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PCL Nanofibers Induce Lens Fiber Formation of Mouse Lens Epithelial Cells

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Introduction

Current cataractous lens replacement therapies require frequent medical checkups due to the potential formation of secondary cataracts resulting from the trans-differentiation of residual lens epithelial cells into mesenchymal myofibroblast cells (EMT). To prevent EMT, current treatments include laser therapy or the administration of anti-fibrotic drugs. Recently, Poly-ε-caprolactone (PCL) has become a popular material for tissue replacement therapy due to its relative durability compared to other biomaterials. For instance, the use of PCL as a nanofibrous scaffold offers a novel tool to model the complex architecture of different tissue types including skin, bone, cartilage, muscle, and brain cells. This study examines the suitability of PCL nanofibers for lens tissue engineering and lens replacement therapies. In an attempt to create a more organized lens fiber alignment without the risk of EMT, this study tests the use of aligned PCL nanofibers as a potential artificial lens matrix for cellular ingrowth and lens epithelial cell differentiation into lens fiber cells.

METHODS

Cell Culture

Staining of primary mouse lens epithelial cells (MLE) with vital dye CFDA-SE (Invitrogen, Grand Island, NY).

Cells plated at proportional 20,000 cells/well into:

-Control Scaffold: Regular 24-well cell culture plates

-Aligned PCL Nanofiber Scaffold: Culture plates coated with Poly-ε-caprolactone (PCL) nanofibers

(average diameter 700nm, average fiber distance 5-10 μm, average thickness of nanofiber layer 20 μm, Part#9602, Nanofiber Solutions, Columbus, OH)

Cell culture Media:

-MEM20%FBS (control culture medium): Minimum essential medium supplemented with 20% fetal bovine serum (Invitrogen, Grand Island, NY)

-MEM2%FBS (serum starvation medium): Minimum essential medium supplemented with 20% fetal bovine serum (Invitrogen, Grand Island, NY)

Immunohistochemistry and Immunoblotting

Primary antibodies:

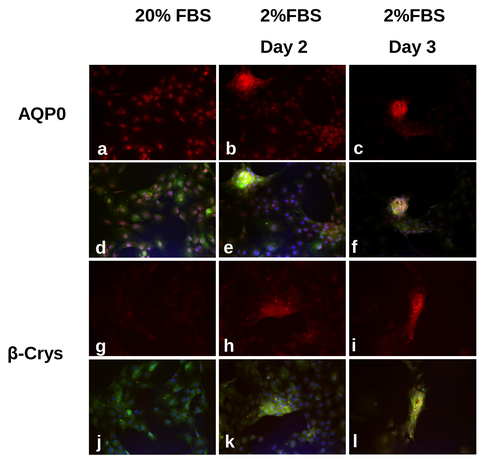
-Aquaporin 0 (AQP0 H-44, sc-99059)

-β-crystallin (β-crys H-3, sc-48335)

(Santa Cruz Biotechnologies, Santa Cruz, CA)

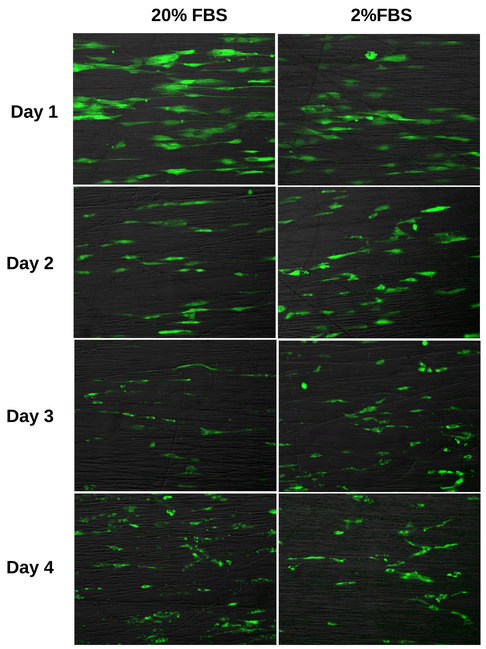
Statistics

For immunoblotting two or three were analyzed in each group. Samples were analyzed by Graph Pad Prism 5 software using students t-test or one-way ANOVA in combination with tukey post-test. p<0.05 was used as criterion for significance.



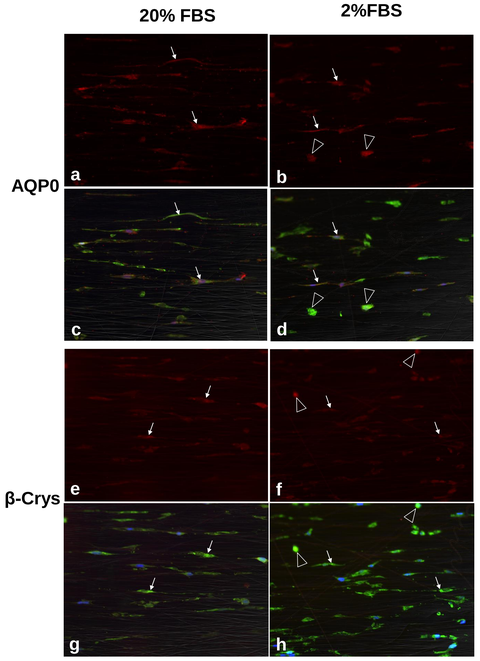
Mouse Lens Epithelial Cells (MLE) Form Lentoids Under Serum Starvation and Demonstrate Expression of Lens-Specific Markers Aquaporin 0 and β-Crystallin

(a,b,c) AQP0 and, (g,h,i) βCrys (red) (d,e,f,j,k,l) overlay with cellular vital dye CFDA-SE (green) and nuclear stain Hoechst (blue).



