The Influence of Stromal Contraction in a Wound Model System on Corneal Epithelial Stratification

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PURPOSE. The healing process of some corneal wounds involves closure by stromal contraction and the renewal of the stratified epithelium. In wound gape injury such stromal contraction occurs with epithelial stratification. In previous in vitro studies of noncontracted and contracted corneal fibroblast-seeded collagen gels (FSCGs) it was shown that initiation of wound contraction by the myofibroblast phenotype (present within the wounded stroma) was dependent on vitronectin and/or fibronectin. This study considers one aspect of the epithelial–stromal interaction that occurs during wounding. The stratification of corneal epithelial cells on noncontracted and contracted corneal FSCGs was compared.

METHODS. Dissociated bovine corneal epithelial cells were seeded on noncontracted and contracted corneal FSCGs, and these assemblies were cultured for 7 days. The epithelium that formed was evaluated using laser confocal microscopy and immunohistochemical markers directed against cytokeratin 3, desmosplakin I and II, integrin α6 subunit, laminin, and collagen VII. The characteristics of the epithelium were compared with stromal carriers comprised of dissociated bovine corneal epithelial cells seeded on intact stroma and basement membrane (stromal carrier biopsies).

RESULTS. The stratified epithelium that developed on contracted corneal fibroblast-seeded collagen gels was similar to that formed on stromal carriers, whereas nonstratified epithelium formed on noncontracted FSCGs.

CONCLUSIONS. These studies showed that the contracted state of fibroblast-seeded gels enhanced the development of well-organized, stratified corneal epithelium. (Invest Ophthalmol Vis Sci. 2001;42:81–89)

Many cellular events occur during corneal wound healing to ensure that the tissue can resume its normal function in light refraction. When both the epithelium and the underlying stroma are wounded, epithelial cells and corneal fibroblasts (CFs) are directly involved in the repair process. In the wounded stromal tissue, CFs are activated and transform into the myofibroblast (MF) phenotype. Previous studies suggest that the presence of CFs/MFs influence epithelial stratification.

The work presented in this article models the type of corneal wound in which both epithelium and stromal damage had occurred. The healing process that follows involves the interaction between corneal epithelial cells and the underlying CFs. The present study focuses on the interaction between corneal epithelial cells and the underlying stromal fibroblasts.

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Previous in vivo studies that examined the healing of gape wounds reported a sequence of events that occurred over a period of several weeks. As the wound bed was reepithelialized, CFs adjacent to the wound disappeared. As the newly migrated epithelium stratified, the CFs repopulated the wounded area and transformed into MFs to effect wound closure by contraction of stromal tissue under repair. Finally, the overlying epithelium was restored to full thickness, and wound healing was completed. In the case of minor scrape wounds, the stroma and basement membrane remain intact. In this study, the wound healing time is significantly reduced when compared with gape wound restoration because no stromal repair is required.

To monitor the development of a stratified epithelium formed in this study, morphologic and biochemical markers were used to demonstrate the degree of corneal epithelial stratification and tissue integrity. The term stratification in the present study is used to describe the process by which corneal epithelial cells differentiate and develop distinctive sublayers that can be identified by morphologic and biochemical markers. This definition differs from the term differentiation, which refers to the process of epithelial cell programming for the expression of a characteristic phenotype.

The markers used in this study target epithelial cell–cell junctions, specific cytokeratins, cell–matrix interactions and the presence of basement membrane components. Cell–cell junctions are an important feature of stratified epithelia, indicating functional communication within the tissue and suggesting the existence of a functional barrier. Desmosplakin I and II are components of the cell–cell junctions known as desmosomes. These cell–cell junctions are a feature of the suprabasal layers of corneal epithelium and are significantly less in number in the basal layer of intact corneal epithelial tissue. The cytokeratins are characteristic components of the corneal epithelial cell cytoskeleton and can be used to identify epithelial cell differentiation status. Cytokeratin expression is reported to be regulated by the extracellular environment as well as epithelial–stromal interactions. One of the cytokeratins, cytokeratin 3, is present only in fully differentiated corneal epithelial cells and is therefore absent from the limbal basal cell layer. The presence of cell–matrix interactions (hemidesmosomes) at the basal aspect of the basal cells confirms the attachment of stratified epithelium to the underlying stroma via the basement membrane. One component of these junctions is the α6 integrin subunit. A characteristic feature of a stratified corneal epithelium is the presence of a basement membrane largely synthesized by the corneal epithelial cells. Two components of the basement membrane that can be used to monitor its presence include laminin and collagen VII.

