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Review Article

Mesenchymal stem cells from biology to therapy

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Mesenchymal stem cells are as fascinating as they are enigmatic. They appear capable of performing a wide array of functions that cross skeletal biology, immunology and haematology. As therapeutics, mesenchymal stem cells or even just their secreted products may be used to regenerate tissue lost through injury or disease and suppress damaging immune reactions. However, these cells lack unique markers and are hard to identify and isolate as pure cell populations. They are often grown in laboratories using basic and undefined culture conditions. We cannot even agree on their name. While mesenchymal stem cells may lack the developmental understanding and defined differentiation hierarchies of their more illustrious stem cell cousins, they offer a compelling scientific challenge. In depth understanding of mesenchymal stem cell biology will enable us to exploit fully one of the most clinically valuable cell sources.

MSC discovery and biology

In 1966, Friedenstein et al. [1] demonstrated that cells derived from mouse bone marrow, as well as other blood-forming organs, contain a subpopulation of stem-like cells that give rise to bone cell precursors. In this seminal paper, these cells were named osteogenic stem cells, although with further study, Friedenstein realised their greater potential to differentiate into fat and cartilage precursors, too [2,3]. In 1991, Caplan [4] coined the term 'mesenchymal stem cell' and the abbreviation 'MSC', which has since remained the most commonly used moniker. The notion that MSCs have trilineage potential, i.e. the capacity to differentiate into bone, cartilage and fat cells, was developed further in the 1999 report by Pittenger et al. [5], where bone marrow cells isolated from iliac crest aspirates were shown to differentiate into these lineages *in vitro* with the addition of differentiation-specific stimuli. Many further studies have since reproduced these methods and built on them.

Expanding research activity and evolution of the field made clear the more complex nature of these cells and that technically limited isolation techniques often failed to select a homogeneous *stem cell* population. It was thought that the name should reflect this, with proposed MSC expansions including 'multipotent stromal cells', 'mesenchymal stromal cells' [6] and even 'medicinal signalling cells' [7]. Some of this nomenclature actually refers to specific subpopulations of cells isolated from tissues by plastic adherence, while others are an attempt at broadening the term. In either case, it can confuse discourse and conflate smaller, more specialised subpopulations, with the overall, heterogeneous cell population. Some authors use the term 'skeletal stem cell (SSC)', recognising that a stem cell population exists in adult bone marrow, capable of forming bone, cartilage, fat, and haematopoietic supporting tissue [8,9]. The SSC term also removes reference to embryonic mesenchyme, which implies the capacity to differentiate in all mesenchyme-derived cells and tissues including blood cells. The naming of these cells continues to be debated [10]. For the remainder of this review, the term 'mesenchymal stromal cells' will be used for MSCs, to refer to the broader population of cells, and to acknowledge their heterogeneity and the fact that not all plastic-adherent cells isolated from sources such as bone marrow and fat have multipotent differentiation capability. This view is in line with the International Society for Cellular Therapy (ISCT) position paper first published in 2005 [6], where MSCs were defined to be plastic-adherent cells, derived from several tissues, such as bone marrow, umbilical cord or fat, with the potential to differentiate into bone, cartilage, and fat cells. They should also express the cell surface proteins CD105, CD73 and CD90, and lack CD45, CD34, CD14 or CD11b, CD79 or

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CD19 and HLA-DR [11], though it is important to note that this statement specifies that these are minimal criteria and a starting point for further study. This publication is often referred to in MSC-related literature to assure the reader that the MSCs used in the study in question met the 'ISCT criteria', though there are pitfalls with this approach, which we discuss later in this review. The ISCT criteria were later expanded [12] to recommend the inclusion of tissue source when referring to particular MSC populations used in experimental work, alongside a robust body of evidence clarifying whether *stem*-like cells or *stromal*-like cells are being presented, with emphasis on the fact that mesenchymal *stem* cells represent self-renewing, multipotent cells, while mesenchymal *stromal* cells describe bulk, unfractionated cells.

While most of this work attempted to define an *in vitro* expanded MSC population, there has been some progress in identifying the *in vivo* location of MSCs, or 'niche', focusing mainly on murine skeletal tissues. The niche is a specialised tissue microenvironment that houses and regulates the function of an adult stem cell [13,14]. Stem and progenitor cells that give rise to osteogenic and chondrogenic lineages have been identified primarily around blood vessels in bone marrow [15–19] and more recently, the outer bone surface [20] and the growth plate of cartilage [21–23]. Much of the work on bone marrow MSCs has analysed stromal cells as *in vivo* regulators of the haematopoietic stem cell (HSC) niche [18,24]. Through these and related studies, MSCs have been identified by their production of HSC-niche regulatory factors, such as CXCL12 and stem cell factor (SCF) and the expression of leptin receptor (LEPR), Nestin and CD146, amongst others [17,19,25,26]. Using in-depth gene profiling techniques, up to 17 different subtypes of related stromal cells have been identified [27]. A clear picture of the *in vivo* 'MSC map' is still developing, which will be aided by the emergence of advanced spatial profiling techniques; see Dolgalev and Tikhonova [28] for a recent extensive review. Further studies of MSCs *in vivo* using human tissues are needed, particularly due to the differences in postnatal mouse and human long bone development [29]. In situ analyses of MSCs in different tissues will also provide better biological understanding and more appropriate terminology linked to tissue-specific subtypes. Effects of factors such as oxygen tension [30] and cell–cell interactions will be of particular interest, as this could shed light on the nature of the *in vivo* MSC environment, which may inform bioengineering approaches to maintaining MSCs *ex vivo* in as natural a state as possible [30,31].

The issue of MSC identity is complicated further as MSC-*like* cells have been isolated from a myriad of tissues, though most commonly from bone marrow and adipose tissue from adults. The umbilical cord and placenta are also accessible sources of MSCs, as these are often considered medical waste. To achieve relevant cell numbers, MSCs are usually culture expanded for both research and clinical applications, which is an easily reproducible procedure in the laboratory. Simple MSC isolation and expansion procedures and their clinically appealing regenerative potential underlie the steady increase in the number of publications and clinical trials using MSCs, especially since the year 2000 (Figure 1).

