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Cell Therapy of Corneal Diseases

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Abstract

Mesenchymal stem cells (MSC) isolated from connective tissues are pluripotent and differentiate to phenotypes of connective tissue cell lineages (osteoblasts, chondrocytes, adipocytes) *in vitro* and *in vivo*. They have been used to treat mouse models of connective tissue disease such as Lumican null (*Lum*^{-/-}), and mucopolysaccharidosis (Gusb) mice. MSC have unique immunosuppressive properties allowing evasion of host rejection; thus, they are valuable tools for cell therapy of congenital and acquired diseases involving immune dysfunction of multiple tissues including ocular surface tissues (cornea). We previously showed that human umbilical mesenchymal stem cells (UMSCs) modulated host immune responses, enabling them to survive xenograft transplantations. *In vitro* UMSCs modulated inflammatory cells by inhibiting adhesion and invasion, and inducing cell death. UMSC also regulated M1/M2 macrophage polarization and induced T-regulatory cell maturation from naïve intraperitoneal cavity lavage cells. UMSCs exposed to inflammatory cells synthesized a rich extracellular glycocalyx composed of hyaluronan (HA) bound to the heavy chains (HCs) of inter-alpha-trypsin inhibitor (HC-HA), which contains tumor necrosis factor- α -stimulated gene 6 (TSG6) that catalyzes the transfer of HCs to HA, versican and pentraxin-3. Our *in vivo* and *in vitro* results showed that glycocalyx regulated inflammatory cells, allowing UMSCs to survive host immune rejection. Administration of antibodies against glycocalyx constituents or digestion with hyaluronidase and chondroitinase ABC abolished the UMSC ability to modulate immune responses. Treatment with anti-CD44 antibodies also diminished modulation of M2 macrophages by UMSC, indicating cell surface CD44 is required for correct UMSC glycocalyx assembly to modulate inflammatory cells.

Keywords

cell therapy; corneal disease; umbilical mesenchymal stem cells; CD44

INTRODUCTION

The identification and isolation of stem cells, i.e., embryonic stem (ES) cells, tissue-specific stem cells, induced pluripotent stem (iPS) cells, and mesenchymal stem cells have spurred enthusiasm for the development of cellular therapy technology to treat congenital and acquired diseases. The conventional aim of cell therapy has focused on the use of cells

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derived from stem/progenitor cells that can assume tissue-specific cell types *in vitro* and/or *in vivo* for the restoration of lost tissue functions caused by diseases and/or trauma.^{1–6}

Mesenchymal stem cells (MSCs) can be isolated from many connective tissues including the bone marrow, umbilical cord, amniotic membrane, cartilage, adipose tissue, cornea, and conjunctiva. These plastic adherent cells are multipotent and capable of differentiation to assume the phenotypes of many connective tissue cell types such as osteoblasts, chondrocytes, and adipocytes *in vitro* and *in vivo*.^{7–10} However, whether these cells are true stem cells that are capable of self-renewal *in situ* is controversial, and they probably should be defined as mesenchymal stromal cells (also abbreviated as MSC).^{6, 11, 12} For example, corneal keratocytes derived from the neural crest are quiescent under normal physiological conditions. They fail to repair damaged corneal stroma, although they proliferate and assume myofibroblast phenotypes of scar tissue.^{13–15} In addition, there is a lack of definitive marker(s) that can aid the identification of MSC.⁶ However, cell surface markers that are characteristic of MSC have been reported and are associated with the identity of MSC; for example CD44, CD105, CD73, and CD90, but not CD45, CD19, CD79, CD14, CD11b or HLA-DR. Currently, unique marker(s) are not available for the identification of MSC. Nonetheless, a large number of markers have been reported for different MSC isolated by different laboratories. Thus, the identification of MSC remains a challenge for their application in cell therapy.