In the present study we examine the role of gel contraction by MFs on the development of a stratified epithelium using a model based on fibroblast-seeded collagen gels (FSCGs). In previous studies using FSCGs, MFs were found to be dependent on fibronectin and/or vitronectin to initiate the contraction process, a process important in the healing of corneal gape wounds. This FSCG-based model system allows control over the stromal contraction process, so that the stromal wound bed can be maintained in either a contracted or noncontracted state. Data presented here demonstrates for the
first time that the contractile nature of the wound bed may affect the stratification of corneal epithelial cells.

**Materials and Methods**

The Stromal Carrier Biopsy Model: Control of Epithelial Stratification In Vitro

This model was established by seeding dissociated bovine corneal epithelial cells onto stromal carriers. Stromal carrier biopsies were prepared as follows: the endothelium, part of the posterior stroma, and the epithelium were mechanically removed from corneas excised from freshly slaughtered steers. The original epithelium was removed by scraping with a blunt scalpel edge, exposing an intact basement membrane. Ten-millimeter-diameter biopsy buttons were then punched from this tissue to form what is termed “stromal carrier biopsies.” To obtain minimally differentiated cells, freshly harvested bovine corneal epithelial cells were cultured in low extracellular calcium (0.09 mM), keratinocyte serum-free medium (KSFM; Gibco BRL Technologies, Grand Island, NY) in 25-cm² tissue culture polystyrene flasks precoated with human collagen type IV (2 ml of 40 µg/ml per flask; Becton Dickinson, Bedford, MA). These primary cells were then seeded, at a density of 1.0 × 10⁵ cells/ml, onto the stromal carriers, and the resulting epithelial–stromal assemblies were submerged in DMEM/F12 + 10% (v/v) fetal calf serum (FCS) and maintained at 37°C in 5% CO₂. After 24 hours, the assemblies were raised to the air–liquid interface, cultured under these conditions for 20 days, with media changes every 2 to 3 days. Twenty days was chosen as the time point because preliminary experiments showed that myofibroblast transformation had occurred in the stromal tissue of the carriers by 15 days under these conditions (data not shown). At days 0, 7, and 20, assemblies were removed from culture and embedded in Tissue-Tek OCT Compound (Sakura Finetek, Torrance, CA), snap-frozen, and sectioned at 6-µm thickness onto gelatin-coated slides. Cryostat sections were either stained with hematoxylin and eosin or stored at −70°C for immunohistochemistry.

FSCGs: Epithelial Assemblies

FSCGs were prepared by seeding collagen gels with passage two corneal fibroblasts in KSFM + 2% (v/v) FCS or KSFM + 2% (v/v) vitronectin- and fibronectin-depleted FCS (DD FCS), respectively. These collagen-based wound model systems were cultured at 37°C in a 5% CO₂-air atmosphere for 20 days, after which time gels had either contracted (+FCS) or remained noncontracted (+DD FCS). These contracted and noncontracted FSCGs were collagen IV coated (1.5 ml of 40 µg/ml per well) and then seeded at a density of 1.0 × 10⁶ cells/ml with corneal epithelial cells in DMEM/F12 + 10% (v/v) FCS where both fibronectin and vitronectin were present. These corneal epithelial cells were from the same culture as those seeded onto stromal carrier biopsies (previously described). Collagen IV-coated (1.5 ml of 40 µg/ml per well) acellular gels (no corneal fibroblasts), seeded with corneal epithelial cells, were constructed as negative controls. All gels seeded with corneal epithelial cells were placed on semipermeable transwell insert membranes (Corning Costar Corporation, Acton, MA) to allow access of nutrients from the basal side of the cultures. These were submerged in DMEM/F12 + 10% (v/v) FCS culture medium at 37°C in 5% CO₂. After 24 hours, the assemblies were raised to the air–liquid interface, maintained in these conditions for 7 days, then embedded in Tissue-Tek OCT Compound (Sakura Finetek), snap-frozen, and sectioned at 6-µm thickness onto gelatin-coated slides. Cryostat sections were stored at −70°C for immunohistochemistry.

