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Differentiation of Morphological Changes Seen in *Anopheles* Cell Lines Over Time

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INTRODUCTION & AIM

Mosquito cell lines such as SuaE1 (An. gambiae), MSQ43 (An. stephensi), and 4a-2 (An. coluzzii) differ from traditional mammalian cell models as these mosquito cell lines were generated through spontaneous immortalization rather than engineered immortalization. Because these cells are not truly immortalized, multiple intrinsic and extrinsic factors influence their growth behavior and phenotypic stability. This presents an inherent challenge at the outset of experimental design, as the cells themselves cannot be fully standardized across experiments. Mosquito cell culture remains relatively uncommon in molecular biology research aimed at reducing vector-borne disease, however, in vitro mosquito cell culture offers important advantages over whole-mosquito studies, including lower cost, faster experimental timelines, and reduced resource and technical requirements. These advantages make mosquito cell culture an attractive and scalable model system for studying vector biology and host-pathogen interactions.

The establishment of cell lines generally benefit from a standardized approach which should allow for consistent repeatable results over time. While this makes them valuable and accessible tools, variability from incomplete immortalization and culture conditions complicates reproducibility. Culture conditions and the length of time a cell line has been maintained in culture can affect gene expression profiles. As a result, measured experimental variables may differ not only due to experimental treatments, but also due to underlying differences in the cells themselves, particularly at the level of gene expression.

In this project, we have a three-fold goal: (1) investigate the core components seen across different cell culture media for mosquito cell lines, (2) determine which media components increase or decrease the viability of the cells, and (3) how cell media may increase or decrease the variability and potential dedifferentiation of SuaE1 (*An. gambiae*), MSQ43 (*An. stephensi*), and 4a-2 (*An. coluzzii*) cell lines. Together, these considerations highlight that common variables, such as media composition, culture conditions, and cell line history, contribute substantially to differences in experimental outcomes observed in the laboratory.

METHOD

To investigate phenotypic stability and differentiation tendencies in mosquito cell lines, we continuously cultured MSQ43 (*Anopheles stephensi*), SuaE1 (*Anopheles gambiae*), and 4a-2 (*Anopheles coluzzii*) cells and documented their morphology changes across serial passages over several months. MSQ43 cells were maintained in Complete Cell Media (CCM) at room temperature in an incubator supplemented with 10% CO_2, whereas SuaE1 and 4a-2 cells were cultured in Schneider's Insect Medium at room temperature without CO_2. Cell morphology was systematically monitored and recorded using bright-field microscopy at 40× magnification throughout the culture period. In parallel, we assessed the influence of media composition and laboratory environmental conditions on observed phenotypic variation and de-differentiation changes among the cell lines.

To contextualize our findings, we conducted a structured literature review using Grok, Google Scholar, and PubMed, prioritizing studies on mosquito cell line origin, spontaneous immortalization, morphological variability, differentiation in immortalized cells, culture reproducibility, and insect cell line stability, with comparative reference to mammalian immortalization strategies.

RESULTS & DISCUSSION

PMID	Cell line	Media Base	% FBS Percentage	Heat-inactivation FBS	CO2 5% added	Temperature (°C)
23407365	A. stephensi (MSQ43)	MEM	5%	Not HI	Y	29
16540402	A. stephensi (MSQ43)	MEM	5%	Not HI	Y	27
36922882	A. stephensi (MSQ43)	MEM	10%	heat-inactivated	Y	28
15845481	A. stephensi (MSQ43)	modified MEM	5%	heat-inactivated	Y	28
15845481	A. stephensi (MSQ43)	modified MEM	5%	heat-inactivated	Y	28
25658622	A. stephensi (MSQ43)	Schneider's medium	10%	Not HI	N	27
17201765	A. stephensi (MSQ43)	Schneider's medium	10%	heat-inactivated	N	25
39405338	A. stephensi (MSQ43)	Schneider's medium	10%	heat-inactivated	N	28

Table 1. Culture conditions used for Anopheles stephensi (MSQ43) mosquito cell lines| The cell lines were grown in either MEM, modified MEM, or Schneider's medium, with fetal bovine serum (FBS) concentrations of 5% or 10%. Some studies used heat-inactivated FBS, while others did not. CO_2 supplementation at 5% was included in all MEM and modified MEM conditions, but not in Schneider's medium. Incubation temperatures varied between 25°C and 29°C

RESULTS & DISCUSSION

We observed significant, fully penetrant morphological changes in two of our three cell lines (SuaE1: high severity; 4a-2: moderate severity), including stratification, shape changes, differences in flask adherence, and cyst-like division. These phenotypes were absent in one line (MSQ-43).

