Generation of a Biomimetic Human Artificial Cornea Model Using Wharton’s Jelly Mesenchymal Stem Cells

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PURPOSE. Human Wharton’s jelly stem cells (HWJSCs) are able to differentiate into skin and oral mucosa epithelial-like cells. In this work, we demonstrate for the first time the capability of HWJSCs to differentiate in vitro into cornea epithelial-like cells in a three-dimensional model.

METHODS. First, primary cell cultures of HWJSCs, corneal epithelial cells, and corneal keratocytes were cultured and three-dimensional orthotypic and heterotypic human cornea models were generated with fibrin-agarose scaffolds. Then, in vitro differentiation of HWJSCs and corneal epithelial cells was performed with keratocytic inductive medium in a three-dimensional system that allowed interaction between stromal and epithelial compartments. Histological, histochemical, and immunofluorescence analyses were used to determine the differentiation status of each sample.

RESULTS. Our results demonstrated that HWJSCs were able to differentiate into corneal epithelial-like cells, with results similar to the native cornea. Heterotypical corneas generated with HWJSCs showed adequate differentiation of the epithelium and stroma, and were similar to orthotypic and native corneas in the expression of epithelial markers (CK3/12, PKC, ZO1, and CX43) and extracellular matrix components (proteoglycans, collagen, elastic and reticular fibers). Immunofluorescence analysis confirmed the presence of crystallins Cry-gA, Cry-gB, Cry-sA, and Cry-sC with moderate or weak expression of Cry-g3 and Cry-g1 (key proteins involved in cornea transparency) in both models.

CONCLUSIONS. Our findings suggest that HWJSCs can be considered an alternative cell source for cornea regeneration and may offer a solution for patients with limbus stem cell deficiency.

Keywords: human umbilical cord Wharton’s jelly cells, corneal epithelial-like cells, tissue engineering, transdifferentiation, cornea.

The human cornea is composed of three main layers: a stratified epithelium, an underlying stroma, and a monolayered corneal endothelium. Homeostasis of corneal epithelial and stromal cells is essential for the integrity of the ocular surface, corneal transparency, and visual function.1 Self-renewal of the corneal epithelium is ensured by a stem cell population located in the cell layer of the limbal region. Stem cell maintenance and function are controlled by several factors provided by the unique microenvironment.2 This microenvironment depends on signaling molecules and growth factors released from neighboring cells and specialized extracellular matrix (ECM) components that may regulate cell phenotype and behavior through cell-cell matrix interactions.3 Aggressive damage to limbal stem cells results in limbal stem cell deficiency (LSD), leading to severe consequences for the ocular surface and visual function, which may in turn lead to loss of visual acuity.4 Limbal stem cell grafting may be used to treat corneal epithelial deficiencies. This can involve the direct transplantation of limbal tissue or the indirect transplantation of in vitro-expanded cells on different biological or synthetic carrier materials.5–7 This strategy always requires a limbal biopsy from the contralateral healthy eye, and is not applicable in patients with bilateral LSD. In these cases, allogeneic limbal stem cells from donors may be used for transplantation with prolonged systemic immunosuppressive therapy.8 This approach has proved to be unsuccessful in the long term compared with autologous transplantation. Therefore, alternative stem cells with immunoprivileged properties for regeneration of the corneal epithelium are still needed. Consequently, alternative stem cell sources with low immunogenicity, such as embryonic stem cells,9 bone marrow mesenchymal stem cells,10 hair follicle stem cells,11 corneal stromal cells,12 immature dental pulp stem cells,13 and oral mucosa epithelial cells,14 have been tested experimentally for corneal epithelium replacement. Our previous findings demonstrated that human umbilical cord Wharton’s jelly stem cells (HWJSCs) can be induced to transdifferentiate into skin and oral mucosa epithelial-like cells.15 Therefore, the differentiation of HWJSCs into corneal epithelium has not been explored to date.
In the present work, we demonstrate that HWJSCs can be efficiently used to generate an artificial anterior human cornea using three-dimensional systems based on biomaterials and tissue-engineering strategies, with results similar to the use of native corneal epithelial cells. Our results strongly support the hypothesis that HWJSCs have potential applications in translational ophthalmology and may serve as an alternative source of stem cells for ocular surface reconstruction.

