MSCohi-O lenses for long-term retention of mesenchymal stem cells on ocular surface as a therapeutic approach for chronic ocular graft-versus-host disease

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SUMMARY

Chronic ocular graft-versus-host disease (oGVHD) is a common complication of allogeneic hematopoietic stem cell transplantation (allo-HSCT) and can lead to vision loss if not diagnosed and treated promptly. Currently, no approved drugs exist for oGVHD treatment. However, umbilical cord-derived mesenchymal stem cells (UCMSCs) have known immunoregulatory properties and have been employed in clinical trials for immune-mediated diseases. To address oGVHD, the application of UCMSCs to the ocular surface is a logical approach. Intravenous administration of UCMSCs poses risks, necessitating topical and local delivery. Retaining UCMSCs on the ocular surface remains a challenge. To overcome this, we invented mesenchymal stem cell-coating high oxygen-permeable hydrogel lenses combining UCMSCs and machinery to enable the long-term retention of UCMSCs on the ocular surface. Animal model experiments demonstrated that these lenses effectively retained UCMSCs, providing therapeutic benefits by decreasing corneal inflammation and damage, and inhibiting immune rejection and response, all crucial aspects in oGVHD treatment.

INTRODUCTION

Ocular graft-versus-host disease (oGVHD) is a complication frequently encountered in patients following allogeneic hematopoietic stem cell transplantation (allo-HSCT) (Nair et al., 2021). According to a joint survey by several major medical institutes in the United States, the prevalence of chronic GVHD in allo-HSCT recipients is 30%–70% (Hill et al., 2021). Several recent studies showed that up to 50%–90% of the chronic GVHD patients had ocular manifestations (Dietrich-Ntoukas et al., 2012; Nassiri et al., 2018). A prospective survey published by the National Institutes of Health in 2021 also confirmed that the primary organ most frequently affected by chronic GVHD was the eye, accounting for 58% of cases (Pietraszkiewicz et al., 2021). In addition, research data from the Schepens Eye Research Institute of the Harvard Medical School showed that the morbidity of oGVHD in allo-HSCT patients is 40%–60% (Shikari et al., 2013). All these data together indicate a high prevalence of oGVHD in patients with allo-HSCT. The symptoms of oGVHD including irritation, itchiness, grittiness, foreign body sensation, burning, excessive tearing, light sensitivity, pain, redness, and blurring of vision (Shikari et al., 2013). Allo-HSCT is a treatment that effectively cures malignant blood disorders like leukemia. This treatment requires the transplantation of hematopoietic stem cells from a healthy donor to a recipient to regenerate the immune and hematopoietic systems of the patient. Following allo-HSCT, oGVHD may develop as the donor T cells recognize the patient’s ocular cells as foreign and attack the entire ocular surface, including the lacrimal gland, meibomian gland, eyelids, corneal, and conjunctiva.

The attack of an overactive donor T cell on an allo-HSCT recipient’s ocular tissues follows a specific mechanism. Initially, the overactive donor T cells provoke an inflammatory response in the recipient’s ocular surface cells. With the resulting immune cascade reactions, the lacrimal gland, meibomian gland, and other ocular tissues are prone to fibrosis. This condition leads to the severe impairment of the glands’ secretory function, resulting in tear deficiency, ocular surface damage (including but not limited to corneal ulcer, ocular surface inflammation, blepharoctis, dry eye, corneal perforation, and atretoblepharia), and, in severe cases, vision loss (Sinha et al., 2021).

oGVHD has a severe impact on the quality of life of affected individuals and, unless diagnosed early and treated promptly, often culminates in vision loss. Its primary clinical features are ocular surface inflammation, dry eye, and corneal ulcer, which in severe cases could lead to an array of secondary complications, posing a threat to visual function and ocular health. Complications include corneal perforation, blepharoctis, and atretoblepharia (Sinha et al., 2021).

There is currently no drug available for the treatment of oGVHD (Zhao Wenxin, 2022). The existing treatment options for oGVHD consist of systemic administrations, topical treatments, and surgical therapies. However, all
three treatment types have their limitations and are unable to prevent or repair corneal scarring effectively (Carreno-Galeano et al., 2021; Dietrich-Ntoukas et al., 2012; Hessen and Akpek, 2012; Inamoto et al., 2015; Plattner et al., 2017; Sabti et al., 2012; Sun et al., 2018; Wolff et al., 2021). Furthermore, oGVHD often does not respond to conventional treatments, resulting in their inadequacy for curing the condition. Hence, new therapies must be developed to address the unmet needs of patients with oGVHD.

The pathogenesis of chronic GVHD is characterized by the attack of acceptor tissues by donor T cells, which belongs to an immune-mediated condition. In contrast, umbilical cord-derived mesenchymal stem cells (UCMSCs) possess natural immune regulatory capabilities. Therefore, several studies have utilized UCMSCs in clinical trials to investigate their potential as treatments for various autoimmune and immune-mediated diseases, including rheumatoid arthritis (Ghoryani et al., 2020), type 1 diabetes (Lu et al., 2021), multiple sclerosis (Petrou et al., 2020), systemic lupus erythematosus (Li et al., 2018), inflammatory bowel disease (Barnhoorn et al., 2020), autoimmune liver disease (Wang et al., 2014), and Sjogren’s syndrome (Xu et al., 2012). Considering the immune-regulatory properties of UCMSCs, a logically sound strategy for the etiological treatment of oGVHD involves using UCMSCs to modulate donor T cells at the ocular surface and prevent them from attacking the recipient’s ocular tissues.

UCMSCs can be administered through internal or external routes for systemic or topical applications. For treating oGVHD, it is necessary to administer UCMSCs topically to ensure their safety. Intravenous or local injection of UCMSCs into the body may disrupt or affect the therapeutic effectiveness of allo-HSCT. A meta-analysis of six significant databases, including PubMed and the Web of Science, up to June 2020, discovered that injecting mesenchymal stem cells (including UCMSCs) caused a decrease in overall survival rates among hematologic malignancy patients who had undergone allo-HSCT (Li et al., 2021). Currently, numerous clinical studies have been conducted to evaluate the efficacy of stem cells in the treatment of acute or chronic GVHD, and these studies have demonstrated promising results (Elgaz et al., 2019; Kelly and Rasko, 2021; von Dalowski et al., 2016). However, as we delve deeper into stem cell therapies for GVHD, it becomes evident that further investigation is warranted, particularly regarding important aspects such as cell resources, quality control measures, and the identification of unknown risk factors (Kelly and Rasko, 2021).

