Review

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Mesothelial cell transplantation: history, challenges and future directions

DOI 10.1515/pap-2016-0014
Received August 5, 2016; accepted August 10, 2016;
previously published online August 31, 2016

Abstract: Mesothelial cells line the surface of the pleura, pericardium, peritoneum and internal reproductive organs. One of their main functions is to act as a non-adhesive barrier to protect against physical damage, however, over the past decades their physiological and pathological properties have been revealed in association with a variety of conditions and diseases. Mesothelium has been used in surgical operations in clinical settings, such as omental patching for perforated peptic ulcers and in glutaraldehyde-treated autologous pericardium for aortic valve reconstruction. Various methods for mesothelial cell transplantation have also been established and developed, particularly within the area of tissue engineering, including scaffold and non-scaffold cell sheet technologies. However, the use of mesothelial cell transplantation in patients remains challenging, as it requires additional operations under general anesthesia in order to obtain enough intact cells for culture. Moreover, the current methods of mesothelial cell transplantation are expensive and are not yet available in clinical practice. This review firstly summarizes the history of the use of mesothelial cell transplantation in tissue engineering, and then critically discusses the barriers for the clinical application of mesothelial cell transplantation. Finally, the recent developments in xenotransplantation technologies are discussed to evaluate other feasible alternatives to mesothelial cell transplantation.

Keywords: cell sheet technology, mesothelial cell, mesothelial cell transplantation, tissue engineering, xenotransplantation

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Introduction

Mesothelial cells (MCs) play a key role in the mesothelium, where one of its basic functions is to form a smooth surface over internal organs. However, MCs also have diverse physiological roles and biological functions in tissue repair, fibrinolysis, inflammatory regulation, epithelial-mesenchymal transition (EMT), mediating the intraperitoneal dissemination of carcinomas and in visceral adipose tissue development and remodeling. These functions have also been described in several pathologies, such as in organismal aging, peritoneal dialysis, tissue injury and repair and cancer progression (Table 1) [1].

The use of mesothelium has been successfully established in clinical settings, and has been used in surgeries for over 50 years, some examples of which include autologous omental patch repair for perforated peptic ulcers [2] and glutaraldehyde-treated autologous pericardium for aortic valve reconstruction [3, 4]. The process of aortic valve replacement with autologous pericardium has improved with the recent development of new devices and methods, based on the concept that the size of the aortic cusp has to be customized for a single patient for a better hemodynamic performance [5, 6]. Alternatively, the use of bioprosthetic aortic valves, composed of porcine aortic valve or calf pericardium (xenograft) and fixed by glutaraldehyde to reduce immunogenicity, have been widely and successfully applied in patients [7]. These techniques have several advantages including increased availability, usability and reasonable costs in a clinical setting.

The use of cultured MCs began in the 1980s, where these cells were seeded onto prosthetic vascular grafts instead of endothelial cells [8, 9], based on the evidence that MCs also produce prostaglandin I2 [10]. This MC transplantation was followed by the injection of enzyme-treated cell suspensions, which were usually trypsinized prior to injection via intraabdominal or intrapericardial route. Clinical applications of this technique include the treatment of peritoneal dialysis-related injury or infections [11–13], postoperative peritoneal adhesions [14] and myocardial infarction [15].

Table 1: Key roles of the biological functions of mesothelial cells in health and disease (*potential role) [1].

Protective barrier (inflammation and cancer)
Fluid and solute transport
Production of cytokines and growth factors
Synthesis of extracellular matrix
Procoagulant and fibrinolytic properties
Immunoactive or regulatory properties
Epithelial to mesenchymal transition
*Visceral adipose tissue development and remodeling

In 2008, Dr. Joanna Witkowicz produced a systematic review on the use of MC transplantation, including this tissue engineering approach [16]. However, tissue engineering has made astonishing progress over the past eight years, including the production of biological scaffolds from natural polymers and extracellular matrix proteins, decellularized scaffolds and scaffold-free "cell sheet technology" in order to construct whole organs. Cell sheet technology can provide an acellular, naturally occurring 3D biological scaffold for cell culture [17, 18], which can be used for MCs as well as other cell types (Figure 1). Recently, Lachaud et al. comprehensively reviewed the use of MCs in tissue engineering and addressed the future directions for MC-based therapeutic applications [19]. According to this review, techniques for the culture of autologous MCs and subsequent tissue engineering processes are available for clinical use. However, there are still at least three crucial barriers for the use of MC transplantation in patients:

(1) the requirement for additional invasive operations, such as abdominal surgery, to obtain autologous MCs for culture;
(2) the difficulty in maintaining enough intact MCs to cover large areas, such as the peritoneal cavity, due to the plasticity of MCs via EMT;
(3) current methodologies are expensive and require prior preparation.