**METHODS**

**Isolation and Cell Culture**

The use of human donor tissues for research was approved by the local ethics and research committee in accordance with the Declaration of Helsinki, including obtaining informed consent from donors included in this study. First, HWJSCs were isolated as previously described. Briefly, human umbilical cord fragments were longitudinally sectioned and treated enzymatically with type-I collagenase (Life Technologies). Isolated keratocytes were expanded in Amniomax (Life Technologies, Carlsbad, CA, USA) and a solution of 0.5 g/L trypsin and 0.2 g/L EDTA (Life Technologies). Then HWJSCs were collected by centrifugation and expanded in Amniomax culture medium (Life Technologies). Isolated cells used in this work fulfilled the minimal criteria for mesenchymal stem cells as determined by the International Society for Cellular Therapy. Second, primary cell cultures of human cornea keratocytes were established using type-I collagenase (Life Technologies) digestion for 6 hours at 37°C. Isolated keratocytes were expanded in culture flasks containing basal culture medium (Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 0.1 mg/mL streptomycin, and 0.25 µg/mL amphotericin B; all from Sigma-Aldrich, Steinheim, Germany) under standard cell culture conditions. For the isolation and culture of corneal epithelial cells, limbal explants were processed according to previously published protocols developed by our research group and cultured in basal culture medium supplemented with epidermal growth factor (EGF) at a final concentration of 10 ng/mL.

**Generation of Orthotypic and Heterotypic Bioengineered Human Anterior Corneas With Tissue-Engineering Strategies**

We generated two different models of human artificial anterior cornea by tissue engineering. These models are based on fibrin–agarosestromal substitutes previously developed by our research group for cornea regeneration. These substitutes contain human keratocytes immersed within and corneal epithelial cells on top (human orthotypic cornea [HOC]) or WHJSCs (human heterotypic cornea [HHC]). Briefly, a hydrogel was generated on Transwell porous inserts (Corning Enterprises, Corning, NY, USA) using human plasma as a source of fibrin, 0.1% agarose, and 250,000 cultured keratocytes. To induce polymerization of the hydrogel, 0.5 mL of a 2% CaCl₂ solution was added to 4.5 mL of the mixture. Once polymerized, artificial stromas were seeded with isolated corneal epithelial cells or HWJSC at a cell density of 70,000 cells per cm² stromal surface and cultured for 7 days immersed in EGF-enriched medium to stimulate epithelial transdifferentiation and favor the three-dimensional coculture system. After 14 and 28 days of culture, the culture medium was removed from the upper part of the inserts to induce stratification and differentiation of epithelial-like cells in an air-liquid culture technique.

**Characterization of Orthotypic and Heterotypic Bioengineered Human Anterior Corneas**

To fully characterize the bioengineered cornea models (HHC and HOC) and compare them with control native human corneas, we used histological, histochemical, and immunohistochemical analyses. For light and electron microscopy, HHC, HOC, and control human corneas were fixed in either 4% paraformaldehyde or 2.5% glutaraldehyde in 0.1 M phosphate buffer. For light microscopy, samples were embedded in paraffin and 4-µm sections were obtained. To analyze the artificial cornea stromas, we used immunohistochemistry for vimentin to detect keratocytes and histochemistry with Alcian blue, PAS, picrosirius red, and Verhoeff methods to identify ECM proteoglycans, glycosaminoglycans, mature collagen fibers, and reticular and elastic fibers, respectively. To characterize the bioengineered corneal stroma and epithelium, we used immunofluorescence with primary antibodies for type-I collagen and cornea cytokeratins (CK3/12), intercellular junction desmosomes (plakoglobin [PKG]), tight junctions (zonula occludens 1 [ZO1]), and gap junctions (connexin 43 [CX43]), respectively. To analyze crystallin expression in both the epithelial and stromal layers of the artificial cornea models, we used immunofluorescence for alpha A (Cry-aA), alpha B (Cry-bB), beta (Cry-b), zeta (Cry-c), beta-gamma 3 (Cry-bγ3), and lambda 1 (Cry-λ1). For this purpose, paraffin-embedded tissue sections were deparaffinized and rehydrated in decreasing concentrations of alcohol, and incubated with the primary antibodies. Then the samples were washed in PBS and a secondary anti-mouse or anti-rabbit antibody labeled with a fluorescent pigment (FITC or Cy3) was applied for 60 minutes, followed by rinsing with PBS. Finally, the nuclei were counterstained with 4,6-diamidino-2-phenylindole and samples were covered with glass coverslips and analyzed in a Nikon Eclipse 90i fluorescent microscope (Nikon, Tokyo, Japan) and a Leica DMi6000 confocal microscope (Leica, Solms, Germany).