Topical and external applications of UCMSCs to the ocular surface of oGVHD patients inhibit only the attack of donor T cells on the ocular surface, without affecting the normal function of the donor T cells in other parts of the patient’s body, as desired after allo-HSCT.

Currently, the primary obstacle is the inability of long-term retention of UCMSCs on the ocular surface. To overcome this challenge, we have developed a unique combination therapy comprising UCMSCs and machinery, mesenchymal stem cell-coating high oxygen-permeable hydrogel lenses (MSCohi-O), to resolve the issue.

There is currently no established animal model for oGVHD. As a result, we selected the corneal alkali-burn model and the corneal transplantation model of New Zealand Rabbits as two suitable complementary animal models for evaluating the efficacy of MSCohi-O lenses for oGVHD treatment. Analysis of results obtained from both models revealed that MSCohi-O lenses effectively inhibit inflammations, promote injury healing, and alleviate transplant rejection. These results indicate that MSCohi-O lenses possess anti-inflammatory properties, hinder immune rejection and response (in immunoregulation by UCMSCs, this involves the suppression of the immune response when host T cells attack transplanted foreign tissue, which are essentially equivalent responses when donor T cells attack host tissue as seen in oGVHD), and promote injury healing, all significant traits of oGVHD. Based on these findings, we believe that MSCohi-O lenses hold high potential for treating oGVHD patients in clinical settings.

RESULTS

MSCohi-O lenses are composed of non-proliferative UCMSCs after irradiation and silicone hydrogel lenses (Figure 1). Detailed information is provided in the Methods. The UCMSCs obtained from three different cell banks were grown in culture and displayed characteristic long and spindle-shaped morphology. Cell identification was confirmed using the cellular short tandem repeat method, which conforms with the standards adopted by the American National Bureau of Standards-ANSI/ATCC ASN-0002-2011 (Organization, 2011). The test revealed 16 human allele markers, identifying a single cellular origin with no trace of contamination from other human cells. The surface markers and relevant quality characters of the UCMSCs were presented in Table 1 and Table S1.

When subjected to trilineage differentiation, UCMSCs showed evidence of calcium salt deposition on the cell surface, resulting in the formation of calcium nodules. These nodules were stained red by chelated calcium ions that were chelated with Alizarin Red. This confirmed the differentiation of UCMSCs into osteoblasts (Figure 2A, left). Additionally, adipocytes could be identified by Oil Red O staining, which highlighted red-dyed oil/fat droplets. Visible red droplets validated the differentiation of UCMSCs into adipocytes (Figure 2A, middle). The
production of matrix and fibers by chondrogenic cells was demonstrated by blue staining with Alcian blue. This signaled the differentiation of UCMSCs into chondrogenic cells (Figure 2A, right).

The indoleamine 2,3-dioxygenase (IDO) expression data indicated that UCMSCs stimulated with interferon-γ (IFN-γ) resulted in a higher expression of IDO compared with the control group. Specifically, the level of IDO expressed by UCMSCs induced by IFN-γ was calculated to be $2.1 \times 10^4$ times higher than that of the control group (Figure 2B). The T cell secretion data demonstrated a decrease in tumor necrosis factor-α (TNF-α) (Figure 2C) and IFN-γ (Figure 2D) levels in the UCMSC+T cells group, in comparison with the control group. Notably, the inhibition rates of UCMSCs on T cell secretion of TNF-α and IFN-γ were found to be 66% and 71%, respectively. As for the T cell proliferation data, the proportion of T cells in the UCMSC + peripheral blood mononuclear cell (PBMC) coculture group was lower than that of the control group (Figure 2E), with a decrease rate of 42%. Additionally, the T cell differentiation data demonstrated an increased proportion of regulatory T (Treg) cells (Figure 2F) and decreased proportions of T helper type 1 (Th1) (Figure 2G) and Th17 (Figure 2H) cells in the UCMSC+PBMC group, with increased or decreased rates of 44%, 49%, and 36%, respectively.

To investigate how UCMSCs modulate the proportions of T cell subsets, including Th1, Th17, and Treg cells, we measured the expression levels of HGF, PGE2, and TGF-β in the co-culture system of UCMSCs and PBMCs. These molecules play crucial roles in promoting Treg cell proliferation and inhibiting the differentiation of native T cells into Th1 and Th17 cells (Rad et al., 2019). Our data revealed that the levels of HGF, PGE2, and TGF-β increased significantly in the co-culture medium of UCMSCs and PBMCs, with fold changes of 1.93, 1.72, and 2.42, respectively, compared with the medium of UCMSCs cultured alone (Figure 2E).

UCMSCs in the 0 Gy and 3 Gy groups exhibited an increase in cell index values (which correlated with cell quantity) over time after the initial cell attachment phase that occurred within the first hour. Conversely, cell index values of UCMSCs in the 10, 15, 30, and 100 Gy groups remained stable. These findings indicate that cell growth occurred over time in the 0 Gy and 3 Gy groups, but no proliferation was observed in the 10, 15, 30, and 100 Gy groups (Figure 3A). The UCMSCs were dissociated from irradiated MSCohi-O lenses (subjected to a 15-Gy X-ray) and assessed for morphology, cell viability and quantity, differentiation, and immunoregulatory function. Results indicated that the UCMSCs maintained normal long and spindle-shaped morphology, with a high cell viability rate of 90% or more and an average of $1.0 - 2.0 \times 10^5$ cells per lens. Importantly, the data of Alizarin Red, Oil Red O, and Alcian blue stains revealed that the UCMSCs were capable of differentiating into osteoblasts (Figure 3B, top), adipocytes (Figure 3B, middle), and chondrogenic cells (Figure 3B, bottom), demonstrating the successful differentiation of UCMSCs after irradiation. Additionally, we found that post-irradiation UCMSCs could induce the expression of IDO, with an average increase of nearly 16,000-fold when stimulated with IFN-γ (Figure 3C). The levels of TNF-α and IFN-γ in the UCMSC+T cells group treated with irradiated UCMSCs were lower than the levels in the control group (Figure 3D). Additionally, radiation treatment did not affect the immunoregulatory functions of UCMSCs, which effectively inhibited the proliferation of T cells, Th1 cells, and Th17 cells, and promoted the proliferation of Treg cells (Figure 3E). The expression levels of HGF, PGE2, and TGF-β by UCMSCs were increased by 1.87-fold, 1.54-fold, and 2.21-fold, respectively, in the co-culture groups. These values...
were consistent with those obtained before irradiation (Figure 3F). Moreover, the cell-surface marker expression levels of the irradiated UCMSCs remained unaltered (Table 1).

