# ECM Protein Shrink-Wrapped Cells Demonstrate Enhanced Integration into Corneal Endothelium

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**Statement of Purpose:** Disease or injury to the corneal endothelium (CE), the posterior cell layer responsible for maintaining the proper hydration level and ultimately transparency of the cornea, is a major cause of blindness. Corneal transplants are successful at restoring vision; however rejection and limited donor supply have motivated development of alternative therapies. One promising approach is the injection of cultured CE cells into the anterior chamber of the eye to integrate in to the damaged CE to increase the cell density and restore vision. Cell injection therapy is desirable to many patients because it is less invasive than a transplant; however, the retention and viability of cells at the injection site as well as integration and efficacy of single cells have generally been poor, driving the need for improved approaches. To address this problem, we have developed a technique to engineer ECM protein nano-scaffolds that support the formation and shrink-wrapping of microscale monolayers of CE cells, termed µMonolayers [1]. The ECM nano-scaffold helps to increase the attachment of the µMonolayers to the existing CE, while the shrink-wrapping process allows the cells to maintain their cell-cell junctions, which are important for cell integration and function. Our preliminary in vivo results show that shrink-wrapped µMonolayers integrate better than single cells indicating they could be an effective alternative to single cell injection and corneal transplants.

**Methods: µMonolayer Formation:** ECM protein nano-scaffolds were fabricated via surface-initiated assembly, based on a previously described method [1]. Briefly, 200x200 µm squares of laminin and collagen IV were microcontact printed onto the thermoresponsive poly(N-isopropyl-acrylamide) (PIPAAm) and sterilized under UV light. The micropatterned samples were heated to 40ºC, seeded with bovine CE cells, and cultured for 24 hours at 37ºC so the cells could form microscale monolayers termed µMonolayers. After 24 hours, the samples were rinsed with warm media and allowed to cool to room temperature to trigger the dissolution of the PIPAAm and release of the µMonolayers. After release, the µMonolayers were collected via centrifugation and resuspended in DMEM for use in *in vivo* studies.

***In Vivo* Mouse Studies:** To allow for tracking of cells after injection, µMonolayers and enzymatically released single CE cells were labeled with CellTracker. Following labeling, cells were injected through a 32G needle in to the anterior chamber of the right eyes of mice. A DMEM only injection served as controls and the contralateral eye of each mouse served as an internal, no procedure control. Mice were observed daily and sacrificed on day 7. The eyes were removed and fixed for 24 hours in paraformaldehyde before excising the cornea. The corneas were then stained for the nucleus and tight junction protein, ZO-1.

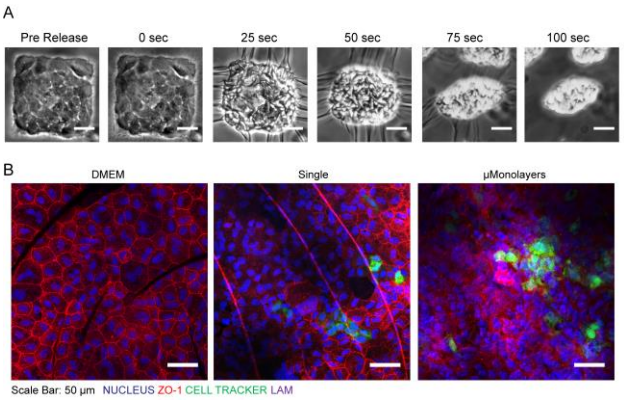


**Figure 1:** A) Phase contrast images showing the release of the µMonolayers from PIPAAm. B) Confocal microscopy images showing the increased integration of injected µMonolayers compared to single cells in mouse eyes.

**Results:** Micro-scale CE monolayers were successfully formed on the micro-patterned ECM squares and shrink-wrapped via the thermal release process (Fig 1A). The cells maintained their tight junctions and cytoskeletal structure through the release and injection process, both of which are important for function and integration. Seven days after injection into a mouse, the corneas were removed, fixed and then imaged by confocal microscopy to detect any green CellTracker labeled cells (Fig. 1B). No green fluorescence was observed in the CE of mice injected with DMEM only, which was expected, indicating any green fluorescence observed in other mice was from injected cells. Very few green cells were observed in the CEs of mice injected with single cells and those that were observed were usually isolated from other injected cells. In contrast, many green cells were observed in the CEs of mice injected with the µMonolayers. The labeled cells were clustered together, indicating they maintained their junctions during attachment and integration, and the ZO-1 staining was continuous between the mice CE cells and the labeled bovine cells, indicating the cells had integrated within the mouse CE and were not on top of the mouse cells creating a multi-layer. Future work will involve larger animal model in vivo studies.

**Conclusions:** These studies indicate that shrink-wrapped cells can achieve better integration into tissues compared to single cells. Clinically, this could enable minimally invasive CE cell delivery to patients as an alternative to corneal transplant.

**References:** [1] Palchesko RN et al. Cell and Mol Bioeng. 2014;7(3):355-368.