For scanning electron microscopy, samples were dehydrated, prepared for critical point, dried and mounted on aluminum stubs, sputter-coated with gold according to preestablished protocols and examined in a Quanta 200 scanning electron microscope (FEI, Eindhoven, The Netherlands).

**RESULTS**

**Histological Structure of HOC and HHC Samples**

As shown in Figure 1, the use of three-dimensional bioactive systems was able to induce differentiation of HOC and HHC bioengineered human corneas into corneal epithelial cells. In HOC cultures, we observed a stratified epithelium with one to three cell layers in samples kept in vitro for 7 and 14 days, and more than seven layers in 28-day samples. In HHC tissues generated with umbilical cord stem cells (HWJSCs), we observed more cell layers than in HOC, with approximately 4, 8, and 12 cell layers at 7, 14, and 28 days of in vitro development, respectively. These HHC tissues more closely resembled human control corneas than did HOC samples. Cells in the top layer of both HOC and HHC tissues were less organized compared with control tissues. This was consistent with the scanning electron microscopy observations. Specifically, samples cultured with both corneal epithelial cells and HWJSCs had an elongated, spindle-like morphology that was especially notable at 7 days and 14 days, whereas native control samples had a typical flat, cobblestone morphology. Samples at 28 days of development showed a tight cell layer covering the entire surface of the biomaterial.
Sequential Development of Stromal ECM Components in HOC and HHC Samples

Staining with PAS (Fig. 1; Table 1) showed a high concentration of glycoproteins in HOC samples, especially at the epithelium-stroma interphase, throughout the period of development. However, HHC samples showed moderate expression at the epithelial layer, which tended to increase after 14 days in culture, becoming similar to control human cornea samples at day 28. Samples stained with Alcian blue (Fig. 2; Table 1) showed the

Table 1. Extracellular Matrix Components and Vimentin Expression in the Three Groups of Samples Analyzed in this Work as Determined by Histochemistry and Immunohistochemistry

<table>
<thead>
<tr>
<th>ECM Proteins and Vimentin</th>
<th>Alcian Blue E</th>
<th>PAS S</th>
<th>Collagen S (HC)</th>
<th>Gomori’s Reticulin S</th>
<th>Verhoeff S</th>
<th>Vimentin S</th>
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HOC generated in vitro by tissue engineering. HHCs were bioengineered samples. Native control indicates normal human native cornea. HOC and HHC samples were analyzed at 7, 14, and 28 days. Both the epithelial (E) and stromal (S) compartments of the artificial and native corneas were analyzed in this study. +++, strong expression; ++, moderate expression; +, weak expression; −, negative expression; *, expression restricted to the epithelial-stromal interface; HC, expression determined by picrosirius red histochemistry; IF, expression determined by immunohistochemistry with collagen type-I antibodies.
presence of acid proteoglycans in HOC samples at 7, 14, and 28 days. Interestingly, after 28 days, an increase in proteoglycans was detected in HHC samples, where proteoglycan levels equalled those seen in control samples at the corneal stroma. Our analysis of collagen fibers in the bioengineered stromas revealed that the presence of well-structured mature collagen fibers (picrosirius staining) was restricted to HHC samples after 28 days; no such collagen fibers were seen in HOC samples. However, collagen expression as shown with anticollagen type I antibodies revealed weak expression of collagen in all samples, with the greatest expression in HOC and HHC samples after 28 days of development (Fig. 3; Table 1). The analysis of samples with

