Chapter 3

"Cell-Free Therapeutics" from Components Secreted by Mesenchymal Stromal Cells as a Novel Class of Biopharmaceuticals

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Abstract

Regenerative medicine is a fast growing multidisciplinary field aiming at the regeneration or replacement of damaged cells, tissues, or organs. Adult multipotent mesenchymal stromal cells (MSCs) are often used as a principal therapeutic tool in this field. Along with differentiation potency, MSCs secrete a wide spectrum of paracrine factors and extracellular vesicles participating in tissue repair and regeneration. Thus, for injuries that require trophic stimulation, cell survival support, and/or resident stem cells activation to be restored, one can apply MSC-conditioned medium, a combination of products and extracellular vesicles in cell culture growth medium, secreted by MSC. It could mediate most of beneficial regenerative effects of MSC without possible side effects of using MSC themselves. However, before the clinical application of this promising biopharmaceutical, several issues such as manufacturing protocols, quality control, and others must be addressed. Subsequently, we highlight the questions considering donor material variability, manufacturing, cell culture medium and auxiliary components selection, and potency tests development.

Keywords: biopharmaceuticals, multipotent mesenchymal stromal cells, conditioned medium, quality control, regenerative medicine

1. Introduction

Regenerative medicine is a fast growing multidisciplinary field aiming at the regeneration or replacement of damaged cells, tissues, or organs. Adult stem and progenitor cells represent

a pivotal tool in regenerative medicine. It is important to highlight mesenchymal stromal cells (MSCs) as the most popular source for cell therapy and tissue engineering. However, a large amount of experimental data indicate that MSC effects on regeneration are mostly mediated by their ability to produce a wide range of bioactive molecules, and the use of MSC-conditioned medium (CM), a complex of the factors secreted to cell culture growth medium, as a distinct biopharmaceutical drug can be a rational alternative to direct MSC therapy. MSC CM could be considered as "cell-free therapeutics" since this product is devoid of MSC themselves in its final formulation, yet possess significant therapeutic potency. It contains components secreted by MSC and reproduced the effects of MSC-based cell therapy.

"Cell-free therapeutics" have various advantages in overcoming the limitations and risks associated with the cell-based therapy. Despite the outstanding preclinical and clinical efficacy of MSC CM, there is still no generally accepted regulating approaches for CM standardization and quality control. This chapter reviews the current state of art in the development of MSC CM-based medicinal products and describes the crucial issues concerning the production and quality control of this promising class of biopharmaceuticals.

2. Physiological activity of MSC

Postnatal stem and progenitor cells are responsible for tissue renewal and regeneration throughout the whole human lifetime. Accumulating evidence indicates that stem cells function only within a specific niche. Stem cell niche is considered as a local tissue microenvironment that maintains stem cells and regulates their function by producing factors that act directly on stem cells [1]. This microenvironment provides structural cues and paracrine signals to support stemness. Mesenchymal stromal cells (MSCs) are found in many niches of tissue-specific resident stem cells. The function of MSC in a niche is often critical for its maintenance. MSC plays an important role in coupling information from the environment with stem cell populations. MSCs react to endocrine and nervous system signals: thus, the stimulation of a specific type of MSC by granulocyte colony-stimulating factor (G-CSF) or beta-3-adrenoreceptor agonists decreases the expression of the panel of genes supporting hematopoietic stem cells (HSCs). Furthermore, upon MSC, withdrawal in niche HSC activity and quantity decrease [2]. MSCs also interact with stem cells and other internal components of the niche for effective control over HSC, ensuring they support hematopoiesis without inducing aberrant proliferation. It should be noted that the maintenance of stem cells in the niche is not the only function of MSC. These cells are also involved in the formation and maintenance of a structural component of the niche. MSC isolated from a subendothelial layer of bone marrow stroma can form a microenvironment alike a miniature bone organ, similar to the HSC niche, under heterotopic transplantation. The establishment of subendothelial stromal cells in developing heterotopic BM in vivo occurs via specific, dynamic interactions with developing sinusoids. Subendothelial stromal cells are major producers of angiopoietin-1 (Angpt-1), the principal factor of HSC niche involved in vascular remodeling [3].

