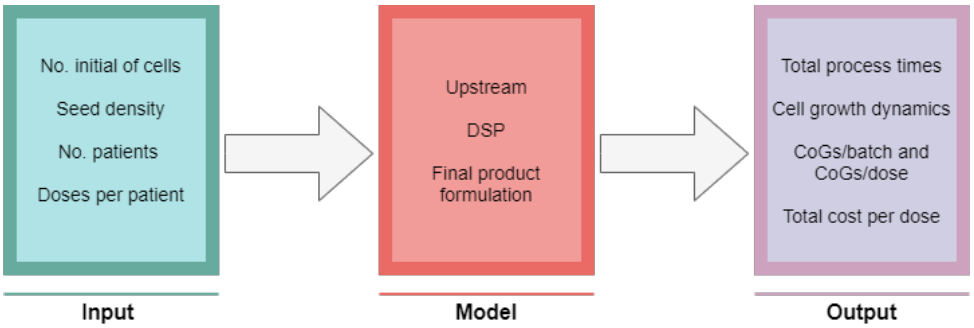

Figure 3.5: Relevant inputs and outputs of the model.

Figure A.2 portrays a simple schematic of the Monte Carlo model developed in Python. The model describes biological features of producing the desired MSC-derived secretome. In this part, stochasticity is introduced in the form of a distribution of cell growth rates. Parallelly, costs and economic aspects are captured by equations (adapted from [33]) described in the following section.

3.2.2.1 Model Equations adapted from TESSEE

Fixed Capital Investment FCI is determined by summing up facility installation costs C_{fi} and equipment installation costs C_{ei}.

$$FCI = C_{fi} + C_{ei} \tag{3.3}$$

It is assumed that for a known area of GMP installations - Table A.1, the total costs of the installations depend on the ratio of GMP installations occupied by clean rooms, with a fixed cost per clean room area and non clean room areas [33].

Thus, C_{fi} is determined by:

$$C_{fi} = C_{cr/a} r_{cr} + C_{ncr/a}(1 - r_{cr}) \tag{3.4}$$

- C_{fi} - facilities total installation cost (€)
- $C_{cr/a}$- clean space cost per unit of area (€/m^2)
- r_{cr} - ratio of total area of GMP facilities occupied by clean rooms
- $C_{ncr/a}$- average cost per area of the installation, except clean rooms.

Equipment dimensioning With a fixed installed capacity - Table A.1, the costs of the installed equipment are simply calculated multiplying the acquisition cost of each equipment by the number of equipment in this facility [33] , as follows:

$$C_{ei} = \sum C_i \, N_i \ , \ \forall \, i \in Equipment \tag{3.5}$$

$Equipment = \{BSC, inc, ctrf, DSP\}$

- C_{BSC} - Cost of installed biosafety cabinets
- N_{BSC} - Number of installed biosafety cabinets
- C_{inc} - Cost of installed incubators
- N_{inc} - Number of installed incubators
- C_{ctrf} - Cost of installed centrifuges
- N_{ctrf} - Number of installed centrifuges
- C_{DSP} - Cost of utilised purification/downstream processing equipment
- N_{DSP} - Number of utilised purification/downstream processing equipment

Equipment and Facility depreciation Costs related with equipment and facility depreciation are obtained by simply dividing the acquisition costs of these assets by the time of depreciation of these assets [33] - Table A.1, considering a linear depreciation, which is calculated through:

$$CoG_{fe,dep} = \left(\frac{C_{fi}}{t_{f,dep}} + \frac{C_{ei}}{t_{e,dep}} \right) t_{operation} \tag{3.6}$$

- $CoG_{fe,dep}$ - total cost of goods associated with the facility and equipment depreciation
- $t_{f,dep}$ - period of time (in days) considered for the GMP facility is depreciation
- $t_{e,dep}$ - period of time (in days) considered for the installed culture equipment depreciation
- $t_{operation}$ - total time (in days) of the duration of the process

Equipment and Facility operation costs Costs associated to the facility and equipment also have an additional component related to the operations necessary to keep the plant running [33].

$$CoG_{fe,op} = t_{operation} \sum C_j \, , \; \forall \, j \in Components \tag{3.7}$$

$$Components = \{gases, supls, reqlf, maint, clean, garm\}$$

- C_{gases} - costs related to gases supply for cell culturing in incubators. Those gases include oxygen, carbon dioxide, liquid nitrogen, and water vapour.
- C_{supls} - costs including disposable office supplies, laboratory supplies not related to manufacturing, and utilities.
- C_{reqlf} - requalification is an annual process for new testing of air quality and compliance with GMP manufacturing norms.
- C_{maint} - maintenance cost is related with predicted costs of preventive and corrective procedures in the equipment and facility. The costs are derived from annual estimates.
- C_{clean} - cleaning costs are associated with the annual costs of cleaning and disinfection of the clean rooms.
- C_{garm} - garment costs include all materials used in clean room gowning.

The total facility and equipment cost contribution are the sum of depreciation and operation components [33].

$$CoG_{fe} = C_{fe,dep} + C_{fe,op} \tag{3.8}$$

Labor costs The labor costs are considered based on the total operation time.

Consumables Within unit operations such as expansion, different technologies for cell culture may be selected [33]. The model admits several planar technologies - set T with different areas and volumes - See Table 3.4.

Table 3.4: Characteristics of expansion technologies used in this work

Expansion Technology	Area (cm^2)	Media Volume (mL)	Work Volume (mL)	Harvesting Volume (mL)	References
T-flask25	25	5	7	1.75	[33]
T-flask75	75	15	25	3.5	[33]
T-flask175	175	35	55	7	[33]
T-flask225	225	45	70	9	[33]

The type of expansion technology (ET) selected for each step is done through an algorithm which minimizes the number of technologies to be seeded. The type of flasks is determined through the Equation 3.9:

$$ET = min\ \left(\frac{N_{cells,\,total}}{N_{cells,\,technology}}\right)\ ,\ technology \in T \tag{3.9}$$

$T = \{Tflask\,25, Tflask\,75, Tflask\,175, Tflask\,225\}$

The number of cells to seed per T-flask is then determined in the basis of the available expansion area.

$$N_{cells,\,technology} = Sd\ A_{technology} \tag{3.10}$$

- Sd - seeding density per culture (cells/cm^2).
- $A_{technology}$ - total culture seeding area available per unit of the culture system (cm^2).

The number of technologies of a given type is selected through Equation 3.9 and rounded up to the nearest integer. The number of cells seeded is limited by the maximum number of flasks of a given type that can be seeded, taking into account capacity limitations of the incubator.

$$N_{technology} = min\left(\frac{N_{cells,\,total}}{N_{cells,\,technology}}\ , N_{technology/equi} N_{equi}\right) \tag{3.11}$$

The number of final product containers (i.e., vials) per storage step are calculated using Equation 3.12 and rounded to the lowest nearest integer:

$$N_{containers} = \frac{N_{exos,\,final}}{c_{exos}\ V_{container}} \tag{3.12}$$

- $N_{exos,\,final}$ - final number of exosomes coming from DSP.
- c_{exos} - goal concentration of exosomes in each container.
- $V_{container}$ - maximum volume of each container.

Yield The number of exosomes obtained after DSP (volume reduction and purification) - $N_{exos,\,final}$ and the fill-finish of the product to the final formulation is obtained by:

$$N_{exos,\,final} = N_{cells,\,initial}\ N_{exos/cell}\ Y_{vrp}\ Y_{ff} \tag{3.13}$$

- $N_{cells,\,initial}$ - number of cells entering the downstream processing.
- $N_{exos/cell}$ - number of exosomes secreted by each cell.
- Y_{vrp} - yield of volume reduction and purification.
- Y_{ff} - yield of final formulation.

Cost of consumables The disposable culture technologies consumable CoGs associated with cell culture are calculated by:

$$CoG_{\,technologies} = \sum N_{technology,\,i}\ C_{technology,\,i} \tag{3.14}$$

- $N_{technology,\,i}$ - number of flasks of each category.
- $C_{technology,\,i}$ - costs of each flask.

Consumable costs associated with storage $CoGs_{\,storage}$ are also considered [33], as well as the purification disposable consumables $CoGs_{\,purification}$ as follows:

$$CoG_{\,storage} = \sum N_{container,\,i}\ C_{container,\,i} \tag{3.15}$$

$$CoG_{\,purification} = N_{ctrf}\ C_{ctrf} \tag{3.16}$$

The total consumable costs of a process are then the sum of these three cost components:

$$CoG_{\,consumables} = CoG_{technologies} + CoG_{storage} + CoG_{purification} \tag{3.17}$$

Reagents The key reagent for cell culture is the culture media used for expansion. The volume of culture media per vessel in a given cell culture passage is calculated through:

$$V_{cm,\,technology} = V_{cm,\,seeding} + V_{cm,\,feeding}\ N_{exchanges,\,media} + V_{cm,\,harvesting} \tag{3.18}$$

- $V_{cm,\,seeding}$ - volume used for cell seeding.
- $V_{cm,\,feeding}\ N_{exchanges,\,media}$ - volume used in media exchanges during each passage
- $V_{cm,\,seeding}$ - volume used for formulation and inactivation of the harvesting reagents.

The total volume of culture media can be calculated by Equation 3.19.

$$V_{cm} = \sum V_{cm,\,technology,\,i}\ N_{technology,i} \tag{3.19}$$

- $V_{cm,\,technology,\,i}$ - volume of the culture media required regarding each type of technology.
- $N_{technology,\,i}$ - number of technologies of the type used in bioprocessing.

It should be noted that culture media can be unit operation specific, as the media used for stem cell culture is different from the media used for cell harvesting, cell wash between operations, and cryopreservation [33].

In the end of each passage, a given volume for cell detachment from the cell culture technologies is used. The total volume of harvesting reagent is calculated in the same way as in Equation 3.19.

$$V_{hr} = \sum V_{hr,\,technology,\,i}\ N_{technology,\,i} \tag{3.20}$$

Volume reduction and purification after cell culture requires washing cells apart from the culture media. Those cells are washed multiple times with a basal media, having this way a different formulation of the culture media [33].

$$V_{wm} = V_{wm,\,wash}\; N_{washes} \tag{3.21}$$

Finally, cryopreservation buffer is required for final product storage.

$$V_{fb} = V_{cryo,\,buffer}\; N_{cryovials} \tag{3.22}$$

Cost of reagents

$$CoG_{\,reagents} = V_{cm}\; C_{cm} + V_{hr}\; C_{hr} + V_{wm}\; C_{wm} + V_{fb}\; C_{fb} \tag{3.23}$$

Quality control The quality controls are assumed to have a fixed cost per batch produced.

$$CoG_{QC} = C_{QC,\,batch}\; N_{batch} \tag{3.24}$$

Quality controls are always associated with a given batch failure rate [33]. If, for a given batch, the quality controls are passed, all the calculated costs in the other categories are kept and the quality control costs divided by all the doses in that batch [33]. However, if there is a batch failure, all the doses in that batch are discarded, and the full costs of producing that batch are equally divided by the doses in other batches that passed the quality controls.

Total Cost of Goods

$$CoG_{total,\,process} = CoG_{consumables,\,process} + CoG_{reagents,\,process} + CoG_{labor,\,process} + CoG_{fe,\,process} + CoG_{QC,\,process} \tag{3.25}$$

The cost of goods per batch are obtained by summing the costs associated directly with the batch size, such as direct expenditure of consumables and reagents, and the quality controls, with the indirect costs (labor, facility and equipment depreciation and operation, and quality controls) [33].

$$CoG_{direct,\,batch} = CoG_{consumables,\,batch} + CoG_{reagents,\,batch} + \frac{CoG_{QC,\,process}\; N_{doses,\,batch}}{N_{doses,\,process}} \tag{3.26}$$

$$CoG_{indirect,\,batch} = \frac{(CoG_{labor,\,process} + CoG_{fe,\,process})\; t_{operation,\,batch}}{t_{operation,\,total}} \tag{3.27}$$

Finally, the cost of goods per dose is obtained by dividing the total costs per batch by the number of doses produced in this batch [33].

$$CoG_{batch} = CoG_{direct,\,batch} + CoG_{indirect,\,batch} \tag{3.28}$$

3.2.3 Overview of sub-model II

In order to model the process of producing a therapeutic, a stochastic model, resorting to Monte Carlo simulations, was developed. Some aspects are based on TESSEE [33], however many features differ from TESSEE configuration. The objective of these calculations is to estimate the CoGs per dose of the MSC-derived secretome therapy to estimate the price dosage, which will be applied on the CUA model.

Cell expansion, exosome production, downstream processing and final product formulation stages costs were estimated [33]. Biological and costs related variables are tracked during simulations, allowing to assess and record values of the whole duration of each simulation [33].

The Monte Carlo model has been configured for the therapeutic manufacturing process starting with an initial number of cells (sourced from donors) and seeded at an optimal initial density on the selected Expansion Technology (ET). The number and type of ET units are selected to minimization of the cell expansion stage cost. Note that given an initial seeding density several combinations of ETs are possible to be attained - see Table 3.4. Therefore, ET selection is done through an algorithm which reduces the cost and quantity of ETs to be exploited during the process (see Equations 3.9 and 3.10).

A confluence time of 5 days was established for the cell expansion stages. The model calculates the needs in culture medium feedings [33], i.e. renewal of the medium in each T-flask according to Equations Equations 3.18 and 3.19. A yield of 90% is assumed at the end of the 3 passages considered in this work [33].

Thereafter, the model simulates the separation of the conditioned medium (secretome) from the cells, which in turn undergoes successive centrifuging, washing and volume reduction steps to purify the exosomes apart from media components and prepare them for final product (see DSP description); which is described by Equation 3.21. A yield of 80% is considered here, causing 20% of the exosomes to be discarded and fail to pass to the next stage [33].

The production process continues the needed times until the target number of exosomes (or doses, equivalently) is met (Equation 3.13).

The final product is then formulated and cryopreserved after passing a quality control stage (Equations 3.22 and 3.28). This is a step which is required to verify if the therapeutic complies with all regulations defined by laboratory standards, making sure that the results are consistent, safe and suitable for human use [33].

The stochasticity incorporated in the current model captures the biological behaviour of cells grow, observed in real conditions. Therefore, the estimations portray more accurately the variability of possible outcomes. The stochastic model here presented follow a triangular distribution of P1 and P2 cell growth (Table 3.1) and the probability of product release of 90% (Table 4.1). Figure A.2 presents a simplistic flowchart for the implementation of MSC-derived secretome therapy.

The costs incurred presented in this work are taken from literature in USD and then converted into EUR according to the currency exchange rates as shown in Table 3.5.

Table 3.5: Exchange rate of 1 EUR to USD, AUD and GBP. Derived from [87], accessed on 20.11.2020.

Euro (EUR)	US Dollar (USD)	Australian Dollar (AUD)	Pound sterling (GBP)
1	1.18456	1.62699	0.89487

It should be noted that the model receives as inputs all the variables present in Table A.1 - concerning the facility itself and the equipment used and Table 4.1 - the parameters corresponding to the experimental procedure. In short, for the effective implementation of the model, several assumptions are formulated - see Table A.2, which then allow us to draw conclusions.

3.2.4 Sensitivity analysis

eHTA has a high degree of associated uncertainty and it is carried out before decisions to invest in a specific technology to understand whether such investment is worthwhile [1]. The use of Monte Carlo simulations and produce information and forecasted results that can incorporate such uncertainty o [88]. Therefore, Monte Carlo simulations can be further used in CUA, as well as in MCDA model in order to develop different scenarios to handle the variability of existing evidences reported in the literature. In this way, the use of stochasticity in a process as Biological process modelling helps to provide meaningful results and closer to a real situation [88]. After implementing the Monte Carlo model and taking into account a baseline scenario (see Chapter 4), several analyses are performed in order to understand how the model behaves and the effects on the final results of considering variations of certain assumptions and parameters values. Namely, it was made sensitive analysis for (i) the decrease of the initial number of cells isolated (Section 4.2.1), assuming 1x10^4 cells or 2x10^4 cells as inputs; (ii) different cost of quality control (Section 4.2.2); and reducing the facility cost by half (see Section 4.2.3). These analyses are of extreme importance, given that in the scope of a bio-economic process, a difference in the final cost may represent an adoption or not of the envisaged therapeutic product.