However, it is important to bear in mind that cultured MSCs differ substantially from their native physiological state, due to the vastly different environmental conditions. Culture expansion removes tissue-specific biological cues of the niche, including the presence of different cell types, extracellular matrix (ECM)
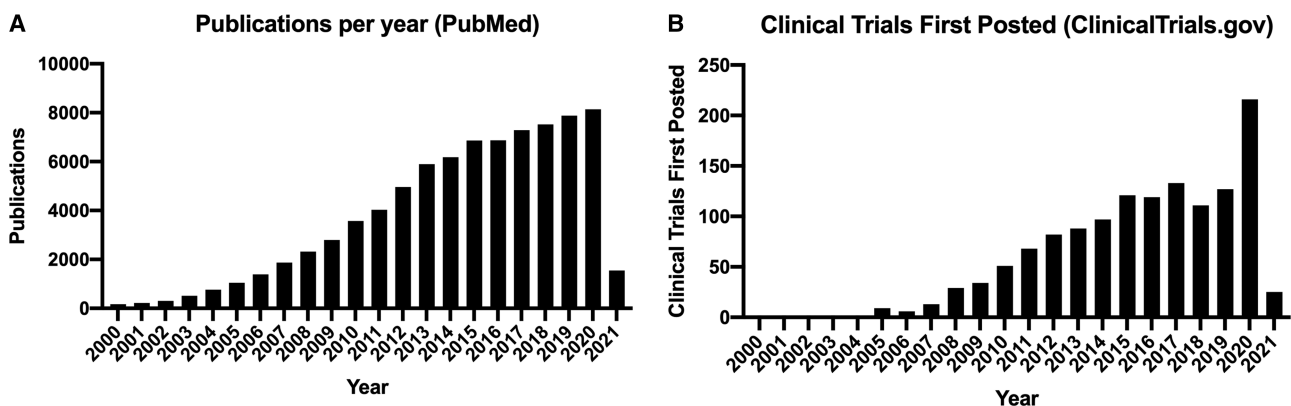

Figure 1. Activity in the research and clinical trial fields using MSCs.
(**A**) Number of publications listed on PubMed database (search term: 'mesenchymal stem cells' OR 'mesenchymal stromal cells') by year from 2000.
(**B**) Clinical trials first posted (search term on ClinicalTrials.gov: 'mesenchymal stem cells' OR 'mesenchymal stromal cells') by year from 2000.

components and oxygen gradients, and may disguise true *in vivo* function. The question of whether the therapeutic potential we observe in the laboratory is a product of the process these cells undergo when they are isolated from their tissues and expanded, or in fact reflects their natural function in the organism is a matter of further research. Indeed, if considering the bone marrow niche as an example, which appears varied and complex based on the evidence from single cell and spatial profiling studies described above, tissue culture conditions differ substantially. The presence of animal serum allows for colony formation and expansion of cells, with abundant nutrition and stimulation to remain in culture for extended periods of time. It is, however, understood that within the bone marrow, MSCs maintain their stem-like properties, at least in part, through specific cell–cell interactions. These are comparatively less abundant once the cells are introduced into a culture vessel. The serum contains ECM proteins, such as fibronectin and collagens, which prompt the formation of extensive cytoskeletal networks as cells attach and spread on a rigid, flattened surface. The cells interact with their substrate through integrin-mediated focal adhesions, which is thought to influence their fate [32,33].

Integrin-based interactions are also involved in directing MSC function through substrate stiffness. On stiff surfaces, MSCs were shown to exhibit a tendency for osteogenic differentiation, based on alkaline phosphatase activity, osteogenic gene marker expression, and calcium staining. In addition to increased expression of several integrins, increased activation of downstream signalling events, for example via focal adhesion kinase (FAK), phosphorylated extracellular signalling regulated kinase (pERK), phosphorylated Akt, glycogen synthase kinase 3β (GSK3β), and β-catenin, have been observed, indicating a complex mechanotransduction cascade mediating the effect of substrate stiffness on cell fate [34–37]. Soft surfaces, on the other hand, have been shown to maintain MSC self-renewal capacity and appear to promote adipogenic differentiation [38,39]. This property of soft hydrogels, thought to be via Yes-associated protein-1 (YAP) signalling, is being investigated as a strategy for maintaining MSC surface marker expression patterns associated with their regenerative properties, which are lost over time in culture [40]. In general, material stiffnesses mimicking those of certain tissues tends to condition MSCs to adapt to this and induces gene expression patterns consistent with corresponding MSC niches (reviewed in [41]), explaining to some extent the propensity for certain lineages on particular substrates.

While *in vitro* analyses may offer only an interpretation of the true biological nature of MSCs, it is clear from this work that MSCs have substantial clinical potential and that there are opportunities to use these cells as therapeutics in a broad range of applications.

MSC therapeutic approaches

Autologous and allogeneic sources of MSCs have been used as cell therapies for many years and form the vast majority of clinical trials identified in Figure 1B. Recently, interest in the use of MSC-derived bioactive products — those secreted by MSCs into the extracellular environment — has increased markedly. We will cover both these approaches under 'Cell-based therapies' and 'Cell-derived therapies' below (see also Figure 2).

Cell-based therapies

Therapeutic approaches exploiting MSC biology focus on their ability to differentiate into new tissues and act as modulators of the immune system. Early work investigating MSCs for their therapeutic utility demonstrated that MSCs have certain immunomodulatory characteristics that allow them to persist in a xenogeneic environment. To demonstrate this, Liechty et al. [42] introduced human MSCs into sheep foetuses both before and after the foetuses were expected to develop immune-competence. The cells successfully engrafted in both cases and integrated into the developing tissues, undergoing site-specific differentiation. This immunomodulatory capability, coupled with their tissue-forming capacity, provides MSCs with their unique therapeutic value. Clinical targets for MSC therapies include inflammatory indications such as graft versus host disease (GvHD) [43] and rheumatoid arthritis [44], as well as for the purposes of tissue regeneration, such as osteogenesis imperfecta [45,46] and large bone defects [47,48]. In our recent analysis of all published clinical trials (2009–2019) using MSCs, we identified 35 different indications, most commonly those affecting the nervous, cardiovascular and musculoskeletal systems [49].

