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Concise Reviews: Can Mesenchymal Stromal Cells Differentiate into Corneal Cells? A Systematic Review of Published Data

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ABSTRACT

The majority of stem cell therapies for corneal repair are based upon the use of progenitor cells isolated from corneal tissue, but a growing body of literature suggests a role for mesenchymal stromal cells (MSC) isolated from noncorneal tissues. While the mechanism of MSC action seems likely to involve their immuno-modulatory properties, claims have emerged of MSC transdifferentiation into corneal cells. Substantial differences in methodology and experimental outcomes, however, have prompted us to perform a systematic review of the published data. Key questions used in our analysis included: the choice of markers used to assess corneal cell phenotype, the techniques used to detect these markers, adequate reporting of controls, and tracking of MSC when studied in vivo. Our search of the literature revealed 28 papers published since 2006, with half appearing since 2012. MSC cultures established from bone marrow and adipose tissue have been best studied (22 papers). Critically, only 11 studies used appropriate markers of corneal cell phenotype, along with necessary controls. Ten out of these eleven papers, however, contained positive evidence of corneal cell marker expression by MSC. The clearest evidence is observed with respect to expression of markers for corneal stromal cells by MSC. In comparison, the evidence for MSC conversion into either corneal epithelial cells or corneal endothelial cells is often inconsistent or inconclusive. Our analysis clarifies this emerging body of literature and provides guidance for future studies of MSC differentiation within the cornea as well as other tissues. STEM CELLS *2015;33:785–791*

INTRODUCTION

The cornea has been extensively studied as a tissue for stem cell therapies. To date, the majority of this research has focused on corneal epithelial progenitor cells located at the peripheral edge, or so-called limbus, where the cornea adjoins the sclera [1, 2]. As such, cultivated epithelial autografts have become widely used as a standard treatment for repairing the ocular surface [3]. Exploring deeper, a number of groups have more recently identified corneal/limbal stromal cells with stem cell properties [4–10] and similar studies are also being pursued for the innermost cellular layer, the corneal endothelium [11]. Nevertheless, the limited availability and sensitive location of corneal tissue present significant challenges for autologous corneal stem cell therapies, particularly in cases of bilateral disease.

Given the limited availability of a patient's own corneal stem cells, a number of noncorneal tissues have been investigated as potential sources of epithelial progenitor cells for repairing the ocular surface including the oral mucosa [12]. More recently, however, several groups have evaluated the potential of mesenchymal stromal cell (MSC) cultures derived from tissues of noncorneal origin [13–16]. While much of this research has centered on exploiting the immuno-regulatory properties of MSC to encourage corneal healing, claims have emerged that mesenchymal cells of noncorneal origin have the capacity to transdifferentiate into corneal cells [17–24]. Such a conclusion, if confirmed, would not only have important implications for the treatment of corneal diseases, but would have a significant impact on our understanding of general MSC biology. Upon initial engagement with this literature, however, we have noted substantial differences in experimental design and reported outcomes that hamper a clear interpretation of the data. The goal of this concise review, therefore, is to systematically evaluate this recent body of literature for evidence of noncorneal MSC differentiation into corneal cells.

Literature Search, Terms of Reference, and Method of Analysis

Our study design is based upon published guidelines for the conduct of systematic reviews [25]. The initial "identification stage" consisted of searching the PubMed database for studies where the terms "mesenchymal" and "cornea" had been used. This search retrieved 296 studies published since 1950 (as of July 25th, 2014). We subsequently screened these publications for studies where cultures of nontransformed MSC, established from tissues of noncorneal origin, had been examined either in vitro or in vivo for their ability to transdifferentiate into corneal cells. Reports of efficacy alone, while interesting, were nonetheless excluded from our subsequent analysis. All literature pertaining to these terms of reference were included in this systematic review, irrespective of the primary language in which the article was published. The evidence presented in each study was evaluated using four standard questions.