MSCs provide their supporting functions through secretory activity in physiological conditions. MSCs secrete a number of factors that are critical for the maintenance of stem cells in their niches [4, 5]. Consistent with other reports, we have shown that the largest functional

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cluster in MSC secretome is composed of extracellular matrix (ECM) proteins [6]. Such protein profile is in line with the stromal characteristics of adipose MSC. Adequate production of ECM components is necessary for tissue homeostasis and regeneration, because these molecules not only provide a scaffold for cells and soluble molecules but also regulate angiogenesis, neurogenesis, and inflammation. In addition, a large amount of data indicates that apart from soluble factors MSCs secrete regulatory non-coding RNA (e.g., micro RNA) within extracellular vesicles (EVs). The release of these small RNA by MSC can play a role in stem cell niche maintenance by controlling and tuning proliferation, differentiation, and homing. Particularly, microRNA regulates diverse biological processes, including growth and differentiation of stem cells [7, 8].

The function of adult stem cells includes the local or remote replacement of senescent or damaged cells along with maintaining their own pool. Stem cells supported by other niche components can participate in the repair of small lesions of a skin, liver, intestines, kidney, and bone marrow. However, stem cells could not cope with more serious injuries without more substantial support [9]. MSCs are important for maintaining the niche of stem cells; therefore, they can participate in stem cell potentiation to respond to damage, stimulate the survival of stem cells, and, thereby, maintain the structural and functional integrity of the niche. For example, in such serious damage as myocardium ischemia/reperfusion injury, MSC mediated its cardioprotective paracrine effect by secreting exosomes which reduced infarct size in a mouse model [10]. As in majority of animal models and clinical studies, only limited or no engraftment at all was often observed, one should consider paracrine MSC function as principal effector for tissue regeneration after, at least, systemic MSC injection for different injuries [11].

3. Rationale for use of MSC and their cell-free derivatives in regenerative medicine

MSCs are the most commonly used cells for cell-based therapy as they do not form teratomas, confer low immunogenicity, and are free of strict ethical concerns [12]. Despite these reasons, the difficulty to trace cell fate and survival in recipient has been a significant obstacle for understanding the mechanisms of the clinical efficacy that can be variable [13]. Furthermore, MSCs are highly heterogeneous and cannot be fully characterized in the context of identity and, finally, potency. At the same time, MSC CM was shown to mediate most of MSC beneficial effects. MSC CM includes various components such as cytokines, growth factors, ECM proteins and factors involved in ECM remodeling, different types of EV, and others. Using cell-free products based on biologically active factors secreted by stem and progenitor cells allows to significantly reduce the risks associated with a direct cell injection, while maintaining efficacy under wide manufacturing scalability and modification potential like fractionation, concentration, and combination with various carriers [14]. Therefore, the application of "cell-free therapeutics" based on the components secreted by MSC as a novel class of biopharmaceuticals represents a rapidly developing and promising approach in regenerative medicine.

4. Harnessing cell-free therapeutics' variability to improve their safety and efficacy

4.1. Age-related variability of MSC properties

Since MSCs are used as a starting material for CM manufacturing, it is necessary to pay attention to the standardization of MSC. The effects of this biopharmaceutical are multifactorial, and even the minimal variability of its composition can strongly affect its activity. As properties of MSC influence CM composition crucially, the variability of MSC donor characteristics and manipulations during manufacturing should be taken into account.

One of the key factors affecting MSC CM composition and therapeutic effects is a donor's age. It is generally accepted that stem cell number and/or function decline with advanced age during the replacement and the turnover of damaged cells in compromised renewable tissues. Several studies showed that MSCs derived from old donors exhibit reduced proliferative capacity, differentiation potential, and, most importantly, impaired specific activity [15]. Older adipose-derived MSC showed impaired angiogenic properties in vitro and in vivo. The production of key pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and others by adipose-derived MSC, decreased with age [16]. It is important to note that factors involved in ECM remodeling like urokinase and its receptor, plasminogen activator inhibitor-1 (PAI-1), metalloproteinases 2 and 9 were overexpressed in adipose MSC from aged donors compared to younger ones, both in human and in mice [16, 17].