Meanwhile, recent advances in genetic editing technologies, including the use of clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system, transcription activator-like effector nucleases (TALENs) or zinc-finger nucleases (ZNFs), enable easier and more precise modification of genomes [20, 21]. Simultaneously, xenotransplantation technology has also been rapidly developed to produce genetically modified animals using the technologies (Figure 2A) [22].

The development of xenotransplant cell therapies using porcine tissue culture are ongoing, based on evidence that the human immune response is milder compared to the use of xeno-solid organs. One example of this is xeno-insulin-producing islet cells, which use this technology to encapsulate porcine islet cells with poly-L-ornithine and an outer coating of alginate to protect the xeno-islets from the human immune system [23]. Patients implanted with these cells are reported to have survived more than nine years without any evidence of immune rejection or infection. This xeno-islet therapy is currently in late-stage clinical trials in several countries [24].

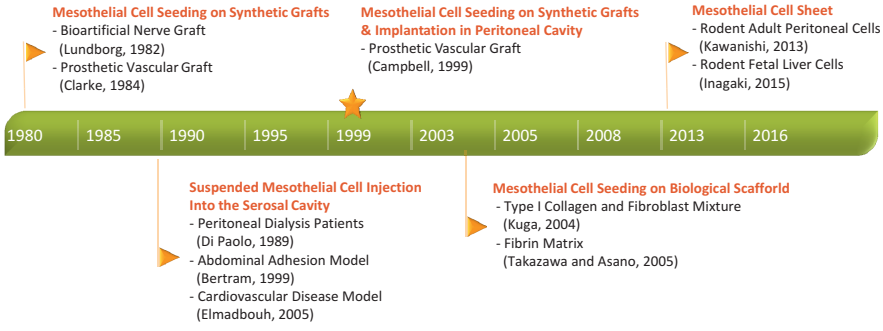

Figure 1: Historical timeline and milestones in the advancement of mesothelial cell transplantation.

Xenotransplantation technologies can be used as an alternative solution to overcome the barriers posed by the clinical application of MC transplantation. This review summarizes the history and current techniques used for MC transplantation, then the barriers for MC transplantation in clinical applications. Finally, the future directions for MC transplantation are discussed in relation to their combination with other xenotransplantation technologies.

History of MC transplantation

Seeding MCs onto synthetic grafts

The use of tissue cultured MCs begun with the idea of an "autologous cell-coated vascular graft", achieved by seeding MCs onto a prosthetic vascular graft instead of endothelial cells [8, 9, 25–28] (Figure 2B). This idea was based on the evidence that MCs produce prostaglandin I2, similar to endothelial cells [10]. However, in practice the outcomes differed, possibly due to the adhesion capacity of MCs to the different prosthetic materials, such as expanded polytetrafluoroethylene (ePTFE) (Dacron® or GoreTex®) [29]. A novel approach was to produce an autologous artificial vessel graft within the recipient's own peritoneal cavity [30]. This method included implanting a silastic tube into the peritoneal cavity of rats or rabbits for a period of 2 weeks, with the harvested grafts composed of MCs and myofibroblasts, in addition to extracellular matrix (ECM) including collagen type I, type IV and elastin. This idea came from the use of a foreign body reaction (granulation) in the peritoneal cavity, which allowed for these artificial vessel grafts to be successfully transplanted as a vessel graft for 3 months in the same animal recipients [30]. MCs have also been used in peripheral nerve reconstruction as a bioartificial nerve graft, which cover the inside of a synthetic nerve guide [31].