**Hematoxylin and Eosin Staining**

Air-dried frozen sections were fixed by immersion in formalin-acetic alcohol (10%:85%:5% v/v) solution for 30 seconds. Slides were stained with hematoxylin and eosin, mounted in Gurr’s Mounting Medium (BDH, Germany) and viewed using a Leica DMLB light microscope (Leica, Germany).

**Immunohistochemistry**

Sections of epithelial–stromal assemblies were blocked with 2% (w/v) bovine serum albumin in phosphate-buffered saline (PBS) for 1 hour. Thereafter, sections were incubated with primary monoclonal antibodies at a 1:20 dilution in PBS for 60 minutes at room temperature. Primary antibodies used included cytokeratin 3 (AE5; ICG Biomedical, Aurora, OH), desmoplakin I and II (Chemicon, Temecula, CA), α-6 integrin (Chemicon), laminin 1 (CSIRO, Sydney, Australia), collagen type VII (Chemicon), or α-smooth muscle actin (α-SMA; Sigma, St. Louis, MO). Normal mouse, rat, rabbit, or human sera (CSIRO) were used in place of the primary antibody as a negative control. Sections were washed three times with PBS and stained with a species-appropriate FITC-conjugated secondary antibody (DAKO, Copenhagen, Denmark) for 60 minutes at room temperature and in the dark. Sections were washed three times in PBS and mounted in Fluorosave (Calbiochem, La Jolla, CA). Sections were viewed using laser confocal microscopy with a Leica TCS-40 scanning laser confocal microscope fitted with a 100× objective and a krypton/argon mixed gas laser with an excitation wavelength of 494 nm.

**Results**

**Morphology**

Morphologic examination of hematoxylin and eosin-stained sections of all assemblies compared the number of epithelial cell layers.
cell layers and sublayers to determine the degree of stratification of corneal epithelium. Cell layers were counted in six fields of view at a magnification of ×1000. The mean values of these counts are summarized in Table 1. Corneal epithelial cells seeded onto the noncontracted FSCGs showed a loosely organized, multilayered epithelium after 7 days (see Figs. 2B to 6B). In contrast, the epithelium formed when corneal epithelial cells were seeded onto the contracted FSCGs showed greater epithelial stratification and integrity of tissue after the same period (see Figs. 2A to 6A). Acellular collagen gels (no fibroblasts) seeded with corneal epithelial cells showed little or no epithelial formation (data not shown). The stromal carrier biopsies with intact basement membrane (used to test epithelial stratification) seeded with corneal epithelial cells showed the formation of stratified epithelial tissue within 20 days (Fig. 1C) that was similar to intact tissue (Fig. 1B). This was characterized by sublayers comprised of cuboidal basal cells, with overlying polygonal wing cells and squamous cells together forming the suprabasal layers. No epithelium formed on the negative controls of stromal carrier biopsies (Fig. 1A). Preliminary experiments (data not shown) involving the stromal carrier biopsies seeded with epithelial cells for 7 days showed only three to four layers of nonstratified epithelium. In addition, no difference in fibroblast distribution was observed between intact tissue, stromal carriers, and noncontracted and contracted seeded FSCGs (data not shown).

Immunohistochemistry

Immunohistochemistry was performed to identify specific features of a stratified epithelium. Desmoplakin and cytokeratin 3 were expressed in the suprabasal epithelial layers formed on contracted FSCGs (Figs. 2A and 3A). In contrast, corneal epithelium formed on noncontracted FSCGs showed intense staining of desmoplakin and cytokeratin 3 among all layers of the epithelium (Figs. 2B and 3B). The epithelium in intact tissue (Figs. 2C and 3C) and that formed on stromal carrier biopsies containing basement membrane (Figs. 2D and 3D) showed only suprabasal expression of desmoplakin and cytokeratin 3, as observed on contracted FSCGs (Figs. 2A and 3A). These results are summarized in Table 1.