Many MSC-based interventions rely on MSCs homing to the target site following systemic injection. MSC homing is thought to be cytokine and surface antigen regulated, and refers to the idea that MSCs, when injected systemically into the bloodstream or administered locally, preferentially migrate toward sites of injury [50]. While systemic administration has its benefits, such as being the least invasive means of delivering MSCs, it has been shown that homing to the desired tissue can be very inefficient [51–53], resulting in low levels of engraftment, mainly due to entrapment in the lung microvasculature [54]. Many strategies are being
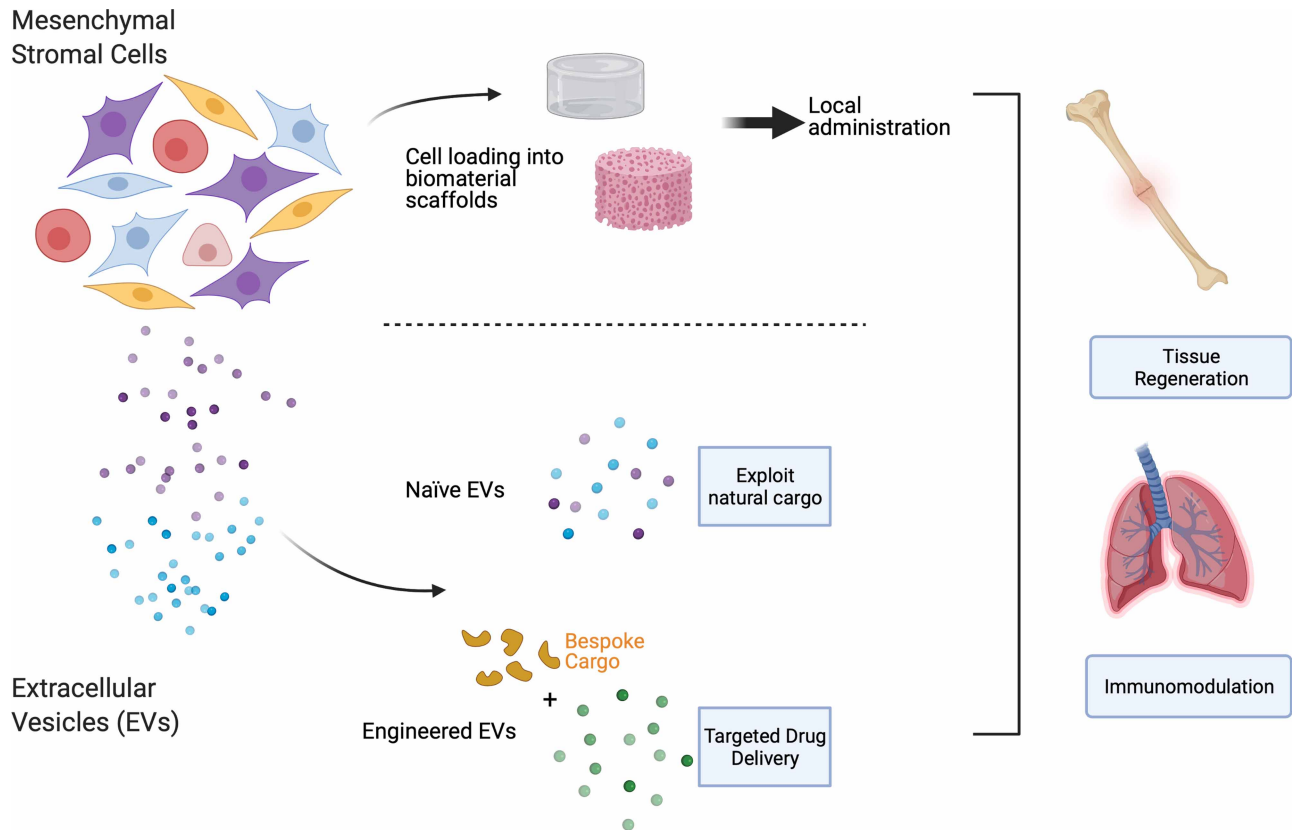
Figure 2. Approaches to therapeutic exploitation of MSCs and their products.

MSCs can be applied by direct injection of cell suspensions or seeded onto biomaterial scaffolds as adhesion sites for local administration. MSC-derived EVs can be used in their naïve, unaltered state or engineered to carry specific cargos and/or cell-targeting motifs. Both modalities are applicable in tissue regeneration or immunomodulatory therapies.

investigated to improve MSC homing, focusing on making patients more receptive to MSCs [55,56] or engineering the MSCs to avoid problems related to patient responses to systemically administered cells [57,58]. It should be noted that in order to translate this approach into viable therapies, scale-up must be addressed. MSC doses are generally in the region of 1–2 million cells/Kg [59], which poses a particular challenge related to culture-associated loss of specific MSC markers used as critical quality attributes in the manufacture of MSC therapeutics [60].

MSCs can be administered in a more targeted manner by local administration using scaffolds (Figure 2). To address the issue of low engraftment, a biomaterial scaffold is often used to provide a three-dimensional (3D) structure with a high surface area for cell adhesion, especially when large areas of damaged tissue need to be replaced and/or mechanical strength is required, for example in bone and cartilage replacements, where much of the activity in this area has focused. The aim of this approach is to mimic the tissue microenvironment. Biomimetic scaffolds can range from simply imitating the stiffness or general architecture of the tissue in question, to being doped with specific growth factors and coated with matrix proteins to coax MSCs into a particular lineage. For example, this concept can be applied to critical size bone defects, where a physical structure is required to administer MSCs. Persson and colleagues describe an 80 : 20 mixture of polylactic acid (PLA) and hydroxyapatite (HA), which was used to fabricate a woven scaffold with specific porosity and pore size. These scaffolds were shown to promote MSC proliferation, as well as supporting osteoblastic differentiation and mineralised bone matrix formation in critical size defects [61]. Scaffolds can also be more complex composites, and even can be personalised, by combining state-of-the-art engineering techniques with current knowledge of MSC biology. An example of this was demonstrated by Kuss et al., where a 3D-printed polycaprolactone (PCL)/HA composite scaffold was constructed, then coated with a complex, cell-laden hydrogel, with the aim

of improving vascularisation. The hydrogel contained a mixture of adipose-derived MSCs and human umbilical vein endothelial cells. This essentially prevascularized the construct, demonstrating the possibility of creating an already vascularised scaffold, made to fit unique anatomical structures [62]. For further information on the use of 3D scaffolds for MSC delivery, tissue regeneration, directing cell function, immunomodulation and genetic modification, please refer to recent reviews [63–66].

These studies are promising steps toward regenerative solutions to tissue repair by effectively engaging in multidisciplinary research to advance our understanding of how materials integrate into and interact with tissues to achieve optimal regeneration.

Cell-derived therapies

While cell-based therapies are proving encouraging, there has been growing interest in the use of cell-derived material for therapeutic purposes. Bioactive factors produced by cells, extracellular vesicles (EVs) in particular, can reflect the functions of the cell from which they originate. EVs are nanoscale, membranous particles secreted from cells, containing diverse cargo including nucleic acids, such as miRNA, and proteins. It has been shown that EVs mediate cell-to-cell communication by shuttling biomolecules to influence the microenvironment [67–70]. Given that the function of EVs is to act as signalling particles for surrounding cells, it follows that the signals carried by the EVs could be harnessed to deliver desirable biological factors to target cells, affording them innate therapeutic utility (Figure 2). The EV field has grown hugely in recent years and several recent reviews describe in more detail EV biogenesis, function and clinical possibilities [71–73].

EVs are also being viewed as delivery vehicles (see Figure 2). Engineering EVs to transport specific cargo is an attractive prospect as they carry surface molecules which could aid in targeted delivery [74,75]. EVs can be loaded with proteins, nucleic acids, or small molecules by either modifying the producing cell or by directly loading the EVs, making this a versatile platform for drug delivery [76].