In the corneal alkali burn model, neovessels grew at the fastest rate in the alkali-burn + blank lenses group, covering areas of 125 mm². Neo-vessel growth was also rapid in the alkali-burn and blank lenses groups, covering areas of 81 mm² and 60 mm², respectively. In contrast, neovessels grew slowly in the alkali-burn + MSChoI-O lenses high-dose group and stopped growing later in the observation period, with a calculated area of 14 mm² (Figure 4A). In the alkali-burn + MSChoI-O lenses high dose group, the calculated fluorescein staining areas of the corneas slowly declined during the observation period, with calculated values of 1,446 pixels. In contrast, the fluorescein staining area of corneas in the alkali-burn and alkali-burn + blank lenses groups remained unchanged from day 7 to day 17, stabilizing at 36,488 and 24,768 pixels, respectively. Meanwhile, the calculated fluorescein staining area of corneas in the blank lenses group increased to 41,203 pixels (Figure 4B). Images of neovascularization and fluorescein staining in different groups are displayed in Figure 4C. Regarding the expression of inflammatory factors, the alkali-burn + blank lenses group showed the highest level of expression for vascular endothelial growth factor (VEGF), followed by the alkali-burn group, the blank control group, and the alkali-burn + MSChoI-O lenses high-dose group (Figure 4D). Conversely, the alkali-burn + MSChoI-O lenses high-dose group displayed the highest expression level of the anti-inflammatory factor, interleukin-4 (IL-4), followed by the blank control group, the alkali-burn group, and the alkali-burn + blank lenses group (Figure 4E). Notably, all tested pro-inflammatory factors, including TNF-α, IL-6, IL-23, matrix metalloproteinase (MMP1), MMP2, and MMP9, exhibited similar trends of reduced expression in the alkali-burn + MSChoI-O lenses group, as observed with VEGF expression (Figure 4F).

In the corneal transplantation model, graft rejection was evaluated by assessing corneal opacity and smoothness using a slit lamp. The cornea in the MSChoI-O lenses group exhibited opaque and smooth characteristics on days 7 and 14, respectively. In contrast, the blank lenses group showed an opaque character only on day 3, and no smooth character was observed (Figure 5A). Furthermore, the score results of the corneal opacity, inflammation, and rejection index revealed that the cornea in the MSChoI-O lenses group had lower values of opacity, inflammation and rejection on days 7, 10, and 14 as compared with the blank lenses group and control group (Figures 5B–5D). The corneal survival period was postponed to day 15 in the MSChoI-O lenses group, longer than that in untreated group and blank lenses group, which were days 7 and 10, respectively (Figure 5E). To further determine the mechanism by which MSChoI-O lenses inhibit inflammation and rejection, relevant cytokines were measured, and inflammatory cell infiltration was detected. All groups showed corneal thickening after transplantation surgery (Figure 6A). However, the MSChoI-O lenses group

### Table 1. UCMSCs characterization prior to and after irradiation

| Irradiation treatment | Cell surface markers (Positive cells proportion) | Methods | Acceptance criteria | Test results |
|-----------------------|-------------------------------------------------|---------|---------------------|-------------|
| Prior to              | CD73 flow cytometry                             | ≥ 95%   | ≥ 95%               |             |
|                       | CD44                                             | ≥ 95%   | ≥ 95%               |             |
|                       | CD90                                             | ≥ 95%   | ≥ 95%               |             |
|                       | CD105                                            | ≥ 95%   | ≥ 95%               |             |
|                       | CD29                                             | ≥ 95%   | ≥ 95%               |             |
|                       | CD166                                            | ≥ 95%   | ≥ 95%               |             |
|                       | CD45                                             | ≤ 2%    | ≤ 2%                |             |
|                       | CD31                                             | ≤ 2%    | ≤ 2%                |             |
|                       | CD326                                            | ≤ 2%    | ≤ 2%                |             |
|                       | CD14                                             | ≤ 2%    | ≤ 2%                |             |
|                       | CD34                                             | ≤ 2%    | ≤ 2%                |             |
|                       | CD19                                             | ≤ 2%    | ≤ 2%                |             |
|                       | CD79a                                            | ≤ 2%    | ≤ 2%                |             |
|                       | CD11b                                            | ≤ 2%    | ≤ 2%                |             |
|                       | HLA-DR                                           | ≤ 2%    | ≤ 2%                |             |
| After                 | CD73 flow cytometry                             | ≥ 95%   | ≥ 95%               |             |
|                       | CD44                                             | ≥ 95%   | ≥ 95%               |             |
|                       | CD90                                             | ≥ 95%   | ≥ 95%               |             |
|                       | CD105                                            | ≥ 95%   | ≥ 95%               |             |
|                       | CD29                                             | ≥ 95%   | ≥ 95%               |             |
|                       | CD166                                            | ≥ 95%   | ≥ 95%               |             |
|                       | CD45                                             | ≤ 2%    | ≤ 2%                |             |
|                       | CD31                                             | ≤ 2%    | ≤ 2%                |             |
|                       | CD326                                            | ≤ 2%    | ≤ 2%                |             |
|                       | CD14                                             | ≤ 2%    | ≤ 2%                |             |
|                       | CD34                                             | ≤ 2%    | ≤ 2%                |             |
|                       | CD19                                             | ≤ 2%    | ≤ 2%                |             |
|                       | CD79a                                            | ≤ 2%    | ≤ 2%                |             |
|                       | CD11b                                            | ≤ 2%    | ≤ 2%                |             |
|                       | HLA-DR                                           | ≤ 2%    | ≤ 2%                |             |

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exhibited fewer infiltrated inflammatory cells when compared with the untreated and blank lenses groups (Figure 6A). mRNA expression levels of pro-inflammatory factors such as IL-17A, MMP2, MMP9, TNF-α, and IL-1β were decreased in the corneas of the MSCohi-O lenses group (0.2, 0.0, 0.03, 0.1, and 0.05, respectively) as compared with the blank lenses group (0.9, 0.98, 0.51, 0.4, and 0.97, respectively) normalized to control (untreated) group (Figure 6B). In addition, the expression level of VEGF mRNA was reduced to 0.2 in the MSCohi-O lenses group compared with that to 1.2 in the blank lenses group when normalized to the control group (Figure 6B). ELISA results of aqueous humor indicated that, as compared with the control group, the MSCohi-O lenses group had
lower secretion of pro-inflammatory factor IFN-γ and IL-1β, at 400 and 1,000 pg/mL, respectively. In contrast, the blank lenses group did not show any significant regulation function on IFN-γ and IL-1β secretion, for each of them being measured at 790 and 1,900 pg/mL, respectively (Figures 6C and 6D).