MSCs are generally recognized as multipotent and can differentiate into various progenitor cells that form connective tissues such as bone, bone marrow, cartilage, adipose tissue, and corneal stroma keratocytes, *in vitro* and *in vivo*.⁶ Thus, there has been increasing interest in the application of MSC transplantation for regenerative medicine, to repair and restore the normal functions of diseased and injured tissues caused by autoimmune diseases, solid organ allograft survival, hepatic cirrhosis, kidney diseases, neuro- and muscle-degenerative diseases, myocardial infarction, or spinal cord injury.^{6, 16–20} However, it should be noted that the potential of MSC for curing diseases depends greatly upon the tissue source of MSC as well as the age of donors. Moreover, passaging MSC in culture gradually diminishes their tri-lineage differentiation potential into adipogenic, chondrogenic, and osteogenic cells.^{11, 21} Reports using stem cells isolated from individuals have also demonstrated that the number of cells, as well as the differentiation and proliferation potential of MSCs, decrease with the donor's age.²² Umbilical cord-derived MSC (UMSCs) are presumably young cells and therefore are likely to have a more potent proliferation and differentiation capability. The other advantages of UMSCs are that the availability of umbilical cords from newborn babies is almost unlimited and these cells can be expanded and stored in liquid nitrogen in a tissue bank and thawed when required. However, the utilization of human UMSCs for treating disease is not as common as that of bone marrow MSCs (BMSC).

The discovery that MSC are capable of both regenerating tissue-specific cell types for restoring tissue function and modulating host inflammation and immunity has expanded their use for treating congenital and acquired diseases. This review will focus on the use of UMSCs for treating congenital and acquired corneal diseases.

USE OF UMSC AND BMSC FOR TREATING CONGENITAL CORNEAL DISEASES[‡]

Restoration of corneal transparency of *Lum*^{-/-} mice

Lumican, belonging to the family of small leucine-rich proteoglycans, is one of the major keratan sulfate (KS) proteoglycans in the corneal stroma required for maintaining corneal transparency via its regulatory functions on collagen fibrillogenesis.²³ Lumican null (*Lum*^{-/-}) mice manifest thin and cloudy corneas caused by disorganization of the stromal extracellular collagen matrix and downregulated expression of keratocan and aldehyde dehydrogenase (a corneal crystalline).²⁴ In our previous study, as a test of principle, we investigated whether human UMSCs transplanted into mouse cornea might differentiate and assume keratocyte phenotypes to restore the corneal transparency of *Lum*^{-/-} mice.²⁵ We intrastromally injected approximately 10⁴ UMSCs in a volume of 2 µL into the corneal stroma of *Lum*^{-/-} mice (Fig. 1). The corneal clarity was examined weekly using *in vivo* confocal microscopy with the HRT-II Rostock Cornea Module up to 12 weeks after transplantation. The results in Figure 2 show that intrastromal transplantation of UMSCs resulted in the gradual restoration of corneal transparency and increased corneal stroma thickness of the treated *Lum*^{-/-} mice. Figure 2 shows a representative image of treated versus untreated corneas. Nonlinear optical imaging using second harmonic analysis showed that the stromal collagen of *Lum*^{-/-} mice was reorganized after the transplantation of UMSCs.²⁵ These observations suggested that a xenograft of human UMSCs into the mouse cornea was capable of improving corneal transparency and stromal thickness in *Lum*^{-/-} mice. Histological and immunofluorescence analyses revealed that the transplanted UMSCs (1) assumed a dendritic morphology typical of keratocytes, (2) established intercellular connections between themselves and with host keratocytes (Fig. 3), and (3) expressed CD34 (a unique human keratocyte marker) and KS proteoglycans; i.e., KS-Lum and KS-Kera (Fig. 4). The transplanted human UMSCs underwent proliferation for about 4 weeks after which they became quiescent, similar to recipient's stromal keratocytes, and remained in the mouse cornea for at least 12 weeks.²⁵ In addition, western blotting and immunohistochemistry further indicated that the transplanted human UMSCs in the murine corneal stroma assumed a unique keratocyte phenotype based on the synthesis of lumican and keratocan containing KS sidechains. Moreover, the expression of aldehyde dehydrogenase was upregulated (Fig. 5). This observation is consistent with the suggestion that lumican, in addition to being a component of the extracellular matrix (ECM), also displays matrikine functions by inducing gene expression profiles of keratocytes, e.g., upregulation of keratocan and aldehyde dehydrogenase that contributes to the maintenance of corneal transparency and thickness.^{23, 25, 26}