1. Have appropriate markers been used to determine transformation to a corneal phenotype?
2. By what methods has the expression of these corneal markers been evaluated?
3. Have appropriate controls been reported to validate these results? For example, positive results obtained by immunostaining should be validated through demonstration of a negative control. Likewise, a negative result should be validated through demonstration of a positive control.
4. In the case of in vivo studies, has the provenance of observed "corneal cells" been traced back to the MSC of noncorneal origin using some form of marker?

Definition of Corneal Cell Phenotype

Our definition of corneal cell phenotype is based upon the following considerations. To begin, the transcription factor paired box 6 (Pax-6) is widely regarded as the canonical marker of eye tissue development and is retained to varying degrees by mature corneal cells. A variety of molecules including the transcription factor p63 [26] and cytokeratin 19 [27] have been used as markers for corneal epithelial progenitor cells, but neither protein is specific to the cornea. Corneal epithelium is therefore defined by expression of the tissue-specific differentiation markers cytokeratin 3 (K3) and cytokeratin 12 (K12) [28]. On a technical note, while K3 expression in corneal epithelial cells can be reliably studied using the monoclonal antibody AE5, care must be taken when applying this antibody to detect K3 in other cell types, since it is known to cross-react with cytokeratin 2p/76 (K2p/76). Corneal stromal cells (keratocytes) are generally defined by expression of CD34, keratocan, lumican, and aldehyde dehydrogenase (ALDH), but during wound healing are known to differentiate into wound repair fibroblasts ($CD34^{-}$/$CD90^{+}$) and myofibroblasts (expressing alpha-smooth muscle actin or α-sma) [29]. Nevertheless, since both CD90 and α-sma are expressed by MSC cultures [30], they have been excluded as valid markers of corneal differentiation. While specific markers for the corneal endothelium are emerging [31], the expression of N-cadherin, zonula occludens-1 (ZO-1), and sodium/potassium ATPase is more generally used to identify these cells [32]. Of the three markers used, the presence of sodium/potassium ATPase is perhaps most important given the role of this protein in maintaining the pump function of corneal endothelial cells required for corneal transparency.

Overview of Published Literature

Our literature search identified 28 papers published between January 2006 and June 2014, with half of these having been reported since January 2012 [13, 14, 17–20, 22–24, 33–51]. The essential details for each study are summarized in the first five columns of Table 1. The majority of studies have been performed using MSC derived from either bone marrow (13 studies) [13, 14, 17, 22–24, 38–40, 43, 47, 48, 50] or adipose tissue (9 studies) [19, 33, 35, 42, 44–46, 49, 51], with the remainder using MSC extracted from either umbilical cord tissues (4 studies) [20, 34, 36, 41] or dental pulp (2 studies) [18, 37]. Most studies have used cultures of MSC established from human tissues (19 studies) [13, 18–20, 23, 24, 33–39, 41, 42, 45, 46, 49, 51], with the balance having been established from rabbits (5 studies) [14, 17, 43, 44, 48], rats (3 studies) [40, 47, 50], and mice (1 study) [22].

A large proportion of studies (19 studies) [13, 14, 17, 18, 20, 22, 24, 33–35, 37, 38, 40, 42–44, 47–49] have involved in vivo experiments, with 11 studies involving administration of human MSC into rabbits (6 studies) [18, 33, 35, 37, 38, 42], rats (3 studies) [13, 24, 49], or mice (2 studies) [20, 34]. Routes of administration include topical application (10 studies) [13, 17, 18, 24, 37, 38, 40, 47–49], typically following alkali burn (9 studies) [13, 14, 17, 18, 24, 38, 40, 42, 49], with or without carrier/adjunct materials including amniotic membrane (7 studies) [13, 18, 24, 37, 38, 47, 48] and fibrin (1 study) [17]. Other methods used include direct injection into the cornea (four studies) [20, 22, 33, 34] or adjacent conjunctiva (one study) [42], stromal implants consisting of MSC cultured within synthetic scaffolds (two studies) [35, 44], and intravenous injection (one study) [14]. Significantly, the fate of administered MSC was traced using some form of temporary (DiI or BrdU) or permanent marker (green fluorescent protein [GFP], human nuclear antigen or sex-linked DNA marker) in only 15 out of 19 studies conducted in vivo [13, 14, 17, 18, 20, 22, 33–35, 37, 38, 43, 44, 47, 49].