3.3 Economic Process modelling

In this section, two levels of action were defined, methods covering CUA and MCDA.

3.3.1 Sub-model III: Cost-Utility Analysis

The manufacturing costs required to set up a CUA were estimated through Monte Carlo simulations (concerning MSC-derived secretome therapy) and based on literature (concerning APS). In order to obtain the health gains of the MSC-secretome therapy, WOMAC scores were used in accordance to literature's patients benefits [42, 44].

van Drumpt et al. [44] report the results of health gains in WOMAC scores for pain, function and stiffness, spread out across six moments over the whole study duration: at the baseline, and then 1, 2, 4, 12 and 26 weeks after administration of the therapy.

QALYs are intended to be assessed in years rather than weeks as in [44]. To overcome this constraint

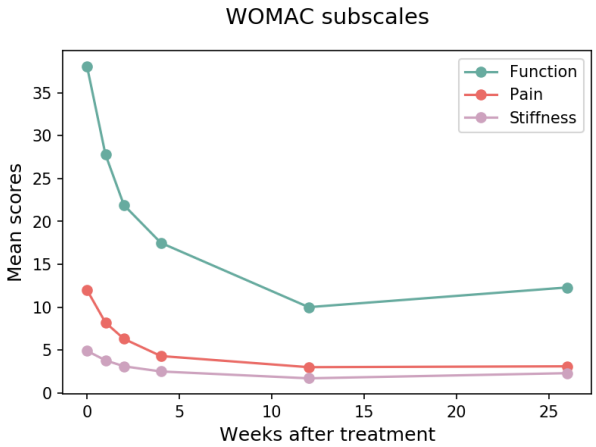

Figure 3.6: Plot of mean WOMAC Function, Pain and Stiffness Subscales scores as a function of time post-treatment. Adapted from [44].

it was plotted a graph based on those WOMAC scores - Figure 3.6. Five different slopes (between points with known coordinates) were taken out of this graph and a wider range of WOMAC scores was estimated - extrapolation of the scores (including mid scores). WOMAC values were calculated in a two-week frequency throughout a year which is twice the time for which the study was conducted. It should be noted that it is assumed that the slope between week 12 and week 26 will be observed for the following weeks (until de 52th week). The WOMAC estimated scores for 52 weeks were then converted into utilities in the form of EQ-5D - a standardised instrument able to measure the health status of an individual or a sample of individuals, through a web tool provided by Wailoo et al. [43] - Figure 3.7. In Wailoo et al. [43] were registered 7072 observations in which OA patients completed both WOMAC and EQ-5D questionnaires. The information was cross-checked and a relationship between utilities EQ-5D and the WOMAC scores (function, pain, stiffness) was established. After one year (52 weeks) of the hypothesised study, the gains in QALY were calculated through Equation 3.29, namely the difference between the estimated utility for week 52 and the initial utility.

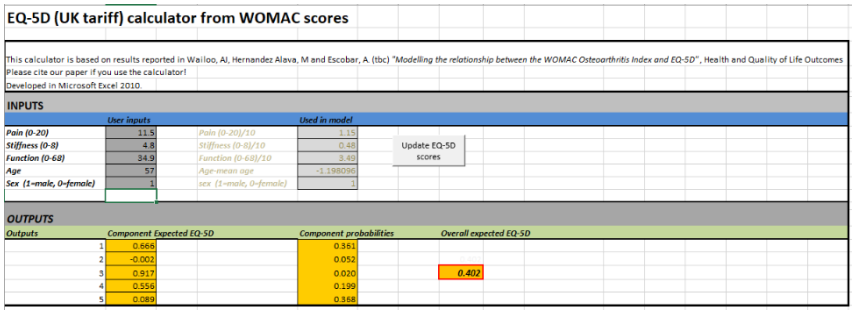

Figure 3.7: Example of the tool used to convert WOMAC scores into EQ-5D utilities. Adapted from [43].

$$QALY_t = (Utility_{\ t} - Utility_{\ t-1}) \tag{3.29}$$

At the end of an analysis, the total cumulative QALYs of a given individual is obtained by adding up the QALY, measured at the end of each year of follow-up.

3.3.2 Sub-model IV: Initialization of a Multi-criteria Decision Analysis

eHTA study was carried out in an environment of uncertainty, and based on a large variety of assumptions, as the therapy (MSC-derived secretome) has not yet been clinically assessed and the hypothetical benefits were assumed. Therefore, it is useful in determining how the variation of a given parameter is reflected in the process, and in turn in supporting decision making.

As mentioned above, there are other aspects besides the CUA that are considered in decision-making in several countries. In this way, a MCDA model is structured to help visualising the extent to which the CUA considers all relevant aspects to the assessment. This prepares a framework for when reliable data on MSC-derived secretome is available to make use of it and draw the appropriate conclusions. Value trees - Figures 4.6 and 4.7, as well as the relevant aspects explanation Tables 4.6 and 4.7 are presented in Chapter 4.

Chapter 4

Results

This chapter presents the sub-model II results obtained for the baseline scenario - Section 4.1 and the sensitivity analysis, given several cases mentioned in the Chapter 3. It is also presented the sub-model III results: cost-utility analysis itself, which combines three levels of analysis - Sections 4.3.1, 4.3.2, and 4.3.3. In addition, the results of the aspects gathered in sub-model IV: MCDA are presented - Section 4.4.

4.1 Baseline scenario

A baseline scenario was simulated, using Monte Carlo simulations (sub-model II), to calculate the costs to obtain the MSC-derived secretome doses, following the envisaged manufacturing process, needed to support a phase II of a clinical trial.

The results on-wards presented were obtained after running the model for 100 runs for each case scenario - Table 4.2. It was observed that, by performing several sets of simulations, a higher number of simulations would not translate into better confidence intervals nor change the mean value of most parameters. Therefore, having a higher number of simulations would only contribute for longer model run times, i.e. for 100 runs the simulation time was around 4 minutes, for 1000 was approximately 40 minutes and finally for 10000 runs it took approximately 22 hours, meaning that it would only increase the time that the model is running without adding significant changes to the results, while having to wait more time for the results do be produced.

The main selected inputs to the model were 1x10^5 for the initial cell number and a seeding density of 3000 cells/cm^2 - Table 4.1. For clinical expansion, it is unfeasible to use very low seed densities since it translates into higher times-to-confluence, higher frequency of medium changes and more ETs utilisation - about 75% of the most recent clinical trials use a seeding density of 3000 cells/cm^2 to further reduce the cost/work trade-off [33].

In this chapter one should remind that 'random' stands for simulation type in which cell growth rate was modelled by a triangular distribution and 'fixed' stands for simulation type in which it was not used a distribution of values for cell growth rates but instead only the mean values. The model does not ask

for the running time which each simulation should last, but rather for patients (N=200) and doses (=5) per patient targeted. Given that, the model assumes a fixed capacity for simulations and no batches can take place concurrently, these parameters (number of patients and number of doses, as well as the initial number of cells and seed density) will define the total duration of each simulation. The pass release ratio on quality control stages (Table 4.1) will play a major role here. In the current configuration, baseline scenario, the average number of batches to obtain doses for target patients was about 18 and 21, for 'random' and 'fixed' simulation type, respectively - Table 4.2.

Table 4.1: Cell processing parameters used to implement the Baseline scenario.

Parameter	**Value**	**References**
Common across stages		
Culture media supplement/mL	0.628€	[89]
DPBS/mL	0.056€	[90]
TrypLE (Harvesting agent)/mL	0.09€	[33]
Expansion		
Initial number of cells	1x10^5	Assumption
Maximum no. passages	3	[33]
Seeding density/passage (cells/cm^2)	3000	[33]
Time to confluence (days)	5	[33]
Harvesting yield	0.9	[33]
Harvesting time	14 min	[33]
Downstream processing		
No. of washes	2	[33]
Volume reduction and washing time	4h	[33]
Volume reduction yield	0.8	[33]
Fill finish time	2h	[33]
Cryovial volume	2mL	[33]
Unit price cryovial	1.1€	[33]
Cryomedium/mL	2.3€	[33]
Ratio cryomedium/basal medium	0.5	[33]
Final product formulation		
Pass/release ratio	0.9	[33]
Price quality control testing/batch	8,441.95€	[33]
Time release testing	2h	[33]
Cleaning up		
Preparation time for the next batch	2h	Assumption

The model starts at the expansion stage, just after thawing the initial cells number, not having isola-

tion stage into account, as it relies on the use of a cell bank to obtained the cells to be seeded to the first expansion. As a consequence, one should just take into account the costs of obtaining a given initial number of MSC and include it as model input, in order to be finally added to CoGs.

Table 4.2: Simulation parameters of the first level of screening according to prob0 simulation type.

Parameter	**Random**	**Fixed**
No. cells at P3/Batch	1.55x10^7	1.34x10^7
No. final exos/Batch	7.8x10^9	6.64x10^9
Doses/Batch	58.45	49.8
Patients/successful Batch	12.9	11.2
Batches to obtain target Patients	17.84	20.24
Batch duration (days)	15.57	15.55
Process duration (days)	277.77	314.76
ETs:		
P1	2 $Tflask25$	2 $Tflask25$
P2	2 $Tflask225$	2 $Tflask225$
CoGs/Batch	21,380.4€	21,295.79€
CoGs Expansion/Batch	351.99€	298.1€
CoGs DSP/Batch	230.28€	199.52€
CoGs FPF/Batch	8,441.95€	8,441.95€
CoGs/Dose	367.59€	429.35€
CoGs/Patient	1,837.95€	2,146.75€

Table 4.2 shows the summary output of the analysis considered as the baseline scenario. It is important to note that the values shown in Table 4.2 refer to the average values per run, in a total of 100 runs, corresponding to prob0 simulation type.

In agreement with Figure 4.1, a set of outputs related to the biological side of the process is portrayed. Overall, analysis after simulations shows that 'random' simulation type yield higher number of cells than the 'fixed' simulation type - Figure 4.1 (b). This result is related to the exponential relation between the cell number and growth rate. Therefore, using a triangular distribution of cell growth rates with the same mean value as the growth rate used for the 'fixed' simulation type - Figure 4.1 (a), generates a higher cell number outputs. An exponential does not preserve the linear relationship observed between the arguments – cell growth rates – so, by using a triangular distribution of cell growth rates with the same mean value will result in a higher distribution of obtained cells in comparison to using only a deterministic value – 'fixed' simulation type, despite having the same mean values for both simulation types.

Figure 4.1 (c) results are in accordance to the modelled condition that each cell will contribute with around 700 exosomes to the secretome.

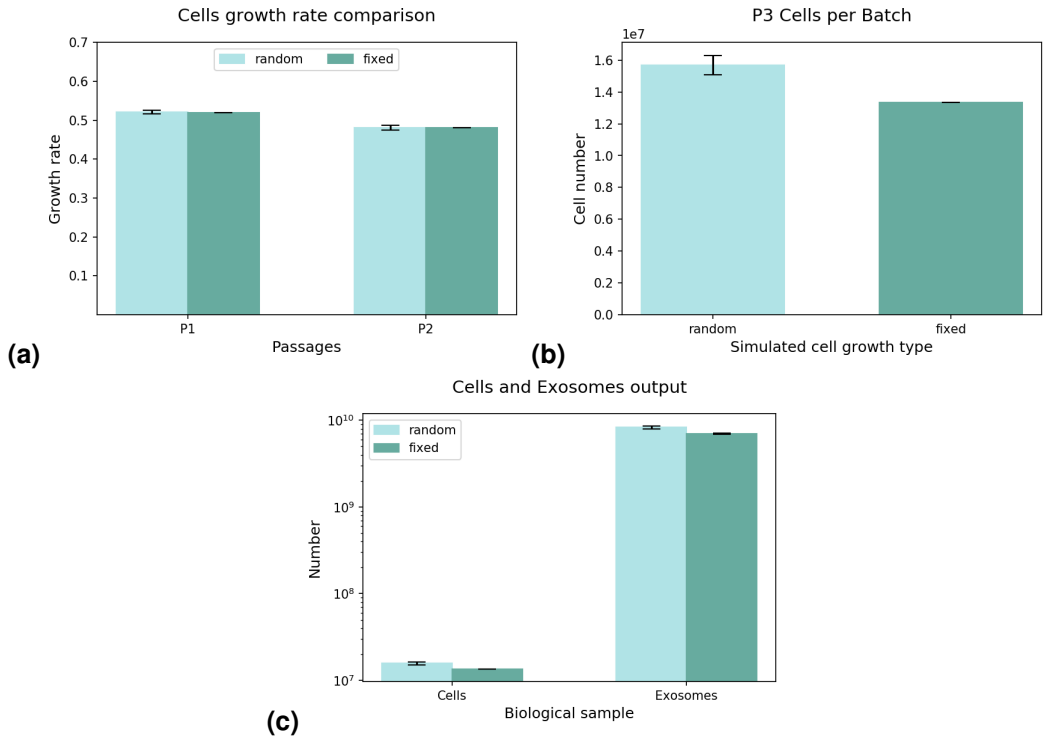

Figure 4.1: **(a)** Mean cell growth rate. **(b)** Cells after the expansion of the P3. **(c)** Cells at the end of Expansion (P3) and Exosomes estimated output by the end of DSP - Baseline scenario - 'random' and 'fixed' simulation types.

Literature points out that the 'pass release ratio' on quality control testing is about 90% [33]. In this step a dose is sacrificed to be tested against the required criteria of quality [33]. When a batch does not pass the quality control testing, all doses of such batch are discarded, but its production cost still need to be incorporated on total dose costs [33].

Figure 4.2 (a) shows the total CoGs per Batch for each simulation type 21,380.5€ and 21,295.8€ ('random' and 'fixed' respectively).

Figure 4.2 (c) portrays the CoGs per Batch of Figure 4.2 (a) broken down into resource categories, which means that the sum of the bars of Figure 4.2 (c) makes up the CoGs per Batch of Figure 4.2 (a), for each simulation type.

Figure 4.2 (b) shows consumables, reagents, and QC CoGs per Batch broken down into process units (Expansion, DSP and FPF - which corresponds to QC resource category). This means that for a simulation type the sum of its bars is equal to the sum of consumables, reagents and QC of Figure 4.2 (c). In Figure 4.2 (b), the Expansion and DSP bars in 'random' simulation type are larger than those corresponding to 'fixed' simulation type. Consequently, the bars for consumables and reagents - Figure 4.2 (c) are also larger than the bars for the 'fixed' scenario. This is because in 'random' simulation type more cells have to be processed, spending more on the overall process (CoGs per Batch). Figure 4.3 (a) shows the total CoGs per Dose for each simulation type 367.59€ and 429.35€ ('random' and 'fixed' respectively). This plot is analogous to the one in Figure 4.2 (a) in the sense that to obtain it the CoGs per Batch are divided by the respective doses produced in that batch. Dose CoGs breakdown are presented either per process unit - Figure 4.3 (b), or per resource category - Figure 4.3 (c). The

qc and facility in Figure 4.3 (c) are the main CoGs drivers (biggest ratio in comparison to the remaining categories) as it was seen in Figure 4.2 (c). Because the objective is to obtain the lowest possible costs, these two categories might have room for improvement.

