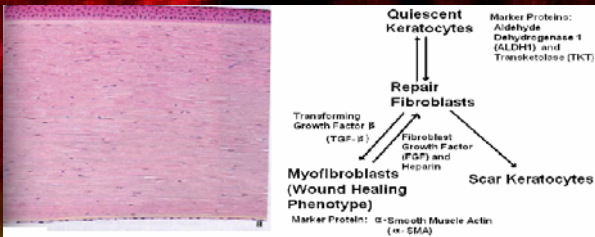


# Effect of Keratocyte-Endothelial Co-culture on Fibroblast Expression of $\alpha$ -SMA, TKT, and ALDH

Stacy Sun, Rebecca Walmsley, Elizabeth Orwin, and Marta Bechtel

## Background

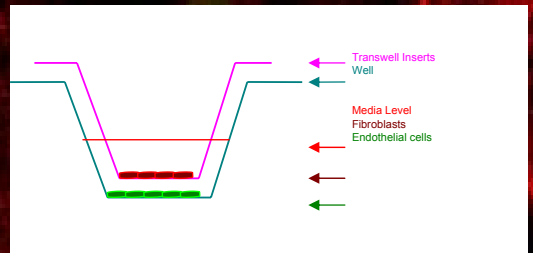
In a healthy cornea, the primary cell type in the stroma is the quiescent keratocyte (Fini, 1999). When the cornea is wounded or grown in vitro, the keratocytes differentiate into the repair fibroblast phenotype and thus upregulate  $\alpha$  smooth muscle actin ( $\alpha$ -SMA) and downregulate ALDH1 and TKT. This differentiation creates corneal haze contributing to a loss of transparency (Berryhill, 2002) (Fig. 1b).



## Experimental Goal

The endothelial-fibroblast co-culture experiment was repeated and the effects of fibroblast expression of alpha-SMA, TKT, and ALDH1 were investigated.

## Method/Setup

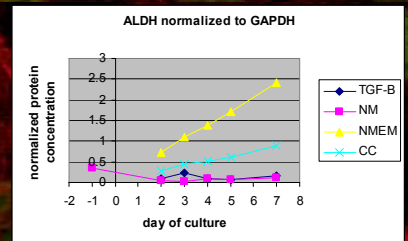
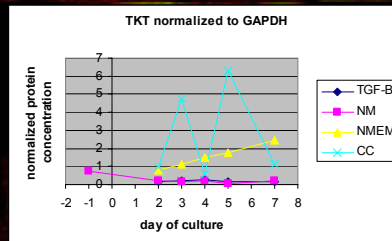
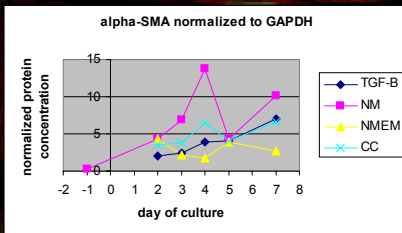


Endothelial cells are plated in six-well plates while fibroblasts are plated in transwell inserts. After one day of culture, the inserts are transferred to the endothelial cell plate for co-culture and fed with 5 mL of NMEM media to cover both cell cultures. Media is able to flow through the membrane of the transwell insert.

## Main Goal

To maximize transparency- revert the differentiation of the myofibroblast phenotype to the quiescent keratocyte phenotype.

## Results



Fibroblasts grown in co-culture with endothelial cells have a sharp increase in levels of  $\alpha$ -SMA at day 4. Fibroblasts cultured in TGF- $\beta$  media had a significant increase in  $\alpha$ -SMA over time. Furthermore, levels of  $\alpha$ -SMA for cells in NM was significantly greater and spiked at day 4 while cells grown in NMEM had little to no change in  $\alpha$ -SMA levels.

Fibroblasts grown in co-culture with endothelial cells display sharp oscillations in protein concentration of TKT, showing high levels on days 3 and 5 and a low level on day 4. TGF- $\beta$  and NM show little change in TKT levels while NMEM increases steadily over the days of culture.

Fibroblasts grown in co-culture with endothelial cells display a slight increase in ALDH levels over the days of culture. NMEM increases in concentration of TKT at a quicker rate than the co-culture samples. TGF- $\beta$  and NM show little changes in ALDH levels and remain constantly low.

## Conclusions

- Validated that TGF-B upregulates alpha-SMA
- Levels of alpha-SMA remain low in co-culture until day 4 in passage 0 experiment and day 3 in passage 1 experiment.
- This may suggest that co-culture delays differentiation from myofibroblastic to quiescent keratocyte phenotype
- Multiple runs of co-culture experiments show large variation and may indicate a unknown variable