FIGURE 2. Histochemical analysis of proteoglycans in control and bioengineered human corneas with Alcian blue staining. (A–C) HOC developed by tissue engineering and kept in vitro for 7, 14, and 28 days, respectively. (D–F) HHC developed by tissue engineering and kept in vitro for 7, 14, and 28 days, respectively. The human native cornea control is shown on the left. Scale bars: 100 μm.

FIGURE 3. Detection of collagen fibers and cells showing positive expression of vimentin in the stromal compartment of control and bioengineered human corneas. Top: Histochemical analysis of collagen type I in control and bioengineered human corneas with picrosirius red staining. Immunofluorescent staining for collagen I is presented as images (in inserts) taken at higher magnification. Bottom: Analysis of vimentin expression in control and bioengineered human corneas by immunohistochemistry. HOC developed by tissue engineering and kept in vitro for 7, 14, and 28 days ([A], [B], and [C], respectively, for picrosirius and [G], [H], and [I], respectively, for vimentin). HHC developed by tissue engineering and kept in vitro for 7, 14, and 28 days ([D], [E], and [F], respectively, for picrosirius and [J], [K], and [L], respectively, for vimentin). The human native cornea controls are shown on the left. Scale bars: 100 μm.
Verhoeff and Gomori’s reticulin staining confirmed the lack of elastic and reticular fibers in human corneas, given the negative expression in HOC and HHC samples and control corneas (Table 1). To study the mesenchymal profile of the stromal cells (keratocytes), vimentin expression was analyzed in bioengineered and native samples. The results showed that human keratocytes from HOC and HHC samples retained vimentin expression in a manner similar to human cornea controls (Fig. 3; Table 1).

**Sequential Development of Epithelial Markers in HOC and HHC Samples**

Our analysis of key epithelial markers in the human corneal epithelium (CK 3/12, PKG, ZO-1, and CX 43) revealed that CK 3/12 expression tended to increase from the first week of development in culture, with strong expression in HOC and HHC constructs after 14 and 28 days (Fig. 4; Table 2). Interestingly, the expression of these cytokeratins tended to remain at basal levels in HHC. In addition, CX43 expression was positive in HOC and HHC samples maintained in vitro after different periods of development, with the strongest expression appearing in HHC, particularly after 14 and 28 days (Fig. 4; Table 2). Regarding the expression of the tight junction protein ZO-1, HOC tissues were weakly positive for this marker after all in vitro development periods, whereas in HHC samples the expression of this protein tended to increase with time in culture, and was most intense on day 28 (Fig. 5; Table 2). The analysis of the desmosome protein PKG showed similar expression levels in HOC and HHC at 7 and 14 days, with stronger expression in HOC samples on day 28 (Fig. 5; Table 2).

**Table 2. Analysis of the Expression of the Key Epithelial Markers CK 3/12, PKG, ZO1, and CX43 in Tissue-Engineered Corneas and Native Controls**

<table>
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<tr>
<th>Epithelial Markers</th>
<th>CK3/12</th>
<th>PKG</th>
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HOC generated in vitro by tissue engineering. HHCs were bioengineered samples. Native control indicates normal human native cornea. HOC and HHC samples were analyzed at 7, 14, and 28 days. ++++, strong expression; ++, moderate expression; +, weak expression; ±, very weak expression; −, negative expression.
Analysis of Crystallin Expression in HOC and HHC Samples