Furthermore, it was shown that age and age-associated conditions such as type 2 diabetes and atherosclerosis decrease the immunomodulatory capacity of human MSCs. MSCs from elderly subjects with atherosclerosis have impaired T-cell suppression compared to their non-elderly adult counterparts. Possibly, the coexistence of age and these age-related conditions could compromise MSC immunomodulatory function [18] and influence MSC CM functional properties.

Bioinformatic data also suggest that age-related changes in MSCs result in impaired therapeutic potential of aged progenitor cells [19]. In one of the studies among the most highly differentially expressed genes, transforming growth factor alpha (TGFα) overexpression in MSC from young donors was detected. TGFα has been shown to mediate the secretion of vascular endothelial growth factor A (VEGFA) by MSC, positively contributing to processes like wound healing and injury response [20]. Some other differentially expressed genes participate in MSC differentiation. As it is revealed that during early MSC differentiation, secretome of MSC can be changed, one should take into account age-related differentiation predisposition.

4.2. Sex-related variation of MSC paracrine activity

In an attempt to overcome MSC donor variation, one can explore donor sex variability. Analysis of human BM-MSC preparations to identify statistically robust influence of donor sex on MSC functional properties identified minor differences among MSC isolated from both the sexes. It was shown that high-clonogenic BM-MSCs divided more rapidly and were

more frequent in BM-MSC preparations from female donors. What is more important, no correlation of donor age to adipogenic, osteogenic, and chondrogenic differentiation in vitro as confirmed by an extended panel of lineage specific markers was found. In addition, the secretion rate of molecules important for tissue regeneration and immunomodulation was analyzed. The evidence of a critical role of factors such as VEGF, platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), fibroblast growth factor 2 (FGF2), and angiopoietin-1 (Angpt-1) in contribution to male and female MSC variability was lacking [21]. As MSC-secreted factors are principal active components of MSC CM, donor sex might not influence MSC CM efficacy substantially. However, in vitro data concerning the impact of donor sex to variability of donor-derived MSC effects are controversial [22].

4.3. Variability determined by the origin of starting material for MSC isolation

MSC isolated from the most common sources can fit minimal characteristic criteria and share majority of biological properties. However, some studies demonstrated that MSCs from the popular sources such as adipose tissue and bone marrow have different transcription profiles. Among differentially expressing or expressing by only one cell type, one can find components involved in cellular communication and differentiation. Therefore, the secretory profile of different MSC types also varies. In addition, not only a spectrum of secreted paracrine factors differs but also nucleic acid, particularly microRNA composition, which might influence EV contents. The variability between EV of two types of MSC suggests that prior tissue-specific microenvironment might influence the exosomal sorting of micro-RNA. It is important as micro-RNA transferred via exosomes might be functional in repressing their targets in vitro and in vivo if transferred in high amounts [7, 23].

Taken together a plethora of donor-associated factors can influence MSC CM composition and effects. Thus to enhance the effect of this biopharmaceutical and harness lot-to-lot variability one can isolate MSC from selected donors that are optimal for MSC CM manufacturing for certain pathology.

4.4. Variability determined by manufacture process

Manufacturing features can also substantially affect the safety and efficacy of MSC CM. Among them, one can name at least optimal cell isolation protocol, growth medium selection, and cell expansion approach as well as the use of proper raw materials.

Although isolated by the same way, MSCs often demonstrate different properties when cultured in different media. The choice of medium determines the potential of MSC for adhesion, growth, and also for maintaining a population of clonogenic cells: both a basal medium and a supplement influence these parameters. It is important to note that ready-to-use commercial media fit to MSC obtained from various sources differently. Media can cause negative effects up to early cell aging or termination of growth in the earliest passages for certain MSC type. In addition, some cell culture media can be positively selective for specific MSC subpopulations. This feature is important as the composition of MSC population might affect regenerative potential of MSC [24, 25]. The media can also influence MSC CM composition significantly.