**DISCUSSION**

oGVHD poses a significant threat to vision and remains an area of unmet medical need because of the lack of targeted medicines and effective therapies. Although the U.S. Food and Drug Administration has approved three drugs for the...
Figure 4. The effects of MSCohi-O lenses on alkali-burn corneal
(A) Time course of the area of neovascularization.
(B) Time course of injury zone stained by fluorescein.
(C) Images of neovascularization and injury (fluorescein staining) recovering level of corneas in deferent groups at day 17.
(D) The VEGF expression level of each group was normalized to that of the alkali-burn group.
(E) The IL-4 expression level of each group was normalized to that of the alkali-burn group.
(F) The expression levels of pro-inflammatory factors, as indicated in each panel, from each group was normalized to that of the alkali-burn group. Five animals were included in each group for experiment, (N = 5).
treatment of chronic GVHD—ibrutinib, belumosudil, and ruxolitinib—they are not designed to address the symptoms of oGVHD and, thus, cannot provide a suitable treatment option (Martini et al., 2022).

The pathogenesis of oGVHD is rooted in the excessive attack of the recipient’s ocular tissues by reactive donor T cells. Therefore, a logical approach to treat the cause of oGVHD is to prevent the overreactive donor T cells from

Figure 5. The effects of MSCohi-O lenses on corneal transplantation model
(A) Images of corneal opacity and smoothness in deferent groups detected by slit lamp from day 3 to day 14.
(B–D) The clinical scoring of opacity index (B), inflammation index (C) and rejection index (D) in corneas.
(E) The survival period of transplanted corneas from day 0 (transplantation) to day 14. Five animals were included in each group for experiment, (N = 5).
Figure 6. Mechanisms of MSCohi-O lenses rescuing graft rejection and inflammation in corneal transplantation model
(A) Hematoxylin and eosin staining of cornea tissues in deferent groups at day 17. The infiltrated inflammatory cells were stained with dark blue of nuclear.
(B) The relevant mRNA expression level of each group normalized to that of the untreated group, including VEGF, MMP2, MMP9, TNF-α, IL-1β, and IL-17A.
(C and D) The secretion levels of IFN-γ (C) and IL-1β (D) detected in the aqueous humor. There were five animals in each group to collect samples for each individual assay (N = 5).
attacking the recipient’s lacrimal gland, meibomian gland, and other ocular tissues at the local level.

UCMSCs exhibit distinct immunomodulatory properties, primarily by secreting soluble cytokines and regulating the release of various anti-inflammatory factors. Additionally, they can inhibit the proliferative response of T cells when allogeneic antigens stimulate them. UCMSCs also facilitate the differentiation of T cells into Treg cells, and activate Treg cells, thus promoting the development of immunological tolerance and the maintenance of immunological homeostasis (Jasim et al., 2022; Ozgul Ozdemir et al., 2021). Briefly, IDO secreted by UCMSCs with stimulation of IFN-γ play a major role in inhibiting T cell proliferation: UCMSCs secrete IDO, which catalyzes the breakdown of tryptophan, resulting in the production of kynurenine, which subsequently inhibits the proliferation of T cells and promotes the apoptosis of T cells (Song et al., 2020). UCMSCs also promote T cell differentiation into Treg cells, while hindering the differentiation of T cells into pro-inflammatory T helper cells, such as Th1 and Th17 cells. This leads to a reduction in the expression of pro-inflammatory cytokines, including TNF-α, IFN-γ, IL-6, and IL-23, and an increase in the expression of anti-inflammatory cytokines, such as IL-4. Our data detailed in the Results demonstrate that UCMSCs also inhibit the expression of MMPs, which contribute to the regulation of the inflammatory response. Overall, these immunomodulatory effects of UCMSCs can decrease inflammatory damage in the body, maintain self-tolerance, facilitate a shift toward an anti-inflammatory environment, and foster immunological tolerance (Zhao et al., 2020).

Therefore, the immunomodulatory effects of UCMSCs offer the potential for their use in the clinical and etiological treatment of oGVHD that is initiated from inflammation by overactive T cells from a donor. As UCMSCs in systemic circulation decreased overall survival of allo-HSCT recipients with hematologic malignancies (Li et al., 2021), to ensure safety, the treatment of oGVHD requires the topical, external administration of UCMSCs to the ocular surface, as they only inhibit the attack of donor T cells on recipient’s ocular surface tissues without interfering with the normal function of donor T cells in other areas of the patient’s body after allo-HSCT.

Despite their efficacy, UCMSCs are unable to remain on the ocular surface on their own. To achieve stable and long-term local application of UCMSCs on the ocular surface, we have developed a novel method that utilizes lenses to carry UCMSCs for prolonged local administration. This is the design principle of MSCohi-O lenses.

To ensure stable attachment of viable UCMSCs to the inner surface of the lens, we utilized irradiation to inhibit cell proliferation, consequently maintaining a stable state suitable for local administration. This approach eliminates the potential negative impact of cell shedding caused by proliferation and ensures an uninterrupted therapeutic effect.

The unique structure of the blood-eye barrier (Rudraraju et al., 2020) limits the entry of blood-derived cells and molecules into the micro-vessels present in ocular tissues, setting it apart from other blood vessels present in the body (Wong and Silver, 2020). Therefore, UCMSCs loaded on MSCohi-O lenses and irradiated to prevent proliferation are unlikely to accidentally shed and cross the blood-ocular barrier, thus minimizing the possibility of entering the systemic circulation. As a result, the use of MSCohi-O lenses for ocular surface treatment of oGVHD does not interfere with or impact the therapeutic efficacy of allo-HSCT.