In another series of experiments, the efficacy of mouse BMSC was examined for treating corneal defects caused by the ablation of the *Kera* and *Lum* genes. Bone marrow (BM) cells have been shown to infiltrate into injured and/or diseased tissues, and it has been hypothesized that these BM cells differentiate into residential cells to repair the diseased/

[‡]All animal procedures adhered to the ARVO statement for the use of animal in vision research and were approved by the IACUC of the University of Cincinnati.

damaged tissue. To examine this possibility, we used chimeric mice generated by the transplantation of enhanced green fluorescent protein (EGFP) BM cells into keratocan null (*Kera*^{-/-}) and *Lum*^{-/-} mice. Upon corneal epithelium debridement, EGFP BM cells invaded into the cornea and assumed dendritic cell morphology; however, they failed to synthesize unique corneal KS proteoglycans such as KS-lumican and KS-keratocan.²⁷ EGFP BM cells directly transplanted into the corneal stroma also assumed a dendritic morphology typical of a keratocyte phenotype. Interestingly, 3 days after corneal and/or conjunctival transplantation of these EGFP BM cells into *Kera*^{-/-} mice, green keratocan-positive cells were found in the cornea, but not in the conjunctiva. However, the transplanted BM cells were rejected within 4 weeks. In further experiments, when BMSC were maintained in culture prior to the intrastromal transplantation into recipient *Kera*^{-/-} mice, they assumed a keratocyte phenotype, expressed KS-keratocan, and survived in the cornea without triggering any immune response, thereby evading host rejection, similar to that observed with UMSC transplantation as described above.^{25, 27}

Restoration of corneal transparency of *Gusb* mice

Mucopolysaccharidosis (MPS) are a family of lysosomal storage diseases caused by a mutation in one of the lysosomal exoglycosidases which is required for the sequential digestion of glycosaminoglycans (GAGs).^{28, 29} Loss of this enzyme activity leads to the accumulation of GAGs *in situ*. MPS VII is caused by a mutation in β -glucuronidase, and patients manifest hepatomegaly, skeletal dysplasia, short stature, corneal clouding, and developmental delays. Similar to other lysosomal storage diseases, current treatment regimens for MPS (including MPS VII) are enzyme infusion and bone marrow transplantation; however, these are not effective for treating impaired mental development and corneal clouding caused by the blood–brain barrier and corneal avascularity, respectively. To develop alternative treatment regimens, we speculated that intrastromal transplantation of human UMSCs might provide an alternative treatment to restore corneal transparency caused by the accumulation of GAGs. Thus, UMSCs could be used to treat MPS. As a test of principle, human UMSCs were transplanted into the corneal stroma of *Gusb* mice, which have a spontaneous nonsense deletion mutation of β -glucuronidase (*Gusb* allele).³⁰ UMSC transplantation restored the dendritic and hexagonal morphology of host keratocytes and endothelial cells, respectively, and *in vivo* confocal microscopy (HRT-II) revealed reduced corneal haze. Whole-mount confocal microscopy illustrated the dramatic morphological improvement of keratocytes and endothelial cells in the *Gusb* mouse corneas treated with UMSCs (Fig. 6). Immunohistochemistry using antibodies against heparan sulfate and chondroitin sulfate chains, as well as lysosomal associated membrane protein 2, revealed a decrease in GAG content and lysosomal number and size in the treated corneas. Labeling UMSC intracellular compartments prior to transplantation revealed the distribution of UMSC vesicles throughout the corneal stroma and endothelium. An *in vitro* co-culture assay between skin fibroblasts isolated from MPS VII mice and UMSCs demonstrated that neutral vesicles released by the UMSCs were taken up by the fibroblasts and fused with the acidic lysosomes (Fig. 7). Thus, as shown in Figure 8, we hypothesize that transplanted UMSCs participate in both extracellular (intercellular space) and intracellular (lysosome) GAG turnover via the secretion of exosomes containing functional β -glucuronidase, enabling host *Gusb* keratocytes and endothelial cells to catabolize accumulated GAG