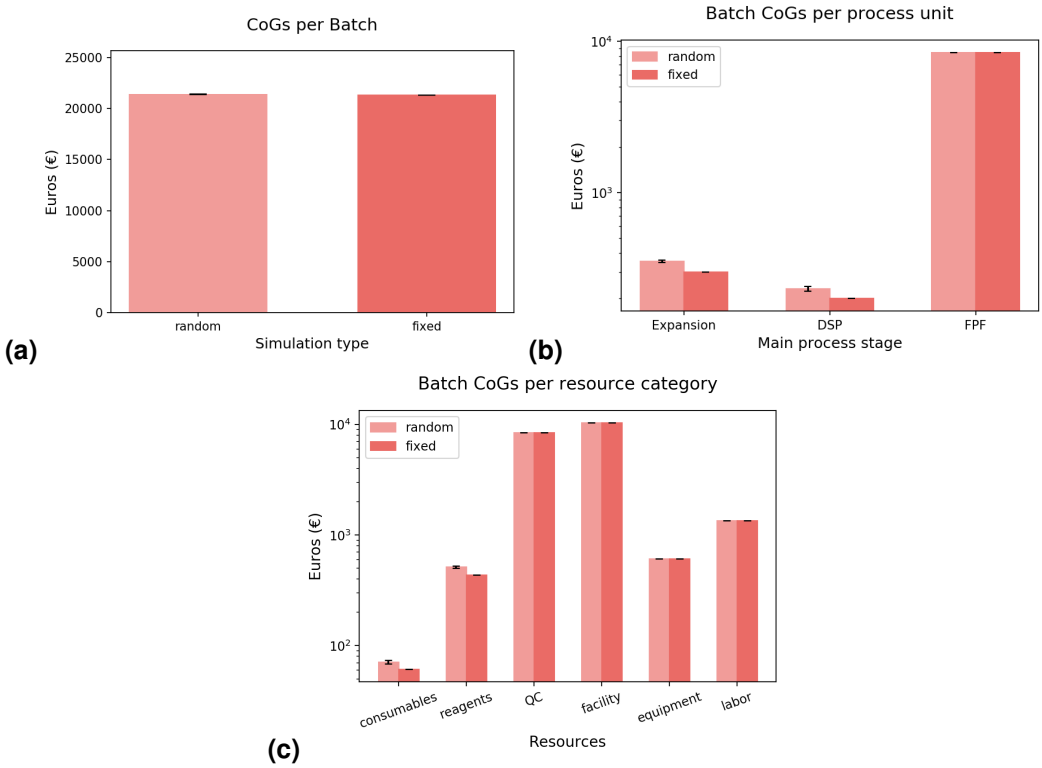

Figure 4.2: **(a)** Direct and indirect CoGs per Batch. **(b)** CoGs per process unit (FPF stands for Final Product Formulation and DSP for Downstream Processing). **(c)** CoGs per resource category, (qc stands for quality control) - Baseline scenario - 'random' and 'fixed' simulation types.

To investigate the influence of 'pass release ratio' on results, it was simulated a scenario in which this parameter was 100%, for both 'random' and 'fixed' simulation types. Scenarios with 100% 'pass release ratio' were called prob1 whilst scenarios in which this variable was kept on 90% were called prob0. The results can be seen in Figure 4.4. Analysing Figure 4.4 (a), at a first sight there is no relevant effect on CoGs per Batch either having a pass release ratio of 100% or 90%, prob1 and prob0 scenarios, respectively. However, in Figure 4.4 (b), comparing 'fixed' CoGs per dose in prob1 (381.76€) with 'fixed' prob0 (429.35€), and analogously 'random' prob1 (325.43€) with prob0 (367.59€), there is a small difference regarding CoGs per dose, where the 'random' simulation type is more profitable. It should be noted that having such small number of runs it is not sufficient to observe the effect of varying the pass release ratio from 90 to 100% (respectively prob0 and prob1).

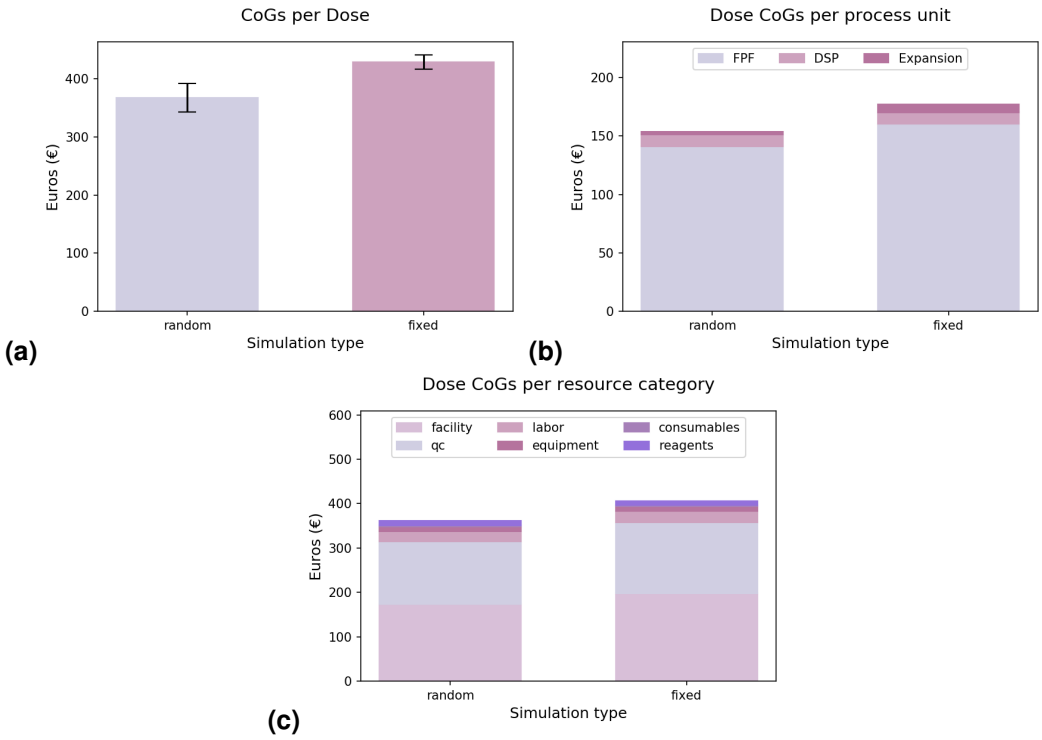

Figure 4.3: **(a)** Direct and indirect CoGs per Dose. **(b)** CoGs per Dose per single main stages of the process (FPF stands for Final Product Formulation and DSP for Downstream Processing). **(c)** CoGs per Dose per resource category throughout the manufacturing process (qc stands for quality control) - Baseline scenario - 'random' and 'fixed' simulation types.

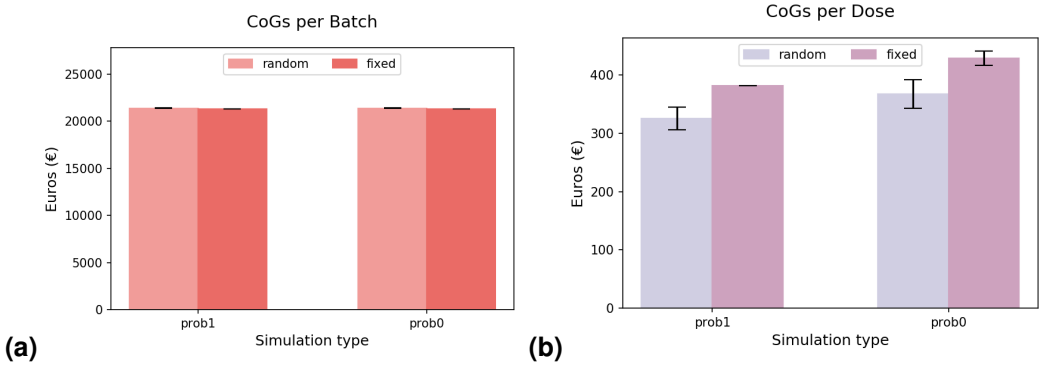

Figure 4.4: **(a)** CoGs per Batch when comparing prob1 vs prob0 **(b)** CoGs per Dose when comparing prob1 vs prob0 - Baseline scenario - 'random' and 'fixed' simulation types.

4.2 Sensitivity analysis outcomes

4.2.1 Decreasing initial number of cells

A sensibility analysis was also done to some of the parameters, to select the most appropriate number of initial cells to be seeding. It was observed that starting with a low initial number of cells would negatively impact the overall costs. By starting with such a low number (10^4), the needed batches to obtain enough doses for a given number of patients would be higher, resulting in higher operating times at the facility and, consequently higher costs. A low initial number of cells would also increase simulation time. In

order to determine the optimal number of initial cells, several scenarios were done - Sensitivity analysis.

For instance, starting with 1x10^4 cells - Figure A.3 (b) - the number of cells at the end of expansion in each batch is on average 1.56x10^6 cells in a 'random' simulation type and around 1.34x10^6 in a 'fixed' simulation type, which is almost ten times less in comparison with the scenario which starts with 1x10^5 cells. Not surprisingly, using such a low number of initial cells (1x10^4) results in CoGs per dose of the order of 4,000€ - Figure A.5 (a). The reason behind observing such an increase in costs is due to the increase in the amount of batches that need to be performed.

When starting with 2x10^4 cells - Figure A.7 (a) - the obtained cells at the end of each passage 3 are about 3.11x10^6 for the 'random' and 2.67x10^6 for the 'fixed' simulation types. Figure A.8 exhibits the costs for this scenario. One can therefore conclude that starting with a higher number of cells (doubling) promotes lower CoGs.

4.2.2 Halving the cost of quality control

In the context of the present work, the number of cells needed to produce the 5 doses of secretome per patient (10^5) is not so high in comparison with literature (10^7) that consider cells as the therapeutic product. Therefore, the quality control price is not dependent on the potential variation of the cells that need to be processed in the present work, having a fixed value independently of the therapeutic that undergoes quality testing. Furthermore, the value used for the quality control stage may correspond to an over estimation for a scenario of non-parallel batches manufacture of an 'off-the-shelf' product for only 200 patients.

For those reasons mentioned above, it was decided to perform a sensitivity analysis. To assess the outcomes if lower costs could be obtained, it was simulated a scenario in which one would have the costs regarding that category (quality control costs) reduced in half. As the quality control step does not directly depend on biological parameters, such as number of cells to be processed - no difference is observed between the baseline scenario - Figure 4.1 (a) and Figure A.11 (a).

QC decreases from around EUR 8,000 (Figure 4.2 (c)) to EUR 4,000 (Figure A.12 (c)). QC is a parameter that greatly influences the CoGs per Batch. Therefore, CoGs per Batch corresponding to this scenario also differ when compared to the CoGs/Batch in baseline scenario.

Table 4.2 shows that CoGs/Batch in baseline scenario are 21,380.95€ and 21,295.8€ ('random' and 'fixed', respectively). On the contrary, when reducing by half the quality control costs - scenario under study, it is estimated CoGs per Batch at values of 17,156.3€ and 17,074.8€, for 'random' and 'fixed', respectively.

It follows that for both scenarios (baseline scenario and half the cost of qc) the average CoGs/Batch values corresponding to the 'random' simulation types are slightly higher than the values estimated in the 'fixed' simulation type. In short, reducing the cost of qc by half, also batch costs corresponding to it will be reduced by approximately 4,224€ ('random') or about 4,221€ ('fixed').

To illustrate, when in the presence of the baseline scenario, according to Table 4.2, for 'prob0' simulation type the CoGs/Dose is 367.59€ or 429.35€ ('random' and 'fixed', respectively). On the other hand, when qc is halved, the cost per dose is 297.61€ or 343.95€ ('random' and 'fixed', respectively). It

follows that costs per dose decrease by approximately 70€ in 'random' and around 85€ in 'fixed'. Thus, per patient cost there is a reduction of about 350€ or 425€.

As such, as quality control costs do not change with the number of doses produced (within the ranges considered), a reduction on quality control costs will be reflected on lower dose CoGs - Figure 4.3 and Figure A.13.

4.2.3 Halving the cost of facility

Following the same line of thought, the same was performed for facility costs: a scenario to have half its value. The results are shown in Figures A.15, A.16 and A.17. One should bear in mind that facility costs will greatly depend on the country, or even the city that the facility plant is located and consequently the labor price will vary accordingly too.

4.3 Cost-Utility Analysis

This section presents three levels of analysis: (i) CoGs per patient of the MSC-derived secretome, estimated from 'random' simulation type, remain the same (1,837.95€), and APS price is taken out of literature (690€) [19]; (ii) a reduction of 50% of CoGs per patient of the new therapy (from 1,837.95€ to 919€); and (iii) a reduction of 75% of CoGs per patient of the new therapy (from 1,837.95€ to 459.5€). The analysis was carried out by price clusters and three hypotheses were analysed: effectiveness of the new therapy same as APS, effectiveness of the new therapy 1.5 (0.6 QALY), and 2 times (0.8 QALY) higher than APS' effectiveness, respectively - Table 4.3.

Table 4.3: Incremental costs and incremental effectivenesses of MSC-derived secretome and APS therapies for three levels of analysis. CoGs per patient derived from 'random' simulation type were established as MSC-derived secretome price.

Level of analysis	**Price**			**Outcomes**			
	MSC-derived secretome	APS	Δ Cost (€)	MSC-derived secretome	APS	Δ Effectiveness (QALY)	**ICER**
	1,837.95	690	1,147.95	0.4	0.4	0	0
(i)	1,837.95	690	1,147.95	0.6	0.4	0.2	5,739.8
	1,837.95	690	1,147.95	0.8	0.4	0.4	2,869.9
	919	690	229	0.4	0.4	0	0
(ii)	919	690	229	0.6	0.4	0.2	1145
	919	690	229	0.8	0.4	0.4	572.5
	459.5	690	-230.5	0.4	0.4	0	0
(iii)	459.5	690	-230.5	0.6	0.4	0.2	-1,152.5
	459.5	690	-230.5	0.8	0.4	0.4	-576.3

The early assessment comprises a sub-model II with a cost-utility analysis associated (sub-model III). MSC-derived secretome therapy costs were calculated through sub-model II - Monte Carlo simulations and then connected to the clinical cost-effectiveness by providing the CoGs of therapy per patient, including the 5-dose series.

In this study, it is assumed that the average CoGs of therapy per patient is the price of the new therapy, because there is no information about this therapy regarding cost and effectiveness. On the other hand, the price of APS considered here is the cost of nSTRIDE APS kit, since is the only value available in literature [19].

A first case of 200 patients, in 'random' simulation type was considered. In this Baseline scenario is estimated that each batch produces enough doses to treat an average of 13 patients and to deliver the 5 doses to the 200 patients it is needed 18 total batches which will take 278 days. On the other hand, the fixed simulation estimates that each batch produces therapeutic sufficient to treat an average of 12 patients per batch and to fulfil the same demand of 1000 doses is required a total of 21 batches, implying a total operation time of 315 days. These calculations do not consider the possible processing of several batches in parallel.

The goal was not to produce as much therapeutic as possible in the lowest period of time. Instead, the objective was to provide the demand for a phase two clinical trial in which an 'off-the-shelf' product was iteratively administered to a set of patients during a pre-established time span, with proper follow-up, assessing outcomes, safety, biological value and activity; in other words to assess safety and effectiveness of the new ATMP for OA management.

After the follow-up period, manufacturing costs and the sum of the utilities per year were estimated, allowing to calculate the total of QALYs. The cost effectiveness of the new treatment was assessed as an ICER, calculated according to Equation 2.1. MSC-derived secretome therapy is only cost-effective if it is below a certain threshold of WTP in cost/QALY.

4.3.1 (i) First level of analysis

This analysis is presented in the first three rows of the Table 4.3.

Estimated Costs

The analysis was made using CoGs derived from 'random' simulation type. CoGs obtained are lower and, importantly, capture biological diversity. Note that for each patient, it is envisaged a 5 dose posology. Therefore, the costs hereby considered regarding MSC-derived secretome therapy are the average CoGs per patient previously calculated.

According to Section 4.1, on average, the novel therapy costs per patient 1,837.95€ - Table 4.2. On the other hand, APS (current treatment) costs approximately 690€ [19]. With this information the Incremental Cost (IC) calculations, i.e the difference of MSC-derived secretome cost to nSTRIDE APS kit cost was estimated at 1,147.95€ - Expression 4.1.

$$IC = 1,837.95 - 690 = 1,147.95 \tag{4.1}$$

Equal Effectiveness In this scenario is considered that effectiveness in QALYs of MSC-derived secretome therapy is equal to APS (both 0.4 QALY) - first row of Table 4.3.