Reports of MSC displaying evidence of corneal phenotype in vitro (15 studies) [17–19, 23, 24, 36, 39–41, 45–48, 50, 51] have used a variety of induction methods including coculture in the presence of corneal cells (2 studies) [40, 51], treatment with ocular cell conditioned media (3 studies) [17, 41, 46], cultivation in either specialized epithelial cell growth media or keratocyte growth media (4 studies) [19, 23, 24, 42], and corneal organ culture (2 studies) [39, 41]. Only two studies have used specialized enrichment techniques such as magnetic-assisted cell sorting (for stage-specific embryonic antigen-4 [23]) or flow cytometry (side population cells [19]) prior to cultivation (under epithelial or keratocyte growth conditions, respectively). Surprisingly, three studies [18, 45, 48] have claimed evidence of corneal phenotype markers being expressed when MSC were apparently maintained in their standard growth medium.

Table 1. Results of systematic review for prior studies of noncorneal MSC conversion into corneal cells

Tissue of MSC origin	Study	Species of MSC origin	Study design	Host species	Correct markers used	Adequate controls used	Origin of cells tracked	Our conclusions	Result
Bone marrow	[38]	Human	In vivo	Rabbit	Yes	Yes	Yes (Human antigen)	Transplanted cells formed epithelial-like structure on ocular surface. Cells were immunoreactive for K12 and human nuclear antigen	√Ep
	[14]	Rabbit	In vivo	Rabbit	No (α-sma)		Yes (DiI)	Evidence of MSC survival and conversion into myofibroblasts	Inc. S
	[13]	Human	In vivo	Rat	Yes	Yes	Yes (Human antigen)	Transplanted cells formed epithelial-like structure on ocular surface, but staining for cytokeratin was negative	XEp
	[43]	Rabbit	In vivo	Rabbit *Auto*	No (Morph.)	No	Yes (BrdU)	Some evidence of ability to substitute for corneal endothelial cell function, but poor evidence of transdifferentiation	?En
	[17]	Rabbit	In vivo In vitro	Rabbit	Yes Yes	No Yes	Yes (BrdU)	Evidence of MSC survival in vivo 10% of cells express K3 in vitro	√Ep
	[40]	Rat	In vivo In vitro	Rat	Yes Yes	Yes No	No	Poor evidence without tracking of cells Poor evidence without control data	Inc. Ep
	[39]	Human	Organ culture	Pig	Yes	Yes	No	Cultures grown on denuded pig corneas displayed positive staining for K12 but origin of cells is unclear	Inc. Ep
	[48]	Rabbit	In vivo In vitro	Rabbit	Yes Yes	No No	No	Poor evidence without control/tracking Cultured MSC reported to express K3/K12, but data not shown	Inc. Ep
	[50]	Rat	In vitro		Yes	Yes		K3 expression (IHC, no controls) supported by RT-PCR and WB	√Ep
	[22]	Mouse	In vivo	Mouse *Kera−/−*	Yes	Yes	Yes (DiI)	Evidence of conversion into keratocytes	√S
	[24]	Human	In vivo In vitro	Rat	Yes Yes	Yes Yes	No	Poor evidence without tracking Evidence of K3 in vitro	√Ep
	[23]	Human	In vitro		Yes	Yes		K3 expression confirmed by ICC and RT-qPCR, but less than for control tissue	√Ep
	[47]	Rat *Male*	In vivo In vitro	Rat *Female*	No (K19) No (K19)	No No	Yes (male DNA)	Traces of male DNA detected in vivo, but K19 not specific for corneal cells	Inc. Ep
Adipose tissue	[33]	Human	In vivo	Rabbit	Yes	Yes	Yes (DiI)	IHC data inconclusive due to high background. Faint band for human keratocan detected by RT-PCR	?S
	[19]	Human	In vitro		Yes	Yes		Keratocan expression confirmed by WB, RT-PCR and RT-qPCR	√S
	[45]	Human	In vitro		Yes	Yes		Inconsistencies are reported with respect to K3 expression when measured by ICC, WB, RT-PCR and RT-qPCR	?Ep
	[35]	Human	In vivo	Rabbit	Yes	No	Yes (DiI)	Poor evidence without control data	Inc. S
	[42]	Human	In vivo	Rabbit	No (H&E)		No	Poor evidence without appropriate markers and tracking of cells	Inc. Ep
	[46]	Human	In vitro		Yes	Yes		Inconclusive owing to expression of K3/K12 throughout all cultures tested including limbal fibroblasts	Inc. Ep
	[44]	Rabbit	In vivo	Rabbit *Auto*	Yes	No	Yes (GFP)	Colocalization of GFP with keratocan and ALDH difficult to interpret without adequate controls	Inc. S
	[49]	Human	In vivo	Rat	No (H&E)		Yes (CFSE)	Traces of label detected, but data Inconclusive without exploring expression of cell phenotype	Inc. Ep
	[51]	Human	In vitro		Yes	Yes		Inconsistencies are reported with respect to measurement of ALDH and keratocan by RT-qPCR and flow cytometry	?S
Umbilical cord	[20]	Human	In vivo	Mouse *Lum−/−* *Kera−/−*	Yes	Yes	Yes (DiI)	Good evidence provided of cells differentiating into functioning keratocytes	√S
	[34]	Human	In vivo	Mouse	No (F-actin)	No	Yes (DiI)	Evidence of cell survival and appropriate morphology. No markers of phenotype assessed	Inc. S
	[36]	Human	In vitro		Yes	No		Poor evidence without control data	Inc. Ep