MSCs seem to be a particularly good source of EVs. Studies have shown that EVs derived from MSCs are more stable than those derived from other cell types [77], and the capabilities that MSCs exhibit in terms of their differentiation and immunomodulation potential leads to naturally clinically potent EVs. It could also be more efficient to use EVs from MSCs over the MSCs themselves, as EVs are produced constantly, so could be harvested as MSCs are expanded. Cell therapy on the other hand, would generally require cells to be expanded up to the point where they are used in that therapy. EV production can be assisted by MSC immortalisation, which has already been demonstrated by some groups [78–80], which gives rise to an inexhaustible source of therapeutically useful MSC-EVs, effectively eliminating batch variability; a problem inherent to the use of primary donor cells.

While there are currently no approved treatments available using EVs, there is an increasing body of published data pointing toward the clinical utility of EVs for many indications. The function of EVs in fracture healing, for example, was demonstrated in CD9 knockout mice, which were shown to have impaired EV biogenesis [81], as well as lowered rates of bone repair, as exhibited by retardation of callous formation [82], compared with wild type. Furuta and colleagues showed that this retardation was rescued by injection of EVs isolated from the conditioned medium of bone marrow-derived MSCs, but not from EV-free conditioned medium. Work by Qin et al. [83] further demonstrated that EVs from bone marrow-derived MSCs could enhance bone formation in calvarial defects of Sprague Dawley rats, with miR-196a identified as critical in regulating osteoblastic differentiation and osteogenic gene expression.

There has also been a lot of interest in the use of EVs for the attenuation of the after-effects of COVID-19, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The mechanism(s) of action is yet to be fully understood but revolve around dampening the aggravated inflammatory effects of the respiratory system and repairing tissue damage. It has been suggested in work completed before the onset of this novel coronavirus that inflammation in the lungs could be controlled using MSC-derived EVs by immune cell modulation [84], the notion of which has been put under more intense investigation as a result of the COVID-19 pandemic, though appropriately controlled trials are required [85].

Current challenges

It is an accepted truth that cultured cells termed 'MSCs' are vastly heterogeneous, as MSCs differ depending on donor/tissue source, isolation/culture technique, and inherent heterogeneity. With the ISCT position statement in 2006, there has been an attempt at harmonisation across groups, which is a positive step forward, but the lack of standardised criteria for the identification and classification of MSC subpopulations presents a

substantial obstacle to the development of MSC therapies. More work is required to further our understanding of MSC identity to move the field forward effectively.

A further challenge in the current approach to MSC therapy is the reliance on donor-derived cells for MSC-based therapy scale-up. Whether relying on MSCs themselves to deliver a therapeutic effect, or harvesting MSC-derived factors, MSCs will have to be culture expanded *ex vivo* to produce clinically usable doses. Using donor-derived cells, which will differ from donor-to-donor, introduces an extra quality control step into production, where there is the potential for many batches to be rejected. Additionally, there is an overwhelming reliance on animal-derived culture additives to produce the quantity of cells required for therapeutic use, which is both ethically and scientifically challenging. The most commonly used additive, foetal bovine serum (FBS), is unsustainably sourced, with the global demand of FBS increasing and the supply struggling to keep up [86,87]. FBS is a complex, undefined mixture, suffering from batch variability. Sources of variability cause major problems in the development of therapeutics, where consistency is key to overcoming regulatory burdens and successfully scaling up production. Xeno-free medium solutions are available, but there is a tendency for life science companies to develop proprietary formulations to protect commercial interests. As far as the research community is concerned, commercially available media are still undefined while being very costly. A chemically defined, non-proprietary medium would aid standardisation across MSC research groups and assist the development and manufacture of MSCs, and their secreted products, for clinical use.

The field of EV therapeutics is an emerging one and we still find ourselves in the early stages of developing and determining a gold standard set of processes by which EVs can be produced, harvested, isolated, and characterised. The problems are similar to those plaguing MSCs currently, as EVs are broadly characterised based on their size and how they were formed, often using marker expression and imaging as readouts. One example of how this problem becomes evident is the fact that the method by which EVs are isolated generally determines the identity of the resulting EV preparation. Currently, the most commonly used isolation technique involves differential ultracentrifugation, which is effective, but fairly crude and time-consuming. To address this, many researchers have developed other isolation techniques and the EV size distribution and yield, quality, and function differs between techniques [88,89].

The International Society for Extracellular Vesicles (ISEV) has published a position statement, similar to the ISCT in 2006, outlining a list of suggested protocols and recommendations on specific criteria to be reported in order to aid in the advancement of the field as a whole with a unified vocabulary. These guidelines also point out that it is an evolving document, and that new technologies are arising regularly, and that the aim is to enhance communication between researchers [90]. Communication is key.

With regard to EV functionality and their use to address the COVID-19 pandemic, ISCT and ISEV issued a joint statement encouraging investigations into MSC-derived EVs, as well as possibly other cell sources, as treatments for COVID-19, recognising their potential in this area, but stressing that they do not currently endorse their use without sufficient evidence of their safety and efficacy, alongside several more provisions related to clinical studies, manufacture, and regulation [85]. EVs are a rapidly growing, exciting field of research but careful consideration needs to be given to their mechanisms of action to ensure that these are used in a targeted manner, for maximal efficacy. Our currently limited understanding of factors underlying COVID-19 complications, as well as the complex mechanisms of action of EV interventions are an obstacle to good clinical trial design [91]. Further work into understanding the very nature of EVs is required to effectively design EV therapeutics.

Conclusions and future directions

MSCs are an exciting cell population. A vast amount of work is attempting to translate MSCs and related technologies into viable therapeutics for an enormous range of applications. In this review, we touched on some of the key target tissues, bone in particular, but the research is being developed in many more areas, including nerve, heart, cartilage, liver, kidney and, as we discussed above, virally induced inflammatory lung disorders. There are new and improved delivery methods in the pipeline, such as hydrogels for cells [92] and intranasal aerosols for EVs [93]. The emergence of EVs as a therapeutic modality has opened the doors to cell-free regenerative medicines, with great versatility and utility. That is not to say that cell therapies will be surpassed by EVs, but EVs are a powerful offshoot of traditional cell therapies with the potential to disrupt the regenerative medicine space. It is important to remember that while excitement continues to grow for MSC-based therapies, clinical development must always follow scientific understanding. There is much we still need to do in order to decipher the enigmatic MSC.

Summary

- Mesenchymal *stem* cells are frequently studied for research and clinical use as heterogeneous cell populations, giving rise to the term mesenchymal *stromal* cells (MSCs).

- MSCs have wide-ranging therapeutic applications but aspects of MSC biology require further work in order to maximise their potential.

- MSC-derived EVs are an emerging therapeutic modality.

- A harmonised approach to defining and analysing MSCs and MSC-EVs is essential for effective communication within the research community to facilitate progression within the field.

Conflicts of Interest

D.K. and P.G. have no conflicts of interest.

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Author Contributions

D.K. and P.G. wrote and revised the manuscript, and approved the final version.