We observed a substantial difference between several growth factor concentrations in MSC CM manufactured with two different media. Importantly, the variability of factor concentrations between two MSC CM reflected on their potency in vitro [26]. The presence or absence of a certain biologically active component in the culture medium can also affect the function of MSC. The addition of FGF2 to bone marrow MSC culture medium influences the expression of some membrane proteins, which contributes to morphology and differentiation potential changes [27]. Another challenging feature is an impact of starting material or sample processing during CM preparation. Such a routine procedure as washing cells with PBS can change the secretion profile of cells dramatically. Thus, the attention to auxiliary component and adequate selection of them are necessary [28].

Apart from materials used for MSC expansion and isolation, we might note the selection of cell culture approaches as a substantial factor affecting the variability of MSC functional properties. The use of bioreactors has been suggested as a promising alternative to conventional static culture flasks for MSC expansion. The advantage of 3D cultivation is more complete modeling of the natural microenvironment of MSC, which allows to retain the proliferation and differentiation potential of MSC for longer time. Besides, selected cell culture method can affect the secretion profile of MSC directly. Three-dimensional growth of bone marrow MSC culture influenced the expression of such factors as pigment epithelium-derived factor (PEDF), Galectin-1, brain-derived neurotrophic factor (BDNF), VEGF, nerve growth factor (NGF), insulin-like growth factor 1 (IGF-1), and miR-16, which are considered to be important regulators/modulators of the neurogenic and neural differentiation processes. Using CM from 3D cultured MSC induced the differentiation of a significantly higher number of human neural progenitors into neurons at different stages of maturation compared with human MSC secretome collected under 2D conditions [29].

5. Developing approaches to assess the potency of cell-free therapeutics

5.1. Legal aspects of potency tests development

The efficacy of a drug means a biological response caused by this drug in a certain dose. As part of a drug development, clinical research, and certified manufacturing, there is a need to develop and apply a standardized rapid method for assessing the potential efficacy of the drug. This technique is called a potency test. By definition of International Conference on Harmonization (ICH) [30], potency is the quantitative measure of biological activity based on the attribute of the product, which is linked to the relevant biological properties. The assay demonstrating the biological activity should be based on the intended biological effect which should ideally be related to the clinical response. This definition has been implemented by Food and Drug Administration (FDA) and European Medicines Agency (EMA).

At the step of research and development, the availability of an approved potency test ensures consistency among the results obtained by different scientific groups. While conducting clinical trials, postulating a presumptive potency test is necessary as one of the evaluated factors

for determining the drug efficacy. At the registration stage, the presence of a clearly defined potency test is required by regulators. An established and valid potency test is required for drug manufacturing to check how scaling affects drug's therapeutic properties as well as the in-process testing for checking production lines as a factor that actually determines the lot release capability. The choice of a potency test should also be economically feasible. A potency test has to be quick and unambiguous in the context of drug efficacy determination at the earliest stages of lot release.

5.2. Development of potency tests for CM-based biopharmaceuticals

It should be emphasized that the development of an appropriate technique for potency tests of biopharmaceuticals based on cells and their secreted products is complicated (**Table 1**). In particular, the recently released "Alofisel" product faced the greatest difficulties at registration stage due to justifying the choice of potency test. "Early in the procedure a major objection was raised in relation to the potency assay. During the procedure the applicant provided additional data to support the suitability of the potency assay and the major" [31]. However, the parties managed to come to a common opinion on this issue, and the very first recommendation from the EMA was "The Applicant will undertake to review the data generated for the potency assay from clinical experience after suitable experience has been generated and to follow any recommendation that is issued following the review of the data assessment report objection is considered to be resolved."