To evaluate the integrity of the corneal epithelium, hemidesmosome (α-6-integrin) and basement membrane components (laminin and collagen VII) were compared. These results are summarized in Table 1. Light punctate expression of α-6 integrin was present in the basal cell layer of epithelium formed on contracted FSCGs (Fig. 4A). Only trace amounts of α-6 integrin were expressed where corneal epithelial cells were seeded onto noncontracted FSCGs (Fig. 4B). In the intact corneal epithelium, continuous linear expression of α-6 integrin was seen in corneal epithelial cells localized to the basement membrane zone of the basal cell layer (Fig. 4C). α-6 integrin staining showed a discontinuous and moderate pattern of expression in the epithelium formed on stromal carrier biopsies (Figs. 4D). The α-6 integrin observed on FSCGs and stromal carriers biopsies were newly synthesized as preliminary immunohistochemical staining revealed an absence of α-6 integrin components on the FSCGs and debrided stromal carrier biopsies before epithelial seeding (data not shown).

Minimal expression of the basement membrane components, laminin and collagen VII, were detected where corneal epithelial cells were seeded onto contracted FSCGs after 7 days (Figs. 5A and 6A). These basement membrane components were absent where corneal epithelial cells were seeded onto noncontracted FSCGs (Figs. 5B and 6B). Sections of intact bovine corneas (Figs. 5C and 6C) showed positive expression of both laminin and collagen VII components that was continuous and localized to the basement membrane.

The transformation of CFs into MFs was monitored using α-SMA expression. MFs were identified in both the contracted

![Figure 1](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933218/ on 06/24/2017)

**Figure 1.** Bovine corneal epithelial cells seeded onto freshly prepared stromal carriers with intact basement membrane. Bar, 50 μm. (A) Denuded stroma (no corneal epithelial cells). (B) Intact limbal bovine cornea. (C) Day 20 of culture. Magnification, ×40. Hematoxylin and eosin-stained frozen sections.
DISCUSSION

The data presented in this article used an in vitro model system to show that the contracted state of stromal equivalents enhanced the development of stratified corneal epithelium. The term “stratified epithelium” refers to epithelial tissue comprised of a basal layer of columnar epithelial cells in contact with the basement membrane and layers of suprabasal layers containing flattened wing and squamous cells. Hence, two distinct sublayers (basal and suprabasal) were identified. This is clearly different from epithelial tissue consisting of multiple layers characterized by disorganization and a lack of distinguishable sublayers associated with a stratified corneal epithelium. The level of epithelial stratification observed on the contracted FSCGs was similar to that found on the stromal carrier biopsies, in which dissociated corneal epithelial cells were cultured on stromal carriers with an intact original basement membrane. Disorganized multilayers of epithelial cells formed when similarly prepared corneal epithelial cells were seeded onto intact stromal carriers, in which dissociated corneal epithelial cells were cultured on stromal carriers with an intact original basement membrane. Disorganized multilayers of epithelial cells formed when similarly prepared corneal epithelial cells were seeded onto noncontracted FSCGs. Overall, the epithelium formed on the contracted FSCGs was superior in terms of the number of cell layers and the degree of stratification, compared with that formed on noncontracted FSCGs.

When corneal epithelial cells were seeded onto the stromal carriers (a control for epithelial formation), morphologic and immunohistochemical examination of the resultant epithelium showed the formation of stratified tissue. Despite this, a slightly lower number of cell layers \( n = 8 \sim 9 \) was observed compared with intact tissue \( n = 10 \sim 12 \). The reasons for this may be threefold. First, the number of cell layers may have increased if the culture time was extended beyond 20 days. The second reason may relate to the absence of an endothelial layer. The corneal endothelium has a major function of controlling the fluid flow into the cornea, and it may also serve to maintain the basement membrane components; hence, its absence may have limited the number of epithelial layers formed. Third, the diffusion of other factors from surrounding tissue, such as those relating to a functional nervous system, may have interrupted or prevented epithelial development. Despite the lesser number of epithelial layers (compared with intact tissue), the stromal carrier biopsies provided a good control, which confirmed that dissociated corneal epithelial cells are capable of forming stratified epithelium in these circumstances. This was evident from a pattern of expression of desmosomes (cell-cell), cytokeratin 3, α-6 integrin (hemidesmosome component), and laminin/collagen VII (basement membrane components) similar to that found in intact tissue.

