

Supplementary Information

In Vitro Expansion of Corneal Endothelial Cells on Biomimetic Substrates

Rachelle N. Palchesko^{1,2,3}, Kira L. Lathrop^{2,3}, James L. Funderburgh^{2,3}, Adam W. Feinberg^{1,4*}

¹ *Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA 15213*

² *Department of Ophthalmology, University of Pittsburgh, Pittsburgh PA, 15213*

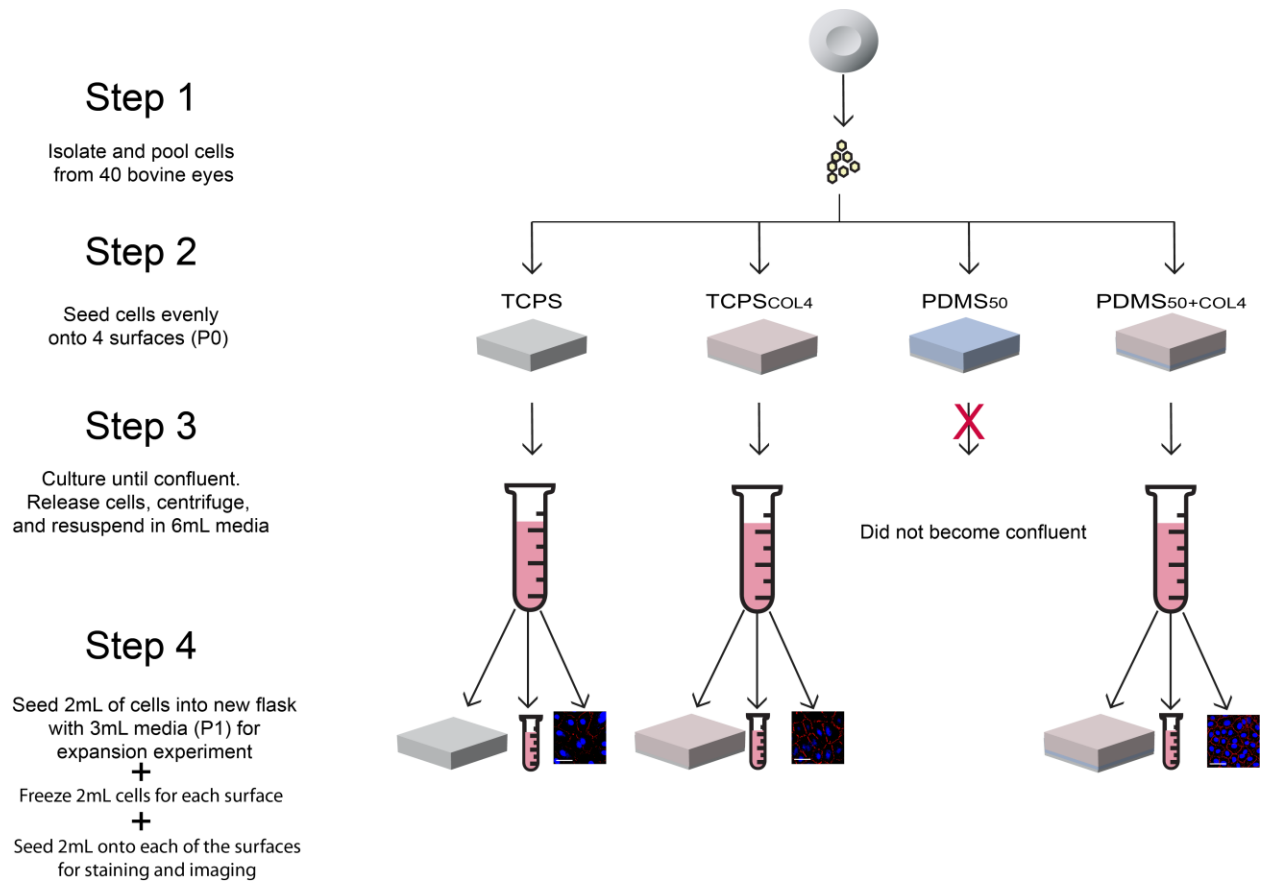
³ *Louis J. Fox Center for Vision Restoration, Pittsburgh PA 15213*