Here, we will not dwell on the complexities of developing a potency test for cellular products, including those based on MSC; however, we note that FDA and EMA have at least a clear classification and regulatory framework for this category of therapeutics [32, 33]. At the same time, biopharmaceuticals based on MSC CM have not been classified by these agencies. Moreover, since the general rule for pharmaceutical certification is the presence of a defined, clearly characterized active substance, further progress in the development and registration of this category of therapeutics is bound to attention from regulatory agencies. According to many experts' opinions working in the field of MSC CM-based drug development, the most correct way is the approval of a new group of drugs called "cell-free therapeutics" with a less strict attitude to the issue of the multicomponent active substance [34]. The main obstacle for establishing the adequate potency tests for such biopharmaceuticals is an elusive nature of their mechanism of action (MOA) as well as the challenging choice of one active components between multiple cell-secreted factors. Nevertheless, the factors from CM composition might be isolated as single ones and classified as biological products. Therefore, it seems reasonable to apply the similar standards for characterization, safety, and dosage testing as well as potency evaluation for both the biologicals and CM, at least with any exceptions. Similar to biological drugs, potency test can be used for dose clarification from lot to lot. For complex biological medicinal products that cannot be fully characterized by physicochemical means, the established concept is to assign potency in units of biological activity based on the use of an international standard for biological activity. The units of biological activity are mostly traced back to an internationally adopted reference preparation (International Standard, IS). The quantitative composition and dosing recommendation of biological medicinal products for which an IS exists are expressed in international units (IUs) [35]. IS analogs could also be

Challenges in development and validation of PT	Possible strategies to overcome
Pleiotropic mode of action	1. One should choose the main mode of action (MOA) depending on pathological process. 2. PT must be disease-relevant and reflect preferred MOA. 3. Other MOAs specific to particular CM-based therapeutic must be taken into account. 4. Reproducible *in vitro* PT should be preferred. This model must reflect preferred MOA as accurate as possible.
Complex active substance	1. CM composition and its batch-to-batch variability should be carefully controlled. 2. Depending on preferred MOA, the most crucial components can be enriched.
"Batch-to-batch variability"	1. Complex of the most ubiquitous and crucial components should be defined at R&D phase to mitigate donor-to-donor variability. 2. Compliance to cGMP/GTP and cGLP.
Time consumption and high cost	Replacement of *in vivo* PT by *in vitro* PT or analysis of crucial components concentration (surrogate PT).
Lack of corresponding specific category in existing regulations (EMA, FDA, etc.)	Development of specific approach for regulation of multi-MOA and multicomponent CM-based therapeutics. Regulatory approach may take into account existing classes of innovative products: • CGTs/ATMPs (in aspect of usage of stem cells in manufacturing) • Biopharmaceuticals (in aspect of actual composition) • Blood plasma-derived therapeutics (in aspect of high heterogeneity and variability)

Abbreviations: PT, potency test; MOA, mode of action; CM, conditioned medium; R&D, research and development; cGMP, current good manufacturing practice; GTP, [current] good tissue practice; cGLP, current good laboratory practice; EMA, European Medicines Agency (European Union); FDA, Food and Drug Administration (USA); CGT, cellular and gene therapy; ATMP, advanced therapy medicinal product.

Table 1. Key challenges in the development of valid PT for CM-based cell-free therapeutics and possible ways to overcome them.

developed for the biopharmaceuticals derived from MSC CM. An important advantage of this approach is applicability of the direct rapid potency tests as well as the use of surrogate analytical and instrumental assays.

5.3. Challenges in the development of potency tests for CM-based products

However, MSC CM-based biopharmaceuticals represent not only a mixture of defined bioactive factors but a complex of multiple components produced by a specific type of cells. MSC CM mimics the beneficial effects of MSC cell therapy, and potency tests applied for corresponding cell and gene therapy products/advanced therapy medicinal products (CGT/ATMP) could also be relevant. For example, the measure of a defined cytokine like IL-10 in MSC secretome may serve as a potency test for a cell-based product with immunosuppressive

activity and also could be used as a potency test for MSC CM-based biopharmaceutical with similar indications.

Thus, the new category of drugs to which MSC CM will belong should be based on its definitions at the junction of two existing regulating categories: biomedical drugs and CGT/ATMP.

