PERSPECTIVE

Mesenchymal stem cells secretome: current trends and future challenges

Mesenchymal stem cells (MSCs) secretome: a good candidate for medical biotechnology? Medical biotechnology is currently defined as the application of biotechnological tools for producing multiple technologies and products to health care, becoming an important bridge between different fields, namely neuroscience, regenerative medicine, pharmacology and bio-engineering (Pham, 2018). The use and manipulation of stem cells can potentially represent a medical biotechnology breakthrough that brings regenerative medicine to a new era. Actually, over the last decade, the use of stem cells has remarkably been proposed as a regenerative tool, and within it, MSCs have emerged as a promising therapeutic option. As a consequence, they currently represent an effective tool in the treatment of several diseases due to their tissue protective and reparative mechanisms (Phelps et al., 2018). Indeed, MSCs are the most frequently stem cell population used in clinical trials (as of the end of 2018, a total of 861 trials were registered according to the US National Institute of Health – https://clinicaltrials.gov), presenting key advantages such as (1) be isolated from a patient and used for autologous transplantation; (2) not divide uncontrollably and form teratomas; and (3) have the capability to differentiate into multiple lineages (Kusuma et al., 2017). The initial therapeutic rationale of the use of MSCs in regenerative medicine as mostly attributed to their capability of homing to injury sites and differentiates into different cell types, leading to tissue repair. Although promising, this theory was revisited at the beginning of the 20th century when, for the first time, Gnecchi and colleagues revealed that MSCs mediated its therapeutical effects by the secretion and release of trophic molecules, nowadays known as secretome (Gnecchi et al., 2005). Indeed, cell tracking analysis has revealed that when transplanted, MSCs (and stem cells in general) do not commonly become a part of the injured site, whereby accumulating pieces of evidence indicate that the secretome is considered the primary attribute of MSC-mediated repair and regeneration (Teixeira et al., 2013).

MSC's secretome has been described as a complex mixture of soluble products composed by a proteic soluble fraction (constituted by growth factors and cytokines), and a vesicular fraction composed by microvesicles and exosomes, which are involved in the transference of proteins and genetic material (e.g., microRNAs) to other cells, with promising therapeutic effects (Teixeira et al., 2013). Notably, MSCs secretome itself is starting to be considered a potential active pharmaceutical component, in which its vesicular portion has been revealing promising characteristics to be used as drug delivery system, mainly due to its homing capabilities, thereby opening an opportune window to specific and targeted compounds (drugs, proteins, etc.) release into damaged lesions (Bari et al., 2018) (**Figure 1**).

The current view using cell transplantation-free strategies such as the one proposed by MSC's secretome provides key advantages over (stem) cells transplantation. First, the secretome strategy overcomes the cells survival upon transplantation; second, secretome compounds have lower cell surface proteins expression, providing less immunogenicity when compared to living and proliferative cells (Vizoso et al., 2017); third, using the secretome as a ready-to-