⁴ *Department of Materials Science and Engineering, Carnegie Mellon University, Pittsburgh PA 15213*

* Corresponding author: feinberg@andrew.cmu.edu

	Sylgard 184 1.72 MPa	5:1 1.34 MPa	1:1 830 kPa	1:5 130 kPa	1:10 50 kPa	Sylgard 527 5 kPa
Uncoated						
Fibronectin						
Collagen I						
Laminin						
Collagen IV						
Lam + Col4						

Supplementary Figure S1: Diagram showing the 36 different substrate conditions that were screened to determine the effect of CE cell morphology and structure. Each column represents a different PDMS formulation with a unique elastic modulus and each row represents a different ECM protein coating.



Supplementary Figure S2: Schematic diagram of the layout for the CE cell expansion

experiment. This schematic shows the serial expansion process followed from isolation of the cells from the cornea, through passage 10.

Supplementary Table S1. Details of statistical analysis performed on normalized cell density as a function of culture time (Fig. 3b).

Passage	Power of α	ANOVA P-value	Bonferroni P-values
0	0.999	<0.001	PDMS _{50+COL4} vs. TCPS P < 0.001 TCPS _{COL4} vs. TCPS P = 0.003
1	0.962	0.002	PDMS _{50+COL4} vs. TCPS P = 0.002
2	0.969	0.002	PDMS _{50+COL4} vs. TCPS P = 0.001 TCPS _{COL4} vs. TCPS P = 0.040
3	0.987	<0.001	PDMS _{50+COL4} vs. TCPS P < 0.001
4	1.000	<0.001	PDMS _{50+COL4} vs. TCPS P < 0.001 TCPS _{COL4} vs. TCPS P = 0.039 PDMS _{50+COL4} vs. TCPS _{COL4} P = 0.005
5	0.989	<0.001	PDMS _{50+COL4} vs. TCPS P < 0.001 PDMS _{50+COL4} vs. TCPS _{COL4} P = 0.013
6	1.000	<0.001	PDMS _{50+COL4} vs. TCPS P < 0.001 TCPS _{COL4} vs. TCPS P = 0.018 PDMS _{50+COL4} vs. TCPS _{COL4} P < 0.001
7	0.997	<0.001	PDMS _{50+COL4} vs. TCPS P < 0.001 PDMS _{50+COL4} vs. TCPS _{COL4} P = 0.004
8	0.999	<0.001	PDMS _{50+COL4} vs. TCPS P < 0.001 PDMS _{50+COL4} vs. TCPS _{COL4} P < 0.001
9	0.999	<0.001	PDMS _{50+COL4} vs. TCPS P < 0.001 PDMS _{50+COL4} vs. TCPS _{COL4} P = 0.001
10	0.992	<0.001	PDMS _{50+COL4} vs. TCPS P = 0.001 PDMS _{50+COL4} vs. TCPS _{COL4} P = 0.003

The three groups were statistically compared at each passage to determine any differences in cell density. Data was tested for normality using the Shapiro-Wilk normality test ($P > 0.050$) and passed at each passage. Statistical analysis was done using a one-way ANOVA (α set to 0.050) with Bonferroni post-hoc test to determine statistical significance. The above table shows the exact α and P values reported for the ANOVA test, as well as the P values for each comparison found to statistically significant by the Bonferroni post-hoc test. For each passage, TCPS n=4, TCPS_{COL4} n=5, PDMS_{50+COL4} n=5.

Supplementary Table S2. Details of statistical analysis performed on cell area as a function of culture time (Fig. 3d).