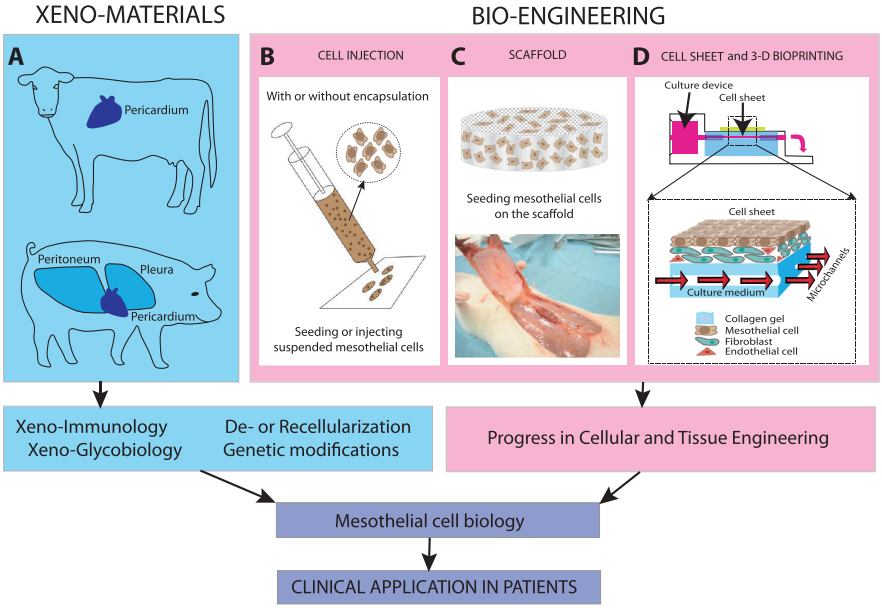

Figure 2: Future directions of mesothelial cell transplantation for clinical applications.
(A) Recent xenotransplantation technologies can reduce the risk of infection or rejection due to the development of immunological, glycobiological and genetic editing technologies. (B) Mesothelial cell (MC) seeding on synthetic grafts has been investigated for use in vessel and nerve regeneration. Suspended MC injection has been tested in some clinical settings and experimental models, such as postoperative abdominal adhesion or complications of peritoneal dialysis. Allo- or xeno-MC transplantation using encapsulation technology may also be applicable for peripheral vessel or nerve regeneration therapies, such as peripheral artery disease and physical impediments after injuries by providing growth factors. (C) Scaffold or (D) cell sheet technologies have been tested for MC transplantation, mainly in postoperative adhesion models. Combining xenotransplantation and tissue engineering technologies may enable progress in MC transplantation for clinical applications. Created using data from [17, 43].

Injection of suspended MCs into the serosal cavity

At the end of the 1980s, Di Paolo et al. began using autologous MC transplantation via intraperitoneal injection as a new therapeutic application for peritoneal dialysis-related infection or injury, not only in a murine model, but also in patients with chronic renal failure under peritoneal dialysis therapy. Four of these patients were diagnosed with acute peritonitis, approximately 300 million autologous peritoneal MCs were implanted after culture and congelation and the transplanted MCs coated the damaged tissue, as confirmed by biopsy [11–13]. Bertram et al. used a similar approach for preventing postoperative adhesions in an animal model [14]. In 2005, Hekking et al. tested autologous rodent peritoneal MCs injection in thioglycollate- induced peritonitis model, however, it caused submesothelial thickening of peritoneum and increased inflammation and microvascular permeability [32]. In 2014, Kanda et al. used temperature-sensitive immortalized mesothelial cell (TSMC) lines, harvested from temperature sensitive SV40 large T antigen gene transgenic rats [33], for evaluating the effect of MCs injection in a peritoneal fibrosis model induced with chlorhexidine gluconate. The TSMC transplantation aggravated peritoneal fibrosis, and lead elevation of mRNA expressions of EMT and RAS/MAP kinase was significantly higher than in the control group [34]. Kitamura et al. used "epithelial-like or fibroblastic-like" MCs cultured from the peritoneal dialysis patients in a mechanical peritoneal injury mice model, and epithelial-like MCs transplantation showed peritoneal adhesion prevention, but fibroblast-like MCs transplantation did not [35]. These last three different reports indicate that the phenotype and function of MC could be switched to a mesenchymal state via EMT, depending on the culture condition or inflammation, and further research is necessary to evaluate a feasible protocol for suspended MCs transplantation.

Meanwhile, genetically modified MC transplantation through intraperitoneal injection could be acceptable as gene therapy, as demonstrated using murine [36, 37] or human [38, 39] peritoneal MCs. Injection of MCs has also been used in several studies in a model of cardiac regeneration and repair for cardiac vascular disease (CVD), and might be useful for biological revascularization in CVD [15, 40].