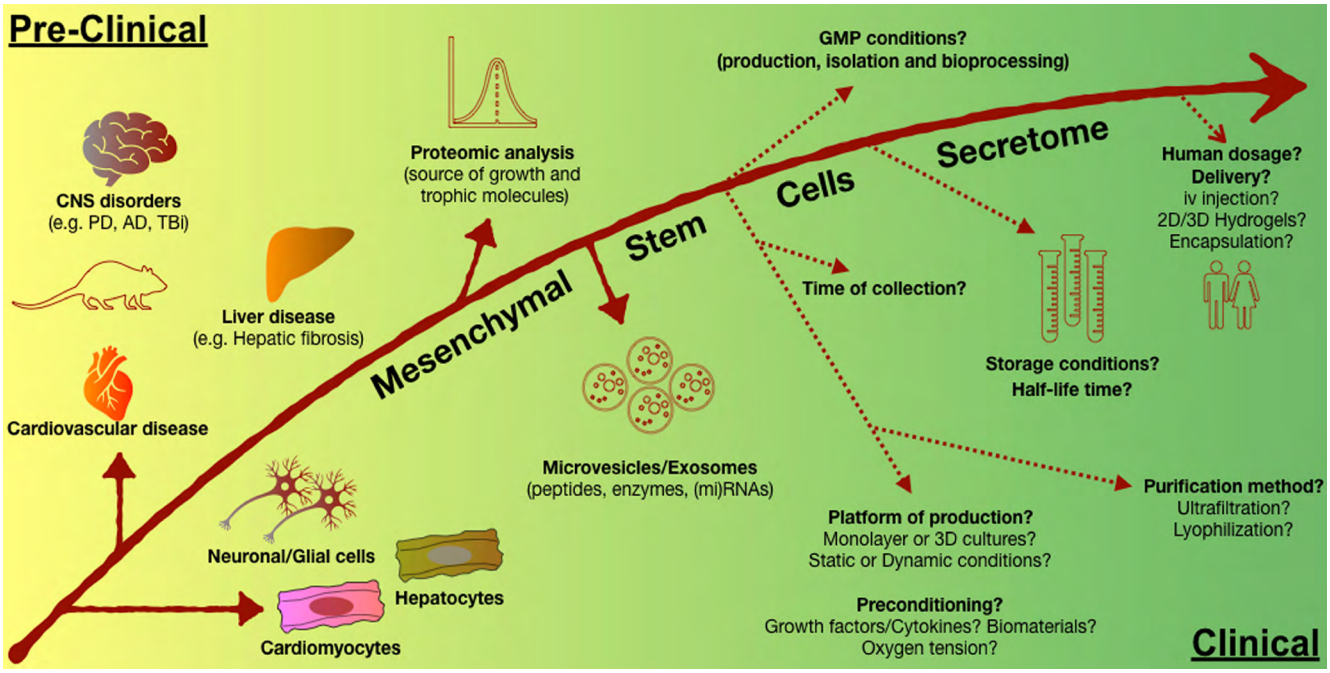

Figure 1 MSCs secretome overview.
A convergence in the scientific community is being formed on the close relation between MSCs therapeutic potential and their secretome. Indeed, pre-clinical studies using *in vitro* and *in vivo* models of disease have demonstrated promising results, with MSCs' secretome potentiating cell proliferation, survival, and differentiation, as well as physiological/motor performance. Actually, it has been based on such results that points of discussion are being established regarding the development of guidelines to secretome production. Due to its highly dynamic profile, MSCs may be "educated/modulated" to produce the best secretome "cocktail" possible, according to the desired therapeutic goals. Although the efficacy of MSCs secretome has been notably established in numerous pre-clinical models, the development of a large-scale GMP secretome-based product is required, thereby representing a crucial step to make MSCs secretome closer to the widens the gap that has been existing for decades between MSCs experimental research and its clinical use. 3D: 3-Dimensions; AD: Alzheimer's disease; GMP: good manufacturing protocol; Iv: intravenous injection; MSC: mesenchymal stem cell; PD: Parkinson's disease; TBi: traumatic brain injury.

use product remarkably reduce the significant high number of cells needed for transplantation (7×10^6 cells/kg), as well as possible phenotypic and therapeutic potential alterations due to long periods of expansion of MSCs *in vitro* prior to transplantation; fourth, higher rates of production are possible through the use of dynamic controlled laboratory conditions (e.g., bioreactors (Teixeira et al., 2016)), providing a convenient source of bioactive factors; fifth, in the form of conditioned media, MSCs secretome use is more economical and practical for clinical applications, as it avoids invasive cell collection procedures; sixth, MSCs secretome obtained for therapeutic applications could be modified to desired cell-specific effects; seventh, time and cost of expansion and maintenance of cultured stem cells could be greatly reduced and off-the-shelf secretome therapies could be immediately available for treatment; eighth, MSCs secretome may be evaluated for safety, dosage and potency in a similar manner to the conventional pharmaceutical compounds; and finally, MSCs secretome storage can be performed with safety and without losing product potency, discarding the use of potential toxic cryoprotectant agents (Vizoso et al., 2017).

Thus, the use of secretome as whole or its components *per se*, has advantages over the transplantation of cells themselves, as the few clinical trials already performed so far by using this concept revealed safety and feasibility, with no adverse effects being reported (US National Institutes of Health, http://ClinicalTrials.gov), thereby indicating secretome as a source of bioactive agents that can be efficiently stored and transported as a ready to use biological product.

What are still the constraints in the development and processing of MSCs secretome? Although it has been hypothesized that MSCs secretome could be a great biomedical product, as it contains highly biological compounds that may be easily manufactured, on the other hand, it has been very hard to fully define the biochemical composition of MSCs secretome, as well as to measure the activity and half-life time of its components (Vizoso et al., 2017). In fact, so far, there is still not a fulfilled and standard list of biomolecules or (mi)RNAs to be quantified, which have led to a range of studies that selected their own or appropriated molecules of interest, instead of considering secretome composition as a whole (Phelps et al., 2018). Gender, donor age, and phenotype have all been found as important factors contributing to the diversity of MSCs function and derivates. The metabolic state of each individual is also being indicated as a factor influencing MSCs and its secretome-derived products, with the vesicular fraction, in particular, being associated with metabolic organ crosstalk (Phelps et al., 2018). Nevertheless, the main concern pointed by the literature remains in the inexistence of a standard protocol for MSCs isolation/expansion, secretome production, collection, and bioprocessing, which have generated uncertainty about secretome (products) biological effects that seem to be strongly influenced by the preparation method (Kusuma et al., 2017). The basis of such uncertainty relies on the use of several culture conditions through the articulation of different culture parameters (e.g., seeding cell density, pH, oxygen tension, shear stress, (bio)mechanical forces, electromagnetic and chemical stimulus) together with different types of culture systems, namely monolayer/static (plates, cell-culture flasks) *versus* 3-dimensions/dynamic cell culture conditions (spheroids, bioreactors), which has resulted in a range of different protocols and outcomes (Kusuma et al., 2017). In line with this, the absence of well-defined and standard culture medium is still another critical point in secretome production and bioprocessing that has also contributed to heterogeneity of results as well (Phelps et al., 2018). In fact, the precise mechanism by which the secretome is changed/modulated by the microenvironment remains unknown. As so, as soon as we can (in early stages) realize the differing effects of these multiple conditions and platforms, closer we will be to ensure a homogenous, scalable and effective secretome quality production.