products. These findings suggest that UMSCs could be a novel alternative for treating MPS and other congenital metabolic disorders.

Mechanism of UMSCs in the suppression of host rejection

It is well recognized that UMSCs have unique immunosuppressive properties, enabling them to evade host rejection and making them valuable cell therapy tools for treating diseases related to immune dysfunctions, e.g., graft versus host disease. However, the molecular and cellular mechanisms of UMSCs that modulate inflammation and host immune response remain elusive.^{5, 6} We previously showed that human UMSCs survived xenograft transplantation, caused low inflammatory responses and successfully corrected corneal clouding defects associated with congenital lysosomal storage disease MPS VII (*Gusb*) and in *Lum^{-/-}* mice.^{25, 27, 30} However, the precise mechanism by which UMSCs suppress the immune system remains elusive. Attempts were made to determine the key components involved in how UMSCs modulate the inflammatory system and to identify the inflammatory cells that are regulated by the UMSCs. Our results showed that human UMSCs transplanted into the corneal stroma of mice 24 h after a chemical injury (alkali burn to the cornea) suppressed severe inflammatory response and enabled the recovery of corneal transparency within 2 weeks. However, UMSCs subjected to chondroitinase ABC treatment were unable to prevent exacerbated inflammatory responses and failed to regenerate the transparent cornea (Fig. 9).³¹ We further demonstrated *in vitro* that UMSCs inhibited the adhesion and invasion of inflammatory cells and induced the cell death of inflammatory cells via both necrosis and/or apoptosis. We also demonstrated that UMSCs modulated the polarization of M1/M2 macrophages *in vitro* by co-culturing UMSCs and naïve macrophages isolated from peritoneal lavage (Fig. 10). UMSCs also induced the maturation of T-regulatory cells and led to inflammatory cell death (Fig. 11). Furthermore, UMSCs exposed to inflammatory cells synthesized a rich extracellular glycocalyx composed of chondroitin sulfate-proteoglycan versican bound to a heavy chain (HC)-modified hyaluronan (HA) matrix (HC-HA). This glycocalyx also contained tumor necrosis factor (TNF)-stimulated gene 6 (*TSG6*) that catalyzes the transfer of HCs to HA and stabilizes the HC-HA matrix, termed the hyaluronan raft (Fig. 12). Interestingly, pretreatment of UMSCs with hyaluronidase and chondroitinase ABC abolished all the anti-inflammatory properties of UMSCs, both *in vivo* and *in vitro*. Therefore, our results clearly demonstrate that glycocalyx mediates the ability of UMSCs to regulate inflammatory cells, thereby enabling them to evade host rejection.³¹ Taken together, these observations suggest that the presence of the glycocalyx containing HC-HA (hyaluronan raft) and chondroitin sulfate plays a pivotal role in endowing UMSCs with the unique capability to suppress host inflammatory responses.