Table 1. *Continued*

Tissue of MSC origin	Study	Species of MSC origin	Study design	Host species	Correct markers used	Adequate controls used	Origin of cells tracked	Our conclusions	Result
Umbilical cord blood	[41]	Human	Organ culture	Human	Yes	Yes	Yes (GFP)	A shift observed toward a phenotype similar to corneal endothelial cells	?En
Dental pulp	[18]	Human	In vivo	Rabbit	Yes	Yes	Yes (Human antigen)	Coexpression of K3 and human antigen	√Ep
			In vitro		Yes	Yes		Traces of K3/K12 in a few cells	
	[37]	Human	In vivo	Rabbit	Yes	Yes	Yes (Human antigen)	Coexpression of K3 and human antigen	√Ep

Abbreviations: Host species: *Auto*, autologous transplant; *Kera−/−*, keratocan null mutant mice; Lum−/−, lumican null mutant mice. Markers of cell phenotype: α-sma, alpha smooth muscle actin; ALDH, aldehyde dehydrogenase; F-actin, filamentous actin; H&E, hematoxylin and eosin staining; K3, cytokeratin 3; K12, cytokeratin 12; K19, cytokeratin 19; Morph., morphology assessed. Cell tracker reagents: BrdU, bromo deoxyuridine; CFSE, carboxyfluorescein succinimidyl ester; DiI, a fluorescent lipophilic dialkylcarbocyanine dye; GFP, green fluorescent protein. Conclusions comments: ICC, immunocytochemistry; IHC, immunohistochemistry; MSC, mesenchymal stromal cells of noncorneal origin; RT-qPCR, quantitative (real-time) reverse transcriptase polymerase chain reaction; RT-PCR, reverse transcriptase polymerase chain reaction; WB, Western blotting. Result comments:?En, partial evidence of differentiation into corneal endothelial cells;?Ep, partial evidence of epithelial differentiation; √Ep, positive evidence for epithelial differentiation; Inc., inconclusive evidence; √S, positive evidence for stromal cell (keratocyte) differentiation;?S, partial evidence of stromal differentiation; XEp, negative evidence for epithelial differentiation.