Another complexity of creating and validating the potency test for MSC CM is the heterogeneity of the product from batch to batch. Many factors such as heterogeneity of donors, in vitro cell population during cultivation, and soluble factors that MSC can secrete under different conditions influence this phenomenon. In addition, one might develop rules for this biopharmaceutical by partial borrowing of available legal documents for preparations based on blood plasma [36] as these drugs are also highly heterogeneous, have uncertain active components, and their potency is difficult to be assessed. However, the most important hurdle in a potency test development is a variety of biological activities of factors secreted by MSC. Hence, there is a diversity of MOA, where extent and type depends on the area of application of MSC CM-based biopharmaceutical. For example, the angiogenic properties of MSC secretome are mediated by well-known pro-angiogenic factors; however, some of them may have another action. In addition, it is necessary to achieve the pleiotropic action of MSC CM, since many different mechanisms are involved in the regeneration of injuries.

It is also worth considering that in some cases, specific effects of MSC CM are contraindicated. For example, angiogenic effects necessary for the restoration of ischemic tissues may promote tumor development. Thus, the creation of a universal method for developing a potency test of MSC CM is questionable. The selection of a method for testing this substance should be disease-focused [37].

One can suggest the following mechanism for selection, testing, and validating the potency test for MSC CM (**Figure 1**). First, based on pathology nature, the most potentially effective MOA should be chosen. It is important to note that potency tests are available for the majority of MOA, and it might be possible to apply them for CM potency testing with minimal modifications.

5.4. Overcoming existing challenges in potency testing

Since there is no "gold standard" or sufficiently defined regulations in this field, developers of MSC CM-based therapeutics use a variety of approaches for determining the potency. To evaluate the immunomodulatory effect of MSC-secreted EV, an in vitro test based on a dose-dependent inhibition by vesicles of the proliferation of phytohemagglutinin-activated T lymphocytes was successfully used [38]. For the surrogate analysis of the immunomodulatory activity of MSC CM in the model of inflammatory organ failure, the effect of MSC CM on bacterial LPS-activated PBMC was tested. The degree of modulation correlated well with the level of IL-10 secreted by PBMC in this experimental model [39]. The subsidiary company of SteMedica, StemProtein, has successfully used two potency tests for its unique product, human fibroblast secretome dried by proprietary technology "Preservation by Vaporization." Because tissue regeneration critically depends on adequate blood supply, they used in vitro angiogenesis assays to evaluate the therapeutic potency of stem cell factors. They routinely monitored the concentrations of VEGF, HGF, interleukin 6 (IL-6), chemokine C-C motif ligand 2 (CCL2), C-X-C motif chemokine 5 (CXCL5),

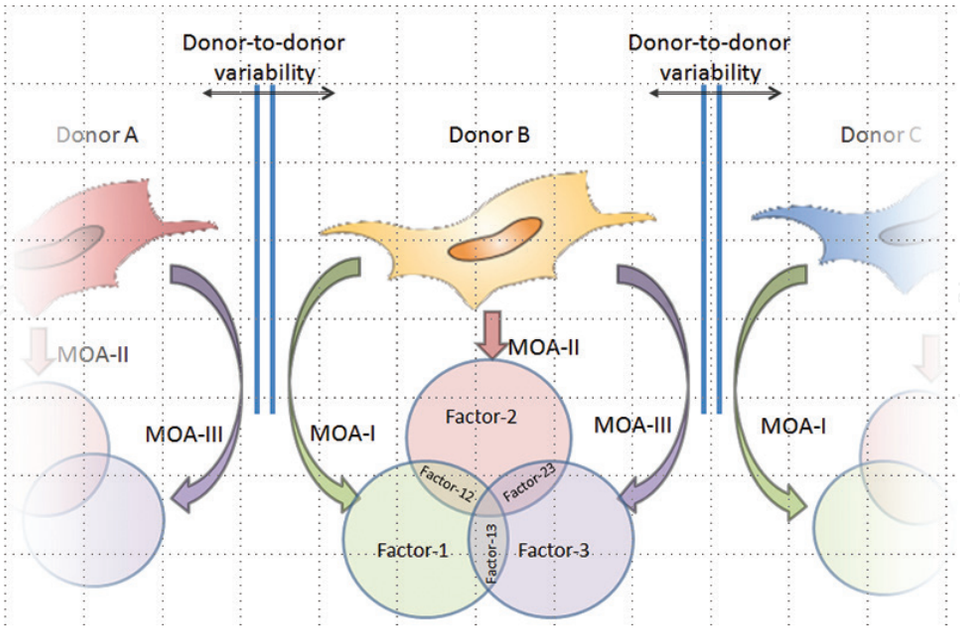

Figure 1. Schematic reflecting multicomponent multi-MOA nature of MSC and effects of factors secreted by them. A detailed description is provided in the subsequent text. Abbreviations: MOA, mode of action; MSC, mesenchymal stromal cells.