Analysis of the main crystallins (CRY-αA, CRY-βB, CRY-βC, CRY-β3, CRY-λ1), key proteins involved in corneal transparency, showed that these proteins were present in both the stroma and the epithelial layer of normal human native corneas. The expression of Cry-αA was very intense in control corneas, particularly in the epithelial layer, and was similar to the expression seen in HOC and HHC samples after 14 days of in vitro development. The expression of Cry-βB was similar in HOC and HHC samples, and expression was strongest in the epithelial layer (Fig. 6; Table 3). This expression pattern resembled control samples at days 7 to 28. The immunofluorescence patterns for Cry-β showed moderately positive expression in HOC and HHC samples in both the epithelial and stromal compartments, and expression tended to resemble that of control corneas after 14 days in culture. Likewise, Cry-λ1 expression was moderately positive in both tissue layers of human control samples, and this expression pattern also was seen in HOC samples at early stages of development (7 and 14 days) and HHC samples after 14 and 28 days of development (Fig. 7; Table 3). Staining for Cry-β3 was weakly positive in human cornea controls, and a similar level of expression was observed in 7-day HOC samples and in all HHC samples. Finally, Cry-λ1 expression was strongly positive in the epithelium of human native corneas and negative in the stromal compartment of controls. Staining also was strongly positive in HOC samples from day 7 to day 28, when the highest level of expression was found in the epithelial component. In contrast, HHC samples on days 14 and 28 showed very weak epithelial signals for this crystalline, and moderate expression in the stroma. Expression in the epithelial layer was diffuse throughout the entire period of in vitro development (Fig. 8; Table 3).

DISCUSSION

Several models of bioengineered human cornea have been described to date, however, these models are normally based on the use of corneal limbus stem cells, and very few have explored the use of alternative cell sources for cornea regeneration. In the present work, we explored the usefulness of HWJSCs to generate a bioengineered human cornea model, and compared this model with an orthotypic artificial human cornea generated with limbal stem cells. Our choice of HWJSCs as an alternative for cornea tissue engineering is based on the accessibility, abundance, and lack of ethical issues regarding these cells, which have previously been shown to transdifferentiate into epithelial cells. Our results showed that the use of HWJSCs allowed the efficient generation of heterotypic corneas, and these corneas were analogous to orthotypic corneas generated with limbal cells. However, the differentiation level in vitro was lower in both HOC and HHC tissues compared with native corneas, suggesting that in
vivo interaction between different cell types is necessary for full cell differentiation and tissue morphogenesis.24,25

Our analysis of the stroma of heterotypic and orthotypic corneas with vimentin immunohistochemistry showed similar stromal cell patterns in both cornea types; the pattern was similar to that seen in control corneas. Staining of the elastic and reticular fibrillar components of the stromal layer revealed that artificial corneas (HOC and HHC) were negative for these stains.

**TABLE 3.** Analysis of the Expression of Crystallins Cry-αA, Cry-αB, Cry-β, Cry-ζ, Cry-βγ3, and Cry-λ1 in the Epithelium (E) and the Stroma (S) in Bioengineered Human Corneas and Native Controls