Seeding MCs on biological scaffold

Another approach for MC transplantation is to seed them onto a scaffold. In the recent years, the development of scaffolds with biomaterials showing high degradability, biocompatibility and biomechanical properties has progressed considerably. These scaffolds were made from acellular animal tissue (extracellular matrix, ECM), natural proteins and/or polysaccharides. These biomaterials demonstrated improved cell adhesion, growth and colonization outcomes [19]. Using this approach, MCs have been used in postoperative adhesion models. Studies have reported that the two-step seeding of MCs on a type I collagen-fibroblast mixture [41] and one-step seeding on gel-like fibrin matrix [42–44] were successful in preventing peritoneal adhesion in both murine and canine models (Figure 2C). One of the major differences of scaffold cell transplantation with suspended cell injection is that the harvested biomaterials can only be transplanted on a specific, anatomically limited lesion. Moreover, these techniques can also be used to make a MC monolayer on the lesion, similar to *in vivo*. A recent study by Lachaud et al. reported that mouse adipose tissue MCs (ATMCs) share morphologic and functional similarities with mouse corneal epithelial cells (CECs), such as $Na+/K+-$ATPase, β-catenin, zona occludens-1 and N-cadherin [45]. In addition, ATMCs seeded on the decellularized basal membrane of human anterior lens capsules (HALCs) were found to form a monolayer with their original phenotype, suggesting that MCs, as ATMCs, could potentially be used for corneal repair [46]. The static cell-seeding technique, consisting of the manual pipetting of a concentrated cell suspension onto the scaffold, appears to be the most reliable approach for establishing MCs layers and maintaining their epithelial cell-like characteristics [19].

Cell sheet technology (CST)

Cell sheet technology (CST) is a scaffold-free tissue engineering method that uses a temperature-responsive cell culture surface. In this method, cultured cells are harvested as an intact contiguous cell sheet, then transplanted onto the host tissue by reducing the temperature from 37 to 20 °C. In the 1990s, Yamada et al. and Okano et al. successfully grafted poly-N-isopropylacrylamide (PIPAAm) onto a commercially available tissue culture polystyrene dish using electron beam irradiation [18, 47]. PIPAAm is a thermo-responsive high molecular compound which has a lower critical solution temperature of 32 °C. Its properties are hydrophobic at higher temperatures and hydrophilic at lower temperatures. Therefore, cultured cells can first attach to the hydrophobic culture base at 37 °C, and then be detached

from the hydrophilic culture base at 20 °C, without any need for enzymes such as trypsin or EDTA.

The harvested "cell sheet" has several unique advantages when compared with cell suspension injection or other scaffolding techniques including:

- intact cell surface components, such as cell-cell junctions and ECM, are preserved, enabling easy engraftment without suturing;
- no contamination by foreign materials;
- *in vivo* cell function can be maintained, even after transplantation.

Various cells such as oral mucosal epithelial cells, renal epithelial cells, epidermal keratinocytes, periodontal ligament cells, chondrocytes, middle ear mucosal cells, pancreatic islet cells, hepatocytes and thyroid cells have been successfully cultured on a temperature-responsive surface to form cell sheets [18]. Our research group synthesized MC sheets by two-step cell seeding using CST, the lower layer of which was composed of peritoneal fibroblast cells and the upper monolayer of MCs, and these cell sheets were shown to successfully prevent intraabdominal postoperative adhesions in a rodent model [48]. Inagaki et al. made cell sheets with CST using rodent fetal liver MCs (FL-MCs), and this FL-MCs cell sheet not only prevented postoperative adhesion, but also promoted liver regeneration, in a hepatectomy model [49]. Although various cell sheets can be harvested with CST, detached cell sheets usually shrink and fold themselves during the detachment procedure. When cell sheets are manipulated using hydrogel, their recovery is possible without any shrinking and folding. Moreover, these cell sheets are thinner and have a larger surface area than cell sheets that spontaneously detach [50]. Notably, the recovered cell sheet can be moved to another CST culture dish, where it can be attached to the new substrate (building up a cell sheet laminate). One example of this is the fabrication of 3D cardiac tissues by stacking cardiac cell sheets, which can pulsate spontaneously, synchronously and macroscopically [51]. Furthermore, a culture device containing a collagen gel patch with microchannels, made using 3D bioprinting technology, can provide the artificial vascular bed for the cell sheet [17]. If the cell sheet is previously cultured with vascular endothelial cells and growth factors, like vascular endothelial growth factor (VEGF), this device enables endothelial cell migration into the lower collagen gel, where it can form vascular networks based on the micro-channel roots (Figure 2D).