In terms of target tissue, 17 studies [13, 17, 18, 23, 24, 36–40, 42, 45–50] have presented data relevant to epithelial differentiation, 9 studies [14, 19, 20, 22, 33–35, 44, 51] have examined differentiation into corneal stromal cells (keratocytes), and 2 studies [41, 43] have examined the potential of MSC to produce corneal endothelium.

The last five columns of Table 1 present the results from our analysis of published data using the standard set of four questions. For convenience, the highlights from this analysis are discussed below according to MSC tissue of origin and are summarized in Table 2.

Corneal Differentiation of Bone Marrow–Derived MSC

Of the 13 studies involving bone marrow-derived MSC (BM-MSC), only 7 papers contain data that according to our analysis have been validated through use of appropriate markers and controls [13, 17, 22–24, 38, 50]. Six of these papers have addressed conversion of MSC into corneal epithelium with the remaining paper exploring keratocyte differentiation in vivo.

While one study found negative evidence of human BM-MSC differentiation into corneal epithelium when applied to the ocular surface of rats (validated by positive control) [13], the combined data from an additional five studies involving cells from rabbits [17], rats [50], or human subjects [23, 24, 38] provide partial evidence of K3 and/or K12 expression under either in vitro or in vivo conditions. Nevertheless, the level of K3 or K12 expression observed in these papers is consistently less than that for corneal epithelium and often limited to a subset of cells. At the very least, therefore, it appears that BM-MSC have some ability to produce low levels of cornea-specific keratins, but it remains unclear as to whether this level of expression represents true conversion to a functional corneal epithelial cell phenotype.

The single paper containing evidence of BM-MSC differentiation into keratocytes in vivo is convincing given that the cells were implanted into *kera−/−* null mutant mice [22]. Thus, the subsequent observation of keratocan expression in conjunction with DiI-labeled cells is justifiably explained by conversion of BM-MSC into keratocytes. Nevertheless, it is unclear as to what percentage of administered MSC adopted a keratocyte phenotype.

Only one study has examined the potential of BM-MSC to transdifferentiate into corneal endothelial cells [43]. In this study (published in Chinese language), the authors report improvements in corneal clarity and thickness when autologous BM-MSC cultured on gelatin membranes are adhered to the posterior surface of corneal buttons implanted into rabbits. While these results are encouraging in terms of efficacy, the phenotype of implanted cells was only examined using a combination of morphological techniques (live confocal imaging and scanning electron microscopy).

Corneal Differentiation of MSC–Derived from Adipose Tissue

Reports of MSC-derived from adipose tissue (A-MSC) conversion into corneal cells are, according to our criteria, also often lacking appropriate markers of cell phenotype and necessary

Table 2. Summary of validated evidence for MSC conversion to corneal phenotype

	BM-MSC	A-MSC	UC-MSC	DP-MSC
Corneal epithelium	X √√√√√[a]	?		√√
Corneal stromal cells	√	√??	√	
Corneal endothelium	?		?	

Source of mesenchymal stromal cells (MSC): A-MSC, derived from adipose tissue; BM-MSC, derived from bone marrow; DP-MSC, derived from dental pulp; U-MSC, derived from umbilical cord. Scoring: X, study reporting validated negative evidence; √, study reporting validated positive evidence;?, partial evidence of conversion.

[a]Note: In the majority of these studies, the levels of expression reported for corneal-specific keratins (K3/K12) are substantially lower than that seen in corneal epithelial cells (positive control) and immunoreactivity is often limited to a small subset of cells.

controls (five out of nine studies). Moreover, the results for a further three studies are clouded by either inconsistent or unclear data.