Kon et al. [42] report that EQ-5D utility of an OA patient, without any treatment, is 0.402 (man) and 0.394 (woman). Cumulative effectiveness was estimated, for a sample of patients with average age of 57, during 3 years of follow-up and according to Table 4.4, at a value of 0.293 (man) and of 0.292 (woman) QALYs.

Table 4.4: WOMAC scores converted in EQ-5D utilities and QALYs. Derived from [42].

	WOMAC scores			**EQ-5D utilities**			**QALYs**	
Year	Pain	Stiffness	Function	Man	Woman	Time	Man	Woman
0	11.5	4.8	34.9	0.402	0.394			
1	4.3	2.7	15.6	0.735	0.727	0-1 year	0.333	0.333
2	4.5	2.4	14.4	0.74	0.732	1-2 year	0.005	0.005
3	5.7	2.8	18	0.695	0.686	2-3 year	-0.045	-0.046

van Drumpt et al. [44] point out that an OA patient without treatment at 57.5 years of age, QALY is estimated to be at 0.35 (man) and 0.34 (woman) - the baseline utility. The resulting gained QALY was estimated at 0.4 (for man and woman) - Table 4.5.

Table 4.5: Extrapolation of WOMAC scores converted in EQ-5D utilities and QALYs. Derived from [44].

	WOMAC scores			**EQ-5D utilities**	
Week	Pain	Stiffness	Function	Man	Woman
0	12	4.9	38.1	0.35	0.34
2	6.3	3.1	21.9	0.66	0.65
4	4.3	2.5	17.5	0.72	0.71
6	3.975	2.3	15.625	0.74	0.73
8	3.65	2.1	13.75	0.76	0.76
10	3.325	1.9	11.875	0.79	0.78
12	3	1.7	10	0.81	0.81
14	3.014	1.782	10.328	0.81	0.80
16	3.028	1.868	10.657	0.81	0.80
18	3.043	1.953	10.985	0.80	0.80
20	3.057	2.039	11.314	0.80	0.79
22	3.071	2.125	11.642	0.80	0.79
24	3.085	2.211	11.971	0.80	0.79
26	3.1	2.3	12.3	0.79	0.79
28	3.114	2.382	12.628	0.79	0.78
30	3.128	2.468	12.957	0.79	0.78
32	3.143	2.553	13.285	0.78	0.78
34	3.157	2.639	13.614	0.78	0.77
36	3.171	2.725	13.942	0.78	0.77
38	3.185	2.811	14.271	0.78	0.77
40	3.2	2.896	14.6	0.77	0.76
42	3.214	2.982	14.928	0.77	0.76
44	3.228	3.068	15.257	0.77	0.76
46	3.243	3.153	15.585	0.76	0.75
48	3.257	3.239	15.914	0.76	0.75
50	3.271	3.325	16.242	0.76	0.75
52	3.285	3.411	16.571	0.75	0.74

Therefore, it is estimated an increase of 0.4 QALY per patient on average during one year follow-up,

in the following calculations.

The IC of MSC-derived secretome is 1,147.95€ when compared with the current treatment. Here, patient's outcomes of both MSC-derived therapy and APS are the same. This initial scenario was selected to address the challenges regarding scarce information and lack of available data, at this point, in clinical trials in human for OA treatments based MSC secretome. Therefore, it is not possible to compare those two treatments regarding effectiveness. Still, it is possible to compare them, regarding costs, where the estimations of the current study point out that MSC- derived secretome therapy is, overall, a more costly treatment than APS due to high upfront costs. Therefore, unless there is clinical data showing that the new envisaged product provides a significant increase on therapeutic effectiveness or its manufacture is possible at significant lower costs, APS should be continued and no investment should be made in the new therapy.

Effectiveness of 1.5-fold This scenario assumes that effectiveness of MSC-derived secretome is 1.5 times higher than APS, (0.6 QALY and 0.4 QALY, respectively) - second row of Table 4.3.

$$ICER = \frac{1,837.95 - 690}{0.6 - 0.4} = 5,739.8 \tag{4.2}$$

ICER is computed by dividing the value of 4.1 by incremental effectiveness. Therefore, ICER is 5,739.8€/QALY - Expression 4.2.

Effectiveness of 2-fold Here, it is assumed that effectiveness of MSC-derived secretome is 2 times higher than APS, (0.8 QALY and 0.4 QALY, respectively) - third row of Table 4.3.

$$ICER = \frac{1,837.95 - 690}{0.8 - 0.4} = 2,869.9 \tag{4.3}$$

This results in 2,869.9€/QALY for the introduction of the therapy as calculated in Expression 4.3.

4.3.2 (ii) Second level of analysis

To assess the level of effectiveness that the new therapy would need to reach to provide a cost-effectiveness strategy to treat OA a second analysis was carried out. This analysis can inform on the target endpoints levels for a clinical trial to be considered successful. The ICER value was estimated to new scenarios. This analysis is presented in the second three rows of the Table 4.3.

Estimated Costs - Reduction of 50%

$$1,837.95 \times 0.5 = 919 \tag{4.4}$$

$$IC = 919 - 690 = 229 \tag{4.5}$$

Table 4.2 shows that the average of CoGs per patient of MSC-derived secretome is estimated to be 1,837.95€, and a reduction of 50% makes up a total of 919€ - Expression 4.4. Here, the IC is estimated

at 229€ - Expression 4.5.

Equal Effectiveness In this scenario, the outcomes (in QALYs) of MSC-derived secretome therapy and APS are equal (both 0.4 QALY) - fourth row of Table 4.3. Considering the same outcomes, it is not possible to compare those two treatments regarding effectiveness. Still, it is possible to compare them regarding costs, even when the new therapy is halved in cost, it is still more expensive (919€) than APS (690€).

Effectiveness of 1.5-fold In this scenario, it is assumed that effectiveness of MSC-derived secretome is 1.5 times higher than APS, (0.6 QALY and 0.4 QALY, respectively), when a price reduction of 50% is also considered - fifth row of Table 4.3.

$$ICER = \frac{919 - 690}{0.6 - 0.4} = 1145 \tag{4.6}$$

1145€/QALY corresponds to the introduction of the new therapy in this case scenario - Expression 4.6.

Effectiveness of 2-fold The effectiveness of MSC-derived secretome is assumed to be 2 times higher than APS, (0.8 QALY and 0.4 QALY, respectively), at the same time as a price reduction of 50% is considered - sixth row of Table 4.3.

ICER of this case study is calculated, taking the previously IC value - Expression 4.5, and dividing it by the incremental effectiveness.

$$ICER = \frac{919 - 690}{0.8 - 0.4} = 572.5 \tag{4.7}$$

This results in 572.5€/QALY for the introduction of the MSC-derived secretome - Expression 4.7.

Despite the reduction in costs by 50%, the biotherapeutic remains more expensive than APS. Therefore, the adoption of therapy is questionable.

4.3.3 (iii) Third level of analysis

This analysis is presented in the last three rows of the Table 4.3.

Estimated Costs - Reduction of 75%

MSC-derived secretome price is estimated to be 1,837.95€, and a reduction of 75% makes up a total of 459.5€ - Expression 4.8. The IC would then be calculated - Expression 4.9.

$$1,837.95 \times 0.25 = 459.5 \tag{4.8}$$

$$IC = 459.5 - 690 = -230.5 \tag{4.9}$$

Equal Effectiveness In this scenario, it is considered that effectiveness in QALYs of MSC-derived secretome therapy is equal to APS (both 0.4 QALY) - seventh row of Table 4.3. Once again, since the effectiveness of APS and MSC-derived secretome therapy is the same, a comparison can only be made in terms of costs. With the 75% reduction MSC therapy becomes more affordable (459.5€) than APS (690€).

Effectiveness of 1.5-fold A possible strategy for the new therapy to compete with APS would be to increase therapeutic outcomes allied to a cost reduction. It is established a scenario where the effectiveness of MSC-derived secretome is 1.5 times higher than APS, (0.6 QALY and 0.4 QALY, respectively), considering a price reduction of 75% - eighth row of Table 4.3.

ICER is calculated, taking the previously IC value - Expression 4.9, and dividing it by the incremental effectiveness.

$$ICER = \frac{459.5 - 690}{0.6 - 0.4} = -1,152.5 \tag{4.10}$$

ICER for this scenario is estimated at -1,152.5€/QALY according to Expression 4.10. So, 1,152.5€ could express the saving for each QALY gained.

Effectiveness of 2-fold A scenario where the effectiveness of MSC-derived secretome is 2 times higher than APS, (0.8 QALY and 0.4 QALY, respectively), is also assumed while a price reduction of 75% is considered - ninth row of Table 4.3.

ICER is calculated, taking the previously IC value - Expression 4.9, and dividing it by the incremental effectiveness.

$$ICER = \frac{459.5 - 690}{0.8 - 0.4} = -576.3 \tag{4.11}$$

For this scenario, ICER is -576.3€/QALY which means that the implementation of the novel therapy could represent a saving of 576.3€ for every each QALY gained - Expression 4.11.

Either in Section 4.3.1 (5,739.8€/QALY and 2,869.9€/QALY) or Section 4.3.2 (1145€/QALY and 572.5€/QALY) the incremental cost and effectiveness are both positive - Figure 4.5. Therefore, MSC-derived secretome therapy is plotted in the NE quadrant. In this quadrant, therapies, i.e. the new biotherapeutic, are more effective, yet more expensive than comparator therapy - APS. To interpret the results obtained, ICER needs to be compared with a specified threshold - WTP. Except when the effectiveness of the two therapies (MSC-derived secretome and APS) is equal, the novel therapy is cost-effective because it lies below the WTP. In Section 4.3.3 were calculated negative ICERs at values of -1,152.5€/QALY or - 576.3€/QALY, respectively for 1.5-fold or doubled the effectiveness scenarios - Figure 4.5. The negative ICER is associated to additional effectiveness, with the values for the novel therapy falling on the SE quadrant of the Figure 4.5, and this being more cost effective than the current treatment (APS), since interventions in this quadrant are less expensive and more effective.

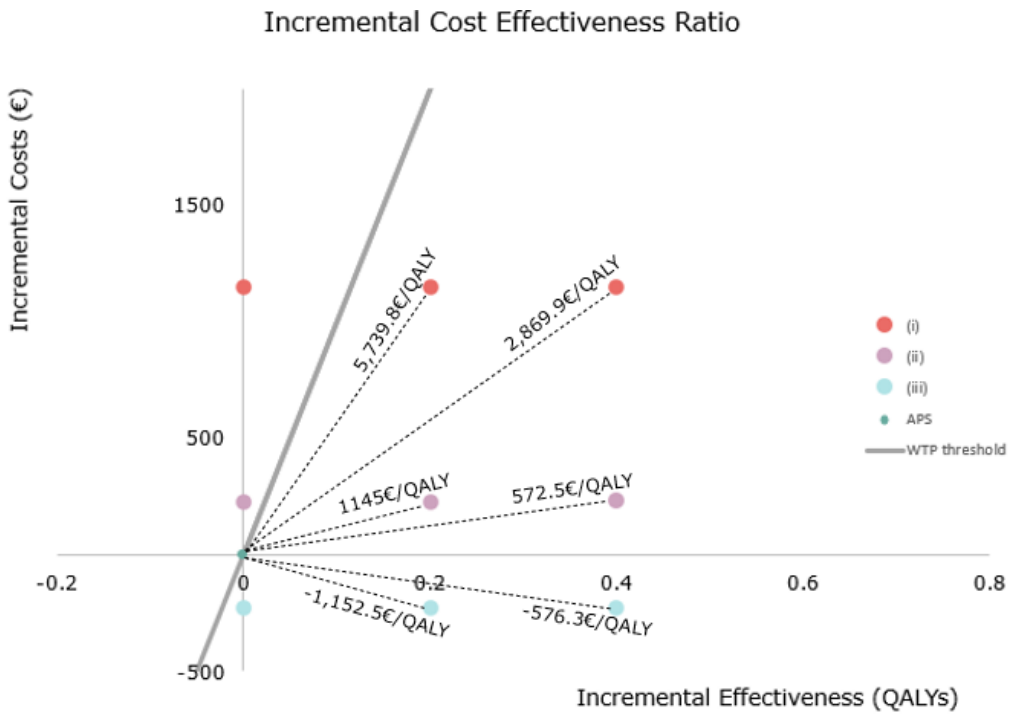

Figure 4.5: Cost-effectiveness plane with incremental effectiveness in QALYs on the x-axis and incremental costs in EUR on the y-axis. (i) corresponds to the first level; (ii) - second level and (iii) - third level of analysis.

In Figure 4.5, (i), (ii), and (iii) points represent the MSC-derived secretome therapy. Points on the y-axis represent the scenarios where the effectiveness of MSC-derived secretome is equal to the APS. Dashed lines connecting MSC-derived secretome therapy to the origin (APS therapy) represent the ICERs for each scenario. In this study, is established a WTP of 10,000€/QALY as reported in [67].

According to previously mentioned assumptions of new therapy ICER, the reasoning is not always so straightforward. If the therapy is too costly, the decision-maker may sense that the decision to invest is too uncertain. This uncertainty level is subjective and it will change depending on each biotherapeutics' adoption and stakeholders who decide to uptake or not. The uncertainty associated with economic evaluations is highly significant in the case of MSC-derived secretome, once there is lack of supporting evidence on its benefits (effectiveness), costs and long-term results.

4.4 Multi-criteria Decision Analysis

MCDA aims to survey other aspects (patient lost wages, ease of therapy administration, etc.), important to be considered at the time of decision, which are not fully captured by CUA. Namely, it is important to consider not only the the impact for the patient but also for impact for the manufacturer and the health system administrator. In this way, MCDA is used as a reminder of the eHTA associated uncertainty and is then adopted in such a way as to contemplate variables (not exclusively related to the OA patients) that the previous analyses do not offer. Figure 4.6 shows a value tree which sets out aspects that have been considered pertinent to assess in this study, based on advantages of the biotherapeutic, presented in literature. In addition to the manufacturing costs per patient of MSC-derived secretome and the pa-

tient outcomes measured in QALYs, other potential aspects should also be considered in order to have a broader view of the study, i.e. following a societal perspective. In Section 4.3, the incremental costs, incremental effectiveness, and ICER were calculated allowing to compare the new therapy against the comparator therapy. In this Section is investigated the socioeconomic impact of OA, highlighting that decisions are not limited to manufacturing costs alone - Table 4.6 shows the direct costs of healthcare use in OA context (e.g. treatment acquisition and administration in patients), as well as indirect costs, which are not related to healthcare (e.g. lost wages of patients who suffer from OA) [13]. Additionally to those costs, administration and monitoring costs incurred by healthcare providers should also be covered in MCDA model - see Figure 4.6. Apart from the outcomes studied only for the patient (Pain release, Function increase and Stiffness improvement), the new therapy can also bring benefits to the medical personnel, since the MSC-derived secretome therapy could be easy to administer and does not involve invasive procedures. In the value tree presented, the patient's risks are, among others, also accounted - it is crucial to take into account side effects when adopting a novel health therapy. Health risks can include both individual and public health risks affecting the wider population. Hence, the assessment of patient risks - health side-effects of the new ATMP should cover both short-term and long-term physical and psychological aspects [91].