Increasing evidence suggests that HA, similar to other ECM components, has diverse biological functions. In addition to serving as a constituent of ECM, it is also an active participant in disease pathogenesis pathways such as inflammation, angiogenesis, fibrosis, and cancer-promoting processes, via its association with numerous binding proteins. Moreover, evidence suggests HA has a dual role in inflammation. HA is present in tissues as high-molecular weight (HMWHA) and low-molecular weight (LWMHA) forms, which play contradictory roles in inflammation. HMWHA has a tendency to be anti-inflammatory while

LWMHA is proinflammatory.^{32, 33} Moreover, the association of HA with proteins may also alter or enhance its physiological properties. Amniotic membranes have been used in the clinic to treat corneal inflammation for many years. It is now known that the anti-inflammatory effects of the amniotic membrane are due to the secretion of a soluble form of HC-HA/PTX3 (pentraxin 3) complex (HC is the heavy chain of IaI, inter-alpha inhibitor).^{34, 35} Recently, Abbadi et al. reported that lung epithelial cells also synthesize cell surface glycocalyx of HC3-HA (HA-Raft) that traps leukocytes; thus, it might have a role in host defense.³⁶ Our observation of the presence of the HC-HA/PTX3 complex on UMSCs is consistent with the idea that such complexes may account for the ability of UMSCs to modulate host inflammation and immune responses.

CONCLUSIONS

UMSCs, similar to MSCs isolated from other connective tissues (bone marrow, adipose tissue, corneal stroma) are pluripotent and capable of differentiating into specific cells types under the influence of specific tissue niches. In addition, these cells possess a cell surface glycocalyx rich in anti-inflammatory molecules including HC-HA, TSG6, pentraxin 3, and versican, which are essential for modulating host inflammatory and immune responses. Therefore, the application of UMSCs might be beneficial for treating diseases of chronic and acute inflammation, e.g., graft versus host disease, and dry eye disease.

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A

B

Figure 1. Intrastromal transplantation of umbilical mesenchymal stem cells (UMSCs) into the cornea of *Lum*^{-/-} mice

Following the thawing of UMSCs from liquid nitrogen in a 37°C water bath, cells were washed by centrifugation and resuspended in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen Corporation, Grand Island, NY, USA). UMSCs were labeled by incubation in 1:200 diluted DiO (green fluorescence) in DMEM for 3 hours at room temperature or DiI (red fluorescence) in DMEM for 30 min at 37°C. After washing, UMSCs were then resuspended in phosphate-buffered saline and 10 μ L of cell suspension was mixed with an equal volume of 0.4% trypan blue to determine the total number of viable cells using a hemacytometer. The fluorescence intensity decreased if the labeled cells proliferated. Experimental mice were anesthetized by combined ketamine and xylazine administration. Panel A shows a tunnel is created in the corneal stroma with a 32-gauge needle. In panel B, the left panel shows an image prior injection, and the right panel shows the image after

injecting 2 μ L of UMSC suspension ($\sim 10^4$ cells) using a 33-gauge needle. After injection, the eyes were treated once with ophthalmic antibiotic ointment. The clouding caused by hydration disappeared in a couple hours.

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Figure 2. Corneal stromal thickness and transparency in *Lum*^{-/-} mice are improved by umbilical mesenchymal stem cell (UMSC) transplantation

Twelve *Lum*^{-/-} mice (6–8 weeks old) were used. The left corneas (OS) received $\sim 10^4$ cells in 2 μL of UMSC cell suspension, and the right corneas (contralateral controls) were treated with 2 μL phosphate-buffered saline. Representative HRT2 images are shown. (A) Before UMSC transplantation, there were similar levels of corneal stromal light scattering and thickness in the right (OD, a) and left (OS, b) eyes. Panel c shows histograms of light scattering and stromal thickness. (B) Panels a and b show 3-dimensional images of the HRT-II confocal microscopy analyses 12 weeks after UMSC transplantation. Lower light scattering and increased thickness are present in the transplanted corneal stroma (b) compared with untransplanted cornea (a). The histograms show increased thickness and less light scattering (more transparency) in the stroma of transplanted corneas compared with the untransplanted corneas after 12 weeks²⁵. α and α' , beginning of superficial corneal epithelium surface; β and β' , end of endothelium. (Reproduced from Liu, et al. [Fig. 2], PLOS ONE 2010; 5(5): e10707, with permission).

