A Decellularized Dehydrated Human Amniotic Membrane-Derived Biomaterial Supports Human Corneal Epithelial Cell Function and Inflammatory Response

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Statement of Purpose: Successful application of decellularized tissue-based biomaterials for wound healing requires matrix components that support cell function and differentiation. Amniotic membrane (AM) is a naturally derived biomaterial from human placental tissue with unique biological and mechanical properties that render it suitable for use in ocular healing (1,2). The purpose of this study is to evaluate the effects of sidedness and AM processing methodology on human corneal epithelial cell (HCEC) function in vitro. Experimental variables include AM sidedness (epithelial [E] and stromal [S]) and AM processing methodology (decellularized and dehydrated [DDHAM], dehydrated [DHAM], and cryopreserved [CHAM]). Dependent variables include HCEC viability, migration, and inflammatory response.

Methods: Three differently processed, commercially available ocular AMs were selected: Biovance3L Ocular (DDHAM), Ambio2® (DHAM), and AmnioGraft® (CHAM). HCECs were seeded onto the E and S sides of AMs and incubated for 1, 4 and 7 days. Cell viability was measured at each time point on the AMs using alamarBlue assay. Conditioned media from HCECs cultured on the AMs were collected, and the effect of conditioned media on the migration of HCECs was evaluated using a scratch wound assay. An inflammatory response was induced by TNFα treatment. The effect of AM on the expression of pro-inflammatory genes in HCECs was compared using quantitative polymerase chain reaction (qPCR). The significance level for all statistical tests was set at $p = 0.05$. Cell viability was analyzed with a two-way analysis of variance (ANOVA), cell proliferation with a three-way ANOVA, and mRNA expression with a one-way ANOVA. Tukey's and unpaired t-tests were used for post-hoc analyses.

Results: On day 1, cell viability was significantly higher on DDHAM-E&S than CHAM-E&S ($p < 0.001$) and DHAM-E ($p \leq 0.002$). On day 4, cell viability was significantly higher on DDHAM-S than all other variables ($p \leq 0.004$, Fig. 1). In addition, on day 4, cell viability was comparable between DDHAM-E and DHAM-S ($p = 0.147$) and significantly higher than DHAM-E ($p \leq 0.004$), CHAM-S&E ($p \leq 0.017$). On day 7, cell viability was significantly higher on DDHAM-S than DHAM-E ($p = 0.028$) and CHAM-S&E ($p \leq 0.049$). Cell viability was similar between DDHAM-E and all other variables ($p \geq 0.097$). HCEC migration in the presence of conditioned media from cells cultured on DDHAM and DHAM was comparable ($p = 0.885$) and significantly greater than cells grown on CHAM ($p \leq 0.005$). Interestingly, HCECs cultured on DDHAM adapted a cobblestone morphology (Fig. 2), which mimics the morphology of ocular epithelial cells in situ (3). The migration of HCEC in the presence of conditioned media from cells cultured on ocular scaffolds was significantly greater than control conditioned media from cells grown on tissue culture plastic ($p < 0.001$). Moreover, in response to inflammatory stimulation by TNFα, the gene expression of pro-inflammatory cytokines (IL-6, IL-8, and TNFα) in HCECs on DDHAM showed an initial increase followed by a decline across time (Fig. 3).

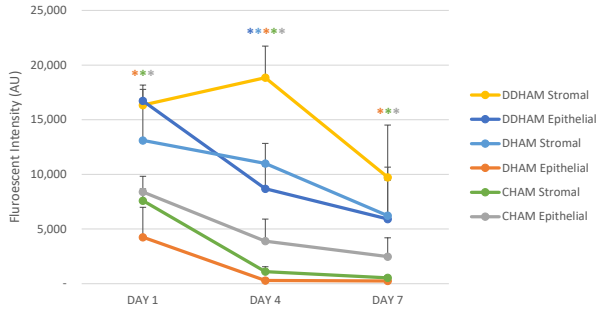

Figure 1. Cell viability on the E&S sides of AMs over 7 days. *$p \leq 0.05$, compared with DDHAM-S. Asterisk color indicates comparator and corresponds with legend.

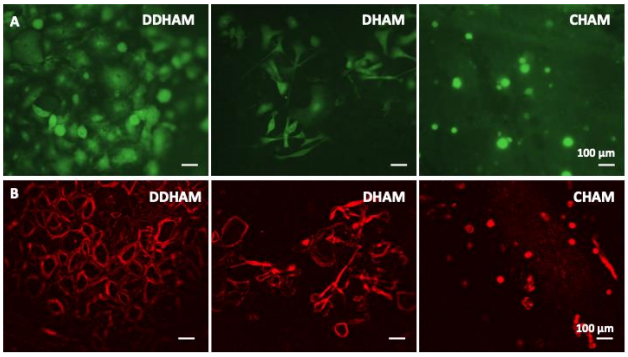

Figure 2. After 4 days, cells on the S side of AMs were stained with CalceinAM to visualize viable cells (A) and with phalloidin to visualize actin (B).

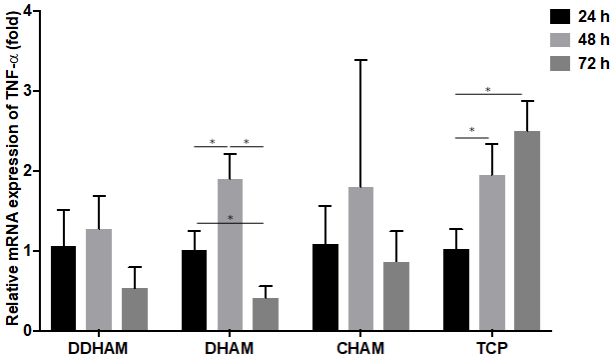

Figure 3. Gene expression of TNFα in HCECs cultured on AMs for 24h, 48 h and 72 h. *$p \leq 0.05$.

Conclusion: In this in vitro study, DDHAM-S best supported HCEC viability and migration. The presence of DDHAM also attenuated the inflammatory response of HCECs over time.

References

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