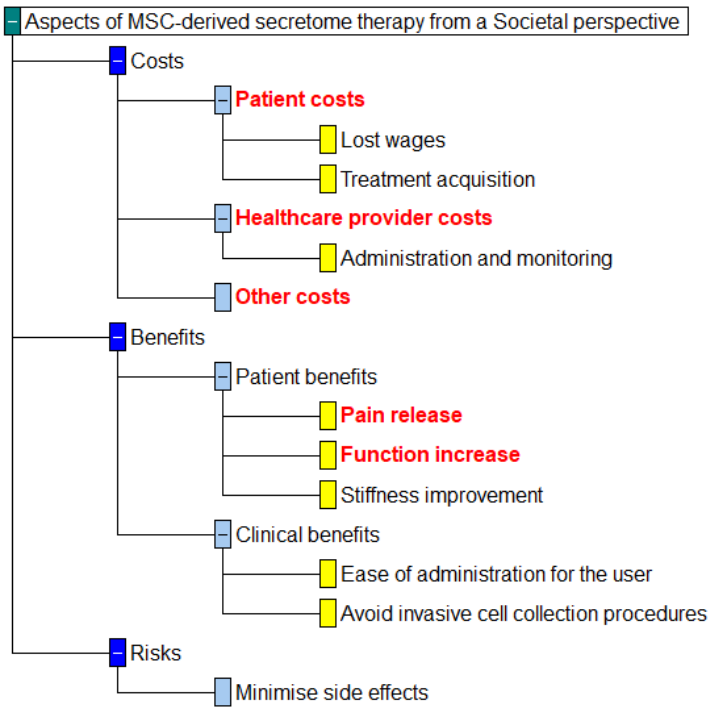

Figure 4.6: Value tree of aspects from a societal perspective which complement the analysis previously made.

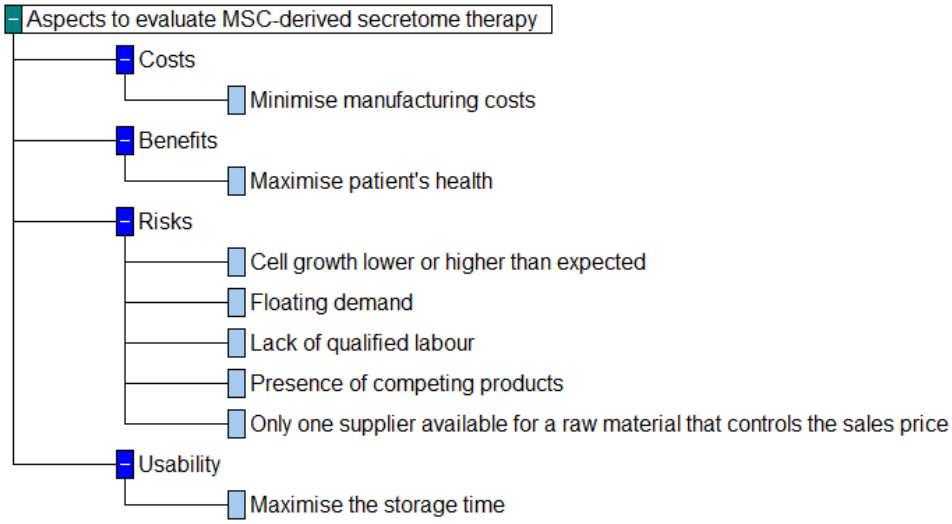

Figure 4.7: Value tree of aspects from a societal perspective, manufacturer's component.

A reminder should also be made regarding the factors inherent to the company, which will produce the therapy. As it seeks to receive the reimbursement of the product it will also be adopted a societal evaluation perspective - Figure 4.7. As one would expect, at this level of evaluation the company that will produce the new MSC-derived secretome must also take into account costs, benefits, risks and also usability as relevant aspects that are present in the initial stage of preparation of this MCDA tool in order to assist decision making. The manufacturers' objective are to produce the therapy at the lowest possible cost for maximum effectiveness in the patient's health (which is assessed through the endpoints aforementioned). It is also presented a set of risks to which the company is subject throughout the biotherapeutic development process - See Table 4.7.

Table 4.6: Explanation of aspects presented in the value-tree in order to evaluate MSC-derived secretome therapy from a Societal perspective.

Criteria	Description	References
Patient costs	It includes lost wages which are the cost of taking time off from work and treatment acquisition costs (including out of pocket expenditures). In the absence of licensed products being available, there are currently no commercially available estimates of the acquisition cost of MSC-derived secretome therapy. Informal sources have shown that future acquisition costs may be between of 4,220.98€ - 8,441.95€ range.	[5, 17]
Healthcare provider costs	Administration and monitoring costs include all costs incurred by the provider in delivering health service to a patient. Consisting of health care professionals fees, costs of medications, equipment, consumables, etc.	[17]
Other costs	The medical (hospitalisation, long-term care) and non-medical costs (home care, social welfare services) and also productivity losses which might be the leaves taken by employee.	[17]
Pain release Function increase	Due to the fact that this therapy is at a very early stage and given the lack of information. An assumption is made where the result regarding to the patient's benefits relative to the therapy in hand is the same as the comparator. OA symptoms such as pain and function were significantly improved. So, those two symptoms are the study endpoints captured by WOMAC scores.	[42, 44]
Risks	It addresses several safety considerations potentially associated with the transplantation of both living and proliferative cell populations, including immune compatibility, and the transmission of infections. This therapy then aims to minimise the side effects.	[38, 92]

Table 4.7: Explanation of aspects to evaluate MSC-derived secretome therapy, manufacturer's component.

Criteria	Description	References
Costs	The manufacturing costs are minimised since the therapy uses a considerably low number of cells, so the cost of expansion and maintenance of cultured MSC could be greatly reduced.	[38]
Benefits	As biological factors can be modified, it is possible to adapt therapy to the needs of individuals in order to maximise their health	[38]
Risks	There are several risks with internal causes such as variations in key parameters of the process or the cells' properties. On the other hand, there are external causes such as fluctuations in demand, the emergence of competing products or failure of suppliers.	[92]
Usability	Storage can be maximised (-20ºC for 6 months or -80ºC for up to 2 years) without the use of potentially toxic cryopreservation for a long period and without losing the biological activity of the product.	[38]

To sum up, the point here is to consider either costs, benefits or risks not only to patient itself but also to their families, manufacturers and society as a whole [12]. Along with the CUA model, one of the targets of this exploratory study is achieved here - to create awareness of what it will take for the biotherapeutic to be adopted or not, as well as, contribute to its research and development.

Chapter 5

Discussion

The production of the therapeutic product based on the MSC scretome, which include the exosomes and extra vesicular proteins carried out to the final formulation has been envisaged on this project, considering the production of doses enough to support a phase two clinical trial with 200 patients, each receiving 5 doses [37]. An envisaged manufacture process comprising cell expansion, secretome production and secretome/exosome isolation. Here, it is proposed successive centrifugations until the exosomes are purified and ready to be included in the final formulation of the MSC-derived secretome product, Figure 3.4 [80]. This work aims to outline and simulate the whole manufacturing process by understanding the strengths and weaknesses of this approach. APS was selected as comparator for this analysis, assuming on a central scenario analysis that the therapeutic effect on the patient should be similar for APS and the new secretome/exosome based product [40].

According to the results obtained for the utilities measured in EQ-5D, in Table 4.4 whose follow-up time is 3 years, APS is assumed to be a therapy with short-term benefit, since the greatest increase in QALYs is at the end of the first year (0.333) and then in the year after the increase in utilities is minimal (0.005). Lastly, as opposed to before, the third year represents a decrease estimated at 0.045 QALYs. Therefore, after three years follow-up the cumulative effectiveness is 0.293 QALYs. Nonetheless, on the assumption of values presented by van Drumpt et al. [44], the utility gain at the end of the first year is 0.41 which diverges from 0.333, the result presented by Kon et al. [42] at the end of the first year of study.

Taking into account previous clinical information on MSC cell based and MSC secretome based therapies, as well as the concentration factors of the secretome achieved during the bioprocess, it was established as objective to administer 10 mL of the new therapeutic. However such a high volume should not be administered at once to the patient, that is why volume reduction step is required [79]. Therefore, it is chosen to split up the administration (a series of five doses with 2 mL each), also with the aim to extend the effect of the therapy on the patient. Contrarily to what can be seen in literature [44, 21, 45] regarding APS where the patient is injected only once with 2.5 mL, see Table 3.3.

Cost-effectiveness analyses are generally designed to assist decision makers choosing between existing treatments and a new therapy. When several options exist, then an optimal cost-effectiveness

analysis will compare them and conclude which one is best [93]. The present study was conducted from societal perspective, using a WTP of 10,000€/QALY. As previously reported [14], before to decide on investing on a therapy the manufacturing company and health payment provided must to calculate the cost-effectiveness and confirm is below the WTP threshold, ranging between 10,000€/QALY, and 100,000€/QALY for knee OA patients, established at approximately 50,000€/QALY in the US [68]. In this study, a conservative WTP of 10,000€/QALY, is used as in Russo et al. [67]. The higher the WTP, the more likely it is that the therapy will be accepted as cost-effective. According to Salmon et al. [66] studies that adopt a societal perspective may include WTPs ranged between 305€/QALY to 53,225€/QALY. The CUA reveals that either MSC-derived secretome therapy or APS injections for the treatment of knee OA would be considered cost-effective (lower than 10,000€/QALY), depending on the level of screening that is being studied. According to the level of screening where the effectiveness is assumed to be double, both new and current therapies are cost-effective. If the new therapy would bring reduced costs and increased quality of life, then it should be adopted [93]. Assuming the scenario where incremental effectiveness between MSC-derived secretome and APS is zero - Equal Effectiveness in quality of life for the OA patients; and MSC-derived secretome's price is higher than APS' price - Section 4.3.1. Therefore, for this scenario the choice to maintain the APS therapy and do not invest on a MSC-derived secretome based therapy becomes clear. Still other hypotheses - Sections 4.3.2 and 4.3.3, show would make sense to invest on the new therapy, for scenarios where (i) the price of the MSC-derived secretome is reduced 75% (in comparison with the baseline scenario) and provide the same therapeutic outcome or (ii) when cost of the new therapy is reduced 50% (in comparison with the baseline) and provides the double of the effectiveness than APS on treating OA therapeutic. Those results point out the need to reduce CoGs and be more ambitious on clinical trial target endpoints.

Indeed, the results of in the Chapter 4, indicate that a scenario where the new therapy would be competitive in comparison with APS is achieved in the third level of analysis, where a 75% cost reduction is achieved, which is quite challenging.

Still, according to information in Appendix A, an important driver for reduction of CoGs per dose is to reduce facility price, and opt for cheaper quality control assessments, which are the major drivers for the high cost per dose of MSC secretome therapy. The yields used and the losses considered throughout the product manufacturing process are also factors that contribute to costs increase. In order to reduce CoGs per dose, a scale-up approach, where higher number of doses are produced in each batch. For example, scale-up of batch size will imply a lower cost in quality control by dose, but probably larger amounts of doses will be discarded. Again the increase on scale will allow to produce more doses per m^2 of facility and per human resource, but will imply the use of different equipment (e.g. cell factories, rather than T-Flasks) or even to replace 2D planar technologies by the use of bioreactors [33]. Those options of project will reduce the cost per dose, but such economy of scale will be only possible for higher demand.

It is also interesting to discuss the use of MSC-derived secretome therapy in comparison of therapies where the MSCs themselves are the therapeutic agent [33]. In the case of cell-based therapy, it is required, on average, $70x10^6$ cells per patient (per dose) through 2D cell expansion technologies and

the production costs are around 20,000€ [33]. On the other hand, in the case of this novel therapy, for the particular case considered of producing doses for 200 patients, around 1.16x10^8 cells would be needed, and the equivalent number of cells per patient being around 5.8x10^5 cells. However, the new therapy here envisaged does not use cells but exosomes, it was assumed (based in literature [94]) that for 200 patients around 6.7x10^{10} exosomes would be needed, i.e. 3.4x10^8 exosomes per patient. The production costs, estimated through Monte Carlo were around 367.59€ or 429.35€ per dose and 1,837.95€ or 2,146.75€ per patient (5 doses per patient), for 'random' and 'fixed' simulation types, respectively. Thus, the cell numbers presented for MSC-derived secretome therapy are only a basis for comparison with an existing cell therapy. One should bear in mind that comparisons are only valid to a certain extent and given a set of assumptions, and should only be interpreted according to the available information, given that not often costs are known and publicly available due to commercial interests. Therefore, if per patient in a cell therapy the manufacturing cost is around 17,000€ [33], and in this therapy it will be around 2,000€, it can be said that this therapy could be a new direction of research in regenerative medicine and could even replace an exclusively MSC-therapy, if one can effectively prove that its outcomes are sufficiently beneficial.

This research is not free of constraints, one can point out that the present work does not take into account other costs a patient incurs along the clinical pathway, despite being referred, because they are not publicly available (in case of APS therapy) or there is lack of literature on it (as it has not yet been properly investigated - MSC-derived secretome therapy). Another limitation is the assumption that MSC-derived secretome's cost of manufacturing (CoGs/Patient - Table 4.2) is equal to the final product price. The same applies to APS therapy, where the cost of nSTRIDE (APS) kit [19] is taken as the therapy price in the economic evaluation - Table 4.3. Bandeiras [33] assumes that the manufacturing costs are 20% of the final price of the cell therapies, however the present study of MSC-derived secretome (cell-free therapy) does not take into account this ratio.

Besides that, the costs used in this study for instance price of clean and non clean room space, daily worker pay, annual costs of several procedures (Table A.1), may differ significantly based on location, contractual agreements, insurance coverage, and may not be widespread to the population as a whole [68]. A further constraint is the time involved in manufacturing an APS injection not being publicly available. That way, is not possible to establish a comparison between manufacturing times of APS and MSC-derived secretome injections. Within the course of eHTA of a new therapy, uncertainty typically arises from the fact that the studies on which the decision is based are sample studies [42, 44, 45], with little representativeness. Nonetheless, uncertainty may also be connected with other issues, such as the assumptions of the model - Table A.2, and the lack or poor quality of evidence for both MSC-derived secretome and APS therapies. Thus, all possibilities studied here have a great degree of uncertainty associated, in relation to the context of the uptake therapy leading to the possibility of bias [14].

Besides the CUA model, the exploratory study aims to go further. Therefore, it is discussed broader view on the drivers (e.g. patient costs, healthcare provider costs, clinical benefits, risks) for decision-making on whether or not to implement the new MSC-derived secretome product. Those drivers are referred in Tables 4.6 and 4.7. Additionally to the such drivers, others factors need to be considered,

such as patient satisfaction or preference, the severity of the OA condition, the accessibility to the therapy and even the innovation level [12, 95]. That said, the first three steps in the formulation of a MCDA model are achieved - see Section 2.6.4. However, in order to complete the MCDA process, more steps would have to be included [64]. Those are describing the performance value of each option against the criteria (which might be the aspects suggested in the value trees – Figures 4.6 and 4.7, by determining the scores of the options (MSC-derived secretome and APS) - Scoring; Selecting weights for each of the criteria to measure their relevance in uptaking or not a therapy - Weighting; and including weights and scores for each of the options to derive and overall value [64]. Only then, the stakeholder can be informed leading to the final judgment and uptake or not the novel MSC-derived secretome therapy.

Chapter 6

Conclusion

This investigation include the estimation of the cost associated with an envisaged consisted in a study of the development of a manufacturing process for the production of an ATMP for the treatment of OA. The product was obtained after from the expansion of MSC from bone marrow, of allogeneic origin, in 2D system and downstream and purification of secretome/exosome produced by such cells. The process is operated according to GMP standards, ensuring the appropriate quality for its clinical application. The process aims at to treating of 200 patients per approximately a year, where each patient receives about five doses of the product, each dose containing 6.67x10^7 exosomes. To reach this objective, it was concluded that it is necessary to perform approximately 18 or 21 batches ('random' or 'fixed' respectively). This corresponds to about 13 or 12 patients treated per successful batch produced. In order to treat a steady number of patients, surplus doses are discarded (42 and 8 doses, respectively for 'random' and 'fixed' simulation types). This work contributes to research and development in the context of the new therapy. It is performed to help design scientific studies and even clinical trials related to MSC-derived secretome. It aims to draw attention to how costs can be reduced. It shows a window of potential for the proposed therapy.