We analyzed the characteristics of UCMSCs that were dissociated from MSCohi-O lenses following exposure to 15 Gy irradiation. Our findings demonstrate that UCMSCs retain the ability of trilineage differentiation and possess immunoregulatory functions comparable with UCMSCs obtained from the same cell bank prior to irradiation. These observations confirm the biocompatibility of UCMSCs with the silicone hydrogel base of the MSCohi-O lenses, which serve as the carrier for UCMSCs. Therefore, the entire lens preparation procedure is deemed safe for ocular surface treatment of oGVHD.

At present, there is no validated animal model for oGVHD. Therefore, in our study, to evaluate the efficacy of MSCohi-O lenses for oGVHD treatment, we selected the New Zealand Rabbit corneal alkali-burn model and the New Zealand Rabbit corneal transplantation model as complementary animal models. The former model was used to assess the lenses’ efficacy in terms of inflammation and injury healing of the corneal ulcer (a common occurrence in oGVHD), while the latter model was chosen to examine the lenses’ ability to alleviate immuno-rejection and response. This is because the attacking of host T cells on transplanted foreign tissue, which is seen in corneal transplantation, is conceptually equivalent to the attacking of donor T cells on host tissue, as seen in oGVHD.

Data obtained from the alkali-burn model provide significant insights into the efficacy of MSCohi-O lenses in inhibiting inflammation and healing injury. Results demonstrate that these lenses with an appropriate dose of UCMSCs can effectively inhibit the growth of fundus neovascularization, decrease the extent of corneal damage, and decrease the expression of various pro-inflammatory factors, including VEGF, TNF-α, IL-6, IL-23, MMP1, MMP2, and MMP9. Furthermore, they increase the expression of IL-4, which plays an essential role in stimulating the healing of corneal ulcers resulting from alkali burns.

The corneal transplantation model demonstrated that the use of MSCohi-O lenses maintained the opaque and smooth characteristics of the cornea, leading to lower values of the opacity and rejection index. Furthermore,
MSCohi-O lenses efficiently downregulated the mRNA expression of pro-inflammatory factors such as IL-17A, MMP2, MMP9, TNF-α, and IL-1β in the ocular tissues of the recipients. MSCohi-O lenses also reduced the expression of VEGF and the infiltration of inflammatory cells, which helped in resisting inflammatory conditions such as angiogenesis and recruitment of inflammatory cells in corneas. Accordingly, MSCohi-O lenses decreased the concentration of IFN-γ and IL-1β in aqueous humor, which play important roles in inflammatory mechanisms in ocular tissues.

These findings indicate that MSCohi-O lenses offer a feasible and etiological therapy for treating immune responses and addressing the symptoms associated with oGVHD, underscoring the potential for conducting pivotal clinical trials.

In conclusion, UCMSCs loaded onto MSCohi-O lenses represent a promising therapy for etiologically treating oGVHD by regulating overreactive donor T cells. The mechanism of action includes (1) IDO secretion for locally inhibiting the proliferation of donor T cells in the ocular surface, (2) facilitating T cell differentiation into Treg cells, (3) inhibiting T cell differentiation into Th1 and Th17 cells, (4) inhibiting the secretion of inflammatory and growth factors by T cells, and (5) facilitating the secretion of anti-inflammatory factors by T cells, jointly preventing the attack of donor T cells on the recipient’s lacrimal gland, meibomian gland, and other ocular tissues. The topical, external method of administration of MSCohi-O lenses to the ocular surface, lack of UCMSC proliferation after irradiation, and the distinct blood-ocular barrier structure of the eye ensure the safety of this therapy while treating oGVHD.

**EXPERIMENTAL PROCEDURES**

**Resource availability**

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**Materials availability**

The UCMSCs in this study are available from the corresponding author upon request. All of the protocols of animal experiments were followed the principle of Laboratory Animal Welfare Act, and approved by the Animal Ethics Committee of The Third Affiliated Hospital, Sun Yat-Sen University.

**Data and code availability**

The datasets used and/or analyzed in the present study are available from the corresponding author on request.

**UCMSC cell bank**

According to ICH Q5D, USP, and ISO24603 (the international standard of stem cell), the umbilical cord tissue collection and transportation, UCMSC isolation, and the establishments of a primary cell bank (PCB), a master cell bank (MCB), and a working cell bank (WCB) have been completed, respectively. The UCMSCs from the PCB, MCB, and WCB were comprehensively tested and all results met the established standards.

**Trilineage differentiation**

UCMSCs were induced to differentiate with trilineage differentiation following the standards adopted by the ISCT (Brinkhof et al., 2020). Briefly, UCMSCs were induced using osteogenic, adipogenic, or chondrogenic differentiation medium with medium exchanged every 3 days until desired days, and then stained with Alizarin Red, Oil Red O, or Alcian blue, respectively.

**IDO expression of UCMSCs stimulated by IFN-γ**

UCMSCs were seeded in six-well plates, with 4 μL 20 ng/mL inflammatory factor IFN-γ added to the test group, but not to the control group. After 24 h of culture, the UCMSCs were collected. The expression level of IDO was detected by a qPCR method (Livak and Schmittgen, 2001).

**Inhibition of the T cells secretion of inflammatory factors by UCMSCs**

After co-culture of UCMSCs (2 × 10^5) with T cells (1 × 10^6), inducers, i.e., 10 μL 10 g/mL phorbol ester, 5 μL 4 mg/mL brefeldin A, and 2 μL 1 mg/mL ionomycin were added to induce T cells to secrete inflammatory factors, and the control group contained no UCMSCs. After 5 h culture, T cells were collected in a culture medium and the secretion level of factors IFN-γ and TNF-α was detected by a qPCR method (Master et al., 2021).

**Inhibition of T cell proliferation by UCMSCs**

UCMSCs (2 × 10^5) were co-cultured with PBMCs (1 × 10^6), and control groups were PBMCs (1 × 10^5) cultured alone, wherein culture systems were both 2 mL. Both a positive control group and a test group were added with phytohemagglutinin that is an agent for inducing proliferation of T cells; after 4 days, the quantities of T cells under different conditions were compared (Wang et al., 2022a).

**Facilitation of T cell differentiation to Treg cells by UCMSCs**

UCMSCs were seeded in six-well plates and incubated with mitomycin C (final concentration being 10 mg/L) in a CO₂ incubator for 20 min. PBMCs (1 × 10^6/well), phytohemagglutinin (final concentration being 10 mg/L), and IL-2 (final concentration being 200 IU/mL) were added. Control groups were PBMCs (1 × 10^5) cultured alone. Culture was performed for 16–20 h, centrifugation was performed to remove supernatant, and a fresh medium was added for additional culture of 2 days. The quantities of Treg cells under different conditions were compared (Yang et al., 2016).