What are the challenges for the clinical translation of MSCs secretome based therapies? As recently reviewed by Phelps et al. (2018), the exclusion of transplanted cells means products (i.e., secretome) that can be bioengineered to increase its therapeutic potential, quality control, and scale up to specific dosages, This fact, associated to the nonliving profile of the secretome means that it can be characterized, stored, packaged and safely transported in a more easy way than viable cells, thereby representing a critical statement for the economic viability of new therapeutical strategies. Therefore, steps should be taken to ensure matched culture conditions to result in secretome-derived products valuable to (specific) translational approaches. To achieve such purpose, several challenges need to be overcome to make MSCs secretome clinically available as a cell-free therapy.

Match donor characteristics and tissue sources as a first step will be crucial to a precise development and application of MSCs secretome-derived products (Vizoso et al., 2017). In fact, studies (including from our group) have recently indicated that the secretome of MSCs from different tissues sources has different secretory profiles and distinct exosomal compositions (Pires et al., 2016), thereby indicating that such difference in their secretion pattern may indicate that their secretome or derived vesicles may be specific to a (pathological) condition. For instance, Assunção-Silva et al. (2018) have recently shown that cells isolated from the adipose tissue have a stronger impact on axonal growth when compared to using bone marrow as a source. Therefore, studies should be performed to correlate the impact of different MSCs (secretome) sources towards benefit for various disease models. Also, while much work has been performed in understanding how MSCs change in response to environmental factors, remarkably much less work has been focused on the effect of these factors on resultant MSCs secretome profile, in its characterization and optimization for specific applications (Teixeira et al., 2016; Phelps et al., 2018). Therefore, to assess and properly characterize the therapeutic potential of MSCs secretome and all its components, it should be mandatory the standardization of a protocol since the isolation to the expansion of MSC cultures, secretome production and isolation of its defined components (Teixeira et al., 2016). Although Bari and colleagues (Bari et al., 2018) have recently proposed a pilot study proposing a good manufacturing protocol (GMP) to transform MSCs secretome into a pharmaceutical product, by combining, in an integrative manner ultrafiltration and freeze-drying techniques, the establishment, validation and consequent use of a GMP production process must be a key aspect to the production, isolation, bioprocessing and purification of secretome from MSCs cell culture supernatants, as it will allow obtaining standardized products suitable for clinical applications – **Figure 1**. If achieved, a full characterization of the obtained secretomes (with a special focus on active molecules that may be oncogenic, as we know that MSCs secretome contains proteins and (mi)RNAs able to alter the genome and function of neighboring cells) must be performed to confirm the reproducibility of the production and isolation method, having in mind regulatory requirements and the ultimate goal of defining an optimal secretome "cocktail" for a given therapeutic application (Pires et al., 2016).

Being so, for MSCs secretome reach clinical practice, MSCs must be expanded in defined GMP culture conditions that are reproducible, scalable and well-controlled aiming to limit heterogeneity and enhance the predictability of secretome-derived products in terms of composition and function (Bari et al., 2018). The use of dynamic culture platforms, namely bioreactors has been suggested as a way to improve such approach, offering a higher level of homogeneity and process control, thereby reducing both batch-to-batch and within-batch variability of MSC cell cultures and consequently its

secretome-derived products (Teixeira et al., 2016). Still, the use of biomaterials has also been looking at an add-on to modulate secretome production, and even as a way to (better) deliver it. However, although the potential of this is enormous yet remains the challenge to prove it as such (Cai et al., 2016). As a complement, is also pertinent to assess/establish the optimal timing of secretome collection, as well as protocols of storage, transport, and delivery since MSCs secretome is a highly dynamic product (**Figure 1**). Such features may be used to improve, potentiate or adjust secretome therapeutical effects in a way of personalizing it according to each patient's condition, thereby individualizing the treatment through a specific MSCs conditioning protocol (by the use of a range of different extracellular cues) or choosing MSCs cell lines to more closely meet each patient's needs (Vizoso et al., 2017). Nevertheless, although MSCs secretome appears to be a promising therapy for diverse disease or injury, before its application in humans, molecular and therapeutical mechanisms must be defined. Indeed, and as it was recently indicated, a highly pure ready-off-the-shelf secretome product (for instance powder-based) and group-to-group comparisons must be performed in the near future, as this may represent the missing piece necessary for the scientific progress of this field (Bari et al., 2018).