and interleukin 8 (CXCL8) in the product, as published literature has identified the important biological activity of these growth factors, cytokines, and chemokines [40, 41]. MultiStem also successfully used an analogous in vitro angiogenic test of biological activity, and also showed that three factors—VEGF, CXCL8, and CXCL5—are the crucial factors for the angiogenic activity of the secretome. The depletion of any of them drops the angiogenic effect of the secretome. Concentrations of each of the factors can be used as a threshold for the lot release [42]. Based on the literature data and guidelines as well as on our own experimental results, we provided rationalization for nomenclature and methods of quality control for human adipose-derived MSC CM developed for tissue reparation and regeneration on "Specific activity." As the most important type of cells involved in tissue repair and regeneration after injury are fibroblasts, one of the models for MSC CM potency measurement was the assessment of human skin fibroblasts migration in the model of the scratch assay. In this regard that angiogenesis is also an indispensable process for the successful regeneration of tissues, we additionally used a model of human endothelial cells direct migration upon MSC CM gradient [26].

Despite direct in vitro or in vivo biological activity tests seem to be more informative, they demonstrate several substantial disadvantages. The main challenge is to render some injury-specific conditions in model objects. Many in vivo studies in the fields of oncology and adaptive immunity conducted on rodents have faced this problem. In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and Directive 2010/63/EU on protection of animals used for scientific purposes, the 3R principles (replacement, reduction, and refinement) should be applied to production and control testing of medicinal products. [43]. Last but not the least, it is rational to take into account a high cost of these types of potency test.

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As CM action is mediated by the soluble factors, the development of a surrogate test based on the correlations between defined factor concentration (e.g., measured by immunoassay) and potency seems to be reasonable. It is necessary to analyze secretome profiles of cells from a large number of donors in order to detect the most robust and relevant active factors in CM concerning current MOA. The molecules selected for this analysis should be the most significant for maintenance of the selected MOA, and less significant for another MOA. In **Figure 1**, if someone selects MOA-II as pivotal one, he should firstly consider factor-2 as the candidate for the surrogate test. Factor-12 and factor-23, which apart from MOA-II are involved in MOA-I and MOA-III, respectively, should be chosen only if their levels are crucial for the MOA-II potential.

6. Conclusion

This chapter is dedicated to a novel class of biopharmaceuticals based on secretory components of MSC as products for regenerative medicine. CM-based cell-free therapeutics comprise multicomponent mixture with multiple targets and pleiotropic effects. These biopharmaceuticals reproduce many benefits of the rapidly developing cell therapy products. However, the use of cells might be complicated due to ectopic transplantation, tumorigenesis, and immune system reactions. MSC secretome is devoid of cell therapy side effects and has substantial advantages in manufacture, storage, and standardization making it a promising type of biopharmaceuticals.

We paid attention to safe and effective MSC CM-based cell-free therapeutics manufacturing conception. We have discussed several challenges concerning donor-associated variability, cell isolation procedure, optimal protocols for manufacturing and quality control, and lack of key regulatory decisions that must be overcome prior to the wide-scale clinical translation of such therapeutics. To reach the best safety and efficacy marks, developers could apply a system-wide approach to disease model analysis considering the principal mechanisms of tissue reparation and regeneration processes. In addition, it is rational to develop appropriate donor selection criteria that can help to involve only a suitable starting material to manufacturing process. Preventing the lot-to-lot variability together with improved efficacy could be facilitated by a relevant potency test development and validation required for the drug-quality control. The use of rational approach to the choice of MOA might help to relieve development and conduction of a potency test by transition to more simple surrogate approach. In addition, implementing of these approaches into practice would help to develop novel legal potency test guidelines for cell-free therapeutics with unambiguous rules and examples.

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