Abbreviations

ECM, extracellular matrix; FBS, foetal bovine serum; HA, hydroxyapatite; HSC, haematopoietic stem cell; ISCT, international Society for Cellular Therapy; ISEV, international Society for Extracellular Vesicles; SSC, skeletal stem cell.

References

1 Friedenstein, A.J., Piatetzky-Shapiro, I.I. and Petrakova, K.V. (1966) Osteogenesis in transplants of bone marrow cells. *J. Embryol. Exp. Morphol.* **16**, 381–390 PMID: 5336210

2 Friedenstein, A.J., Petrakova, K.V., Kurolesova, A.I. and Frolova, G.P. (1968) Heterotopic transplants of bone marrow. *Transplantation* **6**, 230 https://doi.org/10.1097/00007890-196803000-00009

3 Friedenstein, A.J., Chailakhjan, R.K. and Lalykina, K. (1970) The development of fibroblast colonies in monolayer cultures of Guinea-pig bone marrow and spleen cells. *Cell Prolif.* **3**, 393–403 https://doi.org/10.1111/j.1365-2184.1970.tb00347.x

4 Caplan, A.I. (1991) Mesenchymal stem cells. *J. Orthop. Res.* **9**, 641–650 https://doi.org/10.1002/jor.1100090504

5 Pittenger, M.F., Mackay, A.M., Beck, S.C., Jaiswal, R.K., Douglas, R., Mosca, J.D. et al. (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* **284**, 143–147 https://doi.org/10.1126/science.284.5411.143

6 Horwitz, E.M., Le Blanc, K., Dominici, M., Mueller, I., Slaper-Cortenbach, I., Marini, F.C. et al. (2005) Clarification of the nomenclature for MSC: the international society for cellular therapy position statement. *Cytotherapy* **7**, 393–395 https://doi.org/10.1080/14653240500319234

7 Caplan, A.I. (2017) Mesenchymal stem cells: time to change the name! *Stem Cells Transl. Med.* **6**, 1445–1451 https://doi.org/10.1002/sctm.17-0051

8 Bianco, P. and Robey, P.G. (2015) Skeletal stem cells. *Development* **142**, 1023–1027 https://doi.org/10.1242/dev.102210

9 De Luca, M., Aiuti, A., Cossu, G., Parmar, M., Pellegrini, G. and Robey, P.G. (2019) Advances in stem cell research and therapeutic development. *Nat. Cell Biol.* **21**, 801–811 https://doi.org/10.1038/s41556-019-0344-z

10 Sipp, D., Robey, P.G. and Turner, L. (2018) Clear up this stem-cell mess. *Nature* **561**, 455–457 https://doi.org/10.1038/d41586-018-06756-9

11 Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F., Krause, D. et al. (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement. *Cytotherapy* **8**, 315–317 https://doi.org/10.1080/14653240600855905

12 Viswanathan, S., Shi, Y., Galipeau, J., Krampera, M., Leblanc, K., Martin, I. et al. (2019) Mesenchymal stem versus stromal cells: International Society for Cell & Gene Therapy (ISCT®) mesenchymal stromal cell committee position statement on nomenclature. *Cytotherapy* **21**, 1019–1024 https://doi.org/10.1016/j.jcyt.2019.08.002

13 Schofield, R. (1978) The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells* **4**, 7–25 PMID: 747780

14 Scadden, D.T. (2006) The stem-cell niche as an entity of action. *Nature* **441**, 1075–1079 https://doi.org/10.1038/nature04957

15 Shi, S. and Gronthos, S. (2003) Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. *J. Bone Miner. Res.* **18**, 696–704 https://doi.org/10.1359/jbmr.2003.18.4.696

16 Crisan, M., Yap, S., Casteilla, L., Chen, C.-W., Corselli, M., Park, T.S. et al. (2008) A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* **3**, 301–313 https://doi.org/10.1016/j.stem.2008.07.003

17 Zhou, B.O., Yue, R., Murphy, M.M., Peyer, J.G. and Morrison, S.J. (2014) Leptin-receptor-expressing mesenchymal stromal cells represent the main source of bone formed by adult bone marrow. *Cell Stem Cell* **15**, 154–168 https://doi.org/10.1016/j.stem.2014.06.008

18 Kfoury, Y. and Scadden, D.T. (2015) Mesenchymal cell contributions to the stem cell niche. *Cell Stem Cell* **16**, 239–253 https://doi.org/10.1016/j.stem.2015.02.019

19 Sacchetti, B., Funari, A., Michienzi, S., Di Cesare, S., Piersanti, S., Saggio, I. et al. (2007) Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. *Cell* **131**, 324–336 https://doi.org/10.1016/j.cell.2007.08.025

20 Debnath, S., Yallowitz, A.R., McCormick, J., Lalani, S., Zhang, T., Xu, R. et al. (2018) Discovery of a periosteal stem cell mediating intramembranous bone formation. *Nature* **562**, 133–139 https://doi.org/10.1038/s41586-018-0554-8

21 Chan, C.K.F., Gulati, G.S., Sinha, R., Tompkins, J.V., Lopez, M., Carter, A.C. et al. (2018) Identification of the human skeletal stem cell. *Cell* **175**, 43–56.e21 https://doi.org/10.1016/j.cell.2018.07.029

22 Mizuhashi, K., Ono, W., Matsushita, Y., Sakagami, N., Takahashi, A., Saunders, T.L. et al. (2018) Resting zone of the growth plate houses a unique class of skeletal stem cells. *Nature* **563**, 254–258 https://doi.org/10.1038/s41586-018-0662-5

23 Newton, P.T., Li, L., Zhou, B., Schweingruber, C., Hovorakova, M., Xie, M. et al. (2019) A radical switch in clonality reveals a stem cell niche in the epiphyseal growth plate. *Nature* **567**, 234–238 https://doi.org/10.1038/s41586-019-0989-6

24 Morrison, S.J. and Scadden, D.T. (2014) The bone marrow niche for haematopoietic stem cells. *Nature* **505**, 327–334 https://doi.org/10.1038/nature12984

25 Ding, L., Saunders, T.L., Enikolopov, G. and Morrison, S.J. (2012) Endothelial and perivascular cells maintain haematopoietic stem cells. *Nature* **481**, 457–462 https://doi.org/10.1038/nature10783

26 Méndez-Ferrer, S., Michurina, T.V., Ferraro, F., Mazloom, A.R., Macarthur, B.D., Lira, S.A. et al. (2010) Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature* **466**, 829–834 https://doi.org/10.1038/nature09262

27 Baryawno, N., Przybylski, D., Kowalczyk, M.S., Kfoury, Y., Severe, N., Gustafsson, K. et al. (2019) A cellular taxonomy of the bone marrow stroma in homeostasis and leukemia. *Cell* **177**, 1915–1932.e16 https://doi.org/10.1016/j.cell.2019.04.040