Corneal epithelial cells seeded onto contracted FSCGs developed a stratified epithelium that was similar to that formed on stromal carrier biopsies, compared with the noncontracted FSCGs. This was evident by the formation of six to nine organized epithelial layers. Characteristic differences between the basal and suprabasal sublayers were confirmed by positive staining for both cytokeratin 3 and desmoplakin I and II only in the suprabasal layers of epithelial tissue that formed on the contracted FSCG. This expression of cytokeratin 3 and desmoplakins I and II was similar to that observed in stromal carrier biopsies compared with noncontracted FSCGs. In contrast, the
dissociated corneal epithelial cells seeded onto noncontracted FSCGs formed less organized, multilayered epithelial tissue. In this case, only three to six epithelial layers were observed with no identifiable sublayer formation (pertaining to stratified epithelium). Additionally, all cells that stained positive for cyto-keratin 3 and desmoplakin I and II revealed the presence of desmosomes between the cells in all layers.

Light, punctate expression of α-6 integrin was evident at the basal aspect of the epithelial cells, indicating the start of polarized deposition of this protein, which is a component of the cell–matrix junctions (hemidesmosomes) of a stratified epithelium. The punctate nature of the staining patterns for these components may have been related to the short culture period (7 days) of these assemblies. Indeed, one corneal explant study showed that deposition of laminin and collagen VII increased between day 7 and day 14 when cultured on contracted FSCGs.36 Data from the present study suggests that the contracted FSCGs supported earlier polarized deposition of laminin and collagen VII, when compared with the expression of these proteins in the noncontracted FSCGs. In fact, the discontinuous deposition of these components seen on the contracted FSCGs was similar to that reported for the appearance of basement membrane 1 month after a stromal gape wound.37 In the noncontracted FSCGs, the expression of α-6 integrin was weaker than that detected in the contracted FSCGs.

Epithelia that formed on the noncontracted and contracted FSCGs showed less intense staining of α-6 integrin, laminin I, and collagen VII than epithelial cells that formed on the stromal carrier biopsies. The reason for this reduced expression may be explained by the significant difference in culture time between the two experiments. Dissociated corneal epithelial cells were seeded onto prepared noncontracted and contracted FSCGs and maintained in culture for 7 days, whereas those seeded onto stromal carrier biopsies were maintained for 20 days. The difference in culture time was due to the fact that all FSCGs begin to initiate contraction beyond 7 days if intact FCS is added. Hence, noncontracted and contracted FSCGs were cultured with corneal epithelial cells for only 7 days; otherwise, the noncontracted FSCGs also would have become contracted. Intact FCS had to be added during this time to maintain corneal epithelial cells in a viable state, as demonstrated in other bovine corneal equivalent studies.38 Despite the reduced culture time of 7 days, corneal epithelial cells seeded onto contracted FSCGs still formed stratified epithelium that could be compared with stromal carriers.

Data showed that the presence of MFs alone did not enhance epithelial stratification. Both the noncontracted and contracted FSCGs showed positive expression of α-SMA, indicating the presence of MFs. We assume that the presence of TGF-β in the intact FCS (added with the corneal epithelial cells) and the fact that the FSCGs were seeded at a density that maintains a certain level of cell–cell contact resulted in MF expression. These two factors have been reported to control MF expression.39–41 As a result, the MF phenotypic expression detected in both the noncontracted and contracted wound model systems was the same, thus supporting the view that contraction itself was the reason for differences in epithelial formation.

It is commonly reported that stromal contraction is a necessary process for the repair of a wound gape injury,7,20 yet there is no evidence to show how this event directly affects corneal epithelial stratification. This question is pertinent when considering epithelial–stromal interactions in the event of repair of gape wounds. This study is the first to compare the difference in epithelial response between a contracted and

**Figure 3.** Cytokeratin 3 (AE5) expression in bovine corneal epithelial cells seeded onto stromal carriers and FSCGs. Bar, 50 μm. (A) Contracted FSCG seeded with corneal epithelial cells (day 7). (B) Noncontracted FSCG seeded with corneal epithelial cells (day 7). (C) Intact limbal bovine cornea. (D) Bovine corneal epithelial cells seeded onto stromal carrier biopsies with basement membrane intact (day 20). Magnification, ×40. Immunostained transverse frozen sections.
noncontracted FSCG where MFs are present in both systems. Previous studies have shown the formation of a stratified epithelium when corneal epithelial cells were seeded onto FSCGs. Although two of these studies involved the use of contracted FSCGs, one study achieved epithelial stratification on noncontracted FSCGs. This result could be a species specific difference or could relate to the inclusion of an endothelial layer to the system. The presence of an endothelial layer may have allowed the build-up of basement membrane components and controlled the metabolite flow rate. Although there may be a role for the endothelium in corneal epithelial stratification, the endothelial layer was not included in the present study to isolate epithelial–stromal interaction.