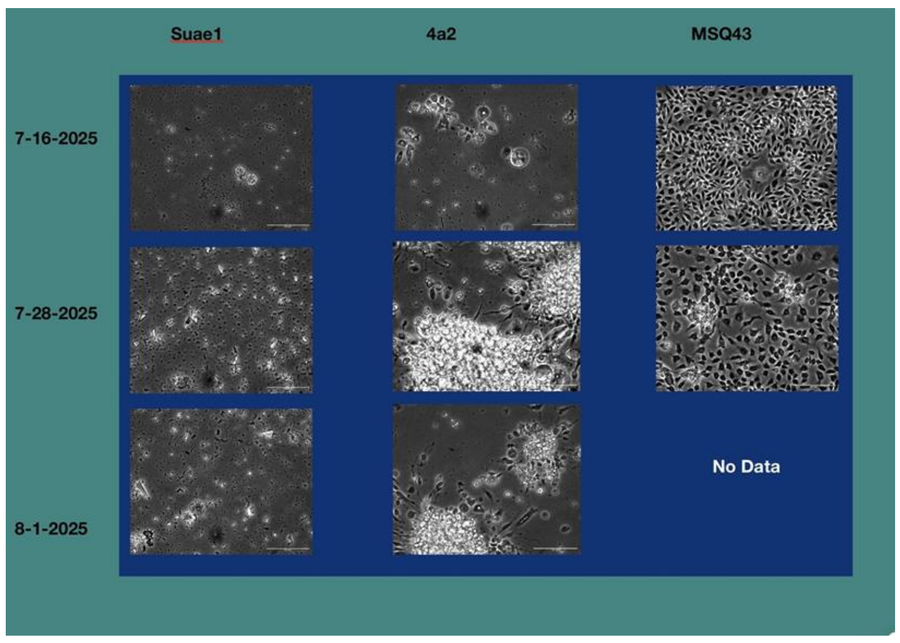

Figure 1. Bright-field microscopy images (40× magnification) of Anopheles cell lines cultured over time. Columns show Sua (left), 4a-2 (middle), and MSQ43 (right) cells, with rows corresponding to different passage dates (7-15-2025, 7-28-2025, and 8-1-2025). Across passages, significant morphological differences emerged, including changes in cell shape, adherence, clustering, and density. These findings highlight the phenotypic variability that develops during continuous culture of mosquito cell lines.

CONCLUSION

Our observations of significant morphological changes over time in SuaE1, MSQ43, and 4a-2 cells are consistent with prior reports describing variability in mosquito cell lines during extended culture and suggest that spontaneous immortalization contributes to ongoing phenotypic instability. Because mosquito cell lines arise through random mutations acquired during continuous passaging, they may diverge substantially from their original tissue of origin, raising important concerns for reproducibility in downstream applications such as transcriptomics, proteomics, and pathogen–interaction studies.

Additional heterogeneity likely stems from the fact that these lines were established from mixed populations of larval cells, making it unclear which specific cell types acquired immortalizing mutations and whether multiple cell types persist within a single culture. Furthermore, repeated passaging and distribution between laboratories and manufacturers can enrich or eliminate subpopulations depending on splitting practices, resulting in line-specific or even batch-specific differences in morphology and behavior; consequently, researchers working with the same cell line may in fact be studying biologically distinct populations.

In this context, standardized culture conditions are critical, as differences in media formulation and supplementation can influence cell growth and contribute to troubleshooting during media preparation, potentially driving perceived differentiation from the expected immortalized phenotype. Both Eagle's MEM and Schneider's medium effectively support mosquito cell growth. MEM requires 5% CO_2 to maintain an acidic pH conducive to insect cells and Schneider's medium is sufficiently buffered to function without CO_2, both require 5–10% fetal bovine serum supplementation. Together, these findings highlight the importance of careful documentation and standardization of culture history and conditions when interpreting experimental results from mosquito cell lines.

FUTURE WORK / ACKNOWLEGEMENTS

- We will next further extend knowledge and awareness of cell culture data variability in mosquitoes using existing data sets of the NIH and our own culture data as we investigate multi-omics of these cell lines.
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