Taken together, in current practices the use of MSCs secretome is gaining a notable consideration, being studied and applied in several experimental disorders (e.g., bone/cartilage lesions, cardiovascular and kidney disease, wound healing and tissue/organ fibrosis) with promising outcomes as it was recently revised by Kusuma et al. (2017). Neurodegeneration is also a popular application of MSCs secretome, as central nervous system has a low regenerative potential, and promising results have been observed in injured animal models of spinal cord, and neurodegenerative disorders as Parkinson's disease (Teixeira et al., 2013, 2017), demonstrating that MSCs (secretome) might be directly engineered to selectively load (certain) factors to treat targeted diseases more effectively (Kusuma et al., 2017). Quite challenging will also be to understand the impact of these (bio)engineering advances in the production of MSCs secretome and how different preconditional (environmental) conditions might be a tool to enhance the feasibility, safety and disease-specific therapeutical potential of MSCs secretome for clinical use.

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References

Assunção-Silva RC, Mendes-Pinheiro B, Patricio P, Behie LA, Teixeira FG, Pinto L, Salgado AJ (2018) Exploiting the impact of the secretome of MSCs isolated from different tissue sources on neuronal differentiation and axonal growth. Biochimie 155:83-91.

Bari E, Perteghella S, Di Silvestre D, Sorlini M, Catenacci L, Sorrenti M, Marrubini G, Rossi R, Tripodo G, Mauri P, Marazzi M, Torre ML (2018) Pilot production of mesenchymal stem/stromal freeze-dried secretome for cell-free regenerative nanomedicine: a validated GMP-compliant process. Cells 7:E190.

Cai L, Dewi RE, Goldstone AB, Cohen JE, Steele AN, Woo YJ, Heilshorn SC (2016) Regulating stem cell secretome using injectable hydrogels with in situ network formation. Adv Healthc Mater 5:2758-2764.

Gnecchi M, He H, Liang OD, Melo LG, Morello F, Mu H, Noiseux N, Zhang L, Pratt RE, Ingwall JS, Dzau VJ (2005) Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med 11:367-368.

Kusuma GD, Carthew J, Lim R, Frith JE (2017) Effect of the microenvironment on mesenchymal stem cell paracrine signaling: opportunities to engineer the therapeutic effect. Stem Cells Dev 26:617-631.

Pham P (2018) Chapter 19 - Medical biotechnology: techniques and applications. In: Omics Technologies and Bio-Engineering (Barh D, Azevedo V, eds), pp 449-469. Ho Chi Minh city, Vietnam: Academic Press.

Phelps J, Sanati-Nezhad A, Ungrin M, Duncan NA, Sen A (2018) Bioprocessing of mesenchymal stem cells and their derivatives: toward cell-free therapeutics. Stem Cells Int 2018:9415367.

Pires AO, Mendes-Pinheiro B, Teixeira FG, Anjo SI, Ribeiro-Samy S, Gomes ED, Serra SC, Silva NA, Manadas B, Sousa N, Salgado AJ (2016) Unveiling the differences of secretome of human bone marrow mesenchymal stem cells, adipose tissue-derived stem cells, and human umbilical cord perivascular cells: a proteomic analysis. Stem Cells Dev 25:1073-1083.

Teixeira FG, Carvalho MM, Sousa N, Salgado AJ (2013) Mesenchymal stem cells secretome: a new paradigm for central nervous system regeneration? Cell Mol Life Sci 70:3871-3882.

Teixeira FG, Carvalho MM, Panchalingam KM, Rodrigues AJ, Mendes-Pinheiro B, Anjo S, Manadas B, Behie LA, Sousa N, Salgado AJ (2017) Impact of the secretome of human mesenchymal stem cells on brain structure and animal behavior in a rat model of Parkinson's disease. Stem Cells Transl Med 6:634-646.

Teixeira FG, Panchalingam KM, Assuncao-Silva R, Serra SC, Mendes-Pinheiro B, Patricio P, Jung S, Anjo SI, Manadas B, Pinto L, Sousa N, Behie LA, Salgado AJ (2016) Modulation of the mesenchymal stem cell secretome using computer-controlled bioreactors: impact on neuronal cell proliferation, survival and differentiation. Sci Rep 6:27791.

Vizoso FJ, Eiro N, Cid S, Schneider J, Perez-Fernandez R (2017) Mesenchymal stem cell secretome: toward cell-free therapeutic strategies in regenerative medicine. Int J Mol Sci 18:E1852.

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