28 Dolgalev, I. and Tikhonova, A.N. (2021) Connecting the dots: resolving the bone marrow niche heterogeneity. *Front. Cell Dev. Biol.* **9**–622519 https://doi.org/10.3389/fcell.2021.622519

29 Kurenkova, A.D., Medvedeva, E.V., Newton, P.T. and Chagin, A.S. (2020) Niches for skeletal stem cells of mesenchymal origin. *Front. Cell Dev. Biol.* **8**, 592 https://doi.org/10.3389/fcell.2020.00592

30 Sayin, E., Baran, E.T., Elsheikh, A., Mudera, V., Cheema, U. and Hasirci, V. (2021) Evaluating oxygen tensions related to bone marrow and matrix for MSC differentiation in 2D and 3D biomimetic lamellar scaffolds. *Int. J. Mol. Sci.* **22**, 4010 https://doi.org/10.3390/ijms22084010

31 Donnelly, H., Salmeron-Sanchez, M. and Dalby, M.J. (2018) Designing stem cell niches for differentiation and self-renewal. *J. R. Soc. Interface* **15**. https://doi.org/10.1098/rsif.2018.0388

32 Pelham, Jr, R.J. and Wang Y, L. (1997) Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc. Natl Acad. Sci. U.S.A.* **94**, 13661–13665 https://doi.org/10.1073/pnas.94.25.13661

33 Clark, A.Y., Martin, K.E., García, J.R., Johnson, C.T., Theriault, H.S., Han, W.M. et al. (2020) Integrin-specific hydrogels modulate transplanted human bone marrow-derived mesenchymal stem cell survival, engraftment, and reparative activities. *Nat. Commun.* **11**, 114 https://doi.org/10.1038/s41467-019-14000-9

34 Mao, A.S., Shin, J.-W. and Mooney, D.J. (2016) Effects of substrate stiffness and cell-cell contact on mesenchymal stem cell differentiation. *Biomaterials* **98**, 184–191 https://doi.org/10.1016/j.biomaterials.2016.05.004

35 Olivares-Navarrete, R., Lee, E.M., Smith, K., Hyzy, S.L., Doroudi, M., Williams, J.K. et al. (2017) Substrate stiffness controls osteoblastic and chondrocytic differentiation of mesenchymal stem cells without exogenous stimuli. *PLoS One* **12**, e0170312 https://doi.org/10.1371/journal.pone.0170312

36 Sun, M., Chi, G., Xu, J., Tan, Y., Xu, J., Lv, S. et al. (2018) Extracellular matrix stiffness controls osteogenic differentiation of mesenchymal stem cells mediated by integrin α5. *Stem Cell Res Ther.* **9**, 52 https://doi.org/10.1186/s13287-018-0798-0

37 Du, J., Zu, Y., Li, J., Du, S., Xu, Y., Zhang, L. et al. (2016) Extracellular matrix stiffness dictates Wnt expression through integrin pathway. *Sci. Rep.* **6**, 20395 https://doi.org/10.1038/srep20395

38 Kureel, S.K., Mogha, P., Khadpekar, A., Kumar, V., Joshi, R., Das, S. et al. (2019) Soft substrate maintains proliferative and adipogenic differentiation potential of human mesenchymal stem cells on long-term expansion by delaying senescence. *Biol. Open* **8**, bio039453 https://doi.org/10.1242/bio.039453

39 Gerardo, H., Lima, A., Carvalho, J., Ramos, J.R.D., Couceiro, S., Travasso, R.D.M. et al. (2019) Soft culture substrates favor stem-like cellular phenotype and facilitate reprogramming of human mesenchymal stem/stromal cells (hMSCs) through mechanotransduction. *Sci. Rep.* **9**, 9086 https://doi.org/10.1038/s41598-019-45352-3

40 Rao, V.V., Vu, M.K., Ma, H., Killaars, A.R. and Anseth, K.S. (2019) Rescuing mesenchymal stem cell regenerative properties on hydrogel substrates post serial expansion. *Bioeng. Transl. Med.* **4**, 51–60 https://doi.org/10.1002/btm2.10104

41 Guimarães, C.F., Gasperini, L., Marques, A.P. and Reis, R.L. (2020) The stiffness of living tissues and its implications for tissue engineering. *Nat. Rev. Mater.* **5**, 351–370 https://doi.org/10.1038/s41578-019-0169-1

42 Liechty, K.W., MacKenzie, T.C., Shaaban, A.F., Radu, A., Moseley, A.M., Deans, R. et al. (2000) Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep. *Nat. Med.* **6**, 1282–1286 https://doi.org/10.1038/81395

43 Zhao, L., Chen, S., Yang, P., Cao, H. and Li, L. (2019) The role of mesenchymal stem cells in hematopoietic stem cell transplantation: prevention and treatment of graft-versus-host disease. *Stem Cell Res Ther.* **10**, 182 https://doi.org/10.1186/s13287-019-1287-9

44 Lopez-Santalla, M., Fernandez-Perez, R. and Garin, M.I. (2020) Mesenchymal stem/stromal cells for rheumatoid arthritis treatment: an update on clinical applications. *Cells* **9**, 1852 https://doi.org/10.3390/cells9081852

45 Horwitz, E.M., Gordon, P.L., Koo, W.K.K., Marx, J.C., Neel, M.D., McNall, R.Y. et al. (2002) Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: implications for cell therapy of bone. *Proc. Natl Acad. Sci. U.S.A.* **99**, 8932–8937 https://doi.org/10.1073/pnas.132252399

46 Infante, A., Gener, B., Vázquez, M., Olivares, N., Arrieta, A., Grau, G. et al. (2021) Reiterative infusions of MSCs improve pediatric osteogenesis imperfecta eliciting a pro-osteogenic paracrine response: TERCELOI clinical trial. *Clin. Transl. Med.* **11**, e265 https://doi.org/10.1002/ctm2.265

47 Agacayak, S., Gulsun, B., Ucan, M.C., Karaoz, E. and Nergiz, Y. (2012) Effects of mesenchymal stem cells in critical size bone defect. *Eur. Rev. Med. Pharmacol. Sci.* **16**, 679–686 PMID:22774411

48 Probst, F.A., Fliefel, R., Burian, E., Probst, M., Eddicks, M., Cornelsen, M. et al. (2020) Bone regeneration of minipig mandibular defect by adipose derived mesenchymal stem cells seeded tri-calcium phosphate- poly(D,L-lactide-co-glycolide) scaffolds. *Sci. Rep.* **10**, 2062 https://doi.org/10.1038/s41598-020-59038-8

49 Wilson, A.J., Rand, E., Webster, A.J. and Genever, P.G. (2021) Characterisation of mesenchymal stromal cells in clinical trial reports: analysis of published descriptors. *Stem Cell Res. Ther.* **12**, 360 https://doi.org/10.1186/s13287-021-02435-1

