Establishment of optimal culture media in human corneal epithelial wound healing models

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Introduction

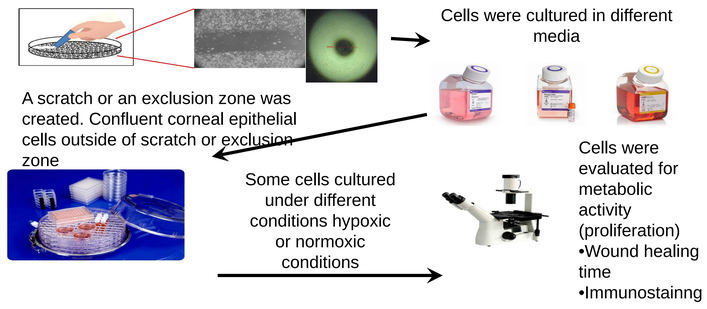
Damage to the corneal epithelium can result in severe vision loss. Epithelial cell damage needs to be quickly repaired to prevent infection and adequate wound healing is required for corneal transplants and recovery from LASIK surgery. To study corneal epithelial wound healing an *in vitro* scratch model and an *in vitro* exclusion zone model are often used. The purpose of this study was to establish the optimum media to use as a control solution in wound healing models.

Purpose

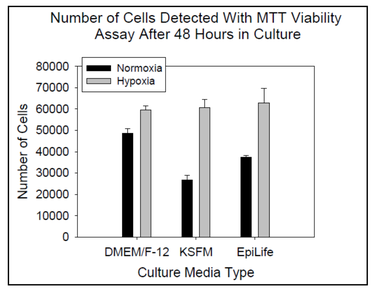
To determine the effect of various media types on the behaviour of corneal epithelial cells, as well as their ability to heal wounds created using a scratch or an exclusion zone *in vitro.*

Methods

Immortalized human corneal epithelial cells were cultured in different growth media. A scratch wound was made on the epithelial cell monolayers and cell recovery was followed for up to 48 hours by measuring the area of the wound. The effect of normoxic and hypoxic conditions on tight junctional integrity and metabolic activity of cells grown in different growth media were also investigated. Using an exclusion zone model, the degree of cell proliferation into the exclusion zone was determined after growth in cell culture media

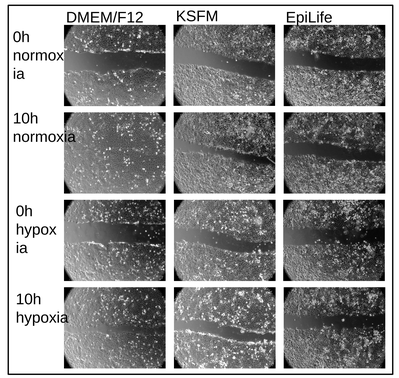


Results



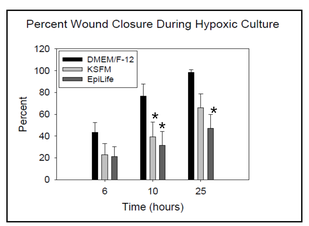
* Significant difference compared to DMEM/F-12 normoxic culture.
† Significant difference compared to KSFM normoxic culture.
§ Significant difference compared to EpiLife normoxic culture.

- The MTT proliferation assay was used to measure the proliferation rate of cells in three media types under normoxic and hypoxic conditions.Under normoxic conditions the highest proliferation rates was seen with DMEM/F-12

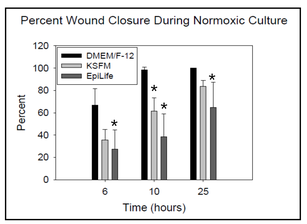


- The % wound closure over time is represented in the graphs below.
- Results suggest that hypoxia slowed healing.
- DMEM/F-12 was significantly better than the other media types at 10 h and better than EpiLife at 25h for both normoxic and hypoxic cultures.

Wound healing with Dulbecco's Modified Eagle Medium: Nutrient mixture F-12 (DMEM/F-12) was significantly faster than both the keratinocyte serum-free medium (KSFM) ($p<0.05$) and EpiLife ($p<0.05$) 10 hours after wounding using the scratch model and nine days after wounding using the exclusion zone technique ($p<0.05$). In addition, hypoxic culture significantly delayed wound healing by an average of 32.4%. In the culture media DMEM/F-12, human corneal epithelial cells stained for abundant zona occludens-1 (ZO-1), connexin 43 (Cx43) and had a high metabolic activity indicating significant epithelial barrier formation, gap junction formation and high cell viability.

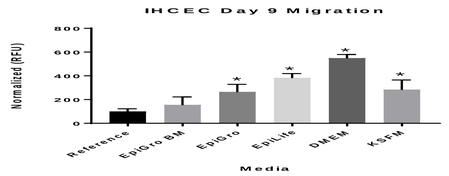


* Significant difference compared to DMEM/F-12 at same time point

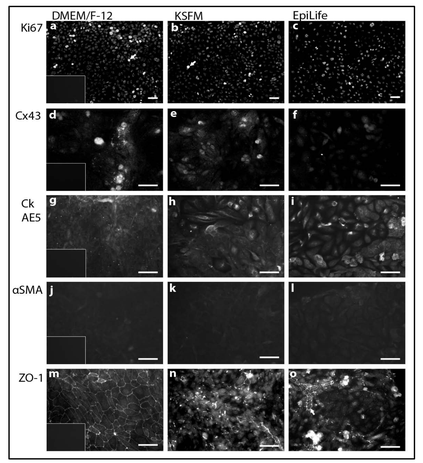


* Significant difference compared to DMEM/F-12 at same time point

Results continued…..



The fluorescence of the cells in the exclusion zone 7 days after culture seeding. *=significantly different from EpiGro BM, $p< 0.05$. DMEM-F12 was also significantly different than EpiLife and KSM media $p<0.05$.



1.) When stained with ki67, more cells were found to be in prophase of the cell cycle (solid nuclear staining; arrow) in DMEM/F-12 (a) than In KSFM (b) or EpiLife (c).
2.) Connexin 43 staining was positive for both DMEM/F-12 (d) and KSFM (e) cultures but was very weak for EpiLife cultures (f).
3.) Cells in all three media types were positive for cytokeratin (g-l; epithelial cell marker) and negative for α-smooth muscle actin (j-l; HeLa cell marker).
4.) ZO-1 staining indicated that there were uniform tight junctions across the monolayer of DMEM/F-12 cultures (m); patchy tight junctions in KSFM cultures were present (n); no complete tight junctions werefound in EpiLife cultures (o).

Conclusions

MEM/F-12 led to superior wound healing under hypoxic and normoxic conditions and in two different wound healing models. DMEM/F-12 appears to be the optimum wound healing control for corneal wound healing models due to superior metabolic activity, wound healing and formation of a greater number of tight junctional proteins in cells grown in this medium over the other media tested.

References

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