**Inhibition of T cell differentiation to Th1 cells and Th17 cells by UCMSCs**

UCMSCs were seeded in trans-well plates (1 × 10^5/well), mitomycin C (final concentration being 10 mg/L) was added next day, incubation was performed in a CO₂ incubator for 20 min,
supernatant was removed, Dulbecco’s phosphate buffered saline (250 μL/well) was used to wash two times, and PBMCs (1 × 10⁶/well) were added. Control groups were PBMCs (1 × 10⁶) cultured alone. Culturing was performed for 48 h, 2 L Leukocyte Activation Cocktail with BD GolgiPlug was added and culturing was continued for 5 h. PBMCs were obtained and analyzed by flow cytometry to determine the proportions of Th1 and Th17 cells (Kamentsky and Kamentsky, 1991). Furthermore, UCMSCs were collected for the quantification of expression levels of HGF, PGE2, and TGF-β using the qPCR method, as described above.

The effect of different irradiation treatments on the proliferation of UCMSCs

A non-invasive and real-time method with the xCELLigence RTCA S16 analyzer was used to detect the growth curve of UCMSCs subjected to irradiation. The UCMSCs were adjusted to a density of 8.0 × 10⁴ cells/mL with an appropriate amount of medium, seeded in a plate at 100 μL cell suspension per well, and irradiated for irradiation doses of 0, 3, 10, 15, 30, and 100 Gy, respectively. After setting a baseline with 50 μL medium in the RTCA analyzer, 50 μL cell suspension of each dose group was added, and analyzer was placed in a CO₂ incubator at 37°C for non-invasive, real-time, and label-free measurement of cell growth.

MSCohi-O lens preparation

MSCohi-O lenses (U.S. patent pending #18/148, 421) are composed of UCMSCs that do not proliferate after irradiation, and silicone hydrogel lenses (Figure 1). Each MSCohi-O lens contains 1.0–2.0 × 10⁵ UCMSCs irradiated with 15 Gy X-ray, and is preserved in 2 mL saline solution without any additional excipient components. UCMSCs lose proliferation capability after irradiation, but maintain secretion and immunomodulatory functions. MSCohi-O lenses were prepared as follows. Hydrogenated silicone oil, allyl methacrylate, and hydroquinone (100:7:0.03) were stirred in a solvent xylene uniformly, then heated under reduced pressure was then performed to obtain the polymer rod. Organosilicone monomer, hydrophilic monomer, cross-linking agent ethylene glycol dimethacrylate, initiator azobisisobutyronitrile, and solvent isopropanol were mixed uniformly as the ratio of 25:58:16:1:30 to obtain polymeric liquid, filled in mold. After heating and curing, the polymer rod was lathed to be a button shape. Finally, the button-shaped material was cut on two sides to produce the bowl-shaped silicone hydrogel carrier (hydrogel lenses). UCMSCs were seeded on the silicone hydrogel lenses as about 2 × 10⁶ cells per lens. Subsequently, the silicone hydrogel lenses loaded with UCMSCs (MSCohi-O Lenses) were stored in incubator at 37°C overnight for UCMSCs adhesion. UCMSCs growth on the lenses was monitored until desired days. Then the MSCohi-O Lenses were gently washed twice with saline solution and irradiated at 15 Gy X-ray.

New Zealand rabbit corneal alkali-burn model

New Zealand rabbits used as acceptors were anesthetized and subjected to alkali burn on the cornea of their left eye by placing a filter paper soaked with NaOH for 30 s, followed by washing the lesion with a sodium chloride solution. This established the New Zealand rabbit corneal alkali-burn model, which mimics corneal injury in a clinical setting (Wang et al., 2022b). The left eyes were sutured and applied with tobramycin eye cream, and the rabbits returned to rearing cages. The study included six groups: (1) the alkali-burn group, where the eye injury treatment was conducted without the wearing of any lenses, (2) the alkali-burn + blank lenses group, where the eye injury treatment was performed, followed by the use of blank lenses, (3) the alkali-burn + MSCohi-O lenses groups with low (1.0 × 10⁴ cells), (4) mid (5.0 × 10⁴ cells), and (5) high (2.0 × 10⁵ cells) doses of UCMSCs, where the eye injury treatment was conducted, followed by using MSCohi-O lenses, and (6) the blank lenses group, where no eye injury treatment was performed, and blank lenses were used. There were five rabbits in each group. The rabbits required surgical anesthesia and suturing when MSCohi-O lenses were worn on day 0, and fresh lenses were changed once on the days 3, 7, 10, and 14, respectively, with a total of five times of lenses wearing. On days 3, 7, 10, 14, and 17 after wearing MSCohi-O lenses, corneal neovascularization was tested (the formula for calculating corneal neovascularization: area A (mm²) = (C/12) × π(r² – (r – h)²), where C is the number of corneal circumferential points involved in neovascularization, r is the corneal radius = 7 mm, and l is the length of the longest neovascular branch invading the cornea from the corneal limbus. Corneal injury area was examined by corneal fluorescence staining after lenses removal. The corneas of the New Zealand rabbits were taken on day 17 to detect inflammatory neovascularization and injury area. Real-time qPCR was performed to detect the expressions of anti-inflammatory factor IL-4, pro-inflammatory factors TNF-α, IL-6, IL-23, MMP1, MMP2, MMP9, and VEGF.

New Zealand rabbit corneal transplantation model

The surgical cornea transplantation in New Zealand rabbits was performed following the protocol of a previous study (Koizumi et al., 2000). Briefly, after the aforementioned alkali-burn treatment, the New Zealand rabbits serving as donors were anesthetized and given proparacaine hydrochloride ophthalmic solution to provide anesthesia to the ocular surface. The peripheral zone of the cornea was then obtained via keratectomies with 8.5 mm corneal trephine and kept in an aseptic dish. The grafts were sutured circumferentially onto the acceptor’s corneal surface using 10-0 nylon sutures, followed by covering the transplanted ocular surface with either MSCohi-O lenses or normal blank lenses as a material control. Corneal neovascularization was tested on days 3, 7, 10, and 14 after wearing the MSCohi-O lenses, using the formula as described above. On day 14, the corneas of the New Zealand rabbits were extracted to detect the infiltration of inflammatory cells and measure the expression of relevant genes, including IL-17A, TNF-α, MMP2, MMP9, and VEGF via qPCR, or proteins such as IFN-γ and IL-1RA through ELISA.

