Supplemental Section S1: Details on Device Fabrication

Additional Details of the basal microfluidic network construction (Figure 1A). After spin coating PDMS over a silanized mold (step 1), the layer was heated at 80°C for 4 minutes, allowing partial curing. Two featureless PDMS blocks (Support Layer), were then cut and partially bonded to the thin film by heating the components for 8 minutes (step 2, 80°C). The smaller of these blocks contained the access ports for the basal microfluidic network, while the larger block served as a temporary support for the thin PDMS film during fabrication. Access holes for cell culture were punched through this thin PDMS film covering the basal microfluidic network (step 3), prior to bonding with a glass coverslip using oxygen plasma treatment (step 4). The resulting permanent plasma bond was stronger than the partially cured interface between the thin PDMS film and the supporting block, so that the latter could easily be removed (step 5). This uncovered the thin PDMS film containing the microfluidic channels and left the holes punched into the culture chambers exposed for sealing with the collagen vitrigel material.

Each culture unit within the basal microfludic network contained a previously described shear protection wall, dubbed "C"-shaped ring.[2] The "C"-shaped culture chambers[2] were created using two layer photolithography with both the 1 mm diameter interior chambers and 50 μm wide outer channels at a 75 μm height, while the high fluidic resistance "C"-shaped ring remained at the 2 μm height. In our application, this protective wall sheilds both the CV and cells from excessive forces during an enzymatic etching step. In addition, the opening in the protective wall provides a controlled method for loading stromal cells to create bi-layered tissue patches. In the final assembly, each basal culture unit is covered by the CV and an apical culture well.

Additional Details of the apical vacuum network construciton (Figure 1B). Figure 1B shows a schematic of the fabrication steps for this top layer of the device, which contains both the apical culture chambers and a vacuum network. First, PDMS for the apical plate is cast over the mold (step 1) and then holes are punched for both access to the vacuum network and creation of the apical wells (step 2). A ~100 μm PDMS wall seperates the vacuum network and the wells, thus ensuring a completely sealed culture chamber.

Additional Details of the final device assembly and CV integration (Figure 1C). Figure 1C shows final integration of the CV and device assembly. Upon drying the CV onto the basl microfluidic network (step 1 and 2), the assembled device now contains arrays of exposed collagen vitrigel patches of 500 μm diameter with fluidic access from the top (apical culture wells) and bottom (microfluidic channels, step 3), which can be used to rehydrate those selective portions of the CV (step 4). During overnight incubation a spring-loaded, 10 mL syringe applied sufficient vacuum to hold the CV and maintain leakage-free, continuous microfluidic perfusion. Apical vacuum networks were also fabricated containing microchannels instead of punched culture wells, and used to test integration of the CV between two opposing microfluidic networks (see supplemental figure S3).

Additional Details of the Bilayered Cell Culture. Each channel within the device was seeded individually resulting in a uniform loading rate as shown in figure 2. After seeding cells in the basal microfluidic "C"-shaped chamber, separate inlets to the apical and basal surfaces of the CV allow two different media types to be used to bathe both the apical epithelium and basal keratocytes. However, bulk culture studies showed little difference in growth rates or morphology for keratocytes cultured in keratinocte media (data not shown); therefore the supplemeted keratinocyte media was used in both the apical wells and microfluidic perfusion channels.

Supplemental Section S2: Details and results from the enzymatic etch process.

During the enzymatic etch epithelial cells surrounding the 500 μm access hole were protected by the PDMS channel, resulting in selective thinning of the CV within the central portion of the apical epithelial cell sheet. This protected the surrounding portions of the CV from the enzyme, minimizing the chance for a catastrophic rupture in the membrane. Still, as the supporting CV was thinned due to enzymatic degradation, this overlying cell layer became more susceptible to damage. Figure S1A shows a series of images of epithelial cells grown under different growth conditions with (iv.-vi.) or without (i.-iii.) sacrificial etching. Outlets to the microfluidic channels within these devices were plugged and inlets were lifted to a specified height to apply a back pressure to the epithelial cell layer (0.5 – 7 inch H_2O). In the non-etched devices, protection from the CV resulted in an increased resistance to cell damage; however, cell death occurred at relatively low pressures within the etched devices, as the epithelial layer was left exposed to a greater portion of the hydrostatic pressure. This difference is apparent in the figure as etched devices show an increased susceptibility to cellular damage, completely losing viability after 24 hours of culture at 3 inch H_2O

compared to 7 inch H_2O in the non-etched wells. However, within this limited pressure range cells remained viable over the entire range of media flow rates, upon opening of the microchannel outlet (0.05 nL/min – 0.31 nL/min; figure S1B).