It can be said that the main objective was fulfilled – evaluate the role of the new biotherapeutic, which has never been studied. It was produced information about costs of the new therapy, but also regarding its benefits (assuming different effectiveness).

When information is available in the literature about the effectiveness of this therapy regarding patients' quality of life, and also about its costs, it will be possible to reduce the level of uncertainty presented throughout this exploratory study - eHTA. One will be able to consult this study and understand which should be the key drivers - e.g. patient costs, healthcare provider costs, clinical benefits, risks, that were involved in MCDA mapping.

In the future, when the maturation of this preliminary model is completed, it will allow to support decisions on whether to adopt a new MSC-derived secretome, the specific target CoGs, and clinical endpoints that need to be reached to ensure cost-effectiveness competitiveness of the new therapy. Furthermore, this thesis points out the way forward until a final decision is reached. Within the approaches established in this work, it is clear that the adoption of the new OA therapies could save money and

time resources, since *a priori* key features for the commitment of the entities to decision making have been revealed. This will help to speed up the decision making process, which in turn may lead to a high standard of care for OA patients.

6.1 Limitations and future work

The results of this work have exploratory nature and need further validation. There are several limitations that can also be considered in future work. Another cell source than bone marrow could have been used, with less invasive extraction processes. The area of the facility used could be reduced in order to reduce the final manufacturing costs.

For future work, it would be interesting to make this study more comprehensive in that it would be possible to apply the stochastic model hereby developed to other diseases which also entail major burdens on HS as COVID-19. Thus, it would be possible to calculate the costs as a whole and not just estimated manufacturing costs. In addition to further analyse the results we already have and perform more simulations (runs) in order to get a more reliable final outcome. It would also be of relevance to simulate the gain of benefits through QALYs according to Markov models in order to obtain states and probabilities that in turn would be applied to the phase of the clinical trial mimicked here. Therefore, it would allow a more robust study of the improvement of the patients' quality of life when they possibly engage in this therapy. Finally, when a better comprehension of the therapy itself and the influence in its outcomes would be present in the literature, a whole new analysis could be carried out. Not only with two strategies to be compared, but a more general range in order to make the concept broader and even more challenging.

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Appendix A

Appendix chapter

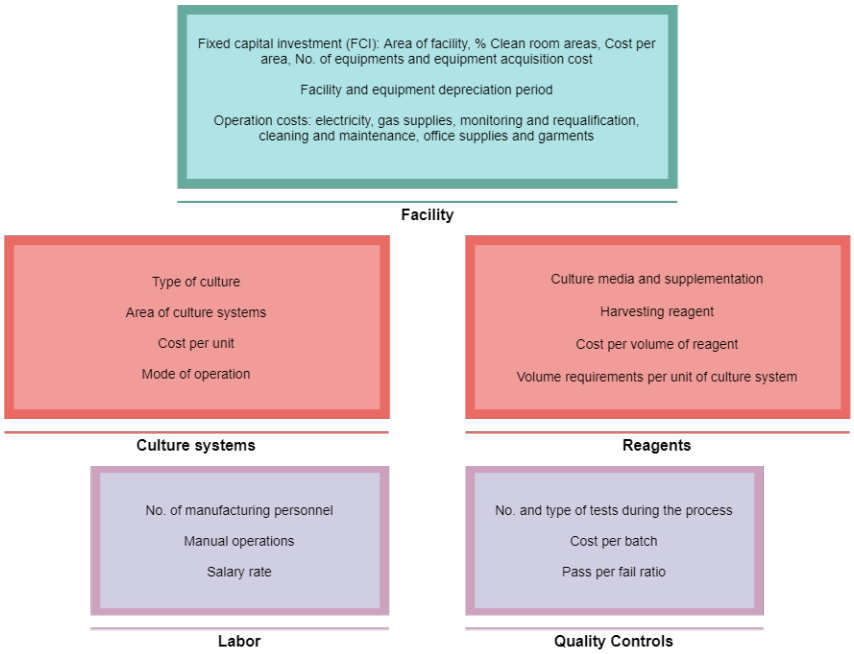

Figure A.1: Main process components in cell-free therapeutic manufacturing. Adapted from [33].

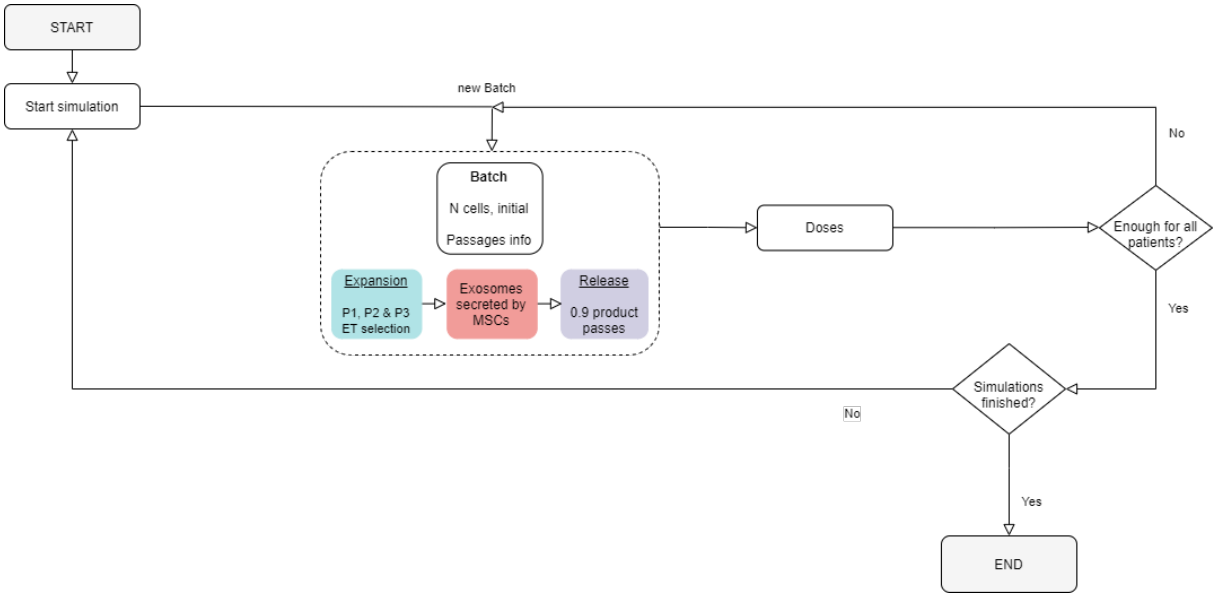

Figure A.2: Developed bio-economic model workflow for the production of MSC-derived secretome.

Table A.1: GMP facility and equipment related parameters - Baseline scenario.

Parameter	Value	References
Area of GMP facility	400 m^2	[96]
No. of workers	1	[96]
No. of incubators	2	[96]
No. of BSCs	1	[96]
No. of centrifuges	2	[96]
Fraction of clean room space	20%	[33]
Price of clean room/ m^2	4,908.41€	[33]
Price of non clean room/ m^2	2,863.17€	[33]
Daily worker pay	84.41€	[33]
Price of unit incubator	15,054.40€	[33]
Price of unit BSC	14,349.60€	[33]
Price of unit centrifuge	10,129.10€	[33]
Annual cost of CO_2 supply	5,064.57€	[33]
Annual cost of other gases supply	13,167.90€	[33]
Annual cost of additional lab supplies	6,668.35€	[33]
Annual cost of requalification	55,203.80€	[33]
Annual cost of maintenance	44,568.20€	[33]
Annual cost of cleaning	23,634.70€	[33]
Annual cost of garments	1,688.19€	[33]
Equipment depreciation period	5 years	[33]
Facility depreciation period	15 years	[33]

Table A.2: Assumptions of the Monte Carlo model in manufacturing MSC-derived secretome therapy.

Description	References
In this work are considered large cells with a radius of 2.5x10^{-3} cm	Assumption
Cost of initial investment in MSCs is 738€	Assumption
It has been estimated that for each MSC there are 700 exosomes	[94]
Cells do not die during expansion	Assumption
Cells undergo a 2 day lag phase before start growing	Assumption
The time to confluence is about 5 days	[33]
The yield of expansion harvesting is 90%	[33]
The ETs used in P3's are the same as applied in P2's	Assumption
ETs are bought at the beginning of the process and reused in the remaining batches	Assumption
In this work there are not considered parallel processes	Assumption
The yield of volume reduction is 80%	[33]
Final product formulation requires sacrificing one dose/vial for testing	[33]
The final product is fifty percent cryomedium (basal medium)	[33]
Surplus doses produced are discarded	Assumption
Final product formulation is independent of doses to be processed.	[33]

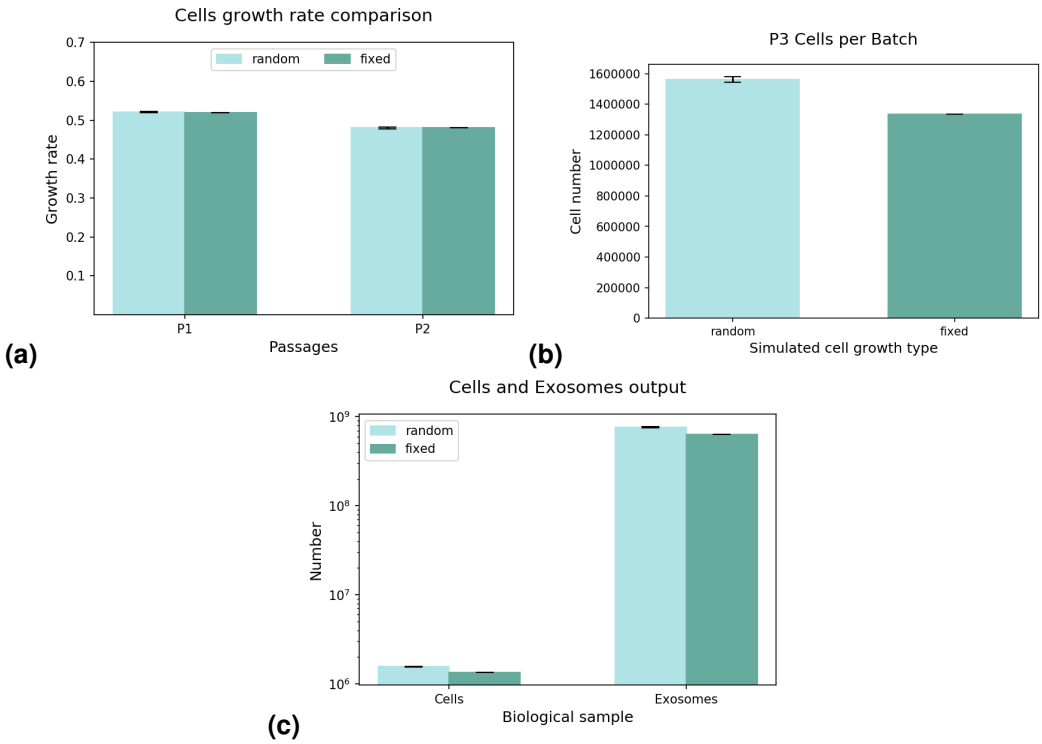

Figure A.3: **(a)** Mean of growth cell rate. **(b)** cells after the expansion of the passage 3. **(c)** Cells at the end of Expansion and Exosomes estimated output by the end of DSP - Scenario with 1x10^4 as input cells - random and fixed simulation types.

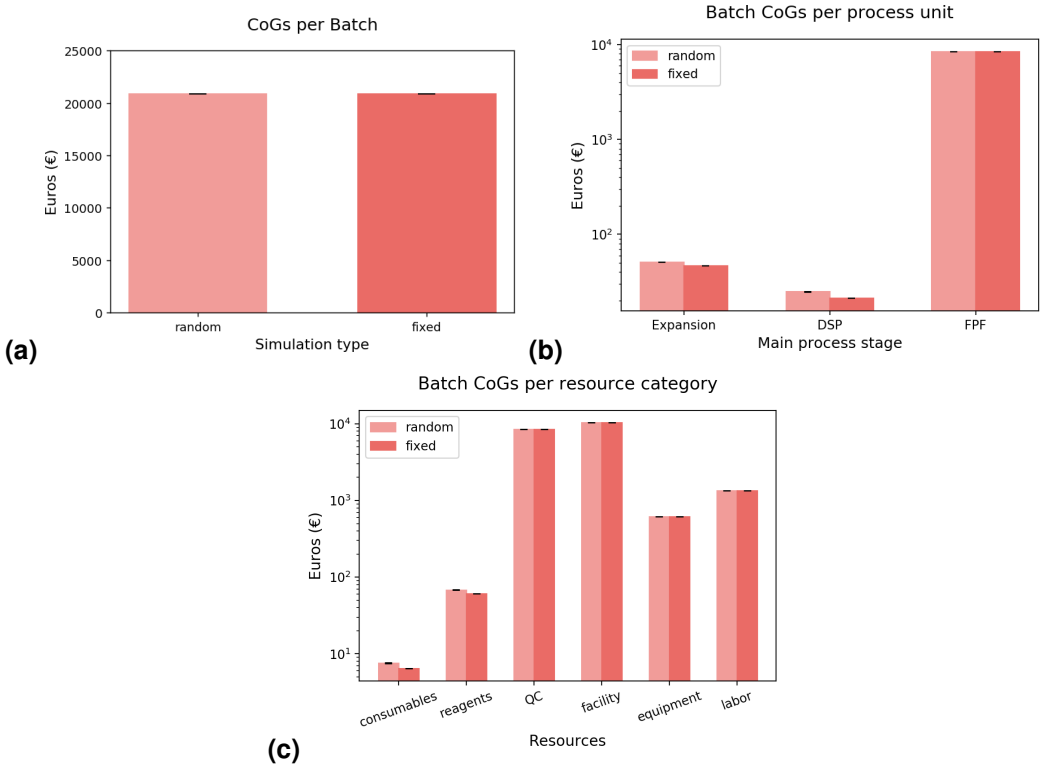

Figure A.4: **(a)** Direct and indirect CoGs per Batch. **(b)** CoGs per process unit. **(c)** CoGs per resource category - Scenario with 1x10^4 as input cells - random and fixed simulation types.

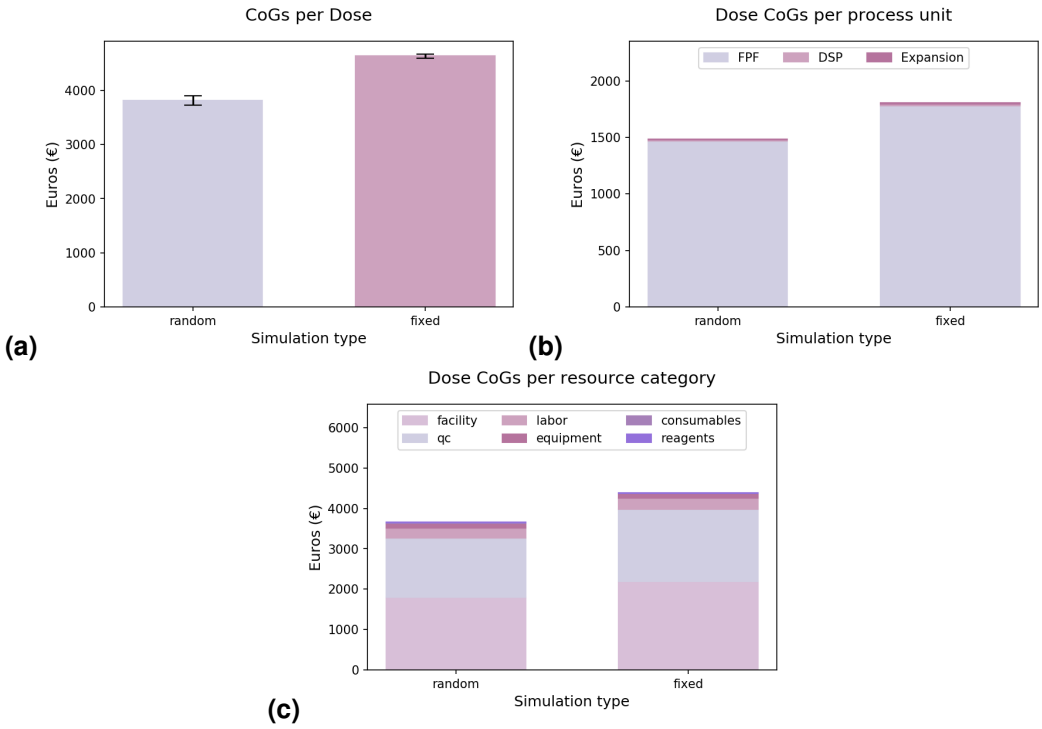

Figure A.5: **(a)** Direct and indirect CoGs per Dose. **(b)** CoGs per Dose per single main stages of the process. **(c)** CoGs per Dose per resource category throughout the manufacturing process - Scenario with 1x10^4 as input cells - random and fixed simulation types.