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<tr>
<th>Crystallins</th>
<th>Cry-αA</th>
<th>Cry-αB</th>
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HOC generated in vitro by tissue engineering. HHCs were bioengineered samples. Native control indicates normal human native cornea. HOC and HHC samples were analyzed at 7, 14, and 28 days. ++++, strong expression; ++, moderate expression; +, weak expression; ±, very weak expression; −, negative expression.
fibers, as were the control native corneas. On the other hand, the expression of collagen type I as determined by immunofluorescence was present in all samples from the first week of culture. Mature collagen fibers marked by picrosirius red staining were detected only in advanced stages of differentiation in artificial corneas, especially in HHC samples, although they were more weakly expressed than in native corneas. This may support the idea that corneas developed in vitro are not fully differentiated; in vivo studies will be needed to determine the role of in vivo signaling between host cells and implanted corneas in corneal differentiation. In addition, the greater presence of mature collagen fibers in HHC than in HOC samples may mean that HWJSCs have the capability to efficiently interact with stromal keratocytes and induce collagen synthesis in a manner different from the mechanism that takes place in the corneal epithelium. Regarding the nonfibrillar components of the stromal layer, the expression of proteoglycans (Alcian blue staining) was weaker than in native corneas. However, HHC samples showed larger amounts of proteoglycans after 28 days of in vitro development, likely because HWJSCs belong to a mucosal embryonic-like connective tissue, and these cells are able to synthesize large amounts of proteoglycans in the umbilical cord. The higher production of both collagen type I and proteoglycans in HHC samples may favor the biomechanical properties of these artificial corneas. In addition, the greater synthesis of proteoglycans may be associated with a more favorable alignment of collagen fibrils, which is a crucial issue in corneal transparency, as previous reports demonstrated that collagen fibrils are separated at specific distances by the influence of proteoglycans. Furthermore, the presence of glycosaminoglycans (PAS staining) was evident in native and tissue-engineered corneas. Together, these results suggest that the development of a bioengineered corneal stroma with all key fibrillar and nonfibrillar components of the ECM and an adequate keratocyte cell population is feasible in vitro, and the results in HHC samples were similar to or even better than those in HOC samples.

Our analysis of typical corneal epithelial markers (CK3/12, PKG, ZO1, and CX43) disclosed the presence of intermediate filaments, desmosomal junctions, tight junctions, and gap junctions in both HOC and HHC samples, especially after longer in vitro development periods; the number of epithelial cell layers was greater in HHC. These results confirm the ability of HWJSCs to differentiate into corneal epithelial-like cells and to form (especially after 14 days of in vitro development) a multistratified epithelial layer potentially able to provide an appropriate barrier. These findings also confirm the hypothesis that HWJSCs can be used for corneal epithelium replacement without the need to obtain corneal epithelium stem cells from the corneal limbus. The efficient differentiation of HWJSCs is probably a consequence of adequate epithelial–keratocyte interaction in a three-dimensional artificial tissue and the use of

![Figure 7](https://example.com/fig7.png)

**Figure 7.** Analysis of the expression of crystallins Cry-β and Cry-ζ in control and bioengineered human corneas. **Top:** Analysis of Cry-β expression in control and bioengineered human corneas by immunohistochemistry. **Bottom:** Analysis of Cry-ζ expression in control and bioengineered human corneas by immunohistochemistry. HOC developed by tissue engineering and kept in vitro for 7, 14, and 28 days ([A], [B], and [C], respectively, for Cry-β and [D], [E], and [F], respectively, for Cry-ζ). HHC developed by tissue engineering and kept in vitro for 7, 14, and 28 days ([G], [H], and [I], respectively, for Cry-β and [J], [K], and [L], respectively, for Cry-ζ). Positive signals are shown in red and all cell nuclei are stained blue. The human native cornea controls are shown on the left. Scale bars: 100 μm.
specific air–liquid culture systems, as previously demonstrated.6,28

Crystallins are involved in remodeling and protecting the cytoskeleton, the inhibition of apoptosis, and resistance to stress, and play an important role in corneal transparency. These proteins act as structural factors that control the cell cycle, cell proliferation, density, and transparency in the cornea by supporting the accumulation of large amounts of crystallins in the corneal epithelial and stromal cells. Furthermore, crystallins play an important role in remodeling and protecting the cytoskeleton, a role that is crucial for reducing light scattering and improving transparency, one of the main issues in cornea regeneration.29 For these reasons, the study of crystallins in artificial corneas contributes to a better characterization of these model corneas for clinical use. Crystallins are expressed in the lens from the beginning of embryonic development, when the expression of Cry-αA is especially abundant. The first crystallin to be expressed during embryonic development is Cry-αB, followed by Cry-αA, which reaches maximum levels at birth and tends to decrease during the first weeks after birth.30