Figure 3. Transplanted umbilical mesenchymal stem cells (UMSCs) undergoing morphological changes resemble host dendritic keratocytes

(A) Images of *in vivo* fluorescent stereomicroscopy show that DiI-labeled UMSCs (red) were localized in the area of the injection tunnel (not visible in red fluorescence) and had a round cell shape within the first week of transplantation. Later, the cells migrated outward and became dendritic in shape. At 8 weeks, the cells were homogeneously distributed throughout the entire cornea. (B) Confocal microscopy shows that transplanted UMSCs had a round-like cell shape after phalloidin staining of the whole-mount cornea in the first week. Afterwards, the cells extended their protrusions and had a flat and dendritic cell shape (scale

bars, 50 μm ; blue, nuclear staining by DAPI). (C) Following phalloidin (green) staining of whole-mount *Lum*^{-/-} mouse corneas 5 weeks after UMSC transplantation, confocal images show that DiI-labeled UMSCs had a dendritic and flat cell shape and formed a 3-dimensional network between the host stromal cells and the donor cells via their extensive dendritic processes, which were similar to those of host keratocytes.²⁵ Scale bar, 20 μm ; blue, nuclear staining by DAPI, *DiI-labeled UMSCI. (Reproduced from Liu, et al. [Fig. 4], PLOS ONE 2010; 5(5): e10707, with permission).

Figure 4. Synthesis of keratan (KS)-keratocan, KS-lumican, and expression of CD34 by transplanted umbilical mesenchymal stem cells (UMSCs)

(A) Immunostaining with anti-human keratocan antibody shows that keratocan (red) was distributed around the transplanted UMSCs (green) in the anterior stroma of *Kera*^{-/-} mice. (B) Western blotting shows that human keratocan (45 kD) was present after enzymatic digestion to remove keratan sulfate glycosaminoglycan (KS-GAG) in the *Kera*^{-/-} cornea transplanted with UMSCs, but not in untransplanted corneas of *Kera*^{-/-}, *Lum*^{-/-}, and wild type mice (n = 5). (C) Immunostaining with anti-mouse lumican antibodies (cross reacts to both human and mouse lumican) shows that lumican (red) was present in *Lum*^{-/-} mouse corneas after UMSC (green) transplantation. (D) Western blotting shows that lumican (45 kD) was detected after enzymatic removal of KS-GAG in *Lum*^{-/-}, *Kera*^{-/-}, and wild type mouse corneas transplanted with UMSCs, but not in the *Lum*^{-/-} mouse cornea (n = 4). (E) Immunostaining shows that transplanted human UMSCs (red) were stained by anti-CD34 (green) in *Lum*^{-/-} mouse corneas 6 weeks after transplantation.²⁵ Scale bars, 50 μm; blue, DAPI. (Reproduced from Liu, et al. [Fig. 8], PLOS ONE 2010; 5(5): e10707, with permission).

Figure 5. Upregulation of keratocan and ALDH3A1 in *Lum*^{-/-} mouse corneas after umbilical mesenchymal stem cell (UMSC) transplantation

Semiquantitative western blot analyses show increased synthesis of keratocan and aldehyde dehydrogenase by recipient keratocytes in corneas transplanted with UMSCs. Panels A and B show that the keratocan expression level was significantly increased after UMSC transplantation compared to untransplanted corneas. Panels C and D show that the expression of Aldh3 is significantly increased in *Lum*^{-/-} mice after UMSC transplantation (n = 4).²⁵ (Reproduced from Liu, et al. [Fig. 9], PLOS ONE 2010; 5(5): e10707, with permission).