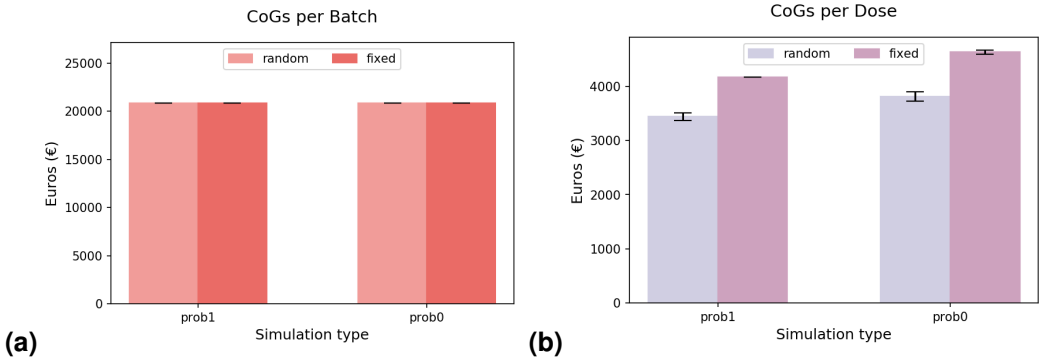

Figure A.6: **(a)** CoGs per Batch when comparing prob1 vs prob0 **(b)** CoGs per Dose when comparing prob1 vs prob0 - Baseline scenario but starting with 1x10^4 cells - random and fixed simulation types.

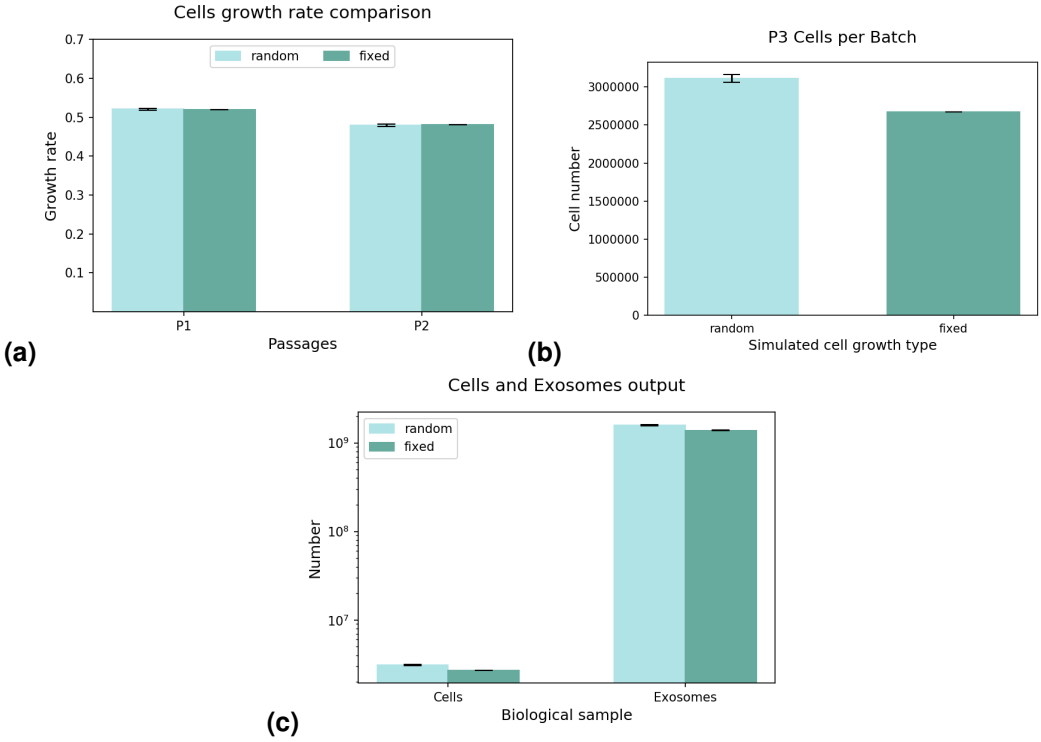

Figure A.7: **(a)** Mean of growth cell rate. **(b)** cells after the expansion of the passage 3. **(c)** Cells at the end of Expansion and Exosomes estimated output by the end of DSP - Scenario with 2x10^4 as input cells - random and fixed simulation types.

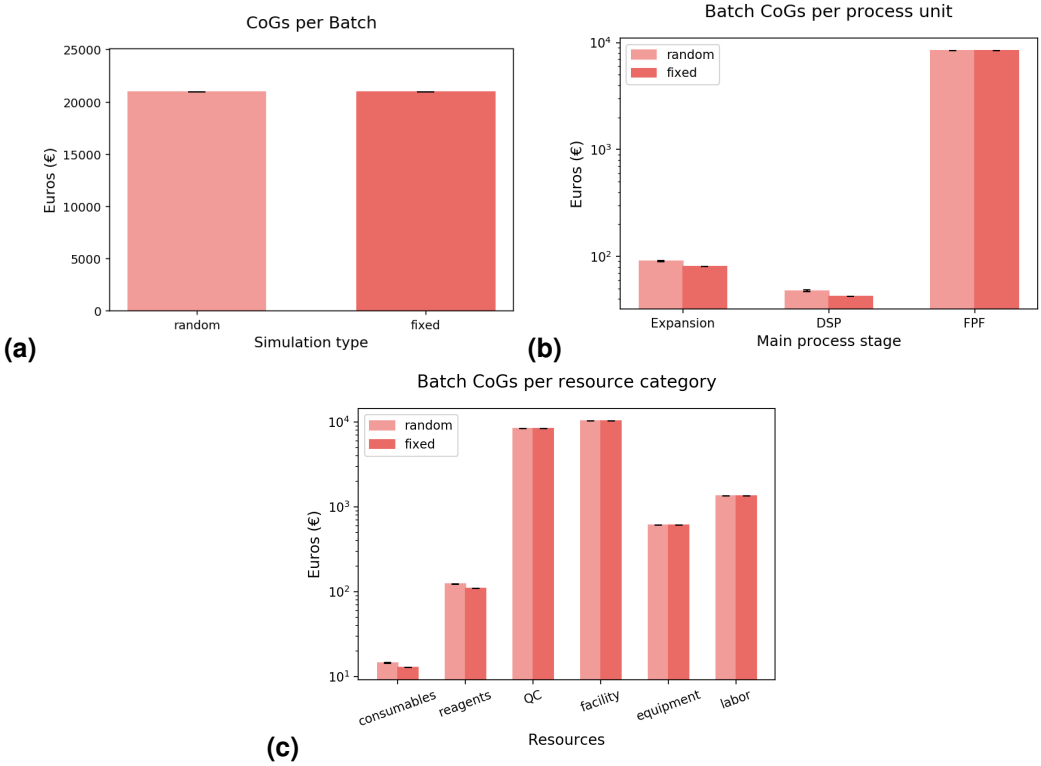

Figure A.8: **(a)** Direct and indirect CoGs per Batch. **(b)** CoGs per process unit. **(c)** CoGs per resource category - Scenario with 2x10^4 as input cells - random and fixed simulation types.

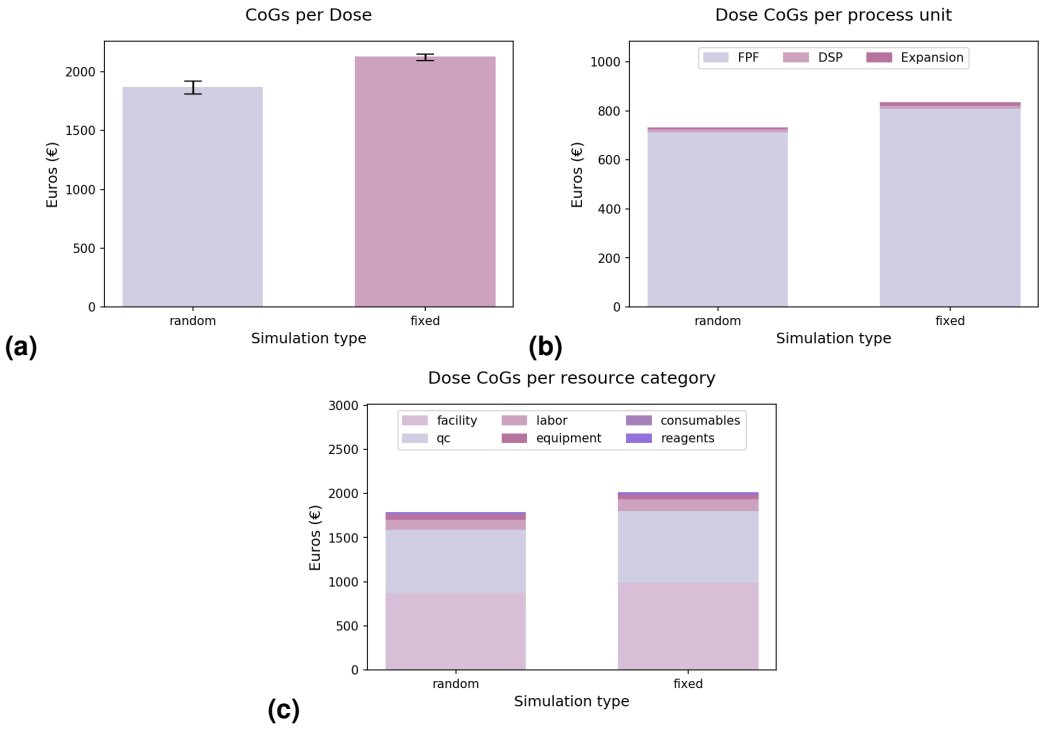

Figure A.9: **(a)** Direct and indirect CoGs per Dose. **(b)** CoGs per Dose per single main stages of the process. **(c)** CoGs per Dose per resource category throughout the manufacturing process - Scenario with 2x10^4 as input cells - random and fixed simulation types.

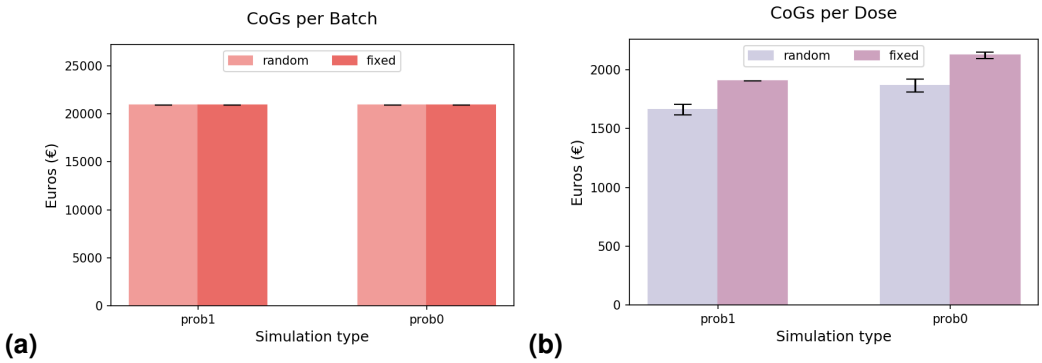

Figure A.10: **(a)** CoGs per Batch when comparing prob1 vs prob0 **(b)** CoGs per Dose when comparing prob1 vs prob0 - Scenario with 2x10^4 as input cells - random and fixed simulation types.

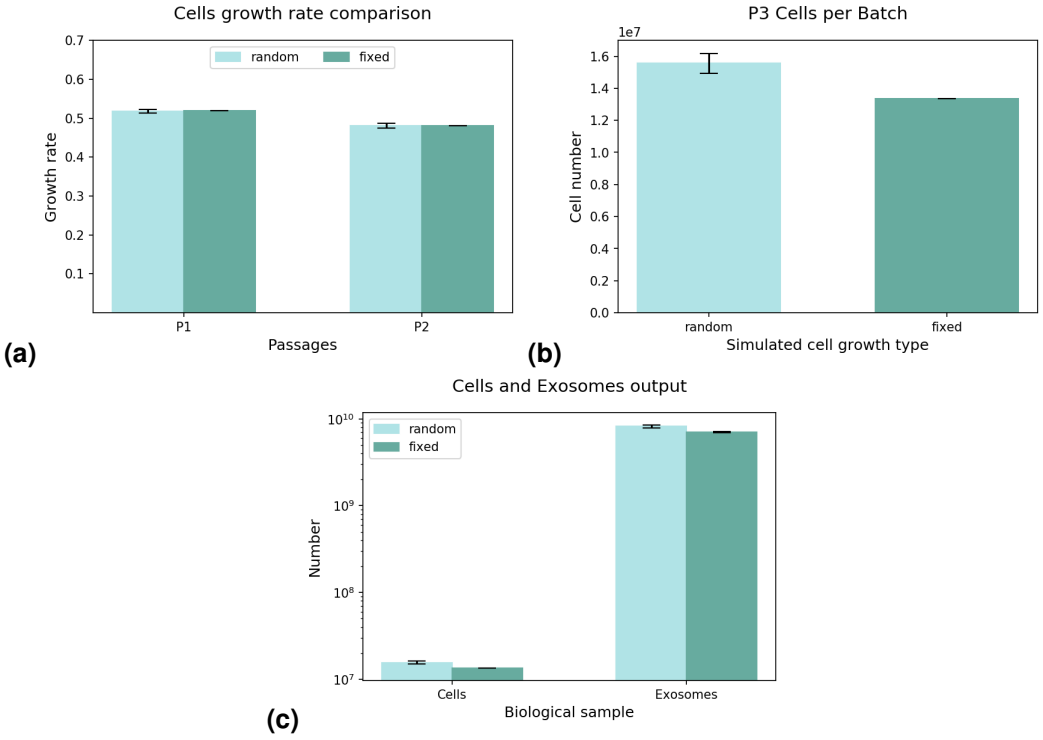

Figure A.11: **(a)** Mean of growth cell rate. **(b)** cells after the expansion of the passage 3. **(c)** Cells at the end of Expansion and Exosomes estimated output by the end of DSP - Scenario with half of quality control cost as input - random and fixed simulation types.