In the present work, we analyzed for the first time a wide range of crystallins in control and bioengineered human corneas. Interestingly, our artificial corneas showed high levels of expression of Cry-αA and Cry-αB, and Cry-αA was the crystallin with the strongest level of expression in both artificial and native corneas. This is consistent with the idea that α-family crystallins are abundant both during corneal development and also in mature, fully differentiated corneas.31 The presence of Cry-αA and Cry-αB in HOC and HHC suggests that three-dimensional systems based in fibrin–agarose scaffolds may behave in a manner similar to native corneas. The second family of crystallins that we analyzed is the Cry-β family. In this connection, it was previously shown that Cry-β expression is important in the control of lens and cornea development, and its expression is always dependent on the previous expression of Cry-αA, which is maximally expressed after birth.32,33 Strikingly, we observed positive expression of Cry-β from day 7 of development in vitro, with an increase at days 14 or 28, suggesting that bioengineered human corneas may attain higher levels of differentiation at 28 days, and that expression of Cry-β may occur sooner in HHC than in HOC cultures. In this connection, it is worth noting that beta-gamma crystallins are typical of mature, well-differentiated eye tissues, including the cornea and lens, and their expression is always preceded by Cry-α and Cry-β family expression. Our results showed that the strongest expression levels in HOC samples tended to appear at days 14 and 28, whereas expression in HHC samples tended to remain constant. This may suggest that the epithelium generated from cornea cells attains greater differentiation and functionality than transdifferentiated epithelium generated from HWJSCs, at least in terms of Cry-β expression at this stage of in vitro development. Finally, we analyzed Cry-ζ and Cry-λ, which have been little studied in

![FIGURE 8. Analysis of the expression of crystallins Cry-βγ3 and Cry-λ1 in control and bioengineered human corneas. Top: Analysis of Cry-βγ3 expression in control and bioengineered human corneas by immunohistochemistry. Bottom: Analysis of Cry-λ1 expression in control and bioengineered human corneas by immunohistochemistry. HOC developed by tissue engineering and kept in vitro for 7, 14, and 28 days ([A], [B], and [C], respectively, for Cry-βγ3 and [G], [H], and [I], respectively, for Cry-λ1). HHC developed by tissue engineering and kept in vitro for 7, 14, and 28 days ([D], [E], and [F], respectively, for Cry-βγ3 and [J], [K], and [L], respectively, for Cry-λ1). Positive signals are shown in red and all cell nuclei are stained blue. The human native cornea controls are shown on the left. Scale bars: 100 μm.](image)
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cornea development, and found that Cry-ζ was constantly expressed by HOC, HHC, and control human corneas, with minor differences between the two types of artificial cornea. Notably, both HOC and HHC samples were able to express Cry-ζ in a pattern that was very similar to native corneas. However, Cry-λ1 tended to increase in HOC, thus mimicking the expression pattern in control corneas, whereas HHC samples had consistently low levels of this crystallin. Although the exact function of Cry-λ1 in the cornea is not well understood, the lower expression of Cry-λ1 in HHC samples compared with controls and HOC samples suggests that HWJSCs may require longer development times or even in vivo grafting to express high amounts of this crystallin.

In summary, the use of HWJSCs to develop functional substitutes for the human cornea is an innovative strategy based on transdifferentiation techniques with umbilical cord mesenchymal stem cells. Under appropriate conditions, HWJSCs were able to reproduce the human cornea epithelium in vitro, with adequate differentiation levels in the cornea stroma and epithelium. Although the expression of the crystallins Cry-β3 and Cry-λ1 was weaker in our biomimetic samples than in normal cornea samples, it should be noted that the role of these proteins in corneal development has not been elucidated to date. Although our results await confirmation at the protein, gene expression, and functional levels, including ex vivo and in vivo analyses, they support the usefulness of HWJSCs in cornea tissue engineering. Previous reports from our research group documented the efficiency and potential usefulness of orthotopic corneas. However, in this study, we used enhanced cell-based strategies with alternative cell sources that may reduce the culture time required to obtain a viable cornea substitute and may obviate some of the problems associated with harvesting limbal stem cells. These findings can support translational progress toward regenerative ophthalmology and may offer more rapid and effective solutions for patients.

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