Critical issues associated with MC transplantation

The first critical issue associated with autologous MC transplantation is the requirement for further invasive surgeries, such as greater omentum biopsy under general anesthesia, in order to obtain MCs for culture, even if laparoscopic methods have been already established. This is clearly different from other available cell therapies like blood-derived or oral skin-derived cell sources, for example, peripheral blood stem cell transplantation (PBSCT) [52] and oral epithelial cell sheet therapy for cornea [53] or upper gastro tract regeneration [54].

The second issue regarding MC transplantation is the difficulty in maintaining a sufficient number of intact MCs to cover large areas such as the peritoneal cavity. The predominant clinical sources of MCs are from the greater omentum [55] followed by the avascular mesenteric membrane [42] and visceral adipose tissue for ATMCs [45, 46]. Parietal tunica vaginalis is a unique MC source [43, 44], which is accessible by a minimally invasive biopsy under local anesthesia, however, it has not yet been deemed acceptable as a reliable clinical source. The peritoneal fluid, particularly the effluent peritoneal dialysis fluid from peritoneal dialysis patients, has been considered as an alternative source of MCs. However, cultured MCs that are derived from the dialysis effluent of patients can be separated into epithelial-like MCs or fibroblastic-like MCs [35, 56], and transplant experiments conducted in a peritoneal injury nude mice model revealed that epithelial-like MCs showed inhibition of peritoneal adhesion or thickening, but fibroblast-like MCs did not [35]. Despite this, the ability of MCs to transition between having epithelial and mesenchymal phenotypes under specific *in vitro* culture conditions suggests that "myofibroblastic MCs" could be forced back to their original mesothelial phenotype with hepatocyte growth factor (HGF) or bone morphogenic protein-7 (BMP-7) [57–59], and therefore, could potentially be useful for the manufacture of bioartificial serosal mesothelial membranes. Other cell sources that may be applicable for MC repair include bone marrow-derived cells [60], mesenchymal stem cells (MSCs) [61, 62] and adipose-derived stem cells (ASCs) [63]. Finally, MCs could be differentiated from induced pluripotent stem (iPS) cells, however, there is currently no published data in the literature.

The last issue associated with MC transplantation is its cost. Current technologies for MC transplantation are not immediately available for the clinical use, as it takes about 2 weeks to establish transplantable materials in the

cell processing center under the clinically applicable standards of quality, including the Good Manufacturing Practice (GMP) guidelines. Moreover, the cost issues associated with the long-term preservation of MCs will probably not be solved while the appropriate time course of MC transplantation remains undetermined. Taken together, autologous MC transplantation is technically feasible, but it is still far from clinical application.

Future directions of MC transplantation

Xenotransplantation

Xenotransplantation of solid organs, such as the heart and kidney, has been encouraged due to the growing number of people awaiting transplantation and organ shortage. Porcine is considered to be the prime candidate for organ donation, due to its easy breeding and similar size of vital organs to those of humans. In addition, there are fewer ethical issues as a result of our history of using pig organs for other applications, including skin and heart valves replacement. Two main problems that remain to be resolved are the occurrence of xenorejection and xenogeneic infections after xenotransplantation.