Statistical analysis

All statistical analysis using two-tailed unpaired Student’s t-test, one-way ANOVA, or two-way ANOVA as desired, was conducted with GraphPad Prism 9 software (GraphPad Software). All data were expressed as mean ± SD with the value of n specified in text.
or figure legends, and p values of less than 0.05 were deemed statistically significant.

**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at https://doi.org/10.1016/j.stemcr.2023.10.010.

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**AUTHOR CONTRIBUTIONS**

S.L. and H.Z conceived and designed the project; Yuanyue Liu, S.S., Youyu Liu, T.F., Y.G., R.L., J.C., Yanchun Lin, Y.C., Yun Li, and T.G performed the experiments and the data analysis; T.G. and H.Z. wrote the manuscript; S.L. and H.Z. approved the final version to be submitted.

**DECLARATION OF INTERESTS**

The authors declare that they have no competing interests.

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**REFERENCES**

Barthoorn, M.C., Wasser, M., Roelofs, H., Maljaars, P.W.J., Molendijk, I., Bonsing, B.A., Oosten, L.E.M., Dijkstra, G., van der Woude, C.J., Roelen, D.L., et al. (2020). Long-term Evaluation of Allogeneic Bone Marrow-Derived Mesenchymal Stromal Cell Therapy for Crohn’s Disease Perianal Fistulas. J Crohns Colitis

Dijkstra, I., Bonsing, B.A., Oosten, L.E.M., Dijkstra, G., van der Woude, B.N., Barnhoorn, M.C., Wasser, M., Roelofs, H., Maljaars, P.W.J., Molenkamp, M. (2020). The Sufficient Immunoregulatory Effect of Autologous Bone Marrow-Derived Mesenchymal Stem Cell Transplantation in Patients with Refractory Rheumatoid Arthritis. J. Immunol. Res. 2020, 3562753.

Hessen, M., and Akpek, E.K. (2012). Ocular graft-versus-host disease. Curr. Opin. Allergy Clin. Immunol. 12, 540–547.

Hill, G.R., Betts, B.C., Tkachev, V., Kean, I.S., and Blazar, B.R. (2021). Current Concepts and Advances in Graft-Versus-Host Disease Immunology. Annu. Rev. Immunol. 39, 19–49.

Inamoto, Y., Sun, Y.C., Flowers, M.E.D., Carpenter, P.A., Martin, P.J., Li, P., Wang, R., Chai, X., Storer, B.E., Shen, T.T., and Lee, S.J. (2015). Bandage Soft Contact Lenses for Ocular Graft-versus-Host Disease. Biol. Blood Marrow Transplant. 21, 2002–2007.

Jasim, S.A., Yumashev, A.V., Abdelbasset, W.K., Margiana, R., Markov, A., Sukatan, W., Pineda, B., Thangavelu, L., and Ahmadi, S.H. (2022). Shining the light on clinical application of mesenchymal stem cell therapy in autoimmune diseases. Stem Cell Res. Ther. 13, 101.

Kametisky, L.A., and Kametisky, L.D. (1991). Microscope-based multiparameter laser scanning cytometer yielding data comparable to flow cytometry data. Cytometry 12, 381–387.

Kelly, K., and Rasko, J.E.J. (2021). Mesenchymal Stromal Cells for the Treatment of Graft Versus Host Disease. Front. Immunol. 12, 761616.

Koizumi, N., Inatomi, T., Quantock, A.J., Fullwood, N.J., Dotta, A., and Kinoshita, S. (2000). Amniotic membrane as a substrate for cultivating limbal corneal epithelial cells for autologous transplantation in rabbits. Cornea 19, 65–71.

Li, T., Luo, C., Zhang, J., Wei, L., Sun, W., Xie, Q., Liu, Y., Zhao, Y., Xu, S., and Wang, L. (2021). Efficacy and safety of mesenchymal stem cells co-infusion in allogeneic hematopoietic stem cell transplantation: a systematic review and meta-analysis. Stem Cell Res. Ther. 12, 246.

Liang, J., Zhang, H., Kong, W., Deng, W., Wang, D., Feng, X., Zhao, C., Hua, B., Wang, H., and Sun, L. (2018). Safety analysis in patients with autoimmune disease receiving allogeneic mesenchymal stem cells infusion: a long-term retrospective study. Stem Cell Res. Ther. 9, 312.

Livak, K.J., and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25, 402–408.

Lu, J., Shen, S.M., Ling, Q., Wang, B., Li, L.R., Zhang, W., Qu, D.D., Bi, Y., and Zhu, D.L. (2021). One repeated transplantation of allogeneic umbilical cord mesenchymal stromal cells in type 1 diabetes: an open parallel controlled clinical study. Stem Cell Res. Ther. 12, 340.

Martini, D.J., Chen, Y.B., and DeFilipp, Z. (2022). Recent FDA Approvals in the Treatment of Graft-Versus-Host Disease. Oncol. 27, 685–693.

Master, A., Kontzias, A., Huang, L., Huang, W., Tsoulias, A., Zarabi, S., Wolek, M., Wollocock, B.M., Honkanen, R., and Rigas, B. (2021). The transcriptome of rabbit conjunctiva in dry eye disease: Large-scale changes and similarity to the human dry eye. PLoS One 16, e0254036.

Nair, S., Vanathi, M., Mukhiya, R., Tandon, R., Jain, S., and Ogawa, Y. (2021). Update on ocular graft-versus-host disease. Indian J. Ophthalmol. 69, 1038–1050.