The case for A-MSC differentiation into corneal epithelial cells is particularly weak. Only one in vitro study contains validated data in support of this hypothesis [45] and even in this article there are inconsistencies with respect to the measurement of corneal phenotype markers by immunocytochemistry, Western blotting, and reverse transcriptase polymerase chain reaction (RT-PCR). In short, while "moderate" levels of K3 were reported using immunocytochemistry and Western blotting, the mRNA transcripts required for producing this protein were not detected by RT-PCR. Nevertheless, weak expression was reported by RT-PCR for K12 and this was confirmed by sequencing. These results were apparently obtained for A-MSC grown in their standard expansion medium. Further studies are therefore required to clarify whether A-MSC have potential to produce corneal epithelium and ideally this research should be extended to in vivo models as well.

One study has reported elevated levels of both ALDH and keratocan by flow cytometry when A-MSC are cocultured in the presence of corneal stromal cells, but no change in transcript numbers for either protein was detected by quantitative (real-time) reverse transcriptase polymerase chain reaction [51]. Likewise, while a faint band for human keratocan was detected by Western blot after injecting human A-MSC into rabbit corneas, background fluorescence hampers a clear interpretation of the images reported to show DiI-labeled cells [33]. Nevertheless, a separate study using the side population fraction of human A-MSC isolated by flow cytometry provides good evidence of keratocan and ALDH expression using a combination of techniques [19]. It therefore seems quite plausible that A-MSC could be used as a source of keratocytes, but this case could also be strengthened by more data including studies in vivo.

Corneal Differentiation of MSC–Derived from Umbilical Cord

Research into stem cells derived from umbilical cord is a complex topic as mesenchymal cells with progenitor cell properties have been isolated from the cord blood as well as the surrounding primitive connective tissue. In addition, epithelial progenitor cells have been isolated from umbilical cords. All three sources of progenitor cells have been examined as tools for corneal reconstruction. For the purpose of this systematic review, however, we have focused on the evidence arising from studies using the mesenchymal cell populations obtained from umbilical cord tissues.

Out of the four studies identified by our literature search, only one report contains evidence that has been validated through use of appropriate markers and controls [20]. In this study, human MSC-derived from umbilical cord (UC-MSC) loaded with DiI were injected into dysfunctional corneas of *lum−/−* and *kera−/−* null mutant mice. The labeled cells subsequently became integrated within the host tissue and adopted a morphology similar to that expected for keratocytes. Evidence of transdifferentiation was provided by colocalization of the DiI tracker dye with observed patterns of immunohistochemistry for keratocan, lumican, and CD34, with lumican and keratocan expression being confirmed by Western blotting. A subsequent study by this research group using a mouse model of lysosomal enzyme dysfunction has produced similar results, but specific markers of corneal phenotype were not used on this occasion [34].

One additional paper requires mention since it is one of the few studies to have evaluated the potential of MSC derived from any noncorneal tissue to adopt a corneal endothelial cell phenotype [41]. In this study, a subculture of UC-MSC was examined for evidence of ZO-1 and N-cadherin expression prior to and following treatment with lens epithelium conditioned medium. GFP-labeled cells were also examined for these same markers following 2 weeks cultivation upon the wounded posterior surface of donor human corneas. While both ZO-1 and N-cadherin were detected in untreated cultures (validated by controls) these proteins reportedly became more distributed to cell boundaries (both in standard and organ cultures) in the presence of conditioned medium. Moreover, the results from a microarray analysis of 250 genes indicated a shift toward a phenotype closer to that of corneal endothelial cells when treated with conditioned medium. Interestingly, the integration of labeled UC-MSC with the organ cultured endothelial cells was best encouraged by attachment to the surface of damaged cells rather than by contact with exposed areas of Descemet's membrane. These findings support the theory of "licensing" whereby MSC are activated or primed by local signals such as those produced by damaged and necrotic cells. While these results are somewhat encouraging, the presence of ZO-1 and N-cadherin in untreated cultures raises questions about the degree of transdifferentiation. When taken together with the functional data observed using BM-MSC [43], however, these findings suggest that MSC might at very least provide a partial surrogate for corneal endothelial cells in the event that transdifferentiation does not occur.