Two of the well-known xenoantigens being the Galactose α1-3 galactose (αGal) and N-glycolylneuraminic acid (Neu5Gc) epitopes [64, 65]. Immunosuppressive strategies after human allograft transplantations could also be potentially applicable as a recipient-based strategy for preventing xenorejection [22, 66, 67]. Elimination of the antigenic epitopes from the donor organ is another strategy for successful pig-to-human xenotransplantation. It is nowadays possible to breed genetically modified pigs lacking several xenoantigens. For example, double knockout pigs lacking both cytidine monophospho-N-acetylneuraminic acid hydroxylase (CMAH) and α_1, α_3-galactosyltransferase (GGTA1) gene activities for elimination of αGal and Neu5Gc, reduced the humoral barrier to xenotransplantation, further than pigs lacking only *GGTA1* [68]. Otherwise, glycans produced by β1, 4 N-acetylgalactosaminyl transferase (β4GalNT2) have been known as another potential candidate xenoantigen [69]. Studies in this field are increasing rapidly, supported by resources such as xeno-glycomics databases [70]. Further technological advances might facilitate the development of genetically modified, complex porcine donors. Powerful genome editing technologies, such as CRISPR/Cas9, TALENs or ZNFs, allow researchers to reduce xenoantigens and xenoinfections [22]. For example, Estrada et al. successfully bred *CMAH/GGTA1/β4GalNT2* knockout porcine via CRISPR/Cas9 [71]. Yang et al. achieved disrupting all copies (63 copies) of porcine endogenous retroviruses (PERVs) at the same time using CRISPR/Cas9 [72]. Finally, donor porcine can also be genetically modified to express human complement regulators (CD46 and CD59), human decay-accelerating factor (CD55) and human antithrombotic genes (CD39 and thrombomodulin) [22, 67, 73, 74].

Cellular xenotransplantation

Cellular level xenotransplantation may be more easily applicable in the clinical setting than solid organs xenotransplantation, as the host immune response is expected to be milder. One example is porcine islet transplantation [23, 24]: genetically modified porcine islets provide an advantage as they reduce xenorejection or xenogeneic infection, including PERVs. Other novel approaches are encapsulating techniques using immunoisolation devices. The goals of encapsulation technologies are to release growth factors, peptides, proteins or hormones in a precise location, and to keep these protected from immune system attack. One of these products was tested in phase I/IIa clinical studies and was not associated with any adverse reactions [23, 75]. This product is currently undergoing additional phase I/IIa trials. Several different cell types have been tested for encapsulation, including fibroblasts, myoblasts, kidney cells, ovary cells, parathyroid cells, hepatocytes, chondrocytes, Leydig cells, adrenal chromaffin cells and stem cells, among others. [76]. MCs can also be candidates for encapsulation technology.

Cellular xenotransplantation technologies are not following the same usual purposes than MC transplantation, for example, establishing a physical barrier for postoperative adhesion prevention or tumor cell implantation. However, cellular xenotransplantation could be an alternative tool for the use of MCs in regeneration of damaged peripheral vessels or nerves in patients suffering from peripheral artery disease (PAD) or peripheral neuropathy. In particular, encapsulated MCs from genetically modified porcine may be useful as a regenerative therapy by providing helpful growth factors, such as VEGF or HGF, to the lesions. It may also have a lower cost than autologous MCs or stem cells, depending on the availability of xeno-materials in the future. Moreover, it could even be more acceptable than autologous MC transplantation, due to reduced risk factors in recipients relating to the number of surgeries required to obtain the cells.

Decellularization and recellularization

Decellularization is defined as removing cells from an organ, leaving the ECM scaffold with the functional and structural proteins. The ECM scaffold alone has been shown to influence cell migration to the scaffold, and their subsequent proliferation and differentiation. Decellularized organs can then be recellularized or reseeded with autologous cells, which can then be transplanted into the recipient's body without rejection [77]. Decellularized porcine mesothelium has been commercially available (Meso BioMatrix®), which is characterized by its strength and high composition of ECM or cytokines, including collagen IV, fibronectin, laminin, VEGF and transforming growth factor β (TGF-β). The decellularized mesothelium has also been shown to stimulate human fibroblasts to produce more VEGF than fibroblasts grown by usual tissue culture methods [78].

The challenges of decellularization are related to determining optimal protocols that are specific to their intended purpose, mainly via the use of chemical agents, physical agents and enzymatic treatments. Another limitation of decellularized organs is the remaining residual DNA and other cell components (xenoantigens). However, the rapid progression of xenotransplantation technologies aimed at reducing xenoantigen or xenogeneic infection, as described above, could also be applicable to MC transplantation (Figure 2).

Conclusions

MC transplantation has been an attractive treatment for the last 30 years, with astounding progress observed in the areas of MC biology and tissue engineering, however, further advances are required before its use in patients. Further translational studies and efforts are needed in order to make MC transplantation acceptable for clinical applications.

Acknowledgments: The author greatly appreciates the critical reading and helpful comments from Marc A. Reymond.
Author contributions: The author has accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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