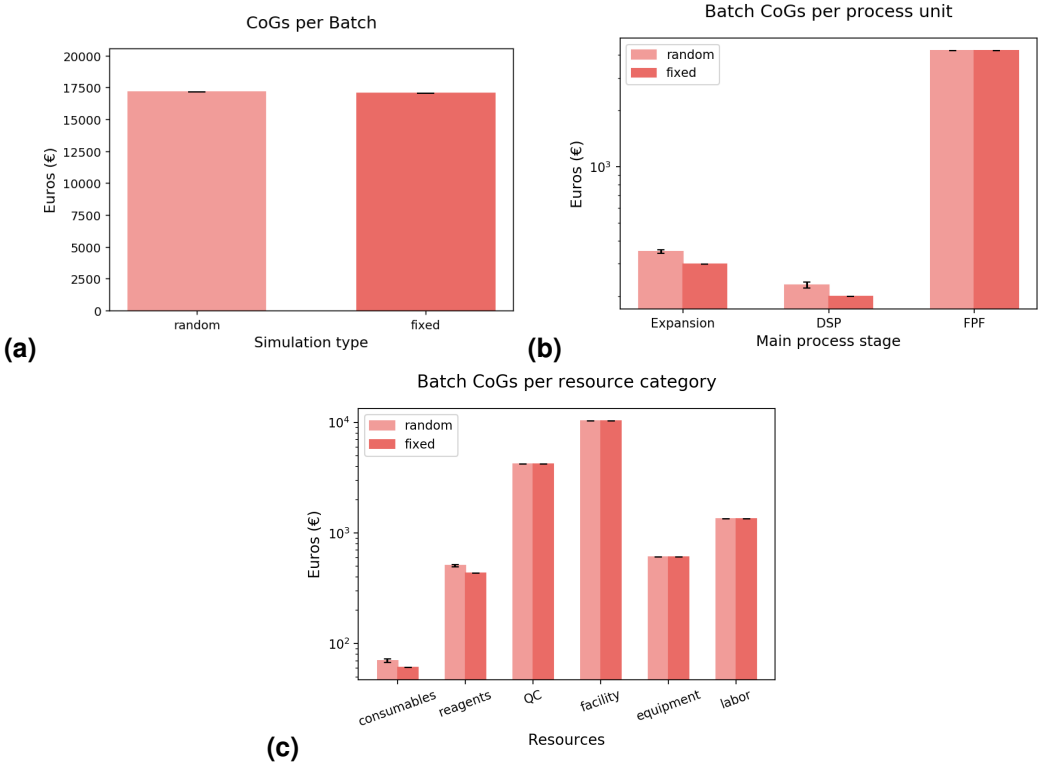

Figure A.12: **(a)** Direct and indirect CoGs per Batch. **(b)** CoGs per process unit. **(c)** CoGs per resource category - Scenario with half of quality control cost as input.

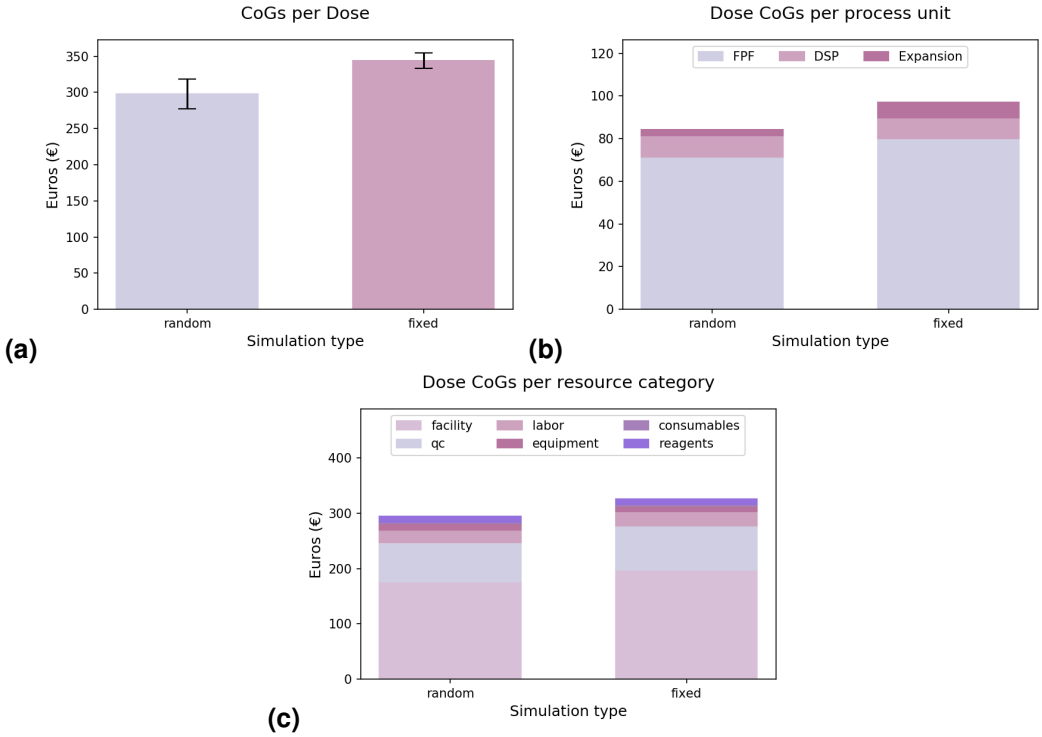

Figure A.13: **(a)** Direct and indirect CoGs per Dose. **(b)** CoGs per Dose per single main stages of the process. **(c)** CoGs per Dose per resource category throughout the manufacturing process - Scenario with half of quality control cost as input - random and fixed simulation types.

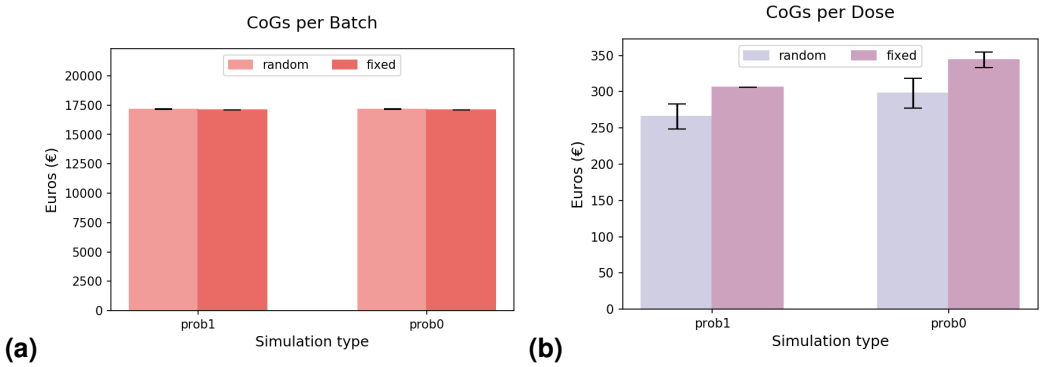

Figure A.14: **(a)** CoGs per Batch when comparing prob1 vs prob0 **(b)** CoGs per Dose when comparing prob1 vs prob0 - Scenario with half of quality control cost as input - random and fixed simulation types.

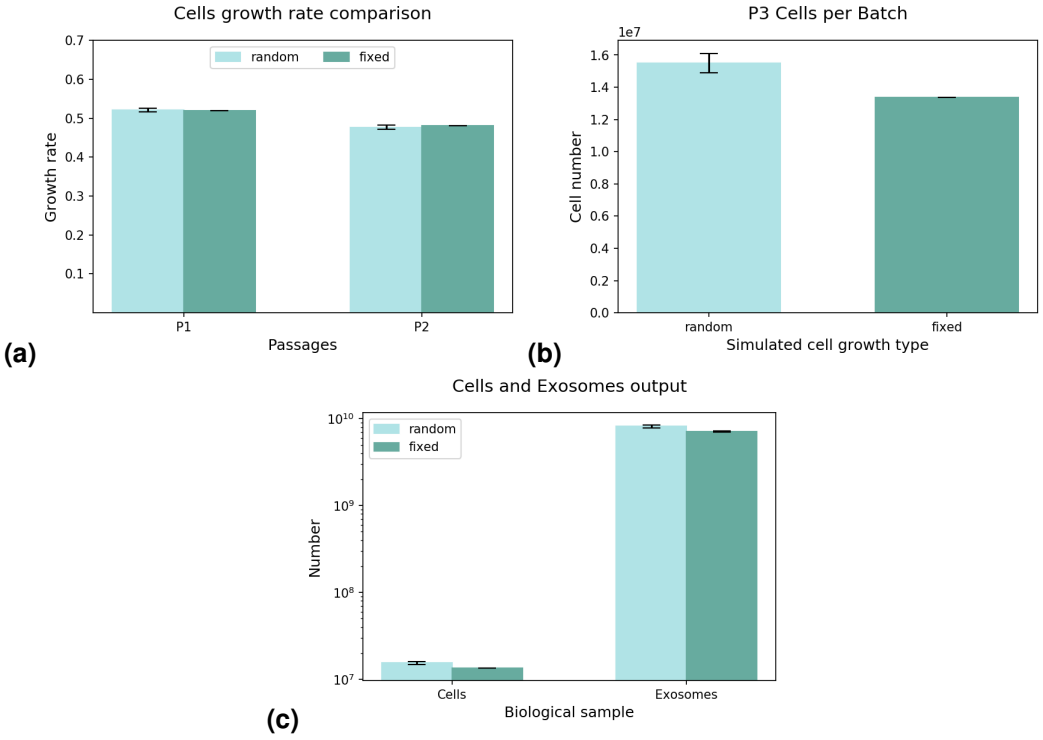

Figure A.15: **(a)** Mean of growth cell rate. **(b)** cells after the expansion of the passage 3. **(c)** Cells at the end of Expansion and Exosomes estimated output by the end of DSP - Scenario with half of facility cost as input - random and fixed simulation types.

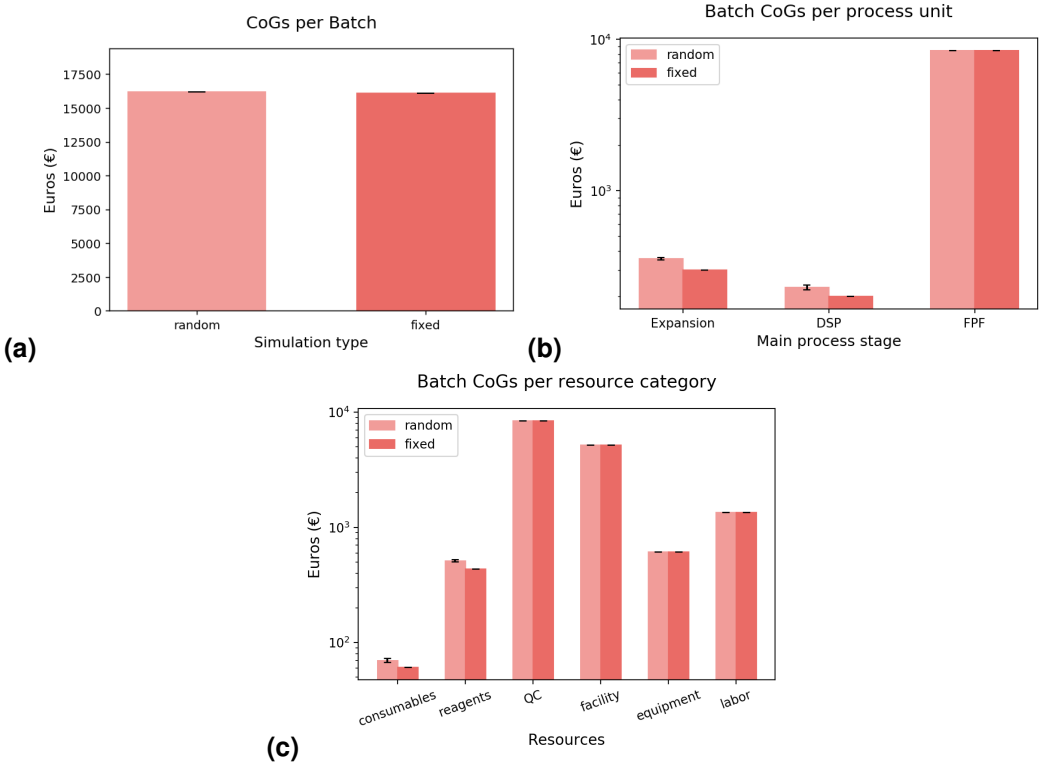

Figure A.16: **(a)** Direct and indirect CoGs per Batch. **(b)** CoGs per process unit. **(c)** CoGs per resource category - Scenario with half of facility cost as input - random and fixed simulation types.

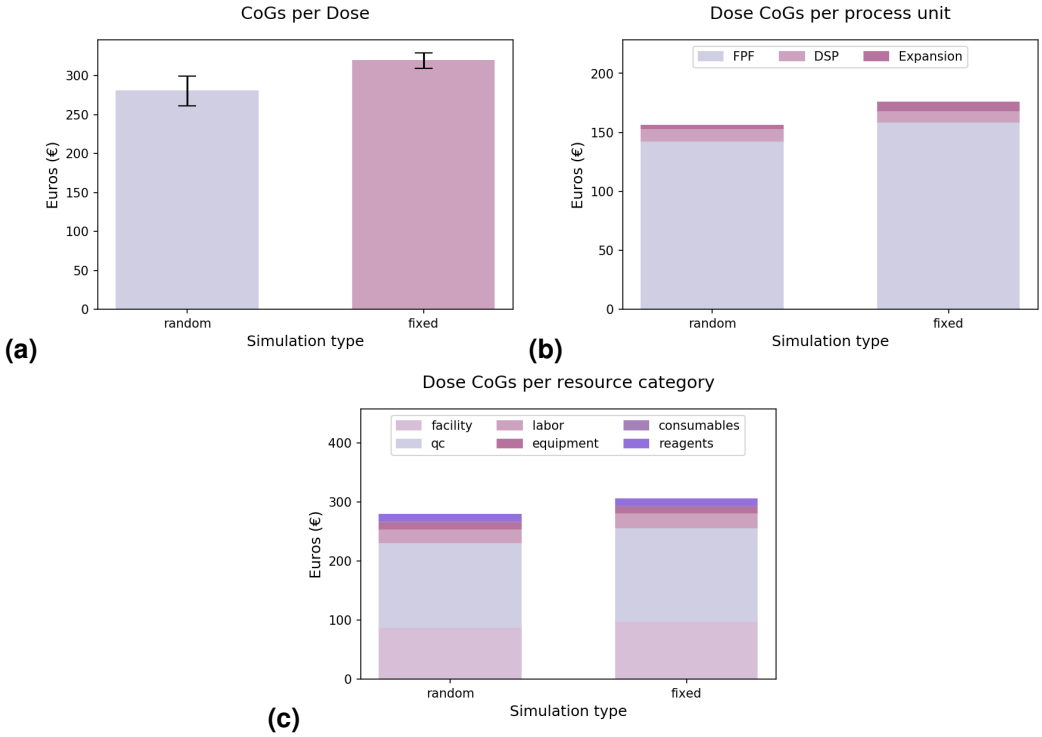

Figure A.17: **(a)** Direct and indirect CoGs per Dose. **(b)** CoGs per Dose per single main stages of the process. **(c)** CoGs per Dose per resource category throughout the manufacturing process - Scenario with half of facility cost as input - random and fixed simulation types.

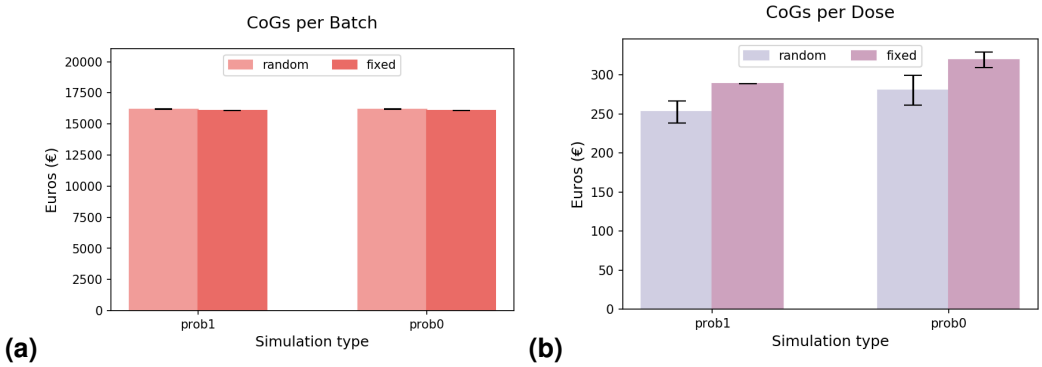

Figure A.18: **(a)** CoGs per Batch when comparing prob1 vs prob0 **(b)** CoGs per Dose when comparing prob1 vs prob0 - Scenario with half of facility cost as input - random and fixed simulation types.