Corneal Differentiation of MSC–Derived from Dental Pulp

While only two studies by one group have explored the potential of MSC-derived from dental pulp (DP-MSC) as a source of corneal tissue [18, 37], both papers contain validated evidence of cornea-specific keratin expression. In the first study [18], while only traces of K3/K12 were detected in

cultures of human DP-MSC by immunostaining (with transcripts for K12 detected by RT-PCR), coexpression of K3 and human nuclear antigen was detected by immunostaining following application to the wounded ocular surface of rabbits. These in vivo findings were essentially confirmed a year later in the group's second study [37].

Conclusions and Recommendations

While there is evidence that MSC derived from tissues of noncorneal origin have some ability to produce proteins associated with corneal phenotype, only 10 out of the 28 papers that we analyzed presented validated data in support of this hypothesis (summarized in Table 2). The strongest evidence exists with regard to expression of markers associated with corneal stromal cells (keratocytes), largely owing to the use of null mutant animal models and multiple sources of MSC having been tested with positive results. Although a greater number of studies have observed expression of markers associated with corneal epithelium, there are often significant gaps in the evidence provided, with many studies relying solely on immunohistochemistry data and several reports indicating that only a subpopulation of MSC may be involved. Despite some interesting preliminary data, convincing evidence of MSC conversion into corneal endothelial cells has yet to be published.

On weight of evidence, we must therefore conclude that there is indeed value in pursuing the use of MSC from tissues of noncorneal origin as a potential source of corneal cells, and especially in the case of stromal tissue reconstruction. Nevertheless, the evidence for MSC differentiation into either corneal epithelial cells or corneal endothelial cells is relatively less clear. With regard to future studies, a number of key recommendations can be made.

1. First and foremost, specific markers of corneal phenotype should be used and ideally the expression of Pax-6, in conjunction with its recently identified regulator Wnt7A [52], should also be examined as the canonical marker of ocular tissue development. In doing so, these studies will be consistent with the best evidence-based strategies being used for studying directed differentiation of induced pluripotent stem cells.
2. Given the technical limitations of some antibodies used for detection of corneal cell phenotype (e.g., AE5 antibody to K3/K2p/76), the expression of corneal-specific markers should always be confirmed at the transcriptional level. Moreover, reporting of experimental controls should be mandatory.

Failure to comply with either of these first two recommendations, risks production of data that would be unlikely to pass quality control measures required under good manufacturing practice for clinical applications.

3. In the case of studies being conducted in vivo, it is essential that the provenance of observed corneal cells be traced back to the MSC administered to the animal.
4. Finally, while the majority of positive evidence has been obtained for MSC cultures established from bone marrow, this trend no doubt arises from the wider availability of this resource and thus should not be taken to indicate an optimal MSC type for corneal reconstruction. Indeed, it could well be argued that MSC isolated from craniofacial tissues provide a more appropriate source, given their shared embryonic origin with corneal stromal cells and corneal endothelial cells (cranial neural crest).

In drawing our conclusions, we must stress that the findings of this systematic review in no-way detract from the broader potential use of MSC as a therapeutic agent for corneal repair through their proven abilities to modulate immune responses. Indeed, in the course of reviewing the 28 papers we noted several claims of improvements in corneal structure and/or function following administration of MSC [18, 20, 34, 42, 47, 49]. Moreover, there have been case reports of clinical efficacy outside the scope of our systematic review [53]. The question of whether or not transdifferentiation is necessary for a therapeutic effect is therefore perhaps academic, but we trust that our analysis provides some much needed clarity with respect to the mechanism of MSC action within the cornea.

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Author Contributions

D.H.: conception and design, data analysis and interpretation, manuscript writing, and final approval of manuscript; L.F.: administrative support, collection and assembly of data, and data analysis and interpretation; L.B., A.S., and B.C.: data analysis and interpretation, manuscript writing, and final approval of manuscript; F.L.: data analysis and interpretation.

Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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