Figure 6. Phalloidin staining after umbilical mesenchymal stem cell (UMSC) treatment of *Gusb* mice

Corneas excised from the eyeballs were stained with phalloidin (green) to analyze the integrity of stromal keratocytes and endothelium. *Gusb* mice were treated with UMSCs as described in Figures 1 and 2. (A and B) Phalloidin staining shows significantly improved keratocyte morphology in treated corneas compared to untreated corneas. (A) Untreated corneas (OS, left eyes) display strong phalloidin staining revealing small rounded keratocytes as well as keratocytes with an amoeboid shape, while treated corneas (OD, right eyes) contain dendritic host keratocytes. (B) DiI-labeled UMSCs (red) are present throughout the stroma of treated corneas. DiI-positive cells (arrow) are present in the stroma

and established cell–cell contact with host keratocytes (*). DiI speckles are present in host keratocytes surrounding DiI-positive UMSCs.³⁰ Scale bar, 20 μ m. (Reproduced from Coulson-Thomas, Caterson, Kao [Fig. 3], STEM CELLS 2013; 31:2116–2126, with permission).

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Transplantation of MPSVII with
FM 1-43 pre-labeled UMSC

MPSVII fibroblast co-cultured with
DND-160-labeled UMSC

LysoSensor™ Yellow/Blue DND-160

Figure 7. Lysosomal content and cell–cell trafficking between umbilical mesenchymal stem cells (UMSCs) and mucopolysaccharidosis (MPS) VII keratocytes and fibroblasts
(A) UMSCs (*) were labeled with FM 1–43FX (red) prior to intrastromal administration, the eyeballs were excised after 3 days, and the corneas processed for whole-mounts. (B) MPS VII skin fibroblasts were exposed to UMSCs previously labeled with LysoSensor Yellow/Blue DND-160 and seeded in Transwell® inserts with 0.44- μ m pores for 15, 30, and 60 minutes. The live cells were analyzed using a Zeiss LSM710 confocal microscope. The nuclei of the UMSC and fibroblasts were labeled with SYTO 59 (red). Neutral organelles are observed as blue and acidic organelles as yellow/green. Scale bar, 20 μ m. There was a statistically significant decrease in lysosome content in MPS VII fibroblasts exposed to UMSC.³⁰ * P <0.05. (Reproduced from Coulson-Thomas, Caterson, Kao [Fig. 5], STEM CELLS 2013; 31:2116–2126, with permission).

Figure 8. Schematic of the mechanism by which mesenchymal stem cells (UMSCs) (red) aid corneal glycosaminoglycan (GAG) turnover in mucopolysaccharidosis (MPS) VII mice. (i) Endocytosis of accumulated extracellular GAGs and proteoglycans by UMSCs; (ii) UMSCs catabolize the GAGs; (iii) intercellular trafficking of active β -glucuronidase between UMSCs and host keratocytes; (iv) sequential degradation of accumulated GAGs in host keratocytes. (Reproduced from Coulson-Thomas, Caterson, Kao [Fig. 6], *STEM CELLS* 2013; 31:2116–2126, with permission).

Figure 9. Umbilical mesenchymal stem cells (UMSCs) suppress the *in vivo* inflammatory response in a glyocalyx-dependent manner

Two weeks after alkali burn, corneas were subjected to whole-mount analysis of inflammatory cell infiltration. The number of F4/80+ (top left) and CD11b+ (bottom left) cells/cornea were counted. * $P < 0.05$, Mean $\pm$ standard deviation (SD) versus phosphate-buffered saline (PBS)-treated and ** $P < 0.05$, Mean $\pm$ SD versus UMSC-treated corneas. The corneas were analyzed by *in vivo* confocal microscopy 2 weeks after alkali burn to evaluate corneal haze. UMSCs were prelabeled with DiI prior to transplantation to evaluate their presence initially and then treated without enzyme (right top panel) or alkali-burned corneas were treated with PBS, heparinase (second from bottom), or Chase AC+B (bottom) prior to transplantation. Two weeks after transplantation, the ability of corneas to survive host rejection was assessed.³¹ (Reproduced from Coulson-Thomas, et. al [Fig. 1], J. Biol. Chem., 2014; 289, 34:23465–23481, with permission).