Why and how contracted FSCGs enhance epithelial stratification is open to speculation. We consider three possible explanations. First, those FSCGs that have contracted present a different mechanical structure to the overlying epithelium than those FSCGs that are noncontracted. As a result, the collagen in the contracted state provides a denser concentration of fibrils than those in the noncontracted collagen, which may be favorable for the stratification of overlying basal cells. Second, the contracted FSCG may provide epithelial cells with a different topographical surface that enhances stratification compared with a noncontracted FSCG. Third, although the transformation of CFs to MFs is essential for the contraction process, the activity of MFs after contraction may involve cell signaling via trophic factor(s) that affects the overlying epithelium. In light of the third suggestion, the endothelium may also present trophic factor(s) and/or provide a “seal” at the base of the equivalent by which to accumulate trophic factor(s). As a result, a contracted FSCG contributes to epithelial stratification by presenting a high concentration of trophic factor(s) through direct contact with the epithelium. A noncontracted stroma without an endothelial seal contains a comparatively lower level of trophic factors because the MFs present have failed to initiate the contraction process. Speculation as to whether the use of double-depleted serum used to specifically prepare noncontracted FSCGs contributed to the differences in the epithelium formed is questionable, although it is believed to be highly unlikely. This is based on the fact that when epithelial cells were seeded onto these constructs, intact FCS was present in both cases, hence equilibrating any deficiency.

Certainly, it is likely that MFs (after initiating the contraction process) release trophic factor(s) that affect the overlying epithelium and enhance stratification. The literature suggests that fibroblasts assist with basement membrane deposition in a process involving the release of trophic factor(s). In addition, Jester et al. and Zieske et al. have observed that fibroblasts seeded into a stromal component of an assembled corneal equivalent migrated toward the epithelial layer. This agrees with in vivo reports from Garana et al. and Jester et al. (using feline and rabbit tissue, respectively) where after epithelial coverage of a wound bed, resident fibroblasts migrate back to the wound site and take part in the contraction process that follows. MFs were present in wounded stromal tissue with concurrent deposition of basement membrane components, and epithelium was renewed to full thickness.

Epithelial stratification may also be enhanced by the presence of metalloproteinases (MMPs). MMPs have been reported to be expressed during the wound healing of the stroma and
**FIGURE 5.** Laminin I expression in corneal epithelial cells seeded onto stromal carriers and FSCGs. Bar, 50 μm. (A) Contracted FSCG seeded with corneal epithelial cells (7 days). (B) Noncontracted FSCG seeded with corneal epithelial cells (7 days). (C) Intact limbal bovine cornea. Magnification, ×40. Immunostained transverse frozen sections.

**FIGURE 6.** Collagen VII expression in corneal epithelial cells seeded onto stromal carriers and FSCGs. Bar, 50 μm. (A) Contracted FSCG seeded with corneal epithelial cells (7 days). (B) Noncontracted FSCG seeded with corneal epithelial cells (7 days). (C) Intact limbal bovine cornea. Magnification, ×40. Immunostained transverse frozen sections.
corneal epithelium within 1 day of injury. Future work endeavoring to observe any differences in the expression of MMPs between noncontracted and contracted FSCGs may help explain why contracted FSCGs enhance the development of a stratified epithelium.

Together, our results demonstrated that contracted FSCGs enhanced the ability of primary corneal epithelial cells to form a stratified epithelium compared with that formed on a noncontracted FSCGs within a 7-day culture period. The stratified epithelium formed on contracted FSCGs included the development of sublayers, including a basal cell layer (lacking desmoplakin I and II and cytokeratin 5), the synthesis and deposition of polarized basement membrane components (laminin and collagen VII) and the hemidesmosomal component, α-6 integrin associated with long-term adhesion. These findings suggest that the contracted FSCGs present the overlying corneal epithelial cells with a mechanical and/or topographical and/or biological factor(s) that enhanced the stratification of corneal epithelial cells. The comparison of a noncontracted versus contracted stromal equivalent recombined with basal corneal epithelial cells provides a better understanding of epithelial-stromal interaction and corneal epithelial development during wound healing.

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