PCL Nanofibers Induce Alignment and Lens Fiber Differentiation of Mouse Lens Epithelial Cells.

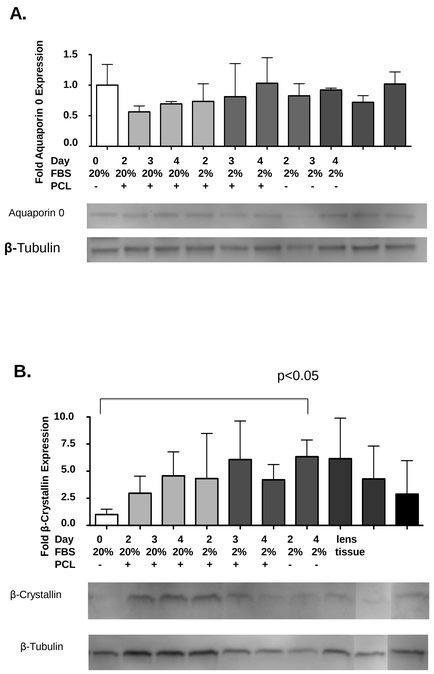
Temporal changes of mouse lens epithelial cell (MLE) morphology over 4 days. Overlay of CFDA-SE vital dye stained MLE (green) with phase contrast image of PCL nanofibers.



PCL Nanofibers Stimulate Lens Fiber Formation with Loss of Nuclei, and Expression of Lens-Specific Markers Aquaporin 0 (AQP0) and β-Crystallin (β-Crys)

(a,b) AQP0 expression (red, open arrows) following 3 days in culture on PCL Nanofibers. (c,d) overlay of AQP0 (red) with cellular vital dye CFDA-SE (green), nuclear stain hoechst (blue) and phase-contrast image of PCL nanofiber scaffold (e,f) β-Crys expression (red, open arrows) following 3 days in culture on PCL Nanofibers.

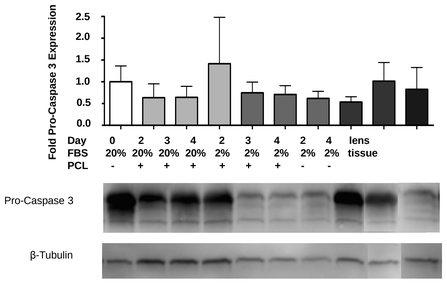
(g,h) overlay of β-crystallin staining (red) with cellular vital dye CFDA-SE (green), nuclear stain hoechst (blue)and phase-contrast image of PCL nanofiber scaffold Fiber formation indicated with arrows.



PCL Nanofiber Induced Changes in Protein Expression of Lens-Specific Markers Aquaporin 0 and β-Crystallin

A.Immunoblot detected Aquaporin 0 expression. Graph shows densitometric changes of MLE cells grown on PCL nanofibers (PCL +) or regular culture dishes (PCL -) in regular culture medium (FBS 20%) or serum-starved medium (FBS 2%). Immunoblot detected Aquaporin 0 levels were normalized against expression of the β-Tubulin housekeeping gene (as demonstrated below graph). Graph shows measurements of samples in duplicates with standard deviations. One-way Anova followed by Bonferroni post-test and students t-test demonstrated no significant changes in Aquaporin 0 Expression. p<0.05 was used as a criterion for significance.

B.Immunoblot detected β-Crystallin expression. Graph shows densitometric changes in Immunoblot detected β-Crystallin expression of MLE cells grown on PCL nanofibers (PCL +) or regular culture dishes (PCL -) in regular culture medium (FBS 20%) or serum-starved medium (FBS 2%). Immunoblot detected β-Crystallin levels were normalized against expression of the β-Tubulin housekeeping gene (as demonstrated below graph). Graph shows measurements of samples in duplicates with standard deviations. One-way Anova followed by Bonferroni post-test revealed no changes in β-Crystallin and students t-test demonstrated a significant change in β-Crystallin expression in the serum starved PCL nanofiber group at day 4 compared to the control. p<0.05 was used as a criterion for significance.



Nuclear Loss of MLE Cells Grown on PCL Nanofibers is Non-Apoptotic

Immunoblot detected Pro-Caspase 3 expression. Graph shows densitometric changes of MLE cells grown on PCL nanofibers (PCL +) or regular culture dishes (PCL -) in regular culture medium (FBS 20%) or serum-starved medium (FBS 2%). Immunoblot detected Pro-Caspase 3 levels were normalized against expression of the β-Tubulin housekeeping gene (as demonstrated below graph). Graph shows measurements of samples in duplicates with standard deviations. One-way Anova followed by Bonferroni post-test and students t-test demonstrated no significant changes in Pro-Caspase 3 Expression. p<0.05 was used as a criterion for significance. In addition, no changes in active caspase 3 were detected by immunoblotting (data not shown) supporting that the observed loss of cell nuclei is due to lens fiber differentiation and not due to PCL-induced apoptotic cell death.

Results and Discussion

- **Over a time period of three days on PCL nanofibers, MLE cells elongate into lens fiber cells with increased cell death and loss of nuclei appearing after four day of culture.**
- **In addition, MLE cells grown on PCL nanofibers also demonstrated expression of lens specific markers such as aquaporin 0 and β-crystallin with an increasing trend in β-crystallin expression over time.**
- **The observed nuclear loss of MLE cells grown on PCL nanofibers is not due to apoptosis as revealed by lack of active caspase 3 detection, suggesting PCL nanofiber-induced lens fiber differentiation of MLE cells.**

Conclusion

The study suggests that aligned PCL nanofibers represent a suitable scaffold material for induction of MLE cell differentiation and lens fiber alignment.