50 Ullah, M., Liu, D.D. and Thakor, A.S. (2019) Mesenchymal stromal cell homing: mechanisms and strategies for improvement. *iScience* **15**, 421–438 https://doi.org/10.1016/j.isci.2019.05.004

51 Devine, S.M., Cobbs, C., Jennings, M., Bartholomew, A. and Hoffman, R. (2003) Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates. *Blood* **101**, 2999–3001 https://doi.org/10.1182/blood-2002-06-1830

52 Barbash, I.M., Chouraqui, P., Baron, J., Feinberg, M.S., Etzion, S., Tessone, A. et al. (2003) Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. *Circulation* **108**, 863–868 https://doi.org/10.1161/01.ClR.0000084828.50310.6A

53 Scarfe, L., Taylor, A., Sharkey, J., Harwood, R., Barrow, M., Comenge, J. et al. (2018) Non-invasive imaging reveals conditions that impact distribution and persistence of cells after in vivo administration. *Stem Cell Res. Ther.* **9**, 332 https://doi.org/10.1186/s13287-018-1076-x

54 Eggenhofer, E., Benseler, V., Kroemer, A., Popp, F.C., Geissler, E.K., Schlitt, H.J. et al. (2012) Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. *Front. Immunol.* **3**, 297 https://doi.org/10.3389/fimmu.2012.00297

55 Yukawa, H., Watanabe, M., Kaji, N., Okamoto, Y., Tokeshi, M., Miyamoto, Y. et al. (2012) Monitoring transplanted adipose tissue-derived stem cells combined with heparin in the liver by fluorescence imaging using quantum dots. *Biomaterials* **33**, 2177–2186 https://doi.org/10.1016/j.biomaterials.2011.12.009

56 Wang, G., Zhuo, Z., Yang, B., Wu, S., Xu, Y., Liu, Z. et al. (2015) Enhanced homing ability and retention of bone marrow stromal cells to diabetic nephropathy by microbubble-mediated diagnostic ultrasound irradiation. *Ultrasound Med. Biol.* **41**, 2977–2989 https://doi.org/10.1016/j.ultrasmedbio.2015.07.002

57 Corradetti, B., Taraballi, F., Martinez, J.O., Minardi, S., Basu, N., Bauza, G. et al. (2017) Hyaluronic acid coatings as a simple and efficient approach to improve MSC homing toward the site of inflammation. *Sci. Rep.* **7**, 7991 https://doi.org/10.1038/s41598-017-08687-3

58 Chou, K.-J., Lee, P.-T., Chen, C.-L., Hsu, C.-Y., Huang, W.-C., Huang, C.-W. et al. (2017) CD44 fucosylation on mesenchymal stem cell enhances homing and macrophage polarization in ischemic kidney injury. *Exp. Cell Res.* **350**, 91–102 https://doi.org/10.1016/j.yexcr.2016.11.010

59 Galipeau, J. and Sensébé, L. (2018) Mesenchymal stromal cells: clinical challenges and therapeutic opportunities. *Cell Stem Cell* **22**, 824–833 https://doi.org/10.1016/j.stem.2018.05.004

60 García-Fernández, C., López-Fernández, A., Borrós, S., Lecina, M. and Vives, J. (2020) Strategies for large-scale expansion of clinical-grade human multipotent mesenchymal stromal cells. *Biochem. Eng. J.* **159**, 107601 https://doi.org/10.1016/j.bej.2020.107601

61 Persson, M., Lehenkari, P.P., Berglin, L., Turunen, S., Finnilä, M.A.J., Risteli, J. et al. (2018) Osteogenic differentiation of human mesenchymal stem cells in a 3D woven scaffold. *Sci. Rep.* **8**, 10457 https://doi.org/10.1038/s41598-018-28699-x

62 Kuss, M.A., Wu, S., Wang, Y., Untrauer, J.B., Li, W., Lim, J.Y. et al. (2018) Prevascularization of 3D printed bone scaffolds by bioactive hydrogels and cell co-culture. *J. Biomed. Mater. Res. B Appl. Biomater.* **106**, 1788–1798 https://doi.org/10.1002/jbm.b.33994

63 Li, C., Ouyang, L., Armstrong, J.P.K. and Stevens, M.M. (2021) Advances in the fabrication of biomaterials for gradient tissue engineering. *Trends Biotechnol.* **39**, 150–164 https://doi.org/10.1016/j.tibtech.2020.06.005

64 Li, J., Liu, Y., Zhang, Y., Yao, B., Enhejirigala, Li Z. et al. (2021) Biophysical and biochemical cues of biomaterials guide mesenchymal stem cell behaviors. *Front. Cell Dev. Biol.* **9**, 640388 https://doi.org/10.3389/fcell.2021.640388

65 Chen, Y., Shu, Z., Qian, K., Wang, J. and Zhu, H. (2019) Harnessing the properties of biomaterial to enhance the immunomodulation of mesenchymal stem cells. *Tissue Eng. Part B Rev.* **25**, 492–499 https://doi.org/10.1089/ten.teb.2019.0131

66 Jo, J.-I., Gao, J.-Q. and Tabata, Y. (2019) Biomaterial-based delivery systems of nucleic acid for regenerative research and regenerative therapy. *Regen. Ther.* **11**, 123–130 https://doi.org/10.1016/j.reth.2019.06.007

67 Valadi, H., Ekström, K., Bossios, A., Sjöstrand, M., Lee, J.J. and Lötvall, J.O. (2007) Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat. Cell Biol.* **9**, 654–659 https://doi.org/10.1038/ncb1596

68 Zhang, Y., Liu, J., Wang, X., Zhang, J. and Xie, C. (2021) Extracellular vesicle-encapsulated microRNA-23a from dorsal root ganglia neurons binds to A20 and promotes inflammatory macrophage polarization following peripheral nerve injury. *Aging* **13**, 6752–6764 https://doi.org/10.18632/aging.202532

69 Del Campo C, V., Liaw, N.Y., Gunadasa-Rohling, M., Matthaei, M., Braga, L., Kennedy, T. et al. (2021) Regenerative potential of epicardium-derived extracellular vesicles mediated by conserved miRNA transfer. *Cardiovasc. Res.*, cvab054 https://doi.org/10.1093/cvr/cvab054

70 Andaloussi S, E.L., Mäger, I., Breakefield, X.O. and Wood, M.J.A. (2013) Extracellular vesicles: biology and emerging therapeutic opportunities. *Nat. Rev. Drug Discov.* **12**, 347–357 https://doi.org/10.1038/nrd3978

71 Vidal, M. (2019) Exosomes: revisiting their role as "garbage bags.". *Traffic* **20**, 815–828 https://doi.org/10.1111/tra.12687

72 Stahl, P.D. and Raposo, G. (2019) Extracellular vesicles: exosomes and microvesicles, integrators of homeostasis. *Physiology* **34**, 169–177 https://doi.org/10.1152/physiol.00045.2018