Passage	H value	Degrees of Freedom	P-value for ANOVA on the Ranks	Dunn's Comparisons with P values <0.05
1	1051.022	2	P = < 0.001	PDMS _{50+COL4} vs. TCPS TCPS _{COL4} vs. TCPS PDMS _{50+COL4} vs. TCPS _{COL4}
5	313.394	2	P = < 0.001	PDMS _{50+COL4} vs. TCPS TCPS _{COL4} vs. TCPS PDMS _{50+COL4} vs. TCPS _{COL4}
8	204.578	2	P = < 0.001	PDMS _{50+COL4} vs. TCPS PDMS _{50+COL4} vs. TCPS _{COL4}

The three groups were statistically compared at each passage to determine any differences in cell area. Data was tested for normality using the Shapiro-Wilk normality test ($P > 0.050$) and failed at each passage. Statistical analysis was done using a one-way ANOVA on the ranks with Dunn's pairwise comparison to determine statistical significance. The table above shows the H values, degrees of freedom, P value for the ANOVA on the ranks, and those pairwise comparisons that had a $P < 0.050$. (*Ex vivo* cornea n = 2674; TCPS P1 n = 401, P5 n = 353, and P8 n = 135; TCPS_{COL4} P1 n = 846, P5 n = 443, and P8 n = 98; and PDMS_{50+COL4} P1 n = 1503, P5 n = 673, and P8 n = 318.)

Supplementary Table S3. Details of statistical analysis performed the percent of α -SMA positive cells as a function time (Fig. 4c).

Passage	H value	Degrees of Freedom	P-value for ANOVA on the Ranks	Dunn's Comparisons with P values <0.05
1	6.147	2	P = 0.046	PDMS _{50+COL4} vs. TCPS
5	11.942	2	P = 0.003	PDMS _{50+COL4} vs. TCPS PDMS _{50+COL4} vs. TCPS _{COL4}

The three groups were statistically compared at each passage to determine any differences in percent α -SMA positive nuclei. Data was tested for equal variance and failed at P1 and was tested for normality using the Shapiro-Wilk normality test ($P < 0.050$) and failed at P5. Statistical analysis was done using a one-way ANOVA on the ranks with Tukey test to determine statistical significance. The table above shows the H values, degrees of freedom, P value for the ANOVA on the ranks, and those pairwise comparisons that had a $P < 0.050$.

Supplementary Table S4. Details of statistical analysis performed on the hexagon shape factor as a function of time (Fig. 4d).

Passage	H value	Degrees of Freedom	P-value for ANOVA on the Ranks	Dunn's Comparisons with P values <0.05
1	34.852	2	P = <0.001	PDMS _{50+COL4} vs. TCPS _{COL4} TCPS _{COL4} vs. TCPS
5	171.034	2	P = <0.001	PDMS _{50+COL4} vs. TCPS PDMS _{50+COL4} vs. TCPS _{COL4} TCPS _{COL4} vs. TCPS

The three groups were statistically compared at each passage to determine any differences in the hexagon shape factor. Data for normality using the Shapiro-Wilk normality test ($P < 0.050$) and failed at P1 and P5. Statistical analysis was done using a one-way ANOVA on the ranks with Dunn's pairwise comparison to determine statistical significance. The table above shows the H values, degrees of freedom, P value for the ANOVA on the ranks, and those pairwise comparisons that had a $P < 0.050$. (*Ex vivo* cornea $n = 2674$; TCPS P1 $n = 401$, P5 $n = 353$; TCPS_{COL4} P1 $n = 846$, P5 $n = 443$; and PDMS_{50+COL4} P1 $n = 1503$, P5 $n = 673$.)

Supplementary Table S5. Details of statistical analysis performed on the cell density of the engineered CEs (Fig. 5b).

Time point	Power of α	ANOVA P-value	Tukey test P-value	
48 hours	0.895	0.009	PDMS _{50+COL4} vs. TCPS	P = 0.008
			PDMS _{50+COL4} vs. TCPS _{COL4}	P = 0.044

The three groups were statistically compared to determine any differences in cell density. Data was tested for normality using the Shapiro-Wilk normality test ($P > 0.050$) and passed. Statistical analysis was done using a one-way ANOVA (α set to 0.050) with Tukey test to determine statistical significance. The above table shows the exact α and P values reported for the ANOVA test, as well as the P values for each comparison found to statistically significant by the Tukey test. TCPS n=3, TCPS_{COL4} n=3, PDMS_{50+COL4} n=3.