Supplemental Section S3: Details on the laser-induced fluorescence system

Briefly, a 488 argon laser (Melles Griot) was used to excite the fluorescein dye after deflection by a dichroic mirror (505DCXR, Chroma Technologies) and focusing using an oil immersion apochromatic objective (100X, NA 1.3, Olympus). The laser detection volume was positioned at a 10 μm depth within the microfluidic channel using a computer controlled, high resolution piezoelectric flexure stage (P-517.3CL, PI). The fluorescence emitted by the sample was collected using the same objective and passed through a pinhole (Melles Griot) and bandpass filter (520DF40, Chroma Technologeis). Data acquisition was performed using a single-photon avalanche photodiode (APD) controlled by a digital counter (National Instruments) and custom Labview software. Laser power was adjusted using neutral density filters to around 0.03 mW at the back aperature of the objective during all experiments. Fluorescence was measured as the average, dead time corrected photons per millisecond in separate 10 second traces. Data from each sample was presented as the relative fluorescence at the measurement point normalized to equilibrium fluorescence measurements taken from each device. Devices were returned to an incubator between measurements and before taking equilibrium fluorecence measurements after waiting at least 3 hours.

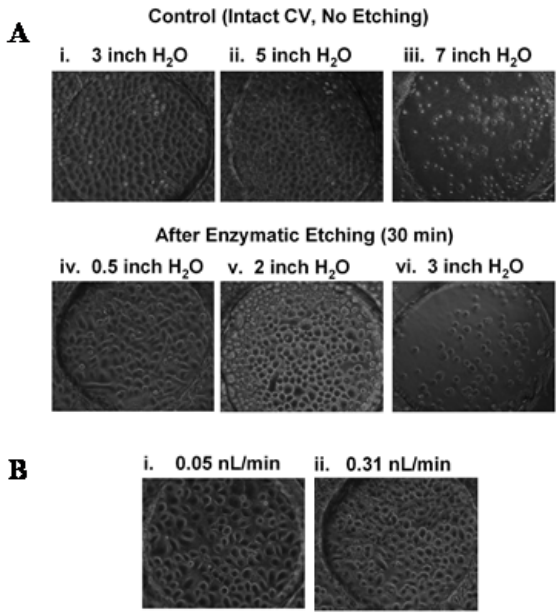
Supplemental Section S4: Details and results on CV vacuum integration

Figure S2 shows leakage-free integration of the CV in devices using the vacuum method. A fully assembled device with apical wells is shown in figure S2A with fluidic seal integrity maintained by keeping all functional features to within 200 μm of the vacuum network and leaving at least a 100 μm PDMS wall between that feature and the vacuum channels (criss-crossing mesh in the inset picture). Figure S2B shows the gross structural properties of the CV (i)[31] and native corneal (ii) . Conventional gels applied in microfluidic systems often lack the mechanical stability present in native tissues. For instance, use of molded, acid solubilized collagen[14] results in comparatively low densities of collagen fibrils.[43] In contrast, the TEM image in S2B shows the characteristic cross-striations of fibrillar collagen within the CV, which has been shown to have a denser reticular architecture than standard collagen gels.[43] In addition, the vitrification process results in a transparent gel (figure S2C) that enables the device to retain the transparency associated with PDMS-based culture platforms.