Nassiri, N., Eslani, M., Panahi, N., Mehrvaran, S., Ziaei, A., and Djallilian, A.R. (2013). Ocular graft versus host disease following
allogeneic stem cell transplantation: a review of current knowledge and recommendations. J. Ophthalmic Vis. Res. 8, 351–358.
Organization, A.S.D. (2011). Authentication of Human Cell Lines: Standardization of STR Profiling. ANSI/ATCC, 1–8.
ÖZgül Özdemir, R.B., Özdemir, A.T., Krmaz, C., Ovalı, E., Ölmez, E., Kerem, H., Evrenos, M.K., and Deniz, G. (2021). Mesenchymal Stem Cells: a Potential Treatment Approach for Refractory Chronic Spontaneous Urticaria. Stem Cell Rev. Rep. 17, 911–922.
Pathak, M., Diep, P.P., Lai, X., Brinch, L., Ruud, E., and Drolsum, L. (2018). Ocular findings and ocular graft-versus-host disease after allogeneic stem cell transplantation without total body irradiation. Bone Marrow Transplant. 53, 863–872.
Petróu, P., Kassis, I., Levin, N., Paul, F., Backner, Y., Benoliel, T., Oertel, F.C., Scheel, M., Hallimi, M., Yaghmour, N., et al. (2020). Beneficial effects of autologous mesenchymal stem cell transplantation in active progressive multiple sclerosis. Brain 143, 3574–3588.
Pietraszkiewicz, A.A., Payne, D., Abraham, M., Garced, A., Devarasetty, K.C., Wall, M., Menezes, S.M., Ugarte, S., Pirsl, F., Goklem, S., et al. (2021). Ocular surface indicators and biomarkers in chronic ocular graft-versus-host disease: a prospective cohort study. Bone Marrow Transplant. 56, 1850–1858.
Plattner, K., Goldblum, D., Halter, J., Kunz, C., Koeppl, R., and Gerber-Hollbach, N. (2017). Osteo-Odonto-Keratoprosthesis in Severe Ocular Graft versus Host Disease. Klin. Monbl. Augenheilkd. 234, 455–456.
Rad, F., Ghorbani, M., Mohammadi Roushandeh, A., and Habibi Roudkenar, M. (2019). Mesenchymal stem cell-based therapy for autoimmune diseases: emerging roles of extracellular vesicles. Mol. Biol. Rep. 46, 1533–1549.
Rudraraju, M., Narayanan, S.P., and Somanath, P.R. (2020). Regulation of blood-retinal barrier cell-junctions in diabetic retinopathy. Pharmacol. Res. 161, 105115.
Sabti, S., Halter, J.P., Braun Fränkl, B.C., and Goldblum, D. (2012). Punctal occlusion is safe and efficient for the treatment of keratoconjunctivitis sicca in patients with ocular GvHD. Bone Marrow Transplant. 47, 981–984.
Shikari, H., Antin, J.H., and Dana, R. (2013). Ocular graft-versus-host disease: a review. Surv. Ophthalmo. 58, 233–251.
Sinha, S., Singh, R.B., Dohlman, T.H., Takteani, Y., Yin, J., and Dana, R. (2021). Prevalence and Risk Factors Associated With Corneal Perforation in Chronic Ocular Graft-Versus-Host-Disease. Cornea 40, 877–882.
Song, N., Scholtemeijer, M., and Shah, K. (2020). Mesenchymal Stem Cell Immunomodulation: Mechanisms and Therapeutic Potential. Trends Pharmacol. Sci. 41, 653–664.
Sun, B.L., Jain, R., Patel, C., and Bhattacharyya, A.K. (2018). Graft-Versus-Host Disease With Early Cytomegalovirus Infection in Gastrointestinal Tract Biopsies. Int. J. Surg. Pathol. 26, 347–352.
von Dalowski, E., Kramer, M., Wermke, M., Wehner, R., Röllig, C., Alakel, N., Stölzel, F., Parmentier, S., Sockel, K., Krech, M., et al. (2016). Mesenchymal Stromal Cells for Treatment of Acute Steroid-Refractory Graft Versus Host Disease: Clinical Responses and Long-Term Outcome. Stem Cell. 34, 357–366.
Wang, J., Gao, S., Zhao, Y., Fan, T., Zhang, M., and Chang, D. (2022a). Manufacture and Quality Control of Human Umbilical Cord-Derived Mesenchymal Stem Cell Sheets for Clinical Use. Cells 11.
Wang, L., Han, Q., Chen, H., Wang, K., Shan, G.L., Kong, F., Yang, Y.J., Li, Y.Z., Zhang, X., Dong, F., et al. (2014). Allogeneic bone marrow mesenchymal stem cell transplantation in patients with UDCA-resistant primary biliary cirrhosis. Stem Cell. Dev. 23, 2482–2489.
Wang, L., Wang, J., Sun, H., Pang, Z., and Mu, G. (2022b). Corneal Collagen Cross-Linking Inhibits Corneal Blood and Lymphatic Vessels Temporarily in Alkali-Burned Rabbits. Curr. Eye Res. 47, 1266–1271.
Wolff, D., Radojic, V., Lajtayis, R., Cinar, R., Rosenstein, R.K., Cowen, E.W., Cheng, G.S., Sheshadri, A., Bergeron, A., Williams, K.M., et al. (2021). National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: IV. The 2020 Highly morbid forms report. Transplant. Cell. Ther. 27, 817–835.
Wong, B.H., and Silver, D.L. (2020). Mfsd2a: A Physiologically Important Lysolipid Transporter in the Brain and Eye. Adv. Exp. Med. Biol. 1276, 223–234.
Xu, J., Wang, D., Liu, D., Fan, Z., Zhang, H., Liu, O., Ding, G., Gao, R., Zhang, C., Ding, Y., et al. (2012). Allogeneic mesenchymal stem cell treatment alleviates experimental and clinical Sjogren syndrome. Blood 120, 3142–3151.
Yang, H., Sun, J., Li, Y., Duan, W.M., Bi, J., and Qu, T. (2016). Human umbilical cord-derived mesenchymal stem cells suppress proliferation of PHA-activated lymphocytes in vitro by inducing CD4+(+CD25(high)CD45RA(+)) regulatory T cell production and modulating cytokine secretion. Cell. Immunol. 302, 26–31.
Zhao, J., Chen, J., Huang, F., Wang, J., Su, W., Zhou, J., Qi, Q., Cao, F., Sun, B., Liu, Z., et al. (2020). Erratum: Human gingiva tissue-derived MSC ameliorates immune-mediated bone marrow failure of aplastic anemia via suppression of Th1 and Th17 cells and enhancement of CD4+Foxp3+ regulatory T cells differentiation. Am. J. Transl. Res. 12, 1167.
Zhao Wenxin, L.L. (2022). New progress on clinical characteristics and related mechanisms of chronic ocular graft-versus-host disease. Transplantation 13, 187–194.