73 Nagelkerke, A., Ojansivu, M., van der Koog, L., Whittaker, T.E., Cunnane, E.M., Silva, A.M. et al. (2021) Extracellular vesicles for tissue repair and regeneration: evidence, challenges and opportunities. *Adv. Drug Deliv. Rev.* https://doi.org/10.1016/j.addr.2021.04.013

74 Wiklander, O.P.B., Nordin, J.Z., O'Loughlin, A., Gustafsson, Y., Corso, G., Mäger, I. et al. (2015) Extracellular vesicle in vivo biodistribution is determined by cell source, route of administration and targeting. *J. Extracell. Vesicles* **4**, 26316 https://doi.org/10.3402/jev.v4.26316

75 Hoshino, A., Costa-Silva, B., Shen, T.-L., Rodrigues, G., Hashimoto, A., Tesic Mark, M. et al. (2015) Tumour exosome integrins determine organotropic metastasis. *Nature* **527**, 329–335 https://doi.org/10.1038/nature15756

76 Fu, S., Wang, Y., Xia, X. and Zheng, J.C. (2020) Exosome engineering: current progress in cargo loading and targeted delivery. *NanoImpact* **20**, 100261 https://doi.org/10.1016/j.impact.2020.100261

77 Frank, J., Richter, M., de Rossi, C., Lehr, C.-M., Fuhrmann, K. and Fuhrmann, G. (2018) Extracellular vesicles protect glucuronidase model enzymes during freeze-drying. *Sci. Rep.* **8**, 12377 https://doi.org/10.1038/s41598-018-30786-y

78 James, S., Fox, J., Afsari, F., Lee, J., Clough, S., Knight, C. et al. (2015) Multiparameter analysis of human bone marrow stromal cells identifies distinct immunomodulatory and differentiation-competent subtypes. *Stem Cell Rep.* **4**, 1004–1015 https://doi.org/10.1016/j.stemcr.2015.05.005

79 Chen, T.S., Arslan, F., Yin, Y., Tan, S.S., Lai, R.C., Choo, A.B.H. et al. (2011) Enabling a robust scalable manufacturing process for therapeutic exosomes through oncogenic immortalization of human ESC-derived MSCs. *J. Transl. Med.* **9**, 47 https://doi.org/10.1186/1479-5876-9-47

80 Simonsen, J.L., Rosada, C., Serakinci, N., Justesen, J., Stenderup, K., Rattan, S.I.S. et al. (2002) Telomerase expression extends the proliferative life-span and maintains the osteogenic potential of human bone marrow stromal cells. *Nat. Biotechnol.* **20**, 592–596 https://doi.org/10.1038/nbt0602-592

81 Chairoungdua, A., Smith, D.L., Pochard, P., Hull, M. and Caplan, M.J. (2010) Exosome release of β-catenin: a novel mechanism that antagonizes Wnt signaling. *J. Cell Biol.* **190**, 1079–1091 https://doi.org/10.1083/jcb.201002049

82 Furuta, T., Miyaki, S., Ishitobi, H., Ogura, T., Kato, Y., Kamei, N. et al. (2016) Mesenchymal stem cell-derived exosomes promote fracture healing in a mouse model. *Stem Cells Transl. Med.* **5**, 1620–1630 https://doi.org/10.5966/sctm.2015-0285

83 Qin, Y., Wang, L., Gao, Z., Chen, G. and Zhang, C. (2016) Bone marrow stromal/stem cell-derived extracellular vesicles regulate osteoblast activity and differentiation in vitro and promote bone regeneration in vivo. *Sci. Rep.* **6**, 21961 https://doi.org/10.1038/srep21961

84 Matthay, M.A. (2017) Extracellular vesicle transfer from mesenchymal stromal cells modulates macrophage function in acute lung injury. Basic science and clinical implications. *Am. J. Respir. Crit. Care Med.* **196**, 1234–1236 https://doi.org/10.1164/rccm.201706-1122ED

85 Börger, V., Weiss, D.J., Anderson, J.D., Borràs, F.E., Bussolati, B., Carter, D.R.F. et al. (2020) International society for extracellular vesicles and international society for cell and gene therapy statement on extracellular vesicles from mesenchymal stromal cells and other cells: considerations for potential therapeutic agents to suppress coronavirus disease-19. *Cytotherapy* **22**, 482–485 https://doi.org/10.1016/j.jcyt.2020.05.002

86 Jochems, C.E.A., van der Valk, J.B.F., Stafleu, F.R. and Baumans, V. (2002) The use of fetal bovine serum: ethical or scientific problem? *Altern. Lab. Anim.* **30**, 219–227 https://doi.org/10.1177/026119290203000208

87 van der Valk, J., Bieback, K., Buta, C., Cochrane, B., Dirks, W.G., Fu, J. et al. (2018) Fetal bovine serum (FBS): past - present - future. *ALTEX* **35**, 99–118 https://doi.org/10.14573/altex.1705101

88 Pavani, K.C., Hendrix, A., Van Den Broeck, W., Couck, L., Szymanska, K., Lin, X. et al. (2018) Isolation and characterization of functionally active extracellular vesicles from culture medium conditioned by bovine embryos in vitro. *Int. J. Mol. Sci.* **20**, 38 https://doi.org/10.3390/ijms20010038

89 Konoshenko, M.Y., Lekchnov, E.A., Vlassov, A.V. and Laktionov, P.P. (2018) Isolation of extracellular vesicles: general methodologies and latest trends. *Biomed. Res. Int.* **2018**, 8545347 https://doi.org/10.1155/2018/8545347

90 Théry, C., Witwer, K.W., Aikawa, E., Alcaraz, M.J., Anderson, J.D., Andriantsitohaina, R. et al. (2018) Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the international society for extracellular vesicles and update of the MISEV2014 guidelines. *J. Extracell. Vesicles* **7**, 1535750 https://doi.org/10.1080/20013078.2018.1535750

91 Xia, X., Yuan, P., Liu, Y., Wang, Y., Cao, W. and Zheng, J.C. (2021) Emerging roles of extracellular vesicles in COVID-19, a double-edged sword? *Immunology* **63**, 416–430 https://doi.org/10.1111/imm.13329

92 Xu, B., Ye, J., Yuan, F.-Z., Zhang, J.-Y., Chen, Y.-R., Fan, B.-S. et al. (2020) Advances of stem cell-laden hydrogels with biomimetic microenvironment for osteochondral repair. *Front. Bioeng. Biotechnol.* **8**, 247 https://doi.org/10.3389/fbioe.2020.00247

93 Kodali, M., Castro, O.W., Kim, D.-K., Thomas, A., Shuai, B., Attaluri, S. et al. (2019) Intranasally administered human msc-derived extracellular vesicles pervasively incorporate into neurons and microglia in both intact and status epilepticus injured forebrain. *Int. J. Mol. Sci.* **21**, 181 https://doi.org/10.3390/ijms21010181