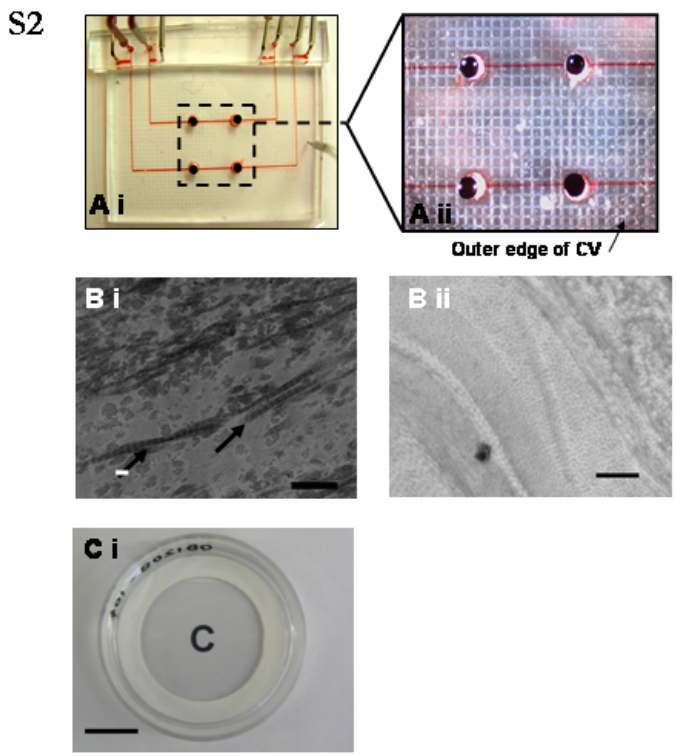
Figure S3A shows an alternate device schematic used to optimize vacuum integration (i). In this design, the central, coiled microfluidic channel was surrounded by a meshed vacuum network. The device was placed upon dried CV and upon application of vacuum the meshed network provided a pulling force to keep the microfluidic chambers flush against the membrane. Three different vacuum channel dimensions and three wall sizes (50, 100 and 200 μm widths) were used, and scored in terms of the number of broken seals or leaks between channels. Figure S3A ii. and iii. show results from the best performing vacuum network (100 μm seperation wall with 200 μm microchannels, 4 out of 5 working leakage-free devices). The 50 μm features failed (1 out of 5 working devies) due to the ability of small debry to bridge the narrow walls between channels. However, both 100 and 200 μm seperation walls gave equal results, thus the denser 100 μm feature were used to all subsequent experiments.

Figure S3B shows selective rehydration of a 500 μm exposed CV patch sandwiched between the two PDMS layers (i). Only the portion of the CV exposed to the underlying microchannel rehydrates, as the majority of the collagen membrane remains protected under the vacuum network. This is demonstrated by the selective uptake of fluorescent tracer dye (Texas Red-conjugated BSA) within the punched access hole. Such molecular uptake stands in sharp contrast to diffusive transport within microdevices fabricated using conventional materials, such as the porous polymer membranes used in current barrier tissue models[27-29], which do not maintain the biomolecular affinity or permeability of a natural protein gel.[43] Exposing the CV patch to a second tracer dye (fluorescein in green, figure S3B ii) via an opposing microchannel resulted in dual-uptake of both dyes at the access hole (overlay in yellow, figure S3B iii). This alternate device geometry shows that the apical wells can be replaced with microfluidics, enabled microfluidic culture on both sides of the CV.