Figure 10. Role of UMSCs in macrophage polarization

The ability of UMSCs to modulate the polarization of macrophages was evaluated. UMSCs were seeded in a Transwell[®] insert with 0.44- μ m pores and treated with Chase AC+B, Hylase, or were untreated. Inflammatory cells were seeded into microplates and placed in co-culture with the UMSCs seeded in Transwell[®] inserts. (A and B) The morphology of macrophages (F4/80⁺ cells) was analyzed (green) and nuclei were stained with DAPI (blue). M1 macrophages are dendritic, while M2 are round. Scale bar in B, 20 μ m. (C) The numbers of F4/80⁺TNF- α ⁺ cells were counted to evaluate the M1 phenotype. (D) The numbers of F4/80⁺IL-10⁺ cells were counted to evaluate the M2 phenotype. (E) IRF5 staining (red) of macrophages is shown, and F4/80⁺ cells are shown in green. The presence of UMSCs promotes M2 macrophage polarization. Pretreatment of UMSCs with Hylase and Chase AC +B abolishes these effects. Scale bar, 20 μ m.³¹ * P <0.05 versus macrophages alone.³¹ (Reproduced from Coulson-Thomas, et. al. [Fig. 4], J. Biol. Chem., 2014; 289, 34:23465–23481, with permission).

Figure 11. Role of UMSCs in the maturation of T-regulatory cells

The ability of UMSCs to activate T-regulatory cells was evaluated. UMSCs were seeded in a Transwell® insert with 0.44-μm pores and were treated with Chase ABC or were untreated. Inflammatory cells were seeded into a microplate and placed in co-culture with UMSCs. (A) The numbers of CD44⁺ cells were counted. (B) The numbers of F4/80-IL-10⁺ cells were counted. (C and D) The numbers of CD25⁺ cells were analyzed and counted. (E) The morphology of macrophages (CD11b⁺ cells) was analyzed in the presence of UMSCs treated with neutralizing anti-IL-10 or anti-TGF-β antibodies. Scale bar, 20 μm. (F) The numbers of stellate CD11b⁺ cells were counted to evaluate the M1 phenotype.³¹ UMSCs promote the maturation of T-reg cells. Pretreatment of UMSCs with chondroitinase ABC, anti-IL10, TGFβ, and CD44 antibodies suppresses the maturation of T-reg cells. **P* < 0.05 versus controls. (Reproduced from Coulson-Thomas, et. al. [Fig. 5], J. Biol. Chem., 2014; 289, 34:23465–23481, with permission).

Figure 12. Immunolocalization of HA, HC1, and HC2, and expression of HAS2, HCs, Bikunin, TSG6, and PTX3 in UMSCs exposed to inflammatory cells

UMSCs were untreated or were treated with Hylase or HylaseS and co-cultured with inflammatory cells seeded in a Transwell® insert with 0.44-µm pores. (A) Localization of HA, HC1, and HC2 by immunocytochemistry. UMSCs were placed in co-culture with inflammatory cells using a Transwell® system and left for 24 h, after which RNA was extracted from the UMSCs in the bottom chamber. Real time polymerase chain reaction analyses verified the expression levels of HC1 (B), HC2 (C), HC3 (D), TSG6 (E), Bikunin (F), RHAMM (H), HAS2 (I), and pentraxin 3 (PTX3) (J). (G) Inflammatory cells were seeded directly over UMSCs to study the adhesion of inflammatory cells to UMSC HA-rich cables (green) that is associated with UMSC. Nuclei were counterstained with DAPI (blue), and * represents the nuclei of UMSCs.³¹ (B–J) * $P < 0.05$. (Reproduced from Coulson-Thomas, et. al. [Fig. 10], J. Biol. Chem., 2014; 289, 34:23465–23481, with permission).