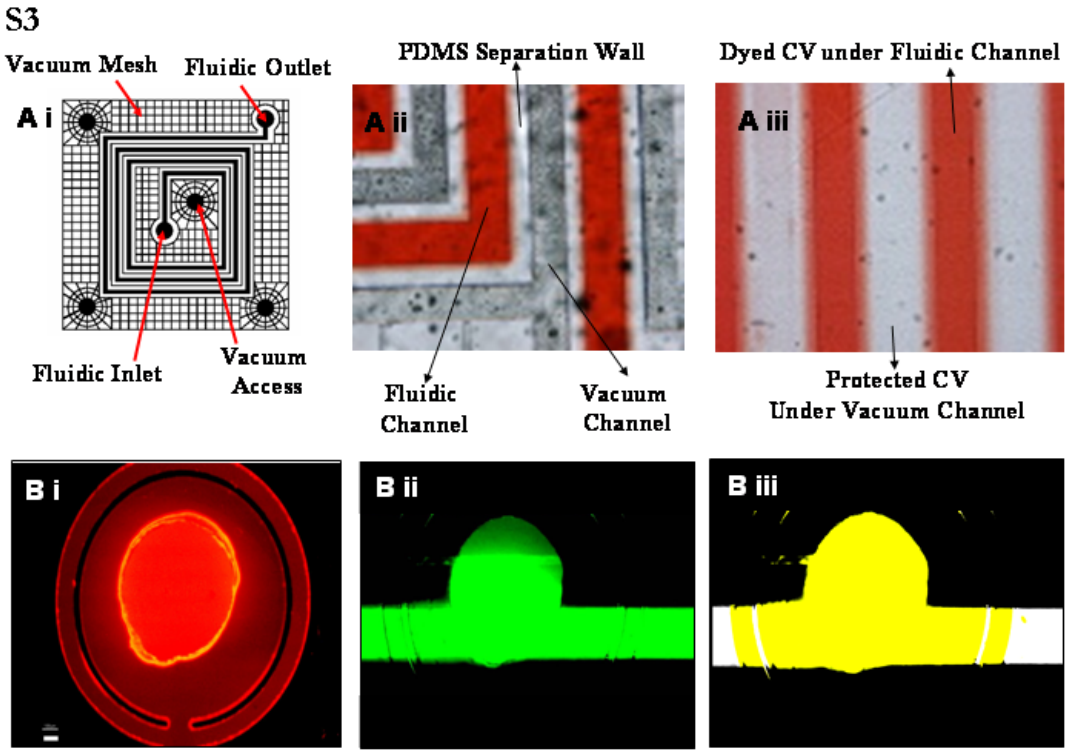
S1



Supplemental Figure S1. Effects of etching on microfluidic culture parameters. **A.** Etching the CV increases the susceptibility of overlying corneal epithelial layers to damage from hydrostatic pressure. While epithelial layers overlying intact or non-etched CV withstand hydrostatic pressures of 3 and 5 inches H_2O (i and ii) and finally succumb to pressures of 7 inches H_2O (iii), corneal epithelial layers overlying etched CV yield to the effects of hydrostatic pressures as low as 3 inches of H_20. **B.** Upon opening the micrcochannel outlets for fluid flow, epithelial cells remained viable for the entire range of media flow rates available for gravity-fed perfusion below the 3 inch H_2O limit. Shown are flow rates of 0.05 nL/min, (i) and 0.31 nL/min (ii).



Supplemental Figure S2: Integration and properties of the Collagen Vitrigel. **A.** Leakage-free integration of the CV into the microfluidic device. The final vacuum-sealed device in which red food dye has been loaded into the basal microfluidic channels maintains seal integrity. **B.i.** Transmission electron microscopy (TEM) of CV showing characteristic banding of fibrillar collagen (arrows). Scale bar: 500 µm. **ii.** TEM of rabbit cornea stroma shows the ordered orthogonal arrangement of collagen fibrils. Scale bar: 1 µm. **C.** An image showing the bulk optical properties of the CV prior to incorporation into the device; note the high transparency as the letter C is clearly identifiable behind the collagen membrane.



Supplemental Figure S3: Optimizing vacuum integration of the CV. **A.i.** A separate device used to optimize vacuum integration of the CV. The surrounding vacuum mesh was used to directly apply fluidic channels to the top of a dried CV. Seperation between the vacuum mesh and fluidic channels was varied between 50 and 200 µm PDMS walls, while vacuum channel widths were varied from 50 to 200 µm. **ii.** Best results were obtained with the 100 µm seperation wall and 200µm vacuum channels. The image shows a leakage free interface with the CV after 20 minutes of flow with red food dye. **iii.** Upon release of the vacuum, the CV remains stained with dye only in areas covered by the fluidic channels in the leakage free devices. **B.i.** Selective rehydration within the device at the access ports results in selective uptake by the CV only in areas exposed to the underlying microfluidic network (as noted by increased fluorescence intensity in the central region overlying the "C"-shaped ring with Texas Red-labeled BSA). **ii.** In separate devices with apical fluidic channels in lieu of punched culture wells, the vacuum network provided a complete seal for exposure to a second tracer dye (fluorescein). **iii.** This seal was maintained when both apical and basal sides of the CV patch were exposed to the separate tracer dyes, resulting in dual-uptake (overlay of i. and ii., shown in iii.). Scale bar: 100 µm.